Chapter Two

Parenteral Drug Delivery

INTRODUCTION

The term parenteral drug delivery covers a number of administration routes, which have little in common other than the fact that they generally involve the use of a hypodermic needle to inject the drug into the body. This route bypasses a number of physiological barriers and hence the constraints on the composition and formulation of the medicine are much more rigorous than for less invasive routes such as oral or transdermal delivery. Despite this a surprising range of materials can be injected into various tissues if the appropriate precautions are taken. We will examine the constraints for specific formulations with respect to the appropriate physiological route, but a number of general principles are common to all routes. The most important is that the formulation must be sterile, since the major defence mechanisms of the body (the skin and mucous membranes) are bypassed, and so any infective agent in the formulation may cause major disease. It is not only necessary to remove live microorganisms; parts of dead organisms can elicit an immune response, and polysaccharides from the bacterial cell wall, known as pyrogens, can cause a substantial increase in body temperature.

The most important routes of parenteral delivery are intravenous and intramuscular, with subcutaneous being widely used for small volumes and for vaccination. There are a number of less important routes, since it is generally possible to inject materials into virtually any part of the body in an attempt to gain a rapid local action. Thus we also have relatively specialized routes such as intrathecal, intraarticular and intracardiac.

INTRAVENOUS DELIVERY

Physiology

Intravenous delivery involves the direct injection of the formulation into the venous circulation. To understand the behaviour of intravenous drugs it is essential to consider the function of the circulatory system. This is shown in diagrammatic form in Figure 2.1. Although the heart is normally described as a pump, it is in fact two pumps, one of which circulates the blood around the tissues, and one which circulates it specifically around the lungs in a separate loop. Oxygenated blood is pumped by the left side of the heart through the aorta and arteries into a network of capillaries which allow transfer of oxygen and nutrients into the tissues, and remove waste products. The blood is then collected by a network of veins and passes into the right side of the heart. It is then passed through the pulmonary circulation into the lungs, where it loses carbon dioxide produced by tissue respiration, and is reoxygenated. It then passes back to the left side of the heart for the cycle to repeat.

Not all of the circulation from the tissues returns directly to the heart. A fraction, in particular that which perfuses the gastrointestinal tract, is pumped to the liver first. The liver performs a wide range of metabolic processes, but for drug delivery it can be rather more of a hindrance since it absorbs a fraction of the drug from the bloodstream and begins the cycle of metabolism and excretion. Consequently drugs which are administered orally will visit the liver before they have the opportunity to reach the target tissue; this effect is called first pass metabolism and can remove a significant and often unpredictable fraction of the dose. When the drug is injected into a vein it passes directly into the heart, and is carried to the tissues before passing on to the liver. Consequently first pass metabolism is avoided and the drug has an opportunity to act on the tissues before it passes to the liver on subsequent cycles around the circulation.

The capillaries are, in general, designed to retain their contents, and so in most tissues the capillary endothelial cells are in close contact without gaps, and a continuous basement membrane underlies them. Capillaries of this type can allow only relatively small solutes, such as water, low molecular weight drugs, and small proteins (maximum

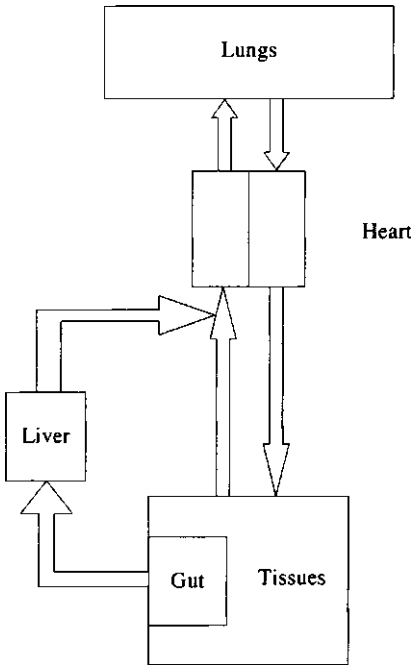

Figure 2.1 Schematic circulation of the blood

molecular weight <10 KDa) to diffuse into the surrounding tissues. This mixture forms the interstitial fluid which bathes the tissues, and under normal conditions there is a slow outflow of this fluid from the capillaries, driven by the blood pressure within the circulation and the osmotic pressure of its solutes. If this fluid accumulated continuously, oedema would result, so it is drained into highly permeable blind-ending capillaries which are the termini of the lymphatic system. These capillaries combine to form the lymph ducts which empty into the bloodstream again in major veins. In certain specialised or diseased areas the capillaries are more permeable and allow larger solutes and particles to leave the circulation. These will be discussed shortly.

Advantages and disadvantages of intravenous delivery

Intravenous delivery of drugs offers significant advantages which cannot easily be achieved by other routes. The most important of these is the rapidity of action which results from the drug being presented directly to the circulation without the need for release from a formulation or absorption through an epithelium. As a result it is possible for the physician to titrate the dose of drug to obtain a desired response, a procedure which is rarely possible with other routes owing to the time lag between administration and action. Dye and tracer experiments have demonstrated that liquids injected into an arm of a human subject appear in the leg within 20–30 seconds, attesting to the rapidity with which the formulation becomes spread around the body.

The second advantage of intravenous administration is a much more predictable response than is obtained from other routes. The uncertainty of poor or incomplete absorption and its variability is eliminated, so that blood levels are more predictable and pharmacokinetics is much simpler, being confined to elimination pathways alone. As a result

new drugs (new chemical entities or NCE's in the trade) are usually studied firstly as intravenous formulations to elucidate their pharmacokinetics and metabolism before oral formulations are developed. Many drugs cannot be usefully administered by any other route because they are degraded in the stomach (for example, peptides) or are unabsorbed due to their insolubility, size, or molecular properties (for example the antifungal amphotericin B). In these cases parenteral delivery is one of the few available options. Finally it should be noted that intravenous delivery can be used when the patient cannot be fed orally (for example they may be comatose or have had gastric resection) or un-cooperative, as in the case of psychiatric patients.

Despite being the most direct and rapid route of delivery, the intravenous route is paradoxically one of the safest for the testing of new drugs. If the drug under test is infused over a period of several minutes, delivery can be stopped instantly should any adverse reaction develop. In comparison, if the drug under test was administered using an oral formulation, the material in the gastrointestinal tract would be absorbed over a period of hours. Hence combating a serious adverse effect would be much more difficult since the unadsorbed material in the small intestine would be almost impossible to remove; absorption would persist and the adverse effect would be more difficult to terminate.

There are a number of disadvantages which generally confine intravenous delivery to situations where its specific advantages are vital. Foremost among these is the need for extensive training of medical staff so that the correct amount of drug goes into the right place with the right technique. As a result this is an expensive procedure and generally only used within a clinic or hospital where appropriate facilities are available. Sterility must be maintained, so the formulation must be prepared and handled in a sterile fashion, although the availability of radiation-sterilized disposable syringes, needles and ancillary equipment has done much to ease this burden. Dosages must always be checked; even so there are still occasional incidences of errors in dosing leading to serious injury or death. There are a number of complications which can have serious consequences. These include:

i) Air embolism, or the injection of air into a vessel. Small air bubbles may be absorbed in the blood but larger amounts (a few ml) of air can prove fatal, particularly if it reaches the brain. To prevent this most infusion pumps are designed to stop pumping and sound an alarm if air is present in the line.

ii) Thrombosis, the formation of a clot in a blood vessel, can be particularly dangerous if the clot circulates in the bloodstream. Certain disease states, or old age, can predispose to thrombosis, but it can also be caused by irritant formulations which are injected too rapidly.

iii) Haemolysis, the breakdown of red cells with the release of haemoglobin, can cause kidney damage if severe. This is normally a problem with strongly hypotonic injections, although certain membrane-active drugs such as amphotericin B can also cause this problem.

iv) Phlebitis is the inflammation of the vein wall due to irritation from the formulation; it can be caused by the formulation itself, or may be due to precipitation of the drug if injection is too rapid.

v) Extravasation, or the leakage of the injection from the vein into the surrounding tissue, can lead to extensive damage since there may be no mechanism to rapidly clear it fom the injection site. This is a particular problem for cytotoxic materials, (e.g. methotrexate or mitomycin) as it can lead to ulceration and necrosis which is slow to heal[1].

Methods for studying these effects have been reviewed by Yalkowsky[2]. As a result of these problems it is essential that injection sites be used with care, particularly if patients are to be maintained on long-term therapy, since it is possible to run out of useable sites over the lifetime of the patient, and maintaining a viable site is essential. The use of implanted

catheters helps considerably; patients undergoing permanent parenteral nutrition have cannulae which must be maintained for several years before replacement. In such cases it is possible to perform treatment in a home setting if the patient can be sufficiently educated in the techniques of sterile handling of total parenteral nutrition mixtures[3].

Formulation considerations

We have already noted that intravenous formulations must be sterile in order to avoid causing an infection. The preferred method of achieving this is by terminal sterilization using an autoclave, but this may not be possible if the formulation is heat sensitive. In this case it can be filtered through a 0.22 μm filter which removes all bacteria and spores, or in some cases it may be possible to sterilize using gamma radiation. If all else fails, the formulation can be prepared in a fully aseptic environment, but this is extremely expensive. Particulate material, such as small fragments of dust, glass, or pieces of rubber closures, must also be rigorously excluded. Pharmacopoeial specifications generally require that no particle larger than 5 μm be present in the formulation; this is largely an unverifiable requirement, but the occurrence of particles in modern infusion fluids is extremely rare.

It might be imagined that the formulation must be very similar in pH and tonicity to blood, but in fact there is considerable latitude, depending on the volume of the formulation and the injection site. Small volume parenterals, defined as those below 100 ml in volume, can be formulated at a pH ranging from 4 to 10, and be considerably hypotonic or hypertonic. Large volume parenterals must be more closely matched to the properties of the blood, and the pH is rarely outside the limits 6–8. Plasma extenders, which are often infused through a peripheral vein, are closely matched in tonicity, but parenteral nutrition mixtures may have a tonicity up to around twice that of blood. Such mixtures are usually administered through an indwelling catheter which empties into the subclavian vein[4]. In this case the infusion is very rapidly diluted, so that variations in its properties are of lesser importance. There is increasing interest in the use of peripheral sites for parenteral nutrition, and in these cases the mixtures should be close to isotonic in order to avoid damage at the infusion site[5].

Ideally all injections would be formulated at pH 7.4 and be isotonic with blood; however it is often necessary to use less physiologically acceptable solvents, especially to aid the solubility of a drug which may be poorly soluble near neutral pH, or to control stability. In certain cases it may also be necessary to add cosolvents such as ethanol or propylene glycol, or surfactant-based solubilizing agents (for example deoxycholate, which is used to solubilize amphotericin B in the injectable Fungizone®). These injections are far from physiological and it is wise to infuse them slowly over several minutes, or ideally with an infusion pump, to ensure that they are rapidly diluted as they enter the blood. However, their use poses an additional problem. If the injection has been formulated under extreme conditions to enhance the drug solubility, when it is injected it becomes diluted in the bloodstream, and the drug may precipitate. This can lead to unpredictable pharmacokinetics as submicron drug particles are processed by the reticuloendothelial system (see below), and cause pain and damage at the injection site. The situation becomes even less predictable if the formulation is added to a concurrent intravenous fluid by an inexperienced clinician, and precipitates in the infusion bag.

Devices and technologies

A wide range of technologies have been developed to reduce the effort and training, and associated cost, of intravenous delivery. In its simplest form the use of sterile disposables minimizes the possibility of infection, and the cost of resterilizing syringes is far greater than that of disposables, even when the cost of proper disposal is taken into account. Long term

infusions are generally administered through catheters, which range in length from a few inches for peripheral use, to much longer devices which can be implanted so that they reach the subclavian vein for the infusion of hypertonic solutions, for example in TPN. Catheters are generally made of PVC, Teflon, polyethylene, silicones or polyurethane, with the latter being preferred for long-term use[6].

Although small volumes are often infused manually, mechanical pumps are used if a slow infusion rate is required. These can be used to drive conventional syringes, or to infuse larger volumes from bags. For long-term administration of small volumes, implantable pumps can be used, and there is now considerable interest in allowing the patient a degree of control over the infusion rate, as in patient-controlled analgesia.

A major source of cost and effort in intravenous therapy is associated with the preparation of the material for administration, particularly if complex mixtures are required. As a result a large number of packaging systems have been devised, including prepacked minibags, premixed drugs in intravenous fluids, and frozen premixes. Reconstitution systems such as the Abbott Add-Vantage and Lilly Faspak allow the drug to be administered from the container in which it was reconstituted, avoiding a transfer step. The increased cost of novel packagings like this can often be justified since it may avoid major expenditure on skilled labour and sterile compounding facilities.

Injected participates

Although pharmacopoeial specifications generally require parenterals to be free of particles, there are occasions in which we need to understand the behaviour of particulate materials in the bloodstream, not least because they may form the basis of potentially useful drug delivery systems. Particles present in intravenous fluids will pass through the heart and circulate around the pulmonary circulation. This represents the first stage of filtration in which any material with a particle size larger than around 5 μm will be trapped in the small pulmonary capillaries. Large amounts of such materials will cause dangerous pulmonary embolisms, although smaller amounts of materials may be used as a useful way of targeting drugs to the lung. This has also been explored as a possible means of targeting tumours, particularly hepatic tumours[7]. Embolisms can also be caused by smaller particles if they aggregate together. There is an extensive body of science concerning the dispersion and aggregation of colloidal particles[8], but despite this it is possible to formulate an injected colloid (for example, a fat emulsion) which while stable alone, becomes unstable in plasma, the large aggregates of droplets depositing in the lungs[9].

Particles which are sufficiently small to pass through the pulmonary capillaries return to the heart, after which they are circulated to the tissues. Large particles which have escaped the pulmonary filter may deposit in the capillaries by blockage but they are more likely to be captured by macrophages. This is particularly important if tissues are inflamed, as extensive macrophage activity can result in considerable uptake of particles in such areas, and has been explored as a possible mechanism of targeting sites of inflammation[10]. Small pores called fenestrae, of diameter 50–60 nm, are present in the exocrine glands, but the capillary basement membrane is continuous in these regions and particles cannot pass through into tissues. Only in a few regions, specifically the liver and spleen, are there pores which are sufficiently large to allow particles to escape the circulation. In these regions particles of size smaller than 100 nm can leave the circulation to be taken up by hepatocytes. However the capillaries in these regions are lined with active macrophages called Kupffer cells, which remove most circulating particles. The majority of injected colloidal particles will end up in the Kupffer cells rather than the hepatocytes. As a result of these combined processes, injected colloids are efficiently removed, largely irrespective of their size (Figure 2.2).

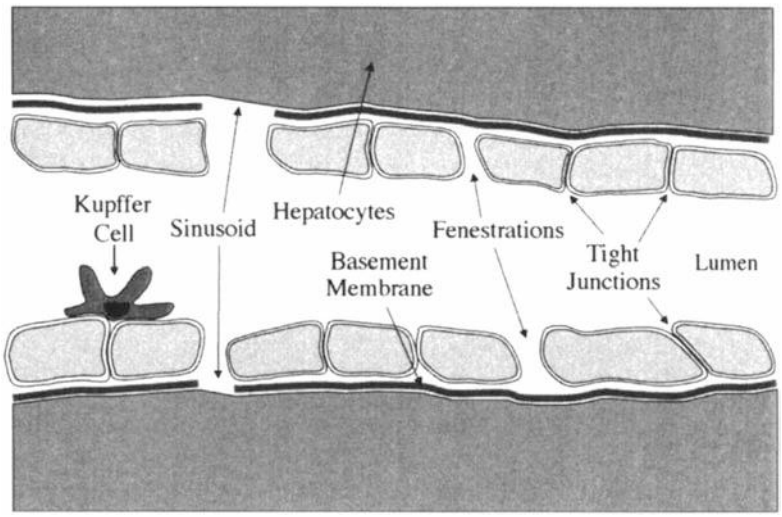

Figure 2.2 Structure of the hepatic blood vessels

This uptake and clearance by the system commonly termed the reticuloendothelial system is a major barrier in the application of colloidal particles for intravenous targeting applications, and considerable effort has been expended in trying to defeat this mechanism. One of the few promising approaches is the use of long-chain hydrophilic polymers (primarily polyethylene glycol) to form a heavily hydrated layer around the particle[11]. The method by which this coating escapes phagocyte surveillance is still uncertain but is thought to be due to the prevention of absorption of serum recognition factors or opsonins which mediate the uptake process. Initally experiments were performed using simple non-degradable polystyrene particles coated with an adsorbed layer of ill-characterized industrial polymer, and much of the early literature in this area is confused and irreproducible. The current state of the art involves the use of highly characterized self-assembling materials such as poly (d, l lactide)—poly (ethylene glycol), and phospholipids with poly (ethylene glycol) grafted to the headgroup to form 'stealth'[12]. Despite the interest these advanced materials, our understanding of their behaviour is largely incomplete.

Intravenous oxygen carriers

There has been a great deal of effort into the possibility of replacing or supplementing blood with an alternative oxygen transport fluid, for example in major trauma, where matched blood may not be available. The increase in AIDS also prompted a transient increase in interest in this area until screening processes were introduced for blood products. There are two main technologies under development:

i) Fluorocarbon emulsions. Fluorocarbons are inert water-immiscible oils which dissolve large quantities of respiratory gases; a well-known demonstration by Clark (1966)[13] showed a hamster breathing while submerged in liquid perfluorodecalin. There have been a number of attempts to develop water-miscible fluorocarbon emulsions which could be used as blood substitutes, of which the Green Cross Fluosol® and the Alliance Oxygen® are the most well-known. The possibility of developing a large-volume blood replacement with this technology seems remote due to the extensive immunological problems which may ensue, coupled with the reticuloendothelial load involved in clearing the emulsion; however

a number of indications for smaller doses, such as myocardial oxygenation during coronary angioplasty, show some promise. The field has been recently reviewed by Krafft and coworkers (1998)[14].

ii) Haemoglobin-based products. Free haemoglobin resulting frim lysis of erythrocytes is cleared rapidly from the circulation; large amounts of haemoglobin will cause kidney damage. Two approaches are being studied to prevent renal excretion; modification and encapsulation. The modified route uses polymerized or cross-linked haemoglobin, usually treated with glutaraldehyde or coated with polyethylene chains[15]. Rabinovici and coworkers[16] and others have studied the possibility of encapsulating haemoglobin in liposomes to make an artificial red cell.

INTRAMUSCULAR DELIVERY

Physiology

Intramuscular delivery involves the injection of the dose form into a muscle, from where it is absorbed due to the perfusion of the muscle by blood. The formulation forms a local depot which is partly mixed with interstitial fluid, and partly forms a bolus within the muscle, particularly if the injected volume is large. As a result it is important to realize that the injection is made into abnormal tissue; this may be particularly important if the formulation is intended to reside in the body for a significant length of time.

The structure of a typical muscle is shown in Figure 2.3. The muscle is wrapped in a connective sheath called the epimysium, within which are bundles of individual fibres, each surrounded by a connective membrane termed the perimysium. A finer matrix of connective fibrous tissue, the endomysium (omitted from Figure 3 for clarity), surrounds the muscle fibres, and the blood capillaries run within the endomysium, largely in a longitudinal manner, with numerous cross-connections. As a result the whole muscle is extremely well perfused. There are also numerous lymphatic vessels, but these lie in the epimysium and perimysium.

The preferred sites for injection are the gluteal, deltoid, triceps, pectoral and vastus lateralis. The deltoid muscle is preferred due to its greater perfusion rate compared to the other muscles, although the vastus lateralis has the advantage of having fewer major blood vessels into which the injection might accidentally be placed. When an intramuscular injection is administered, it is normal practice to withdraw the syringe plunger briefly to see if blood can be withdrawn. Blood indicates that the needle may be in a vessel, and the injection should be repositioned. There is also a minor danger of damaging a nerve fibre during the injection.

Pharmacokinetics

The most significant advantage of intramuscular delivery is the ease with which a wide range of drugs can be administered in a variety of dosage forms, which not only provide rapid absorption, but can also be used for sustained therapy. Intramuscular delivery involves a number of steps (Figure 2.4); i) release of the drug from the dose form into the intercellular fluid (ICF), ii) absorption from the ICF into the blood and lymphatics, iii) transport from the local blood volume into the general circulation, and iv) metabolism. The concentration of drug and kinetic profile are determined by the relative rates of these processes, and we should note that the capillary membrane is highly permeable and in general will not be rate-limiting, but perfusion of the muscle by the blood may be significantly slower. We can distinguish two particular limiting cases of interest:

i). Injection of a bolus of soluble drug. In this case the drug is immediately available in the ICF and is rapidly absorbed into the capillaries. In this case the rate-limiting absorption step is the perfusion of the muscle by the blood. Any factor which influences muscle perfusion (such as movement or exercise) will change the rate of absorption. In

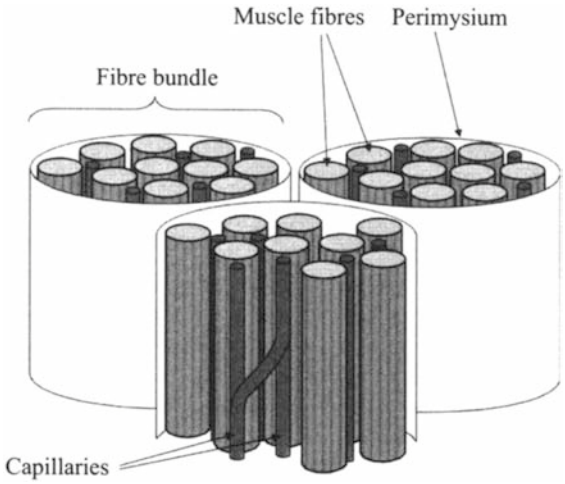

Figure 2.3 Schematic structure of muscle

particular, if cardiac failure has occurred, absorption will be extremely low since the muscle perfusion rate will be small. For this reason intramuscular delivery is contra-indicated if cardiac function is poor.

ii) Injection of the drug in sustained-release form (e.g a solid depot or crystal suspension). In this case release from the formulation is slower than absorption or perfusion, and so the behaviour of the device becomes the rate-limiting step, and the effects of muscle perfusion are not evident. Under these conditions the concentration of drug in the plasma remains approximately constant until the delivery device is exhausted, a period which can be designed to last from several hours to several months.

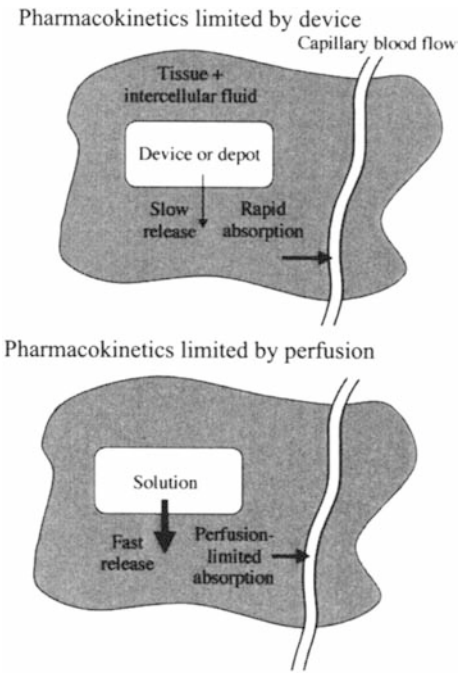

Figure 2.4 Absorption of drugs from intramuscular injections

Formulation considerations

Since the formulation does not have to be miscible with water, it is possible to inject a much wider range of materials than those which can be administered intravenously. The possible formulations include aqueous solutions, aqueous suspensions, oily solutions, oil in water emulsions, water in oil emulsions, oily suspensions, and dispersions in polymer or solid implants. These are listed approximately in order of release rate, as aqueous solutions can be absorbed in minutes, while implants can deliver drugs for several months.

In addition a range of other factors can influence the absorption rate. If the drug is extremely hydrophobic it will not dissolve in the ICF, while if it is strongly ionized or extremely water soluble it will not be able to cross the capillary membrane. Drugs which are strongly protein-bound will also be slowly absorbed since their activity in solution will be reduced. A number of drugs administered in solutions may be absorbed anomalously slowly if the composition of the formulation changes after injection. For example, phenytoin is formulated as an injection at pH 12 due to its low solubility. On injection the ICF quickly reduces the pH to normal levels, and the drug precipitates. As a result it may then take several days for the dose to be fully absorbed.

SUBCUTANEOUS DELIVERY

Physiology

A subcutaneous injection (SC) is made into the connective tissue beneath the dermis, and should be contrasted with an intradermal injection which is made into the dermal layer, often between the dermis and the epidermis (Figure 2.5). This is a critical distinction because the subcutaneous tissues have a significant volume of interstitial fluid into which the drug can diffuse, while the epidermal tissue has relatively little available fluid, nor is it well perfused by blood. As a result an intradermal injection persists at the site for a long period and the available volume for injection is small; it is normally used for antigens (e.g. tuberculin) and vaccines (smallpox).

Drugs injected subcutaneously dissolve in the interstitial fluid and gain entry to the bloodstream by two routes. They may be absorbed directly into blood vessels, but the subcutaneous tissues are often adipose and poorly perfused. Alternatively the interstitial

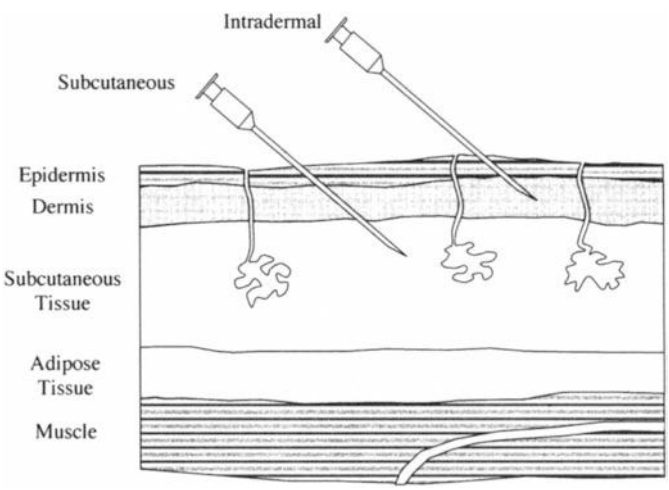

Figure 2.5. Physiology of parenteral administration routes

fluid is collected by lymphatic capillaries and these drain into the regional lymph nodes and then into the bloodstream. These pathways are both relatively slow and depend on the local vascularure, so absorption from subcutaneous sites can be slow and unpredictable. To some extent this allows a sustained release effect to be obtained; however it is not particularly satisfactory to design a dose form in this way because of the inherent variability of the pharmacokinetics. A better strategy is to make the release from the dose form rate-limiting (as is the case for intramuscular depot delivery systems) so that biological variation then has little influence on the drug pharmacokinetics. A large number of delivery systems have been devised which work in this way; probably the best known being Zoladex® (AstraZeneca) which releases the hormone goserelin, a chemical castrating agent used in the treatment of androgen-dependent tumours. The hormone is incorporated into a small rod of biodegradable poly (D, L lactide) polymer about 5mm long and implanted subcutaneously in the abdomen. A single injection lasts for 28 days; the cost (over £100 per injection at the time of writing) reflects the difficulty of manufacturing the device in a totally aseptic environment. Technology of this type is particularly suited to peptide hormones in which the dose is small and the size of the device can be minimized. A number of other interesting examples of this depot technique can be found in the literature, including the use of emulsion depots for methotrexate[17], hydrogels[18] and block copolymer gels[19].

Subcutaneous colloidal delivery systems

It has already been indicated that materials injected subcutaneously may be carried by the lymphatic flow into the regional lymph nodes and then into the blood. Colloidal particles which are injected subcutaneously can follow the same route, although their large size (tens to hundreds of nanometres) relative to drug molecules will reduce their diffusion rate considerably. On reaching the lymph nodes they will be taken up by macrophages rather than passing to the bloodstream.

Most of the research in this area has been performed in rats, with the foot and footpad being the most closely studied injection sites. Ousseren and Storm[20] used this technique to study the lymphatic uptake of liposomes from SC injection, and found relatively high lymphatic localization (60% of injected dose). However, injections into the flank produced only a slight uptake, and the suspicion is that footpad injections cause a large increase in local interstitial pressure due to the small volume of the site, and that this drives the injection into the lymphatic vessels. Consequently the targeting effect may not be so pronounced if the technique were used in man. The same study showed that the liposomes needed to be small (>0.2 μm) or they could not be moved from the injection site, and that highly charged liposomes were taken up more efficiently than weakly charged ones. Interestingly, liposomes made from the 'stealth phospholipid' with grafted PEG-chains were taken up to a similar extent to normal liposomes of the same size. Although this study was performed exclusively with liposomes there seems to be no reason why the results should not broadly apply to other colloidal particles.

TISSUE DAMAGE AND BIOCOMPATABILITY

With any injected formulation damage occurs to the surrounding tissues. In the case of an intravenous injection, this usually has little consequence for drug absorption, but in the case of intramuscular and subcutaneous systems the drug or device is inherently present in a wound site and the body will react accordingly. The reaction depends on the size and composition of the device. Small isolated particles less than 10 μm will be engulfed by macrophages without any major reaction occurring, but larger objects in a microsphere form will gather a layer of macrophages and giant cells adhering to the surface of the particles. Larger devices with large surface areas will elicit a foreign body reaction which

begins with inflammation and the formation of a layer of macrophages and giant cells, and is followed by the formation of granulation tissue. Ultimately a fibroid capsule consisting of fibroblasts and collagen will surround the device. The consequences of these changes for drug delivery have not been widely explored but at present it is thought that they make little difference to drug release and absorption unless the reaction is severe.

DRUG DISTRIBUTION FOLLOWING PARENTERAL ADMINISTRATION

The blood transports the drug to the tissues, however the drug concentration in the tissues is usually not equal to that in the blood. A number of factors influence the drug concentration in tissues, one of the most important being the blood flow per unit mass of the tissue. Tissues can be broadly classified as poorly-perfused, adequately perfused and well-perfused on this basis as shown in Table 2.1. Note how organs with a relatively small mass, such as the heart and brain, only require a modest blood flow to perfuse them well. The blood flow to the heart musculature (not to be confused with the flow *through* the heart) is equal to that through the adipose tissue, but the heart has a much smaller mass and so is correspondingly better perfused.

The blood flow controls the rate at which the drug is supplied to the particular tissue, and will be reflected in the drug concentration profile in that tissue. If the tissue is well-perfused, the tissue pharmacokinetics will reach a maximum value at a similar time to that in the blood. However, if the tissue is less well perfused, the supply of drug to the tissue will be rate-limiting and so the concentration in the tissue will increasingly lag behind that in the blood, as shown in Figure 2.6.

The second important factor determining the tissue pharmacokinetics is the affinity of the tissue for the drug. This can take 2 forms; passive or active. Passive affinity is simply the partitioning of the drug. For example, the partitioning of a lipophilic drug into adipose tissues results in high drug concentrations in that tissue, although this is achieved slowly due to the poor perfusion of the adipose tissue.

Table 2.1 Blood flow through various human organs

Tissue	Blood flow (litres min^{-1})	Tissue mass as (% of body weight)
Blood	5.4	8.0
Poorly Perfused		
Skeleton	0.2	17
Adipose tissue	0.25	14-20%
Adequately perfused		
Skin	0.4	7%
Muscle	0.8	48%
Well perfused		
Kidneys	1.2	0.5%
Heart	0.25	0.5%
Liver	1.55	3.5%
Brain	0.75	2.0%

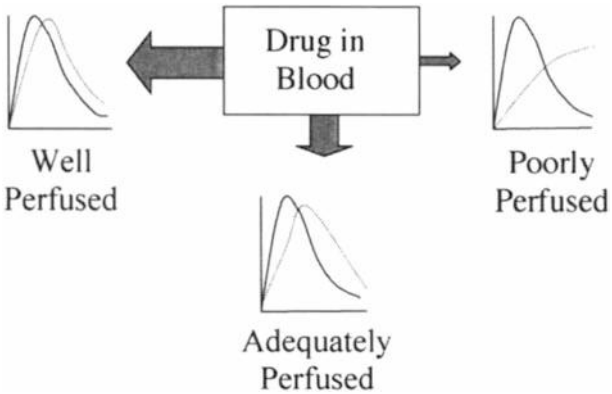

Figure 2.6. Pharmacokinetic profiles of drugs in tissues of varying perfusion. Solid line: concentration in blood; dotted line: concentration in tissue

Active affinity occurs if the drug is taken up by the tissue by a specific transport mechanism. An example of this is guanethidine, which is used in the treatment of hypertension. This drug reaches its site of action by active transport into the heart and skeletal muscle. As a result the drug concentrations in these tissues are much higher than would be expected on the basis of partition from the blood, and due to the affinity of the tissue for the drug, are sustained for a much longer period than those in the bloodstream (Figure 2.7).

PROTEIN BINDING

One of the underlying principles of clinical pharmacology is that only the unbound, free drug is pharmacologically active and that only in this form can a drug cross a biological membrane or interact with a receptor. Interaction with the receptor results in a biochemical change leading to a physiological response.

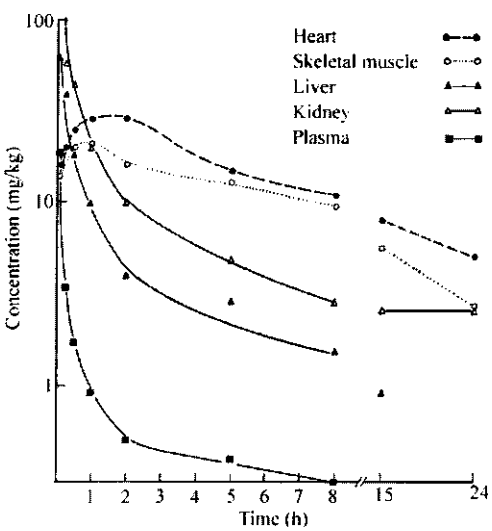

Figure 2.7. Concentrations of guanethidine in tissues following intravenous administration[21]

Drugs bind to a number of plasma proteins including albumin, lipoproteins, and gamma globulins, and the more extensively the drug is bound, the lower will be the drug activity available to exert a pharmacological effect. Since the concentrations of these proteins change in disease, and as a function of nutrition, the effect of the drug may be significantly and unpredictably modulated. For example, most antimicrobials bind primarily to albumin, while basic drugs including erythromycin, clindamycin, and trimethoprim bind to the "acute-phase" serum protein ([[alpha]] 1-acid glycoprotein). Moreover, binding is not limited to proteins in the serum. Because albumin is the principal protein in interstitial fluid, substantial binding to this protein and to other tissue constituents, takes place. Differential concentrations of these proteins, and cell debris, leads to marked alterations in peripheral drug concentrations around an inflamed area such as a wound or abscess.

THE BLOOD-BRAIN BARRIER

Physiology

The ventricles of the brain lie within the ventricular compartment and are surrounded by approximately 150 ml of cerebrospinal fluid (CSF) which is secreted by the choroid plexi and circulates around the brain before being absorbed into the venous circulation. The entire fluid volume surrounding the brain is replaced every five hours in normal subjects. As a result it is rarely practical to deliver drugs by injection of a bolus into the ventricular space, since they are efficiently cleared before they can diffuse into the brain tissue. As a result the only practical means of delivering a drug effectively to the whole brain volume is via the systemic circulation. A significant feature of the blood flow in the brain is that the capillaries are very close together (of the order of 40 micrometres), so that any substance passing into the brain from the circulation will equilibrate within seconds through the whole of the brain tissue.

Unfortunately, delivery of drugs to the brain from the systemic circulation is difficult due to the presence of the so called *blood brain barrier.* This is not so much a physical structure as the absence of the usual mechanisms which allow drugs to be transferred across the capillary wall in other tissues. The junctions separating the capillary endothelial cells are extremely tight, eliminating the possibility of paracellular transport, and there is an almost complete absence of pinocytosis across the endothelial membrane. It also appears that there is a specific transport molecule, P-glycoprotein, which actively back-transports a wide range of drugs out of the apical cells, preventing their passage into the brain tissues[22]. A number of workers have attempted to open channels through the endothelium by infusing osmotic agents such as mannitol. Although this does lead to an increase in permeability, extensive side effects are observed, including seizures in experimental animals, and the practicality of this method seems limited. It is also possible to increase the permeability of the endothelium pharmacologically using bradykinin[23]. However, the most widely studied pathways for drug absorption are those which are part of the normal endothelial function, i.e. simple diffusion in the lipid membrane, and receptor-mediated active transport.

Uptake by diffusion

In order for a molecule to cross the endothelial capillary by diffusion, it must have two properties; it must be highly lipid soluble, and of relatively low molecular weight. A number of studies have examined the dependence of uptake on molecular weight, and an upper limit between 400 and 700 Daltons is generally accepted[24 25]. The absorption of hydrophilic molecules can be increased by administering them as hydrophobic prodrugs. A good example of this is provided by the absorption of morphine, codeine (methylmorphine) and heroin (diacetylmorphine) which have LogP (calculated) of 0.24, 1.14 and 1.86 respectively. Codeine has a blood-brain permeability of approximately 10 times that of morphine, while that of heroin is 10 times greater still.

Receptor-mediated transport

The brain capillary endothelium possesses a number of receptors which allow the transport of specific materials. These include small molecule nutrient receptors, such as those for hexose, amino acids, amines, and a number of peptide and protein receptors, including those for insulin, transferrin and IGF-II[26]. It is possible to take advantage of these pathways for the delivery of mimetic drugs; for example, dopamine is poorly transported through the blood brain barrier, and its administration is of no direct therapeutic value, but the amino-acid analogue L-DOPA is transported through the phenylalanine receptor and can thus exert a useful pharmacological effect[27].

A number of reserchers have attempted to make use of these active pathways to develop blood brain barrier vectors which can improve drug uptake. A typical example is the OX26 monoclonal antibody to the endothelial transferrin receptor[28]. The antibody binds efficiently to the receptor, and is actively transported across the endothelium. The drug can be attached to the antibody using conventional avidin-biotin methods which do not degrade the activity of the drug. This technique has been used to deliver vasoactive intestinal peptide (VIP) attached to OX26 to the brain, resulting in a significant increase in hemispheric brain blood flow; the peptide alone was not significantly absorbed and showed no pharmacological effect[29]. It has also been used in an attempt to deliver antisense gene therapy agents to the brain[30]. Antisense oligonucleotide-OX26 conjugates were not delivered efficiently since they bound to plasma proteins, but peptide nucleic acid—OX26 conjugates did not suffer from this problem and were efficiently transported into the brain. More recently the antibody has been grafted to the surface of liposomes containing daunomycin and successfully used to produce elevated drug levels within the brain tissue.

The most significant difficulty with these vector methods is that the receptors being used in the target tissue occur in a wide range of organs; for example transferrin receptors are present on most cells since they are required for the active uptake of iron. It is thus important to distinguish between brain uptake and brain targeting; vector based conjugates may improve brain uptake but will not produce targeting, with the drug being absorbed in a wide range of organs. Many published studies are particularly vague on this point and one is led to suspect that it is a significant problem.

A number of polyionic macromolecules are also actively transported into the brain capillary endothelium by absorptive transcytosis. This mechanism is distinct from the receptor-mediated pathways discussed above since it involves the physical adsorption of the macromolecule (usually a polycation) to the negatively charged membrane by charge interactions, after which internalization occurs. Molecules transported in this way include lectins such as wheat germ agglutinin and polycationic proteins such as cationized albumin[31]. This approach has been used to deliver radiolabelled antibody diagnostics; for example a monoclonal antibody to the amyloid protein which is characteristic of Alzheimers disease. The antibody itself was not significantly taken up by brain tissue, but localized in the brain in dogs after it had been converted into a polycationic protein by reaction with hexamethylene diamine[32].

Colloidal delivery

We have already mentioned the use of liposomes coated with the OX26 transferrin antibody which successfully increased brain levels of daunomycin. In this case, it was unclear whether or not the liposomes were internalized in the endothelial cells or simply adhered to the surface, thus providing a greater concentration gradient to assist passive diffusion. It has also been found that cyanoacrylate microspheres coated with polysorbates can be used to deliver a number of hydrophilic analgesic peptides[33 34]. The mechanisms of this effect are also uncertain but may be due to a surface receptor recognizing the sorbate block of the surfactant.

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