SECTION 9

# DRUGS ACTING ON KIDNEY

## Chapter 41a Relevant Physiology of Urine Formation

Urine formation starts from glomerular filtration (g.f.) in a prodigal way. Normally, about 180 L of fluid is filtered everyday: all soluble constituents of blood minus the plasma proteins (along with substances bound to them) and lipids, are filtered at the glomerulus. More than 99%

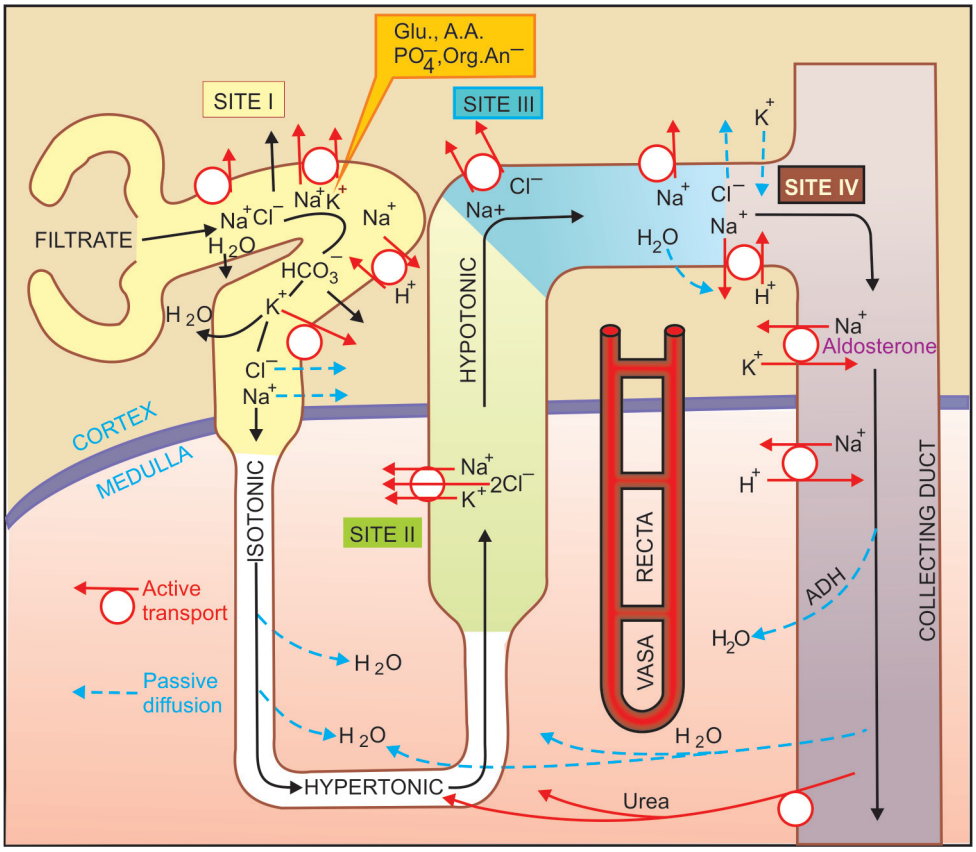



**Fig. IX.1:** Diagrammatic representation of nephron showing the four sites of solute reabsorption. The thick ascending limb of loop of Henle is impermeable to water; Glu.—Glucose; A.A.—Amino acid; Org. An.—Organic anions.

of the glomerular filtrate is reabsorbed in the tubules; about 1.5 L urine is produced in 24 hours. The diuretics act primarily by inhibiting tubular reabsorption: just 1% decrease in tubular reabsorption would more than double urine output.

The mechanisms that carryout ion movement across tubular cells are complex and involve a variety of energy dependent transmembrane pumps as well as channels in between the loose fitting cells of the proximal tubule (PT). All $Na^+$ that enters tubular cells through the luminal membrane is pumped out of it into the renal interstitium at the basolateral membrane by $Na^+K^+$ATPase energised $Na^+$-$K^+$ antiporter (*see* Figs 41.1 and 41.2). Because there is a large intracellular to extracellular gradient for $K^+$, it diffuses out through $K^+$ channels to be recirculated by the $Na^+$-$K^+$ antiporter. For simplification, tubular reabsorption can be divided into four sites (Fig. IX.1).

**Site I: Proximal tubule** Four mechanisms of $Na^+$ transport have been defined in this segment.

(a) Direct entry of $Na^+$ along a favourable electrochemical gradient. This is electrogenic.

(b) Transport of $Na^+$ and $K^+$ coupled to active reabsorption of glucose, amino acids, other organic anions and $PO_4^{3-}$ through specific symporters. Only the glucose coupled $Na^+$ reabsorption is electrogenic.

(c) Exchange with $H^+$: The PT cells secrete $H^+$ with the help of a $Na^+$-$H^+$ antiporter ($Na^+$-$H^+$ exchanger) located at the luminal membrane. This exchange moves $Na^+$ from tubular fluid to inside the cell. The secreted $H^+$ combines with $HCO_3^-$ in the tubular fluid to form carbonic acid (Fig. IX.2). This $H_2CO_3$ is broken into $H_2O$ + $CO_2$ by membrane bound brush border CAse (Type IV enzyme), because spotaneous dissociation of $H_2CO_3$ is very slow. Both $CO_2$ and $H_2O$ diffuse inside the cell and recombine to form $H_2CO_3$ (intracellular soluble type II CAse catalysed reaction) which is the source of $H^+$. The dissociated $HCO_3^-$ in the cell is transported

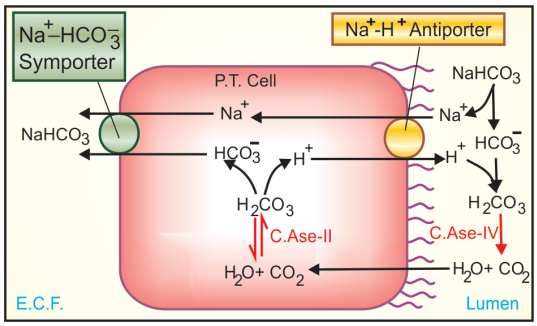



**Fig. IX.2:** The carbonic anhydrase (C.Ase) mediated bicarbonate absorption in proximal tubule (P.T.)

to cortical e.c.f. by basolateral membrane $Na^+$-$HCO_3^-$ symporter resulting in net reabsorption of $NaHCO_3$. Practically all $HCO_3^-$ is reabsorbed in PT by this mechanism, because tubular membrane, as such, is relatively impermeable to $HCO_3^-$.

(d) The disproportionately large $HCO_3^-$, acetate, $PO_4^{3-}$, amino acid and other anion reabsorption create passive driving forces for $Cl^-$ to diffuse through the paracellular pathway (in between tubular cells), particularly in the later PT. This takes $Na^+$ and water along to maintain electrical neutrality and isotonicity; reabsorption in PT is isotonic.

Major part of filtered $K^+$ is reabsorbed in the PT. Thus, an isotonic tubular fluid with major changes in composition enters the thin descending limb of loop of Henle.

**Site II: Ascending limb of loop of Henle (Asc LH)** The thick AscLH can be distinguished into two distinct portions:

(i) Medullary part lined by cuboidal cells.
(ii) Cortical part lined by flattened cells.

Both portions are relatively impermeable to water but absorb salt actively and thus dilute the tubular fluid.

In the *medullary portion* a distinct luminal membrane carrier transports ions in the stoichiometric ratio of $Na^+$-$K^+$-$2Cl^-$ (*see* Fig. 41.1), and is nonelectrogenic. The $Na^+$ that enters the cell is pumped to e.c.f. by $Na^+$ $K^+$ ATPase at the basolateral membrane. In addition, a $Na^+$-$Cl^-$

symporter moves $Cl^-$ down its electrochemical gradient into e.c.f. and carries $Na^+$ along. As the tubular fluid traverses AscLH it progressively becomes hypotonic. Accumulation of NaCl in the medullary interstitium without accompanying water makes it hypertonic: a corticomedullary osmotic gradient is set up. This draws in water from the descending limb of loop of Henle (this thin segment has high osmotic water permeability but lacks active NaCl transport) so that the fluid that enters AscLH becomes hypertonic. A 4 times higher osmolarity of medullary tip (papilla) is maintained by the hairpin structure of the loop of Henle acting as passive *counter current multiplier* and the arrangement of blood vessels as *vasa recti* with shunts that prevents washing away of the osmotic gradient by progressively reducing blood flow to the inner medulla. Because of meagre blood supply, renal papilla is so prone to necrosis and suffers maximum damage when a toxic substance is being excreted.

**Site III: Cortical diluting segment of loop of Henle** This segment, also impermeable to water, continues to absorb salt, but here it is through a $Na^+$-$Cl^-$ symporter (*see* Fig. 41.2). Tubular fluid gets further diluted.

**Site IV: Distal tubule (DT) and collecting duct (CD)** In the late DT and CD, $Na^+$ is again actively reabsorbed; the cation-anion balance being maintained partly by passive $Cl^-$ diffusion and partly by secretion of $K^+$ and $H^+$. Absorption of $Na^+$ at this site occurs through a specific amiloride sensitive $Na^+$ channel and is controlled to a large extent by aldosterone (*see* Fig. 41.3). This provides fine tuning to electrolyte excretion according to body needs.

In common with other cells, the DT and CD cells are rich in $K^+$; a chemical gradient exists for its diffusion into tubular lumen which is aided by the lumen negative transepithelial potential difference in this part of the tubule. The luminal membrane possesses an active secretory pump for $H^+$ which is again governed by movement of $Na^+$ in the reverse direction. Any diuretic acting proximal to the aldosterone sensitive ion exchange site causes an increased delivery of $Na^+$ to the distal nephron—more exchange with $K^+$ takes place. Thus, $K^+$ is reabsorbed in the PT and AscLH, and is secreted in the DT and CD. The net $K^+$ loss is regulated by variations in the secretory process and depends on:

(i) The $Na^+$ load delivered to distal segment
(ii) Presence or absence of aldosterone
(iii) Availability of $H^+$
(iv) Intracellular $K^+$ stores

The characteristic feature of cells lining CD is their responsiveness to antidiuretic hormone (ADH). If ADH is absent, the hypotonic fluid entering CD is passed as such → dilute urine is produced during water loading. If ADH levels are high, CD cells become fully permeable to water → equilibrate with hyperosmotic medulla → concentrated urine is passed, as occurs during water deprivation or hypertonic saline infusion.

The CD and thin AscLH are the only segments permeable to urea. ADH promotes insertion of urea transporter ($UT_1$ or VRUT) into the luminal membrane of CD cells → more urea is accumulated in the medullary interstitium, reinforcing the medullary hypertonicity during water deprivation.

**Free water clearance** It is defined as the volume of urine excreted per unit time in excess of that required to excrete the contained solute isoosmotically with plasma. It is positive when dilute urine is passed in the absence of ADH and negative when concentrated urine is passed in the presence of ADH. If isotonic urine is passed, regardless of its volume, free water clearance is zero.

Both positive and negative free water clearance are dependent on the production of a corticomedullary osmotic gradient; diuretics acting on medullary AscLH depress both.

**Organic ion transport** The PT has non-specific bidirectional active transport mechanism, separately for organic acids and organic bases. However, the magnitude of

transport in the two directions may vary from compound to compound, e.g. reabsorption of uric acid is generally more than its secretion, while in case of penicillin the converse is true. Important diuretics like furosemide, thiazides and amiloride utilize this transport to approach their site of action from the luminal side of the tubule in the AscLH/DT/CD.

### Regulation of renal function

Glomerular filtration rate (g.f.r.) is dependent on the pumping action of heart, the magnitude of renal blood flow and the relative dimensions of afferent and efferent glomerular vessels. Thus, systemic and intrarenal haemodynamic changes can reflect in g.f.r.

About 80% nephrons lie in outer cortex, have short loops of Henle and low $Na^+$ reabsorptive capacity; while 20% or so are juxtamedullary, possess long loops of Henle and are largely responsible for creating the corticomedullary osmotic gradient. Redistribution of blood flow between these two types of nephrons can alter salt and water excretion. Further, haemodynamic changes within different segments of renal vasculature can alter pressure relationships which govern flow of solute and water.

The renin-angiotensin-aldosterone system has a profound bearing on distal tubular reabsorption of $Na^+$ and secretion of $K^+/H^+$. Angiotensin II produced locally in the kidney has direct effects on intrarenal vascular beds as well as on salt and water reabsorption (*see* Ch. 36).

Sympathetic stimulation of kidney results in renin release which would indirectly affect tubular transport. In addition, adrenergic drugs can directly enhance reabsorption of salt and water.

Prostaglandins (PGs) are produced locally in kidney; act as modulators of renal circulation and renin release. $PGE_2$ inhibits the action of ADH and has direct effects on tubular reabsorption.

A natriuretic hormone produced by the atrium (atrial natriuretic peptide: ANP) and may be other sites also has been found to be important in inducing natriuresis in response to salt and volume overload. It mediates 'escape' from long-term aldosterone action.

All nephrons are so arranged that the Asc LH passes close to the early PT of the same nephron. The macula densa cells are thus in close contact with afferent and efferent arterioles. This provides opportunity for feedback regulation of single unit function.

### Relation to diuretic action

The relative magnitudes of $Na^+$ reabsorption at different tubular sites are:

| | |
|---|---|
| PT 65–70%; | Asc LH 20–25%; |
| DT 8–9%; | CD 1–2%. |

The maximal natriuretic response to a diuretic can give a clue to its site of action. It may appear that diuretics acting on PT should be the most efficacious. However, these agents are either too weak or cause distortion of acid-base balance (CAse inhibitors). Moreover, their effect may be obscured by compensatory increase in reabsorption further down the nephron, because the reserve reabsorptive capacity of diluting segments is considerable and can overshadow more proximal actions.

A diuretic having primary action on medullary Asc LH (furosemide) can produce substantial effect because of limited capacity for salt absorption in DT and CD. This also explains why agents acting on DT and CD ($K^+$ sparing diuretics) evoke only mild saluretic effect. Diuretics acting on cortical diluting segment (thiazides) are intermediate between these two.

# Chapter 41b Diuretics

Diuretics (natriuretics) are drugs which cause a net loss of $Na^+$ and water in urine. However, $Na^+$ balance is soon restored, even with continuing diuretic action, by compensatory homeostatic mechanisms of the body, albeit with a certain degree of persisting $Na^+$ deficit and reduction in extracellular fluid volume.

Based on the diuretic action of calomel, organomercurials given by injection were introduced in the 1920s and dominated for nearly 40 years. The CAse inhibitors were developed in the 1950s from the observation that early sulfonamides caused acidosis and mild diuresis. The first modern orally active diuretic *chlorothiazide* was produced in 1957, and by early 1960s its congeners (thiazide diuretics) were already in common use. Availability of *furosemide* and *ethacrynic acid* by mid 1960s revolutionized the pattern of diuretic use. The aldosterone antagonist and other $K^+$ sparing diuretics *spironolactone* and *triamterene/amiloride* were developed in parallel to these.

Diuretics are among the most widely prescribed drugs. Application of diuretics in the management of hypertension has outstripped their use in edema. Availability of diuretics has also had a major impact on the understanding of renal physiology.

## CLASSIFICATION

1. *High efficacy diuretics (Inhibitors of $Na^+$-$K^+$-$2Cl^-$ cotransport)*
   *Sulphamoyl derivatives*
   Furosemide, Bumetanide, Torasemide
2. *Medium efficacy diuretics (Inhibitors of $Na^+$-$Cl^-$ symport)*
   (a) *Benzothiadiazines (thiazides)*
   Hydrochlorothiazide, Benzthiazide, Hydroflumethiazide, Bendroflumethiazide
   (b) *Thiazide like (related heterocyclics)*
   Chlorthalidone, Metolazone, Xipamide, Indapamide, Clopamide
3. *Weak or adjunctive diuretics*
   (a) *Carbonic anhydrase inhibitors*
   Acetazolamide
   (b) *Potassium sparing diuretics*
   (i) *Aldosterone antagonist*: Spironolactone, Eplerenone
   (ii) *Inhibitors of renal epithelial $Na^+$ channel*: Triamterene, Amiloride.
   (c) *Osmotic diuretics*
   Mannitol, Isosorbide, Glycerol

Other high ceiling diuretics, *viz. ethacrynic acid* and organomercurials *(mersalyl)* are only historical.

## HIGH CEILING (LOOP) DIURETICS
(Inhibitors of $Na^+$-$K^+$-$2Cl^-$ Cotransport)

**Furosemide (Frusemide)** Prototype drug

The development of this rapidly acting highly efficacious oral diuretic was a breakthrough. Its maximal natriuretic effect is much greater than that of other classes. The diuretic response goes on increasing with increasing dose: upto 10 L of urine may be produced in a day. It is active even in patients with relatively severe renal failure. The onset of action is prompt (i.v. 2–5 min., i.m. 10–20 min., oral 20–40 min.) and duration short (3–6 hours).

The major site of action is the thick AscLH (therefore called *loop diuretics*) where furosemide inhibits $Na^+$- $K^+$-$2Cl^-$ cotransport (site II, Fig. 41.1). A minor component of action on PT has also been indicated. It is secreted in PT by organic anion transport and reaches AscLH where it acts from luminal side of the membrane. The cortico-medullary osmotic gradient is abolished and positive as well as negative free water clearance is blocked. $K^+$ excretion is increased mainly due to high $Na^+$ load reaching DT. However, at equinatriuretic doses, $K^+$ loss is less than that with thiazides.

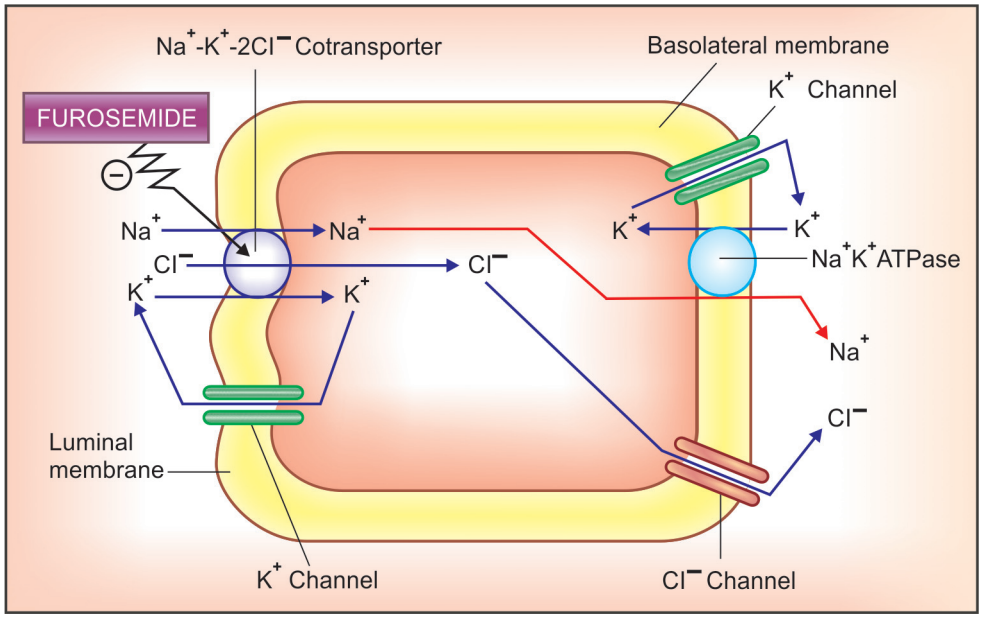



**Fig. 41.1:** Mechanism of salt reabsorption in the thick ascending limb of loop of Henle (AscLH) cell, and site of action of furosemide on the $Na^+$-$K^+$-$2Cl^-$ cotransporter



Furosemide has weak CAse inhibitory action; increases $HCO_3^-$ excretion as well; urinary pH may rise but the predominant urinary anion is $Cl^-$. Therefore, acidosis does not develop. The diuretic action is independent of acid-base balance of the body and it causes little distortion of the same; mild alkalosis occurs at high doses.

In addition to its prominent tubular action, furosemide causes acute changes in renal and systemic haemodynamics. After 5 min of i.v. injection, renal blood flow is transiently increased and there is redistribution of blood flow from outer to midcortical zone; g.f.r. generally remains unaltered due to compensatory mechanisms despite increased renal blood flow. Pressure relationship between vascular, interstitial and tubular compartments is altered, the net result of which is decreased PT reabsorption. The intrarenal haemodynamic changes are brought about by increased local PG synthesis.

Furosemide also sets in motion compensatory mechanisms which tend to limit its diuretic action. Interference with $Na^+$ entry into maculadensa causes marked renin release. Activation of the renin-angiotensin-aldosterone system is the major compensatory mechanism. Reflex sympathetic stimulation of the kidney reinforces renin release. These mechanisms restore $Na^+$ balance after termination of the diuretic action. Because of this phenomenon and short t½ of furosemide, its once daily administration may have less marked overall effect on $Na^+$ status of the body.

Intravenous furosemide causes prompt increase in systemic venous capacitance and decreases left ventricular filling pressure, even before the saluretic response is apparent. This action also appears to be PG mediated and is responsible for the quick relief it affords in LVF and pulmonary edema.

Furosemide increases $Ca^{2+}$ excretion (contrast thiazides which reduce it) as well as $Mg^{2+}$ excretion by abolishing transepithelial potential difference in the thick AscLH which drives reabsorption of these divalent cations. It tends to raise blood uric acid level by competing with its proximal tubular secretion as well as by increasing reabsorption in PT which is a consequence of reduced e.c.f. volume. The magnitude of hyperuricaemia is lower than that with thiazides. A small rise in blood sugar level may be noted after regular use of furosemide, but is again less marked compared to thiazides.

*Molecular mechanism of action*: A glycoprotein with 12 membrane spanning domains has been found to function as the $Na^+$-$K^+$-$2Cl^-$ cotransporter in many epithelia performing secretory/absorbing function, including AscLH. Recently, distinct *absorptive* and *secretory* isoforms of $Na^+$-$K^+$-$2Cl^-$ cotransporter have been isolated. The former is exclusively expressed at the luminal membrane of thick AscLH—furosemide attaches to the $Cl^-$ binding site of this protein to inhibit its transport function. The secretory form is expressed on the basolateral membrane of most glandular and epithelial cells.

*Pharmacokinetics* Furosemide is rapidly absorbed orally but bioavailability is about 60%. In severe CHF oral bioavailability may be markedly reduced necessitating parenteral administration. Lipid-solubility is low, and it is highly bound to plasma proteins. It is partly conjugated with glucuronic acid and mainly excreted unchanged by glomerular filtration as well as tubular secretion. Some excretion in bile and directly in intestine also occurs. Plasma t½ averages 1–2 hour but is prolonged in patients with pulmonary edema, renal and hepatic insufficiency.

*Dose* Usually 20–80 mg once daily in the morning. In renal insufficiency, upto 200 mg 6 hourly has been given by i.m./i.v. route. In pulmonary edema 40–80 mg may be given i.v.

LASIX 40 mg tab., 20 mg/2 ml inj. LASIX HIGH DOSE 500 mg tab, 250 mg/25 ml inj; (solution degrades spontaneously on exposure to light), SALINEX 40 mg tab, FRUSENEX 40, 100 mg tab.

**Bumetanide** It is similar to furosemide in all respects, but is 40 times more potent. It induces very rapid diuresis and is highly effective in pulmonary edema. However, the site of action, ceiling effect, renal haemodynamic changes and duration of action are similar to furosemide. A secondary action in PT has also been demonstrated. Bumetanide may act in some cases not responding to furosemide, and may be tolerated by patients allergic to furosemide. Hyperuricaemia, $K^+$ loss, glucose intolerance and ototoxicity are claimed to be less marked, but it may rarely cause myopathy.

Bumetanide is more lipid-soluble; oral bioavailability is 80–100%. It is preferred for oral use in severe CHF, because its bioavailability is impaired to a lesser extent than that of furosemide. Bumetanide is extensively bound to plasma proteins, partly metabolized and partly excreted unchanged in urine. Its accumulation in tubular fluid is less dependent on active secretion. Plasma t½ ~60 min. It gets prolonged in renal and hepatic insufficiency.

*Dose:* 1–5 mg oral OD in the morning, 2–4 mg i.m./i.v., (max. 15 mg/day in renal failure).

BUMET, 1 mg tab., 0.25 mg/ml inj.

**Torasemide (Torsemide)** Another high ceiling diuretic with properties similar to furosemide, but 3 times more potent. Oral absorption is more rapid and more complete. The elimination t½ (3.5 hours) and duration of action (4–8 hours) are longer. Torasemide has been used in edema and in hypertension.

*Dose:* 2.5–5 mg OD in hypertension; 5–20 mg/day in edema; upto 100 mg BD in renal failure.

DIURETOR 10, 20 mg tabs, DYTOR, TIDE 5, 10, 20, 100 mg tabs, 10 mg/2 ml inj.

### Use of high ceiling diuretics

1. *Edema* Diuretics are used irrespective of etiology of edema—cardiac, hepatic or renal. The high ceiling diuretics are preferred in CHF for rapid mobilization of edema fluid (*see* Ch. 37). Thiazides may be used for maintenance, but often prove ineffective and high ceiling drugs are called in. For nephrotic and other forms of resistant edema, only the high ceiling diuretics are effective, and are the drugs of choice. In chronic renal failure massive doses have to be used, but they continue to be effective while thiazides just do not produce any action. In impending acute renal failure, loop diuretics may decrease the need for dialysis.

2. *Acute pulmonary edema (acute LVF, following MI):* Intravenous administration of furosemide or its congeners produces prompt relief. This is due to vasodilator action that precedes the saluretic action. Subsequently, decrease in blood volume and venous return is responsible for the improvement.

3. *Cerebral edema* Though osmotic diuretics are primarily used to lower intracranial pressure by withdrawing water, furosemide may be combined to improve efficacy.

4. *Hypertension* High ceiling diuretics are indicated in hypertension only in the presence of renal insufficiency, CHF, or in resistant cases and in hypertensive emergencies; otherwise thiazides are preferred (*see* p. 460).

5. Along with blood transfusion in severe anaemia, to prevent volume overload. Infused with hypertonic saline, it may be helpful in hyponatraemia.

6. *Hypercalcaemia of malignancy* This condition may present as a medical emergency with severe volume depletion. Rapid and large volume i.v. saline infusion is the most important measure. Addition of furosemide (10–20 mg/hour) to the i.v. drip after volume replacement, augments $Ca^{2+}$ excretion and prevents volume overload.

Forced diuresis with saline and furosemide infusion is no longer recommended to treat poisonings.



## THIAZIDE AND RELATED DIURETICS
(Inhibitors of $Na^+$-$Cl^-$ symport)

Chlorothiazide was synthesized as a CAse inhibitor variant which (unlike acetazolamide) produced urine that was rich in $Cl^-$, and diuresis occurred in alkalosis as well as acidosis. A large number of congeners were developed subsequently and the thiadiazine ring was replaced by other heterocyclic rings, but the type of activity remained the same. The important features of representative thiazide and thiazide-like diuretics are presented in Table 41.1.

These are medium efficacy diuretics with primary site of action in the cortical diluting segment or the early DT (Site III). Here they inhibit $Na^+$–$Cl^-$ symport at the luminal membrane. They do not affect the corticomedullary osmotic gradient indicating lack of action at the medullary thick AscLH. Positive free water clearance is reduced, because tubular fluid is not maximally diluted (very dilute urine cannot be passed in the absence of ADH), but negative free water clearance (in the presence of ADH) is not affected. This strengthens the view that the site of action is in between thick AscLH and late DT. These drugs gain access to their site of action *via* organic acid secretory pathway in PT and then along the tubular fluid to the early DT, where they bind to specific receptors located on the luminal membrane. Like the $Na^+$-$K^+$-$2Cl^-$ cotransporter, the $Na^+$-$Cl^-$ symporter is also a glycoprotein with 12 membrane spanning domains that binds thiazides but not furosemide or any other class of diuretics. It has been cloned and shown to be selectively expressed on the luminal membrane in the DT. The site of action of thiazide diuretics is shown in Fig. 41.2.

Some of the thiazides and related drugs have additional CAse inhibitory action in PT; intensity of this action differs among different compounds (Table 41.1), but it is generally weak. However, it may confer some proximal tubular action to the compounds, and accounts for the increase in $HCO_3^-$ and $PO_4^{3-}$ excretion.

Under thiazide action, increased amount of $Na^+$ is presented to the distal nephron, more of it exchanges with $K^+$ → urinary $K^+$ excretion is increased in parallel to the natriuretic response. The maximal diuresis induced by different agents falls in a narrow range; though potency (reflected in daily dose) differs markedly. Nevertheless, they are moderately efficacious diuretics, because nearly 90% of the glomerular filtrate has already been reabsorbed before it reaches their site of action. Thiazides have a flat dose response curve; little additional diuresis occurs when the dose is increased beyond 100 mg of hydrochlorothiazide or equivalent. They do not cause significant alteration in acid-base balance of the body.

By their action to reduce blood volume, as well as intrarenal haemodynamic changes, they tend to reduce g.f.r. This is one reason why thiazides are not effective in patients with low g.f.r. They decrease renal $Ca^{2+}$ excretion and increase $Mg^{2+}$ excretion by a direct distal tubular action. Thiazides cause greater reduction in urate

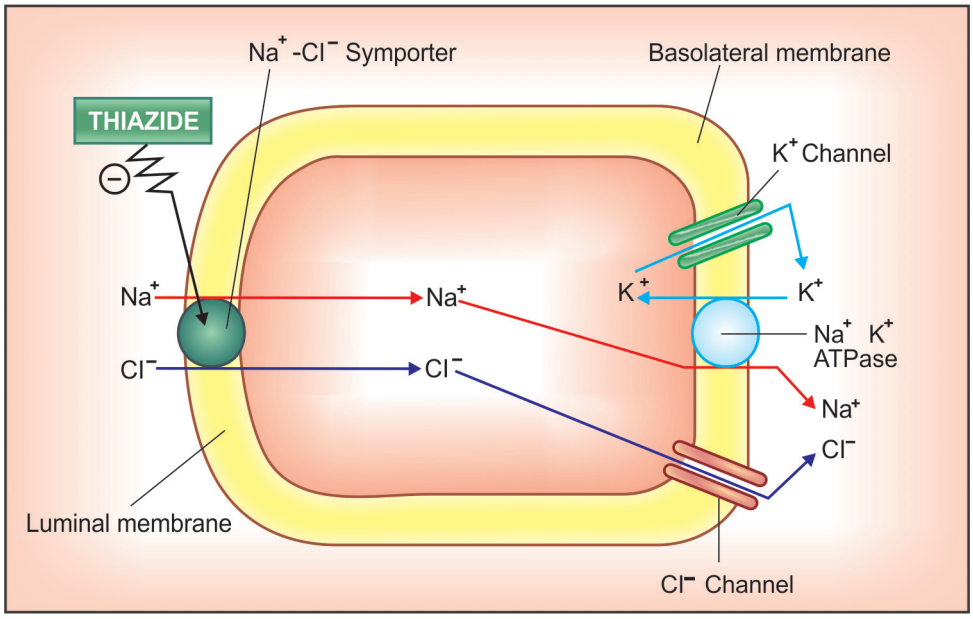



**Fig. 41.2:** Mechanism of salt reabsorption in early distal tubular cell and site of action of thiazide diuretics on $Na^+Cl^-$ symporter

excretion than furosemide, but the mechanism is the same (*see* p. 580).

The *extrarenal actions* of thiazides consist of a slowly developing fall in BP in hypertensives and elevation of blood sugar in some patients due to decreased insulin release which probably is a consequence of hypokalaemia.

**Pharmacokinetics** All thiazides and related drugs are well absorbed orally. There are no injectable preparations of these drugs. Their action starts within 1 hour, but the duration varies from 6–48 hours (Table 41.1). The more lipid-soluble agents have larger volumes of distribution (some are also bound in tissues), lower rates of renal clearance and are longer acting. The protein binding is also variable. Most of the agents undergo little hepatic metabolism and are excreted as such. They are filtered at the glomerulus as well as secreted in the PT by organic anion transport. Tubular reabsorption depends on lipid solubility: the more lipid soluble ones are highly reabsorbed—prolonging duration of action.

The elimination t½ of hydrochlorothiazide is 3–6 hours, but action persists longer (6–12 hours).

*Chlorthalidone* It is a particularly long acting compound with a t½ 40–50 hours, used exclusively as antihypertensive.

*Metolazone* In common with loop diuretics, it is able to evoke a clinically useful response even in severe renal failure (g.f.r. ~15 ml/min), and has marked additive action when combined with furosemide. An additional proximal tubular action has been demonstrated; $PO_4$ reabsorption that occurs in PT, is inhibited. It is excreted unchanged in urine. Metolazone has been used mainly for edema (5–10 mg/day, rarely 20 mg), and occasionally for hypertension (2.5–5 mg/day).

*Xipamide* It has more pronounced diuretic action similar to low doses of furosemide. Though overall reduction in plasma $K^+$ level is similar to thiazides, several instances of severe hypokalemia with ventricular arrhythmias have been reported. It is used both as antihypertensive (10–20 mg/day) and for treatment of edema (40 mg/day, max. 80 mg/day).

*Indapamide* It has little diuretic action in the usual doses, probably because it is highly lipid-

**TABLE 41.1 Thiazides and related diuretics**

| | Drug | Trade name (Tab. strength) (mg) | Daily dose (mg) | CAse inhibition | Duration of action (Hr) |
|---|---|---|---|---|---|
| 1. | Hydrochlorothiazide | AQUAZIDE, HYDRIDE THIAZIDE (12.5, 25, 50 mg) ESIDREX (50) | 12.5–100 | + | 6–12 |
| 2. | Chlorthalidone | HYTHALTON (50,100) HYDRAZIDE, THALIZIDE (12.5, 25) | 50–100 | ++ | 48 |
| 3. | Metolazone | XAROXOLYN (5, 10) DIUREM, METORAL (2.5, 5, 10) | 5–20 | + | 12–24 |
| 4. | Xipamide | XIPAMID (20) | 20–40 | + | 12 |
| 5. | Indapamide | LORVAS (2.5) | 2.5–5 | – | 12–24 |
| 6. | Clopamide | BRINALDIX (20) | 10–60 | ± | 12–18 |

soluble, is extensively metabolized and only small quantity of unchanged drug is present in the tubular fluid. However, it retains antihypertensive action and is used for that purpose only (*see* p. 561).



## Uses

1. *Edema* Thiazides may be used for mild-to-moderate cases. For mobilization of edema fluid more efficacious diuretics are preferred, but thiazides may be considered for maintenance therapy. They act best in cardiac edema; are less effective in hepatic or renal edema. Thiazides are powerless in the presence of renal failure, but metolazone may still act. Cirrhotics often develop refractoriness to thiazides due to development of secondary hyperaldosteronism.

2. *Hypertension* Thiazides and related diuretics, especially chlorthalidone are one of the first line drugs (Ch. 40).

3. *Diabetes insipidus* Thiazides decrease positive free water clearance and are the only drugs effective in nephrogenic diabetes insipidus. However, they reduce urine volume in pituitary origin cases as well (*see* Ch. 42).

4. *Hypercalciuria* with recurrent calcium stones in the kidney. Thiazides act by reducing $Ca^{2+}$ excretion.

## Complications of high ceiling and thiazide type diuretic therapy

Most of the adverse effects of these drugs are related to fluid and electrolyte changes caused by them. They are remarkably safe in low doses used over short periods. Many subtle metabolic effects have been reported in their long-term use as antihypertensives at the relatively higher doses used in the past (*see* p. 560).

1. *Hypokalaemia* This is the most significant problem. It is rare at low doses, but may be of grave consequence when brisk diuresis is induced or on prolonged therapy, especially if dietary $K^+$ intake is low. Degree of hypokalaemia appears to be related to the duration of action of the diuretic; longer acting drugs cause more $K^+$ loss. The usual manifestations are weakness, fatigue, muscle cramps; cardiac arrhythmias are the serious complications. Hypokalaemia is less common with standard doses of high ceiling diuretics than with thiazides, possibly because of shorter duration of action of the former which permits intermittent operation of compensatory repletion mechanisms. Hypokalaemia can be prevented and treated by:
(a) High dietary $K^+$ intake or
(b) Supplements of KCl (24–72 mEq/day) or
(c) Concurrent use of $K^+$ sparing diuretics.

Measures (b) and (c) are not routinely indicated, but only when hypokalaemia has been documented or in special risk situations, e.g. cirrhotics, cardiac patients—especially post MI, those receiving digitalis, antiarrhythmics, or tricyclic antidepressants and elderly patients. Serum $K^+$ levels are only a rough guide to $K^+$ depletion, because $K^+$ is primarily an intracellular ion. Nevertheless, an attempt to maintain serum $K^+$ at or above 3.5 mEq/L should be made.

Combined tablets of diuretics and KCl are not recommended because:

- they generally contain insufficient quantity of $K^+$ (8–12 mEq only).
- may cause gut ulceration by releasing KCl at one spot.
- $K^+$ is retained better if given after the diuresis is over.

$K^+$ sparing diuretics are more efficacious and more convenient in correcting hypokalaemia than are $K^+$ supplements. ACE inhibitors/$AT_1$ antagonists given with thiazides tend to prevent development of hypokalaemia.

Alkalosis may occur with hypokalaemia, because more $H^+$ exchanges with $Na^+$ in DT when less $K^+$ is available for exchange.

2. *Acute saline depletion* Overenthusiastic use of diuretics, particularly high ceiling ones, may cause dehydration and marked fall in BP (especially in erect posture). Haemoconcentration increases risk of peripheral venous thrombosis. Serum $Na^+$ and $Cl^-$ levels remain normal because isotonic saline is lost. It should be treated by saline infusion.

3. *Dilutional hyponatraemia* Occurs in CHF patients when vigorous diuresis is induced with high ceiling agents, rarely with thiazides. Kidney tends to retain water, though it is unable to retain salt due to the diuretic; e.c.f. gets diluted, hyponatraemia occurs and edema persists despite natriuresis. Patients feel very thirsty. Treatment of this distortion of fluid-electrolyte balance is difficult: withhold diuretics, restrict water intake and give glucocorticoid which enhances excretion of water load. If hypokalaemia is present, its correction helps.

4. *GIT and CNS disturbances* Nausea, vomiting and diarrhoea may occur with any diuretic. Headache, giddiness, weakness, paresthesias, impotence are occasional complaints with thiazides as well as loop diuretics.

5. *Hearing loss* Occurs rarely, only with high ceiling diuretics and when these drugs are used in the presence of renal insufficiency. Increased salt content of endolymph and a direct toxic action on the hair cells in internal ear appear to be causative.

6. *Allergic manifestations* Rashes, photosensitivity occur, especially in patients hypersensitive to sulfonamides. Blood dyscrasias are rare; any diuretic may be causative.

7. *Hyperuricaemia* Long-term use of higher dose thiazides in hypertension has caused rise in blood urate level. This is uncommon now due to use of lower doses (*see* Ch. 40). Furosemide produces a lower incidence of hyperuricaemia. This effect can be counteracted by allopurinol. Probenecid is better avoided, because it may interfere with the diuretic response, particularly of loop diuretics.

8. *Hyperglycaemia and hyperlipidemia* Have occurred in the use of diuretics as antihypertensive (*see* p. 560). These metabolic changes are minimal with low dose thiazides now recommended.

9. *Hypocalcaemia* may occur with high ceiling diuretics when these are administered chronically. Thiazides, on the otherhand, tend to raise serum $Ca^{2+}$; may aggravate hypercalcaemia due to other causes.

10. *Magnesium depletion* It may develop after prolonged use of thiazides as well as loop diuretics, and may increase the risk of ventricular arrhythmias, especially after MI or when patients are digitalized. $K^+$ sparing diuretics given concurrently minimise $Mg^{2+}$ loss.

11. Thiazides have sometimes *aggravated renal insufficiency*, probably by reducing g.f.r.

12. Brisk diuresis induced in cirrhotics may precipitate *mental disturbances* and hepatic coma. It may be due to hypokalaemia, alkalosis and increased blood $NH_3$ levels.

13. Diuretics should be avoided in *toxaemia of pregnancy* in which blood volume is low despite edema. Diuretics may further compromise placental circulation increasing the risk of miscarriage, foetal death.

### Interactions

1. Thiazides and high ceiling diuretics potentiate all other antihypertensives. This interaction is intentionally employed in therapeutics.
2. Hypokalaemia induced by these diuretics:
   - Enhances digitalis toxicity.
   - Increases risk of polymorphic ventricular tachycardia due to drugs which prolong Q-T interval (*see* p. 528).
   - Reduces sulfonylurea action.
3. High ceiling diuretics and aminoglycoside antibiotics are both ototoxic and nephrotoxic; produce additive toxicity; should be used together cautiously.
4. Cotrimoxazole given with diuretics has caused higher incidence of thrombocytopenia.
5. Indomethacin and other NSAIDs diminish the action of high ceiling diuretics by inhibiting PG synthesis in the kidney, through which furosemide and related drugs induce intrarenal haemodynamic changes which secondarily affect salt output. Antihypertensive action of thiazides and furosemide is also diminished by NSAIDs.
6. Probenecid competitively inhibits tubular secretion of furosemide and thiazides: decreases their action by lowering concentration in the tubular fluid, while diuretics diminish uricosuric action of probenecid.
7. Serum lithium level rises due to enhanced reabsorption of $Li^+$ (and $Na^+$) in PT.

### Resistance to high ceiling diuretics

Refractoriness (progressive edema despite escalating diuretic therapy) is more common with thiazides, but occurs under certain circumstances with high ceiling diuretics as well. The causes and mechanism of such resistance include:

| *Cause* | *Mechanism* |
|---|---|
| 1. Renal insufficiency (including advanced age) | Decreased access of diuretic to its site of action due to low g.f.r. and low proximal tubular secretion. |
| 2. Nephrotic syndrome | Binding of diuretic to urinary protein, other pharmacodynamic causes. |
| 3. Cirrhosis of liver | Abnormal pharmacodynamics; hyperaldosteronism; mechanism not clear. |
| 4. CHF | Impaired oral absorption due to intestinal congestion, decreased renal blood flow and glomerular filtration, increased salt reabsorption in PT. |

Long-term use of loop diuretics causes distal nephron hypertrophy → resistance. Addition of metolazone, or to some extent a thiazide, which act on distal tubule overcome the refractoriness in many cases. Fractionation of daily dose may prevent operation of compensatory mechanisms and restart diuresis. Bedrest also helps.

## CARBONIC ANHYDRASE INHIBITORS

Carbonic anhydrase (CAse) is an enzyme which catalyses the reversible reaction $H_2O + CO_2 \rightleftarrows H_2CO_3$. Carbonic acid spontaneously ionizes $H_2CO_3 \rightleftarrows H^+ + HCO_3^-$ (Fig. IX.2). Carbonic anhydrase thus functions in $CO_2$ and $HCO_3^-$ transport and in $H^+$ ion secretion. The enzyme is present in renal tubular cell (especially PT) gastric mucosa, exocrine pancreas, ciliary body of eye, brain and RBC. In these tissues a gross excess of CAse is present, more than 99% inhibition is required to produce effects.

### Acetazolamide

It is a sulfonamide derivative which noncompetitively but reversibly inhibits CAse (type II) in PT cells resulting in slowing of hydration of $CO_2$ → decreased availability of $H^+$ to exchange with luminal $Na^+$ through the $Na^+$-$H^+$ antiporter. Inhibition of brush border CAse (type IV) retards dehydration of $H_2CO_3$ in the tubular fluid so that less $CO_2$ diffuses back into the cells. The net effect is inhibition of $HCO_3^-$ (and accompanying $Na^+$) reabsorption in PT. However, the resulting alkaline diuresis is only mild (maximal fractional $Na^+$ loss 5%), because part of the $Na^+$ (but not $HCO_3^-$) rejected in the PT is reabsorbed at the high capacity AscLH.

Secretion of $H^+$ in DT and CD is also interfered. Though $H^+$ is secreted at this site by a $H^+$-ATPase, it is generated in the cell by CAse mediated reaction. As such, this is a subsidiary site of action of CAse inhibitors. When CAse inhibitors are given, the distal $Na^+$ exchange takes place only with $K^+$ which is lost in excess. For the same degree of natriuresis CAse inhibitors cause the most marked kaliuresis compared to other diuretics. The urine produced under acetazolamide action is alkaline and rich in $HCO_3^-$ which is matched by both $Na^+$ and $K^+$. Continued action of acetazolamide depletes body $HCO_3^-$ and causes acidosis; less $HCO_3^-$ (on which its diuretic action depends) is filtered at the glomerulus → less diuresis occurs (self-limiting diuretic action).

The extrarenal actions of acetazolamide are:

(i) Lowering of intraocular tension due to decreased formation of aqueous humour (aqueous is rich in $HCO_3^-$).

(ii) Decreased gastric HCl and pancreatic $NaHCO_3$ secretion: This action requires very high doses—not significant at clinically used doses.

(iii) Raised level of $CO_2$ in brain and lowering of pH → sedation and elevation of seizure threshold.

(iv) Alteration of $CO_2$ transport in lungs and tissues. These actions are masked by compensatory mechanisms.

*Pharmacokinetics* Acetazolamide is well absorbed orally and excreted unchanged in urine. Action of a single dose lasts 8–12 hours.

*Uses* Because of self-limiting action, production of acidosis and hypokalaemia, acetazolamide is not used as diuretic. Its current clinical uses are:

1. Glaucoma: as adjuvant to other ocular hypotensives (*see* Ch. 10).
2. To alkalinise urine: for urinary tract infection or to promote excretion of certain acidic drugs.
3. Epilepsy: as adjuvant in absence seizures when primary drugs are not fully effective.
4. Acute mountain sickness: for symptomatic relief as well as prophylaxis. Benefit occurs probably due to reduced CSF formation as well as lowering of CSF and brain pH.
5. Periodic paralysis.

*Dose:* 250 mg OD–BD; DIAMOX, SYNOMAX 250 mg tab. IOPAR-SR 250 mg SR cap.

*Adverse effects* are frequent.

Acidosis, hypokalaemia, drowsiness, paresthesias, fatigue, abdominal discomfort.

Hypersensitivity reactions—fever, rashes.

Bone marrow depression is rare but serious.

It is contraindicated in liver disease: may precipitate hepatic coma by interfering with urinary elimination of $NH_3$ (due to alkaline urine).

Acidosis is more likely to occur in patients of COPD.

*Methazolamide* and *Dichlorphenamide* are the other systemic CAse inhibitors, while *Dorzolamide* and *Brinzolamide* are topical CAse inhibitors used in glaucoma (*see* Ch.10).

## POTASSIUM SPARING DIURETICS

Aldosterone antagonists and renal epithelial $Na^+$ channel inhibitors indirectly conserve $K^+$ while inducing mild natriuresis, and are called 'potassium sparing diuretics'.

### Aldosterone antagonist

#### Spironolactone

It is a steroid, chemically related to the mineralocorticoid aldosterone. Aldosterone penetrates the late DT and CD cells (Fig. 41.3) and acts by combining with an intracellular mineralocorticoid receptor (MR) → induces the formation of *'aldosterone-induced proteins' (AIPs)*. The AIPs promote $Na^+$ reabsorption by a number of mechanisms (legend to Fig. 41.3) and $K^+$ secretion. Spironolactone acts from the interstitial side of the tubular cell, combines with MR and inhibits the formation of AIPs in a competitive manner. It has no effect on $Na^+$ and $K^+$ transport in the absence of aldosterone, while under normal circumstances, it increases $Na^+$ and decreases $K^+$ excretion.

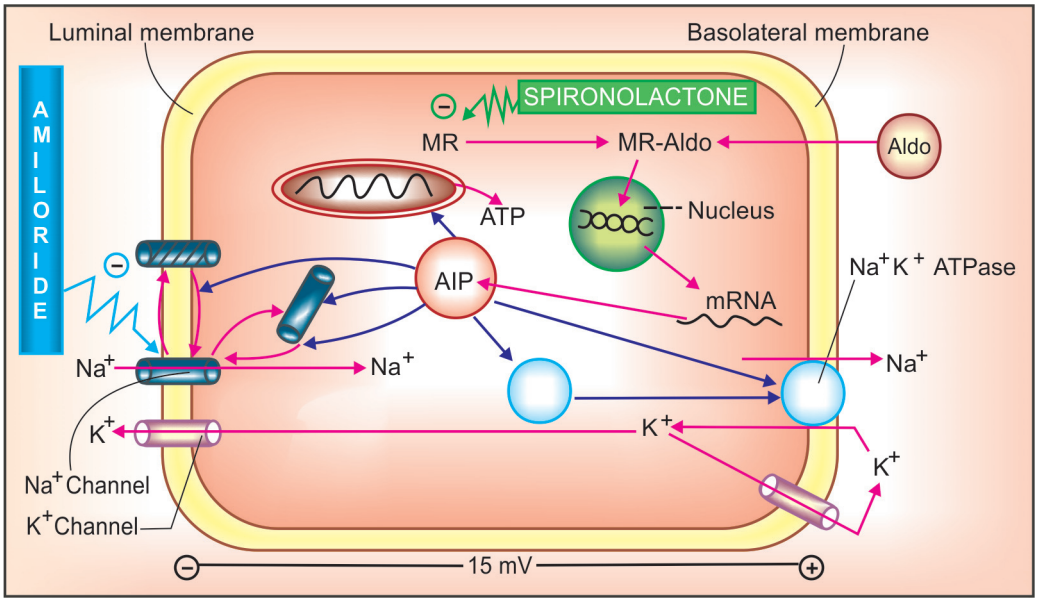



**Fig. 41.3:** Site and mechanism of action of potassium sparing diuretics on the late distal tubule/collecting duct cell Aldosterone (Aldo) penetrates the cell from the interstitial side and combines with the mineralocorticoid receptor (MR). The complex translocates to the nucleus—promotes gene mediated mRNA synthesis. The mRNA then directs synthesis of aldosterone induced proteins (AIPs). The AIPs include $Na^+K^+$ ATPase and renal epithelial (amiloride sensitive) $Na^+$ channels. More of these proteins are synthesized. The AIPs also activate these $Na^+$ channels and, translocate them from cytosolic site to luminal membrane. They also translocate $Na^+K^+$ATPase to the basolateral membrane. AIPs also increase ATP production by mitochondria. All these changes promote $Na^+$ reabsorption. More $K^+$ and $H^+$ is secreted indirectly. Spironolactone binds to MR, prevents Aldo action and produces opposite effects.

Amiloride approaches the $Na^+$ channel from the luminal side and blocks it—reducing the lumen negative transepithelial potential difference which governs $K^+$ and $H^+$ secretion



Spironolactone is a mild saluretic because majority of $Na^+$ has already been reabsorbed proximal to its site of action. However, it antagonises $K^+$ loss induced by other diuretics and slightly adds to their natriuretic effect/reverses resistance to them due to secondary hyperaldosteronism. The $K^+$ retaining action develops over 3–4 days. Spironolactone increases $Ca^{2+}$ excretion by a direct action on renal tubules.

*Pharmacokinetics* The oral bioavailability of spironolactone from microfine powder tablet is 75%. It is highly bound to plasma proteins and completely metabolized in liver; converted to active metabolites, the most important of which is *Canrenone* that is responsible for 1/2–2/3 of its action *in vivo*. The t½ of spironolactone is 1–2 hours, while that canrenone is ~18 hours. Some enterohepatic circulation occurs.

*Dose:* 25–50 mg BD–QID; max 400 mg/day

ALDACTONE 25, 50, 100 mg tabs.

ALDACTIDE: Spironolactone 25 mg + hydroflumethiazide 25 mg tab. LACILACTONE, SPIROMIDE: Spironolactone 50 mg + furosemide 20 mg tab, TORLACTONE Spironolactone 50 mg + torasemide 10 mg tab.

*Use* Spironolactone is a weak diuretic in its own right and is used only in combination with other more efficacious diuretics.

1. To counteract $K^+$ loss due to thiazide and loop diuretics.
2. Edema: It is more useful in cirrhotic and nephrotic edema in which aldosterone levels are generally high. Spironolactone is frequently added to a thiazide/loop diuretic in the treatment of ascitis due to cirrhosis of liver. It breaks the resistance to thiazide diuretics that develops due to secondary hyperaldosteronism and reestablishes the response.

Thus, it is particularly employed in refractory edema.

3. Hypertension: Used as adjuvant to thiazide to prevent hypokalaemia, it may slightly add to their antihypertensive action. More importantly, it may have the potential to attenuate hypertension related renal fibrosis and ventricular/vascular hypertrophy (*see* p. 561).
4. CHF: As additional drug to conventional therapy in moderate to severe CHF; can retard disease progression and lower mortality (*see* p. 524).

### *Interactions*

1. Given together with $K^+$ supplements—dangerous hyperkalaemia can occur.
2. Aspirin blocks spironolactone action by inhibiting tubular secretion of its active metabolite canrenone.
3. More pronounced hyperkalaemia can occur in patients receiving ACE inhibitors/ARBs.
4. Spironolactone increases plasma digoxin concentration.

***Adverse effects*** The side effects are drowsiness, ataxia, mental confusion, epigastric distress and loose motions. Spironolactone interacts with progestin and androgen receptors as well. In addition, it may enhance testosterone clearance or its peripheral conversion to estradiol, producing dose and duration of treatment related hormonal side effects like gynaecomastia, erectile dysfunction or loss of libido in men, and breast tenderness or menstrual irregularities in women.

Most serious is hyperkalaemia that may occur, especially if renal function is inadequate. Acidosis is a risk, particularly in cirrhotics. Peptic ulcer may be aggravated; it is contraindicated in ulcer patients.

## Eplerenone

It is a newer and more selective aldosterone antagonist which has much lower affinity for other steroidal receptors; therefore much less likely to produce hormonal disturbances like gnaecomastia, impotence, menstrual irregularities, etc. This feature makes it particularly suitable for longterm use in the therapy of hypertension and chronic CHF. However, the risk of hyperkalaemia and g.i. side effects are like spironolactone. Other side effects have an incidence similar to placebo, and it has a better tolerability profile.

Eplerenone is well absorbed orally, inactivated in liver by CYP3A4, and excreted in urine ($2/3^{rd}$) as well as faeces ($1/3^{rd}$). The t½ is 4–6 hours. Inhibitors of CYP3A4 (clarithromycin, itraconazole, etc.) increase its blood levels, while inducers like carbamazepine, rifampin, etc. may decrease its efficacy.

Eplerenone is indicated specifically in moderate to severe CHF, post-infarction left ventricular dysfunction and hypertension. It can also be used as alternative to spironolactone.

*Dose:* 25–50 mg BD;
EPLERAN, EPTUS, ALRISTA 25, 50 mg tabs.

## Inhibitors of renal epithelial $Na^+$ channel

*Triamterene* and *amiloride* are two nonsteroidal organic bases with identical actions. Their most important effect is to decrease $K^+$ excretion, particularly when it is high due to large $K^+$ intake or use of a diuretic that enhances $K^+$ loss. This is accompanied by a small increase in $Na^+$ excretion. The excess urinary $Na^+$ is matched by $Cl^-$ and variable amounts of $HCO_3^-$; urine is slightly alkalinized. The effect on urinary electrolyte pattern is superficially similar to spironolactone, but their action is independent of aldosterone. $Ca^{2+}$ and $Mg^{2+}$ exretion is also reduced, but there is no effect on renal haemodynamics.

***Mechanism of action*** The luminal membrane of late DT and CD cells expresses a distinct *'renal epithelial'* or *'amiloride sensitive' $Na^+$ channel* through which $Na^+$ enters the cell down its electrochemical gradient which is generated by $Na^+K^+$ ATPase operating at the basolateral membrane (Fig. 41.3). This $Na^+$ entry partially depolarizes the luminal membrane creating a –15 mV transepithelial potential difference which promotes secretion of $K^+$ into the lumen through $K^+$ channels. Though there is no direct coupling

between $Na^+$ and $K^+$ channels, more the delivery of $Na^+$ to the distal nephron—greater is its entry through the $Na^+$ channel—luminal membrane is depolarized to a greater extent—driving force for $K^+$ secretion is augmented. As such, all diuretics acting proximally (loop diuretics, thiazides, CAse inhibitors) promote $K^+$ secretion. Amiloride and triamterene block the luminal $Na^+$ channels and indirectly inhibit $K^+$ excretion, while the net excess loss of $Na^+$ is minor, because this is only a small fraction of the total amount of $Na^+$ excreted in urine.

The intercalated cells in CD possess an ATP driven $H^+$ pump which secretes $H^+$ ions into the lumen. This pump is facilitated by the lumen negative potential. Amiloride, by reducing the lumen negative potential, decreases $H^+$ ion secretion as well and predisposes to acidosis. Thus, amiloride conserves both $K^+$ and $H^+$ while marginally increasing $Na^+$ excretion.

Both triamterene and amiloride are used in conjunction with a thiazide type or a high ceiling diuretic to prevent hypokalaemia and slightly augment the natriuretic response. The antihypertensive action of thiazide is also supplemented. Risk of hyperkalaemia is the most important adverse effect of amiloride and triamterene. These drugs should not be given with $K^+$ supplements; dangerous hyperkalaemia may develop. Hyperkalaemia is also more likely in patients receiving ACE inhibitors/ARBs, β blockers, NSAIDs and in those with renal impairment.

Both drugs elevate plasma digoxin levels.

**Triamterene** It is incompletely absorbed orally, partly bound to plasma proteins, largely metabolized in liver to an active metabolite and excreted in urine. Plasma t½ is 4 hours, effect of a single dose lasts 6–8 hours.

*Side effects* are infrequent: consist of nausea, dizziness, muscle cramps and rise in blood urea. Impaired glucose tolerance and photosensitivity are reported, but urate level is not increased.

*Dose*: 50–100 mg daily; DITIDE, triamterene 50 mg + benzthiazide 25 mg tab;
FRUSEMENE, triamterene 50 mg + furosemide 20 mg tab.

**Amiloride** It is 10 times more potent than triamterene (dose 5–10 mg OD–BD). At higher doses it also inhibits $Na^+$ reabsorption in PT, but this is clinically insignificant. It decreases $Ca^{2+}$ and $Mg^{2+}$ excretion but increases urate excretion. Thus, hypercalcaemic action of thiazides is augmented but hyperuricaemic action is partly annuled. A mild antihypertensive action is also reported.

Only ¼ of an oral dose is absorbed. It is not bound to plasma proteins and not metabolized. The t½ (20 hours) and duration of action are longer than triamterene.

BIDURET, KSPAR: Amiloride 5 mg + hydrochlorothiazide 50 mg tab, LASIRIDE, amiloride 5 mg + furosemide 40 mg tab.

Usual side effects are nausea, diarrhoea and headache.

Amiloride blocks entry of $Li^+$ through $Na^+$ channels in the CD cells and mitigates diabetes insipidus induced by lithium.

Given as an aerosol it affords symptomatic improvement in cystic fibrosis by increasing fluidity of respiratory secretions.

## OSMOTIC DIURETICS

### Mannitol

Mannitol is a nonelectrolyte of low molecular weight (182) that is pharmacologically inert—can be given in large quantities sufficient to raise osmolarity of plasma and tubular fluid. It is minimally metabolized in the body; freely filtered at the glomerulus and undergoes limited reabsorption: therefore excellently suited to be used as osmotic diuretic. Mannitol appears to limit tubular water and electrolyte reabsorption in a variety of ways:

1. Retains water isoosmotically in PT—dilutes luminal fluid which opposes NaCl reabsorption.
2. Inhibits transport processes in the thick AscLH by an unknown mechanism. Quantitatively this appears to be the largest contributor to the diuresis.

**TABLE 41.2** **Urinary electrolyte pattern and natriuretic efficacy of some diuretics**

| Diuretic | Urinary electrolyte excretion $Na^+$ | $K^+$ | $Cl^-$ | $HCO_3^-$ | Max. % of filtered $Na^+$ excreted | Efficacy |
|---|---|---|---|---|---|---|
| 1. Furosemide | ↑↑↑ | ↑ | ↑↑ | ↑,– | 25% | High |
| 2. Thiazide | ↑↑ | ↑ | ↑ | ↑ | 8% | Intermediate |
| 3. Acetazolamide | ↑ | ↑↑ | ↑,– | ↑↑ | 5% | Mild |
| 4. Spironolactone | ↑ | ↓ | ↑ | –,↑ | 3% | Low |
| 5. Amiloride | ↑ | ↓ | ↑ | –,↑ | 3% | Low |
| 6. Mannitol | ↑↑ | ↑ | ↑ | ↑ | 20% | High |

3. Expands extracellular fluid volume (because it does not enter cells, mannitol draws water from the intracellular compartment)—increases g.f.r. and inhibits renin release.
4. Increases renal blood flow, especially to the medulla—medullary hypertonicity is reduced (due to washing off)—corticomedullary osmotic gradient is dissipated—passive salt reabsorption is reduced.

Though the primary action of mannitol is to increase urinary volume, excretion of all cations ($Na^+$, $K^+$, $Ca^{2+}$, $Mg^{2+}$) and anions ($Cl^-$, $HCO_3^-$, $PO_4^{3-}$) is also enhanced.

*Administration* Mannitol is not absorbed orally; has to be given i.v. as 10–20% solution. It is excreted with a t½ of 0.5–1.5 hour.

MANNITOL 10%, 20%, in 100, 350 and 500 ml vac.

*Uses* Mannitol is never used for the treatment of chronic edema or as a natriuretic. Its indications are:

1. Increased intracranial or intraocular tension (acute congestive glaucoma, head injury, stroke, etc.): by osmotic action it encourages movement of water from brain parenchyma, CSF and aqueous humour; 1–1.5 g/kg is infused over 1 hour as 20% solution to transiently raise plasma osmolarity. It is also used before and after ocular/brain surgery to prevent acute rise in intraocular/intracranial pressure.
2. To maintain g.f.r. and urine flow in impending acute renal failure, e.g. in shock, severe trauma, cardiac surgery, haemolytic reactions: 500–1000 ml of the solution may be infused over 24 hours. However, prognostic benefits in conditions other than cardiac surgery are still unproven. If acute renal failure has already set in, kidney is incapable of forming urine even after an osmotic load; mannitol is contraindicated: it will then expand plasma volume → pulmonary edema and heart failure may develop.
3. To counteract low osmolality of plasma/e.c.f. due to rapid haemodialysis or peritoneal dialysis (dialysis disequilibrium).

Mannitol along with large volumes of saline was infused i.v. to produce *'forced diuresis'* in acute poisonings in the hope of enhancing excretion of the poison. However, this has been found to be ineffective and to produce electrolyte imbalances. Not recommended now.

Mannitol is *contraindicated* in acute tubular necrosis, anuria, pulmonary edema; acute left ventricular failure, CHF, cerebral haemorrhage. The most common side effect is headache. Nausea and vomiting may occur; hypersensitivity reactions are rare.

**Isosorbide and glycerol** These are orally active osmotic diuretics which may be used to reduce intraocular or intracranial tension. Intravenous glycerol can cause haemolysis.

*Dose*: 0.5–1.5 g/kg as oral solution.

## ☞ PROBLEM DIRECTED STUDY

**41.1** A 50-year-old male patient of hepatic cirrhosis with ascitis and pedal edema was treated with tab Furosemide 80 mg twice a day, in addition to bed rest, suitable dietary advice and vitamin supplementation. He started passing larger quantity of urine and the ascitis/edema started regressing. After a week, he was brought with incoherent talking, drowsiness, tremor and ataxia. The relatives informed that for the past 2 days he was no longer passing the increased amount of urine as at the start of medication. Serum $K^+$ measurement found a value of 2.8 mEq/L.

(a) What is the cause of the neurological symptoms and diminution of the diuretic response to furosemide? Was the choice of the diuretic appropriate?

(b) How should this patient be managed at the present stage?

(see Appendix-1 for solution)

# Chapter 42 Antidiuretics

*Antidiuretics* (more precisely 'anti-aquaretics', because they inhibit water excretion without affecting salt excretion) are drugs that reduce urine volume, particularly in *diabetes insipidus* (DI) which is their primary indication. Drugs are:

1. Antidiuretic hormone (ADH, Vasopressin), Desmopressin, Lypressin, Terlipressin
2. Thiazide diuretics, Amiloride.
3. Miscellaneous: Indomethacin, Chlorpropamide, Carbamazepine.

## ANTIDIURETIC HORMONE (Argenine Vasopressin-AVP)

It is a nonapeptide secreted by posterior pituitary (neurohypophysis) along with oxytocin (*see* Ch. 23). It is synthesized in the hypothalamic (supraoptic and paraventricular) nerve cell bodies as a large precursor peptide along with its binding protein 'neurophysin'. Both are transported down the axons to the nerve endings in the median eminence and pars nervosa. Osmoreceptors present in hypothalamus and volume receptors present in left atrium, ventricles and pulmonary veins primarily regulate the rate of ADH release governed by body hydration. Osmoreceptors are also present in the hepatic portal system which sense ingested salt and release ADH even before plasma osmolarity is increased by the ingested salt. Impulses from baroreceptors and higher centres also impinge on the nuclei synthesizing ADH and affect its release. The two main physiological stimuli for ADH release are rise in plasma osmolarity and contraction of e.c.f. volume.

Several neurotransmitters, hormones and drugs modify ADH secretion. It is enhanced by angiotensin II, prostaglandins (PGs), histamine, neuropeptide Y and ACh. GABA and atrial natriuretic peptide (ANP) decrease its release. Opioids have agent-specific and dose dependent action. Low-dose morphine inhibits ADH secretion, but high doses enhance it. Opioid peptides are mostly inhibitory. Nicotine and imipramine stimulate, while alcohol, haloperidol, phenytoin and glucocorticoids decrease ADH release.

The human ADH is *8-arginine*-vasopressin (AVP); 8-*lysine*-vasopressin (lypressin) is found in swine and has been synthetically prepared. Other more potent and longer acting peptide analogues of AVP having agonistic as well as antagonistic action have been prepared.

### ADH (Vasopressin) receptors

These are G protein coupled cell membrane receptors; two subtypes $V_1$ and $V_2$ have been identified, cloned and structurally characterized.

*$V_1$ Receptors* All vasopressin receptors except those on renal CD cells, AscLH cells and vascular endothelium are of the $V_1$ type. These are further divided into $V_{1a}$ and $V_{1b}$ subtypes:

$V_{1a}$ receptors are present on vascular smooth muscle (including that of vasa recta in renal medulla), uterine and other visceral smooth muscles, interstitial cells in renal medulla, cortical CD cells, adipose tissue, brain, platelets, liver, etc. The $V_{1b}$ receptors are localized to the anterior pituitary, certain areas in brain and in pancreas.

The $V_1$ receptors function mainly through the phospholipase C–$IP_3$/DAG pathway—release $Ca^{2+}$ from intracellular stores—causing vasoconstriction, visceral smooth muscle contraction, glycogenolysis, platelet aggregation, ACTH release, etc. These actions are augmented by enhanced influx of $Ca^{2+}$ through $Ca^{2+}$ channels as well as by DAG mediated protein kinase C activation which phosphorylates relevant proteins. $V_1$ receptors, in addition, activate phospholipase A2—release arachidonic acid resulting in generation of PGs and other eicosanoids which contribute to many of the $V_1$ mediated effects. Persistent $V_1$ receptor

stimulation activates protooncogenes (possibly through $IP_3$/DAG pathway) resulting in growth (hypertrophy) of vascular smooth muscle and other responsive cells.

*$V_2$ Receptors* These are located primarily on the collecting duct (CD) principal cells in the kidney—regulate their water permeability through cAMP production. Some $V_2$ receptors are also present on AscLH cells which activate $Na^+K^+2Cl^-$ cotransporter. Vasodilatory $V_2$ receptors are present on endothelium of blood vessels.

The $V_2$ receptors are more sensitive (respond at lower concentrations) to AVP than are $V_1$ receptors.

Selective peptide agonists and antagonists of the subtypes of vasopressin receptors are:

| | *Selective agonist* |
|---|---|
| $V_{1a}$ Receptor | [$Phe^2$, $Ile^2$, $Orn^8$] AVP |
| $V_{1b}$ Receptor | Deamino [D-3 {pyridyl)-$Ala^2$] AVP |
| $V_2$ Receptor | Desmopressin (dDAVP) |
| | *Selective antagonist* |
| $V_{1a}$ Receptor | $d(CH_2)_5$ [Tyr ($Me^2$)] AVP |
| $V_{1b}$ Receptor | dp [Tyr ($me^2$)] AVP |
| $V_2$ Receptor | $d(CH_2)_5$ [D-$Ile^2$, $Ile^4$, Ala-$NH_2{}^9$] AVP |

Some orally active nonpeptide $V_{1a}$, $V_{1b}$ and $V_2$ receptor antagonists have been produced. *Tolvaptan* and *Mozavaptan* are nonpeptide $V_2$ selective antagonists that are now in clinical use.

SECTION 9

## Actions

**Kidney** AVP acts on the collecting duct (CD) principal cells to increase their water permeability—water from the duct lumen diffuses to the interstitium by equilibrating with the hyperosmolar renal medulla (*see* Fig. IX.1). In man, maximal osmolarity of urine that can be attained is 4 times higher than plasma. When AVP is absent, CD cells remain impermeable to water → dilute urine (produced by the diluting segment) is passed as such. Graded effect occurs at lower concentrations of AVP: urine volume closely balances fluid intake.

*Mechanism of action* Vasopressin is instrumental in rapid adjustments of water excretion according to the state of body hydration, as well as in dealing with conditions prevailing over long-term. The $V_2$ subtype of ADH receptors are present on the basolateral membrane of principal cells in CDs (*see* Fig. 42.1). Activation of these receptors increases cAMP formation intracellularly → activation of cAMP dependent protein kinase A → phosphorylation of relevant proteins which promote exocytosis of *'aquaporin-2'* water channel containing vesicles (WCVs) through the apical membrane → more aqueous channels get inserted into the apical membrane. The rate of endocytosis and degradation of WCVs is concurrently reduced. The water permeability of CD cells is increased in proportion to the population of aquaporin-2 channels in the apical membrane at any given time. Continued $V_2$ receptor stimulation (during chronic water deprivation) in addition upregulates aquaporin-2 synthesis through cAMP response element of the gene encoding aquaporin-2.

Other aquaporins like aquaporin-1 (in PT) and aquaporin-3,4 (on basolateral membrane of CD cells) also participate in water transport at these sites.

To achieve maximum concentration of urine, activation of $V_2$ receptors increases urea permeability of terminal part of CDs in inner medulla by stimulating a vasopressin regulated urea transporter (VRUT or UT-1)—which in turn augments medullary hypertonicity. Recently, $V_2$ receptor mediated actions of AVP on AscLH have also been demonstrated which further reinforce medullary hypertonicity by translocating to luminal membrane and activating the $Na^+K^+2Cl^-$ cotransporter in the short-term and increasing its synthesis in the long-term.

The $V_1$ receptors also participate in the renal response to AVP. Activation of $V_1$ receptors constricts vasa recta to diminish blood flow to inner medulla which reduces washing off effect and helps in maintaining high osmolarity in this region. Thus, it contributes to antidiuresis. On the other hand, activation of medullary interstitial cell $V_1$ receptors enhance PG synthesis which attenuate cAMP generation in CD cells and oppose $V_2$ mediated antidiuresis. $V_1$ receptors are also present on CD cells. Their stimulation activates

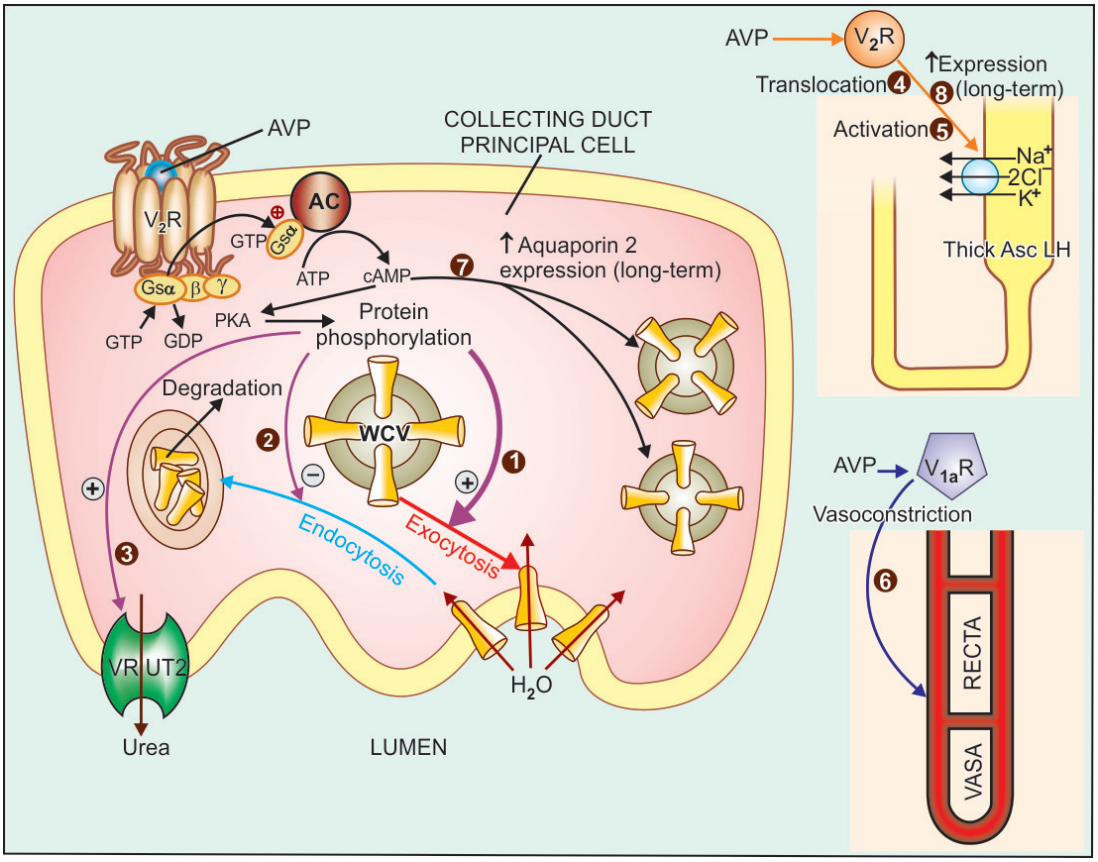



**Fig. 42.1:** Mechanisms of rapid and long-term anti-aquaretic action of vasopressin

All $V_2$ receptor ($V_2R$) mediated actions are exerted through the adenylyl cyclase (AC)-cyclic AMP (cAMP) pathway, while the $V_{1a}$ receptor ($V_{1a}R$) mediated action is exerted *via* the phospholipase C—$IP_3$: DAG pathway.

*Rapid actions*

(1) Translocation of water channel containing vesicles (WCVs) and exocytotic insertion of aquaporin 2 water channels into the apical membrane of principal cells of collecting ducts; the primary action responsible for antidiuresis.

(2) Inhibition of endocytotic removal of aquaporin 2 channels from the apical membrane.

(3) Activation of vasopressin regulated urea transporter (VRUT) at apical membrane of collecting ducts in the inner medulla.

(4) Translocation of $Na^+K^+2Cl^-$ cotransporter to the luminal membrane of cells in thick ascending limb of loop of Henle (AscLH).

(5) Activation of $Na^+K^+2Cl^-$ cotransporter in AscLH cells.

(6) $V_{1a}$ receptor ($V_{1a}R$) mediated vasoconstriction of vasa recta

*Long-term actions*

(7) Gene mediated increased expression of aquaporin 2 channels in collecting duct cells.

(8) Gene mediated increased expression of $Na^+K^+2Cl^-$ cotransporter in AscLH cells.

$PK_A$—cAMP dependent protein kinase.

PKc which directly diminishes responsiveness of CD cells to $V_2$ receptors and restrains $V_2$ mediated water permeability. The logic of this apparent paradox may lie in the fact that these $V_1$ actions are produced at much higher concentrations of AVP, so that physiologically they may serve to restrict $V_2$ effects only when blood levels of AVP are very high.

Lithium and demeclocycline partially antagonize AVP action (probably by limiting cAMP formation), reduce the urine concentrating ability of the kidney, produce polyuria and polydipsia. They have been used in patients with inappropriate ADH secretion. On the other hand NSAIDs (especially indomethacin) augment AVP induced antidiuresis by inhibiting renal PG synthesis. Carbamazepine and chlorpropamide also potentiate AVP action on kidney.

**Blood vessels** AVP constricts blood vessels through $V_1$ receptors and can raise BP (hence the name vasopressin), but much higher concentration is needed than for maximal antidiuresis. The cutaneous, mesenteric, skeletal muscle, fat depot, thyroid, and coronary beds are particularly constricted. Though vasoconstrictor action of AVP does not appear to be physiologically important, some recent studies indicate that it may play a role in CHF, haemorrhage, hypotensive states, etc. Prolonged exposure to AVP causes vascular smooth muscle hypertrophy.

The $V_2$ receptor mediated vasodilatation can be unmasked when AVP is administered in the presence of a $V_1$ antagonist. It can also be demonstrated by the use of selective $V_2$ agonist desmopressin, and is due to endothelium dependent NO production.

**Other actions** Most visceral smooth muscles contract. Increased peristalsis in gut (especially large bowel), evacuation and expulsion of gases may occur.

*Uterus* is contracted by AVP acting on oxytocin receptors. In the nonpregnant and early pregnancy uterus, AVP is equipotent to oxytocin. Only at term sensitivity to oxytocin increases selectively.

*CNS* Exogenously administered AVP does not penetrate blood-brain barrier. However, it is now recognized as a peptide neurotransmitter in many areas of brain and spinal cord. AVP may be involved in regulation of temperature, systemic circulation, ACTH release, and in learning of tasks.

AVP induces platelet aggregation and hepatic glycogenolysis. It releases coagulation factor VIII and von Willebrand's factor from vascular endothelium through $V_2$ receptors.

**Pharmacokinetics** AVP is inactive orally because it is destroyed by trypsin. It can be administered by any parenteral route or by intranasal application. The peptide chain of AVP is rapidly cleaved enzymatically in many organs, especially in liver and kidney; plasma t½ is short ~25 min. However, the action of aqueous vasopressin lasts 3–4 hours.

Aqueous vasopressin (AVP) inj: POSTACTON 10 U inj; for i.v., i.m. or s.c. administration.

## VASOPRESSIN ANALOGUES

**Lypressin** It is 8-lysine vasopressin. Though somewhat less potent than AVP, it acts on both $V_1$ and $V_2$ receptors and has longer duration of action (4–6 hours). It is being used in place of AVP—mostly for $V_1$ receptor mediated actions. PETRESIN, VASOPIN 20 IU/ml inj; 10 IU i.m. or s.c. or 20 IU diluted in 100–200 ml of dextrose solution and infused i.v. over 10–20 min.

**Terlipressin** This synthetic prodrug of vasopressin is specifically used for bleeding esophageal varices; may produce less severe adverse effects than lypressin.

*Dose:* 2 mg i.v., repeat 1–2 mg every 4–6 hours as needed. GLYPRESSIN 1 mg freeze dried powder with 5 ml diluent for inj, T-PRESSIN, TERLINIS 1 mg/10 ml inj.

**Desmopressin** (dDAVP) This synthetic peptide is a selective $V_2$ agonist; 12 times more potent antidiuretic than AVP, but has negligible vasoconstrictor activity. It is also longer acting because enzymatic degradation is slow; t½ 1–2 hours; duration of action 8–12 hours. Desmopressin is the preparation of choice for all $V_2$ receptor related indications. The intranasal route is preferred, though bioavailability is only 10–20%. An oral formulation has been recently marketed with a bioavailability of 1–2%; oral dose

is 10–15 times higher than intranasal dose, but systemic effects are produced and nasal side effects are avoided. Many patients find oral tablet more convenient.

*Dose: Intranasal:* Adults 10–40 µg/day in 2–3 divided doses, children 5–10 µg at bed time.
*Oral:* 0.1–0.2 mg TDS.
*Parenteral* (s.c. or i.v.) 2–4 µg/day in 2–3 divided doses.
MINIRIN 100 µg/ml nasal spray (10 µg per actuation); 100 µg/ml intranasal solution in 2.5 ml bottle with applicator; 0.1 mg tablets; 4 µg/ml inj.

## Uses

**A. Based on $V_2$ actions** (Desmopressin is the drug of choice)

1. *Diabetes insipidus* DI of pituitary origin (neurogenic) is the most important indication for vasopressin. It is ineffective in renal (nephrogenic) DI, since kidney is unresponsive to ADH. Lifelong therapy is required, except in some cases of head injury or neurosurgery, where DI occurs transiently.

The dose of desmopressin is individualized by measuring 24 hour urine volume. Aqueous vasopressin or lypressin injection is impracticable for long-term treatment. It can be used in transient DI and to differentiate neurogenic from nephrogenic DI—urine volume is reduced and its osmolarity increased if DI is due to deficiency of ADH, but not when it is due to unresponsiveness of kidney to ADH. Desmopressin 2 µg i.m. is the preparation of choice now for the same purpose.

2. *Bedwetting in children and nocturia in adults* Intranasal or oral desmopressin at bedtime controls primary nocturia by reducing urine volume. Nocturnal voids are reduced to nearly half and first sleep period in adults is increased by ~2 hr. Fluid intake must be restricted 1 hr before and till 8 hr after the dose to avoid fluid retention. Monitor BP and body weight periodically to check fluid overload. Withdraw for one week every 3 months for reassessment.

3. *Renal concentration test* 5–10 U i.m. of aqueous vasopressin or 2 µg of desmopressin causes maximum urinary concentration.

4. *Haemophilia, von Willebrand's disease* AVP may check bleeding by releasing coagulation factor VIII and von Willebrand's factor. Desmopressin is the preferred preparation in a dose of 0.3 µg/kg diluted in 50 ml saline and infused i.v. over 30 min.

**B. Based on $V_1$ actions**

1. *Bleeding esophageal varices* Vasopressin/terlipressin often stop bleeding by constricting mesenteric blood vessels and reducing blood flow through the liver to the varices, allowing clot formation. Terlipressin stops bleeding in ~80% and has been shown to improve survival. It has replaced AVP because of fewer adverse effects and greater convenience in use. Octreotide (a somatostatin analogue) injected i.v. is an alternative. However, definitive therapy of varices remains endoscopic obliteration by sclerotherapy.

2. *Before abdominal radiography* AVP/lypressin has been occasionally used to drive out gases from bowel.

**Adverse effects** Because of $V_2$ selectivity, desmopressin produces fewer adverse effects than vasopressin, lypressin or terlipressin. However, transient headache and flushing are frequent.

Nasal irritation, congestion, rhinitis, ulceration and epistaxis can occur on local application. Systemic side effects are: belching, nausea, abdominal cramps, pallor, urge to defecate, backache in females (due to uterine contraction). Fluid retention and hyponatraemia may develop. Symptoms of hyponatremia are due to shift of water intracellularly resulting in cerebral edema producing headache, mental confusion, lassitude, nausea, vomiting and even seizures. Children are more susceptible.

AVP can cause bradycardia, increase cardiac afterload and precipitate angina by constricting coronary vessels. It is contraindicated in patients with ischaemic heart disease, hypertension, chronic nephritis and psychogenic polydipsia. Urticaria and other allergies are possible with any preparation.

## THIAZIDES

Diuretic thiazides paradoxically exert an antidiuretic effect in DI. High ceiling diuretics

are also effective but are less desirable because of their short and brisk action. Thiazides reduce urine volume in both pituitary origin as well as renal DI. They are especially valuable for the latter in which AVP is ineffective. However, their efficacy is low; urine can never become hypertonic as can occur with AVP in neurogenic DI. The mechanism of action is not well understood, possible explanation is:

Thiazides induce a state of sustained electrolyte depletion so that glomerular filtrate is more completely reabsorbed iso-osmotically in PT. Further, because of reduced salt reabsorption in the cortical diluting segment, a smaller volume of less dilute urine is presented to the CDs and the same is passed out. That salt restriction has a similar effect, substantiates this mechanism of action. Secondly, thiazides reduce g.f.r. and thus the fluid load on tubules.

Hydrochlorothiazide 25–50 mg TDS or equivalent dose of a longer acting agent is commonly used. Though less effective than AVP, it is more convenient and cheap even for pituitary origin DI; may reduce polyuria to some extent. $K^+$ supplements are needed.

*Amiloride* is the drug of choice for lithium induced nephrogenic DI (*see* p. 590).

*Indomethacin* has also been found to reduce polyuria in renal DI to some extent by reducing renal PG synthesis. It can be combined with a thiazide ± amiloride in nephrogenic DI. Other NSAIDs are less active.

**Chlorpropamide** It is a long-acting sulfonylurea oral hypoglycaemic, found to reduce urine volume in DI of pituitary origin but not in renal DI. It sensitizes the kidney to ADH action; thus its efficacy depends on small amounts of the circulating hormone; it is not active when ADH is totally absent.

**Carbamazepine** It is an antiepileptic (*see* Ch. 30) which reduces urine volume in DI of pituitary origin, but mechanism of action is not clear. Higher doses are needed; adverse effects are marked; it is of little value in treatment of DI.

## VASOPRESSIN ANTAGONISTS

*Tolvaptan* It is an orally active nonpeptide selective $V_2$ receptor antagonist that has been recently introduced for the treatment of hyponatraemia due to CHF, cirrhosis of liver or syndrome of inappropriate ADH secretion (SIADH). It increases free water clearance by the kidney (aquaretic) and helps to correct the low plasma $Na^+$ levels. In clinical trials symptoms of worsening heart failure were improved. However, too rapid correction of hyponatraemia should not be attempted, because thrombotic complications can occur due to haemoconcentration. The most frequent side effect is thirst and dry mouth. Others are fever, g.i. upset and hyperglycaemia. Tolvaptan is metabolized by CYP3A4; should not be given to patients receiving inhibitors of this isoenzyme. The t½ is 6–8 hours, and it is given once daily.

*Mozavaptan* ($V_2$ selective antagonist) and *Conivaptan* ($V_{1a}$+$V_2$ antagonist) are the other vasopressin antagonists that are in clinical use.

SECTION 10

# DRUGS AFFECTING BLOOD AND BLOOD FORMATION

## Chapter 43 Haematinics and Erythropoietin

**Haematinics** These are substances required in the formation of blood, and are used for treatment of anaemias.

Anaemia occurs when the balance between production and destruction of RBCs is disturbed by:

(a) Blood loss (acute or chronic)

(b) Impaired red cell formation due to:
- Deficiency of essential factors, i.e. iron, vitamin $B_{12}$, folic acid.
- Bone marrow depression (hypoplastic anaemia), erythropoietin deficiency.

(c) Increased destruction of RBCs (haemolytic anaemia)

In this chapter essential factors required for normal formation or pigmentation of RBCs will be covered.

### IRON

Iron has for long been considered important for the body. *Lauha bhasma* (calcined iron) has been used in ancient Indian medicine. According to Greek thought Mars is the God of strength, and iron is dedicated to Mars: as such, iron was used to treat weakness, which is common in anaemia. In 1713 iron was shown to be present in blood. In the early 19th century Blaud developed his famous 'Blaud's pill' consisting of ferrous sulfate and potassium carbonate for anaemia. All important aspects of iron metabolism have been learned in the past 60 years.

**Distribution of iron in body** Iron is an essential body constituent. Total body iron in an adult is 2.5–5 g (average 3.5 g). It is more in men (50 mg/kg) than in women (38 mg/kg). It is distributed into:

| | |
|---|---|
| Haemoglobin (Hb) | : 66% |
| Iron stores as ferritin and haemosiderin | : 25% |
| Myoglobin (in muscles) | : 3% |
| Parenchymal iron (in enzymes, etc.) | : 6% |

Haemoglobin is a protoporphyrin; each molecule having 4 iron containing haeme residues. It has 0.33% iron; thus loss of 100 ml of blood (containing 15 g Hb) means loss of 50 mg elemental iron. To raise the Hb level of blood by 1 g/dl—about 200 mg of iron is needed. Iron is stored only in ferric form, in combination with a large protein *apoferritin*.

$$\text{Apoferritin} + Fe^{3+} \rightarrow \text{Ferritin} \xrightarrow{\textit{aggregates}} \text{Haemosiderin}$$

(not reutilized)

Ferritin can get saturated to different extents; at full saturation it can hold 30% iron by weight. The most important storage sites are reticuloendothelial (RE) cells. Parenchymal iron occurs as prosthetic group in many cellular enzymes—

cytochromes, peroxidases, catalases, xanthine oxidase and some mitochondrial enzymes. Though, the primary reflection of iron deficiency occurs in blood, severe deficiency affects practically every cell.

**Daily requirement** To make good average daily loss, iron requirements are:

| | | |
|---|---|---|
| Adult male | : | 0.5–1 mg (13 µg/kg) |
| Adult female (menstruating) | : | 1–2 mg (21 µg/kg) |
| Infants | : | 60 µg/kg |
| Children | : | 25 µg/kg |
| Pregnancy (last 2 trimesters) | : | 3–5 mg (80 µg/kg) |

### *Dietary sources of iron*

*Rich* : Liver, egg yolk, oyster, dry beans, dry fruits, wheat germ, yeast.
*Medium* : Meat, chicken, fish, spinach, banana, apple.
*Poor* : Milk and its products, root vegetables.

## Iron absorption

The average daily diet contains 10–20 mg of iron. Its absorption occurs all over the intestine, but majority in the upper part. Dietary iron is present either as haeme or as inorganic iron. Absorption of haeme iron is better (upto 35% compared to inorganic iron which averages 5%) and occurs directly without the aid of a carrier (Fig. 43.1). However, it is a smaller fraction of dietary iron. The major part of dietary iron is inorganic and in the ferric form. It needs to be reduced to the ferrous form before absorption. Two separate iron transporters in the intestinal mucosal cells function to effect iron absorption. At the luminal membrane the *divalent metal transporter* 1 (DMT1) carrys ferrous iron into the mucosal cell. This along with the iron released from haeme is transported across the basolateral membrane by another iron transporter *ferroportin* (FP). These iron transporters are regulated according to the body needs. Absorption of haeme iron is largely independent of other foods simultaneously ingested, but that of inorganic iron is affected by several factors.

### *Factors facilitating iron absorption*

1. Acid: by favouring dissolution and reduction of ferric iron.

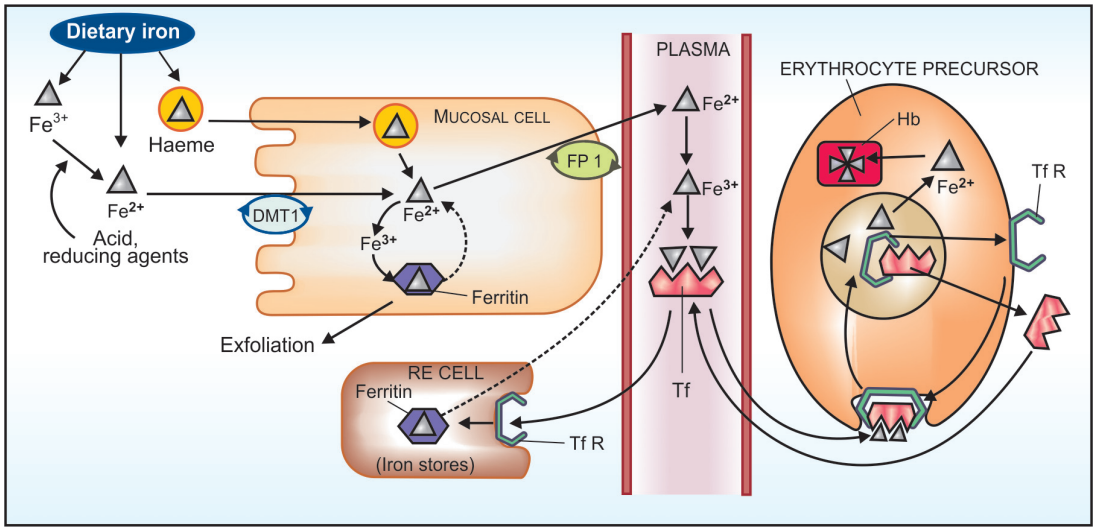



**Fig. 43.1:** Schematic depiction of intestinal absorption, transport, utilization and storage of iron (see text for description) $Fe^{2+}$—Ferrous iron; $Fe^{3+}$—Ferric iron; DMT1—Divalent metal transporter 1; Hb—Haemoglobin; RE cell—Reticuloendothelial cell; FP1—Ferroportin; Tf—Transferrin; TfR—Transferrin receptor

2. Reducing substances: ascorbic acid, amino acids containing SH radical. These agents reduce ferric iron and form absorbable complexes.
3. Meat: by increasing HCl secretion and providing haeme iron.

### *Factors impeding iron absorption*

1. Alkalies (antacids) render iron insoluble, oppose its reduction.
2. Phosphates (rich in egg yolk)
3. Phytates (in maize, wheat)
4. Tetracyclines

(2–4: By complexing iron)

5. Presence of other foods in the stomach.

In general, bioavailability of iron from cereal based diets is low.

*Mucosal block* The gut has a mechanism to prevent entry of excess iron in the body. Iron reaching inside mucosal cell is either transported to plasma or oxidised to ferric form and complexed with apoferritin to form ferritin (Fig. 43.1). This ferritin generally remains stored in the mucosal cells and is lost when they are shed (lifespan 2–4 days). This is called the '*Ferritin curtain*'.

The iron status of the body and erythropoietic activity govern the balance between these two processes, probably through a 'haematopoietic transcription factor', and thus the amount of iron that will enter the body. A larger percentage is absorbed during iron deficiency. When body iron is low or erythropoiesis is occurring briskly, ferritin is either not formed or dissociates soon—the released iron is transported to the blood.

Mucosal block however, can be overwhelmed by gross excess of iron.

## Transport, utilization, storage and excretion

Free iron is highly toxic. As such, on entering plasma it is immediately converted enzymatically to the ferric form and complexed with a glycoprotein *transferrin (Tf)*. Iron circulates in plasma bound to Tf (two $Fe^{3+}$ residues per molecule). The total plasma iron content (~3 mg) is recycled 10 times everyday (turnover of iron is 30 mg/day).

Iron is transported inside erythropoietic and other cells through attachment of transferrin to specific membrane bound transferrin receptors (TfRs). The complex is engulfed by receptor mediated endocytosis. Iron dissociates from the complex at the acidic pH of the intracellular vesicles; the released iron is utilized for haemoglobin synthesis or other purposes, while Tf and TfR are returned to the cell surface to carry fresh loads. In iron deficiency and haemolytic states when brisk erythropoiesis is occurring, erythropoietic cells express more TfRs, but other cells do not. Thus, the erythron becomes selectively more efficient in trapping iron.

After entering the storage cells through TfRs, iron is stored in RE cells (in liver, spleen, bone marrow), as well as in hepatocytes and myocytes as ferritin and haemosiderin. Apoferritin synthesis is regulated by iron status of the body. When it is low—the 'iron regulating element' (IRE) on mRNA is blocked—transcription of apoferritin does not occur, while more Tf is produced. On the other hand, more apoferritin is synthesized to trap iron when iron stores are rich. Plasma iron derived from destruction of old RBCs (lifespan ~120 days), from stores and from intestinal absorption forms a common pool that is available for erythropoiesis, to all other cells and for restorage.

Iron is tenaciously conserved by the body; daily excretion in adult male is 0.5–1 mg, mainly as exfoliated g.i. mucosal cells, some RBCs and in bile (all lost in faeces). Other routes are desquamated skin, very little in urine and sweat. In menstruating women, monthly menstrual loss may be averaged to 0.5–1 mg/day. Excess iron is required during pregnancy for expansion of RBC mass, transfer to foetus and loss during delivery; totalling to about 700 mg. This is to be met in the later 2 trimesters.

## Preparations and dose

### Oral iron

The preferred route of iron administration is oral. Dissociable ferrous salts are inexpensive, have high iron content and are better absorbed than

**TABLE 43.1 Some combination preparations of iron**

| *Trade name* | *Iron compound* | *Other ingredients* |
|---|---|---|
| CONVIRON Cap | Fe. sulfate (dried) 60 mg | $B_{12}$ 15 µg, folic acid 1.5 mg, $B_6$ 1.5 mg, vit. C 75 mg |
| FESOVIT-SPANSULE Cap | Fe. sulfate (dried) 150 mg | $B_{12}$ 15 µg, folic acid 1 mg, nicotinamide 50 mg, $B_6$ 2 mg |
| FERSOLATE-CM tab | Fe. sulfate (dried) 195 mg | Cu sulfate 2.6 mg, Mn. sulfate 2 mg |
| FEFOL SPANSULE Cap | Fe. sulfate 150 mg | Folic acid 0.5 mg |
| HEMGLOB syr (15 ml) | Fe. gluconate 300 mg | $B_{12}$ 15 µg, $B_1$ 5 mg, $B_2$ 5 mg, $B_6$ 1.5 mg, niacinamide 45 mg |
| AUTRIN Cap | Fe. fumarate 300 mg | $B_{12}$ 15 µg, folic acid 1.5 mg |
| DUMASULES Cap | Fe. fumarate 300 mg | $B_{12}$ 7.5 µg, folic acid 0.75 mg, $B_1$ 5 mg, niacinamide 50 mg, vit. C 75 mg, $B_6$ 1.5 mg |
| HEMSYNERAL Cap | Fe. fumarate 200 mg | $B_{12}$ 15 µg, folic acid 1.5 mg |
| ANEMIDOX Cap | Fe fumarate 360 mg | Folic acid 1.5 mg, vit $B_{12}$ 15 µg, Cal. carb. 200 mg, vit C 75 mg, vit D 400 i.u. |
| HEMSI syr. (5 ml) | Fe. fumarate 100 mg | Vit $B_{12}$ 5 µg, folic acid 0.5 mg, Zn 3.3 mg, Cu 0.035 mg, Mn 0.2 mg |
| FERRICARB Cap | Carbonyl iron (100 mg iron) | Folic acid 1.5 mg, vit $B_{12}$ 15 µg, zinc sulfate 88 mg, pyridoxine 3 mg, sod. selenite 60 µg |
| HBFAST tab | Carbonyl iron (100 mg iron) | Folic acid 0.35 mg |
| HEMATRINE Cap | Fe. succinate 100 mg | $B_{12}$ 2.5 µg, folic acid 0.5 mg, vit. C 25 mg, niacinamide 15 mg |
| POLYRON tab, BIOFER tab<br>POLYFER chewable tab | Ferric hydroxy polymaltose (Iron 100 mg) | Folic acid 0.35 mg |
| MUMFER syr (5 ml)<br>drops (1 ml) | Ferric hydroxy polymaltose (50 mg iron)<br>—do—(50 mg iron) | |
| FERROCHELATE syr (5 ml)<br>drops (1 ml) | Ferric ammon. cit. (Iron 60 mg)<br>—do—(Iron 20 mg) | $B_{12}$ 5 µg, folic acid 1 mg<br>$B_{12}$ 4 µg, folic acid 0.2 mg |
| RARICAP tab | Iron cal. complex (Iron 25 mg) | Folic acid 0.3 mg |
| PROBOFEX Cap | Fe. aminoate (60 mg iron) | $B_{12}$ 15 µg, folic acid 1.5 mg, $B_6$ 3 mg |
| DEXORANGE Cap, syrup (15 ml) | Ferric ammon. cit. 160 mg | $B_{12}$ 7.5 µg, folic acid 0.5 mg, Zn 7.5 mg (as sulfate) |

Combination of iron with strychnine, arsenic and yohimbine and all fixed dose combination of haemoglobin in any form are banned in India.

ferric salts, especially at higher doses. Gastric irritation and constipation (the most important side effects of oral iron) are related to the total quantity of elemental iron administered. If viewed in terms of iron content, nearly all preparations have the same degree of gastric tolerance, the limits of which are fairly well defined in individual patients. Some simple oral preparations are:

1. Ferrous sulfate: (hydrated salt 20% iron, dried salt 32% iron) is the cheapest; may be preferred on this account. It often leaves a metallic taste in mouth; FERSOLATE 200 mg tab.
2. Ferrous gluconate (12% iron): FERRONICUM 300 mg tab, 400 mg/15 ml elixer.
3. Ferrous fumarate (33% iron): is less water soluble than ferrous sulfate and tasteless; NORI-A 200 mg tab.
4. Colloidal ferric hydroxide (50% iron): FERRI DROPS 50 mg/ml drops.
5. Carbonyl iron: It is high purity metallic iron in very fine powder form (particle size < 5 μM), prepared by decomposition of iron pentacarbonyl, a highly toxic compound. It is claimed to be absorbed from intestines over a long time, and gastric tolerance may be better. However, bioavailability is about 3/4th that of ferrous sulfate.

Other forms of iron present in oral formulations are:

Ferrous succinate (35% iron)
Iron choline citrate
Iron calcium complex (5% iron)
Ferric ammonium citrate (20% iron)
Ferrous aminoate (10% iron)
Ferric glycerophosphate
Ferric hydroxy polymaltose

These are claimed to be better absorbed and/or produce less bowel upset, but this is primarily due to lower iron content. They are generally more expensive.

A number of oral formulations containing one of the iron compounds along with one to many vitamins, yeast, amino acids and other minerals are widely marketed and promoted. Some of these are listed in Table 43.1, but should be considered irrational.

A Technical Advisory Board (India) has recommended that B complex vitamins and zinc should not be included in iron and folic acid containing haematinic preparations.

Ferric hydroxy polymaltose has been marketed by many pharmaceuticals and vigorously promoted for its high iron content, no metallic taste, good g.i. tolerability and direct absorption from the intestines. Because the complex releases little free iron in the gut lumen—g.i. irritation is minimal. However, the high bioavailability observed in rats has not been found in humans and reports of its poor efficacy in treating iron deficiency anaemia have appeared. Its therapeutic efficacy is questionable.

The elemental iron content and not the quantity of iron compound per dose unit should be taken into consideration. Sustained release preparations are more expensive and not rational because most of the iron is absorbed in the upper intestine, while these preparations release part of their iron content lower down. Bioavailability of iron from such preparations, though claimed to be good, is actually variable. Liquid formulations may stain teeth: should be put on the back of tongue and swallowed. In general, they are less satisfactory.

A total of 200 mg elemental iron (infants and children 3–5 mg/kg) given daily in 3 divided doses produces the maximal haemopoietic response. Prophylactic dose is 30 mg iron daily. Absorption is much better when iron preparations are taken in empty stomach. However, side effects are also more; some prefer giving larger amounts after meals, while others like to give smaller doses in between meals.

*Adverse effects of oral iron* These are common at therapeutic doses and are related to elemental iron content. Individuals differ in susceptibility. Side effects are:

Epigastric pain, heartburn, nausea, vomiting, bloating, staining of teeth, metallic taste, colic, etc. Tolerance to oral iron can be improved by initiating therapy at low dose and gradually escalating to the optimum dose.

Constipation is more common (believed to be due to astringent action of iron) than diarrhoea (thought to reflect irritant action). However, these may be caused by alteration of intestinal flora as well.

## Parenteral iron

Iron therapy by injection is indicated only when:

1. Oral iron is not tolerated: bowel upset is too much.
2. Failure to absorb oral iron: malabsorption; inflammatory bowel disease. Chronic inflammation (rheumatoid arthritis) decreases iron absorption, as well as the rate at which iron can be utilized.
3. Non-compliance to oral iron.
4. In presence of severe deficiency with chronic bleeding.
5. Along with erythropoietin: oral ion may not be absorbed at sufficient rate to meet the demands of induced rapid erythropoiesis.

Parenteral iron therapy needs calculation of the total iron requirement of the patient for which several formulae have been devised. A simple one is:

Iron requirement (mg) =
4.4 × body weight (kg) × Hb deficit (g/dl)

This formula includes iron needed for replenishment of stores. The rate of response with parenteral iron is not faster than with optimal doses given orally, except probably in the first 2–3 weeks when dose of oral iron is being built up. However, iron stores can be replenished in a shorter time by parenteral therapy, because after correction of anaemia, a smaller fraction of ingested iron is absorbed.

The ionized salts of iron used orally cannot be injected because they have strong protein precipitating action and free iron in plasma is highly toxic. Four organically complexed formulations of iron are currently available in India; two of these *Iron-dextran* and *Iron-sorbitol-citric acid* have been in use for over 50 years, while two relatively new ones *Ferrous sucrose* and *Ferric carboxymaltose* have been added in the past few years. The newer formulations are less risky and have improved ease of administration. Few other formulations are marketed elsewhere.

*Iron-dextran* It is a high molecular weight colloidal solution containing 50 mg elemental iron/ml; is the only preparation that can be injected i.m. as well as i.v. By i.m. route it is absorbed through lymphatics, circulates without binding to transferrin and is engulfed by RE cells where iron dissociates and is made available to the erythron for haeme synthesis. In the injected muscle 10–30% of the dose remains locally bound and becomes unavailable for utilization for several weeks. Thus, 25% extra needs to be added to the calculated dose. Iron-dextran is not excreted in urine or in bile. Because dextran is antigenic, anaphylactic reactions are more common than with the newer preparations.

IMFERON, FERRI INJ: iron dextran 100 mg in 2 ml for i.m./i.v. injection.

*Intramuscular:* Injection is given deeply in the gluteal region using Z track technique (to avoid staining of the skin). Iron dextran can be injected 2 ml daily, or on alternate days, or 5 ml each side on the same day (local pain lasting weeks may occur with the higher dose).

*Intravenous:* After a test dose of 0.5 ml iron-dextran injected i.v. over 5–10 min, 2 ml can be injected per day taking 10 min for the injection. Alternatively, the total calculated dose is diluted in 500 ml of glucose/saline solution and infused i.v. over 6–8 hours under constant observation. Injection should be terminated if the patient complains of giddiness, paresthesias or tightness in the chest.

### Adverse effects

*Local* Pain at site of i.m. injection, pigmentation of skin, sterile abscess—especially in old and debilitated patient.

*Systemic* Fever, headache, joint pains, flushing, palpitation, chest pain, dyspnoea, lymph node enlargement.

An anaphylactoid reaction resulting in vascular collapse and death occurs rarely.

*Iron-sorbitol-citric acid* It is a low molecular weight complex which can be injected only i.m., from where absorption occurs directly into circulation and not through lymphatics. No local

binding in muscle occurs, but about 30% of the dose is excreted in urine; the calculated total dose has to be increased accordingly. Patient may be alarmed because the urine turns brown after some time. Iron-sorbitol-citric acid binds to transferrin in plasma and may saturate it if present in large quantity. That is why it is not suitable for i.v. injection or infusion, as the remaining free iron is highly toxic. Even with the recommended i.m. dose, incidence of immediate reaction, including ventricular tachycardia, A-V block, other irregularities, hypotension, flushing is higher. It is contraindicated in patients with kidney disease. This formulation is not favoured now.

*Dose:* 75 mg i.m. (max 100 mg) daily or on alternate days.

FERIMAX: iron-sorbitol-citric acid 75 mg, folic acid 0.75 mg, hydroxocobalamin 75 µg in 1.5 ml amp.

*Ferrous-sucrose* This newer formulation is a high molecular weight complex of iron hydroxide with sucrose, that on i.v. injection is taken up by RE cells, where iron dissociates and is utilized. It is safer than the older formulations and a dose of 100 mg (max 200 mg) can be injected i.v. taking 5 min, once daily to once weekly till the total calculated dose (including that to replenish stores) is administered. However, total dose i.v. infusion is not possible. The solution is highly alkaline ruling out i.m./s.c. injection.

The incidence of hypersensitivity reaction is very low. Though, some consider a test dose to be unnecessary, the British guidelines recommend it before the first dose. Other side effects are also milder. This preparation is particularly indicated for anaemia in kidney disease patients, but reports of kidney damage are on record. Oral iron should not be given concurrently and till 5 days after the last injection.

UNIFERON, ICOR, MICROFER: ferrous sucrose 50 mg in 2.5 ml and 100 mg in 5 ml amp. for i.v. inj.

*Ferric carboxymaltose* It is the latest formulation of iron in which a ferric hydroxide core is stabilized by a carbohydrate shell. The macromolecule is rapidly taken up by the RE cells, primarily in bone marrow (upto 80%), as well as in liver and spleen. Iron is released and delivered subsequently to the target cells. It is administered either as daily 100 mg i.v. injection, or upto 1000 mg is diluted with 100 ml saline (not glucose solution) and infused i.v. taking 15 min or more. Infusion may be repeated after a week. It should not be injected i.m. In clinical trials, it has caused a rapid increase in haemoglobin level in anaemia patients and replenished stores. The incidence of acute reaction is very low. Pain at injection site, and rashes have occurred, but anaphylaxis is rare. Headache, nausea, abdominal pain are generally mild. Hypotension, flushing and chest pain are infrequent. Due to lack of safety data, it is not recommended for children <14 years.

ENCICARB INJ: Ferric carboxymaltose 50 mg/ml in 2 ml and 10 ml vials.

## Use

1. *Iron deficiency anaemia* It is the most important indication for medicinal iron. Iron deficiency is the commonest cause of anaemia, especially in developing countries where a sizable percentage of population is anaemic. The RBC are microcytic and hypochromic due to deficient Hb synthesis. Other metabolic manifestations are seen when iron deficiency is severe. Apart from nutritional deficiency, chronic bleeding from g.i. tract (ulcers, inflammatory bowel disease, hookworm infestation) is a common cause. Iron deficiency also accompanies repeated attacks of malaria and chronic inflammatory diseases. The cause of iron deficiency should be identified and treated. Iron should be normally administered orally; parenteral therapy is to be reserved for special circumstances. A rise in Hb level by 0.5–1 g/dl per week is an optimum response to iron therapy. It is faster in the beginning and when anaemia is severe. Later, the rate of increase in Hb% declines. However, therapy should be continued till normal Hb level is attained (generally takes 1–3 months depending on the severity) and 2–3 months or more thereafter to replenish the stores.

*Prophylaxis*: The amount of iron available from average diet and the absorptive processes in the intestine place a ceiling on iron absorption of ~3 mg/day. Thus, iron balance is precarious in most menstruating women. Later half of pregnancy and infancy are periods when iron deficiency will develop unless medicinal iron is supplemented. In these situations as well as others (chronic

illness, menorrhagia, after acute blood loss, etc.) prophylactic use of iron is indicated.

2. *Megaloblastic anaemia* When brisk haemopoiesis is induced by vit $B_{12}$ or folate therapy, iron deficiency may be unmasked. The iron status of these patients should be evaluated and iron given accordingly.

## ACUTE IRON POISONING

It occurs mostly in infants and children: 10–20 iron tablets or equivalent of the liquid preparation (> 60 mg/kg iron) may cause serious toxicity in them. It is very rare in adults.

Manifestations are vomiting, abdominal pain, haematemesis, diarrhoea, lethargy, cyanosis, dehydration, acidosis, convulsions; finally shock, cardiovascular collapse and death. In few cases death occurs early (within 6 hours), but is typically delayed to 12– 36 hours, with apparent improvement in the intervening period. The pathological lesion is haemorrhage and inflammation in the gut, hepatic necrosis and brain damage.

**Treatment** It should be prompt.

*To prevent further absorption of iron from gut*

(a) Induce vomiting or perform gastric lavage with sodium bicarbonate solution—to render iron insoluble.

(b) Give egg yolk and milk orally: to complex iron. Activated charcoal does not adsorb iron.

*To bind and remove iron already absorbed*

Desferrioxamine (an iron chelating agent—*see* Ch. 66) is the drug of choice. It should be injected i.m. (preferably) 0.5–1 g (50 mg/kg) repeated 4–12 hourly as required, or i.v. (if shock is present) 10–15 mg/kg/hour; max 75 mg/kg in a day till serum iron falls below 300 µg/dl. Early therapy with desferrioxamine has drastically reduced mortality of iron poisoning.

Alternatively DTPA or calcium edetate (*see* Ch. 66) may be used if desferrioxamine is not available. BAL is contraindicated because its iron chelate is also toxic.

*Supportive measures* Fluid and electrolyte balance should be maintained and acidosis corrected by appropriate i.v. infusion. Respiration and BP may need support. Diazepam i.v. should be cautiously used to control convulsions, if they occur.

### Miscellaneous/Adjuvant haematinics

**1. Copper** Haeme synthesis is interfered in copper deficiency. However, copper is a trace metal for man and clinical deficiency is very rare. Its routine use is, therefore, not justified. However, when copper deficiency is demonstrated, 0.5–5 mg of copper sulphate/day may be given therapeutically; prophylactic dose is 0.1 mg/day. It is present in some haematinic combinations (*see* Table 43.1).

**2. Pyridoxine** (*see* Ch. 67) Pyridoxine responsive anaemia is a rare entity. It is due to inherent abnormality in haeme synthesis. Sideroblastic anaemia associated with isoniazid and pyrazinamide (which interfere with pyridoxine metabolism and action) therapy needs to be treated with pyridoxine. Some other sideroblastic anaemias show partial improvement with large doses of pyridoxine. However, routine use of pyridoxine in anaemia is wasteful.

**3. Riboflavin** (*see* Ch. 67) Hypoplastic anaemia occurs in riboflavin deficiency which is generally a part of multiple deficiencies in protein-calorie malnutrition. In the absence of specific deficiency, use of riboflavin in anaemia is of no value.

## MATURATION FACTORS

Deficiency of *vit $B_{12}$* and *folic acid*, which are B group vitamins, results in megaloblastic anaemia characterized by the presence of large red cell precursors in bone marrow and their large and shortlived progeny in peripheral blood. Vit $B_{12}$ and folic acid are therefore called maturation factors. The basic defect is in DNA synthesis. Apart from haemopoietic, other rapidly proliferating tissues also suffer.

## VITAMIN-$B_{12}$

Cyanocobalamin and hydroxocobalamin are complex cobalt containing compounds present in the diet and referred to as vit $B_{12}$.

Thomas Addison (1849) described cases of anaemia not responding to iron. This was later called 'pernicious' (incurable, deadly) anaemia and its relation with atrophy of gastric mucosa was realized. Minot and Murphy (1926)

treated such patients by including liver in diet and received Nobel prize. Castle (1927–32) propounded the hypothesis that there was an *extrinsic factor* present in diet which combined with an *intrinsic factor* produced by stomach to give rise to the *haemopoietic principle*. Vit $B_{12}$ was isolated in 1948 and was shown to be the extrinsic factor as well as the haemopoietic principle, the intrinsic factor only helped in its absorption.

Vit $B_{12}$ occurs as water soluble, thermostable red crystals. It is synthesized in nature only by microorganisms; plants and animals acquire it from them.

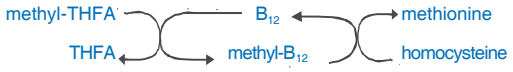
*Dietary sources* Liver, kidney, sea fish, egg yolk, meat, cheese are the main vit $B_{12}$ containing constituents of diet. The only vegetable source is legumes (pulses) which get it from microorganisms harboured in their root nodules.

Vit $B_{12}$ is synthesized by the colonic microflora, but this is not available for absorption in man. The commercial source is *Streptomyces griseus*; as a byproduct of streptomycin industry.

*Daily requirement* 1–3 µg, pregnancy and lactation 3–5 µg.

**Metabolic functions** Vit $B_{12}$ is intricately linked with folate metabolism in many ways; megaloblastic anaemia occurring due to deficiency of either is indistinguishable. In addition, vit $B_{12}$ has some independent metabolic functions as well. The active coenzyme forms of $B_{12}$ generated in the body are *deoxyadenosyl-cobalamin* ($DAB_{12}$) and *methyl-cobalamin* (methyl $B_{12}$).

(i) Vit $B_{12}$ is essential for the conversion of homocysteine to methionine




Methionine is needed as a methyl group donor in many metabolic reactions and for protein synthesis. This reaction is also critical in making tetrahydrofolic acid (THFA) available for reutilization. In $B_{12}$ deficiency THFA gets trapped in the methyl form and a number of *one carbon* transfer reactions suffer (*see* under folic acid).

(ii) Purine and pyrimidine synthesis is affected primarily due to defective 'one carbon' transfer because of 'folate trap'. The most important of these is inavailability of thymidylate for DNA production.

(iii) Malonic acid $\xrightarrow{DAB_{12}}$ Succinic acid

is an important step in propionic acid metabolism. It links the carbohydrate and lipid metabolisms. This reaction does not require folate and has been considered to be responsible for demyelination seen in $B_{12}$ deficiency, but not in pure folate deficiency. That myelin is lipoidal, supports this contention.

(iv)Now it appears that interference with the reaction:

Methionine $\xrightarrow{DAB_{12}}$ S-adenosyl methionine

may be more important in the neurological damage of $B_{12}$ deficiency, because it is needed in the synthesis of phospholipids and myelin.

(v) Vit $B_{12}$ is essential for cell growth and multiplication.

**Utilization of vit $B_{12}$** Vit $B_{12}$ is present in food as protein conjugates and is released by cooking or by proteolysis in stomach facilitated by gastric acid. Intrinsic factor (a glycoprotein, MW60,000) secreted by stomach forms a complex with $B_{12}$—attaches to specific receptors present on intestinal mucosal cells and is absorbed by active carrier mediated transport. This mechanism is essential for absorption of vit $B_{12}$ ingested in physiological amounts. However, when gross excess is taken, a small fraction is absorbed without the help of intrinsic factor.

Vit $B_{12}$ is transported in blood in combination with a specific β globulin *transcobalamin II* (TCII). Congenital absence of TCII or presence of abnormal protein (TCI or TCIII, in liver and bone marrow disease) may interfere with delivery of vit $B_{12}$ to tissues. Vit $B_{12}$ is especially taken up by liver cells and stored: about 2/3 to 4/5 of body's content (2–8 mg) is present in liver.

Vit $B_{12}$ is not degraded in the body. It is excreted mainly in bile (3–7 µg/day); all but 0.5–1 µg of this is reabsorbed—considerable enterohepatic circulation occurs. Thus, in the absence of intrinsic factor or when there is

malabsorption, $B_{12}$ deficiency develops much more rapidly than when it is due to nutritional deficiency. It takes 3–5 years of total absence of $B_{12}$ in diet to deplete normal body stores.

Vit $B_{12}$ is directly and completely absorbed after i.m. or deep s.c. injection. Normally, only traces of $B_{12}$ are excreted in urine, but when pharmacological doses (> 100 µg) are given orally or parenterally—a large part is excreted in urine, because the plasma protein binding sites get saturated and free vit $B_{12}$ is filtered at the glomerulus. Hydroxocobalamin is more protein bound and better retained than cyanocobalamin.

**Deficiency** Vit $B_{12}$ deficiency occurs due to:

1. Addisonian pernicious anaemia: is an autoimmune disorder which results in destruction of gastric parietal cells → absence of intrinsic factor in gastric juice (along with achlorhydria) → inability to absorb vit $B_{12}$.It is rare in India.
2. Other causes of gastric mucosal damage, e.g. chronic gastritis, gastric carcinoma, gastrectomy, etc.
3. Malabsorption (damaged intestinal mucosa), bowel resection, inflammatory bowel disease.
4. Consumption of vit $B_{12}$ by abnormal flora in intestine (blind loop syndrome) or fish tape worm.
5. Nutritional deficiency: is a less common cause; may occur in strict vegetarians.
6. Increased demand: pregnancy, infancy.

### *Manifestations of deficiency are:*

(a) Megaloblastic anaemia (generally the first manifestation), neutrophils with hypersegmented nuclei, giant platelets.

(b) Glossitis, g.i. disturbances: damage to epithelial structures.

(c) Neurological: subacute combined degeneration of spinal cord; peripheral neuritis—diminished vibration and position sense, paresthesias, depressed stretch reflexes; mental changes—poor memory, mood changes, hallucinations, etc. are late effects.

## Preparations, dose, administration

*Cyanocobalamin*: 35 µg/5 ml liq.
*Hydroxocobalamin*: 500 µg, 1000 µg inj.
In India both oral and injectable vit $B_{12}$ is available mostly as combination preparation along with other vitamins, with or without iron. The leading ones are listed in Tables 43.1 and 67.2. Some selected brands with their vit $B_{12}$ content are:

NEUROBION FORTE (1000 µg/3 ml inj; 15 µg/tab.), OPTINEURON (1000 µg/3 ml inj.), NEUROXIN-12 (500 µg/10 ml inj.), POLYBION (15 µg/cap), BECOSULES (5 µg/cap), FESOVIT (15 µg/cap), AUTRIN (15 µg/cap) FERRICARB (15 µg/cap).

Because of higher protein binding and better retention in blood, hydroxocobalamin is preferred for parenteral administration to treat vit $B_{12}$ deficiency. In Britain it has totally replaced cyanocobalamin, which is restricted to oral use. However, professionals in USA consider that hydroxocobalamin may induce antibody formation in some patients and its blood level may decline as rapidly (within 1 month) as that of cyanocobalamin. Therefore, they use cyanocobalamin orally as well as parenterally.

*Prophylactic dose*: 3–10 µg/day orally in those at risk of developing deficiency.

*Therapeutic dose*: Oral vit $B_{12}$ is not dependable for treatment of confirmed vit $B_{12}$ deficiency because its absorption from the intestine is unreliable. Injected vit $B_{12}$ is a must when deficiency is due to lack of intrinsic factor (pernicious anaemia, other gastric causes), since the absorptive mechanism is totally non-functional. Various regimens are in use. The one followed in Britain is—hydroxocobalamin 1 mg i.m./s.c. daily for 2 weeks or till neurological symptoms (when present) abate, followed by 1 mg injected every 2 months for maintenance. The regimen popular in USA is—cyanocobalamin 100 µg i.m./s.c. daily for 1 week, then weekly for 1 month, and then monthly for maintenance indefinitely.

***Methylcobalamin*** (methyl $B_{12}$) is the active coenzyme form of vit $B_{12}$ for synthesis of methionine and S-adenosylmethionine that is needed for integrity of myelin. This preparation of vit $B_{12}$ in a dose of 1.5 mg/day has been especially promoted for correcting the neurological defects in diabetic, alcoholic and other forms of peripheral neuropathy. However, in USA and many other countries, it is used only as a nutritional supplement, and not as a drug.

*Methylcobalamin* BIOCOBAL, DIACOBAL, METHYLCOBAL 0.5 mg tab.

### Uses

1. Treatment of vit $B_{12}$ deficiency: vit $B_{12}$ is used as outlined above. It is wise to add 1–5 mg of oral folic acid and an iron preparation, because reinstitution of brisk haemopoiesis may unmask deficiency of these factors. Response to vit $B_{12}$ is dramatic—symptomatic improvement starts in 2 days: appetite improves, patient feels better; mucosal lesions heal in 1–2 weeks; reticulocyte count increases; Hb% and haematocrit start rising after 2 weeks; platelet count normalises in 10 days and WBC count in 2–3 weeks. Time taken for complete recovery of anaemia depends on the severity of disease to start with. Neurological parameters improve more slowly—may take several months; full recovery may not occur if vit $B_{12}$ deficiency has been severe or had persisted for 6 months or more.
2. Prophylaxis: needs to be given only when there are definite predisposing factors for development of deficiency (*see* above).
3. Mega doses of vit $B_{12}$ have been used in neuropathies, psychiatric disorders, cutaneous sarcoid and as a general tonic to allay fatigue, improve growth—value is questionable.
4. Tobacco amblyopia: hydroxocobalamin is of some benefit—it probably traps cyanide derived from tobacco to form cyanocobalamin.

**Adverse effects** Even large doses of vit $B_{12}$ are quite safe. Allergic reactions have occurred on injection, probably due to contaminants. Anaphylactoid reactions (probably to sulfite contained in the formulation) have occurred on i.v. injection: this route should never be employed.

## FOLIC ACID

It occurs as yellow crystals which are insoluble in water, but its sodium salt is freely water soluble. Chemically it is *Pteroyl glutamic acid (PGA)* consisting of pteridine + paraaminobenzoic acid (PABA) + glutamic acid.

Wills (1932–37) had found that liver extract contained a factor, other than vit $B_{12}$, which could cure megaloblastic anaemia. Mitchell in 1941 isolated an antianaemia principle from spinach and called it 'folic acid' (from leaf). Later the Will's factor was shown to be identical to folic acid.

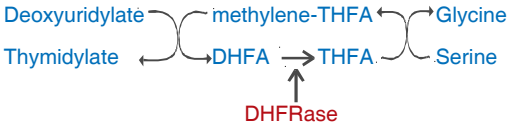
*Dietary sources* Liver, green leafy vegetables (spinach), egg, meat, milk. It is synthesized by gut flora, but this is largely unavailable for absorption.

*Daily requirement* of an adult is < 0.1 mg but dietary allowance of 0.2 mg/day is recommended. During pregnancy, lactation or any condition of high metabolic activity, 0.8 mg/day is considered appropriate.

**Utilization** Folic acid is present in food as polyglutamates; the additional glutamate residues are split off primarily in the upper intestine before being absorbed. Reduction to DHFA and methylation also occurs at this site. It is transported in blood mostly as methyl-THFA which is partly bound to plasma proteins. Small, physiological amounts of folate are absorbed by specific carrier-mediated active transport in the intestinal mucosa. Large pharmacological doses may gain entry by passive diffusion, but only a fraction is absorbed.

Folic acid is rapidly extracted by tissues and stored in cells as polyglutamates. Liver takes up a large part and secretes methyl-THFA in bile which is mostly reabsorbed from intestine: enterohepatic circulation occurs. Alcohol interferes with release of methyl-THFA from hepatocytes. The total body store of folates is 5–10 mg. Normally, only traces are excreted, but when pharmacological doses are given, 50–90% of the absorbed dose may be excreted in urine.

**Metabolic functions** Folic acid is inactive as such and is reduced to the coenzyme form in two steps: FA → DHFA → THFA by folate reductase (FRase) and dihydrofolate reductase (DHFRase). THFA mediates a number of *one carbon* transfer reactions by carrying a methyl group as an adduct (*see* under vit. $B_{12}$ also).
1. Conversion of homocysteine to methionine: vit $B_{12}$ acts as an intermediary carrier of methyl group (*see* p. 607). This is the most important reaction which releases THFA from the methylated form.
2. Generation of thymidylate, an essential constituent of DNA:




3. Conversion of serine to glycine: needs THFA and results in the formation of methylene-THFA which is utilized in thymidylate synthesis.

4. Purine synthesis: *de novo* building of purine ring requires formyl-THFA and methenyl-THFA (generated from methylene-THFA) to introduce carbon atoms at position 2 and 8.

5. Generation and utilization of 'formate pool'.

6. Histidine metabolism: for mediating formimino group transfer.

Ascorbic acid protects folates in the reduced form. Other cofactors, e.g. pyridoxal, etc. are required for some of the above reactions.

**Deficiency** Folate deficiency occurs due to:

(a) Inadequate dietary intake

(b) Malabsorption: especially involving upper intestine—coeliac disease, tropical sprue, regional ileitis, etc. Deficiency develops more rapidly as both dietary and biliary folate is not absorbed.

(c) Biliary fistula; bile containing folate for recirculation is drained.

(d) Chronic alcoholism: intake of folate is generally poor. Moreover, its release from liver cells and recirculation are interfered.

(e) Increased demand: pregnancy, lactation, rapid growth periods, haemolytic anaemia and other diseases with high cell turnover rates.

(f) Drug induced: prolonged therapy with anticonvulsants (phenytoin, phenobarbitone, primidone) and oral contraceptives—interfere with absorption and storage of folate.

*Manifestations of deficiency are:*

(i) Megaloblastic anaemia, indistinguishable from that due to vit $B_{12}$ deficiency. However, folate deficiency develops more rapidly if external supply is cut off: body stores last 3–4 months only. In malabsorptive conditions megaloblastosis may appear in weeks.

(ii) Epithelial damage: glossitis, enteritis, diarrhoea, steatorrhoea.

(iii) Neural tube defects, including spina bifida in the offspring, due to maternal folate deficiency.

(iv) General debility, weight loss, sterility. However, neurological symptoms do not appear in pure folate deficiency.

## Preparations and dose

Folic acid: FOLVITE, FOLITAB 5 mg tab;

Liquid oral preparations and injectables are available only in combination formulation (*see* Tables 43-1 and 67-2). Oral therapy is adequate except when malabsorption is present or in severely ill patient—given i.m.

*Dose:* therapeutic 2 to 5 mg/day, prophylactic 0.5 mg/day.

Folinic acid; CALCIUM LEUCOVORIN 3 mg/ml inj. FASTOVORIN 3 mg, 15 mg amps, 50 mg vial; RECOVORIN 15 mg tab, 15 mg, 50 mg vial for inj.

## Uses

1. *Megaloblastic anaemias* due to:

(a) Nutritional folate deficiency; manifests earlier than vit $B_{12}$ deficiency. Oral folic acid 2–5 mg/day is adequate, but in acutely ill patients, therapy may be initiated with injection of folic acid 5 mg/day. Response occurs as quickly as with vit $B_{12}$.

(b) Increased demand: pregnancy, lactation, infancy, during treatment of severe iron deficiency anaemia, haemolytic anaemias.

(c) Pernicious anaemia: folate stores may be low and deficiency may be unmasked when vit $B_{12}$ induces brisk haemopoiesis. Folic acid has only secondary and adjuvant role in this condition.

Folic acid should never be given alone to patients with vit $B_{12}$ deficiency, because haematological response may occur, but neurological defect may worsen due to diversion of meagre amount of vit $B_{12}$ present in body to haemopoiesis.

(d) Malabsorption syndromes: Tropical sprue, coeliac disease, idiopathic steatorrhoea, etc.

(e) Antiepileptic therapy: Megaloblastic anaemia can occur due to prolonged phenytoin/phenobarbitone therapy (*see* Ch. 30). This is treated by folic acid, but large doses should be avoided as they may antagonize anticonvulsant effect.

2. *Prophylaxis* of folate deficiency: only when definite predisposing factors are present. Routine folate supplementation (1 mg/day) is recommended during pregnancy to reduce the risk of neural tube defects in the newborn.

3. *Methotrexate toxicity* Folinic acid (Leucovorin, citrovorum factor, 5-formyl-THFA) is an active coenzyme form which does not need to be reduced by DHFRase before it can act. Methotrexate is a DHFRase inhibitor; its toxicity is not counteracted by folic acid, but antagonized by folinic acid (3.0 mg i.v. repeated as required).

Folinic acid is expensive and not needed for the correction of simple folate deficiency for which folic acid is good enough.

4. *Citrovorum factor rescue* In certain malignancies, high dose of methotrexate is injected i.v. and is followed within ½ –1 hour with 1–3 mg i.v. of folinic acid to rescue the normal cells. It is ineffective if given > 3 hours after methotrexate.

5. *To enhance anticancer efficacy of 5-fluorouracil (5-FU)* Folinic acid is now routinely infused i.v. along with 5-FU (*see* p. 864), because THFA is required for inhibition of thymidylate synthase by 5-FU.

**Adverse effects** Oral folic acid is entirely nontoxic. Injections rarely cause sensitivity reactions.

## ERYTHROPOIETIN

Erythropoietin (EPO) is a sialoglycoprotein hormone (MW 34000) produced by peritubular cells of the kidney that is essential for normal erythropoiesis. Anaemia and hypoxia are sensed by kidney cells and induce rapid secretion of EPO → acts on erythroid marrow and:

(a) Stimulates proliferation of colony forming cells of the erythroid series.

(b) Induces haemoglobin formation and erythroblast maturation.

(c) Releases reticulocytes in the circulation.

EPO binds to specific receptors on the surface of its target cells. The EPO receptor is a JAK-STAT-binding receptor that alters phosphorylation of intracellular proteins and activates transcription factors to regulate gene expression. It induces erythropoiesis in a dose dependent manner, but has no effect on RBC lifespan.

The recombinant human erythropoietin (Epoetin α, β) is administered by i.v. or s.c. injection and has a plasma t½ of 6–10 hr, but action lasts several days.

*Use* The primary indication for epoetin is anaemia of chronic renal failure which is due to low levels of EPO. Only smptomatic patients with Hb ≤ 8 g/dl should be considered for EPO therapy. Epoetin 25–100 U/kg s.c. or i.v. 3 times a week (max. 600 U/kg/week) raises haematocrit and haemoglobin, reduces need for transfusions and improves quality of life. It is prudent to start with a low dose and titrate upwards to keep haematocrit between 30–36%, and Hb 10–11 g (max 12 g) per dl. Trials have found higher mortality if Hb level was raised to normal (13.5 g/dl). Some recent studies have indicated that dose reduction by about 30% is possible when epoetin is given s.c. compared to i.v. Exercise capacity and overall wellbeing of the patients is improved. Most patients have low iron stores; require concurrent parenteral/oral iron therapy for an optimum response. Other uses are:

1. Anaemia in AIDS patients treated with zidovudine.
2. Cancer chemotherapy induced anaemia.
3. Preoperative increased blood production for autologous transfusion during surgery.

*Adverse effects* Epoetin is nonimmunogenic. Adverse effects are related to sudden increase in haematocrit, blood viscosity and peripheral vascular resistance (due to correction of anaemia). These are—increased clot formation in the A-V shunts (most patients are on dialysis), hypertensive episodes, serious thromboembolic events, occasionally seizures. Flu like symptoms lasting 2–4 hr occur in some patients.

HEMAX 2000 IU/ml and 4000 IU/ml vials; EPREX 2000 IU, 4000 IU and 10000 IU in 1 ml prefilled syringes; ZYROP (epoetin β) 2000 IU and 4000 IU vials.

Recently, a hyperglycosylated modified EPO Darbepoetin has been introduced that has a t½ >24 hours, is longer acting and can be administered once every 2–4 weeks.

## ☞ PROBLEM DIRECTED STUDY

**43.1** A lady aged 40 years consults you for treatment of her anaemia that is not improving with medicine prescribed by a local doctor. She told that she is suffering from weakness, fatigue and occasional giddiness for the last 4–5 months. She went to a local doctor 2 months ago who got her blood tested, which showed Hb was 7.5 g/dl. A liquid medicine was prescribed, that she has been taking 1 tablespoonful daily without any benefit. The medicine was found to be syrup Ferric ammonium citrate 160 mg/15 ml along with folic acid 0.5 mg and vit $B_{12}$ 7.5 µg. She also revealed that she suffers from heart burn, and has been taking a tablet (Rabeprazole 20 mg) once daily for the last 2–3 years. Repeat blood testing showed Hb to be 7.6 g/dl, haematocrit was 27%, RBCs were microcytic-hypochromic, and other values were consistent with iron deficiency anaemia. Her periods were normal and detailed examination showed no evidence of bleeding from any site.

(a) What could be the reason for her failure to improve with oral iron therapy that she has been taking?

(b) Can she still be treated with oral iron, or does she require parenteral iron therapy? What treatment would be appropriate for her?

(see Appendix-1 for solution)

# Chapter 44 Drugs Affecting Coagulation, Bleeding and Thrombosis

Haemostasis (arrest of blood loss) and blood coagulation involve complex interactions between the injured vessel wall, platelets and coagulation factors. A cascading series of proteolytic reactions (Fig. 44.1) is started by:

(i) Contact activation of Hageman factor: *intrinsic system*, in which all factors needed for coagulation are present in plasma. This pathway is responsible for clotting when blood is kept in a glass tube, and for amplification of the common pathway. This is slow and takes several minutes to activate factor X.

(ii) Tissue thromboplastin: *extrinsic system*, needs a tissue factor, but activates factor X in seconds. In the normal course, coagulation after injury to vessel wall occurs by this pathway.

The subsequent events are common in the two systems and result in polymerization of fibrinogen to form fibrin strands. Blood cells are trapped in the meshwork of fibrin strands producing clot.

Two *in vitro* tests 'activated partial thromboplastin time' (aPTT) and 'prothrombin time' (PT) are employed for testing integrity of the intrinsic, extrinsic and common pathways of the coagulation cascade. The results are interpreted as:

| | PT | aPTT |
|---|---|---|
| Intrinsic pathway interfered | Normal (12–14S) | Prolonged |
| Extrinsic pathway interfered | Prolonged | Normal (26–32S) |
| Common pathway interfered | Prolonged | Prolonged |

Most clotting factors are proteins present in plasma in the inactive (zymogen) form. By partial proteolysis they themselves become an active protease and activate the next factor. In addition to its critical role in cleaving and polymerizing fibrinogen, thrombin activates many upstream factors (especially f. XI, VIII and V) of the intrinsic and common pathways—amplifying its own generation and continuation of clot formation. It is also a potent activator of platelets.

On the other hand, factors like *antithrombin*, *protein C*, *protein S, antithromboplastin* and the *fibrinolysin system* tend to oppose coagulation and lyse formed clot. Thus, a check and balance system operates to maintain blood in a fluid state while in circulation and allows rapid haemostasis following injury.

## COAGULANTS

These are substances which promote coagulation, and are indicated in haemorrhagic states.

Fresh whole blood or plasma provide all the factors needed for coagulation and are the best therapy for deficiency of any clotting factor; also they act immediately. Other drugs used to restore haemostasis are:

### 1. *Vitamin K*

$K_1$ (from plants, fat-soluble) : Phytonadione (Phylloquinone)

$K_3$ (synthetic)

—Fat-soluble : Menadione, Acetomenaphthone

—Water-soluble : Menadione sod. bisulfite

: Menadione sod. diphosphate

### 2. *Miscellaneous*

Fibrinogen (human)
Antihaemophilic factor
Desmopressin
Adrenochrome monosemicarbazone
Rutin, Ethamsylate

## VITAMIN K

It is a fat-soluble dietary principle required for the synthesis of clotting factors.

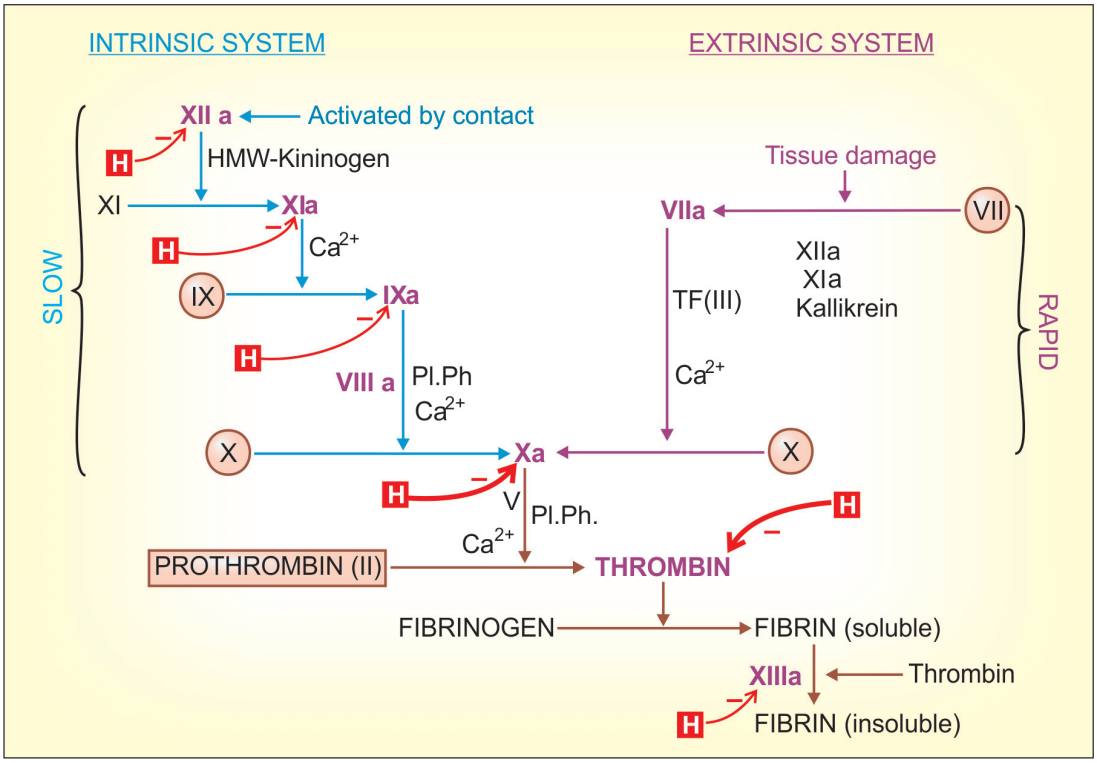
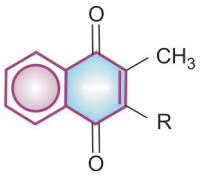



**Fig. 44.1:** The coagulation cascade. The vit. K dependent factors have been encircled, Factors inactivated by heparin (H) in red; the more important inhibited steps are highlighted by thick arrow. a—activated form; Pl.Ph.—Platelet phospholipid; HMW—High molecular weight; TF—Tissue factor (factor III)

SECTION 10

Dam (1929) produced bleeding disorder in chicken by feeding deficient diet. This was later found to be due to decreased concentration of prothrombin in blood and that it could be cured by a fat soluble fraction of hog liver. This factor was called *Koagulations vitamin* (vit K) and soon its structure was worked out. A similar vitamin was isolated in 1939 from alfalfa grass and labelled vit $K_1$, while that from sardine (sea fish) meal was labelled $K_2$. Synthetic compounds have been produced and labelled $K_3$.




VITAMIN K

*Chemistry and source* Vit K has a basic naphthoquinone structure, with or without a side chain (R) at position 3. The side chain in $K_1$ is *phytyl*, in $K_2$ *prenyl*, while in $K_3$ there is no side chain.

Dietary sources are—green leafy vegetables, such as cabbage, spinach; and liver, cheese, etc.

*Daily requirement* It is uncertain, because a variable amount of menaquinone (vit $K_2$) produced by colonic bacteria becomes available. Even 3–10 µg/day external source may be sufficient. However, the total requirement of an adult has been estimated to be 50–100 µg/day.

**Action** Vit K acts as a cofactor at a late stage in the synthesis by liver of coagulation proteins—prothrombin, factors VII, IX and X. The vit K dependent change (γ carboxylation of glutamate residues of these zymogen proteins; *see* Fig. 44.2) confers on them the capacity to bind $Ca^{2+}$ and to get bound to phospholipid surfaces—properties essential for participation in the coagulation cascade.

**Utilization** Fat-soluble forms of vit K are absorbed from the intestine *via* lymph and require

bile salts for absorption, while water-soluble forms are absorbed directly into portal blood. An active transport process in the jejunum has been demonstrated for $K_1$, while $K_2$ and $K_3$ are absorbed by simple diffusion. Vit K is only temporarily concentrated in liver, but there are no significant stores in the body. It is metabolized in liver by side chain cleavage and glucuronide conjugation; metabolites are excreted in bile and urine.

**Deficiency** Deficiency of vit K occurs due to liver disease, obstructive jaundice, malabsorption, long-term antimicrobial therapy which alters intestinal flora. However, deficient diet is rarely responsible. The most important manifestation is bleeding tendency due to lowering of the levels of prothrombin and other clotting factors in blood. Haematuria is usually first to occur; other common sites of bleeding are g.i.t., nose and under the skin—ecchymoses.

### Preparations

Phytonadione: VITAMIN-K, KVI, K-WIN 10 mg/ml for i.m. injection.
Menadione: 0.66 mg in GYNAE CVP with vit C 75 mg, ferrous gluconate 67 mg, Cal. lactate 300 mg and citras bioflavonoid 150 mg per cap:
Acetomenaphthone: ACETOMENADIONE 5, 10 mg tab; KAPILIN 10 mg tab.
Menadione sod. bisulfite: 20 mg, in CADISPER-C with vit C 100 mg, adrenochrome monosemicarbazone, 1 mg, rutin 60 mg, methylhesperidin 40 mg, Cal. phosphate 100 mg per tab.
STYPTOCID 10 mg with adrenochrome monosemicarbazone 0.5 mg, rutin 50 mg, vit C 37.5 mg, vit D 200 i.u., Cal. phosphate 260 mg per tab.

**Use** The only use of vit K is in prophylaxis and treatment of bleeding due to deficiency of clotting factors in the following situations:
(a) *Dietary deficiency:* of vit K is very rare in adults. However, when it occurs 5–10 mg/day oral or parenteral vit K rapidly corrects the defects.
(b) *Prolonged antimicrobial therapy:* treat in the same way as dietary deficiency of vit K.
(c) *Obstructive jaundice or malabsorption syndromes* (sprue, regional ileitis, steatorrhoea, etc.): vit K 10 mg i.m./day, or orally along with bile salts.
(d) *Liver disease* (cirrhosis, viral hepatitis): associated bleeding responds poorly to vit K. Because of hepatocellular damage, synthesis of clotting factors is inadequate despite the presence of vit K. However, vit K may be of some use if its absorption had been affected due to lack of bile salts.
(e) *Newborns:* All newborns have low levels of prothrombin and other clotting factors. Further decrease occurs in the next few days. The cause is both lower capacity to synthesize clotting factors as well as deficiency of vit K. The defect is exaggerated in the premature infant. Vit K 1 mg i.m. soon after birth has been recommended routinely. Some prefer administering 5–10 mg i.m. to the mother 4–12 hours before delivery. Haemorrhagic disease of the newborn can be effectively prevented/treated by such medication.

Menadione ($K_3$) should not be used for this purpose (*see* below).
(f) *Overdose of oral anticoagulants:* This is the most important indication of vit K. Phytonadione ($K_1$) is the preparation of choice, because it acts most rapidly; dose depends on the severity of hypoprothrombinaemia (measured INR) and bleeding. Unnecessary high dose is to be avoided because it will render the patient unresponsive to oral anticoagulants for several days.

*Severe*: 10 mg i.m. followed by 5 mg 4 hourly; bleeding generally stops in 6–12 hours, but normal levels of coagulation factors are restored only after 24 hr. This dose of vit K will block anticoagulant action for 7–10 days.

*Moderate*: 10 mg i.m. followed by 5 mg once or twice according to response.

*Mild*: Just omit a few doses of the anticoagulant.

(g) Prolonged high dose salicylate therapy causes hypoprothrombinemia; vit K should be given prophylactically. If bleeding occurs—treat as for oral anticoagulants.

**Adverse effects** Phytonadione injected i.m. or given orally hardly produces any adverse effect; allergic reactions are rare. Severe anaphylactoid

reactions can occur on i.v. injection of emulsified formulation; this route should not be used.

Menadione and its water-soluble derivatives can cause haemolysis in a dose-dependent manner. Patients with G-6-PD deficiency and neonates are especially susceptible. In the newborn menadione or its salts can precipitate kernicterus: (a) by inducing haemolysis and increasing bilirubin load. (b) by competitively inhibiting glucuronidation of bilirubin. Glucuronide conjugation is, as such, inadequate in neonates.

Because of poor efficacy and higher toxicity, there is little justification to use menadione and its water soluble salts for any indication.

**Fibrinogen** The fibrinogen fraction of human plasma is employed to control bleeding in haemophilia, antihaemophilic globulin (AHG) deficiency and acute afibrinogenemic states; 0.5 g is infused i.v.
FIBRINAL 0.5 g/bottle for i.v. infusion.

**Antihaemophilic factor** It is concentrated human AHG prepared from pooled human plasma. It is indicated (along with human fibrinogen) in haemophilia and AHG deficiency. It is highly effective in controlling bleeding episodes, but action is short-lasting (1 to 2 days).
*Dose:* 5–10 U/kg by i.v. infusion, repeated 6–12 hourly.
FIBRINAL-H, ANTIHAEMOPHILIC FACTOR: 150 U or 200 U + fibrinogen 0.5 g/bottle for i.v. infusion.

**Desmopressin** It releases factor VIII and von Willebrand's factor from vascular endothelium and checks bleeding in haemophilia and von Willebrand's disease (*see* p. 597).

**Adrenochrome monosemicarbazone** It is believed to reduce capillary fragility, control oozing from raw surfaces and prevent microvessel bleeding, e.g. epistaxis, haematuria, secondary haemorrhage from wounds, etc. Its efficacy is uncertain.
*Dose:* 1–5 mg oral, i.m.
STYPTOCHROME 3 mg/2 ml inj., STYPTOCID: 2 mg/2 ml inj.

**Rutin** It is a plant glycoside claimed to reduce capillary bleeding. It has been used in a dose of 60 mg oral BD–TDS along with vit C which is believed to facilitate its action. Its efficacy is uncertain.
In CADISPER-C 60 mg tab.

**Ethamsylate** It reduces capillary bleeding when platelets are adequate; probably exerts antihyaluronidase action or corrects abnormalities of platelet adhesion, but does not stabilize fibrin (not an antifibrinolytic). Ethamsylate has been used in the prevention and treatment of capillary bleeding in menorrhagia, after abortion, PPH, epistaxis, malena, hematuria and after tooth extraction, but efficacy is unsubstantiated. Side effects are nausea, rash, headache, and fall in BP (only after i.v. injection).
*Dose:* 250–500 mg TDS oral/i.v.; ETHAMSYL, DICYNENE, HEMSYL, K. STAT 250, 500 mg tabs; 250 mg/2 ml inj.

## LOCAL HAEMOSTATICS (STYPTICS)

After injury to arterioles and other smaller blood vessels, normal haemostasis occurs successively by contraction of injured vessel wall (lasting few minutes), adhesion and aggregation of platelets to form a plug, formation of a blood clot, and finally in due course dissolution of the clot by fibrinolysis. External bleeding is usually stopped by manual pressure, cotton-gauze pressure pack or by suturing. Control of bleeding may be aided by *local haemostatics (styptics)* that are substances used to stop bleeding from a local and approachable site. They are particularly effective on oozing surfaces, e.g. tooth socket, abrasions, etc. Absorbable materials like *fibrin* (prepared from human plasma and dryed as sheet or foam), *gelatin foam, oxidized cellulose* (as strips which can be cut and placed in the wound) provide a meshwork which activates the clotting mechanism and checks bleeding. Left *in situ* these materials are absorbed in 1–4 weeks and generally cause no foreign body reaction. *Thrombin* obtained from bovine plasma may be applied as dry powder or freshly prepared solution to the bleeding surface in haemophiliacs. *Vasoconstrictors* like 0.1% Adr solution may be soaked in sterile cotton-gauze and packed in the bleeding tooth socket or nose in case of epistaxis to check bleeding when spontaneous vasoconstriction is inadequate. *Astringents* such as tannic acid or metallic salts are occasionally applied for bleeding gums, bleeding piles, etc.

### SCLEROSING AGENTS

These are irritants, cause inflammation, coagulation and ultimately fibrosis, when injected into haemorrhoids (piles) or varicose vein mass. They are used only for local injection.

1. *Sod. tetradecyl sulfate (3% with benzyl alcohol 2%): 0.5–2 ml at each site.* SETROL 2 ml inj.
2. *Polidocanol (3% inj): 2 ml;* ASKLEROL 2 ml inj.

## ANTICOAGULANTS

These are drugs used to reduce the coagulability of blood. They may be classified into:

**I. Used in vivo**

A. *Parenteral anticoagulants*

(i) *Indirect thrombin inhibitors*: Heparin, Low molecular weight heparins, Fondaparinux, Danaparoid

(ii) *Direct thrombin inhibitors*: Lepirudin, Bivalirudin, Argatroban

B. *Oral anticoagulants*

(i) *Coumarin derivatives*: Bishydroxycoumarin (dicumarol), Warfarin sod, Acenocoumarol (Nicoumalone), Ethylbiscoumacetate

(ii) *Indandione derivative*: Phenindione.

(iii) *Direct factor Xa inhibitors*: Rivaroxaban

(iv) *Oral direct thrombin inhibitor*: Dabigatran etexilate

**II. Used in vitro**

A. Heparin:
150 U to prevent clotting of 100 ml blood.

B. Calcium complexing agents:
Sodium citrate: 1.65 g for 350 ml of blood; used to keep blood in the fluid state for transfusion;
ANTICOAGULANT ACID CITRATE DEXTROSE SOLUTION 2.2 g/100 ml (75 ml is used for 1 unit of blood).
Sodium oxalate: 10 mg for 1 ml blood
Sodium edetate: 2 mg for 1 ml blood
} used in blood taken for investigations

## HEPARIN

McLean, a medical student, discovered in 1916 that liver contains a powerful anticoagulant. Howell and Holt (1918) named it 'heparin' because it was obtained from liver. However, it could be used clinically only in 1937 when sufficient degree of purification was achieved.

**Chemistry and occurrence** Heparin is a non-uniform mixture of straight chain mucopolysaccharides with MW 10,000 to 20,000. It contains polymers of two sulfated disaccharide units:

D-glucosamine-L-iduronic acid
D-glucosamine-D-glucuronic acid
} chain length and proportion of the two disaccharide units varies. Some glucosamine residues are N-acetylated.

Heparin carries strong electronegative charges and is the strongest organic acid present in the body. It occurs in mast cells as a much bigger molecule (MW ~75,000) loosely bound to the granular protein. Thus, heparin is present in all tissues containing mast cells; richest sources are lung, liver and intestinal mucosa. Commercially it is produced from ox lung and pig intestinal mucosa.

### ACTIONS

**1. Anticoagulant** Heparin is a powerful and instantaneously acting anticoagulant, effective both *in vivo* and *in vitro*. It acts indirectly by activating plasma antithrombin III (AT III, a serine proteinase inhibitor). The heparin-AT III complex then binds to clotting factors of the intrinsic and common pathways (Xa, IIa, IXa, XIa, XIIa and XIIIa) and inactivates them but not factor VIIa operative in the extrinsic pathway. At low concentrations of heparin, factor Xa mediated conversion of prothrombin to thrombin is selectively affected. The anticoagulant action is exerted mainly by inhibition of factor Xa as well as thrombin (IIa) mediated conversion of fibrinogen to fibrin.

Low concentrations of heparin prolong aPTT without significantly prolonging PT. High concentrations prolong both. Thus, low concentrations interfere selectively with the intrinsic pathway, affecting amplification and continuation of clotting, while high concentrations affect the common pathway as well.

Antithrombin III is itself a substrate for the protease clotting factors; binds with the protease to form a stable complex (suicide inhibitor). However, in the absence of heparin, the two interact very slowly. Heparin enhances the action of AT III in two ways:

(a) Long heparin molecule provides a scaffolding for the clotting factors (mainly Xa and IIa) as well as AT III to get bound and interact with each other.

(b) Heparin induces conformational change in AT III to expose its interactive sites. A specific pentasaccharide sequence, which is present in only some of the heparin molecules, binds to AT III with high affinity to induce the conformational change needed for rapid interaction with clotting factors. This has been synthesized and named *fondaparinux*.

Inhibition of IIa requires both the mechanisms, but Xa inhibition can occur by mechanism 'b' alone. This probably explains why low molecular weight heparin, which is insufficient to provide a long scaffolding, selectively inhibits factor Xa.

Higher doses of heparin given for some time cause reduction in AT-III levels, probably a

compensatory phenomenon. Sudden stoppage of conventional-dose therapy may result in rebound increase in coagulability for few days.

**2. Antiplatelet** Heparin in higher doses inhibits platelet aggregation and prolongs bleeding time.

**3. Lipaemia clearing** Injection of heparin clears turbid post-prandial lipaemic plasma by releasing a lipoprotein lipase from the vessel wall and tissues, which hydrolyses triglycerides of chylomicra and very low density lipoproteins to free fatty acids. These then pass into tissues and the plasma looks clear. This action requires lower concentration of heparin than that needed for anticoagulation.

Facilitation of fatty acid transport may be the physiological function of heparin; but since, it is not found in circulating blood and its storage form in tissues is much less active, this seems only conjectural.

## PHARMACOKINETICS

Heparin is a large, highly ionized molecule; therefore not absorbed orally. Injected i.v. it acts instantaneously, but after s.c. injection anticoagulant effect develops after ~60 min. Bioavailability of s.c. heparin is inconsistent. Heparin does not cross blood-brain barrier or placenta (it is the anticoagulant of choice during pregnancy). It is metabolized in liver by heparinase and fragments are excreted in urine.

Heparin released from mast cells is degraded by tissue macrophages—it is not a physiologically circulating anticoagulant.

After i.v. injection of doses < 100 U/kg, the t½ averages 1 hr. Beyond this, dose-dependent inactivation is seen and t½ is prolonged to 1–4 hrs. The t½ is longer in cirrhotics and kidney failure patients, and shorter in patients with pulmonary embolism.

**Unitage and administration** Because of variable molecular size of unfractionated heparin (UFH), it is standardized only by bioassay: 1 U is the amount of heparin that will prevent 1 ml of citrated sheep plasma from clotting for 1 hour after the addition of 0.2 ml of 1% $CaCl_2$ solution. Heparin sod. 1 mg has 120–140 U of activity.
HEPARIN SOD., BEPARINE, NUPARIN 1000 and 5000 U/ml in 5 ml vials for injection.

Heparin should not be mixed with penicillin, tetracyclines, hydrocortisone or NA in the same syringe or infusion bottle. Heparinized blood is not suitable for blood counts (alters the shape of RBCs and WBCs), fragility testing and complement fixation tests.

**Dosage** Heparin is conventionally given i.v. in a bolus dose of 5,000–10,000 U (children 50–100 U/kg), followed by continuous infusion of 750–1000 U/hr. Intermittent i.v. bolus doses of UFH are no longer recommended. The rate of infusion is controlled by aPTT measurement which is kept at 50–80 sec. or 1.5–2.5 times the patient's pretreatment value. If this test is not available, whole blood clotting time should be measured and kept at ~2 times the normal value.

Deep s.c. injection of 10,000–20,000 U every 8–12 hrs can be given if i.v. infusion is not possible. Needle used should be fine and trauma should be minimum to avoid haematoma formation. Haematomas are more common with i.m. injection—this route should not be used.

*Low dose (s.c.) regimen* 5000 U is injected s.c. every 8–12 hours, started before surgery and continued for 7–10 days or till the patient starts moving about. This regimen has been found to prevent postoperative deep vein thrombosis without increasing surgical bleeding. It also does not prolong aPTT or clotting time. However, it should not be used in case of neurosurgery or when spinal anaesthesia is to be given. The patients should not be receiving aspirin or oral anticoagulants. It is ineffective in high-risk situations, e.g. hip joint or pelvic surgery.

## ADVERSE EFFECTS

1. Bleeding due to overdose is the most serious complication of heparin therapy. Haematuria is generally the first sign. With proper monitoring, serious bleeding occurs only in 1–3% patients.
2. Thrombocytopenia is another common problem. Generally it is mild and transient; occurs due to aggregation of platelets. Occasionally serious thromboembolic events result. In some patients antibodies are formed to the heparin-platelet complex and marked depletion of platelets occurs—heparin should be discontinued in such cases. Even low molecular weight (LMW) heparins are not safe in such patients.
3. Transient and reversible alopecia is infrequent. Serum transaminase levels may rise.

4. Osteoporosis may develop on long-term use of relatively high doses.
5. Hypersensitivity reactions are rare; manifestations are urticaria, rigor, fever and anaphylaxis. Patients with allergic diathesis are more liable.

### Contraindications

1. Bleeding disorders, history of heparin induced thrombocytopenia.
2. Severe hypertension (risk of cerebral haemorrhage), threatened abortion, piles, g.i. ulcers (risk of aggravated bleeding).
3. Subacute bacterial endocarditis (risk of embolism), large malignancies (risk of bleeding in the central necrosed area of the tumour), tuberculosis (risk of hemoptysis).
4. Ocular and neurosurgery, lumbar puncture.
5. Chronic alcoholics, cirrhosis, renal failure.
6. Aspirin and other antiplatelet drugs should be used very cautiously during heparin therapy.

## Low molecular weight (LMW) heparins

Heparin has been fractionated into LMW forms (MW 3000–7000) by different techniques. LMW heparins have a different anticoagulant profile; i.e. selectively inhibit factor Xa with little effect on IIa. They act only by inducing conformational change in AT III and not by providing a scaffolding for interaction of AT III with thrombin. As a result, LMW heparins have smaller effect on aPTT and whole blood clotting time than unfractionated heparin (UFH) relative to antifactor Xa activity. Also, they have lesser antiplatelet action—less interference with haemostasis. Thrombocytopenia is less frequent. A lower incidence of haemorrhagic complications compared to UFH has been reported in some studies, but not in others. However, major bleeding may be less frequent. They are eliminated primarily by renal excretion; are not to be used in patients with renal failure. The more important advantages of LMW heparins are pharmacokinetic:

- Better subcutaneous bioavailability (70–90%) compared to UFH (20–30%): Variability in response is minimized.
- Longer and more consistent monoexponential t½: (4–6 hours); making possible once daily s.c. administration.
- Since aPTT/clotting times are not prolonged, laboratory monitoring is not needed; dose is calculated on body weight basis.
- Risk of osteoporosis after long term use is much less with LMW heparin compared with UFH.

Most studies have found LMW heparins to be equally efficacious to UFH except during cardiopulmonary bypass surgery, in which high dose UFH is still the preferred anticoagulant, because LMW heparin and fondaparinux are less effective in preventing catheter thrombosis and their effects are not fully reversed by protamine. Indications of LMW heparins are:

1. Prophylaxis of deep vein thrombosis and pulmonary embolism in high-risk patients undergoing surgery; stroke or other immobilized patients.
2. Treatment of established deep vein thrombosis.
3. Unstable angina and MI: they have largely replaced continuous infusion of UFH.
4. To maintain patency of cannulae and shunts in dialysis patients.

A number of LMW heparins have been marketed. They differ in composition, pharmacokinetics and dosage.

*Enoxaparin:* CLEXANE 20 mg (0.2 ml) and 40 mg (0.4 ml) prefilled syringes; 20–40 mg OD, s.c. (start 2 hour before surgery).

*Reviparin:* CLIVARINE 13.8 mg (eq. to 1432 anti Xa IU) in 0.25 ml prefilled syringe; 0.25 ml s.c. once daily for 5-10 days.

*Nadroparin:* FRAXIPARINE 3075 IU (0.3 ml) and 4100 IU (0.4 ml) inj., CARDIOPARIN 4000 anti Xa IU/0.4 ml, 6000 anti Xa IU/0.6 ml, 100, 000 anti Xa IU/10 ml inj.

*Dalteparin:* 2500 IU OD for prophylaxis; 100 U/Kg 12 hourly or 200 U/Kg 24 hourly for treatment of deep vein thrombosis. FRAGMIN 2500, 5000 IU prefilled syringes.

*Parnaparin:* 0.6 ml s.c. OD for unstable angina and prophylaxis of DVT; FLUXUM 3200 IU (0.3 ml), 6400 IU (0.6 ml) inj.

*Ardeparin:* 2500-5000 IU OD; INDEPARIN 2500 IU, 5000 IU prefilled syringes.

**Fondaparinux:** The pentasaccharide with specific sequence that binds to AT III with high

affinity to selectively inactivate factor Xa without binding thrombin (factor IIa), has been recently produced synthetically and given the name *fondaparinux*. It is being increasingly used and has been marketed in India as well. The bioavailability of fondaparinux injected s.c. is 100% and it is longer acting (t½ 17 hours). Metabolism is minimal, and it is largely excreted unchanged by the kidney. As such, it is not to be used in renal failure patients. Fondaparinux is less likely to cause thrombocytopenia compared to even LMW heparins. Risk of osteoporosis after prolonged use is also minimal. Fondaparinux does not require laboratory monitoring of aPTT, and is a longer acting alternative to LMW heparins with the above advantages.

*Dose:* 5–10 mg s.c. once daily. FONDAPARINUX, ARIXTRA 5 mg/0.4 ml, 7.5 mg/0.6 ml and 10 mg/0.8 ml prefilled single dose syringe.

**Danaparoid** is a preparation containing mainly heparan sulfate which is a heparin-like substance found in many tissues, having less potent anticoagulant action than heparin. Danaparoid is obtained from pig gut mucosa, and is used in cases with heparin induced thrombocytopenia.

### DIRECT THROMBIN INHIBITORS

Unlike heparin, these recently developed anticoagulants bind directly to thrombin and inactivate it without the need to combine with and activate AT III.

**Lepirudin** This recombinant preparation of hirudin (a polypeptide anticoagulant secreted by salivary glands of leech) binds firmly to the catalytic as well as the substrate recognition sites of thrombin and inhibits it directly. Injected i.v., it is indicated only in patients who are at risk of heparin induced thrombocytopenia. On repeated/prolonged administration, antibodies against the lepirudin-thrombin complex may develop resulting in prolonged anticoagulant effect and possibility of anaphylaxis. Its action cannot be reversed by protamine or any other antidote.

**Bivalirudin** It is a smaller peptide prepared synthetically which has actions and uses similar to lepirudin. However, its action is slowly reversible due to cleavage of its peptide bonds by thrombin itself.

**Argatroban** This is a synthetic nonpeptide compound which binds reversibly to the catalytic site of thrombin, but not to the substrate recognition site. As such, it produces a rapid and short-lasting antithrombin action. Administered by i.v. infusion, it can be used in place of lepirudin for short-term indications in patients with heparin induced thrombocytopenia.

## HEPARIN ANTAGONIST

**Protamine sulfate** It is a strongly basic, low molecular weight protein obtained from the sperm of certain fish. Given i.v. it neutralises heparin weight for weight, i.e. 1 mg is needed for every 100 U of heparin. For the treatment of heparin induced bleeding, due consideration must be given to the amount of heparin that may have been degraded by the patient's body in the mean time. However, it is needed infrequently because the action of heparin disappears by itself in a few hours, and whole blood transfusion is needed to replenish the loss when bleeding occurs. Protamine is more commonly used when heparin action needs to be terminated rapidly, e.g. after cardiac or vascular surgery. Protamine does not neutralize fondaparinux, and it only partially reverses the anticoagulant effect of LMW heparins.

In the absence of heparin, protamine itself acts as a weak anticoagulant by interacting with platelets and fibrinogen. Being basic in nature it can release histamine in the body. Hypersensitivity reactions have occurred. Rapid i.v. injection causes flushing and breathing difficulty.

PROTA, PROTAMINE SULFATE 50 mg in 5 ml inj.

## ORAL ANTICOAGULANTS

A haemorrhagic disease was described in cattle in 1924 which was due to feeding them on spoiled sweet clover hay. The disorder was found to be due to prothrombin deficiency and the toxic principle was identified as *bishydroxycoumarin* in 1939. It was cured by feeding alfalfa grass. First clinical use of bishydroxycoumarin was made in 1941 and many congeners were added later. *Warfarin* was initially used as rat poison; demonstration of its safety led to clinical trial; it is now a commonly employed oral anticoagulant.

### Action and mechanism

Warfarin and its congeners act as anticoagulants only *in vivo,* not *in vitro*. This is so because they act indirectly by interfering with the synthesis of vit K dependent clotting factors in liver. They apparently behave as competitive antagonists of vit K and lower the plasma levels of functional clotting factors in a dose-dependent manner. In fact, they inhibit the enzyme vit K epoxide

reductase (VKOR) and interfere with regeneration of the active hydroquinone form of vit K (Fig. 44.2) which acts as a cofactor for the enzyme γ-glutamyl carboxylase that carries out the final step of γ carboxylating glutamate residues of prothrombin and factors VII, IX and X. This carboxylation is essential for the ability of the clotting factors to bind $Ca^{2+}$ and to get bound to phospholipid surfaces, necessary for the coagulation sequence to proceed.

Factor VII has the shortest plasma t½ (6 hr), its level falls first when warfarin is given, followed by factor IX (t½ 24 hr), factor X (t½ 40 hr) and prothrombin (t½ 60 hr). Though the synthesis of clotting factors diminishes within 2–4 hours of warfarin administration, anticoagulant effect develops gradually over the next 1–3 days as the levels of the clotting factors already present in plasma decline progressively. Thus, there is always a delay between administration of these drugs and the anticoagulant effect. Larger initial doses hasten the effect only slightly.

Therapeutic effect occurs when synthesis of clotting factors is reduced by 40–50%.

Protein C, protein S (both having anticoagulant property) and osteocalcin contain glutamate residues that require vit. K dependent γ carboxylation. These are also inhibited by oral anticoagulants, but density of adult bone is not affected, though new bone formation may be depressed.

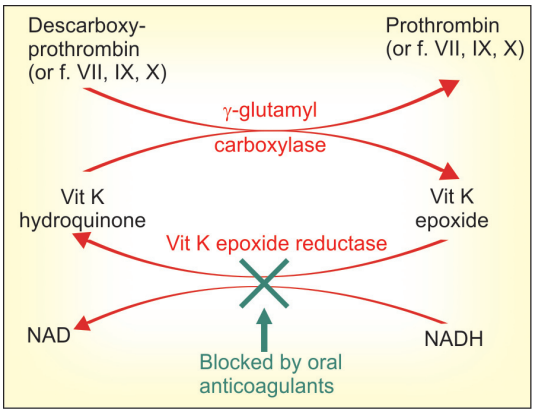



**Fig. 44.2:** Mechanism of action of oral anticoagulants
NAD—Nicotinamide adenine dinucleotide; NADH—its reduced form

The differences between different oral anticoagulants are primarily pharmacokinetic and in the adverse side effects produced by them. These are summarized in Table 44.1.

*Racemic Warfarin sod.* It is the most popular oral anticoagulant. The commercial preparation of warfarin is a mixture of R (dextrorotatory) and S (levorotatory) enantiomers. The S form is more potent and is metabolized relatively faster by ring oxidation carried out by CYP2C9, while R form is less potent and degraded by side chain reduction carried out by CYP1A and CYP3A4.

**TABLE 44.1** **Pharmacokinetic and adverse effect profile of oral anticoagulants**

| *Drug* | *t½ (hour)* | *Duration of action (days)* | *Dose (mg)* | | *Adverse side effects (other than bleeding)* |
|---|---|---|---|---|---|
| | | | *Loading* | *Maintenance** | |
| 1. Bishydroxycoumarin | 25–100 (dose dependent) | 4–7 | 200 for 2 days | 50–100 | Frequent g.i.t. disturbances |
| 2. Warfarin sod. | 36–48 | 3–6 | 5–10 | 2–10£ | Alopecia, dermatitis, diarrhoea |
| 3. Acenocoumarol (Nicoumalone) | 18–24 | 2–3 | 8–12 | 2–8 | Oral ulceration, g.i.t. disturbances, dermatitis, urticaria, alopecia |
| 4. Ethylbiscoumacetate | 2 | 1–3 | 900 | 300–600 | Alopecia, bad taste |
| 5. Phenindione | 5 | 1–3 | 200 | 50–100 | Orange urine, rashes, fever, leukopenia, hepatitis, nephropathy, agranulocytosis |

* Daily maintenance dose: to be adjusted by measurement of prothrombin time (INR).
£ To be taken in a single dose at the same hour (usually bed time) each day.

Both are partially conjugated with glucuronic acid and undergo some enterohepatic circulation; finally excreted in urine.

Warfarin is rapidly and completely absorbed from intestines and is 99% plasma protein bound. It crosses placenta and is secreted in milk; however, quantity of active form is generally insufficient to affect the suckling infant.

UNIWARFIN 1, 2, 5 mg tabs; WARF-5: 5 mg tab.

*Bishydroxycoumarin (Dicumarol)* It is slowly and unpredictably absorbed orally. Its metabolism is dose dependent—t½ is prolonged at higher doses. Has poor g.i. tolerance; not preferred now.

DICOUMAROL 50 mg tab.

*Acenocoumarol (Nicoumalone)* The t½ of acenocoumarol as such is 8 hours, but an active metabolite is produced so that overall t½ is about 24 hours. Acts more rapidly.

ACITROM, 1, 2, 4 mg tabs.

*Ethyl biscoumacetate* It has a rapid and brief action; occasionally used to initiate therapy, but difficult to maintain.

*Phenindione* Apart from risk of bleeding, it produces more serious organ toxicity; should not be used.

DINDEVAN 50 mg tab.

**Adverse effects** Bleeding as a result of extension of the desired pharmacological action is the most important problem causing ecchymosis, epistaxis, hematuria, bleeding in the g.i.t. Intracranial or other internal haemorrhages may even be fatal. Bleeding is more likely if therapy is not properly monitored, or when INR exceeds 4, or interacting drugs/contraindications are present.

*Treatment*: of bleeding due to oral anticoagulants consists of:

- Withhold the anticoagulant.
- Give fresh blood transfusion; this supplies clotting factors and replenishes lost blood. Alternatively fresh frozen plasma may be used as a source of clotting factors.
- Give vit $K_1$ which is the specific antidote (*see* p. 615), but it takes 6–24 hours for the clotting factors to be resynthesized and released in blood after vit K administration.

Adverse effects unrelated to anticoagulation are given in Table 44.1. Cutaneous necrosis is a rare complication that can occur with any oral anticoagulant.

Phenindione produces serious toxicity; should not be used.

Warfarin and acenocoumarol are considered to be the most suitable and better tolerated drugs.

**Dose regulation** The dose of oral anticoagulant must be individualised by repeated measurement of *prothrombin time*; the aim is to achieve a therapeutic effect without unduly increasing the chances of bleeding.

The optimum ratio of PT during treatment with the oral anticoagulant to the normal value (of the testing laboratory) has been defined for various indications. But this value differs depending on whether rabbit brain or human brain thromboplastin (Tp) has been used for the test. A standardized system called the International Normalized Ratio (INR) based on the use of human brain Tp has been developed by WHO and adopted in all countries.

**Recommended INR for various indications of oral anticoagulants**

| | *INR* |
|---|---|
| 1. Prophylaxis of deep vein thrombosis and similar indications | 2–2.5 |
| 2. Treatment of deep vein thrombosis, pulmonary embolism, TIAs, hip surgery | 2–3 |
| 3. Recurrent thromboembolism, arterial disease (MI), prosthetic heart valves | 3–3.5 |

*Factors enhancing effect* of oral anticoagulants are:

- Debility, malnutrition, malabsorption and prolonged antibiotic therapy: the supply of vit K to liver is reduced in these conditions.
- Liver disease, chronic alcoholism: synthesis of clotting factors may be deficient.
- Hyperthyroidism: the clotting factors are degraded faster.
- Newborns: have low levels of vit K and clotting factors (there should be no need of these drugs in neonates anyway).

*Factors decreasing effect* of oral anticoagulants are:

- Pregnancy: plasma level of clotting factors is higher.
- Nephrotic syndrome: drug bound to plasma protein is lost in urine.

- Genetic warfarin resistance: the affinity of warfarin (as well as of vit K epoxide) to bind to the reductase (VKOR) enzyme, which generates the active vit K hydroquinone, is low. Dose of oral anticoagulant is 4–5 times higher.

**Contraindications** All contraindications to heparin (*see* p. 619) apply to these drugs as well. Factors which enhance the effect of oral anticoagulants (*see* above) should also be taken into consideration.

Oral anticoagulants should not be used during pregnancy. Warfarin given in early pregnancy increases birth defects, especially skeletal abnormalities. It can produce *foetal warfarin syndrome*—hypoplasia of nose, eye socket, hand bones, and growth retardation. Given later in pregnancy, it can cause CNS defects, foetal haemorrhage, foetal death and accentuates neonatal hypoprothrombinemia.

**Drug interactions** A large number of drugs interact with oral anticoagulants at pharmacokinetic or pharmacodynamic level, and either enhance or decrease their effect. These interactions are clinically important (may be fatal if bleeding occurs) and may involve more than one mechanism; the exact mechanism of an interaction is not always definable.

### A. *Enhanced anticoagulant action*

1. Broad-spectrum antibiotics: inhibit gut flora and reduce vit K production.
2. Newer cephalosporins (ceftriaxone, cefoperazone) cause hypoprothrombinaemia by the same mechanism as warfarin —additive action.
3. Aspirin: inhibits platelet aggregation and causes g.i. bleeding—this may be hazardous in anticoagulated patients. High doses of salicylates have synergistic hypoprothrombinemic action and also displace warfarin from protein binding site.
4. Long acting sulfonamides, indomethacin, phenytoin and probenecid: displace warfarin from plasma protein binding.
5. Chloramphenicol, erythromycin, celecoxib, cimetidine, allopurinol, amiodarone and metronidazole: inhibit warfarin metabolism.
6. Tolbutamide and phenytoin: inhibit warfarin metabolism and *vice versa*.
7. Liquid paraffin (habitual use): reduces vit K absorption.

### B. *Reduced anticoagulant action*

1. Barbiturates (but not benzodiazepines), carbamazepine, rifampin and griseofulvin induce the metabolism of oral anticoagulants. The dose of anticoagulant determined during therapy with these drugs would be higher: if the same is continued after withdrawing the inducer—marked hypoprothrombinemia can occur—fatal bleeding is on record.
2. Oral contraceptives: increase blood levels of clotting factors.

## DIRECT FACTOR Xa INHIBITORS

Recently some orally active drugs have been produced which directly bind to and inactivate factor Xa, instead of inhibiting its synthesis. They, therefore, act rapidly without a lag time (as in case of warfarin, etc.), and have short-lasting action.

### Rivaroxaban

It is an orally active direct inhibitor of activated factor Xa which has become available for prophylaxis and treatment of DVT. Its anticoagulant action develops rapidly within 3–4 hours of ingestion and lasts for ~24 hours. It is largely metabolized, but also excreted unchanged in urine; plasma t½ is 7–11 hours. Another advantage is that it requires no laboratory monitoring of PT or aPTT, and is recommended in a fixed dose of 10 mg once daily starting 6–10 hours after surgery for prophylaxis of venous thromboembolism following total knee/hip replacement. In comparative trials, its efficacy has been found similar to a regimen of LMW heparin followed by warfarin. Rivaroxaban has also been found equally effective as warfarin for preventing stroke in patients with atrial fibrillation. Side effects reported are bleeding, nausea, hypotension, tachycardia and edema.

## ORAL DIRECT THROMBIN INHIBITOR

**Dabigatran etexilate** It is a prodrug which after oral administration is rapidly hydrolysed to *dabigatran*, a direct thrombin inhibitor which reversibly blocks the catalytic site of thrombin and produces a rapid (within 2 hours) anticoagulant action. Though oral bioavailability is low, the anticoagulant effect is consistent, and no laboratory monitoring is required. The plasma t½ is 12–14 hours and duration of action 24 hours. In the UK, Canada and Europe it is approved for prevention of venous thromboembolism following hip/knee joint replacement surgery. Administered in a dose of 110 mg (75 mg for elderly > 75 years) once

**TABLE 44.2 Some comparative aspects of heparin and oral anticoagulants**

| | *Heparin* | *Warfarin* |
|---|---|---|
| 1. Chemistry | Mucopolysaccharide | Coumarin derivative |
| 2. Source | Hog lung, pig intestine | Synthetic |
| 3. Route of admin. | Parenteral (i.v., s.c.) | Oral |
| 4. Onset of action | Immediate | Delayed (1–3 days) |
| 5. Duration of action | 4–6 hrs | 3–6 days |
| 6. Activity | *In vitro* and *in vivo* | *In vivo* only |
| 7. Mechanism | Blocks action of factor X and thrombin | Inhibits synthesis of clotting factors |
| 8. Antagonist | Protamine sulphate | Vit K |
| 9. Variability in response | Little | Marked |
| 10. Lab. control | aPTT/clotting time (desirable) | Prothrombin time/INR (essential) |
| 11. Drug interactions | Few and not significant | Many and significant |
| 12. Use | To initiate therapy | For maintenance |

daily, it has been found comparable to warfarin. In another large trial dabigatran etexilate 150 mg twice daily has yielded superior results to warfarin for prevention of embolism and stroke in patients of atrial fibrillation. In the USA it is approved for this indication. Adverse effects are bleeding and less commonly hepatobiliary disorders.

## USES OF ANTICOAGULANTS

The aim of using anticoagulants is to prevent thrombus extension and embolic complications by reducing the rate of fibrin formation. They do not dissolve already formed clot, but prevent recurrences. Heparin is utilized for rapid and short-lived action, while oral anticoagulants are suitable for maintenance therapy. Generally, the two are started together; heparin is discontinued after 4–7 days when warfarin has taken effect.

The important features of heparin and oral anticoagulants are compared in Table 44.2.

1. *Deep vein thrombosis (DVT) and pulmonary embolism (PE)* Because venous thrombi are mainly fibrin thrombi, anticoagulants are expected to be highly effective. The best evidence of efficacy of anticoagulants comes from treatment and prevention of venous thrombosis and pulmonary embolism. Prophylaxis is recommended for all high risk patients including bedridden, elderly, postoperative, postpartum, poststroke and leg fracture patients. When deep vein thrombosis/pulmonary embolism has occurred, immediate heparin/LMW heparin followed by warfarin therapy should be instituted. Three months anticoagulant therapy (continued further if risk factor persists) has been recommended by American College of Chest Physicians (2001).

Introduction of low dose s.c. heparin prophylaxis for patients undergoing elective surgery has considerably reduced the incidence of leg vein thrombosis and pulmonary embolism in the postoperative period. It has been extended to other situations needing prolonged immobilization. It is based on the premise that inhibition of small amount of activated factor X prevents further amplification of active products—particularly thrombin. This is the regimen of choice which does not need laboratory monitoring; spontaneous bleeding does not occur. LMW heparin/fondaparinux have now practically replaced UFH, except in case of major surgery and in high risk cases, because action of UFH can be terminated rapidly.

Anticoagulants are of little value in chronic peripheral vascular diseases.

2. *Myocardial infarction (MI)* Arterial thrombi are mainly platelet thrombi; anticoagulants are of questionable value. Their use in acute MI has declined. They do not alter immediate mortality of MI. It was hoped that anticoagulants will

prevent extension of the thrombus and ward off a recurrent attack. This has not been supported by the collected statistics. They may benefit by preventing mural thrombi at the site of infarction and venous thrombi in leg veins. Thus, anticoagulants may be given for a short period till patient becomes ambulatory. For secondary prophylaxis against a subsequent attack—anticoagulants are inferior to antiplatelet drugs.

Heparin (i.v.) or preferably LMW heparin/fondaparinux s.c. once or twice daily for 2–8 days followed by oral anticoagulants for 3 months or continuation of LMW heparin for 2–3 months are generally given after recanalization of coronary artery by fibrinolytic therapy. Heparin is also used during coronary angioplasty and stent placement.

3. *Unstable angina* Short-term use of heparin has reduced the occurrence of MI in unstable angina patients; aspirin is equally effective. Current recommendation is to use aspirin + heparin/LMW heparin followed by warfarin.

4. *Rheumatic heart disease; Atrial fibrillation (AF)* All atrial fibrillation patients should be protected against thromboembolism from fibrillating atria and the resulting stroke. For this purpose, the effective options are warfarin/low dose heparin/low dose aspirin. The 'Stroke prevention in Atrial Fibrillation' trial and a metaanalysis have shown warfarin to be more effective than aspirin. Current guideline is to give warfarin to a target INR of 2–3 in AF patients with high risk for stroke (elderly, heart failure, etc.), and to reserve aspirin for low risk patients or for those unable to take warfarin. Anticoagulants are given for 3–4 weeks before and after attempting conversion of AF to sinus rhythm.

5. *Cerebrovascular disease* Anticoagulants are of little value in cerebral thrombosis. They have been used with the aim of preventing clot propagation, but all the trials conducted, including International Stroke Trial (IST), have failed to demonstrate significant benefit. Neurological sequelae are similar whether anticoagulants are used or not. Moreover, in the initial stages it is difficult to rule out cerebral haemorrhage (unless CAT scan is done) in which they can be devastating. They may be used in cerebral embolism, because showers of emboli are often recurrent and can be prevented by anticoagulants. A late start (after one week) anticoagulant therapy is advocated by many in case of large embolic stroke. Oral anticoagulants may be beneficial in transient ischaemic attacks (TIAs), but antiplatelet drugs are simpler to use and probably better.

6. *Vascular surgery, prosthetic heart valves, retinal vessel thrombosis, extracorporeal circulation, haemodialysis* Anticoagulants are indicated along with antiplatelet drugs for prevention of thromboembolism.

Heparin flushes (200 U in 2 ml) every 4–8 hr are used to keep patent long-term intravascular cannulae/catheters.

7. *Defibrination syndrome* or 'disseminated intravascular coagulation' occurs in abruptio placentae and other obstetric conditions, certain malignancies and infections. The coagulation factors get consumed for the formation of intravascular microclots and blood is incoagulable. Heparin paradoxically checks bleeding in such patients by preserving the clotting factors. However, in some cases heparin may aggravate bleeding.

## FIBRINOLYTICS (Thrombolytics)

These are drugs used to lyse thrombi/clot to recanalize occluded blood vessels (mainly coronary artery). They are therapeutic rather than prophylactic and work by activating the natural fibrinolytic system (Fig. 44.3).

Haemostatic plug of platelets formed at the site of injury to blood vessels is reinforced by fibrin deposition to form a thrombus. Once repair is over, the fibrinolytic system is activated to remove the fibrin. The enzyme responsible for digesting fibrin is a serine protease *Plasmin* generated from *plasminogen* by tissue plasminogen activator (t-PA), which is produced primarily by vascular endothelium. Plasminogen circulates in plasma as well as remains bound to fibrin. The t-PA selectively activates fibrin-bound plasminogen within the thrombus, and any plasmin that leaks is inactivated by circulating antiplasmins. Fibrin bound plasmin

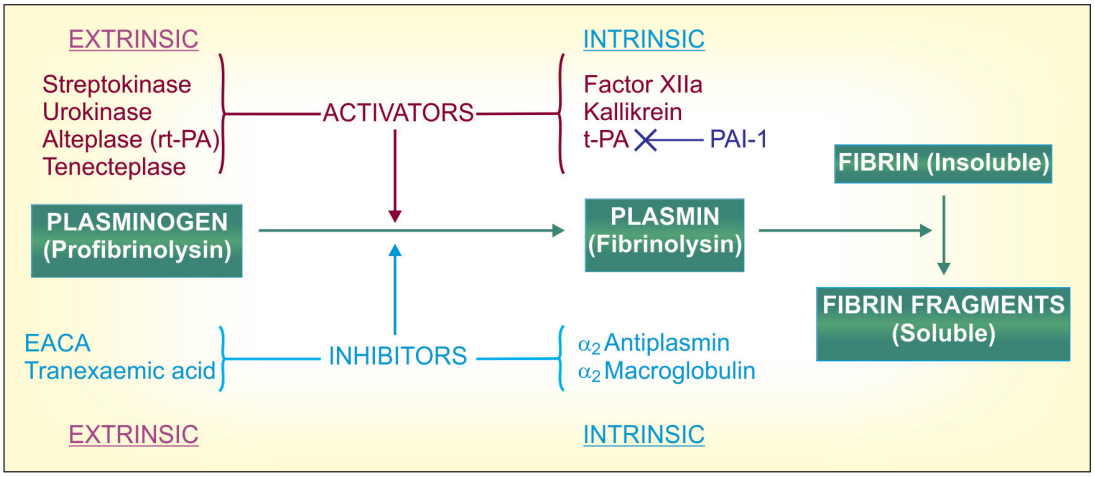



**Fig. 44.3:** The plasminogen-plasmin system
t-PA—Tissue plasminogen activator; rt-PA—Recombinant t-PA; PAI-1—Plasminogen activator inhibitor-1

is not inactivated by antiplasmins because of common binding site for both fibrin and antiplasmin.The t-PA itself is inactivated by plasminogen activator inhibitor-1 and -2 (PAI-1, PAI-2).

When excessive amounts of plasminogen are activated (by administered fibrinolytics), the $\alpha_2$ antiplasmin is exhausted and active plasmin persists in plasma. Plasmin is a rather nonspecific protease: degrades coagulation factors (including fibrinogen) and some other plasma proteins as well. Thus, activation of circulating plasminogen induces a lytic state whose major complication is haemorrhage. Even selective activation of thrombus bound plasmin can cause bleeding by dissolving physiological thrombi.

In general, venous thrombi are lysed more easily by fibrinolytics than arterial, and recent thrombi respond better. They have little effect on thrombi > 3 days old. The clinically important fibrinolytics are:

| | |
|---|---|
| Streptokinase | Alteplase (rt-PA) |
| Urokinase | Reteplase |
| | Tenecteplase |

**Streptokinase (Stk)** Obtained from β haemolytic *Streptococci* group C, it is the first fibrinolytic drug to be used clinically, but is not employed now except for considerations of cost. Streptokinase is inactive as such; combines with circulating plasminogen molecules to form an activator complex which then causes limited proteolysis of other plasminogen molecules to generate the active enzyme plasmin. Stk. is non-fibrin specific, i.e. activates both circulating as well as fibrin bound plasminogen. Therefore, it depletes circulating fibrinogen and predisposes to bleeding. Compared to newer more fibrin-specific tissue plasminogen activators (alteplase, etc.) it is less effective in opening occluded coronary arteries, and causes less reduction in MI related mortality.

There are several other disadvantages as well with Stk. Antistreptococcal antibodies due to past infections inactivate considerable fraction of the initial dose of Stk. A loading dose therefore is necessary. Plasma t½ is estimated to be 30–80 min. Stk is antigenic—can cause hypersensitivity reactions; anaphylaxis occurs in 1–2% patients. It cannot be used second time due to neutralization by antibodies generated in response to the earlier dose. Fever, hypotension and arrhythmias are reported. However, being less expensive, it is still used in resource poor areas, but not in Europe or USA.

STREPTASE, (freeze dried powder in vials) 2.5 lac, 7.5 lac and 15 lac IU/vial, ESKINASE, CARDIOSTREP 7.5 lac, 15 lac IU/vial.

*For MI*: 7.5–15 lac IU infused i.v. over 1 hr.

*For deep vein thrombosis and pulmonary embolism*: 2.5 lac IU loading dose over ½–1 hr, followed by 1 lac IU/hr for 24 hr.

**Urokinase** It is an enzyme isolated from human urine; but commercially prepared from cultured human kidney cells. It activates plasminogen directly and has a plasma t½ of 10–15 min. It is nonantigenic. Fever occurs during treatment, but hypotension and allergic phenomena are rare. Urokinase is Indicated in patients in whom streptokinase has been given for an earlier episode, but is seldom used now.

UROKINASE, UROPASE, 2.5 lac, 5 lac, 7.5 lac, 10 lac IU per vial inj.

*For MI*: 2.5 lac IU i.v. over 10 min followed by 5 lac IU over next 60 min (stop in between if full recanalization occurs) or 6000 IU/min for upto 2 hr.

*For venous thrombosis and pulmonary embolism*: 4400 IU/kg over 10 min i.v. followed by 4400 IU/kg/hr for 12 hr.

**Alteplase** *(recombinant tissue plasminogen activator (rt-PA)* Produced by recombinant DNA technology from human tissue culture, it is moderately specific for fibrin-bound plasminogen, so that circulating fibrinogen is lowered only by ~ 50%. It is rapidly cleared by liver and inactivated by plasminogen activator inhibitor-1 (PAI-1). The plasma t½ is 4–8 min. Because of the short t½, it needs to be given by slow i.v. infusion and often requires heparin co-administration. It is nonantigenic, but nausea, mild hypotension and fever may occur. It is expensive.

ACTILYSE 50 mg vial with 50 ml solvent water.

*For MI*: (accelerated regimen) 15 mg i.v. bolus injection followed by 50 mg over 30 min, then 35 mg over the next 1 hr. (total 90 min).

*For pulmonary embolism*: 100 mg i.v. infused over 2 hr.

*For ischaemic stroke:* 0.9 mg/kg by i.v. infusion over 60 min, with 10% of the dose injected in the first minute.

**Reteplase** It is a modified form of rt-PA that is longer acting, but somewhat less specific for fibrin-bound plasminogen. The longer duration of action enables bolus dose administration (10 mg over 10 min repeated after 30 min).

**Tenecteplase** This genetically engineered substitution mutant of native t-PA has higher fibrin selectivity, slower plasma clearance (longer duration of action) and resistance to inhibition by PAI-1. It is the only fibrinolytic agent that can be injected i.v. as a single bolus dose over 10 sec, while alteplase requires 90 min infusion. This feature makes it possible to institute fibrinolytic therapy immediately on diagnosis of ST segment elevation myocardial infarction (STEMI), even during transport of the patient to the hospital. Several randomized multicentric trials have assessed its efficacy in STEMI and found it to be at least equally efficacious to alteplase. Risk of noncerebral bleeding may be lower with tenecteplase, but cranial bleeding incidence is similar.

*Dose*: 0.5 mg/kg single i.v. bolus injection.

ELAXIM 30 mg, 50 mg per vial inj.

### Uses of fibrinolytics

1. *Acute myocardial infarction* is the chief indication. Fibrinolytics are an alternative first line approach to emergency percutaneous coronary intervention (PCI) with stent placement. Recanalization of thrombosed coronary artery has been achieved in 50–90% cases. Time lag in starting the infusion is critical for reducing area of necrosis, preserving ventricular function and reducing mortality. The benefits of i.v. thrombolytic therapy have been established by large randomised studies. Aspirin with or without heparin is generally started concurrently or soon after thrombolysis to prevent reocclusion.

Alteplase has advantages over streptokinase, including higher thrombolytic efficacy. However, incidence of haemorrhage is not lower. Its stronger lytic effect on physiological haemostatic plugs may compensate for the lesser systemic fibrinolytic state.

2. *Deep vein thrombosis* in leg, pelvis, shoulder etc.; up to 60% patients can be successfully treated. Thrombolytics can decrease subsequent pain and swelling, but the main advantage is preservation of venous valves and may be a reduced risk of pulmonary embolism, though at the risk of haemorrhage. Comparable results have been obtained with Stk, urokinase and alteplase.

3. *Pulmonary embolism* Fibrinolytic therapy is indicated in large, life-threatening PE. The lung function may be better preserved, but reduction in mortality is not established.

4. *Peripheral arterial occlusion* Fibrinolytics recanalise ~40% limb artery occlusions, especially those treated within 72 hr. However, it is indicated only when surgical thrombectomy is not possible. Regional intraarterial fibrinolytics have been used for limb arteries with greater success. Peripheral arterial thrombolysis is followed by short-term heparin and long-term aspirin therapy.

Fibrinolytics have no role in chronic peripheral vascular diseases.

5. *Stroke:* Thrombolytic therapy of ischaemic stroke is controversial. Possibility of improved neurological outcome is to be balanced with risk of intracranial haemorrhage. No net benefit was concluded by the ATLANTIS trial in patients treated at 3–5 hours of stroke onset. However, alteplase is approved for use in ischaemic stroke, and current opinion strongly recommends use of i.v. alteplase in carefully selected patients who

**Contraindications to thrombolytic therapy**

1. H/o Intracranial haemorrhage
2. H/o Ischaemic stroke in past 3 months
3. H/o Head injury in past 3 months
4. Intracranial tumour/vascular abnormality/ aneurysms
5. Active bleeding/bleeding disorders
6. Peptic ulcer, esophageal varices
7. Any wound or recent fracture or tooth extraction
8. H/o major surgery within 3 weeks
9. Uncontrolled hypertension
10. Pregnancy

can be treated within 3 hours of onset, and in whom intracranial haemorrhage is ruled out along with all risk factors for bleeding (*see* contraindications in box).

**Evaluation** All patients with STEMI are candidates for reperfusion therapy. No consistent benefit of fibrinolytics has been demonstrated in non-STEMI cases, while possibility of haemorrhage is increased. Only selected cases of NSTEMI may be treated with fibrinolytics. Both short-term and long-term outcome is determined by early restoration of flow in the occluded artery, regardless of whether it is achieved by thrombolysis or by PCI. Best results are obtained if perfusion can be restored within the first hour (the golden hour). While the efficacy of fibrinolytics in dissolving the thrombus diminishes with passage of time (little benefit after 6 hours of MI onset), reperfusion by PCI is affected to a lesser extent by the time lapse. Thrombolysis may be favoured if it can be started within 1–2 hours of onset. After 3 hours, PCI is favoured. Moreover, PCI has the advantage of lower bleeding risk, higher grade of flow in the reperfused artery and reduction in the rate of nonfatal recurrent MI compared to thrombolysis. As such, PCI has yielded superior results compared to fibrinolytics and is being preferred at centres where it can be performed swiftly with requisite expertise. Primary PCI is the procedure of choice for patients with contraindications to thrombolytics (*see* box). Fibrinolytic therapy requires careful patient selection, but often can be instituted with less delay, and even at centres not well equipped for PCI.

Another approach is 'facilitated PCI' wherein full or reduced dose fibrinoytic therapy is followed at the earliest by PCI. The results of this approach are comparable to those of primary PCI. The European as well as American (ACC, AHA) guidelines provide that STEMI patients should be treated with primary PCI or with fibrinolytic drugs followed by immediate rescue PCI, if reperfusion fails with the fibrinolytic. Aspirin and heparin are continued after thrombolysis.

## ANTIFIBRINOLYTIC DRUGS

These are drugs which inhibit plasminogen activation and dissolution of clot, and are used to check fibrinolysis associated bleeding.

**Epsilon amino-caproic acid (EACA)** It is a lysine analogue which combines with the lysine-binding sites of plasminogen and plasmin so that the latter is not able to bind to fibrin and lyse it. It is a specific antidote for fibrinolytic agents and has been used in many hyperplasminaemic states associated with excessive intravascular fibrinolysis resulting in bleeding. The primary indication is to counteract the effect of fibrinolytic drugs and bleeding due to their use. In haemophiliacs, it has adjunctive value for controlling bleeding due to tooth extraction, prostatectomy, trauma, etc.

In haematuria it can cause ureteric obstruction by the unlysed clots. Therefore, fibrinolysis must be established firmly before using it. It can cause intravascular thrombosis. Rapid i.v. injection results in hypotension, bradycardia and may be arrhythmias. It should be used cautiously when renal function is impaired. Myopathy occurs rarely. The large dose needed is a limitation, and tranexamic acid is mostly preferred.

Initial priming dose is 5 g oral/i.v., followed by 1 g hourly till bleeding stops (max. 30 g in 24 hrs).

AMICAR, HEMOCID, HAMOSTAT 0.5 g tab., 1.25 g/5 ml syr., 5 g/20 ml inj.

**Tranexamic acid** Like EACA, it binds to the lysine binding site on plasminogen and prevents its combination with fibrin leading to fibrinolysis. It is 7 times more potent than EACA, and is

preferred for prevention/control of excessive bleeding due to:

- Fibrinolytic drugs.
- Cardio-pulmonary bypass surgery.
- Tonsillectomy, prostatic surgery, tooth extraction in haemophiliacs.
- Menorrhagia, especially due to IUCD.
- Recurrent epistaxis, hyphema due to ocular trauma, peptic ulcer.

Main side effects are nausea and diarrhoea. Thromboembolic events, disturbed colour vision and allergic reactions are infrequent. Thrombophlebitis of injected vein can occur.

*Dose:* 10–15 mg/kg 2–3 times a day or 1–1.5 g TDS oral, 0.5–1 g TDS by slow i.v. infusion.

DUBATRAN, PAUSE, TRANAREST 500 mg tab, 500 mg/5 ml inj.

## ANTIPLATELET DRUGS (Antithrombotic drugs)

These are drugs which interfere with platelet function and are useful in the prophylaxis of thromboembolic disorders.

Platelets express several glycoprotein (GP) integrin receptors on their surface. Reactive proteins like collagen are exposed when there is damage to vascular endothelium, and they react respectively with platelet $GPI_a$ and $GPI_b$ receptors. This results in platelet activation and release of proaggregatory and vasoconstrictor mediators like $TXA_2$, ADP and 5-HT. The platelet $GPII_b/III_a$ receptor undergoes a conformational change favouring binding of fibrinogen and vonWillebrand factor (vWF) that crosslink platelets inducing aggregation and anchorage to vessel wall/other surfaces. Thus, a 'platelet plug' is formed. In veins, due to sluggish blood flow, a fibrinous tail is formed which traps RBCs 'the red tail'. In arteries, platelet mass is the main constituent of the thrombus. Antiplatelet drugs are, therefore, more useful in arterial thrombosis, while anticoagulants are more effective in venous thrombosis.

Prostacyclin ($PGI_2$), synthesized in the intima of blood vessels, is a strong inhibitor of platelet aggregation. A balance between $TXA_2$ released from platelets and $PGI_2$ released from vessel wall appears to control intravascular thrombus formation. Platelets also play a role in atherogenesis.

In the above scheme, various drugs act on different targets to interfere with platelet function. Therefore, given together, their actions are synergistic. The clinically important antiplatelet drugs are:

**Antiplatelet Drugs**

| | |
|---|---|
| Aspirin | |
| Dipyridamole | |
| *$P2Y_{12}$ Receptor Blockers* | *$GPII_b/III_a$ Antagonists* |
| Ticlopidine | Abciximab |
| Clopidogrel | Eptifibatide |
| Prasugrel | Tirofiban |

**Aspirin** It acetylates and inhibits the enzyme COX1 and TX-synthase—inactivating them irreversibly. Because $TXA_2$ is the major arachidonic acid product generated by platelets, and that platelets are exposed to aspirin in the portal circulation before it is deacetylated during first pass in the liver, and because platelets cannot synthesize fresh enzyme (have no nuclei), $TXA_2$ formation is suppressed at very low doses and till fresh platelets are formed. Thus, aspirin induced prolongation of bleeding time lasts for 5–7 days. Effect of daily doses cumulates and it has now been shown that doses as low as 40 mg/day have an effect on platelet aggregation. Maximal inhibition of platelet function occurs at 75–150 mg aspirin per day. However, aspirin may not effectively inhibit platelet aggregation in some patients.

Inhibition of COX-1 by aspirin in vessel wall decreases $PGI_2$ synthesis as well. However, since intimal cells can synthesize fresh enzyme, activity returns rapidly. It is possible that at low doses (75–150 mg/day or 300 mg twice weekly), $TXA_2$ formation by platelets is selectively suppressed, whereas higher doses (> 900 mg/day) may decrease both $TXA_2$ and $PGI_2$ production.

Aspirin inhibits the release of ADP from platelets and their sticking to each other, but has no effect on platelet survival time and their adhesion to damaged vessel wall.

ASA 50 mg tab., COLSPRIN, DISPRIN CV-100: aspirin 100 mg soluble tab, LOPRIN 75 mg tab, ASPICOT 80 mg tab, ECOSPRIN 75, 150 mg tab.

*Other NSAIDs* are reversible inhibitors of COX, produce short-lasting inhibition of platelet function—are not clinically useful.

**Dipyridamole** It is a vasodilator that was introduced for angina pectoris (*see* Ch. 39). It inhibits

phosphodiesterase as well as blocks uptake of adenosine to increase platelet cAMP which in turn potentiates $PGI_2$ and interferes with aggregation. Levels of $TXA_2$ or $PGI_2$, are not altered, but platelet survival time reduced by disease is normalized.

Dipyridamole alone has little clinically significant effect, but improves the response to warfarin, along with which it is used to decrease the incidence of thromboembolism in patients with prosthetic heart valves.

Dipyridamole has also been used to enhance the antiplatelet action of aspirin. This combination may additionally lower the risk of stroke in patients with transient ischaemic attacks (TIAs), but trials have failed to demonstrate additional benefit in prophylaxis of MI.

*Dose:* 150–300 mg/day. PERSANTIN 25, 100 mg tabs, THROMBONIL 75, 100 mg tabs, DYNASPRIN: dipyridamole 75 mg + aspirin 60 mg e.c. tab., CARDIWELL PLUS: dipyridamole 75 mg + aspirin 40 mg tab.

**Ticlopidine** It is the first thienopyridine which alters surface receptors on platelets and inhibits ADP as well as fibrinogen-induced platelet aggregation. The Gi coupled $P2Y_{12}$ (also labelled $P2Y_{AC}$) type of purinergic receptors which mediate adenylyl cyclase inhibition due to ADP are blocked irreversibly by the active metabolite of ticlopidine. As a result, activation of platelets is interfered. Fibrinogen binding to platelets is prevented without modification of $GPII_b/IIIa$ receptor. There is no effect on platelet $TXA_2$, but bleeding time is prolonged and platelet survival in extra-corporeal circulation is increased. Because of different mechanism of action, it has synergistic effect on platelets with aspirin. Their combination is a potent platelet inhibitor.

Ticlopidine is well absorbed orally, is converted in liver to an active metabolite, and is eliminated with a plasma t½ of 8 hours. However, because it causes irreversible blockade of $P2Y_{12}$ receptors, the effect on platelets cumulates; peak platelet inhibition is produced after 8–10 days therapy, and the effect lasts 5–6 days after discontinuing the drug.

Ticlopidine has produced beneficial effects in stroke prevention, TIAs, intermittent claudication, unstable angina, PCI, coronary artery bypass grafts and secondary prophylaxis of MI. Combined with aspirin, it has markedly lowered incidence of restenosis after PCI and stent thrombosis.

Because of its potential for serious adverse reactions, use of ticlopidine has markedly declined in favour of clopidogrel.

*Side effects*: Diarrhoea, vomiting, abdominal pain, headache, tinnitus, skin rash. Serious adverse effects are bleeding, neutropenia, thrombocytopenia, haemolysis and jaundice. Several fatalities have occurred.

*Dose*: 250 mg BD with meals; effect persists several days after discontinuation; TYKLID, TICLOVAS, TICLOP, 250 mg tab; ASTIC ticlopidine 250 mg + aspirin 100 mg tab.

**Clopidogrel** This newer and more potent congener of ticlopidine has similar mechanism of action, ability to irreversibly inhibit platelet function and range of therapeutic efficacy, but is safer and better tolerated (CLASSICS study). The clopidogrel *vs* aspirin in patients at risk of ischaemic events (CAPRIE) trial has found clopidogrel recipients to have a slightly lower annual risk of primary ischaemic events than aspirin recipients. Combination of clopidogrel and aspirin is synergistic in preventing ischaemic episodes, and is utilized for checking restenosis of stented coronaries.

Like ticlopidine, clopidogrel is also a prodrug. About 50% of the ingested dose is absorbed, and only a fraction of this is slowly activated in liver by CYP2C19, while the rest is inactivated by other enzymes. It is a slow acting drug; antiplatelet action takes about 4 hours to start and develops over days. Since CYP2C19, exhibits genetic polymorphism, the activation of clopidogrel and consequently its antiplatelet action shows high interindividual variability. Some patients are nonresponsive. Omeprazole, an inhibitor of CYP2C19, reduces metabolic activation of clopidogrel and its antiplatelet action. However, like ticlopidine, the action of clopidogrel lasts 5–7 days due to irreversible blockade of platelet $P2Y_{12}$ receptors.

The most important adverse effect is bleeding. Addition of aspirin to clopidogrel has been found

to double the incidence of serious bleeding among high risk stroke patients (MATCH study). However, neutropenia, thrombocytopenia and other bone marrow toxicity is rare. Side effects are diarrhoea, epigastric pain and rashes.

*Dose:* 75 mg OD; CLODREL, CLOPILET, DEPLATT 75 mg tab.

**Prasugrel** This is the latest, most potent and faster acting $P2Y_{12}$ purinergic receptor blocker, that is being increasingly used in acute coronary syndromes (ACS) and when strong antiplatelet action is required. Like its predecessors, it is also a prodrug, but is more rapidly absorbed and more rapidly as well as more completely activated, resulting in faster and more consistent platelet inhibition. Though CYP2C19 is involved in activation of prasugrel as well, genetic polymorphism related decrease in response, or interference by omeprazole treatment has not been prominent.

Because of rapid action, prasugrel is particularly suitable for use in STEMI. It is the preferred thienopyridine for ACS to cover angioplasty with or without stent placement. The TRITON trial compared prasugrel with clopidogrel in STEMI and NSTEMI. There was 19% greater reduction in death from cardiovascular causes in the prasugrel group. Superior clinical outcomes and reduction in stent thrombosis have been obtained with prasugrel. Bleeding complications are also more frequent and more serious. Patients with history of ischaemic stroke and TIAs are at greater risk of intracranial haemorrhage. Prasugrel is contraindicated in such patients.

*Dose*: 10 mg OD; elderly or those <60 kg body weight 5 mg OD; a loading dose of 60 mg may be given for urgent action.

PRASULET, PRASUSAFE, PRASUREL 5 mg, 10 mg tabs.

### Glycoprotein (GP) $II_b/III_a$ receptor antagonists

GP $II_b/III_a$ antagonists are a newer class of potent platelet aggregation inhibitors which act by blocking the key receptor involved in platelet aggregation. The $GPII_b/III_a$ is an adhesive receptor (integrin) on platelet surface for fibrinogen and vWF through which agonists like collagen, thrombin, $TXA_2$, ADP, etc. finally induce platelet aggregation. Thus, GP $II_b/III_a$ antagonists block aggregation induced by all platelet agonists.

**Abciximab** It is the Fab fragment of a chimeric monoclonal antibody against GP $II_b/III_a$, protein, but is relatively nonspecific and binds to some other surface proteins as well. Given along with aspirin + heparin during PCI it has markedly reduced the incidence of restenosis, subsequent MI and death. In the ISAR-REACT2 trial addition of abciximab to clopidogrel (600 mg oral loading dose) for PCI in high-risk ACS patients, reduced ischaemic events by 25%. After a bolus dose, platelet aggregation remains inhibited for 12–24 hr, while the remaining antibody is cleared from blood with a t½ of 10–30 min.

*Dose:* 0.25 mg/kg i.v. 10–60 min before PCI, followed by 10 µg/min for 12 hr. REOPRO 2 mg/ml inj.

Abciximab is nonantigenic. The main risk is haemorrhage, incidence of which can be reduced by carefully managing the concomitant heparin therapy. Thrombocytopenia is another complication. It should not be used second time, since risk of thrombocytopenia increases. Constipation, ileus and arrhythmias can occur. It is expensive, but is being used in unstable angina and as adjuvant to coronary thrombolysis/PCI with stent placement.

**Eptifibatide** It is a synthetic cyclic peptide that selectively binds to platelet surface $GPII_b/III_a$ receptor and inhibits platelet aggregation. Though its plasma t½ (2.5 hours) is longer than that of abciximab, platelet inhibition reverses in a shorter time (within 6–10 hours) because it quickly dissociates from the receptor. Infused i.v., eptifibatide is indicated in:

*Unstable angina:* 180 µg/kg i.v. bolus, followed by 2 µg/kg/min infusion upto 72 hours.

*Coronary angioplasty:* 180 µg/kg i.v. bolus, immediately before procedure; follow with 2 µg/kg/min for 12–24 hours.

CLOTIDE, COROMAX, UNIGRILIN 20 mg/10 ml and 75 mg/100 ml vials.

Aspirin and heparin are generally given concurrently.

Bleeding and thrombocytopenia are the major adverse effects. Rashes and anaphylaxis are rare.

**Tirofiban** This is a nonpeptide but specific $GPII_b/III_a$ antagonist that is similar in properties

to eptifibatide. Its plasma t½ is 2 hours, and it dissociates rapidly from the receptors. The indications and adverse effects are also similar to eptifibatide.

Acute coronary syndromes: 0.4 µg/kg/min for 30 min followed by 0.1 µg/kg/min for upto 48 hours. If angioplasty is performed, infusion to continue till 12–24 hours thereafter.

AGGRAMED, AGGRITOR, AGGRIBLOC 5 mg/100 ml infusion.

### Uses of antiplatelet drugs

The aim of using antiplatelet drugs is to prevent intravascular thrombosis and embolization, with minimal risk of haemorrhage. The intensity of antiplatelet therapy is selected according to the thrombotic influences present in a patient. For indications like maintenance of vascular recanalization, stent placement, vessel grafting, etc. potent inhibition of platelet function is required. This is now possible and is achieved by combining antiplatelet drugs which act by different mechanisms.

1. *Coronary artery disease* On the basis of trials in post-MI patients as well as in those with no such history, it is recommended that aspirin 75–150 mg/day be given to all individuals with evidence of coronary artery disease and in those with risk factors for the same, but routine use in the whole population is not warranted. Primary prevention of ischaemia with aspirin is of no proven benefit. It reduces the incidence of fatal as well as nonfatal MI, but increases the risk of cerebral haemorrhage. Clopidogrel is an alternative to aspirin in symptomatic patients of ischaemia. Continued aspirin/clopidogrel prophylaxis in post-MI patients clearly prevents reinfarction and reduces mortality.

2. *Acute coronary syndromes (ACSs)* These comprise of a range of acute cardiac ischaemic states from unstable angina (UA) to non-ST elevation myocardial infarction (NSTEMI) to STEMI (*see* p. 556).

The coronary obstruction in UA and NSTEMI is partial, while that in STEMI is total. UA and NSTEMI are differentiated on the basis of absence or presence of laboratory markers of cardiac myocyte necrosis (myoglobin, CK, troponin I, etc.). The ischaemic status is often dynamic and the patient may rapidly shift from one category to the next.

Soluble aspirin (325 mg oral) and a LMW heparin (s.c.) are given at presentation to all patients with ACS.

**Unstable angina** Aspirin reduces the risk of progression to MI and sudden death. Clopidogrel is generally combined with aspirin, or may be used as alternative if aspirin cannot be given. For maximum protection the antiplatelet drugs are supplemented with heparin followed by warfarin.

The 'Clopidogrel in unstable angina to prevent recurrent events' (CURE) trial has found that addition of clopidogrel to aspirin further reduced cardiovascular mortality, nonfatal MI and stroke by 20%.

**NSTEMI** Patients of NSTEMI who are managed without PCI/thrombolysis are generally put on a combination of aspirin + clopidogrel, which is continued upto one year.

**STEMI** Primary PCI with or without stent placement is the procedure of choice for all STEMI as well as high risk NSTEMI patients who present within 12 hours. Prasugrel + aspirin is the antiplatelet regimen most commonly selected for patients who are to undergo PCI. Prasugrel acts rapidly and more predictably than clopidogrel. Prasugrel is also perferred over clopidogrel in diabetics. The $GPII_b/III_a$ antagonists are the most powerful antiplatelet drugs; are combined with aspirin for high risk patients undergoing PCI. Abciximab/eptifibatide/tirofiban infused i.v. along with oral aspirin and s.c. heparin markedly reduce incidence of restenosis and subsequent MI after coronary angioplasty. The $GPII_b/III_a$ antagonists are infused for a maximum of 72 hours.

Aspirin and/or clopidogrel are routinely given to ACS patients treated with thrombolysis. Coronary artery bypass surgery is also covered by intensive antiplatelet regimen including aspirin + $GPII_b/III_a$ antagonists/prasugrel.

The patency of recanalized coronary artery or implanted vessel is improved and incidence of reocclusion is reduced by continuing aspirin + clopidogrel/prasugrel almost indefinitely. Dual antiplatelet therapy is recommended after stent placement. Prasugrel is used when stent thrombosis occurs during clopidogrel treatment.

3. *Cerebrovascular disease* Antiplatelet drugs do not alter the course of stroke due to cerebral thrombosis. However, aspirin has reduced the incidence of TIAs and of stroke in patients with TIAs. Occurrence of stroke is also reduced in patients with persistent atrial fibrillation and in those with history of stroke in the past. Aspirin or clopidogrel is recommended in all such individuals. The European stroke prevention study-2 (ESPS) has found combination of dipyridamole with low dose aspirin to be synergistic in secondary prevention of stroke.

4. *Prosthetic heart valves and arteriovenous shunts* Antiplatelet drugs, used with warfarin reduce formation of microthrombi on artificial heart valves and the incidence of embolism. Aspirin is clearly effective but increases risk of bleeding due to warfarin. Dipyridamole does not increase bleeding risk, but incidence of thromboembolism is reduced when it is combined with an oral anticoagulant. Antiplatelet drugs also prolong the patency of chronic arteriovenous shunts implanted for haemodialysis and of vascular grafts.

5. *Venous thromboembolism* Anticoagulants are routinely used in DVT and PE. Trials have shown antiplatelet drugs also to have a prophylactic effect, but their relative value in comparison to, or in addition to anticoagulants is not established; they are infrequently used.

6. *Peripheral vascular disease* Aspirin/clopidogrel may produce some improvement in intermittent claudication and reduce the incidence of thromboembolism.

## ☞ PROBLEM DIRECTED STUDY

**44.1** A 35-year-old woman was on maintenance therapy with warfarin for leg vein thrombosis that she had developed during a complicated delivery 2 months back. The dose was adjusted by repeated measurement of INR, and for the last one month it was maintained between 2.4–2.8 with 4 mg taken daily at bed time. She developed a pelvic infection for which she was admitted to the hospital and given Inj. Ceftriaxone 1 g i.v. 8 hourly. On the 3rd day she started bleeding per-vaginum and reported passing dark urine. The haemoglobin level fell to 9.0 g/dl, while on admission 3 days back, it was 11.0 g/dl. The INR was measured to be 5.4.

(a) What could be the cause of bleeding per-vaginum, passing dark urine; fall in Hb level and rise in INR value? Could this complication be prevented?

(b) How should this patient be managed?

(see Appendix-1 for solution)

# Chapter 45 Hypolipidaemic Drugs and Plasma Expanders

## HYPOLIPIDAEMIC DRUGS

These are drugs which lower the levels of lipids and lipoproteins in blood.

The hypolipidaemic drugs have attracted considerable attention because of their potential to prevent cardiovascular disease by retarding the accelerated atherosclerosis in hyperlipidaemic individuals.

### Lipid transport

Lipids are carried in plasma in lipoproteins after getting associated with several apoproteins; plasma lipid concentrations are dependent on the concentration of lipoproteins. The core of lipoprotein globules consists of triglycerides (TGs) or cholesteryl esters (CHEs) while the outer polar layer has phospholipids, free cholesterol (CH) and apoproteins. The lipoproteins have been divided into 6 classes on the basis of their particle size and density. They also differ in the nature of apoproteins, the ratio of TG and CHE, tissue of origin and fate. These are given in Table 45.1.

Dietary lipids are absorbed in the intestine with the help of bile acids. Chylomicrons (Chy) are formed and passed into lacteals—reach blood stream *via* thoracic duct. During their passage through capillaries, the endothelium bound lipoprotein lipase hydrolyses the TGs into fatty acids which pass into muscle cells to be utilized as energy source and in fat cells to be reconverted into TGs and stored. The remaining part—chylomicron remnant (Chy. rem.) containing mainly CHE and little TG is engulfed by liver cells, which have receptors for the surface apoproteins of Chy. rem., and digested. Free CH that is liberated is either stored in liver cells after reesterification or incorporated into a different lipoprotein and released in blood or excreted in bile as CH/bile acids.

Liver secretes *very low density lipoproteins (VLDL)* containing mainly TG and some CHE into blood. VLDL is acted upon by endothelial lipoprotein lipase in the same way as on Chy and the fatty acids pass into adipose tissue and muscle; the remnant called *intermediate density lipoprotein* (IDL) now contains more CHE than TG. About half of the IDL is taken back by the liver cells by attachment to another receptor (LDL receptor), while the rest loses the remaining TGs gradually and becomes *low density lipoprotein* (LDL) containing only CHE. The LDL circulates in plasma for a long time; its uptake into liver and other tissues is dependent on the need for CH. The rate of LDL uptake is regulated by the rate of LDL receptor synthesis in a particular tissue.

**TABLE 45.1** **Characteristics and function of plasma lipoproteins**

| | *Lipoprotein class* | *Diameter (nm)* | *Lipid contained* | *Source of lipid* | *Function* |
|---|---|---|---|---|---|
| 1. | Chy. | 100–500 | TG >> CHE | Diet | Dietary TG transport |
| 2. | Chy. rem. | 30–50 | CHE >> TG | Diet, Chy. | Dietary CH transport |
| 3. | VLDL | 40–80 | TG >> CHE | Liver | Endogenous TG transport |
| 4. | IDL | 30–35 | CHE ≥ TG | VLDL | Transport CHE & TG to liver, source of LDL |
| 5. | LDL | 20–25 | CHE | IDL | Transport CH to tissues and liver |
| 6. | HDL | 5–10 | Phospholipid, CHE | Tissues, cell memb. | Removal of CH from tissues |

Chy—Chylomicrons; Chy. rem.—Chylomicron remnant; VLDL—Very low density lipoprotein; IDL—Intermediate density lipoprotein; LDL—Low density lipoprotein; HDL—High density lipoprotein; CHE—Cholesteryl esters; TG—Triglyceride; CH—Cholesterol

**TABLE 45.2 Types of primary hyperlipoproteinaemias**

| *Type* | *Disorder* | *Cause* | *Occurrence* | *Elevated plasma lipoprotein* | *Plasma lipids* | |
|---|---|---|---|---|---|---|
| | | | | | *CH* | *TG* |
| I | Familial lipoprotein lipase deficiency | G | Very rare | Chylomicron | ↑↑ | ↑↑↑ |
| IIa | Familial hypercholesterolaemia | G | Less common | LDL | ↑↑ | N |
| IIb | Polygenic hypercholesterolaemia | MF | Commonest | LDL | ↑ | N |
| III | Familial dysbetalipoproteinaemia | G | Rare | IDL, Chy. rem. | ↑ | ↑ |
| IV | Hypertriglyceridaemia | MF, G | Common | VLDL | N | ↑↑ |
| V | Familial combined hyperlipidaemia | G | Less common | VLDL, LDL | ↑ | ↑ |

CH—Cholesterol; TG—Triglycerides; G—Genetic; MF—Multifactorial; Chy. rem.—Chylomicron remnants; VLDL—Very low density lipoprotein; IDL—Intermediate density lipoprotein; LDL—Low density lipoprotein.
The genetic defect in some of the monogenic disorders is:
Type I : absence of lipoprotein lipase—TG in Chy cannot be utilized.
Type IIa : deficiency of LDL receptor—LDL and IDL are taken up very slowly by liver and tissues.
Type III : the apoprotein in IDL and Chy. rem. (apoE) is abnormal, these particles are cleared at a lower rate.
Type IV : this type of hypertriglyceridaemia is both multifactorial and monogenic, the former is more prevalent than the latter.

The CHE of LDL is deesterified and used mainly for cell membrane formation. The CH released into blood from degradation of membranes is rapidly incorporated in *high density lipoproteins* (HDL), esterified with the help of an enzyme lecithin: cholesterol acyltransferase (LCAT) and transferred back to VLDL or IDL, completing the cycle.

The excess lipoproteins in plasma are phagocytosed by macrophages for disposal. When too much of lipoproteins have to be degraded in this manner, CH is deposited in *atheromas* (in arterial walls) and *xanthomas* (in skin and tendons). Raised levels of VLDL, IDL and LDL (rarely Chy and Chy. rem. also) are atherogenic, while HDL may be protective, because HDL facilitates removal of CH from tissues.

**Hyperlipoproteinaemias** can be:
(i) *Secondary*: associated with diabetes, myxoedema, nephrotic syndrome, chronic alcoholism, drugs (corticosteroids, oral contraceptives, β blockers) etc.
(ii) *Primary*: due to:
(a) A single gene defect: is familial and called 'monogenic' or *genetic*.
(b) Multiple genetic, dietary and physical activity related causes: 'polygenic' or *multifactorial*.

On the whole, LDL is the primary carrier of plasma CHE, and VLDL that of TGs. The important features of major types of hyperlipoproteinaemias are given in Table 45.2.

## CLASSIFICATION

1. *HMG-CoA reductase inhibitors (Statins)*: Lovastatin, Simvastatin, Pravastatin, Atorvastatin, Rosuvastatin, Pitavastatin
2. *Bile acid sequestrants (Resins)*: Cholestyramine, Colestipol
3. *Lipoprotein lipase activators (PPARα activators, Fibrates)*: Clofibrate, Gemfibrozil, Bezafibrate, Fenofibrate.
4. *Lipolysis and triglyceride synthesis inhibitor:* Nicotinic acid.
5. *Sterol absorption inhibitor:* Ezetimibe.

The mechanism of action and profile of lipid lowering effect of important hypolipidaemic drugs is summarized in Table 45.3.

**TABLE 45.3 Mechanism of action and pattern of lipid lowering effect of important hypolipidaemic drugs**

| *Drug (daily dose)* | *Mechanism of action* | *Effect on lipids (%)* |
|---|---|---|
| *HMG-CoA reductase inhibitors* | | |
| Lovastatin (10–80 mg)<br>Simvastatin (5–40 mg)<br>Atorvastatin (10–80 mg)<br>Rosuvastatin (5–20 mg) | ↓ CH synthesis by inhibition of rate limiting HMG-CoA reductase | LDL ↓ 20–55<br>HDL ↑ 5–15<br>TG ↓ 10–35 |
| *Bile acid sequestrants* | | |
| Cholestyramine (4–16 g)<br>Colestipol (5–30 g) | ↓ bile acid absorption, ↑ hepatic conversion of CH to bile acids, ↑ LDL receptors on hepatocytes | LDL ↓ 15–30<br>HDL ↑ 3–5<br>TG not affected, may ↑ in some |
| *Fibric acid derivatives* | | |
| Gemfibrozil (1200 mg)<br>Bezafibrate (600 mg)<br>Fenofibrate (200 mg) | ↑ Activity of lipoprotein lipase, ↓ release of fatty acids from adipose tissue | LDL ↓ 5–20*<br>HDL ↑ 10–20<br>TG ↓ 20–50 |
| *Nicotinic acid (2–6 g)* | ↓ Production of VLDL, ↓ lipolysis in adipocytes | LDL ↓ 15–25<br>HDL ↑ 20–35<br>TG ↓ 20–50 |

* Gemfibrozil may ↑ LDL-CH when TG levels are high; bezafibrate and fenofibrate not likely to raise LDL-CH

## HMG-CoA REDUCTASE INHIBITORS (Statins)

Introduced in the 1980s, this class of compounds are the most efficacious and best tolerated hypolipidaemic drugs. They competitively inhibit conversion of 3-Hydroxy-3-methyl glutaryl coenzyme A (HMG-CoA) to mevalonate (rate limiting step in CH synthesis) by the enzyme HMG-CoA reductase. Therapeutic doses reduce CH synthesis by 20–50%. This results in compensatory increase in LDL receptor expression on liver cells → increased receptor mediated uptake and catabolism of IDL and LDL. Over long-term, feedback induction of HMG-CoA reductase tends to increase CH synthesis, but a steady-state is finally attained with a dose-dependent lowering of LDL-CH levels.

Different statins differ in their potency and maximal efficacy in reducing LDL-CH. The daily dose for lowering LDL-CH by 30–35% is lovastatin 40 mg, pravastatin 40 mg, simvastatin 20 mg, atorvastatin 10 mg, rosuvastatin 5 mg and pitavastatin 2 mg. Moreover, at their maximum recommended doses simvastatin (80 mg) causes 45–50% reduction, while atorvastatin (80 mg) and rosuvastatin (40 mg) can reduce LDL-CH by upto 55%. The ceiling effect of lovastatin and pravastatin is 30–40% LDL-CH reduction. All statins produce peak LDL-CH lowering after 1–2 weeks therapy. Hepatic synthesis of VLDL is concurrently reduced and its removal from plasma is enhanced.

A dose-dependent effect is seen with all statins. With lovastatin a mean reduction of LDL-CH by 25% at 20 mg/day, 32% at 40 mg/day and 40% at 80 mg/day has been measured. Atorvastatin is more potent; the corresponding figures of LDL-CH reduction are 33% at 10 mg/day, 40% at 20 mg/day, 45% at 40 mg/day and 50–55% at 80 mg/day. A concurrent fall by 10–30% in plasma TG level, probably due to reduction of VLDL occurs. A modest rise in HDL-CH by 5–15% is also noted. Simultaneous use of bile salt sequestrant augments the LDL lowering effect upto 60% and addition of nicotinic acid to this combination may boost the effect to 70% reduction in LDL-CH. Statins are effective in secondary hypercholesterolaemias also. The more efficacious statins (simvastatin, atorvastatin, rosuvastatin) given at their higher doses effectively reduce TGs (by 25% to 35%) when they are moderately raised, but not when they are markedly raised.

Because HMG-CoA reductase activity is maximum at midnight, all statins are administered at bed time to obtain maximum effectiveness. However, this is not necessary for atorvastatin and rosuvastatin, which have long plasma t½.

All statins, except rosuvastatin are metabolized primarily by CYP3A4. Inhibitors and inducers of this isoenzyme respectively increase and decrease statin blood levels.

*Lovastatin* It is the first clinically used statin; is lipophilic and given orally in the precursor lactone form. Absorption is incomplete and first pass metabolism is extensive. Metabolites are excreted mainly in bile. The t½ is short (1–4 hours).

*Dose:* 10–40 mg/day (max. 80 mg).
ROVACOR, AZTATIN, LOVAMEG 10, 20 mg tabs.

*Simvastatin* It is twice as potent as lovastatin; also more efficacious. A greater rise in HDL-CH (when low) has been noted with simvastatin than lovastatin or pravastatin. Like lovastatin, it is lipophilic and given in the lactone precursor form. Oral absorption is better and first pass metabolism extensive; t½ is 2–3 hr.

*Dose:* 5–20 mg/day (max. 80 mg)
SIMVOTIN, SIMCARD, ZOSTA 5, 10, 20 mg tabs.

*Pravastatin* It is hydrophilic and given in the active form. At low doses it is equipotent to lovastatin, but at higher dose (40 mg/day), CH lowering effect is less. It can be employed when reduction of LDL-CH by ≤ 25% is contemplated. An additional action of decrease in plasma fibrinogen level has been observed. The t½ is 1–3 hours.
PRAVATOR 10, 20 mg tabs.

*Atorvastatin* This newer and most popular statin is more potent and appears to have the highest LDL-CH lowering efficacy at maximal daily dose of 80 mg. At this dose a greater reduction in TGs is noted if the same was raised at baseline. Atorvastatin has a much longer plasma t½ of 18–24 hr than other statins, and has additional antioxidant property.
*Dose:* 10-40 mg/day (max. 80 mg)
AZTOR, ATORVA, ATORLIP 5, 10, 20 mg tabs.

*Rosuvastatin* This is another newer, commonly used and potent statin (10 mg rosuvastatin ≃ 20 mg atorvastatin), with a plasma t½ of 18–24 hours. Greater LDL-CH reduction can be obtained in severe hypercholesterolaemia; partly due to its longer persistence in the plasma. In patients with raised TG levels, rosuvastatin raises HDL-CH by 15–20% (greater rise than other statins).
*Dose:* Start with 5 mg OD, increase if needed upto 20 mg/day, (max 40 mg/day)
ROSUVAS, ROSYN 5, 10, 20 mg tabs.

*Pitavastatin* This is the latest and dose-to-dose the most potent statin. However, no specific advantages compared to other statins have been demonstrated, and experience with its use is limited. A ceiling response of 40% LDL-CH reduction with the maximum recommended daily dose of 4 mg is noted. The plasma t½ is 12 hours. Use of pitavastatin in combination with gemfibrozil should be avoided, as the latter decreases its clearance.
*Dose:* 1–4 mg per day; FLOVAS 1.0, 2.0 mg tabs.

**Adverse effects** All statins are remarkably well tolerated; overall incidence of side effects not differing from placebo. Notable side effects are:

Gastrointestinal complaints and headache are usually mild. Rashes and sleep disturbances are uncommon. Rise in serum transaminase can occur, but liver damage is rare. Monitoring of liver function is recommended.

Muscle aches are the commonest (10%) side effect. Rise in CPK levels occurs infrequently. Myopathy is the only serious reaction, but is rare (< 1 per 1000). Few fatalities due to rhabdomyolysis are on record. Myopathy is more common when nicotinic acid/gemfibrozil or CYP3A4 inhibitor—ketoconazole/ erythromycin/ cyclosporine/HIV protease inhibitor is given concurrently. Gemfibrozil inhibits the hepatic uptake of statins by the organic anion transporter OATP2. Fenofibrate interferes the least with statin uptake/metabolism and should be preferred for combining with them. A lower dose of statin is advisable when a fibrate is given concurrently. Statins should not be given to pregnant women, since there is no data on their safety.

**Use** Statins are the first choice drugs for primary hyperlipidaemias with raised LDL and total CH levels, with or without raised TG levels (Type IIa, IIb, V), as well as for secondary (diabetes, nephrotic syndrome) hypercholesterolaemia.

Efficacy of statins in reducing raised LDL-CH associated mortality and morbidity is now well established. Since the dose-response relationship of each statin is quite well documented, the initial dose of selected statin should aim to bring down the LDL-CH to the target level. It should then be adjusted by LDL-CH measurements every 3–4 weeks.

In the 'Scandinavian Simvastatin Survival Study' (4S study, 1994), patients with history of MI (80%) or angina (20%) and raised serum CH level (> 212 mg/dl) were treated with simvastatin or placebo. Simvastatin reduced total CH by 25%, LDL-CH by 35%, raised HDL-CH by 8%. Over a period of 6 years coronary artery disease (CAD) mortality was less by 42%, overall mortality by 30% and cerebrovascular events by 30% in the simvastatin group. Similar results have been obtained with other statins, e.g. the West of Scotland Coronary Prevention Study (WOSCOPS) in men with no history of MI has found pravastatin to lower risk of MI by 31% and all cause mortality by 22%.

Subsequent studies like Long-term intervention with pravastatin in ischaemic disease (LIPID-1998), Airforce/Texas coronary atherosclerosis prevention study (AFCAPS/TexCAPS-1998), Cholesterol and recurrent events (CARE-1998), and trials conducted by Heart Protection Study Collaborative Group (2002, 2004) in over 20,000 patients have confirmed the mortality and morbidity benefits of statins, including stroke prevention.

Beneficial effects in subjects who have raised CH levels but no evidence of CAD may relate to improved coronary artery compliance and atheromatous plaque stabilization due to suppression of macrophage mediated inflammation, reducing chances of plaque rupture and thrombus formation. Improvement in endothelial function due to increased NO production and reduction in LDL oxidation are proposed as additional mechanisms by which statins may exert antiatherosclerotic action. Recently, a reduction in venous thromboembolism has also been observed with rosuvastatin. On the basis of these results as well as the excellent patient acceptability, the statins are being increasingly used for primary and secondary hypercholesterolaemia with or without raised TG levels. They are the first choice drugs for dyslipidaemia in diabetics. Statin therapy is continued indefinitely, unless adverse effects occur.

### BILE ACID SEQUESTRANTS (Resins)

**Cholestyramine and Colestipol** These are basic ion exchange resins supplied in the chloride form. They are neither digested nor absorbed in the gut: bind bile acids in the intestine interrupting their enterohepatic circulation. Faecal excretion of bile salts and CH (which is absorbed with the help of bile salts) is increased. This indirectly leads to enhanced hepatic metabolism of CH to bile acids. More LDL receptors are expressed on liver cells: clearance of plasma IDL, LDL and indirectly that of VLDL is increased.

Resins have been shown to retard atherosclerosis, but are not popular clinically because they are unpalatable, inconvenient, have to be taken in large doses, cause flatulence and other g.i. symptoms, interfere with absorption of many drugs and have poor patient acceptability.

## LIPOPROTEIN-LIPASE ACTIVATORS (Fibrates)

The fibrates (isobutyric acid derivatives) primarily activate lipoprotein lipase which is a key enzyme in the degradation of VLDL resulting in lowering of circulating TGs. This effect is exerted through peroxisome proliferator-activated receptor α (PPARα) that is a gene transcription regulating receptor expressed in liver, fat and muscles. Activation of PPARα enhances lipoprotein lipase synthesis and fatty acid oxidation. PPARα may also mediate enhanced LDL receptor expression in liver seen particularly with second generation fibrates like bezafibrate, fenofibrate. Fibrates decrease hepatic TG synthesis as well. A peripheral effect reducing circulating free fatty acids has also been shown.

Drugs in this class primarily lower TG levels by 20–50%, generally accompanied by 10–15% decrease in LDL-CH and a 10–15% increase in HDL-CH. In some patients with hypertriglyceridaemia LDL-CH may rise, partly because of inability of LDL receptor to clear the excess number of LDL particles generated by enhanced VLDL catabolism. The increase in HDL-CH is at least in part due to transfer of surface lipid components from catabolized VLDL to HDL, and partly due to increased production of HDL apoproteins (apo A-I, apo A-II) by liver. Gemfibrozil also appears to reduce VLDL secretion by liver.

LDL composition may be altered. Gemfibrozil and bezafibrate have been shown to shift small dense LDL particles (believed to be more atherogenic) to larger less dense particles.

**Clofibrate** It was a widely used hypolipidaemic drug, but later evidence showed that it does not prevent atherosclerosis, therefore has gone out of use.

**Gemfibrozil** This fibric acid derivative effectively lowers plasma TG level by enhancing breakdown and suppressing hepatic synthesis of TGs. Besides high efficacy in type III hyperlipoproteinemia, gemfibrozil has shown action in subjects with raised blood CH in addition. In the 'Helsinki Heart Study' men without known CAD treated with gemfibrozil had a 34% reduction in fatal and nonfatal MI, though overall mortality was not affected. That these benefits extend to secondary prevention of coronary events in men with existing CAD and low HDL-CH, has been demonstrated in another trial. Additional actions to decrease the level of clotting factor VII-phospholipid complex and promotion of fibrinolysis have been observed, which may contribute to the antiatherosclerotic effect.

*Pharmacokinetics* Gemfibrozil is completely absorbed orally, metabolized by glucuronidation and undergoes some enterohepatic circulation. It is excreted in urine; elimination t½ is 1–2 hr.

*Adverse effects* Common side effects are epigastric distress, loose motions.
Skin rashes, body ache, eosinophilia, impotence, headache and blurred vision have been reported.
Myopathy is uncommon. Gemfibrozil + statin increases risk of myopathy.
Incidence of gallstone is not increased as was seen with clofibrate.

It is contraindicated during pregnancy.

GEMPAR, NORMOLIP 300 mg cap. LOPID 300 mg cap, 600 mg and 900 mg tabs.

*Use* In a dose of 600 mg BD taken before meals, gemfibrozil is a first line drug for patients with markedly raised TG levels, whether or not CH levels are also raised. Episodes of acute pancreatitis are prevented in patients with chylomicronaemia and severe hypertriglyceridaemia. It is most effective in type III hyperlipoproteinaemia; also beneficial in type IV and type V disease. Patients with raised TG and low HDL-CH levels (as is the case with metabolic syndrome, type 2 diabetes) are the most suitable to be treated with fibrates. Fibrates may also be used to supplement statins.

**Bezafibrate** This second generation fibric acid derivative is an alternative to gemfibrozil in mixed hyperlipidaemias (type III, IV and V). Though it has also been indicated in hypercholesterolaemia (type II), it is inferior to statins and resins. Bezafibrate has not shown propensity to increase LDL-CH in hypertriglyceridaemic patients and appears to have greater LDL-CH lowering action than gemfibrozil. Circulating fibrinogen and glucose levels may decrease. The 5 year 'Bezafibrate Coronary Atherosclerosis Intervention Trial' (BECAIT) in young male post-MI subjects showed an atherosclerosis slowing effect and reduction in coronary events. The Bezafibrate Infarction Prevention (BIP) registry has also noted reduction in coronary events in subjects with high TG and low HDL-CH levels.

Adverse effects and contraindications are similar to other fibrates. Main side effects are g.i. upset, myalgia, rashes. Dose reduction is needed in elderly and in renal insufficiency. Action of oral anticoagulants may be enhanced.

In contrast to gemfibrozil, combination of bezafibrate with a statin has not so far been found to increase the incidence of rhabdomyolysis.

*Dose:* 200 mg TDS with meals.

BEZALIP 200, 400 mg tab.

**Fenofibrate** Another 2nd generation prodrug fibric acid derivative which has greater HDL–CH raising and greater LDL-CH lowering action than other fibrates: may be more appropriate as an adjunctive drug in subjects with raised LDL-CH levels in addition to raised TG levels. No rise in LDL-CH has been observed in patients with high TG levels. Its t½ is 20 hr. Adverse effects are myalgia, hepatitis, rashes. Cholelithiasis and rhabdomyolysis are rare. Fenofibrate appears

to be the most suitable fibrate for combining with statins, because statin metabolism is minimally affected and enhancement of statin myopathy risk is lower. Indications of fenofibrate are similar to that of gemfibrozil.

*Dose:* 200 mg OD with meals.

FENOLIP, LIPICARD 200 mg cap.

## LIPOLYSIS AND TRIGLYCERIDE SYNTHESIS INHIBITOR

### Nicotinic Acid (Niacin)

It is a B group vitamin (*see* Ch. 67) which in much higher doses reduces plasma lipids. This action is unrelated to its vitamin activity and not present in nicotinamide. When nicotinic acid is given, TGs and VLDL decrease rapidly, followed by a modest fall in LDL-CH and total CH. A 20–50% reduction in plasma TGs and 15–25% reduction in CH levels has been recorded. Nicotinic acid is the most effective drug to raise HDL-CH, probably by decreasing rate of HDL destruction; a 20–35% increase is generally obtained. Relatively lower dose suffices to raise HDL–CH. It also reduces lipoprotein Lp (a), which is considered more atherogenic.

Nicotinic acid reduces production of VLDL in liver by inhibiting TG synthesis. Indirectly the VLDL degradation products IDL and LDL are also reduced. No direct effect on CH and bile acid metabolism has been found. It inhibits intracellular lipolysis in adipose tissue and increases the activity of lipoprotein lipase that clears TGs.

A cell surface Gi-protein coupled receptor which negatively regulates adipocyte adenylyl cyclase has been found to selectively bind nicotinic acid, and has been called 'niacin receptor'. Nicotinic acid appears to inhibit lipolysis in adipose tissue by decreasing hormone stimulated intracellular cAMP formation through this receptor. Hepatic VLDL production is believed to be decreased due to reduced flow of fatty acids from adipose tissue to liver.

*Adverse effects* The large doses needed for hypolipidaemic action are poorly tolerated. Only about half of the patients are able to take the full doses.

Nicotinic acid is a cutaneous vasodilator: marked flushing, heat and itching (especially in the blush area) occur after every dose. This is associated with release of $PGD_2$ in the skin, and can be minimized by starting with a low dose taken with meals and gradually increasing as tolerance develops. Use of sustained release (SR/ER) tablet also subdues flushing. Aspirin taken before niacin substantially attenuates flushing by inhibiting PG synthesis. *Laropiprant* is a specific antiflushing drug with no hypolipidaemic action of its own, that has been combined with nicotinic acid to minimize flushing. An ER tablet containing 1.0 g nicotinic acid and 20 mg laropiprant is used in UK and Europe.

Dyspepsia is very common; vomiting and diarrhoea occur when full doses are given. Peptic ulcer may be activated.

Dryness and hyperpigmentation of skin can be troublesome. Other long-term effects are:

Liver dysfunction and jaundice. Serious liver damage is the most important risk.

Hyperglycaemia, precipitation of diabetes (should not be used in diabetics).

Hyperuricaemia and gout, atrial arrhythmias.

It is contraindicated during pregnancy and in children.

*Interaction* Postural hypotension may occur in patients on antihypertensives when they take nicotinic acid.

Risk of myopathy due to statins is increased.

*Dose:* Start with 100 mg TDS, gradually increase to 2–4 g per day in divided doses. It should be taken just after food to minimize flushing and itching.

NIALIP, NEASYN-SR 375, 500 mg tabs.

*Use* Nicotinic acid is a wide spectrum hypolipidaemic drug. It is highly efficacious in hypertriglyceridaemia (type III, IV, V) whether associated with raised CH level or not. It is mostly used to lower VLDL and raise HDL levels, and as an adjunctive drug to statins/fibrates.

Nicotinic acid is the most effective drug in reducing plasma TG levels. Its most important indication is to control pancreatitis associated with severe hypertriglyceridaemia, mostly in genetic type IV and type V disorders. Long-term use prevents further attacks of pancreatitis. Given over

long-term in post-MI patients, it has been found to reduce recurrences of MI and overall mortality. However, doses above 2 g/day are poorly tolerated; should seldom be exceeded for maintenance purposes. Because of potential toxicity, use of nicotinic acid is restricted to high-risk cases only.

## STEROL ABSORPTION INHIBITOR

**Ezetimibe** It is a novel drug that acts by inhibiting intestinal absorption of cholesterol and phytosterols. It interferes with a specific CH transport protein NPC1L1 in the intestinal mucosa and reduces absorption of both dietary and biliary CH. There is compensatory increase in hepatic CH synthesis, but LDL-CH level is lowered by 15–20%. The enhanced CH synthesis can be blocked by statins, and the two drugs have synergistic LDL-CH lowering effect.

Due to very poor aqueous solubility, ezetimibe is not absorbed as such. A fraction is absorbed after getting conjugated with glucuronic acid in the intestinal mucosa. This is secreted in bile and undergoes enterohepatic circulation to be mainly excreted in faeces. A plasma t½ of 22 hours has been calculated.

Used alone, ezetimibe is a weak hypocholesterolaemic drug; LDL-CH lowering beyond 15–20% is not obtained by increasing the dose. Though it may be used alone in mild hypercholesterolaemia when a statin is contraindicated/not tolerated, its main value is to supplement statins without increasing their dose. The combination of ezetimibe + low dose of a statin is as effective in lowering LDL-CH as high dose of statin alone. Upto 60% decrease in LDL-CH level has been obtained with a combination of simvastatin + ezetimibe. The ENHANCE trial has found that though addition of ezetimibe to simvastatin further decreased LDL-CH, it caused little reduction in carotid artery intima : media thickness (IMT) ratio, a measure of subintimal CH deposition. While this could be due to the fact that the subjects were on long-term statin therapy and had relatively low basal IMT ratio, the actual clinical benefit of adding ezetimibe to a statin needs confirmation. Another study has found statin + niacin to cause greater reduction in IMT of carotid than statin + ezetimibe.

No specific adverse effect, except reversible hepatic dysfunction and rarely myositis has been noted with ezetimibe.

*Dose:* 10 mg OD; ZETICA, EZEDOC 10 mg tab. BITORVA, LIPIVAS-EZ, LIPONORM-EZ: Atorvastatin 10 mg + ezetimibe 10 mg tab; SIMVAS-EZ, STARSTAT-EZ: Simvastatin 10 mg + ezetimibe 10 mg tabs.

### CETP-INHIBITORS

The cholesteryl ester transfer protein (CETP) facilitates exchange of CHEs with TGs between HDL particles and chylomicrons, VLDL, LDL, etc. It plays an important role in the disposal of HDL-associated CH. Inhibitors of this protein, *torcetrapib*, *anacetrapib*, etc. markedly raise HDL-CH and lower LDL. They were presumed to have antiatherosclerotic action. However, during a large randomized clinical trial, torcetrapib was found to increase cardiovascular events like angina, MI, heart failure and death. The trial and further development of the drug was stopped in 2007. Whether other CETP inhibitors will have therapeutic value is being investigated, but appears doubtful.

## Summary guidelines on the use of hypolipidaemic drugs

Raised plasma CH is a major risk factor for coronary artery disease (CAD); higher the CH level, greater is the risk of CAD. Abundant data has confirmed that lowering the level of LDL-CH, when the same is high, results in lowering of cardiovascular mortality and morbidity. More recent evidence (HPS, 2002; ASCOT-LLA, 2003 studies) has indicated that prophylactic use of a statin in CAD/hypertensive patients even with average or lower than average CH levels lowers coronary and stroke events. With the availability of effective, well tolerated and safe hypolipidaemic drugs, it has become a standard practice to prescribe statin therapy after an acute coronary event irrespective of lipid levels. Evidence that elevated plasma TG level or low plasma HDL-CH level poses independent high risk of CAD and stroke is also quite strong now.

Whereas raised LDL-CH is atherogenic, a higher HDL-CH level is either itself protective or indicates a low atherogenic state.

**TABLE 45.4 Interpretation of plasma lipid levels***

| | Plasma lipid levels (mg/dl) | | | |
|---|---|---|---|---|
| | *Total CH* | *LDL-CH* | *HDL-CH* | *TGs* |
| 1. Optimal/desirable | < 200 | < 100 (< 70 for CAD pts) | > 40 (men) > 50 (women) | < 150 |
| 2. Borderline high | 200–239 | 130–159 | — | 150–199 |
| 3. High | ≥ 240 | 160–189 | > 60 | 200–499 |
| 4. Very high | — | ≥190 | — | ≥ 500 |

* Adopted from NCEP (2001)

The US National Cholesterol Education Programme (NCEP) in its third report (2001) delineated the optimal levels of plasma lipids and various grades of hyperlipidaemias (Table 45.4) and revised the guidelines for use of hypolipidaemic drugs (Adult Treatment Panel III or ATP III).

Subsequently, the results of some large randomized controlled trials like HPS (2002, 2004), ASCOT-LLA (2003), PROVE-IT (2004) became available and necessitated further revision of the treatment guidelines. A 2004 revision of the ATP III guidelines has been published (Grundy *et al*, 2004). These guidelines are likely to be revised soon in NCEP-ATPIV. The salient features of the current ATPIII guidelines are incorporated in the following summary.

Lifestyle modification, such as low fat, low cholesterol diet, limitation of saturated and trans-fats, regular exercise, body weight control, smoking cessation, restriction of alcohol are the primary approach, whether drugs are used or not.

The decision to prescribe hypolipidaemic drugs depends not only on the LDL-CH level and the type of lipid abnormality, but also on associated CAD risk factor(s) or existing CAD or its equivalent like diabetes, peripheral/cerebral vascular disease, etc. in an individual patient (*see* box).

**Risk factors for coronary artery disease***

- Men > 45 years, women > 55 years
- Family history of MI/sudden cardiac death before 55 year (men), 65 year (women) age in first degree relative
- Smoking
- Hypertension (BP > 140/90 or use of anti-hypertensive medication)
- Diabetes mellitus£
- Low HDL-CH (< 40 mg/dl in men, < 50 mg/dl in women)
- High LDL-CH (≥ 160 mg/dl) or total CH ≥ 240 mg/dl
- Obesity (BMI > 25 Kg/m²)† or waist > 40″ (men), > 35″ (women)

* Adopted from the NCEP-ATP III (2001)
£ Diabetes is considered equivalent to existing CAD
† Not included in NCEP guideline (2001)

*Treatment based on LDL-CH level* The revised NCEP-ATP III guidelines are summarized in Table 45.5. All subjects should receive statin (or statin-based combination) therapy if LDL-CH is ≥ 190 mg/dl. The dose should be titrated to achieve the goal LDL-CH level or 30–40% reduction, which-ever is lower. This degree of lipid lowering has been found to yield optimum prognostic benefit. For subjects who already have CAD or CAD equivalent, there is no lower threshold LDL-CH level; all subjects should receive lipid lowering drug. Though, LDL-CH value upto 100 mg/dl is considered optimal for

1. HPS (2002, 2004): Heart protection study. *Lancet* (2002) **360**, 7-22, and *Lancet* (2004) **363**, 757-767.
2. ASCOT-LLA (2003): Anglo-Scandinavian cardiac outcomes trial–Lipid lowering arm. *Lancet* (2003) **361**, 1149-1158.
3. PROVE-IT (2004): Intensive versus moderate lipid lowering with statins after acute coronary syndromes. *NEJM* (2004), **350,** 1495-1504.
4. NCEP-ATP III (2004 revision). Grundy. SM *et al. Circulation* (2004) **110**, 227-239.

**TABLE 45.5 LDL-CH lowering treatment guidelines***

| | Risk category | LDL-CH goal (mg/dl) | LDL-CH (mg/dl) level for initiation of Lifestyle modifications | Drug therapy |
|---|---|---|---|---|
| 1. | Very high risk (CAD/CAD equivalent£ + one$) | <70† | All subjects | All subjects |
| 2. | High risk (CAD/CAD equivalent) | < 100† | All subjects | All subjects |
| 3. | Moderately high risk (≥ 2 CAD risk factors + 10 yr CAD risk° 10–20% | < 130 (or < 100#) | ≥ 100ψ | ≥ 130 (or 100–129#) |
| 4. | Moderate risk (≥ 2 CAD risk factors + 10 yr CAD risk° < 10%) | < 130 | ≥ 130ψ | ≥ 160 |
| 5. | Low risk (0–1 CAD risk factor) | < 160 | ≥ 160ψ | ≥ 190 (or 160–189#) |

* Adopted from the US National Cholesterol Education Programme (NCEP); 2004 revision of adult treatment panel III (ATP III)
£ CAD equivalent includes—diabetes mellitus; 10 yr CAD risk > 20%; peripheral vascular disease; abdominal-aortic aneurysm; symptomatic carotid artery disease
$ One additional feature from (i) ≥ 2 CAD risk factors (ii) Single uncontrolled CAD risk factor (iii) diabetes mellitus (iv) metabolic syndrome (v) acute coronary syndrome
° As per risk assessment tables from the Framingham Heart Study
† When LDL-CH is near or below the goal value, then a statin dose to lower LDL-CH by 30–40% should be employed
# Patients with a severe risk factor or multiple risk factors.
ψ Any subject who has life-style related risk factor(s), such as obesity, physical inactivity, smoking, etc. is a candidate for life-style change of the risk factor(s) regardless of the LDL-CH level.

non-CAD subjects, the goal for CAD patients has been lowered to 70 mg/dl. These decisions are based on the findings of recent studies which have compared mortality as well as CAD and stroke prevention benefits of standard *vs* intensive CH lowering regimens. Metaanalysis by Cholesterol Treatment Trialists collaborators and others conclude that standard statin therapy lowering LDH-CH by 30–40% reduces cardiovascular events by 30–35%, while intensive LDL-CH lowering by ~50% curtails cardiovascular events by nearly 50%. The JUPITER trial (2008) demonstrated a 44% reduction in combined end-point of stroke, MI, unstable angina and cardiovascular death by using high potency rosuvastatin. Moreover, the criteria for grading the cardiovascular disease risk as 'very high' to 'low' have been defined, and threshold as well as goal LDL-CH levels have been demarcated for each category of risk (*see* Table 45.5).

The primary drugs to lower LDL-CH are statins. Statin therapy should be commenced at the dose estimated to attain target LDL-CH lowering. In case of inadequate response, dose should be doubled at 6 week intervals (till max recommended doses are reached), or another drug (fibrate/nicotinic acid/ezetimibe) should be added to achieve the target LDL-CH level. Intensive lipid lowering by adequate dose of statin is now considered to imporove endothetial function and stabilize plaques in addition to the antiathero-sclerotic effect; all of these leading to reduction in CAD, stroke and death.

*Treatment of low HDL-CH level* Epidemio-logical data has shown that most patients with premature CAD have low HDL-CH level. The total CH: HDL-CH ratio has been recognized as a more important determinant of CAD risk. While a ratio of ≤ 3.5 is considered desirable, a ratio

of >4.5 is associated with higher risk. Recent trials have shown that statin therapy reduces CAD endpoints in subjects with low HDL-CH even though LDL-CH may be in the normal range. Most low HDL-CH subjects have metabolic syndrome (obesity, hypertriglyceridaemia, insulin resistance/diabetes, hypertension). Therapy directed towards components of this syndrome often helps to normalise HDL-CH. In addition to these measures, the primary approach of therapy in subjects with low HDL-CH is to reduce LDL-CH to the target level as per their LDL-CH risk category or to achieve a total CH: HDL-CH ratio of ≤ 3.5, whichever is more intensive. This may require reduction of total CH even to <150 mg/dl and LDL-CH to < 100 mg/dl. None of the currently available lipid modifying drugs has a marked effect to raise HDL-CH, but nicotinic acid has the highest efficacy followed by fibrates. These drugs may be usefully combined with the statin, watching for signs of myositis.

*Treatment of raised TG level:* On the basis of metaanalysis of studies, the NCEP have recognized elevated TGs to be an independent CAD risk factor. Treatment strategy for hypertriglyceridaemia depends on its cause (obesity, physical inactivity, smoking, alcohol, high carbohydrate diet, diabetes, renal failure, drugs like corticosteroids, estrogens, high dose β blockers and genetic disorders) and its severity. Initial treatment is directed to achieving the target LDL-CH level appropriate for the patient's CAD risk category (by using a statin). This may itself lower the TG level. The primary TG lowering drugs are fibrates and nicotinic acid. In case of failure to reduce serum TG to < 200 mg/dl, a fibrate (preferably fenofibrate) or nicotinic acid may be added to the statin regimen, with extra vigilance to guard against the increased risk of myopathy.

**Therapeutic intervention for raised TG level**

a. *Plasma TG < 150 mg/dl (normal)*
No TG lowering needed; treat as per CH levels

b. *Plasma TG 200-499 mg/dl (high)*
- Lifestyle modification
- Treatment of cause if identified
- Statin therapy to achieve the goal LDL-CH level as per CAD risk category
- Specific TG lowering drug (fibrate/nicotinic acid) may be considered if—
  (i) CAD present
  (ii) family history of premature CAD
  (iii) non-HDL-CH ≥ 190 mg/dl
  (iv) HDL-CH < 40 mg/dl
  (v) genetic dysbetalipoproteinaemia (type III) or familial combined hyperlipidaemia (type v)
  (vi) multiple risk factors present

c. *Plasma TG > 500 mg/dl (very high)*
Vigorous measures to lower TG level needed since risk of acute pancreatitis is high
- control diabetes and other causes
- institute very low fat diet
- reduce weight; curtail alcohol
- specific TG lowering drugs strongly indicated.

## PLASMA EXPANDERS

These are high molecular weight substances which exert colloidal osmotic (oncotic) pressure, and when infused i.v. retain fluid in the vascular compartment. They are used to correct hypovolemia due to loss of plasma/blood.

Human plasma or reconstituted human albumin would seem to be the best. However, the former carries risk of transmitting serum hepatitis, AIDS, etc., and the latter is expensive. Therefore, synthetic colloids are more often used. The desirable properties of a plasma expander are given in the box.

**Desirable properties of plasma expander**

1. Should exert oncotic pressure comparable to plasma.
2. Should remain in circulation and not leak out in tissues, or be too rapidly disposed.
3. Should be pharmacodynamically inert.
4. Should not be pyrogenic or antigenic.
5. Should not interfere with grouping and cross-matching of blood.
6. Should be stable, easily sterilizable and cheap.

*Substances employed are:*

Human Albumin
Dextran
Polygeline
Hetastarch

**Human albumin** It is obtained from pooled human plasma; 100 ml of 20% human albumin solution is the osmotic equivalent of about 400 ml of fresh frozen plasma or 800 ml of whole blood. It can be used without regard to patient's blood group and does not interfere with coagulation. Unlike whole blood or plasma, it is free of risk of transmitting serum hepatitis because the preparation is heat treated. There is also no risk of sensitization with repeated infusions.

The 20% solution draws and holds additional fluid from tissues: crystalloid solutions must be infused concurrently for optimum benefit. Apart from burns, hypovolemia, shock, etc., it has been used in acute hypoproteinaemia, acute liver failure and dialysis. Dilution of blood using albumin and crystalloid solutions can be used before cardiopulmonary bypass. Febrile reaction to human albumin occurs occasionally. It is expensive.

Human albumin 20%: ALBUDAC, ALBUPAN 50, 100 ml inj., ALBUMED 5%, 20% infusion (100 ml)

**Dextran** It is a polysaccharide obtained from sugar beat, and is available in two forms.

Dextran-70 (MW 70,000): DEXTRAN-70, LOMODEX-70; 6% solution in dextrose or saline, 540 ml vac.
Dextran-40 (MW 40,000; low MW dextran): LOMODEX 10% solution in dextrose or saline, 540 ml vac.

The more commonly used preparation is dextran-70. It expands plasma volume for nearly 24 hours, and is slowly excreted by glomerular filtration as well as oxidized in the body over weeks. Some amount is deposited in RE cells. Dextran has nearly all the properties of an ideal plasma expander except:

(a) It may interfere with blood grouping and cross-matching.
(b) Though the dextran used clinically is not antigenic, its structure is similar to other antigenic polysaccharides. Some polysaccharide reacting antibodies, if present, may cross react with dextran and trigger anaphylactic reaction. Urticaria, itching, bronchospasm, fall in BP occur occasionally; anaphylactic shock is rare.
(c) It can interfere with coagulation and platelet function, and thus prolong bleeding time; should not be used in hypofibrinogenaemia, thrombocytopenia or in presence of bleeding.

*Dextran-40* It acts more rapidly than dextran-70. It reduces blood viscosity and prevents RBC sludging that occurs in shock by coating them and maintaining their electronegative charge. Microcirculation may improve. However, it is rapidly filtered at the glomerulus: expands plasma volume for a shorter period, and may get highly concentrated in the tubule if oliguria develops—tubular obstruction may occur. The total dose should not exceed 20 ml/kg in 24 hr.

Dextrans can be stored for 10 years and are cheap. They are the most commonly used plasma expanders.

**Polygeline (Degraded gelatin polymer)** It is a polypeptide with average MW 30,000 which exerts oncotic pressure similar to albumin and is not antigenic; hypersensitivity reactions are rare, but should be watched for. It does not interfere with grouping and cross-matching of blood and remains stable for three years. It is not metabolized in the body; excreted slowly by the kidney. Expansion of plasma volume lasts for 12 hours. It is more expensive than dextran. It can also be used for priming of heart-lung and dialysis machines.

Hypersensitivity reactions like flushing, rigor, urticaria, wheezing and hypotension can occur.

HAEMACCEL,SERACCEL 500 ml vac. (as 3.5% solution in balanced electrolyte medium).

**Hetastarch** It is a complex mixture of ethoxylated amylopectin of various molecular sizes; average MW 4.5 lac (range 10,000 to 1 million). The colloidal properties of 6% hetastarch approximate those of human albumin. Plasma volume expands slightly in excess of the volume infused. Haemodynamic status is improved for 24 hour or more. Hetastarch is incompatible with many drugs; no injectable drug should be added to the infusion. Blood grouping and cross matching may be vitiated.

Smaller molecules (MW < 50,000) are excreted rapidly by kidney; 40% of infused dose appears in urine in 24 hr. Larger molecules are slowly broken down to smaller ones and eliminated with a t½ of 17 days.

*Adverse effects* are vomiting, mild fever, itching, chills, flu like symptoms, swelling of salivary glands. Urticaria, periorbital edema and bronchospasm are the anaphylactoid reactions.

It has also been used to improve harvesting of granulocytes because it accelerates erythrocyte sedimentation.

EXPAN 6% inj (100, 500 ml vac)

## USE OF PLASMA EXPANDERS

These colloidal solutions are used primarily as substitutes for plasma in conditions where plasma has been lost or has moved to extravascular compartment, e.g. in burns (acute phase only), hypovolemic and endotoxin shock, severe trauma and extensive tissue damage. They can also be used as a temporary measure in cases of whole blood loss till the same can be arranged: but they do not have $O_2$ carrying capacity. Apart from albumin, other plasma expanders should not be used for maintenance of plasma volume in conditions like burns, where proteins leakout with fluids for several days.

*Contraindications* to plasma expanders are—severe anaemia, cardiac failure, pulmonary edema, liver disease, renal insufficiency.

## ☞ PROBLEM DIRECTED STUDY

**45.1** The routine medical checkup of a 50-year-old male, asymptomatic, non-smoker business executive with sendentary job and no family history of premature cardiac death has yielded the following findings:

Body mass index—27, waist circumferance—92 cm (38"), BP—130/86 mm Hg, fasting blood glucose—98 mg/dl, total serum cholesterol (CH) 268 mg/dl, LDL-CH 198 mg/dl, HDL-CH 38 mg/dl, serum triglyceride 160 mg/dl. Liver, kidney and thyroid function test values and ECG are within normal limits. There are no remarkable findings on physical examination.

(a) Apart from counselling on life-style modification, does this person require any medication?

(b) In case he needs medication, which drug and dose would be appropriate? What should be the goal of drug therapy?

(see Appendix-1 for solution)