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Frijlink, Henderik; Lagarce, Frederic; Touw, Daan; Woerdenbag, Herman

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Henderik Frijlink, Frederic Lagarce, Daan Touw, and Herman Woerdenbag

Contents

Abstract

Biopharmaceutics, in connection with pharmacokinetics, is the field that investigates and describes everything that happens with a medicinal product and the active substance between the moment of administration, the moment it exerts its action and the moment it is eliminated from the body. Biopharmaceutics connects the physico-chemical properties of an active substance and its dosage form, especially through the drug release characteristics, with its fate and action in the living organism. Actually biopharmaceutics describes how medicine formulation technologies can affect pharmacokinetics and pharmacodynamics. The route of administration, the way the active substance is released from the dosage form, and the way the body handles (absorbs, distributes, metabolises and excretes) the active substance, together determine its (duration of) action, its efficacy and the occurrence of adverse effects. This chapter explains general principles of biopharmaceutics and its relation to pharmacokinetics in the light of their implications on the design of medicines. It describes the general biopharmaceutical principles that are relevant to the major routes of administration: parenteral, oromucosal, oral, rectal, dermal, nasal, pulmonary and ocular. Topics discussed include solubility and dissolution, bioavailability, partition coefficient and pH partition theory, the biopharmaceutical classification system (BCS), excipient-, food-, drug- and herb-drug interactions, first-pass effects and drug metabolism, bioequivalence and new developments in the field of advanced drug delivery systems.

What Is New?

Minor parts of the chapter have been revised. Attention is given to new developments.

Learning Objectives

The reader knows that:

- Pharmacotherapy can only be optimal if the pharmaceutical formulation (the medicinal product) and its functionalities are appropriate for the chosen route of administration and the intended therapeutic objective.
- The technical and biopharmaceutical functionalities of a medicinal product are determined by its qualitative and

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H. Frijlink (✉) · H. Woerdenbag
Department of Pharmaceutical Technology and Biopharmacy, University of Groningen, Groningen, The Netherlands
e-mail: h.w.frijlink@rug.nl; h.j.woerdenbag@rug.nl

F. Lagarce
Department of Pharmacy, Faculty for Health Sciences, University of Angers and University Hospital of Angers, Angers, France
e-mail: frederic.lagarce@univ-angers.fr

D. Touw
Department of Clinical Pharmacy and Pharmacology, University Groningen, University Medical Center Groningen, Groningen, The Netherlands
e-mail: d.j.touw@umcg.nl

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quantitative composition, as well as by the structure of the different components in the product.
- From a biopharmaceutical point of view, a pharmaceutical formulation should be tailored to the route of administration, the physico-chemical properties of the active substance, the desired mode of action, the onset and duration of the therapeutic effect, the site of action, and the intrinsic pharmacokinetic and pharmacodynamic properties of the active substance.
- An active substance can only pass the absorptive membranes when it is dissolved in the aqueous fluid adjacent to these membranes.
- The bioavailability of an active substance is defined as the fraction of the active substance that reaches the systemic circulation intact. It equals the pharmaceutical availability *in vivo*, minus the loss of the active substance during the absorption into the systemic circulation.
- Active substances undergoing substantial metabolism when passing the intestine and/or liver (first-pass effect) show a limited bioavailability, often with large intra- and inter-individual differences.
- Drug-drug interactions on the level of metabolising enzymes (cytochrome P450, P-glycoproteins) may cause significant changes in bioavailability and therefore cause undesired effects.
- Each route of administration for an active substance brings along specific requirements for the pharmaceutical formulation to ensure optimal bioavailability.
- The oral, parenteral and rectal routes are the most widely used routes to achieve a systemic effect of a medicine.
- Locally administered medicines may exert a systemic effect as well, due to active substance absorption.
- Quantitative data on bioavailability and safety data are required when two different pharmaceutical products with the same active substance are compared to determine (bio)equivalence (e.g., a branded product versus a generic product or two different generic products).

5.1 From Medicinal Product to Effect and Beyond

5.1.1 Design of a Medicinal Product

A medicinal product (also called medicine or drug product, formulation or dosage form) is characterised by the quantitative composition (encompassing both the active substance(s) and excipients), the physico-chemical state of the active substance, the excipients and the medicinal product as a whole, as well as the structure in which the different components are present in the medicinal product. These aspects together will determine the functionality of the medicinal product and will make it more or less suitable for its intended use. Because the final physico-chemical aspects and structure of a medicinal product are often determined by the process and process conditions used during production, a reliable, robust and reproducible production is of paramount relevance for pharmaceutical preparations.

The functionality of the medicinal product can be described both in technical and in biopharmaceutical terms. The technical terms encompass aspects such as stability, uniformity of dosage and microbiological quality of the product. The biopharmaceutical functionality of a medicinal product relates to aspects such as the drug release profile, suitability of the medicinal product for administration via the intended route and ability of the active substance to reach the site of action. In this chapter the basics of biopharmaceutics are described and explained in relation to the different aspects of formulation, routes of administration and therapeutic objectives (in relation to pharmacokinetics; consult Rowland and Tozer's Clinical pharmacokinetics and pharmacodynamics: concepts and applications [1] as textbook for this topic).

Medicinal products exist in a variety of forms, to be used for different routes of administration, aiming at either a systemic or a local effect. To obtain a systemic effect, the oral and parenteral routes are the most frequently used. Alternatively, medicinal products can be given through rectal, transdermal, nasal or pulmonary administration to achieve a systemic effect. Rectal and pulmonary administration may also be applied for a local effect. Medicinal products administered to the eye, nose and ear are mostly used for a local effect.

5.1.2 Pharmaceutical Availability and Bioavailability

Biopharmaceutics describe the interrelationship between the physicochemical properties of the active substance and its interaction with the biological environment in relation to its formulation. The biopharmaceutical phase is the first step describing the whole process of drug delivery to the body. After this phase, leading to the absorption of the API, the steps included in pharmacokinetics describe the fate of the drug in the body. Those steps are absorption, distribution, metabolism and elimination. In general, not all administered medicine will reach its pharmacological target(s) and exert its action, this latter process is described by pharmacodynamics.

In most cases, an active substance is not administered directly at its site of action. As a consequence, transport is necessary from the site of administration to the site of action. The first step in this process is the release of the active substance from the dosage form. The delicate interaction between the physico-chemical properties of the active sub-

stance and its dosage form as well as the physiological conditions at the site of release, determine the extent and rate at which release will occur. However, a medicinal product can be designed in such a way that the extent and rate of drug release are determined by the physico-chemical characteristics of the dosage form. Controlled release being obtained in this way may affect the intensity, duration and moment of the effect. It may also affect (preferably reduce) the occurrence of adverse effects.

Often, the active substance is released from its administration form in a dissolved state. If this is not the case, the active substance must first dissolve in an aqueous environment after it has been released. Only in the dissolved state, an active substance can pass biological membranes separating the site of administration from the systemic circulation (the blood circulation) via which transport to the site of action occurs. The fraction of the administered active substance that dissolves in the aqueous fluid adjacent to the biological membranes and thereby becomes available for passing them is called the pharmaceutical availability. The fraction of the total amount of the administered active substance that ultimately reaches the systemic circulation in an unchanged form is called bioavailability.

Bioavailability is defined as the rate and extent to which the active ingredient or active moiety is absorbed from a drug product and becomes available at the site of action. Since, in practice, it is generally impossible to measure the active substance concentration at the site of action, it is assumed that when a substance is taken up in the systemic blood circulation, it will become active at the site of action. This translates the definition of bioavailability into "the rate and extent to which the active ingredient or active moiety is absorbed from a medicinal product and becomes available in the systemic blood circulation".

An intravenously injected medicine is considered to have a bioavailability of 1.0 (or 100%) meaning that all injected substance is available at the fastest rate for pharmacological action. Strictly speaking only intra-arterial administration should be considered as 100% bioavailable as some drug fraction can be eliminated by the lung (pulmonary first-pass effect) after intravenous injection and before reaching the arteries, thus the targeted organs. When a medicine is administered via a different route, its bioavailability can be reduced because of, for example, incomplete dissolution or loss during the transport of dissolved active substance to the systemic circulation (due to e.g. metabolism in the gut or the liver).

The transport of the dissolved active substance over the membranes to the blood circulation is called absorption. The extent and rate of absorption are determined by several factors, including the size and charge of the active substance molecule, its lipophilicity, the volume available for active substance dissolution, the surface and permeability of the absorbing membrane, the presence of metabolising enzymes and, in the case of active transport, the presence of transporters or efflux pumps such as P-glycoproteins (P-gp). As a consequence, poor bioavailability may be caused by incomplete dissolution of the active substance, by poor permeation over the absorbing membrane, or by metabolism during absorption.

In general, the bioavailability of an active substance is largely determined by the characteristics of the active substance and the formulation, structure and performance of the dosage form. (which may, for example, determine the dissolution rate), the chosen route of administration (different membranes show different permeability) and the administration conditions (e.g. the concomitant intake of food with oral medicine administration). If, for example, a high dose of a poorly soluble active substance is administered, the concomitant intake of two glasses of water or a meal with high lipid content may increase its bioavailability. If, however, an active substance is metabolised in the liver to a large extent, the oral route may be less suitable, making it worthwhile to investigate whether a sublingual (under the tongue) or nasal route of administration may be a better alternative. In contrast to the intestines, blood vessels from the tongue and the nose do not end up in the portal blood vessel system that immediately transports the active substance to the liver where it is exposed to metabolising enzymes.

After absorption into the systemic circulation, transport to the site of action occurs, first through the veins and then through the arteries. Once arrived, the active substance can exert its action. During and after transport as well as during and after exerting its action, distribution, metabolism and excretion of the active substance occur. These processes, together defined as the pharmacokinetic behaviour of the active substance, largely determine to what extent a medicine will be effective. The rate of absorption and elimination plus the volume of distribution determine the time and height of the peak concentration of the active substance in the blood. Therefore, they often also determine the occurrence of adverse effects.

The following medicine-related physico-chemical aspects may influence the release and absorption (bioavailability) of an active substance and thereby its final efficacy:

- The chemical form of the active substance (e.g., free acid or base, a salt, ester or other type of prodrug);
- The physical state of the active substance (e.g., particle size, crystal type or amorphic state);
- The nature and quantity of excipients and their interaction with the active substance;
- The route of administration (e.g., oral, parenteral, rectal, etc.);
- The dosage form (e.g., a solid dosage form or a solution);
- The pharmaceutical formulation (e.g. the medicinal product, the quantitative composition or the structure in which

the active substance and excipients are present in the dosage form).

Next to these factors other aspects, related to human physiology and external factors, may affect release and absorption of the active substance too. These include, amongst others:

- The interaction with food or concomitantly administered other medicines;
- The presence of enzymes that metabolise the active substance before absorption;
- The volume of the fluid, available at the site where the medicine dissolves;
- The presence of endogenous substances (such as bile salts) that affect the solubility of an active substance;
- The presence of efflux transporters that limit absorption;
- Variations in gastro-intestinal motility;
- Blood flow variations at the site of absorption.

In summary, biopharmaceutics relates to the physicochemical characteristics of an active substance, to the functionalities of a medicinal product and to the route of administration, to the performance in the living organism and to the efficacy and safety of the medicinal product. The biopharmaceutical characteristics are important aspects in the design of new medicinal products. They should be considered during the different stages of research and development as well as during the full subsequent lifetime of a medicine.

To achieve the desired therapeutic effect of an active substance, an adequate administration form in relation to the chosen route of administration must be used. The current pharmaceutical-technological knowledge offers possibilities to achieve optimal absorption of an active substance. Optimal in this context means: reliable, with a reproducible fraction absorbed (desirably the highest possible) and, if necessary, with a desired control of the release profile. Already small variations in excipients may significantly influence the pharmaceutical and biological availability and hence the therapeutic and adverse effects of a medicine. This applies to systemic as well as local administration. Furthermore, it is to be realised that the equivalence of these aspects may be of paramount importance for generic substitution.

5.1.3 Pharmacokinetics, Pharmacodynamics and Toxicology

Several aspects of the pharmacodynamic and toxicological behaviour of an active substance are highly relevant to the desired biopharmaceutical characteristics of a medicinal product. The efficacy of an active substance is determined by the intrinsic receptor affinity of the compound and the receptor occupancy. Since the last parameter is difficult to measure in man, drug blood concentrations (as measured in whole blood, serum or plasma) are usually taken as a surrogate parameter. This is based on the assumption that the active substance concentration in the blood of a patient is related to the active substance concentration at the site of action (and thereby the receptor occupancy). Next to the relationship between blood levels and therapeutic action, also blood levels and toxic action of a medicine are related. Based on this, the concept of the therapeutic window has been defined.

The therapeutic window of an active substance is the blood concentration range within which the desired therapeutic effect will occur without serious side effects. The lower limit of the therapeutic window is the so-called minimal effective concentration (MEC). This is the lowest blood concentration of the active substance that exerts a therapeutic effect. The upper limit of the therapeutic window is the maximal tolerable concentration (MTC). When the MTC is reached or exceeded, unacceptable adverse effects of the active substance are likely to occur.

Figure 5.1 shows the blood concentration (can be either on a linear as well as on a log-scale) versus time curve of an orally administered medicine (the same kind of curve can be obtained with other routes of administration including an absorption phase, such as an intramuscular injection or a rectal administered dose). It also shows the MEC and the MTC. During the initial phase of the curve the active substance is absorbed from the intestinal lumen into the blood. At time T_{max} the maximum blood concentration (C_{max}) is reached. At that time the absorption rate has reduced (due to depletion of the active substance from the site of absorption) to such an extent that the elimination rate of the active substance from the body equals the absorption rate. From this moment on the blood drug concentration will decrease because the elimination rate will exceed the absorption rate. At the end of this process we can consider that only elimina-

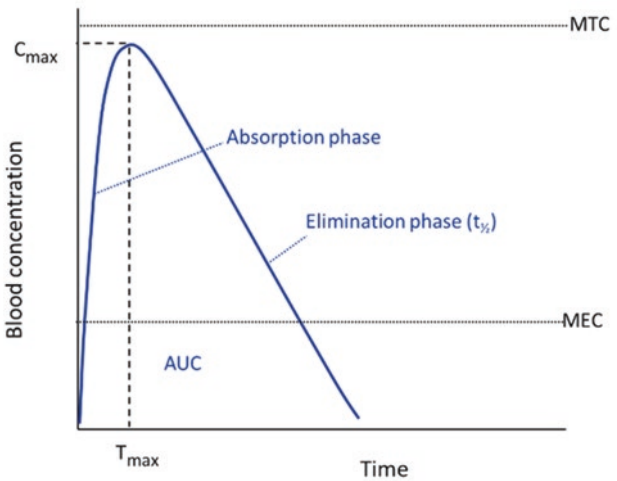

Fig. 5.1 Blood concentration (log-scale) versus time curve of an orally administered medicine

tion occurs because absorption has ended; this phase of pure elimination is used to determine the elimination half-life of the active substance ($t_{½}$). The duration of drug action is determined by the period during which the blood concentration is above the MEC. The total amount of absorbed active substance (bioavailability) is characterised by the area under the curve of the blood concentration versus time curve, relative to the total administered dose.

It is the major objective of any medicinal product to yield blood levels of the active substance within the therapeutic window for the period during which the therapeutic effect is desired. At the same time C_{max} should not exceed the MTC and the medicine administration frequency should remain reasonable, preferably not more than two to three times a day. However, this objective is often not easily reached, since the degree of absorption, the absorption and elimination rates, the therapeutic window as well as the intrinsic pharmacological and toxicological activities of an active substance may vary and interactions may occur.

For example, an active substance with a longer elimination half-life may have the advantage of a lower dosing frequency. However, due to the lower elimination rate significant amounts of active substance may still be present in the body when the next dose is administered. This is why an administration schedule based on elimination half-life is advised. Therefore it usually takes five subsequent administrations before the pharmacokinetic equilibrium (the 'steady state') is reached and both peak and trough concentration levels will be within the therapeutic window. For medicines that are administered once daily this may take several days and a loading dose may be needed to obtain a faster therapeutic effect. Active substances possessing a narrow therapeutic window often require therapeutic drug monitoring (TDM). Alternatively, their rate of absorption may need to be regulated through the use of technologically advanced controlled release products. If an active substance has to be administered frequently because of fast elimination, the use of a slow release product may be considered. This technology may also be considered when toxic effects occur due to peak concentration levels above the MTC.

Concentration versus time relationships (Fig. 5.1) can be translated into concentration versus effect relationships, both for desired and for adverse drug effects (Fig. 5.2). Within the therapeutic window, a low drug blood concentration will generally lead to a low efficacy and increasing the blood concentration will increase efficacy. Moreover, it is considered advantageous when the maximum therapeutic effect is reached already at a concentration that is significantly below the MTC since this would yield a broad safety margin for the patient. This is reflected in an increased margin between the therapeutic and the adverse effect in the curve.

Whether or not a concentration-effect curve is desired to be steep or flat depends on the drug action and on the intended therapy. For example, for an antihypertensive medicine it may be desirable to tune the effect and a somewhat flatter profile of the curve is preferred. For other situations, such as anti-migraine therapy or infections, tuning the effect is not or less relevant. A steeper curve may have the advantage that the desired effect will be obtained sooner, provided that the maximum effect is reached at concentrations considerably below the MTC.

The impact of variations in the bioavailability on the efficacy and safety of an active substance can be easily understood from these figures. The effect of individual variations in drug sensitivity in relation to variations in drug absorption can also be demonstrated. Examples are given in the box.

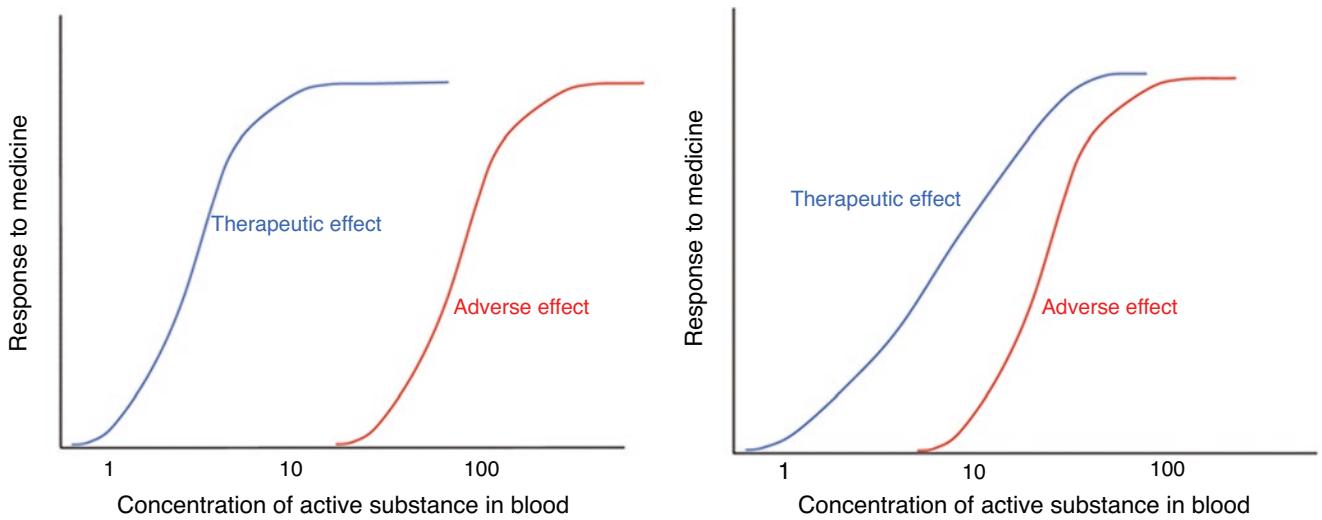

Fig. 5.2 Concentration versus effect relationship of different active substances. On the *left hand side* an active substance with a steep dose response and a large therapeutic window is shown and on the *right hand side* an active with a less steep response curve and a smaller safety margin

Effect of Variations in Bioavailability on Drug Efficacy and Safety and the Relevance of Variations in Drug Sensitivity

Figure 5.3 left panel, shows the effects of an increased bioavailability of an active substance on safety and efficacy. This may happen when a given active substance ('drug A') interacts with another active substance ('drug B') on the level of transporting enzymes. If, for instance, drug B has a P-gp inhibiting effect and drug A is excreted back into the intestinal lumen by P-gp after absorption, this interaction will lead to an increase in the blood concentration and the bioavailability of drug A. However, the efficacy of drug A hardly increases as a result of this interaction, since the efficacy was already at its maximum before the interaction with drug B occurred (already at the lower concentration the occupancy of receptors causing the therapeutic effect was complete). Conversely, more adverse effects of drug A will occur, because of the increased blood levels leading to an increased occupancy of those receptors that are causing the adverse effects.

In Fig. 5.3 right panel, the effect of a change in bioavailability is shown in relation to individual differences in drug sensitivity. The drug blood concentration versus effect relationships of the same active substance in two different patients are given in this figure. Patient A is more sensitive to the active substance than patient B. Due to an interaction between the medicine and food less active substance is absorbed (e.g. the effect of milk on tetracycline) in both patients. The bioavailability becomes reduced and the lower blood concentrations are reduced by about 30%. For patient A this will not result in a significant change in efficacy. For the less sensitive patient B however, this interaction will lead to a more than 50% reduction in efficacy. Such a reduction may, for example, lead to the emergence of drug resistant bacteria in the case of antibiotic therapy.

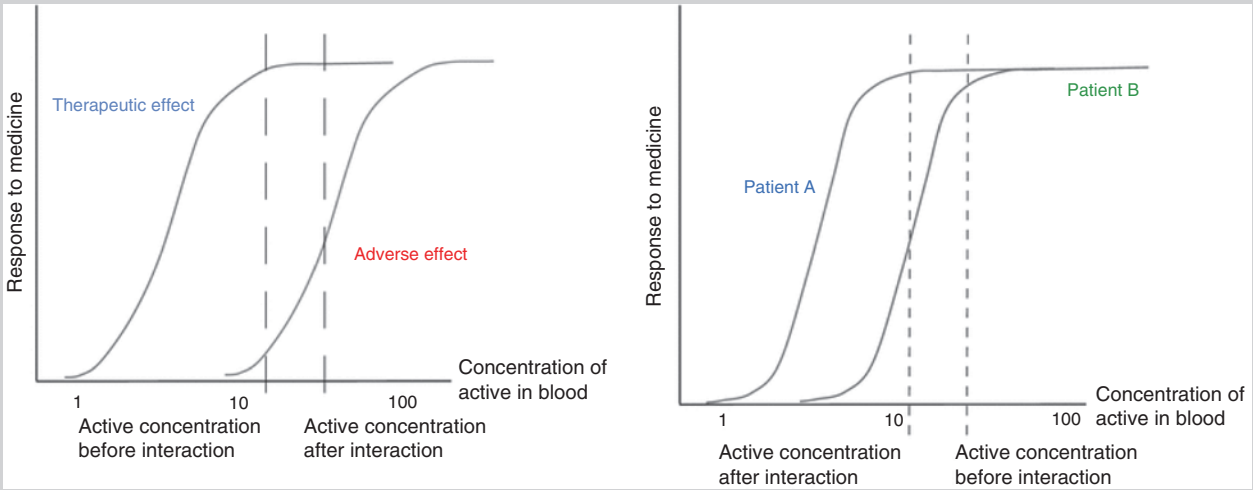

Fig. 5.3 Relationship between response to a medicine and blood concentration. The *left* figure shows the effect of an increased absorption on efficacy and safety. The *right* figure shows the effect that variation in bioavailability may have in patients with different sensitivity for an active substance

5.1.4 Solubility, Dissolution and Partition Coefficient

Solubility (in aqueous and lipophilic solvents), dissolution (in aqueous solvents) and partition coefficient are major determinants of the performance of a medicinal product in terms of bioavailability, efficacy and safety. The fundamentals of solubility and dissolution (rate) are described in Chap. 6.

For many medicinal products, dissolution in an aqueous environment (e.g. the fluids of the gastro-intestinal tract or mucosal lining fluids in the airways) is the major release mechanism of the active substance. The dissolved concentration of the active substance is the driving force for all diffusion-based drug transport mechanisms, since only the dissolved active substance is able to pass the absorbing membranes. Therefore the aqueous solubility of an active substance and the dissolution rate of the active substance from a medicinal product are considered to be highly rele-

vant characteristics. Because many biological fluids (such as the content of the intestinal lumen) are aqueous and the (mucosal) membranes possess a lipophilic nature, the active substance should present amphiphilic properties. The active substance must first dissolve into a hydrophilic environment and then diffuse through the lipid membranes. This route of absorption is called the transcellular route. Next to this route, several small highly hydrophilic drugs can pass membranes via the tight junctions that occur in the mucosal membrane. This so-called paracellular route offers a fully aqueous pathway for drug absorption, but is limited to molecules with a molecular mass below 600 Da and absorption via this route is generally incomplete.

The solubility of an active substance and the dissolution rate of a medicinal product can be varied by changing either the characteristics that relate to the active substance (such as particle size, salt form, crystalline form, the use of a pro-drug like an ester) or the characteristics of the medicinal product (such as the use of disintegrants, complex-forming agents like cyclodextrins or polymers that form highly viscous gels, and the application of diffusion limiting coatings). See the box for examples.

There are numerous examples of medicinal products in which dissolution rate enhancing technologies are applied to increase the bioavailability or absorption rate of an active substance. Cardiac glycosides should be given as micronised particles in a solid oral dosage form because otherwise their dissolution rate and hence their bioavailability is too low. Piroxicam was shown to be absorbed faster when given as a β-cyclodextrin complex, which increases the dissolution rate. Similarly, the bioavailability of albendazole as a cyclodextrin complex was increased compared to crystalline non-complexed albendazole, based on the same mechanism. And finally, the bioavailability of amorphous chloramphenicol is higher than that of crystalline chloramphenicol.

Biological factors may change the solubility or dissolution rate of the active substance from the medicinal product as well. The residence time in the stomach may increase the dissolution of poorly water-soluble active substances and change their bioavailability. The pH in the stomach or the intestine may influence the dissolution rate of acidic or basic active substances whose solubility is pH-dependent. Bile salts may increase the dissolution rate and thereby the absorption of poorly water-soluble active substances such as ciclosporine, phenytoin, levothyroxine and tacrolimus. Though, it has been shown that the association with bile acids reduces the absorption of the hydrophilic beta-blocker atenolol.

It is not only the solubility in aqueous solutions that may affect the biopharmaceutical behaviour of an active substance. The solubility in non-polar solvents is of importance too. The solubility in a lipid phase is of relevance for the passive transport of the substance over lipid membranes, a process that plays an important role in the absorption of many drug compounds. In order to quantify the lipophilicity of an active substance in relation to its aqueous solubility the concept of the partition coefficient was developed. The partition coefficient is defined as the quantitative distribution ratio of a dissolved substance over two immiscible liquids at equilibrium. In the pharmaceutical sciences the ratio of the concentrations in an aqueous phase (water or aqueous buffer solutions) and a lipid phase (e.g. n-octanol) is often considered. For this purpose the so called log P value has been defined. The partition coefficient between an aqueous and a lipid phase (log $P_{o/w}$ value) of non-ionised substances is defined according to Eq. 5.1.

$$\log\ P_{o/w} = \log\ \left(C_{so} / C_{sw}\right) \tag{5.1}$$

In this equation C_{sw} is the saturation concentration of the active substance in the aqueous phase and C_{so} is the saturation concentration in the lipid phase. In general, the partition coefficient between an aqueous buffer or water and n-octanol is determined.

For active substances that can be ionised the distribution coefficient (log D) has been defined. The oil-water distribution coefficient (log $D_{o/w}$ value) for a substance is presented in Eq. 5.2.

$$\log\ D_{o/w} = \log\left(C_o / \left(C_w^{\ i} + C_w^{\ n}\right)\right) \tag{5.2}$$

In this equation C_o is the concentration of the substance in the lipid phase, $C_w^{\ i}$ is the concentration of the ionised substance in the aqueous buffer at a specific pH and $C_w^{\ n}$ is the concentration of the non-ionised substance at the same pH. From the definition it follows that the distribution coefficient varies with the pH of the aqueous buffer. Thus, log D is always calculated for a given pH and can be calculated from the log P of the drug.

$$\text{For a weak acid}: \log D_{pH} = \log P - \log\left(1 + 10^{(pH\ pKa)}\right) \tag{5.3}$$

$$\text{For a weak base}: \log D_{pH} = \log P - \log\left(1 + 10^{(pKa\ pH)}\right) \tag{5.4}$$

5.1.5 Absorption and Bioavailability

Poor aqueous solubility or a low dissolution rate or both may be the cause of poor bioavailability. For poorly water-soluble active substances, the amount and composition of endogenous aqueous fluid present at the site of absorption will play an important role. In the stomach and in the small intestine there is ample aqueous fluid available, but in the mouth, the nose or the rectum a volume of only a few millilitres is available at most.

5.1.5.1 Absorption

After being released from the formulation and dissolved, the next step for the active substance is the absorption, passing a biological membrane. The surface area and type of membrane are major determinants for the extent and rate of absorption. Active substances may be transported over the membrane passively or actively. Absorption via passive transport may occur as paracellular transport through the interstitial spaces (tight junctions) between the cells lining the absorptive membrane. For absorption by passive diffusion across a biological membrane, the concentration gradient is the driving force according to Fick's diffusion law [2]. A high external (or luminal) concentration (dissolved active substance at the site of absorption), will increase the absorption rate over the biological membrane. The continuous blood flow in combination with protein binding of the active substance will keep the internal concentration low and maintain the concentration gradient. During a meal the blood flow is physiologically increased, having a positive effect not only on the absorption of nutrients but also on the absorption of medicines.

This transport mechanism is typical for the absorption via mucosal membranes such as the intestinal tract, the intra-oral (sublingual or buccal), nasal or pulmonary membranes. The size of the interstitial spaces in mucosal membranes may vary in size between around 0.4 nm for the duodenum up to about 4 nm for the alveolar membranes in the lung. In general, the absorption through the interstitial space is limited to small molecules that are soluble in water such as nitroglycerin after sublingual administration. The absorption of smaller proteins (up to a molecular mass of 20 kDa) via the alveolar membranes in the lung is an exception to this rule. The larger interstitial spaces between the alveolar type I cells (covering 93% of the alveolar surface) enable what may be the only non-parenteral route for protein administration.

Passive transport of lipophilic substances may occur via the fluid bilayer membrane of the cells lining the membranes. This transcellular route is the most important route for membrane passage of active substances. This transport mechanism is not only driven by the concentration gradient over the absorptive membrane, but also by the oil to water partition coefficient of the active substance (expressed as the octanol-water partition coefficient, describing the ratio of the substance's solubility in aqueous and fatty phases). More lipophilic substances will be transported faster over the lipophilic absorption membrane than those with a more hydrophilic character. In general, lipophilic active substances will not accumulate into the lipid parts of the absorption membrane because the continuous blood flow in combination with protein binding of the active substance will keep the internal concentration low. This will also maintain the concentration gradient at a maximum, causing rapid uptake of the substance into the systemic circulation.

The lipophilicity of an active substance, and thereby its tendency to pass through lipophilic membranes, can be described in terms of the octanol-water partition coefficient and the polar surface area. The octanol-water partition coefficient, the log P value, should be within the 0.4–5.6 range and preferably between 1 and 3. The polar surface area of a compound is the total sum of the surface of the polar atoms in a molecule. In general this will refer to the oxygen and nitrogen atoms in a molecule and include the hydrogen atoms attached. Co-inclusion of sulfur and phosphor atoms in the calculations was shown to add little to the predictive value of the results. When the polar surface area of a molecule exceeds 140 $Å^2$ the molecule is too hydrophilic and considered to be unsuitable for absorption after oral administration. For special barriers such as the highly lipophilic blood brain barrier the threshold is lower, 60 $Å^2$.

Apart from the partition coefficient and the polar surface area, several other molecular characteristics predict membrane permeation; major determinants are:

- Molecular mass, which is preferably below 300–500 Da;
- Number of H-bond donors and H-bond acceptors, not more than 3 donors and not more than 3 acceptors;
- Number of rotatable bond bonds, not more than 3;
- Number of non-hydrogen atoms.

Furthermore, a limited number of active substances are absorbed by active transcellular transport mechanisms. These processes are energy-requiring and may even occur opposed to a concentration gradient. The mechanism is characterised by the involvement of transporter enzymes able to transport the molecules of the active substance over the intestinal membrane. An example is the absorption of angiotensin converting enzyme inhibitors such as lisinopril and enalapril, which is mediated by the di/tri-peptide transporter protein (PepT1). Competition for or induction of transport enzymes may have a significant effect on the absorption of active substances serving as a substrate. This makes these processes sensitive for drug-drug or drug-food interactions that may significantly affect drug absorption, and thereby drug efficacy and safety.

5.1.5.2 Bioavailability

The bioavailability of an active substance is defined as the fraction of the administered active substance that enters the systemic circulation (for oral administration the circulation beyond the liver) in unchanged form. The absolute bioavailability of an active substance is in simplified form (see Sect. 5.1.2) calculated from the area under the curve (AUC) of the blood concentration of unchanged drug versus time profile found upon intravenous administration (by definition 100% bioavailability) and the AUC found following the chosen administration route, corrected for the dose (D). The bioavailability after, for example, oral administration (F_{or}) can be calculated from Eq. 5.5.

$$F_{or} = \left[\left(AUC_{or} \cdot D_{iv}\right) / \left(AUC_{iv} \cdot D_{or}\right)\right] \cdot 100\% \qquad (5.5)$$

Differences in bioavailability of different medicinal products or after administration via different routes can also be deduced from the ratio of the AUCs of the blood concentration versus time curves.

In addition to the total absorbed fraction, the absorption rate is an important aspect of bioavailability. The absorption rate is characterised from the maximum blood concentration and the time at which the maximum concentration occurs in the concentration versus time curve.

The absorption rate of an active substance is relevant to the therapeutic effect for various reasons. If an active substance is used in an acute situation (e.g. epileptic insult, asthmatic attack, sleep induction, pain killing), a high absorption rate is preferred. A formulation design in which the active substance is already dissolved, may be useful in such case. A prolonged effect can be achieved by a dosage form from which an active substance is released slowly (sustained release). Finally, if an active substance has a longer residence time in the absorbing organs, the bioavailability may be negatively influenced by, for example, enzymatic or chemical degradation.

5.1.5.3 Dose Number and Biopharmaceutical Classification System

The solubility of an active substance in the different body fluids and the efficacy of the absorption process, together with the dose, determine the bioavailability of a medicine. The dose number (DN) is a dimensionless parameter that links solubility (C_s) to the maximum dose given in one administration (D) and volume available for dissolution (V) during the absorption process. The dose number can be calculated from (Eq. 5.6):

$$DN = D \,.\, \left(C_s .\, V\right)^{-1} \qquad (5.6)$$

$$DN = \frac{D}{C_s . V}$$

If the dose number below 0.1 dissolution is not expected to affect the absorption process, whereas at a dose number above 10 dissolution is most likely to decrease the absorption (rate) of an active substance. In general, solubilisation technology has to be applied for such compounds. If the dose number is between 0.1 and 10 the effect of dissolution on drug absorption should be investigated, to find out whether bioavailability is affected by the dissolution behaviour.

The corticosteroid dexamethasone has a poor aqueous solubility of 89 microgram/mL. It is given by the oral route for various diseases. When the medicine is given with a glass of water the oral route offers a dissolution volume of about 500 mL in the stomach. When the 4 mg dose used in rheumatic diseases is given by the oral route the dose number will be 0.09 and no major dissolution problems are to be expected. In contrast, when dexamethasone is used in pyodermia gangrenosum the dose has to be as high as 300 mg. At this dose, the dose number will be 6.7. This is close to 10 and the dissolution behaviour of the medicinal product has to be investigated in order to prevent incomplete absorption of the dexamethasone.

The biopharmaceutical classification system (BCS) not only considers solubility but also the permeability of the absorbing membrane. The BCS classifies active substances based on their water-solubility in relation to their dose and the oral route and to their permeability over the absorptive membranes. The BCS divides active substances into four classes:

- Class I: high solubility, high permeability
- Class II: low solubility, high permeability
- Class III: high solubility, low permeability
- Class IV: low solubility, low permeability

This classification system is used to characterise potential problems related to bioavailability and bioequivalence of an active substance [3].

The variable solubility is determined by the intrinsic solubility of the active substance in the aqueous fluid available for dissolution, the dose given and the volume of liquid available for a certain route of administration. A medicine that is administered orally will have a larger volume of liquid available (250–750 mL) for dissolution than a medicine that is administered via the sublingual or rectal route (5–10 mL) and the composition of the different fluids varies. As a result, dissolution problems are to be expected for the sublingual or rectal route at lower doses than for the oral route. In general an active substance is considered to be highly soluble when the highest dose applied dissolves in about one third of the total available volume for dissolution. For the oral route, for example, an active substance is considered highly soluble when the highest dose dissolves in 250 mL of an aqueous liquid over the entire pH range between 1.0 and 7.5.

The variable permeability is determined by the absorption of the dissolved active substance over the membrane. This may be measured by the direct assessment of the mass transfer (rate) over the human intestinal membrane or predicted from relevant animal models or *in vitro* epithelial cell culture models. An active substance is considered highly permeable when the extent of absorption is 90% or more.

Bioequivalence

Knowledge of the bioavailability and absorption rate of an active substance is important because these are important determinants of efficacy and safety. Large variations in bioavailability or absorption rate may, in the case of decreased absorption (rate), result in insufficient efficacy. In the case of unexpectedly high absorption it may lead to toxicity or serious adverse effects. Quantitative data on the bioavailability and absorption rate are necessary to evaluate the equivalence or non-equivalence between different medicinal products with the same active substance. These data may be used as surrogate parameters to establish efficacy and safety of a (generic) product.

According to the EMA two products are considered to be bioequivalent when they contain the same active substance and when their respective bioavailabilities (rate and extent) after administration in the same molar dose and via the same route, lie within acceptable predefined limits. These limits are set to ensure comparable *in vivo* performance, i.e. similarity in terms of safety and efficacy. The design and number of studies that is to be carried out to establish bioequivalence depends on the physico-chemical and pharmacokinetic properties of the active substance. In this respect reference is made to the BCS classification of an active substance. For BCS class I active substances it may even be possible to obtain a waiver for the *in vivo* studies (a so-called biowaiver), whereas for the active substances showing more complex pharmacokinetic behaviour extensive studies are to be carried out. In general bioequivalence will be determined from the parameters C_{max} and AUC. Two products are considered to be bioequivalent when the 90% confidence interval of the ratio of test and reference product falls within the 85–125% acceptance interval. However, for the required design of the bioequivalence study and for statistical evaluation details for a specific active substance reference is made to the appropriate (most recent) guideline on this subject [4, 5].

When in daily practice substitution (e.g. by a generic product) is considered, bioequivalence is of course the first parameter to evaluate. However, other aspects of medicine's use related to both the product and the condition of the patient are to be taken into consideration as well.

Among these are:

- For active substances having a narrow therapeutic window or showing non-linear pharmacokinetics substitution is not advised since even the smallest variations in the product and its performance may result in significant fluctuations in effect or safety. Examples of such active substances include: ciclosporin, tacrolimus, digoxin, ergotamine, levothyroxine, capecitabine and phenytoin.
- Safety issues to be considered include the exact equivalence of the product (especially for 'biologicals' this may pose a problem) and the risk of allergy or intolerance for a specific excipient (e.g. colouring agents).
- When specific administration devices (affecting parameters of relevance to the performance characteristics such as efficacy and safety of a medicinal product) are used, substitution should not occur. Different dry powder inhalers, for example, generate aerosols of significantly different particle size. As a result, relevant variations may occur in lung deposition of the active substance.
- The appearance of the product. When large differences in appearance between both products exist, substitution may confuse the patient and affect patient compliance.
- The performance characteristics and handling of both medicinal products should be similar (e.g. for auto-injectors, or the occurrence of a score on the tablet that allows for dividing a dose).

Medicinal products administered at a specific site to obtain a local effect should preferably not be absorbed systemically. However, significant amounts of active substance can be absorbed, e.g. after application on the skin. Removal of locally acting active substances from the site of action by systemic absorption may result in systemic effects that can be considered as adverse effects. After nasal, pulmonary and rectal administration of active substances for a local effect, absorption into the systemic circulation is likely to occur. This may cause adverse effects and limit the duration of the

desired medicinal effect. Conversely it should be realised that the systemic route is often also the main route for clearance of the active substance from the site of administration. The bioavailability of locally acting medicines is, of course, not determined by the amount of active substance that reaches the systemic circulation. As an alternative, the fraction of the active substance that is dissolved in any aqueous fluid at the site of application (e.g., water phase of a cream, sweat on the skin under a fatty ointment) is usually taken as a measure for the bioavailability.

5.1.6 Excipient and Food Interactions

An active substance, although initially released from its dosage form (and dissolved), may become unavailable for absorption due to reactions with other medicines or food components [6]. An example is the formation of insoluble complexes of tetracycline with calcium or aluminium ions from antacids or dairy products. Interaction (chelation or binding) with iron ions leads to a reduced absorption for a variety of active substances such as doxycycline, penicillamine, methyldopa and ciprofloxacin. The absorption of active substances showing pH-dependent dissolution behaviour may be influenced by medicines that influence the gastric pH, such as H_2-antagonists, proton pump inhibitors and antacids. Antimycotic active substances such as ketoconazole or itraconazole dissolve better in acidic fluids. Therefore their bioavailability may be increased by the concomitant use of an acidic drink like cola, whereas the concomitant use of antacids or proton pump inhibitors is likely to reduce the bioavailability. Concomitant use of milk may increase the dissolution of acidic active substances, whereas fats from food may increase the bioavailability of lipophilic active substances like albendazole and griseofulvin.

In dermal preparations ion-pair formation between ionic surfactants and an active substance may reduce the local availability of the active substance. An example is the interaction between the anion laurylsulfate (from the surfactant sodium laurylsulfate) and the cationic neomycine or tetracycline (as hydrochlorde salts) in creams.

It is important that the (clinical) pharmacist always provides appropriate advice to the patient about how to take a medicine and warns against the concomitant use of other medicines or certain foods, in cases where interactions may occur. The potential risk of interactions may even be of greater clinical importance when they occur at the level of the metabolism of the active substance. This issue is discussed elsewhere in this chapter.

5.1.7 Stability of the Active Substance in the Physiological Environment

Several active substances are unstable in an acidic environment and will degrade when in contact with gastric juice. Examples are omeprazole, pantoprazole, erythromycin and pancreatic enzymes. Such active substances are formulated in a tablet or pellets covered with an acid-resistant layer, a so-called enteric coating. The acidic nature of the polymers used in these coatings prevents dissolution in the gastric environment. The coating will dissolve in the small intestine (with higher pH values), after which the active substance is released. Alternatively, the active substance may be used in the form of a more stable salt, for example erythromycin ethylsuccinate.

Enteric coated products exist in various presentation forms. They include tablets surrounded with the coating, coated pellets in a capsule or coated pellets compressed into a tablet (see Chap. 12). This last form sets specific requirements to the quality of the coating around the pellets since the integrity of this coating must not be compromised during the tablet compaction process. Depending on the presentation form, tablet breaking may or may not be allowed. Tablets covered with an enteric coating may never be broken, whereas controlled release tablets produced from coated pellets may be divided or sometimes even dispersed for a short period of time in liquids or semisolid food (custard, yoghurt). The same can be done with capsules containing coated pellets. However, coated products should never be exposed for longer than 5 min to drinks or food before use. Crushing is not allowed for any controlled release formulation.

Metabolic instability of the active substance in the physiological environment may reduce bioavailability especially after oral administration. Enzymatic degradation of an active substance in the lumen of the stomach or intestine may reduce the amount of active substance available for absorption, whereas after absorption enzymes in the intestinal wall and the liver may reduce the bioavailability. This is called the first-pass effect.

After intramuscular or subcutaneous injection, bioavailability may become reduced because of enzymatic breakdown of the active substance at the site of injection.

5.1.8 First-Pass Effect

Following oral administration and absorption from the intestine, an active substance passes the intestinal wall and the liver. During uptake from the gastro-intestinal tract, enzymes present in the intestinal lumen or in cells of the intestinal

wall can metabolise an active substance. After absorption, the active substance is transported to the liver by the portal vein system. During the first passage through the liver, liver enzymes may metabolise another part of the active substance. The metabolic degradation during the first passage of intestine and liver may significantly reduce the bioavailability since it occurs before the active substance is distributed over the entire body. This particular loss of active substance, after absorption but before distribution, is called the first-pass effect or pre-systemic metabolism.

The extent of a first-pass effect of an active substance depends on the dose and absorption rate in relation to the enzymatic capacity. Moreover, the first-pass effect can be influenced by the concomitant use of food, which may affect the absorption rate or induce or inhibit the enzymatic degradation of certain active substances. From this perspective it is clear that the bioavailability of an active substance can be optimised by taking a medicine at the appropriate time, e.g. before, during or after a meal. Examples of active substances that undergo a high first-pass effect after oral administration include: amiodarone, ciclosporin, 6-mercaptopurin, metoprolol, midazolam, morphine, nifedipine, propranolol, saquinavir, tacrolimus, terbutaline and verapamil.

The hepatic first-pass effect can be circumvented by giving a medicine through a route of administration that does not primarily absorb the active substance via the portal vein system, such as an intramuscular or subcutaneous injection, sublingual or nasal administration or via the pulmonary route. The rectal route is often mentioned as an option too. However, since two of the three rectal veins do end up in the portal vein, the maximum effect of this route will be a reduction of the first-pass effect by only about 30%.

5.1.8.1 First-Pass Metabolism and Controlled Release Products

In order to understand the relevance of first-pass metabolism for the formulation of controlled release products, one should have a basic understanding of enzyme kinetics. Enzyme kinetics are described using the Michaelis-Menten Eq. (5.7). It describes the rate of transformation of a substrate (the active substance) by an enzyme.

$$\nu = V_{max}.[S]/(K_m + [S]) \tag{5.7}$$

In this equation ν is the enzymatic reaction rate, V_{max} represents the maximum transformation rate (the rate achieved at complete saturation of the enzymes), [S] is the substrate concentration and K_m the substrate concentration at $\frac{1}{2}V_{max}$. Figure 5.4 shows the relationship between the substrate (active substance) concentration and the transformation rate.

At low concentrations an enzymatic system is able to efficiently break down the presented active substance. When the concentration of active substance (substrate) rises, the transformation rate will increase proportionally to the increase in concentration of active substance. As a result the relative amount of active substance that is metabolised will remain the same. However, as the concentration increases further (significantly beyond the K_m) the enzymatic system will become saturated and the increase in rate of transformation will no longer be proportional to the increase in concentration. As a result, relatively more active substance will escape from transformation by the metabolic enzymes.

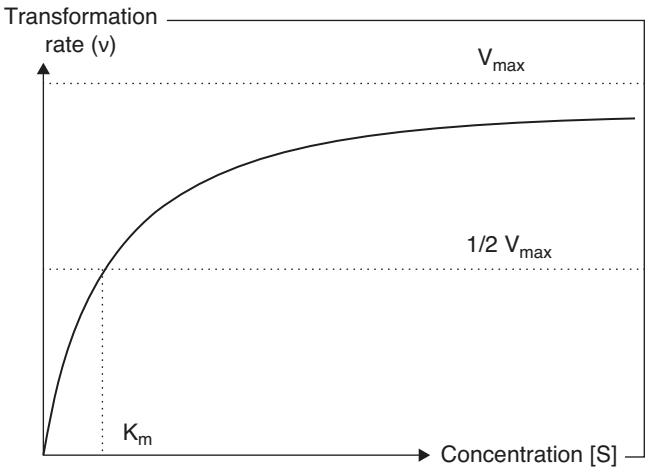

Fig. 5.4 Relationship between the substrate concentration [S] and rate of transformation [ν]

This is exactly what happens when an active substance suffering from high first-pass metabolism is given orally. After absorption from the intestinal tract the active substance is transported with the hepatic blood flow to the liver. The concentration in the hepatic blood determines the transformation rate and thereby the (relative) amount of active substance that will escape the metabolism and will become bioavailable. At lower concentrations the enzymatic system will be far from saturated and bioavailability will thus be low. If, in contrast, higher doses are given, concentrations levels far beyond the K_m may be present in the portal blood and a dose-dependent increase in bioavailability may occur (non-linear absorption kinetics).

If an active substance at a specific dose already generates portal blood concentrations around or even somewhat beyond the K_m even a minor increase in dose may already cause a significant increase in bioavailability. Be aware that even a new formulation with the same dose that dissolves slightly faster may result in a faster absorption and thus higher concentrations in the portal blood, which may lead to an increased bioavailability.

Conversely, if an immediate release product with a given dose yields a reasonable bioavailability (in spite of a significant hepatic first-pass metabolism) because the portal concentrations generated by the immediate release product are far beyond the K_m, one can easily image what the effect of a

slow release formulation would be. Even although the dose may be higher, the release of the active substance and the subsequent absorption are slower. Consequently, significantly lower concentrations will occur in the portal blood, the enzymatic system will be less saturated and lower amounts of active substance will escape from the enzymatic degradation in the liver. This may result in a significant reduction of the bioavailability. It is for this reason that caution should be exercised during the development of slow release products of active substances exhibiting a significant first-pass effect.

In Fig. 5.5 the effect of a variable dose on the relative bioavailability of an active substance with a high first-pass metabolism is presented as well as the effect of absorption rate of a certain dose on the bioavailability of an active substance with a high first-pass metabolism. It is assumed that the dissolution rate of the active substance is not affected by the dose.

The effects of dose and absorption rate on the absolute bioavailability are presented in Fig. 5.6.

The phenomena described above clearly illustrate the significant effects on bioavailability that may be caused by even small variations in absorption rate. Bioavailability is linked to AUC, which also depends on the total clearance. In linear pharmacokinetics bioavailability is considered proportional to the dose (compare Fig. 5.6). However, in non-linear pharmacokinetics, bioavailability increases or decreases faster than the dose rising because elimination is saturated or, on the contrary, is inducted. It should be realised that such small variations may easily occur upon formulation changes or due to food effects on dissolution and/or absorption rate.

5.1.9 Charge and the pH Partition Theory

Since the absorptive membrane is a lipophilic barrier, active substances are generally absorbed in a form that is able to pass lipophilic barriers. Therefore the difference in partition between the oil and water phase is a major driving force for the absorption process. Often the octanol-water partition coefficient is used to quantitate this (see Sect. 5.1.4). Active substances with a high partition coefficient will be absorbed rapidly once they are dissolved. Weak acids and weak bases are usually absorbed in their non-ionised form since this

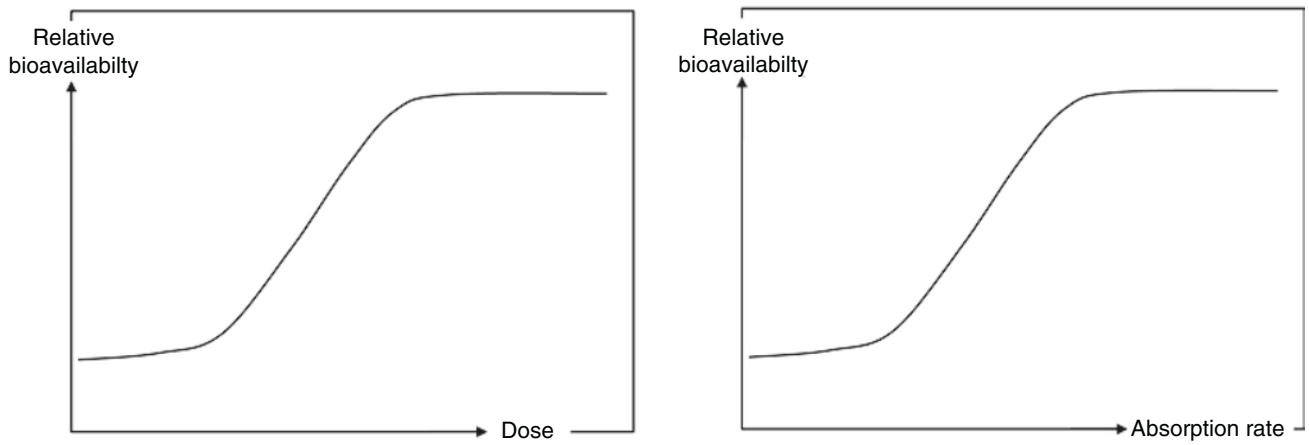

Fig. 5.5 Effect of the dose (*left*) and the absorption rate (*right*) on the relative bioavailability

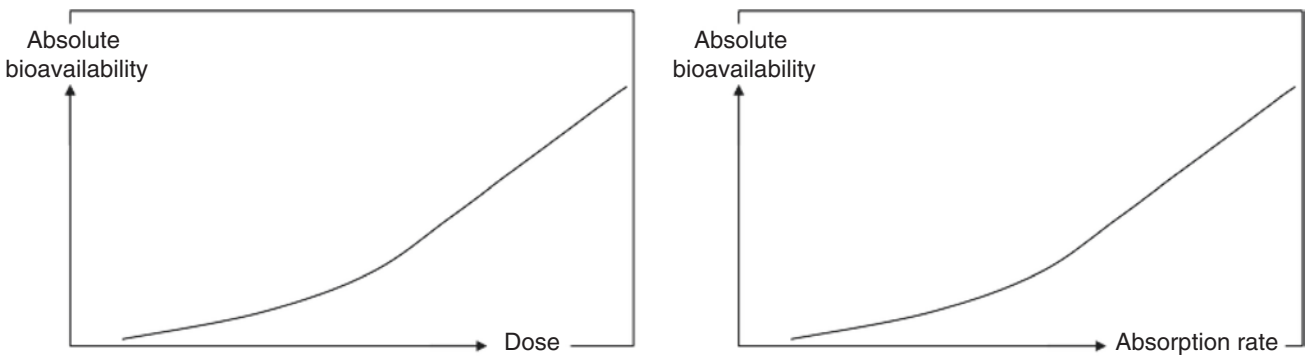

Fig. 5.6 Effect of the dose (*left*) and the absorption rate (*right*) on the absolute bioavailability in case of a linear pharmacokinetic process

form has a much higher partition coefficient than the ionised form as follows from the distribution coefficient. It is however worth to note that in some exceptional cases ionised active substances can be absorbed if they form ion pairs with contra-ions or if they use some active transporters. The driving force of the passive absorption process is the concentration gradient of the non-ionised active substance. The pH partition theory states that the non-ionised fraction of a weak acid or a weak base is determined by the pK_a of the active substance and the pH of the surrounding environment. The non-ionised fraction of an active substance can be calculated using the Henderson-Hasselbalch equation, which after rearrangement looks for an acid as:

$$\text{Non-ionised fraction} = \left[1 + 10^{pH - pKa} \right]^{-1} \tag{5.8}$$

For a base the equation is:

$$\text{Non-ionised fraction} = \left[1 + 10^{pKa - pH} \right]^{-1} \tag{5.9}$$

The Eqs. 5.8 and 5.9 show that for a weak acid the highest non-ionised fractions exist in an acid environment, whereas a more alkaline environment will result in a higher non-ionised fraction of a basic active substance. However, one should realise that the water-solubility of the ionised form is significantly higher than that of the non-ionised form. This contradiction causes one of the major problems in medicine formulation and bioavailability. An environment in which the majority of the active substance is ionised is required in order to obtain a fast dissolution, whereas an environment in which the active substance is largely non-ionised is required for rapid membrane passage.

Fully ionised active substances, for which no specific carrier is available in the membrane, are generally too hydrophilic to be absorbed from the gastro-intestinal tract. Not many quaternary ammonium compounds are available in a non-ionised form in the gastro-intestinal tract after oral administration due to their high pK_a. These active substances are therefore only administered as an injection. Other active substances for which ionisation may hamper absorption after oral administration are those containing multiple H-bond acceptors and H-bond donors with varying pK_a values. Some of these active substances may not exist in a non-ionised form at any of the pH values occurring in the gastro-intestinal tract, which may significantly reduce their bioavailability after oral administration. An example is the angiotensin II inhibitor eprosartan (chemical structure shown in Fig. 5.7): an active substance with two carboxylic acid and two amine functions. The oral bioavailability of eprosartan is only 13–15%. The limited oral bioavailability is explained by both the pH-dependent dissolution behaviour of eprosartan in combination with the degree of ionisation of the dissolved active substance at the different pH values encountered in the gastro-intestinal tract.

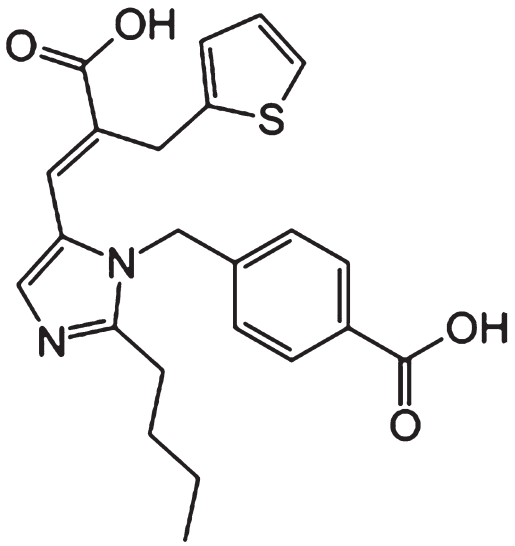

Fig. 5.7 Chemical structure of eprosartan

It is also important to know that the acid-base equilibrium adjusts rapidly. If a non-ionised form of an active substance has a high partition coefficient, this may compensate for the presence of lower fractions of non-ionised active substance at the absorption site. Due to the fast absorption of the small fraction of non-ionised active substance in the vicinity of the absorbing membrane a continuous de-ionisation of ionised active substance will occur as a result of the continuous equilibrium adjustment. In this way even active substances with somewhat lower non-ionised fractions at the absorption site (active substances possessing only a single ionisable group) may still show a considerable absorption rate.

After being taken up over the membrane the second step in the active substance absorption process is the transport of the active substance into the blood. Again the concentration gradient and partition coefficient will be the rate determining factors for passive transport. However, even for lipophilic active substances the transport to the blood will be rather fast. This is caused by the continuous refreshment of the blood (creating so-called sink conditions in most cases (except for e.g. an active compound with a log P over 4), keeping the concentration difference at a maximum, and by the binding of the active substances to proteins and cells in the blood. Therefore transport of active substances from the absorption membrane into the blood will generally not be the rate-limiting step in the absorption process. During meals, the blood flow in the intestine is enhanced, allowing a better passive absorption process by maintaining the gradient between the lumen of the intestine and the blood.

5.1.10 Distribution

Active substances are often distributed over the body and tissues via the blood. Interactions at the level of protein binding in blood are possible, for instance with another active substance competing for binding sites on the protein. This may result in different active substance concentrations in various tissues since the free concentration in the blood determines the transport into the tissue (or to the site of action). If the free concentration increases, the therapeutic efficacy may increase but it may also lead to toxic side effects. However, clearance may also be increased when the free active substance concentration is increased. For active substances with a narrow therapeutic window such as valproic acid or carbamazepine, this type of interaction must be taken into account. However, after having gained a newly established equilibrium, the concentration of free active substance, responsible for the activity and subject to biotransformation, usually, hardly changes (as a result of the increased clearance). Most interactions on the metabolic level are caused by enzyme inhibition or induction (see elsewhere in this chapter).

5.1.11 Clearance

Many active substances are eliminated from the body after biotransformation. The metabolites are subsequently cleared via one of the excretion routes or further metabolised to products that can be excreted. The major routes of excretion encompass glomerular filtration followed by excretion into the urine, excretion of the active substance or its metabolites into the faeces (via the liver and bile, biliary excretion) or via the exhaled breath. Biotransformation and glomerular filtration are the major routes of elimination for most active substances. The bioavailability of an active substance can therefore be influenced by interaction with other active substances (or food components) that inhibit biotransformation enzymes or change the glomerular filtration rate, or by genetic polymorphisms.

In case an active substance is excreted via the biliary route a so-called enterohepatic circulation may occur. This means that the active substance is removed from the circulation by the liver and (after glucuronidation) excreted via the bile into the lumen of the intestinal tract. In the intestinal tract (after enzymatic or microbial deglucuronidation) the active substance can be absorbed again into the systemic circulation via the intestinal membrane. This effect will usually be seen as a second peak in the blood concentration versus time curve. The effect that this phenomenon could have on the AUC of a medicine should be taken into account when the bioavailability is determined since it may seriously compromise the outcome of the calculations. Clearance is proportional to the blood flow entering into the elimination organ and the extraction coefficient. Thus, clearance can be understood as a flow rate of elimination; its unit may be mL/min. For example, a hepatic clearance of 15 mL/min means that 15 mL of blood going across the liver are ‘cleaned’ from the active drug every minute.

5.1.12 P-Glycoproteins

P-glycoproteins (P-gp) are membrane-bound transporters that are able to actively remove active substances and other xenobiotics from the cell. P-gp are energy-dependent and present in many tissues, including the blood-brain barrier, the intestinal wall, the liver and the kidney, and responsible for the active removal of among others, antineoplastics [7]. Typical substrates for P-gp are digoxin, metronidazole, saquinavir, talinol, calcium antagonists.

P-gp are mainly known for their capacity to remove active substances from a cell once they are absorbed. Many active substances are known to be excreted again by P-gp mediated transport into the intestinal lumen after being absorbed first. This may significantly reduce their bioavailability. The bioavailability of such active substances may be significantly increased when they are administered together with other compounds (active substances) that are a substrate for P-gp transporters. For example, the bioavailability of the antiretroviral medicine saquinavir is largely enhanced when it is administered together with a P-gp inhibitor such as ritonavir, also being a substrate for P-gp and competing for its binding places. It should be noted that P-gp substrates are usually also substrates for cytochrome P450 3A4. Furthermore, ritonavir increases the saquinavir tissue concentrations in the central nervous system, since it also inhibits the effects of the P-gp in the blood-brain barrier.

5.1.13 Drug Metabolising Enzymes

Biotransformation of active substances largely occurs through the action of cytochrome P450 enzymes, a large group of mono-oxygenases located primarily in the liver and the intestine. They are responsible for oxidative metabolising steps, often preceding glucuronidation which is followed by urinary or biliary excretion. These enzymes can be inhibited or, conversely, induced by certain active substances (or by food substances). On the one side, enzyme inhibition will increase the bioavailability of active substances that are metabolised by these enzymes, which may cause toxicity. On the other side, enzyme induction will reduce bioavailability and increase systemic clearance, which in turn may reduce the therapeutic efficacy.

The inhibition or induction of cytochrome P450 subtype 3A4 (CYP 3A4) is clinically relevant, because a variety of

active substances and food substances (e.g. grapefruit juice) are able to affect this enzyme. Substances inhibiting CYP 3A4 include: ciclosporin, dihydropyridines, verapamil, midazolam, paclitaxel, simvastatin, lovastatin, atorvastatin, cimetidine, erythromycin, troleandomycin, ketoconazole (and other azoles). Substances inducing CYP 3A4 include: steroids, rifampicin, phenobarbital and St John's wort. CYP 3A4 is present in the enterocytes and in the liver cells. Other isoforms sensitive to induction and inhibition such as 2D6 or 2C19 are only present in the liver.

Drug metabolising enzymes can be over- or underactive in different individuals and between populations of different ethnic backgrounds. Genetic polymorphism may be the underlying feature. Polymorphisms with relevance for drug metabolism are found for N-acetyltransferases and for cytochrome P450 subtypes 2D6 and 2C19.

Herb-Drug Interactions

Herb-drug interactions may be clinically relevant. They may be responsible for failure or regular therapy as well as for toxicity [8, 9]. The dosage and the duration of treatment with herbal preparations should be taken into account to assess the possible interaction risks with regular pharmacotherapy. Special attention should be given to active substances with a narrow therapeutic window when combined with herbal medicines.

Pharmacokinetic herb-drug interactions occur when an herbal preparation changes absorption, distribution, metabolism or excretion of a medicine. Interactions at the level of metabolism, comprise the inhibition or induction of different cytochrome P450 enzymes, glucuronosyltransferases (UGTs) and P-glycoproteins. Herbal drugs rich in saponins and herbal products that alter the gastric pH or influence intestinal motility may influence dissolution and absorption. Finally, absorbed plant constituents may influence plasma protein binding of medicines and hence their distribution over the body and the clearance.

Furthermore, pharmacodynamic herb-drug interactions may occur. These can be the result of a similar or antagonistic action of an herbal preparation and a medicine, changing cellular response or receptor affinity. Knowledge of the pharmacological properties of plant constituents may help to predict such interaction.

Active substances that are considered to be most sensitive to interaction with herbal preparations include oral anticoagulants, cardiac glycosides, oral contraceptives, anti-epileptics, antidepressants and antihypertensives. Typical herbal products that may influence their activity are St John's wort, ginkgo, ginseng, garlic, red sage and laxatives (bulk formers and anthraquinones). Note that the composition of the particular herbal preparation should always be considered as the profiles of different extracts from the same plant may show considerable variation [10].

5.1.14 Slow Release and Flip-Flop Pharmacokinetics

Slow release technology is generally applied to active substances that are connected with:

- Long-term or chronic therapies.
- Large and undesired differences in peak and trough blood levels, leading to adverse effects or to periods with no or suboptimal therapy, respectively.
- Non-linear pharmacokinetic behaviour, meaning that the blood concentration increases are not proportional to increasing dose. This may lead to toxic blood levels. Examples of active substances with non-linear pharmacokinetics include: nitrates, nifedipine, fentanyl, theophylline, paclitaxel and lithium carbonate. Non-linear pharmacokinetic behaviour may be due to saturation of pre systemic enzymatic elimination mechanisms at increasing concentrations of the substrate.
- High dosing frequency (three or more times daily) due to their rapid elimination or excretion. Under normal circumstances the elimination will be slower than the absorption rate of the active substance. As a result of this difference, the elimination is reflected in the descending part of the blood concentration versus time curve. A fast elimination rate however would require it to be administered several (more than three to four) times a day. Slow release of the active substance from the dosage form (e.g. a slow release tablet) will cause the absorption rate to become slower than the elimination rate. As a consequence the elimination phase in the blood concentration versus time curve no longer reflects the elimination rate, but rather the absorption rate of the active substance, whereas the initial rising phase in the curve is a reflection of the elimination rate of the active substance. This phenomenon is known as 'flip-flop pharmacokinetics' [11].

When the occurrence of flip-flop pharmacokinetics is not recognised it may compromise the interpretation of results from studies on the pharmacokinetic behaviour of slow release dosage forms. Flip-flop pharmacokinetics may occur with any extravascularly administered parenteral slow release dosage form. Intramuscular depot injections of antipsychotics such as fluphenazine decanoate, haloperidol decanoate or flupenthixol decanoate show this behaviour. It also occurs after the administration of oral slow release

products of active substances such as isoxuprine, carbamazepine, diclofenac, valproic acid, morphine and theophylline.

5.2 Dosage Forms and Routes of Administration

For the administration of medicines, various routes and dosage forms are available. The choice for the most suitable route and dosage form is complex and affected by a large number of interacting factors. Among these are aspects related to the physico-chemical characteristics of the active substance, the pharmacodynamics and pharmacokinetic characteristics of the active substance and various patient related aspects. This section gives, per route of administration, the basics for handling of the interacting factors. Chapters 12–24 discuss the practical consequences for product design and formulations for the respective administration routes.

5.2.1 Parenteral Administration

Intravenous administration delivers an active substance immediately into the systemic circulation. It is often considered the fastest way to obtain a therapeutic effect. By definition, the bioavailability of an active substance given intravenously is 100%. A typical characteristic of the blood concentration (as measured in whole blood, in serum or in plasma) versus time curve of intravenously administered active substances is the absence of the absorption phase (Fig. 5.8). Any other route of administration would show an absorption phase (see Fig. 5.1) but immediately after intravenous injection the blood concentration is at its maximum. This is usually followed by a short distribution phase and the elimination phase of the active substance; both processes will reduce the blood concentration of the active substances.

The duration of the action depends on the dose, the duration of administration (in case of an intravenous drip or infusion), the distribution, metabolism and excretion of the active substance. Constant blood levels can be achieved by a continuous infusion. By regulating the rate of the infusion, the height of a blood level can be adjusted and maintained at a constant level without fluctuations. This may be important for active substances with a narrow therapeutic window.

The blood concentration versus time profile shown in Fig. 5.8 is typically found after the injection of an aqueous solution of the active substance. When lipophilic compounds are administered as an oil-in-water emulsion the profile may be significantly changed, since the transport of the active substance over the oil-water interface may limit the distribution or elimination rate.

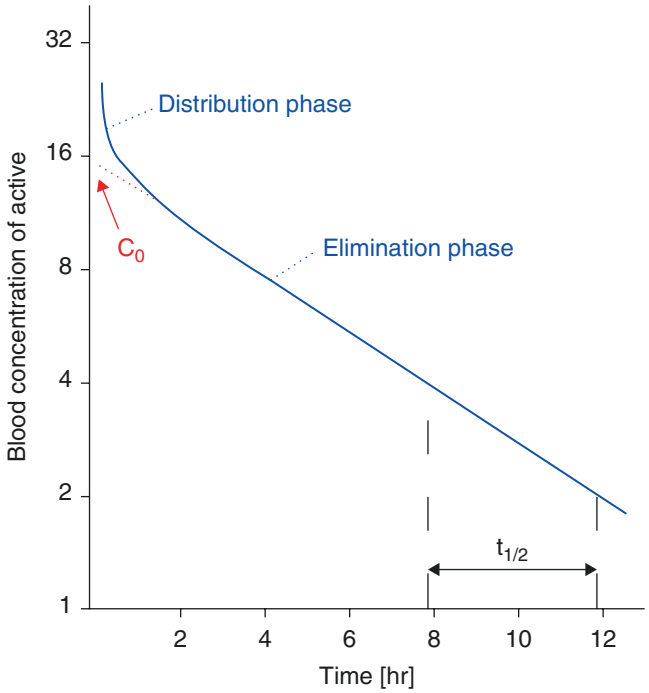

Fig. 5.8 Blood concentration versus time curve obtained after intravenous injection. Extrapolation of the elimination phase to the intersection with the y-axis gives the virtual C_0 value. This is the theoretical concentration of the active substance immediately after administration assuming that it is distributed over the full volume of distribution for the particular active substance. The C_0 can, together with the dose, be used to calculate the volume of distribution ($V_d = D/C_0$)

By intramuscular administration, the medicine is injected into muscle tissue, through the skin and the subcutaneous fat layer. Suitable muscles for intramuscular injection are the upper arm and shoulder muscle (musculus deltoideus), thigh muscle (musculus vastus lateralis) and buttocks muscle (musculus glutaeus maximus). There are differences in blood flow between these muscles and hence also in the extent and rate at which an active substance is absorbed from these sites. Activity of the muscle and physical movements (e.g. horse riding after an injection in the buttock muscle) will also strongly affect the absorption of the active substance from the injection site.

By subcutaneous administration, the medicine is injected into the subcutaneous connective tissue. These injections are experienced as more painful than intramuscular ones. Suitable places are the thigh and the belly pleat. The absorption after subcutaneous injection varies in rate and extent depending on the site of injection and on patient specific factors such as the amount of subcutaneous fat and physical activity. If a solution is injected the diffusion of the active substance through the tissue to the blood vessel will be the rate determining step for the absorption. If a suspension is injected the dissolution rate of the active substance may become rate determining for the absorption process, whereas

for lipophilic active substances formulated as an oily solution or an oil-in-water emulsion the transport over the oil-water interphase may become rate determining. Oily liquids and aqueous or oily suspensions can only be injected intramuscularly or subcutaneously, never intravenously. For suspensions and emulsions the absorption rate can be slowed down to such an extent that it will become rate limiting for the elimination rate of the medicine. In this way slow release injections can be formulated.

The bioavailability of an intramuscularly or subcutaneously administered active substance is determined by:

- Site and depth of the injection;
- Physical form of the active substance in the injection (solution, suspension, emulsion);
- Physico-chemical properties of the active substance (solubility, charge, aggregation);
- Injected volume;
- Thickness of the fat layer in relation to the depth of the injection;
- Muscle activity;
- Excipients used and composition of the injection (formulation: solvent or vehicle, osmotic value, viscosity, pH, surfactants, etc.).

See also Chap. 21 for the practical consequences for parenteral formulations.

5.2.2 Oromucosal Administration

The mucosal membranes in the mouth can be used to administer active substances. The thin and highly vascularised membrane under the tongue is especially suitable for fast absorption. The rate of absorption of the active substance is determined by the size and lipophilicity of an active substance. A high lipophilicity and a small size of the molecule are favourable for rapid penetration of the sublingual membrane. But before an active substance can be absorbed it should be dissolved. Specially designed oromucosal dosage forms should provide dissolution of the active substance in the limited amount of saliva available. Since many persons experience difficulties keeping a product in the mouth for about 2 min, dissolution must be rapid as well [12]. When given in a rapidly dissolving dosage form, lipophilic hormones like testosterone or oestradiol can be absorbed efficiently via the sublingual route (circumventing the hepatic first-pass effect). Nitroglycerin is an example of a small slightly lipophilic compound (log P = 1.62) that is effectively administered (showing a fast absorption within minutes) as a sublingual tablet or spray in acute cardiac insufficiency.

5.2.3 Oral Administration

For orally administered medicinal products the pharmaceutical availability, the rate of absorption and the bioavailability strongly depend on the design and formulation of the dosage form. During the transit through the gastro-intestinal tract, the active substance is exposed to varying conditions. Of importance are the variations in pH, residence time in different parts of the gastro-intestinal tract and digestive (metabolising) enzymes. The gastro-intestinal tract covers the mouth, oesophagus, stomach, small intestine (duodenum, jejunum, ileum) and large intestine (colon).

The oesophagus has only a transport function for active substances. It is important, however, that corroding and irritating active substances, like doxycycline and bisphosphonates, do not stick in the oesophagus as they can damage the tissue and cause ulcerations. Ample water should be taken when swallowing such medicinal products.

Within the stomach an acidic environment exists, with a pH between 1.5 and 3.0 (with extremes between 1.0 and 5.0). In addition, digestive enzymes are present. The residence time in the stomach depends on the nutritional status and on the physical form of the medicine and may be highly variable. An active substance in solution, taken on an empty stomach, will pass the stomach quickly, usually within 30 min. A non-disintegrating large tablet, taken after a high-fat meal, may remain in the stomach for several hours. Absorption from the stomach plays a minor role in the total absorption, because of the relatively small surface of the gastric wall in relation to the stomach volume and the thickness of mucus layer and membrane.

In the duodenum, the first part of the small intestine, the pH of the acidic gastric content rises to values between 6.4 and 6.8, after pancreatic juice is added. This pH is maintained in the jejunum and the proximal part of the ileum. In the terminal part of the ileum, the pH rises to 7.1–7.5. The transit time through the small intestine is about 4.5–6 h. For many active substances the duodenum is the principal site of absorption. This is because of its large surface and the relatively large variation in luminal pH, as a result of which many active substances are present in the duodenum as a non-ionised molecule for some time. However, also in lower parts of the small intestine significant absorption can still occur.

In the colon the pH drops a little, to 6.4–7.4, due to the metabolic activity of the intestinal flora. The residence time in the colon varies between 6 and 12 h. Absorption from the colon is generally of minor importance for orally administered active substances.

The significant variations in pH and residence time in the gastro-intestinal tract strongly affect the solubility and the dissolution rate of many active substances as well as the transport over the intestinal membranes during the absorption process.

Interactions with food in the gastro-intestinal tract can influence the bioavailability of active substances at various levels (see elsewhere in this chapter). Examples are:

- Food components (or active substances) that delay stomach emptying will delay the absorption of active substances that are only absorbed from the small intestine.
- Food (and active substances) that change the pH in the gastro-intestinal tract may influence the absorption of active substances of which the absorption process is pH dependent.
- Food components may form insoluble complexes with active substances, hampering their absorption.
- Swallowing a lot of fluid will increase the amount of liquid in the gastro-intestinal tract available for dissolution. As a consequence, larger fractions of poorly soluble active substances may dissolve and the bioavailability may increase.
- Fat in food stimulates the excretion of bile into the intestinal lumen. Bile contains effective emulsifiers that can increase the solubility of lipophilic active substances.
- Food components (fibres) that increase the motility of the intestine may decrease the absorption of active substances due to a shorter residence time.
- Food increases the blood flow to the intestinal tract. The absorption of active substances will increase, leading to higher portal drug concentrations. Since the higher concentrations may surpass the maximum enzyme capacity of the liver, the first-pass effect may become reduced. The first-pass effect then decreases as a result of less effective extraction by the liver.
- Certain food substances and herbal preparations may interfere with enzymes that play a role in drug metabolism (e.g. inhibition of cytochrome P450 3A4 enzymes by grapefruit juice) or with transporters such as P-pg.

See also Chaps. 12 and 13 for the practical consequences for oral formulations.

5.2.4 Rectal Administration

Rectal administration of medicines aims at a local or a systemic effect. Suppositories and (micro) enemas (3–10 mL) are mainly used to obtain a systemic effect, enemas with a larger volume (up to 100 mL) for a local effect (in the rectum and lower colon).

The rectum is the lowest part of the large intestine. It is 15–20 cm long with a diameter of about 5 cm. In the rectum 1–5 mL of viscous fluid with a pH between 6.4 and 7.4 and a small buffer capacity is present. The temperature (under physiological conditions) is 36.2–37.6 °C. The rectum is a flat tube, because of the pressure of the bowels. After administration of a fluid into the rectum the liquid will spread due to this pressure. Larger volumes (enemas) are spread into the colon as well. A defecation reflex will occur when volumes exceeding 100 mL are applied, which limits the volume of enemas.

In older literature the circumvention of the hepatic first-pass effect is mentioned as an advantage for rectal administration of medicines. This is now known to be a partly valid argument, since two out of three rectal veins end up in the portal vein. Rectal administration can be useful when a patient is vomiting, has swallowing problems, is unconscious, experiences severe gastro-intestinal complaints when taking the medicine orally (e.g. indomethacin), or for active substances with an unpleasant taste (especially for children).

The most commonly used basis for suppositories is hard fat, which melts when brought into the rectum. Most active substances are suspended into the base, but some lipophilic active substances are dissolved. The first step after administration is melting of the base, followed by sedimentation of the suspended active substance particles to the fat-aqueous interface where the active substance is to dissolve in the aqueous rectal fluid. Subsequently, the active substance is absorbed by the rectum membrane after which it enters the systemic circulation. It should be known that due to the viscous nature of the mucus covering the rectal membrane direct transport from the molten fat to the membrane is not possible; the active substance always has to pass the aqueous mucus layer. Therefore, highly lipophilic active substances should not be formulated into a lipid suppository base or in an oily enema. Since the transport from the lipophilic base into the aqueous rectal fluids is an inevitable step in the drug absorption process, the high octanol-water partition coefficient of these lipophilic active substances will make this process inefficient and absorption slow and incomplete. As an alternative to fat, a water-soluble base (macrogol (polyethylene glycol)) can be used for the preparation of suppositories. The aqueous suppository base should especially be applied to those active substances that are highly lipid soluble and will not partition from the molten fat base into the aqueous rectal fluid.

The release of an active substance from a suppository depends on the pH and the buffer capacity in the rectum, the solubility in water and the lipophilicity of the active substance (the solubility in the fat base), the volume of the suppository (usually 2–3 mL for adults), the nature of the suppository base (lipid or aqueous, the viscosity of the melted lipid based, the solubility for the aqueous base) and the particle size of the active substance. To improve the absorption rate of less soluble substances, active substances

can be formulated as a cyclodextrin complex in suppositories. For example, piroxicam is formulated as a beta-cyclodextrin (betadex) complex and cisapride is used as an hydroxypropyl beta-cyclodextrin (hydroxypropylbetadex) complex in fatty suppositories.

Rectal solutions have water or oil as a vehicle. If necessary to enhance the solubility of poorly soluble active substances, aqueous rectal solutions may contain cosolvents, such as ethanol and propylene glycol. However, cosolvents and surfactants should only be used in limited amounts because of the potential irritation and the defecation reflex they may cause. For the rectal absorption of active substances from enemas the same mechanisms as for suppositories apply. A major advantage of a rectal solution over a suppository may be the fact that the active substance is already in a dissolved state which may increase the absorption rate. Increasing the volume of a rectal solution to dissolve a poorly water-soluble active substance will enhance the dissolution rate and thereby increase the absorption rate. Because of the higher volume more active substance will be dissolved and the membrane surface over which absorption occurs, is increased as well.

See also Chap. 19 for the practical consequences for rectal formulations.

5.2.5 Dermal and Transdermal Administration

Medicinal products can be applied on the skin to treat local skin diseases (topical application) or to systemically administer an active substance (transdermal application). In the first case, the active substance should accumulate in or even on the skin and display its effect (only) there. When transdermal administration is intended, the active substance should be transported through the skin followed by absorption into the systemic circulation. In the skin the stratum corneum (the most outer layer of 5–50 layers of dead cells, a horned layer of corneocytes, see Chap. 20) forms the major barrier for absorption of active substances. The layer is highly lipophilic in nature and is fully impermeable for hydrophilic active substances. Lipophilic active substances, when adequately formulated, may be absorbed via the skin. Typical characteristics which make an active substance suitable for transdermal transport are: an octanol-water partition coefficient (expressed as log $P_{o/w}$) between 1 and 3 and a molecular mass below 500 Da [13, 14] Moreover the dose should not exceed 20 mg per day. Even of an active compound with an ideal log P no more than 20 mg can penetrate the skin over a surface of 20 cm^2 per day. Transport through the skin is a slow process and more active substance can only be absorbed when large surfaces of the body are treated, which is for many reasons (convenience, cosmetic etc.) undesirable.

Hydrophilic active substances can be absorbed via the transdermal route when the stratum corneum is damaged, for example by the use of microneedles [15]. However, such action also compromises the natural protective function of the skin. The layers of the skin under the stratum corneum are permeable to both hydrophilic and lipophilic substances. Lipophilic substances may form a depot in the lipid parts of the skin before they are transported into the systemic circulation. The major route of penetration over the stratum corneum is the intercellular route, this route is especially suited for lipophilic compounds that can diffuse through lipids, which are around dead skin cells. The transcellular route through the corneocytes is less relevant; it may be used by amphiphilic compounds (log P between 0 and 1). Penetration may also occur via hair follicles, sebum glands and sweat glands, but because of the smaller surface these routes are of minor importance.

For a good therapeutic effect the choice of the active substance and the choice of the vehicle are important. Physical and chemical factors play an important role. The solubility of the active substance in the vehicle and the concentration, the size of the molecule of the active substance, the partition between vehicle and skin, the particle size (in case of suspensions) and the nature of the vehicle (aqueous or lipid) determine the penetration speed and depth. Hydrocortisone, for example, is more lipid soluble in the ester form (hydrocortisone acetate). The latter will penetrate into the skin faster and more complete. Hydrocarbons, such as soft and liquid paraffin, release lipophilic active substances only very slowly and substances formulated in these bases will penetrate only in limited amounts into the skin. Fatty oils (vegetable oils, triglycerides) are able to pass into the upper layers of the skin. Penetration enhancers (salicylic acid, dimethyl sulfoxide, propylene glycol, urea) increase the penetration of active substances into the skin.

The pH of the vehicle in relation to the skin pH (around 5.5) is very important. Many active substances, also in dermal preparations, are weak acids or bases. The pH partition theory (see elsewhere in this chapter) plays a role here as well and affects the effective partition of the active substance between the vehicle and the skin.

Physiological factors also determine the effectiveness of dermal preparations. The thickness of the skin varies across the body. The penetration rate is higher when applied on thin skin (e.g. behind the ear, on the eyelid or scrotum) than when applied on thick skin (e.g., palm of the hand, sole of the foot). Comparing the skin of babies and adults, the ratio between body surface (skin) and body volume is larger for babies. In addition, skin absorption in term and in preterm new-borns is increased because their stratum corneum is

thinner and the epidermis of children is better perfused and hydrated compared to adults. As a result, the toxicity of active substances applied on a baby's skin can be much higher than on an adult's skin.

The rate of penetration through a damaged skin is higher than that through an intact skin. The state of hydration of the skin is also important. Restoring the skin's hydration state to normal is often part of dermatological therapy and can be achieved by choosing the appropriate base. The rate of penetration is usually higher in a well-hydrated skin. Occlusion (e.g., by covering the part of the skin where the dermatological preparation has been applied) enhances hydration and hence enhances drug penetration of more hydrophilic active substances into the skin.

Technical factors, like rubbing and massage, also enhance the penetration of medicines. Several excipients may act as penetration enhancers, by reducing the thickness of the skin (with salicylic acid or urea), or by changing the coherent structure of the stratum corneum (with dimethyl sulfoxide, propylene glycol, or several surfactants). Whether the active substance is present in the inner or outer phase of an oil-in-water or a water-in-oil cream affects the extent and rate of transdermal absorption of an active substance. When present in the inner phase transport is generally slower, due to the fact that the active substance at first has to pass the outer phase before reaching the skin. When the partition between inner and outer phase is highly in favour of the inner phase only small amounts of active substance will enter the outer phase and transport will be slow. Conversely, when the inner phase consists of small droplets a large interface exists between the inner and outer phase and drug transport may still be relatively fast. As a consequence, active substances that are dissolved in the inner phase may still be faster and more completely absorbed into the skin compared to a suspension-type formulation. This is, for example, the reason why lipophilic corticosteroids are often formulated in oil-in-water creams.

Iontophoresis (see also Chap. 20), finally, is a means to enhance the penetration of charged active substances. When positively charged molecules are administered at the site of an anode and negatively charged active substances under the cathode, electric repulsion will increase the driving force for transport of the active substance into the skin. Variation of the electric charge that is applied will change the rate of transdermal drug transport. This can be used to rapidly adjust the dose for example in pain management: iontophoresis is used for example to control the delivery of lidocaine (as hydrochloride) for local anaesthesia or of fentanyl (as citrate).

Dermatological preparations for local use may exert a systemic effect as well. Especially for corticosteroids this can be a clinically relevant problem. The patient should be informed not to use such preparations for too long, and not more frequently than prescribed. Furthermore, the surface on which the preparation is applied and the thickness of the film should be limited.

See also Chap. 20 for the practical consequences for the dermal formulations.

5.2.6 Nasal Administration

The application of medicines in the nose usually aims at a local effect (e.g., for decongestion of the mucous membrane of the nose, or to administer anti-allergic medicines). In recent years, it has become clear that the nose can be used as a route for systemic therapy as well. Nasal drops and nasal sprays are the most commonly used nasal preparations.

Many active substances, solvents and excipients possess ciliotoxic properties, meaning that they (irreversibly) damage the cilia. Cilia are hair-like projections of the nasal epithelium cells. They facilitate the movement of mucus from the nasal cavity towards the nasopharynx. From there it is swallowed into the gastro-intestinal tract. The cilia play an important role in keeping the nose clean from particles and pathogens. In order not to damage this defense mechanism, special attention should be given to the constituents of nasal preparations, including the active substance, antimicrobial preservatives, antioxidants, salts for adjusting pH and tonicity, solvents and viscosity increasing agents. Also the pH and tonicity of nasal preparations lie within narrow limits.

As a consequence of the mucociliary clearance of the nasal cavity, active substances that are unable to pass the nasal membrane will end up in the oropharynx and are swallowed into the gastro-intestinal tract, from where they may be absorbed. This gastro-intestinal absorption after nasal administration may erroneously be considered as nasal absorption. Such a phenomenon may, for example, occur when the nasal spray of the anti-migraine medicine sumatriptan is used.

The nasal administration was shown to be an effective administration route for lipophilic active substances like fentanyl. Moreover, the nasal route has also been used for the systemic administration of small peptides like buserelin acetate, nafarelin acetate and desmopressin, all of them containing ten or less amino acid residues. However, for these molecules the nasal route forms only a poor non-invasive alternative to injection, since the nasal bioavailability of these peptides is less than 3–5%.

Chapter 16 discusses nasal absorption, mainly the enhancement, in more detail as well as the practical consequences for nasal formulations.

5.2.7 Pulmonary Administration

The human lungs consist of a branching system of airways with 23 bifurcations between the mouth and the alveoli. The transport of active substance-containing aerosol particles by the airflow into the lung is one of the major obstacles in pulmonary medicine administration. Due to their inertia (meaning that due to their mass and velocity the particles show the tendency to 'fly out of the bend') the particles that are transported by the airflow may be unable to pass the throat or subsequent bifurcations of the airways. Inertial impaction, especially in the throat, may significantly reduce the overall efficacy of the drug deposition in the lung. Throat depositions varying between 70% and 90% of the dose are not exceptional for widely used inhalation devices, such as nebulisers, metered dose inhalers or dry powder inhalers.

Since the medicine loss by throat deposition of aerosols is a mechanical phenomenon this problem should also be solved in a mechanical way. This can be done by reducing the particle size of the aerosol particles to a size between 1.5 and 3.0 μm, reducing the inhalation airflow rate and prolonging the breath-hold. The mean density of the particles is also a very important parameter for their diffusion to the deep lungs. In this way, throat and upper airway deposition can be decreased while optimising the lung deposition. It is for this reason that for pulmonary medicine administration the device and the way it is used by the patient are considered to be as important as the choice of the active substance. Once particles have passed the upper airways, particles in the size range between 1.5 and 3.0 μm will deposit by settling on the airways wall and a small fraction will deposit in the alveoli. Most of the particles smaller than 0.5–1.0 μm may penetrate deeply into the lung but sedimentation on the airway wall will be limited due to the limited effect of gravity on these particles. They will therefore to a large extent be exhaled again. This is discussed in more detail in Chap. 14.

The lungs can be considered to consist of two different organs: the airways and the alveoli. These two parts of the lung are physiologically different and the barrier function of their mucosal membranes differs completely. The airways are covered by a ciliated mucosal membrane covered with a mucus layer that is transported to the throat. The airway membrane is not permeable to hydrophilic molecules with a molecular mass over 1400 Da. The alveoli on the other hand, have a completely different structure. The surface of the pulmonary alveoli approximates 125 m^2 and their membrane consists for over 93% of the alveolar type I cell. This is a non-ciliated cell type that is covered with the alveolar lining fluid, an aqueous fluid that contains huge amounts of the surfactant dipalmitoylphosphatidylcholine. Moreover the alveolar membrane is highly permeable, which is explained by the occurrence of intercellular spaces occur with sizes up to 4 nm between the alveolar type I cells. Because of these large intercellular spaces proteins with a molecular mass up to 20 kDa can be absorbed into the systemic circulation after inhalation. This, combined with the low activity of proteolytic enzymes in the alveolar lining fluid, makes the pulmonary route probably a suitable alternative to the parenteral route for small and medium size therapeutic proteins. However, it should be kept in mind that for proteins with a molecular mass over 1400 Da absorption will only occur via the alveoli. Unfortunately the alveoli are found only at the end of the branching airway system and only a fraction of 10–15% of the particles of 1.5–3.0 μm will reach the alveolar region. This limits the final bioavailability of inhaled proteins.

The Pulmonary Route for the Systemic Administration of Medicines

The systemic administration of medicines via the pulmonary route has attracted significant interest over the past decades. The development of an insulin formulation for inhalation is an example. A few years after the discovery of insulin in the 1920s, it was described that after inhalation in dogs systemic blood levels of insulin were obtained. Considering the fact that proteins with a molecular mass of up to 20 kDa can pass the alveolar membrane, it is not surprising that the less than 6 kDa insulin is systemically absorbed after inhalation as well. However, it took more than 60 years before inhalation systems and protein formulation technology had developed to a level that could guarantee a well-controlled and reproducible alveolar deposition of the insulin containing aerosol particles. In 2006 the first insulin dry powder inhaler was launched (Exubera™). This product had a low bioavailability of about 12% and was available in two dose strengths only. After less than 2 years the product was withdrawn from the market because of safety concerns and poor sales performance. Following this withdrawal most other developments regarding inhaled insulin were also stopped.

In spite of this failure, the lungs seem to be a suitable port of entry for active substances that cannot be systemically administered by non-invasive routes such as oral or transdermal. Also for active substances that suffer from a high first-pass metabolism or a highly variable absorption behaviour after oral administration, the pulmonary route could potentially be a better route of administration. For a wide variety of active substances ranging from the therapeutic peptides such as somatropin or gonadorelin agonists to small organic molecules such as fentanyl or ciclosporin, the systemic availability after inhalation has been proved.

(continued)

Furthermore, the lungs seem to offer a suitable organ for needle-free vaccine administration. For several vaccines such as the measles and influenza vaccine, successful vaccination, eliciting an adequate (protective) immune response, has already been shown in humans [16, 17].

Pulmonary administration of active substances is common in the treatment of lung diseases such as asthma and COPD and infectious lung diseases. For these indications different levels of the airways are targeted with different medicines. For the beta-agonists such as formoterol the higher airways (generation 4–11, see Chap. 14) are the primary target, whereas for the anti-inflammatory corticosteroids the smaller airways should be the primary target. Long acting muscarinic antagonists should be targeted at the entire bronchial tree, whereas antibiotics should preferably be directed at the infectious loci in the lung.

Pulmonary drug administration can be considered as the most successful application of the drug targeting concept so far. Locally at the site of action, considerably higher active substance concentrations can be attained and the onset of action is faster than after systemic administration. Following inhalation, blood levels are usually lower and thus the occurrence of adverse effects is limited compared to systemic administration. When a patient is short of breath, he will experience the effect of pulmonary administered medicines fast, within a few minutes. This is of paramount importance when exacerbations are treated.

As described above, the pulmonary route can also be used for systemic administration of active substances. Smaller molecules (<1400 Da) can already be absorbed via the airways and bioavailabilities of over 30% can be reached. For the larger molecules penetration can only occur over the alveolar membrane and their bioavailability will be much smaller. However, no such products are on the market yet.

See also Chap. 14 for the practical consequences for the pulmonary formulations.

5.2.8 Ocular Administration

The ocular administration of medicines is only used for a local effect in, on or around the eye. The local application results in high local concentrations of the active substance. Pharmaceutical dosage forms for the eye encompass eye drops, eye washings, eye ointments, inserts and intraocular injections. In all cases, it is important that the preparations do not cause irritation of the eye. If they do, the medicine will be washed out quickly due to tear production, which may reduce the effect and will limit its duration. In addition, small sharp particles can damage the eye. Strict limits exist for pH and tonicity of ocular preparations.

Active substances administered via the ocular route (with the exception of an intraocular injection) may be absorbed via the different membranes of the eye. When an active substance is active in the eye it should be able to pass the cornea. The epithelial and endothelial layers of the cornea are of a highly lipophilic nature as a result of which only lipophilic and non-charged active substances can pass the epithelium of the cornea. For active substances with a partition coefficient below 0.3 or active substances that are charged at the physiological pH of the eye the driving force for transport over the corneal epithelium (the first step of the absorption process) will be insufficient and absorption will generally be low. To obtain sufficient transport of the active substance over the corneal epithelium it is therefore necessary to administer the active substance in a formulation that has a pH at which a significant part of the active substance is unionised. Examples are carbonic anhydrase inhibitors and beta adrenergic antagonists, used in the treatment of glaucoma.

Absorption into the systemic circulation of active substances administered to the eye may occur to a limited extent, via the conjunctival membrane and the nasal mucosa (after being washed from the eye the active substance may enter the nasal cavity via the nasolacrimal duct). The residence time of medicines in the eye may be prolonged by increasing the viscosity of the eye drops (which reduces the efficacy of lacrimation to wash out the medicine from the eye) or via the application of polymeric ocular inserts slowly releasing the active substance. It should be realised that many ocular inserts are placed in the conjunctival sac, as a result of which a significant fraction of the active substance will not be absorbed via the cornea but rather end up in the systemic circulation due to absorption via the membrane of the conjunctival sac.

The intraocular injection of active substances that are active in the eye leads to a bioavailability of 100%. However, this route of administration is a serious burden to the patient and formulation related safety aspects are of paramount importance since elimination from the site of injection is generally slow. However, this route may be the only way to get an active substance to the site of action, or to get it in a sufficiently high concentration at the site of action (e.g., of an antibiotic). Larger molecules such as monoclonal antibodies (e.g., bevacizumab or ranibizumab used against macular degeneration) are administered in this way into the eyeball. A further advantage of the intraocular route is that the large molecules are cleared slowly from the intraocular fluids and effects may last for days (or even weeks).

See also Chap. 18 for the practical consequences for the ocular formulations.

5.3 New Developments and Advanced Drug Delivery Systems

Over the past years a range of new developments in the field of drug delivery has emerged. Advanced drug delivery systems and nanomedicines have been designed and a few of them made it to the market [18–21]. Examples of such advanced drug delivery systems are liposomal medicinal products, nanocapsules, antibody-drug conjugates, polymer-drug conjugates (e.g. pegylated proteins) and higher drug excipient (polymer) associated medicines. Many of these delivery systems have been developed for active substances that suffer from intrinsic difficulties from a biopharmaceutical point of view such as therapeutic proteins, DNA or RNA as well as small organic molecules that require drug targeting because of their intrinsic toxicity when distributed over the entire body (e.g. antineoplastic medicines). New vaccines based on mRNA delivery have been formulated using lipid nanoparticles [22]. In the case of COVID-19 vaccines the mRNA coding for the spike protein of the virus is encapsulated and thus protected in the lipid nanoparticles injected intramuscularly. Furthermore, advanced delivery systems are being developed to open new (mostly non-parenteral) routes of administration in order to improve the ease and comfort of drug administration for the patient. Certainly, the development of advanced drug delivery forms is not new. Oral slow release products and pro-drugs that target mesalazine to the lower part of the intestinal tract have been available for decades. However, the rapid developments in biotechnology as well as the development of new active substances (especially biologicals) that require site-specific delivery has propelled the development of more advanced drug delivery systems. Without these systems therapeutically successful administration of these new active substances will be impossible. See also Chap. 24.

A common denominator of all advanced drug delivery systems is their aim to change the intrinsic pharmacokinetic behaviour of the active substance they contain. This may either be at the level of absorption or absorption rate, at the level of metabolism or distribution, or by changing the elimination. From a biopharmaceutical point of view these changes may have significant implications, since they may alter bioavailability, efficacy and safety as well as the onset and duration of action. It is for this reason that newly developed advanced drug delivery systems should be considered as completely new medicines, requiring an almost full development programme before they can be licensed. So far the number of advanced drug delivery systems that reached the market is limited. Obviously many of the concepts designed for improved bioavailability or drug targeting appeared to be not successful in real life, in the complex and compartimentalised *in vivo* situation in man. This is also due to the difficulty to properly characterise those very complex nanoformulations and also to produce them in large scale with industrial quality requirements in mind.

Among the advanced drug delivery systems that did reach the market are liposomal formulations of active substances like doxorubicin and amphotericin B. Recently trastuzumab emtansine which is a conjugate of the monoclonal antibody trastuzumab and the antineoplastic mertansine (= emtansine) was introduced onto the market [23]. Since the HER2-receptor is overexpressed in tumour cells a dual action will be obtained from this conjugate. Trastuzumab itself can already stop the growth of tumour cells whereas the antineoplastic mertansine is specifically targeted to the tumour cells by the antibody, increasing efficacy and reducing adverse effects. The active substance is used in HER2-positive metastatic breast cancer. Using this product for the first time monoclonal antibody-based drug targeting has been applied successfully. Another example is Vyxeos, the first nanomedicine used in leukemia being a liposomal formulation of cytarabine and daunoribicine (FDA approved in 2017).

Taking into account the considerable efforts that are still being made in the development of the advanced drug delivery systems, several new advanced drug delivery systems may reach the market in the coming years. mRNA as a platform technology may offer interesting perspectives.

Questions

1. Which aspects of an active substance determine its pharmaceutical availability?
2. Explain the effects of pre-systemic metabolism, P-gp activity and systemic metabolism on active substance pharmacokinetics in general terms.
3. Explain the phenomenon of flip-flop pharmacokinetics.
4. Explain the effect of enzyme saturation on the pharmacokinetics of an active substance.
5. Explain that a locally administered medicine can have a systemic effect and how that can be enhanced or avoided.

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