15

Extrapolation from Animals to Human Beings and Translational Science

15.1 Introduction

Historically, pharmacologists have conducted extensive laboratory studies of drugs *in vitro* reproducing physiological and neurochemical functions, and modifying them with drugs, in isolated animal tissues in organ baths. The qualitative and quantitative phenomena studied in this way were also studied in whole animals. Analogies would be sought in human therapeutics. New drugs were especially studied in mice, rats, and dogs, and a judgement would be made that the risk of initial exposure of humans, volunteers or patients, was warranted, with no pharmacokinetic data.

In the field of drug disposition and pharmacokinetics, a vast amount of valuable information has been discovered using isolated enzymes and functioning cells, liver and other tissue homogenates, perfused organs, and laboratory animals *in vivo*. However, it is rare that our need is to understand the disposition of a drug in the rat or other laboratory animals *per se*, and comparative pharmacokinetics is especially important in the discovery of new therapeutic agents. The transition from animals to human beings remains a critical step, and in its modern form it is facilitated by pharmacokinetics.

When a new chemical entity (NCE) intended for investigation in man as a potential therapeutic agent leaves the laboratory for the clinic, there will be a body of knowledge about its disposition and fate that is considerable in size but limited in scope. Types of information available will commonly include:

- Physical and chemical properties (solubility, pk_a, stability, etc.), and preliminary information on absorption potential, from molecular modelling or simple experiments
- Metabolic reactions in several species including, in part, human, *in vitro*, from enzyme, hepatocyte, and isolated organ (animals only) studies
- Protein binding in several species *in vitro* including human
- Renal clearance from *in vivo* studies in animals in which blood and urine samples were collected in parallel
- Microsomal intrinsic clearance in animals and humans, sometimes called 'metabolic stability'
- *In vivo* single-dose animal pharmacokinetics, sometimes including comparison of oral and i.v. doses, and including calculation of whole body half-life and clearance
- Limited data on pharmacokinetics during long-term dosing from toxicokinetic studies lasting up to 3 months
- Observations on pharmacological effects.

The challenge is to choose doses and blood sampling sequences for the initial human investigations, such that the safety of the exposed subjects is protected, and the maximum amount of data available is obtained from a

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limited number of human exposures. While all of the data that exists is taken into account at this point in the process, the major emphasis is placed on the *in vivo* pharmacokinetics, and an approach called allometric scaling is a valuable tool in this context.

15.2 Allometric scaling

Allometry is the study of the relationships between size and shape. Allometric scaling in biology assumes that because most mammals have similar shapes and utilize the same physiological processes there must be some definable relationship between these processes in different mammalian species. For example, on average, a mammal's heart beats approximately four times for every breath taken no matter how large the animal. Note it is the *ratio* that is constant and this is so because both the breathing rate, BR, and heart rate HR, can be scaled to body weight, W:

$$BR = 0.169W^{-0.28} \tag{15.1}$$

$$HR = 0.0428W^{-0.28} \tag{15.2}$$

using the same power function, or exponent of -0.28. The same exponent has been used to relate life expectancy to body weight suggesting that all mammals will have the same total number of heart beats during a lifetime, although this must be a gross oversimplification. So, generally speaking, smaller animals live their lives at a faster pace. Mice, rats, dogs, monkeys and humans all absorb nutrients and drugs from their stomachs and intestines, they all deliver drug and nutrient molecules to the liver, brain, lungs and other organs, dissolved or solubilized in blood, they all metabolize chemicals in their livers, and they all excrete drugs and their metabolites in bile, urine and other fluids. However, drugs are eliminated much more rapidly in mice (which can rapidly metabolize weight-adjusted amounts of barbiturates that would kill a human being) and by different routes in rabbits and dogs (some rabbits can consume belladonna – deadly nightshade – with impunity without the cardiovascular effects that can kill humans).

Allometric scaling attempts to relate differences in pharmacokinetic parameters, such as systemic clearance (CL), volume of distribution (V) and elimination half-life ($t_{1/2}$) to differences in body weight between species. Equations 15.1 and 15.2 can be written in a general form:

$$Y = aW^b \tag{15.3}$$

where Y is the physiological or pharmacological function, W is body weight, b is the 'allometric exponent' and a is the 'allometric coefficient'. Relatively little use is made of a. Logarithmic transformation of Equation 15.3 gives:

$$\log Y = \log a + b \log W \tag{15.4}$$

showing that a graph of log Y versus log W is a straight line with a slope b and intercept log a. In many cases the slope is positive between 0 and unity, but it can be 0 and it can be negative. Thus:

- A slope of zero indicates that the parameter is the same across the species (e.g. number of eyes).
- A slope of unity indicates that the parameter increases in direct proportion to body weight.
- A positive slope between 0 and unity indicates that the parameter increases with body weight but not in direct proportion to body weight.
- A negative slope indicates that the parameter decreases with increases in body weight.

Body surface area has been related to body weight by the formula:

$$\text{surface area} = 0.1\, W^{0.67} \tag{15.5}$$

and log–log transformations give the expected straight line (Figure 15.1). Drugs, particularly cytotoxic agents used in cancer treatment, are often dosed according to surface area, or in paediatric medicine. If Equation 15.5 is used then one is actually scaling to body weight. More complex formulae for dosage adjustment in human beings usually include height as well as weight.

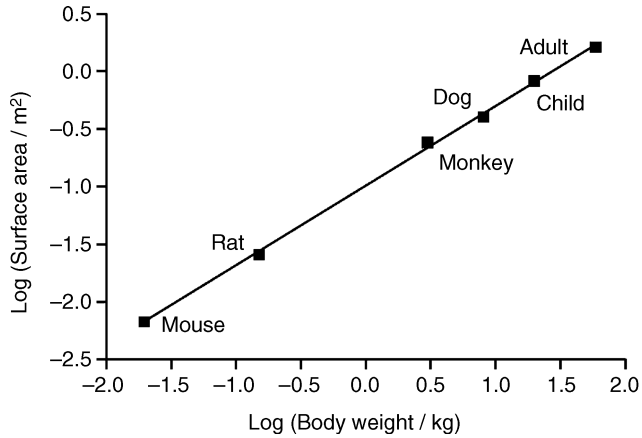

Figure 15.1 Allometric relationship between body surface area and species body weight. (Redrawn from Curry *et al.*, 2007.)

Table 15.1 shows a number of allometric exponents for some physiological and pharmacokinetic phenomena. Drug half-life, systemic clearance of drugs *in vivo*, renal clearance of drugs, and apparent volume of distribution of drugs all increase with body weight, but not proportionally. The rate constant of the terminal phase of elimination, being inversely related to half-life, decreases with body weight, and has a negative exponent.

Table 15.1 Some examples of allometric relationships

Parameter	Power
Heart rate	−0.28
Rate constant of elimination (λ_z)	−0.24
Terminal half-life	0.24
Systematic clearance *in vivo*	0.70
Renal clearance (of drugs)	0.78
Apparent volume of distribution	0.92

The exponent of the apparent volume of distribution is often close to 1, showing as might be expected, that this parameter correlates directly with body weight. Exponents for clearance are often approximately 0.75 whilst those for elimination half-life are about −0.25. This arises because:

$$t_{1/2} = 0.693\,\frac{V}{CL} \tag{4.7}$$

For volume of distribution, Equation 15.3 becomes:

$$V = aW^1 \tag{15.6}$$

and for clearance it is:

$$CL = a'W^{0.75} \tag{15.7}$$

so substitution into Equation 15.7 gives:

$$t_{1/2} = 0.693\frac{aW^1}{a'W^{0.75}} \tag{15.8}$$

Simplifying:

$$t_{1/2} = 0.693\frac{aW^{(1-0.75)}}{a'} = 0.693\frac{aW^{0.25}}{a'} \tag{15.9}$$

Combining, a, a' and 0.693 into a new allometric coefficient, A, Equation 15.9 can be written:

$$t_{1/2} = AW^{0.25} \tag{15.10}$$

which explains why the sum of the exponents for clearance and half-life should equal 1, provided of course that the allomeric exponent for volume of distribution is 1.

Clearly, a research team interested in predicting the half-life of a new drug in humans can obtain data in a variety of species, determine the allometric exponent, and calculate the expected half-life in humans. This will permit the adequate design of a Phase I experiment from the point of view of blood sampling times and overall duration of the experiment. It does not determine the safe dose; the initial dosing level is chosen after multiple-dose safety studies in two mammalian species.

Figure 15.2 shows a set of data of this kind for bosentan, an endothelin receptor antagonist for pulmonary hypertension. It is surprisingly difficult to find data of this kind in the public domain, although it exists

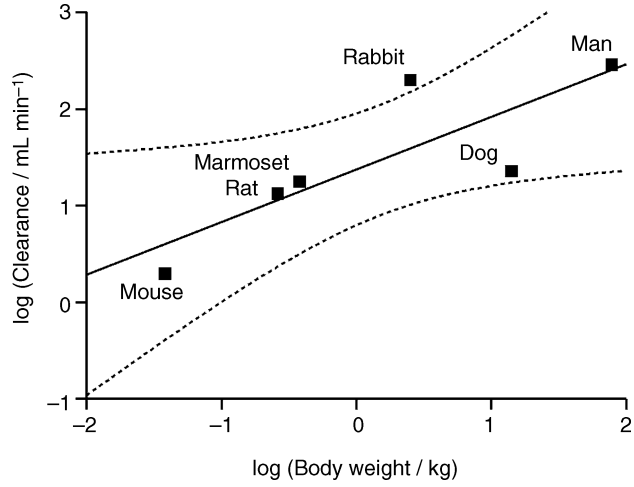

Figure 15.2 Allometric plot showing the relationship between systemic clearance and body weight in six species. The slope of line is the allometric exponent = 0.54. The broken lines represent the 95% confidence limits.

in plentiful supply in the files of research-based industry. In this case, data were collected in five species of laboratory animals. From these data, it would have been predicted that the total body clearance in humans would be in the region of 3 mL min^{-1}. The figure shows that *CL* recorded in humans was somewhat lower, at approximately 2.2 mL min^{-1}, but this would be considered a good result. Note that log–log plotting can give a false sense of validity to the prediction. The 95% confidence intervals of Figure 15.2 show that at high and low body weights the 95% intervals encompass more than 2 log units, that is more than two orders of magnitude in the parameter, *CL*. Regression analysis of the logarithmic values is akin to weighting the original data $1/x^2$ (Section 5.2.2.1).

15.2.1 Refinements to allometric scaling

15.2.1.1 Effect of neoteny

It has been proposed that through some evolutionary adaptation, human beings have an advantage over other mammals, resulting in increased longevity (modern medicine being excluded presumably). A larger brain and reduced metabolic rate (i.e. reduced mixed-function oxidase concentrations), have been suggested as possible reasons. Brain weight (*B*) has been used to compute a maximum life span potential (*MLP*):

$$MLP = a(W^b)(B^c) \tag{15.11}$$

where $a = 185.4$, $b = -0.225$ and $c = 0.636$. Using *MLP* rather than chronological time improves the prediction of phenytoin clearance in man (Figure 15.3). A modification of Equation 15.11 in which *MLP* is replaced by *CL* has been used with drugs with low hepatic clearances, the assumption being that inclusion of a factor for brain weight is also a correction for low mixed-function oxygenase activity.

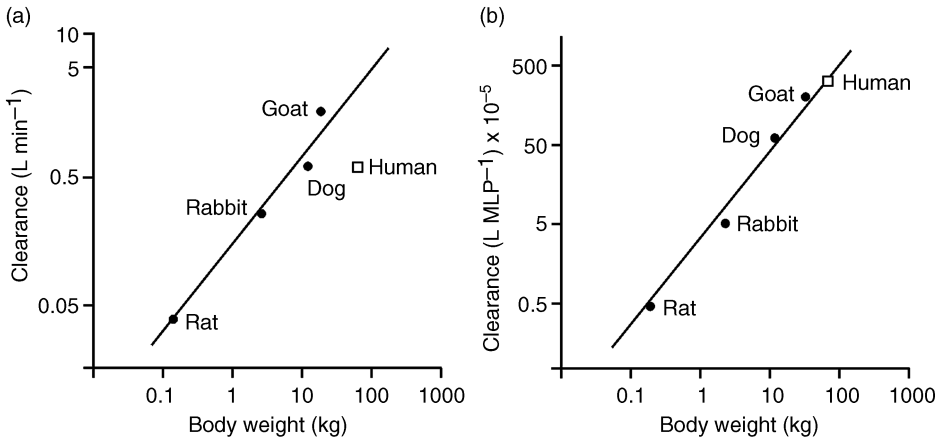

Figure 15.3 Comparison of allometric scaling of phenytoin clearance (a) using chronological time (b) using maximum life-span potential (MLP). See text for details. (Redrawn from the data of Campbell and Ings, 1988.)

15.2.1.2 Pharmacokinetic time

Just as it is possible to use maximum life span potential as a unit of time to calculate pharmacokinetic parameters, such as clearance (Figure 15.3) there is no reason why something analogous to *MLP* cannot be used as a scale for time. Thus, when comparing pharmacokinetic results from different species both dose and time would be scaled as appropriate. In their studies with methotrexate, Dedrick *et al*. (1970), converted

chronological time into 'pharmacokinetic time' by dividing time by $W^{0.25}$. This has become known as the *Elementary Dedrick* plot where:

$$x\text{-}axis\ units = \frac{time}{W^{(1-b)}} \tag{15.12}$$

and the concentration is normalized for dose and body weight:

$$y\text{-}axis\ units = \frac{concentration}{dose/W} \tag{15.13}$$

In Equation 15.12, b is the allometric exponent for clearance whilst the exponent for volume of distribution is assumed to be 1 (see the derivation of Equation 15.10). No such assumption is made in the *Complex Dedrick* plot, where the time axis units are:

$$x\text{-}axis\ units = \frac{time}{W^{(c-b)}} \tag{15.14}$$

c being the exponent for volume of distribution. New terms, *kallynochrons* and *apolysichrons* have been introduced for the time units of elementary and complex plots, respectively. *Dienetichrons* include *MLP* so that the time axis units are:

$$x\text{-}axis\ units = \frac{time}{W^{(c-b)}} \frac{1}{MLP} \tag{15.15}$$

A complex Dedrick plot for an example of rat and human concentration data is illustrated in Figure 15.4. This graph reflects data for an experimental neuroprotective agent (AR-R 15896) that is presented in more detail elsewhere (Curry *et al*., 2007). The axes are normalized concentration, and apolysichrons from Equation 15.13. The close proximity of the two graphing lines illustrates the power of this intellectually-stimulating mathematical approach to data analysis.

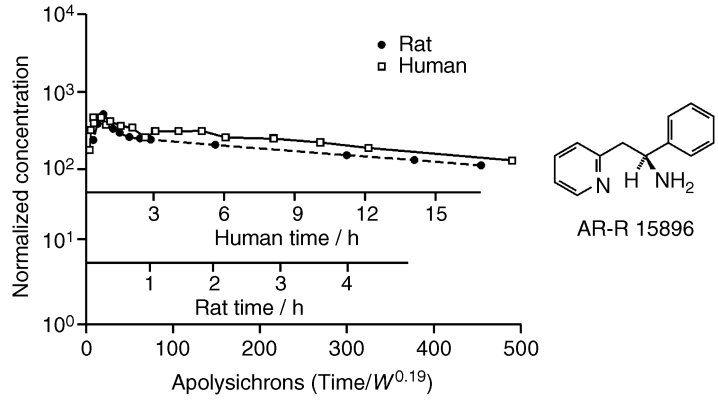

Figure 15.4 Complex Dedrick plot of rat and human data. (Redrawn from Curry *et al.*, 2007.)

However, simple allometric scaling, using Equation 15.3, also proved to be useful in this case in predicting human pharmacokinetic parameters from rat data (Table 15.2). Values of b were assigned for clearance (0.75), half-life (0.25) and volume of distribution (0.938) . The intercept functions a, determined for each parameter from rat data, were then used to compute the pharmacokinetic parameters for a 70 kg man. Three methods

were used to estimate the elimination half-life:

$$t_{1/2\,\text{human}} = \frac{0.693\,V}{CL} \tag{15.16}$$

$$t_{1/2\,\text{human}} = t_{1/2\,\text{rat}}\left(\frac{W_{\text{human}}}{W_{\text{rat}}}\right)^{y-x} \tag{15.17}$$

where y is the exponent for volume of distribution (0.938) and x is the exponent for clearance (0.75). Thus, $y - x = 0.188$.

$$t_{1/2\,\text{human}} = \log a + \log W_{\text{human}} \tag{15.18}$$

Table 15.2 Predicted and actual pharmacokinetic parameters (from Curry *et al*, 2007)

Pharmacokinetic parameter	Predicted	Actual
Clearance ($\text{L h}^{-1}\,\text{kg}^{-1}$)	0.138	0.128
Half-life[a] (h)	14.5	13.6
V_1 (L kg^{-1})	1.01	1.02
V_{ss} (L kg^{-1})	2.14	2.11

[a] Average of three values calculated using Equations 15.16, 15.17 and 15.18.

15.2.2 Practical aspects of allometry

In general, the following holds true:

- Physical phenomena (e.g. tissue distribution measured by apparent volume of distribution) are relatively similar across species (exponent ~ 0.95).
- Processes dominated by physiological phenomena lead some drug related allometric relationships to reflect physiological allometric relationships (e.g. for drug metabolism when controlled by blood flow).
- Half-life and elimination rate constant tend to reflect a complex of factors, both physical and biological, including heart rate and tissue distribution, but also intrinsic metabolic clearance.
- Whole body clearance reflects a hybrid of factors in its allometric scaling, both physical and biological.
- Because *in vitro* clearance represents only a proportion of *in vivo* clearance, it will be expected to have allometric exponents that reflect isolated processes; allometric scaling is less commonly applied to *in vitro* data.

The scientific community is often asked about monkeys in this context. Intuitively, close analogies with humans might be expected. This is a vast topic, but while many scientists can cite individual examples of excellent predictions, as a general rule, data from monkeys are no better and no worse than data from mice, rats and dogs in the process of making predictions for humans. These data obviously can and do make a valuable contribution to the process of allometry, but they do no more than that. For example, some years ago, Chiou and Buehler (2002) showed that for a large number of compounds, by plotting log–log correlation diagrams of human half-life against half-life in monkeys, rats and dogs, success rates for human predictions were approximately the same for monkeys (87%) and rats (83%), and lower (72%) for dogs. One of the reasons why monkeys fail to give a more robust prediction for humans is that, like humans, and for that matter most dogs, they are heterogeneous – they are not as pure bred as are laboratory rats.

Thus, as a general observation, when following the traditional Phase I concept of choosing a single compound on the basis of preclinical data and preparing it for initial human testing, the investigator will, in all probability, want to predict the pharmacokinetic properties of the compound in human beings from animal

data. This can be done quite well from allometric scaling if you first determine CL_{int}, CL, $t_{1/2}$, V, etc. in four to five other species. There are also some convenient rules of thumb based on experience:

- Human *in vivo* pharmacokinetic predictions can be obtained using the simple allometric scaling relationships of
  - CL (human) $= 40\,CL$ (rat) ($L\,h^{-1}$),
  - V_{ss} (human) $= 200\,V_{ss}$ (rat) (L),
  - $t_{1/2}$ (human) $= 4\,t_{1/2}$ (rat).

  (Note that units for CL and V_{ss} are not adjusted on a per kg basis in this rule.)
- Whole body clearance from plasma level decay (CL) is more predictable for compounds eliminated by renal (or biliary) excretion than by metabolism, because renal elimination has a larger physical (diffusion) component in its mechanisms.
- Drugs that are largely excreted unchanged will tend to have half-life values in humans three to four times those in rats; drugs that are largely dependent on metabolism for their elimination will have half-life values in humans that are 8–12 times those in rats.

15.3 Dose-ranging versus microdosing studies

The reality of choosing the dose for an initial human exposure is, however, a little different, because, as stated earlier, it is based on safety studies, not on pharmacokinetics. The first human exposure is likely to be made with a dose 1/50 or 1/100 of the lowest dose showing any effect, desired or undesired, in the laboratory animals, on a $mg\,kg^{-1}$ basis. No effect is anticipated or sought in the human subjects.

Commonly, this dose is below the limit of analytical detection in plasma, preventing any pharmacokinetic studies with these initial doses. As the investigation proceeds, the dose is increased, slowly, step by step until detection in plasma becomes possible, and until pharmacological effects of significance, if any, occur. This is a slow, expensive process, with more exposures than is desirable in an expeditious program. Consequently, this leads us into consideration of the modern idea and practice of human microdosing.

Human microdosing is driven by developments in bioanalysis. Thanks to the use of such techniques as positron emission tomography (PET) and accelerator mass spectrometry (AMS) it is increasingly possible to administer minute doses of new drugs and to study their pharmacokinetic properties. These doses are subthreshold in terms of interest by the medicine control agencies in them as potentially toxic. FDA and other such agencies permit the use of accelerated protocols for human microdosing, with what is called an Exploratory IND, after single doses followed by recovery studies in animals, rather than after one month of continuous exposure followed by necropsy.

This has some useful side effects. It reduces the use of animals, facilitates earlier human exposure, involves less CMC (chemistry and manufacturing controls) requirements, and makes it possible to conduct Phase I trials with less drug material. It becomes possible to compare multiple compounds in Phase I, and to choose the best compound for clinical trials from among several candidates on the basis of data in the ultimate test subject, the human. No longer are investigators optimizing new drugs for rats *per se*. Another objective of human microdosing (which is sometimes called Phase 0), can be to establish that the half-life of the drug is apparently the same over a broad range of doses, providing an early screening mechanism that can reduce the risk of a drug with the non-linear pharmacokinetic characteristics of phenytoin, reaching clinical trials, although this does not guarantee non-linearity at the larger doses likely to be needed in the clinic. Another valuable use of AMS technology is the measurement of concentrations of ^{14}C-labelled drug administered in very small doses intravenously, concurrently with conventional oral doses. Non-labelled drug is assayed by such techniques as high pressure liquid chromatography (HPLC) permitting calculation of absolute bioavailability using simultaneous i.v. and oral doses. However, PET and AMS, while reducing the ancillary costs in a variety of different ways, lead to individual experiments being very expensive.

15.4 Statistical approaches

Another developing idea is the use of statistical approaches in the dose and plasma concentration escalation process during Phase I. Whitehead and his colleagues (2007) have demonstrated how Bayesian decision-making procedures can be applied in choosing the dose to be used in the second and subsequent dosage rounds of the study. Bivariate observations are made of undesirable events and signs of therapeutic benefit (if any). Logistic regression models are used to study the two responses, which are assumed to take a binary form. Accurate definition of the therapeutic window is sought, and with each stage, optimal treatment of the subsequent cohort of subjects is achieved. Thus, using the first set of data for calibration, the probability of a particular response occurring at a particular dose, or plasma drug concentration, is calculated, and that dose, rather than some arbitrarily chosen dose in a predetermined ladder of small-step minor escalations is chosen. At its best, this process could apparently lead to one inactive dose, two individual doses focused separately on desired and undesired effects, and one dose focused on both types of effect – a total of four doses in all – being used to characterize the drug, thus achieving an accurate estimate of the therapeutic window at minimal cost. This has so far only been applied to the search for new anti-cancer drugs. Phase I studies in this field must incorporate a hope of therapeutic benefit and a tolerance of adverse effects not experienced in most other fields of medicine.

15.5 Translational science

'Translational science' is new terminology relevant to the transition from animals to man. It seeks to define a scientific track, not unlike that existing today, but making extensive use of biomarkers, and an administrative track of providing government funding for early clinical development of academic discoveries in academic centres. Biomarkers come in various shapes and sizes. They include imaging (e.g. PET), and assays of biochemical and physiological function including disease, with heavy emphasis on DNA-based gene sequence tests and proteomics. A reasonable objective would be discovery of *in vitro* biomarkers that predict the pharmacokinetic properties of drugs *in vivo* in humans with the target disease. Then, perhaps, it would be possible to define the properties of the drug that we want in human beings, and back-calculate through reverse allometric scaling (prediction of the desired properties in rats from the specification for man and reverse scale-up (from *in vivo* back to *in vitro*), permitting the choice of the right drug from candidate compounds, in the preclinical stage of the process, for the target humans from *in vitro* data. As yet, we are not there.

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16

Peptides and Other Biological Molecules

16.1 Introduction

This chapter is about a collection of important compounds that are characterized as being basically 'biological' in origin. However, no single descriptive term applies to all of them. While many are new, representing the impact of biotechnology on modern medicine, not all of them are new – insulin has been established for several generations as a biological product used in therapeutics. In contrast, monoclonal antibody drugs are very new. Neither are all the drugs in this chapter macromolecules – they include small peptide hormones used as drugs as well as large proteins. They are not all peptides or proteins, as they include a polysaccharide – heparin. They do not include all of the drugs containing peptide bonds, as the 'pril' antihypertensive drugs (such as lisinopril) contain peptide bonds. And they do not include all of the endogenous compounds used as therapeutic agents – there will be no further references to the ions, amino acids, sugars, or vitamins that are both essential nutrients and therapeutic agents, or to non-peptide/polysaccharide small molecule hormones. Thus the most useful classification is as 'compounds of biological origin, either fully or in part,' which serves little purpose! Table 16.1 lists the types of molecule in this category, with some examples and some of their properties. These compounds as a group show all of the properties to be found in 'small molecule' drugs, and more. Some examples of special interest, exemplifying recurring themes, are the subject of Section 16.6.

16.2 Chemical principles

The compounds in this category range in relative molecular mass from approximately 200 (simple dipeptides) up to 30,000 (some of the proteins). They are mostly polar, but some non-polar compounds that can be administered orally are included. Larger molecules behave differently from the relatively low molecular mass (<500), lipophilic, weak electrolyte drugs that have been produced in large numbers in medicinal chemistry laboratories over the last 50–60 years. Peptide and protein drugs contain amide links, and although many of these drugs may contain amine and carboxylic acid groups, as do the smaller molecules, they do not, in the main, fit Lipinski's 'Rules of Five', which for a molecule to be suitable as a drug state that it should have:

- A molecular weight less than 500 g mol^{-1}.
- No more than five hydrogen-bond donors (sum of –OHs and –NHs),
- Log $P \leq 5$,
- No more than 10 hydrogen-bond acceptors (sum of Ns and Os).

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Table 16.1 Examples of peptides, proteins and polysaccharides used as drugs

Group	Example	Route	Uses	Comments
Hormones	Vasopressin and desmopressin	i.n.	Control of urine in diabetes insipidus	Nonapeptides given by any parenteral route; first intranasal drugs
	Insulin	s.c.	Diabetes mellitus	Original model for radioimmunoassay; polypeptide hormone of 51 amino acids
	Oxytocin	i.v.	Induction of labour	Nonapeptide, given by any parenteral route
	Human growth hormone	i.v.	Promoting growth in children	Somatrophin – single polypeptide chain of 191 amino acids
Enzymes	Digestive enzymes	Oral	Promoting digestion of food	Long-established home medicine agent
	Pancreatic enzymes	Oral	Promoting digestion of food	Enteric coated granules – used in cystic fibrosis
	Thrombolytic agents (e.g.streptokinase)	i.v.	Dissolving blood clots	Degrades fibrin and breaks up thrombus
	Asparaginase	i.v.	Leukaemia	Can be PEGylated
Synthetic peptides	Capaxone	s.c	Suppression of autoimmune encephalomyelitis, MS	Mixture of synthetic tetrapeptides
Botanical peptides	Ciclosporin	Oral	Preventing transplant rejection	Natural fungal cyclic peptide – non-polar and P-450 substrate
Protein bound drugs	Paclitaxel/protein complex	i.v./i.m.	Cancer treatment	Paclitaxel is highly insoluble, and must be solubilized to facilitate injection
Polysaccharides	Heparin	i.v.	Anticoagulation	Complex drug used in management of risk of blood clots in several medical conditions

Proteins (natural or modified)	Interferon	i.v.	MS and other conditions	Non-glycosylated proteins with several applications; some products PEGylated.
	Erythropoietin	i.v.	Anaemia of renal failure	Product of recombinant technology – standardized in international units
	Interleukin	i.v.	Prevention of abortion and/or miscarriage	$Rh_o(D)$ immune globulin
Monoclonal antibodies	Trastuzumab	i.v.	Breast cancer (HER2 type)	Model for principles of monoclonal antibody drug production
	Rituximab	i.v.	Non-Hodgkin's lymphoma	Targets phosphoprotein CD 52 on T- and B-lymphocytes
	Adalimumab	s.c	Crohn's disease; and rheumatoid arthritis	IgG_1 monoclonal antibody
	Infliximab	i.v.	Crohn's disease	Inhibits TNF-α
	Basiliximab	i.v.	Acute rejection of kidney transplants	Inhibits IL-2 on activated T-cells

i.m., intramuscular; i.n., intranasal; i.v., intravenous; s.c., subcutaneous; MS, multiple sclerosis; IL-2, interleukin-2.

The above are the specifications for successful oral absorption, and it is recognized that they are irrelevant to drugs that are substrates for biological transporters and/or are polar biological molecules.

Although many of these molecules are biochemically important endogenous compounds, they may be administered in pharmacological doses for therapeutic purposes. Some of them are 'humanized' proteins (Section 1.1.1), that is they are manufactured to resemble the endogenous human protein as closely as possible. Historically, animal proteins of the same type, but with small, relatively unimportant, differences in amino acid sequence, have been used as therapeutic agents; an obvious example being the use of porcine rather than human insulin for the control of diabetes mellitus. More recently, the biotechnology industry has been able to manufacture 'recombinant' versions of these proteins, with the human arrangement of the amino acids, after isolation of the genes responsible for their synthesis. These proteins for human use are manufactured outside the human body, often in suspensions of bacteria such as *Escherichia coli* (Box 16.1).

Box 16.1 Stages in the production of recombinant DNA and its use to manufacture a human polypeptide or protein (e.g. human insulin)

- Identify the human genes required for synthesis of the polypeptide/protein *in vivo*.
- 'Cut' DNA with endonucleases to liberate the genes required.
- Use 'vectors' (e.g. viruses) to transfer these desired DNA fragments into host cells (e.g. bacteria) to be 'sliced' on to plasmid DNA – plasmids are small circular pieces of DNA lying outside the main bacterial chromosome – they themselves are opened up using endonucleases, then re-closed to include the gene that was transferred – other activity of the plasmid is lost.
- This creates a 'hybrid plasmid'.
- Mix the hybrid plasmid with the bacterial host cells to form 'transformed cells'.
- Separate transformed cells from others and grow in cultures.
- Create a fermentation chamber containing cultured cells and amino acids as a 'factory' for production of the polypeptide/protein.
- Separate and purify the human polypeptide/protein.
- Formulate pharmaceutically for human use (e.g. 'Humulin,' replacing animal sourced insulin that was not identical with human insulin).

These drugs include the monoclonal antibody products of the modern biotechnology industry that are revolutionizing medicine in both scientific and financial ways. The stages in a typical production of a monoclonal antibody are listed in Box 16.2.

Box 16.2 Stages in the production of a monoclonal antibody for use against human disease

- Identify an antigenic component of a disease-inducing protein (e.g. the HER2 extracellular domain of one of the breast cancer-inducing proteins).
- Separate the antigen, and inject into mice – separate spleen cells that have developed antibodies.
- Fuse the spleen cells containing the antibodies with myeloma cells, creating an immortal hybridoma; these cells now synthesize purines by means of the 'salvage' pathway.
- Grow clones of these cells in microtitre wells, manufacturing greater quantities of the cells that developed the original antigen.
- Scale up using the peritoneal cavities of mice, or a culture medium (preferred) in a fermentation chamber.
- Separate and purify the antibodies.
- Formulate pharmaceutically for human use (e.g. trastuzumab for the treatment of HER2 breast cancer).

Thus, many, but not all, of the chemical principles documented in earlier chapters do not apply with these compounds. Some of these compounds are sometimes described as 'biological response modifiers'.

16.2.1 PEGylation

This word is used to describe the process by which a polyethylene glycol (PEG) polymer chain is added to another molecule, commonly a therapeutic protein. It is also applicable to peptides, and of course, therefore, to antibody drugs. The PEG is bonded covalently to a region of the molecule not involved in the pharmacological action, and although it increases the molecular weight, this is not significant with such large molecules. This has several beneficial results, including masking of the protein from the host's immune system, increasing size in solution, which can reduce renal clearance, and adding water solubility to hydrophobic drugs and proteins. It can change the conformation, and electrostatic binding of the protein.

Thus apart from improving drug solubility, PEGylation can reduce dose frequency, extend circulating life in the body, and increase drug stability by enhanced protection from proteolytic degradation. This has found greatest application with interferon drugs, L-asparaginase, and recombinant methionyl human granulocyte colony-stimulating factor. It has also been used in formation of PEGylated liposomes containing doxorubicin.

16.3 Assay methods

While it is possible to achieve chromatographic separations of peptides and other biological molecules, these drugs often lack sufficient aromatic structures to facilitate their ultraviolet or fluorescence detection (although some successes have been achieved with electrochemical detection). Consequently, these compounds often need derivatizing before they can be quantified by ultraviolet or fluorescence detection. As this introduces a step into the assay that is difficult to control, less conventional methods of analysis may be needed. Methods commonly employed with these compounds are:

- Chromatographic separation with derivatization to confer absorbance or fluorescence properties; a variant on this can be radioactive derivatization, quantitation being dependent on assessment of the amount of radioactivity incorporated.
- Radioimmunoassay (RIA) using the drug as the antigen to which antibodies are developed. Competition between the drug and radioactively-labelled drug is used for quantification. The very first published application of RIA was for the polypeptide drug, insulin.
- Enzyme-linked immunosorbent assay (ELISA), an antibody binding approach of broad applicability to proteins in particular, and can be calibrated in mass per volume or 'units' per volume terms if the molecular mass of the analyte is not clearly known.
- Biological assays measure effects to indicate the drug concentration present by comparison with appropriate calibration experiments. For example, heparin can be assayed on the basis of the amount of hexadimethrine needed to neutralize heparin activity using thrombin-induced coagulation as an endpoint.
- Imaging assays can be used for monoclonal antibodies. An example is trastuzumab which has been studied by adding 'a new trifunctional chelating agent containing a biotin residue and a radiometal chelation moiety,' known as ^{111}In-1033-BR96 to the molecule and studying the distribution of the radioactivity. Much as PEGylation does not change the pharmacological properties of a protein, combination with this reagent, even though it creates a different molecule, with different physical and chemical properties, is considered not to change the disposition and fate of the drug.

- Another imaging method, also used with trastuzumab, has involved simultaneous use of ^{90}Y-trastuzumab (a β^--emitter, for tissue uptake studies in dissected animals) and ^{86}Y-trastuzumab (a positron emitter) for non-invasive live imaging work using PET scanning.

16.4 Pharmacokinetic processes

16.4.1 Administration and dosage

In some cases, compounds in this category are not only standardized, but also dosed in terms of internationally accepted units, based on biological potency, rather than on the basis of mass. This is because different batches of the drug may vary in chemical constitution, while retaining the required potency. However, standardization and measurement of dose by mass is sought whenever possible.

Compounds in this category are mostly administered by injection because of their lack of potential for absorption from the gastrointestinal tract. This results from instability in the acid of the stomach, or in the presence of peptidases in the intestines and/or the liver during first passage through that organ, or from their relative polarity, molecular size and charges resulting in too little transcellular diffusion. Whether i.v., i.m. or s.c. routes of administration are used will depend on how fast the pharmacological effect is wanted, and also on convenience (insulin is given i.m. or s.c because that provides the most suitable speed of onset of effect, and because the patient can conveniently self-administer it). There may also be a component of procedural need. Intravenous infusions of trastuzumab are supervised by clinic staff, in case of dose-related emergencies such as an allergic response.

In contrast to the examples in the previous paragraph, digestive enzymes given orally for local effects in the gastrointestinal tract survive exposure to acid and to peptidases, as does botulinum toxin and, presumably, polio vaccine. Despite being a peptide, ciclosporin is lipophilic and very poorly soluble in water. It is resistant to acid and peptidases, and is given orally. Peptides in general, and vasopressin in particular, can be given by the intranasal route for systemic action. Insulin has been studied for decades, and chemically modified, in the hope of finding an effective preparation that can be given i.n., by inhalation, by means of suppositories, through the skin, or orally, with little, if any, impact on its use by patients.

The lymphatic system has a significant role in the absorption of proteins. This system is a network of vessels draining from the many tissues of the body into the left and right subclavian veins. Although it is a one-way system, together with the cardiovascular system, it serves to deliver nutrients and oxygen to tissues, and to remove metabolic waste products from them. Simply put, the lymphatic system returns fluid (lymph) and its contents to the circulatory system from intercellular tissue spaces. Because drainage from the intestinal tissue is *towards* the venous system, materials from the intestine become dissolved in lymph and absorbed into the cardiovascular system.

The lymphatic system by-passes the portal system of the liver, and it is important to the absorption of dietary lipids. It also undoubtedly plays a part in the absorption of all orally-administered drugs, although with so much potential for absorption into the cardiovascular system via the mesenteric blood capillaries and the hepatic portal system, this contribution is, in many cases, quite small. However, and in particular, part of the lymphatic system is made up of thin-walled lymphatic capillaries within the tissues of the gastrointestinal tract. These vessels are more permeable than are blood capillaries. Also, the lymphatic vessels are well adapted for the movement of large molecules, particularly proteins, and this includes any peptide and protein drugs that survive the acidity of the stomach and the proteases of the intestine. For example, polypeptides of size greater than 20,000 g mol^{-1} are able to reach the blood via the lymphatic system, even though they are unable to traverse blood capillary membranes. Nevertheless, movement through lymphatics is relatively slow, and absorption in this way is rarely sought in the search for absorption of new drugs of any type.

The lymphatic system also plays a significant part in the absorption of subcutaneously-administered drugs of all kinds.

16.4.2 Bioequivalence

Concepts of bioequivalence have to be applied carefully with these drugs. In some cases, chemical properties and methods of analysis allow standards similar to those applied to small molecules to be applied. However, even different batches within the processes of a single manufacturer can vary with protein drugs, let alone between manufacturers, and regulatory authorities permit a rational protocol of the best available practices in manufacturing, chemical analysis, and bioassay, to be used in ensuring batch-to-batch and manufacturer-to-manufacturer consistency.

16.4.3 Distribution

Because most compounds in this category are polar, their apparent volumes of distribution tend to be low; often similar to the volume of plasma, or at the highest, a little over $1\,L\,kg^{-1}$, indicating occurrence of some specific tissue uptake. Some typical apparent volume of distribution values are: $0.058\,L\,kg^{-1}$ (heparin); $0.08\,L\,kg^{-1}$ (streptokinase); $0.055\,L\,kg^{-1}$ (L-asparaginase); and $1.3\,L\,kg^{-1}$ (ciclosporin).

Distribution can be very specific. After intranasal administration, cholecystokinin is found only at sites in the stomach associated with the neuronal pathways to the brain that are connected with feelings of satiety. Trastuzumab shows specific localization in tumour tissue, because it forms a complex with the extracellular domain of HER-2.

16.4.4 Metabolism

Peptides are mostly metabolized by peptidases in the liver and kidney. Half-life values vary from a few minutes to as much as 28 days, depending on the ease with which the peptide bonds interact with the peptidase active sites. Just exactly why particular peptides and proteins vary so much in their sensitivity to proteases has been the subject of a considerable amount of research. Proteases are broadly classified into endopeptidases, which cleave the internal peptide bonds in substrates, and exopeptidases, which cleave the terminal peptide bonds. Exopeptidases can be further subdivided into aminopeptidases and carboxypeptidases. There is a further subclassification based on models applicable to describing the physical nature of the interactions between the substrate and the active site of the enzyme. Proteases can also be classified as aspartic proteases, cysteine proteases, metalloproteases, serine proteases, and threonine proteases, depending on the nature of the active site. Whether or not a particular substrate interacts with a particular active site will depend on the degree to which the peptide bond(s) in the substrate lack(s) hindrance to a close fit, and whether or not the substrate and enzyme are to be found in close proximity in the same body fluids. Thus, small, straight chain peptides have little chance of survival in the presence of intestinal proteases, while the peptide bonds of ciclosporin are so hindered that this drug barely behaves like a peptide, and is primarily metabolized by cytochrome P-450 enzymes. The therapeutic proteins that are given by intravenous injection do not come into contact with intestinal proteases. Whenever possible, protein drugs will be designed to resist attack by proteases.

16.4.5 Excretion

The standard concepts apply (Section 3.3). In particular, insulin ($M_r \sim 6{,}000$) is filtered at the glomerulus and is then metabolized to its amino acids by enzymes in the brush border of the renal tubular lumen, and/or within luminal cells.

Superoxide dismutase shows saturation of renal excretion as a function of plasma concentration, a phenomenon that is unusual amongst drugs, but familiar to investigators of transport maxima in the kidney with endogenous compounds, as occurs with glucose reabsorption.

16.5 Plasma kinetics and pharmacodynamics

A variety of phenomena familiar to pharmacokineticists is seen with compounds in this category. Thus:

- Many quite conventional studies are conducted: measurement of half-life values, protein binding, growth and decay of plasma concentration curves, renal excretion of metabolites, etc.
- Heparin shows dose-dependent kinetics; this causes difficulty in controlling dosing.
- Trastuzumab also shows dose-dependent kinetics. This is so prominent that it has been turned to advantage in devising optimum dosage regimens for this drug.
- Several of the molecules in this class show bi- or tri-exponential plasma level decay after i.v. bolus or infusion doses. For example, trastuzumab data were fitted to a 'two-compartment linear model,' even though its apparent volume of distribution was just 2.95 L (~plasma volume).
- A close correlation between kinetics and effect does not always occur. For example, erythropoietin has a half-life after i.v. injection of 10 hours, but the response can be delayed for up to 2–6 weeks. Desmopressin shows a biphasic plasma concentration decay after i.v. dosing, with the half-life values of 6.5–9 minutes and 30–117 minutes, but its antidiuretic effect lasts for 6–20 hours. Insulin has a half-life of 5–6 minutes, and its time-course of effect is controlled by its pharmaceutical formulation. Somatotrophin (HGH) has a half-life of 20–30 minutes, although its therapeutic value is measured over a period of months or years.
- It would appear that PK/PD models with effect compartments, or invoking principles similar to those applicable with warfarin are most appropriate with drugs in this class.

16.6 Examples of particular interest

16.6.1 Cholecystokinins

Cholecystokinins are a family of hormones produced by post-translational metabolism of preprocholecystokinin. Individual products are identified by the number of amino acids: for example CCK58, CCK33. CCK8 (Figure 16.1). CCK8 is not a drug *per se*, but it is of importance as a probe for the study of satiety mechanisms. After intravenous or intranasal pharmacological doses it is to be found almost exclusively in the pyloric region of the stomach wall, where there are stretch receptors which send signals to the brain via the

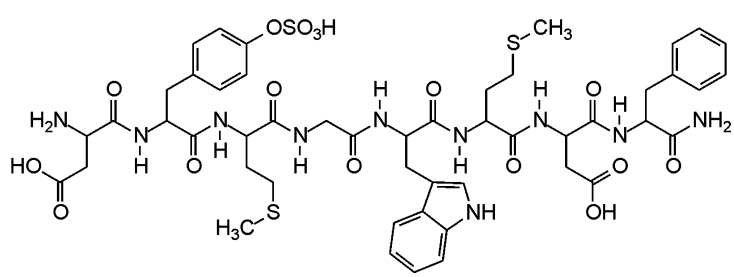

Figure 16.1 Formula of CCK-8.

vagus nerve to indicate that the stomach is full and that appetite should be shut down. This has been explored in human pharmacological investigations by Greenough *et al.* (1998) and others. Cholecystokinin has a half-life measured in minutes when incubated with kidney peptidase preparations. However, it is sufficiently stable *in vivo* for it to be possible to show a clear reduction in meal size in human volunteers given intranasal doses, thus shedding light on satiety mechanisms. There have been attempts to synthesize cholecystokinin analogues that are more stable, and have the same properties, in the search for anti-obesity drugs.

16.6.2 Ciclosporin

Ciclosporin (cyclosporine) is a cyclic polypeptide (Figure 16.2) of fungal origin, which is used as an immunosuppressant in transplant medicine. It is lipophilic and hydrophobic. It can be administered i.v., dissolved in a modified castor oil/ethanol mixture, or orally in a soft capsule when it has systemic availability of 20–50%. Soft capsules containing a micro-emulsion formulation have 10% greater availability. It has an apparent volume of distribution of 1.3 L kg^{-1}, and approximately 30 known metabolites. It is a cytochrome P-450 substrate, and so it is prone to the drug interactions common with small molecule drugs. Its half-life is 5–6 hours. It is mostly excreted as metabolites in bile and hence faeces. Its therapeutic use requires close monitoring of concentrations in plasma.

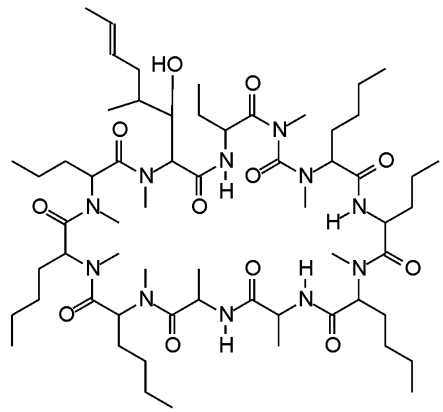

Figure 16.2 Structure of ciclosporin.

16.6.3 Heparin

Heparin (Figure 16.3) is a naturally-occurring negatively charged glycosoaminoglycan, and thus is a polymer of alternating D-glucuronic acid and *N*-acetyl-D-glucosamine residues. It is produced in the body in mast cells where it is stored in granules along with histamine. It is used as an anticoagulant, and it is obtained for

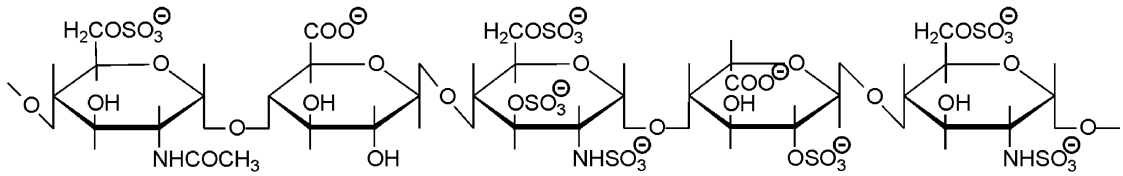

Figure 16.3 Representation of heparin. Heparin consists of repeating disaccharide units which may be sulfated to varying degrees.

medical purposes from biological sources as a by-product of the meat industry. Different batches are not consistent with each other. Its molecular weight varies from 4,000 to 30,000 g mol^{-1} depending on the degree of breakdown of its mucopolysaccharide side chains. The low molecular weight fractions and high molecular weight fractions have effects at different points in the blood clotting cascade.

Heparin is standardized and dosed in internationally recognized potency units, not in mass units, and even plasma concentration measurements use these units. It is given by i.v. injection for rapid effect, or by subcutaneous injection, when the onset time is 1–2 hours. Even with i.v. injection there is a short delay in onset time, and the effect lasts longer than would be expected from the plasma concentration–decay kinetics. This has led to suggestions that 'activation' is needed. The apparent volume of distribution is 0.058 L kg^{-1}. There is a short phase of fast decline of plasma concentrations after a bolus injection, with a half-life of approximately 5 minutes, followed by a slower phase with a half life in the range 1–5 hours. There has been extensive discussion about whether there is or is not dose-dependent decline of plasma concentrations, but the evidence favours dose dependence. Half-lives of the terminal phase of concentration decline in plasma are generally accepted to be 1, 2.5 and 5 h after doses of 100, 400 and 800 units kg^{-1} respectively, showing marked non-linearity in the elimination kinetics of the drug.

Part of the confusion over the kinetics of heparin has undoubtedly been caused by the methods of analysis. While direct chemical assay is desirable, several 'pharmacokinetic' studies have really been studies of the time course of effect, using methods described as the Lee–White clotting time, the activated clotting time, the thrombin clotting time, the anti Xa assay, and the activated thromboplastin time (APTT) assay. Also, there has been inconsistency concerning application of these assays to plasma and/or blood. The APTT assay is probably the most accepted. In this assay:

- Oxalated plasma + a partial thromboplastin time reagent + a surface activator are incubated for 3–5 minutes.
- Calcium chloride is added.
- The coagulation time is measured.

Heparin increases the coagulation time, from a baseline of 25–50 s. A plot of APTT versus heparin concentration may be linear, or log–linear depending on circumstances that are not always fully understood. The range of the assay is 0.1–0.8 units mL^{-1}, with a normal value of around 0.4, and a reasonable expectation of therapy resulting in a 50–75% increase. Thus the apparent non-linearity in kinetics may have been because of a lack of appreciation of the non-linearity of the assays used. However, there is broad acceptance now that a log–linear model is applicable to the effect of heparin, thus:

$$APTT = APTT_0 \exp(mC) \tag{16.1}$$

and as taking logarithms gives:

$$\ln(APTT) = mC + \ln(APTT_0) \tag{16.2}$$

m is the slope of the graph of $\ln(APTT)$ versus concentration, C.

Heparin plasma concentrations are considered to be best described by a Michaelis–Menten type equation in which V_{max} is measured as the maximal rate of heparin elimination (in units h^{-1}), Km is the heparin concentration at half-maximal velocity (units mL^{-1}), there is an apparent volume of distribution term, and R is the rate of heparin infusion (units h^{-1}). This aids the understanding of the relationship between time after the dose, concentration in plasma, and effect, when considered together with the equations for APTT above. The average dose is 1,400 units h^{-1} for males, and 1,100 units h^{-1} for females. V_{max} tends to be 40% greater in males than in females ($3{,}555 \pm 2{,}139$ units h^{-1} in males). Km is 0.35 units mL^{-1} – in the lower part of the normal range for useful therapeutic effect.

A scatter diagram of *APTT* versus plasma concentration of heparin in a population of patients is truly a scatter diagram, showing no meaningful between-patient correlation. Heparin is a complex drug to understand, and a difficult drug to manage in the clinic.

16.6.4 Trastuzumab

This compound is a monoclonal antibody devised by the biotechnology industry for the specific treatment of HER-2-positive breast cancer. It shows marked non-linearity in its elimination kinetics, and as the aim of therapy is to maintain trough concentrations in plasma of 20 $\mu g\,L^{-1}$ over a 25–30 week period, it has been possible to replace the originally proposed dosing regimen of a 4 $mg\,kg^{-1}$ loading dose followed by 2 $mg\,kg^{-1}$ weekly, for about 6 months, with an 8 $mg\,kg^{-1}$ loading dose followed by 6 $mg\,kg^{-1}$ every three weeks for the same time. Because the drug must be given i.v. in an outpatient clinic setting, this has resulted in a much more convenient regimen, with the number of clinic visits reduced by two-thirds. The half-life of trastuzumab is similar to that of the endogenous IgGI immunoglobulin that constitutes the backbone of the drug. Of particular interest is the fact that it forms a complex with the extracellular domain of HER-2 and the clearance of the complex is greater than that of the drug – demonstrating a rational approach to removing an unwanted residue from the body.

16.6.5 Erythropoietin

This compound is typical of the protein drugs formed using recombinant DNA techniques to imitate the normal body constituent. It consists of 193 amino acid residues and has a molecular weight of approximately 30,000 $g\,mol^{-1}$. It is heavily glycosylated, and is used to treat the anaemia of chronic renal disease. There is always a baseline erythropoietin concentration in plasma, so therapy adds 'drug' to the endogenous amount. The recombinant product is epoetin alfa, and it can be given i.v. or s.c. Its half-life is 10 hours, although its t_{max} is often said to be between 5–24 hours; the response can be delayed for 2–6 weeks in some patients – success in treatment is measured over long periods of time.

16.6.6 Vasopressin and desmopressin

Vasopressin, antiduretic hormone (ADH), is a natural nonapeptide (Figure 16.4) that acts in the body to control urine production in the process of homeostasis. Desmopressin (Figure 16.5) is a synthetic peptide

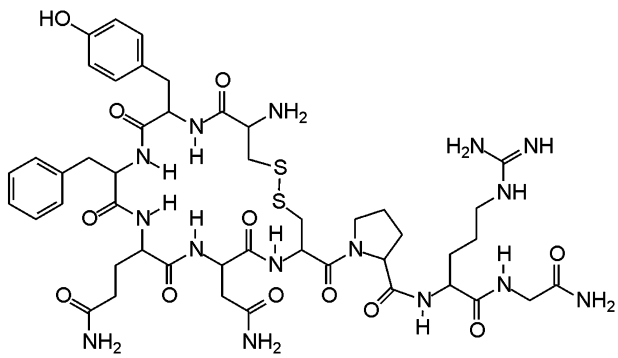

Figure 16.4 Structure of vasopressin.

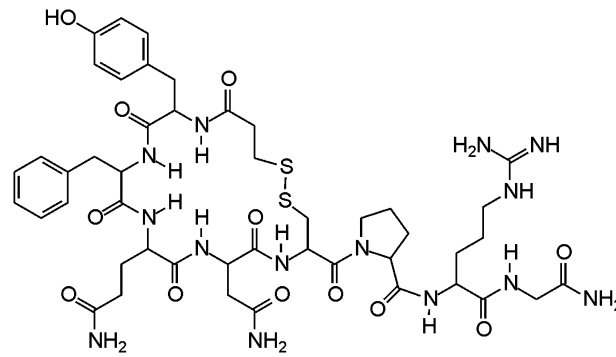

Figure 16.5 Structure of desmopressin.

analogue. Both compounds are used in pharmacological doses to control the excessive water loss of diabetes insipidus. Vasopressin has been used in this way for many years, and it was the first intranasally (i.n.) administered peptide, having been formulated as a powder which was known in the past as 'pitressin snuff'. This route of administration, while being the only feasible method for this drug, does provide a measure of control of dosing for what is a very difficult drug to manage.

16.7 Conclusion

The last 30 years have probably seen the birth of a brave new world of scientifically-conceived highly-specific 'biological' drugs. The examples quoted here are probably just the leading edge of what is to come, in the search for specificity in drug delivery and effect, in freedom from unwanted pharmacological effects, and in what is coming to be called 'personalized medicine.'

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