Drug metabolism and pharmacokinetics

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Abstract
In this article, aspects of absorption, distribution, metabolism, and excretion have been described bearing in mind the pathogenesis of allergic diseases and their possible therapeutic opportunities. The importance of the routes of administration of the different therapeutic groups has been emphasized. The classical aspects of drug metabolism and disposition related to oral administration have been reviewed, but special emphasis has been given to intranasal, cutaneous, transdermal, and ocular administration as well as to the absorption and the subsequent bioavailability of drugs. Drug-metabolizing enzymes and transporters present in extrahepatic tissues, such as nasal mucosa and the respiratory tract, have been particularly discussed. As marketed antiallergic drugs include both racemates and enantiomers, aspects of stereoselective absorption, distribution, metabolism, and excretion have been discussed. Finally, a new and promising methodology, microdosing, has been presented, although it has not yet been applied to drugs used in the treatment of allergic diseases.

Keywords: Compartmental and noncompartmental pharmacokinetics; sites of administration; absorption; distribution; metabolism; excretion; transporters; chronopharmacokinetics; enantiomers; microdosing

Introduction: compartmental and noncompartmental pharmacokinetics

Pharmacokinetics (PK) essentially investigates all transport processes in an organism following drug administration. Not everything that happens to a drug in a complex organism such as the human body can be fully understood. For simplification, different functional units (such as tissues and organs responsible for distribution and elimination via the different pathways: biliary and renal excretion, liver metabolism) can be combined. In this way, the so-called physiological model of pharmacokinetics is obtained; this means a description of the body considering the essential functional units, comprising organs, organ functions, and connections. Figure 1 illustrates the transports of drug molecules in the body system (PK, i.e., what the body does to the drug). Figure 1 illustrates that PK is the input for pharmacodynamics (PD) via potential interaction with a target, receptor, or enzyme (PD, what the drug does to the body system).

This representation is certainly a simplification when one considers that a transport of the total blood volume constantly occurs in parallel through different organs in an organism. Figure 2 illustrates this in the example of the transport processes involving the most important organs implicated in the pharmacokinetics of a drug. The transport processes involved in elimination cannot be limited to elimination by metabolism or renal elimination: Elimination must always be seen as the sum of several processes progressing side by side.

Pharmacokinetics today is an established discipline in drug development and pharmaceutical research. For optimum therapeutic use of any drug, it is essential to know its fate in the body after administration and to characterize its disposition. For that purpose, there are two different approaches: the compartmental pharmacokinetics and the noncompartmental pharmacokinetics.
A compartment in pharmacokinetics describes a closed homogeneous space for the transport processes of a drug. The anatomical tissues to be included in a compartment can be chosen either on the basis of their homogeneity in terms of kinetic properties or on physiological grounds. Some compartments are specified more closely owing to their function. For example, blood, together with the tissues that are rapidly equilibrating with the concentrations in blood, are included in the central compartment; in this compartment, tissues such as muscle, skin, and lungs are included. Peripheral compartments typically include the tissues, which are only slowly equilibrating (e.g., fat or tissues representing a sanctuary, such as brain). Urine as well as feces can be considered in the so-called excretory compartments. For the full definition of this model, it is required to define the direction of the drug transport and, based on the available plasma or blood concentration-time data, the volumes and the rate constants can be estimated.

A basic compartment model of pharmacokinetics is represented in Figure 3A. The three transport processes for absorption, distribution, and elimination are illustrated in the specified model. These transport processes show the direction of the molecule movements by arrows and specify both start and destination of the individual transport processes. This model is appropriate for describing the pharmacokinetics of a drug following oral application of a drug. The model can, however, be used with only minor modifications for the intravenous and intraarterial application of a drug (as a bolus injection or as an infusion), assuming that the drug is placed directly in the central compartment, which includes blood (i.e., without considering that the drug is administered into the GI tract and only later transferred to the central compartment via the absorption rate constant $k_{10}$). Fitting the model, expressed in differential or integral terms, to the plasma concentration-time data allows estimating the parameters of the model (e.g., the transfer rate constants and the volume of the central compartment, or, in alternative, the absorption constant, the two volumes of distribution, the distributional and elimination clearance). The model shown in Figure 3B describes the pharmacokinetics of a drug undergoing enterohepatic cycling.

Noncompartmental PK

Noncompartmental PK describes transports of drug molecules without the definition of compartments. The only assumptions are that the processes are all linear (i.e., clearance is independent of drug concentrations) and that a terminal phase exists, characterized by a constant slope of the log-transformed plasma concentrations vs. time data (Gillespie, 1991).

Parameters are directly derived from drug concentrations in plasma or blood: maximum concentration...
(C_{\text{max}}), time of maximum concentration \( (t_{\text{max}}) \), lag time, trough value, and average concentration. Other parameters are area under the concentration-time curve (AUC) and terminal half-life \( (t_{1/2}) \).

**Sites of administration**

There are several sites at which drugs are commonly administered. These sites may be classified as either intra- or extravascular. Intravascular administration refers to the placement of a drug directly into the blood, either intravenously or intra-arterially. Extravascular modes of administration include the oral, sublingual, buccal, nasal, ocular, intramuscular, subcutaneous, dermal, pulmonary, and rectal routes. To enter the blood, drugs administered extravascularly must be absorbed. No absorption step is required when a drug is administered intravascularly. Tissues susceptible to be adequate for extravascular administration are: muscle, skin, mouth, tongue, GI tract (i.e., stomach, small intestine, and large intestine), nose, eye, urethra, and vagina. Sites for intravascular administrations are: veins, arteries, and heart.

For each of the sites/routes of administration, special formulations are requested. Table 1 summarizes the preferred formulation for the common routes of administration.

**Cutaneous and transdermal administration**

The skin is the largest organ of the integumentary system and is made up of multiple layers of epithelial tissues and guards the underlying muscles, bones, ligaments, and internal organs.

An adult has a skin area of 1.5–2.0 m² and the weight of the skin is 10–12 kg. The thickness of the skin can significantly vary in function of its location: the thinnest part is the face, 0.02 mm, whereas the sole has a thickness of 5 mm. The skin is composed of three primary layers: epidermis, dermis, and hypodermis (subcutaneous adipose layer) (Figure 4A and 4B).

The epidermis provides waterproofing and serves as a barrier to infection, whereas the dermis serves as a location for the appendages of skin.

Epidermis is histopathologically subdivided into the following five sublayers or strata (beginning with the outermost layer): stratum corneum, stratum lucidum (only in palms of hands and bottoms of feet), stratum granulosum, stratum spinosum, and stratum germinativum (also called “stratum basale”) (Figure 4C).

The epidermis contains no blood vessels, and cells in the deepest layers are nourished by diffusion from blood capillaries extending to the upper layers of the dermis. The main types of cells that make up the epidermis are keratinocytes, melanocytes, sensitive cells such as Merkel cells, and antigen-presenting cells, such as Langerhans cells. Cells replicate through mitosis at the basal layer. The daughter cells move up the strata,
changing shape and composition as they die due to isolation from their blood source. The cytoplasm is released and the protein keratin is inserted. They eventually reach the corneum and slough off (i.e., desquamation). This process is called keratinization and takes place within about 27 days. This keratinized layer of skin is responsible for water retention inside the body and for protection against harmful chemicals and pathogens, making skin a natural barrier to infection.

The dermis is the layer of skin beneath the epidermis that consists of connective tissue and cushions the body from stress and strain. The dermis is tightly connected to the epidermis by a basement membrane. It also harbors many mechanoreceptor/nerve endings that provide the sense of touch and heat. It contains the hair follicles, sweat glands, sebaceous glands, apocrine glands, lymphatic vessels, and blood vessels. The blood vessels in the dermis provide nourishment and waste removal to its own cells as well as the stratum basale of the epidermis. The dermis is structurally divided into two areas: a superficial area adjacent to the epidermis, called the papillary region, and a deep, thicker area known as the reticular region.

The hypodermis is not strictly a part of the skin and lies below the dermis. Its purpose is to attach the skin to underlying bone and muscle as well as supplying it with blood vessels and nerves. It consists of loose connective tissue and elastin. The main cell types are fibroblasts, macrophages, and adipocytes (the hypodermis contains 50% of body fat). Fat serves as padding and insulation for the body (Goldsmith, 1991).

From all these histological layers, the outer layer, the stratum corneum (SC), acts as the most efficacious barrier for permeability. Overall, 10–30% of the stratum corneum components are lipids and 5–15% consists of water. The SC can be considered the major limiting structure for drug absorption. As summarized in Table 2, with the drug, hydrocortisone, there are large differences of the relative rate of absorption. Whereas the absorption via foot arch is 7 times less than via the forearm, the absorption through the scrotum is 42 times higher than using the forearm administration of the drug (Feldmann and Maibach, 1967).

**Buccal and sublingual administration**

This site of administration is predominant, if the drug has to be absorbed very rapidly. One of the reasons to choose this site is to avoid first pass through the liver, as blood perfusing the buccal cavity bypasses the liver and enters directly into the superior vena cava. The oral or buccal mucosa consists of three layers: the epithelium, a lining layer, which is in direct contact with the contents of the buccal cavity, the underneath layer, called the lamina propria, which supports epithelium and accessory structures (such as glands and blood vessels), and a thin layer of smooth muscle, the muscularis mucosae (Tortora and Grabowski, 1996).

**Table 2.** Relative rate of hydrocortisone absorption at different sites of administration.

| Site                | Relative rate (forearm normalized to 1) |
|---------------------|----------------------------------------|
| Forearm (ventral)   | 1.0                                    |
| Forearm (dorsal)    | 1.1                                    |
| Palm of the hand    | 0.83                                   |
| Foot arch (plantar) | 0.14                                   |
| Ankle               | 0.42                                   |
| Back                | 1.7                                    |
| Axilla              | 3.6                                    |
| Sculp               | 3.5                                    |
| Forehead            | 6.0                                    |
| Jaw angle           | 13.0                                   |
| Scrotum             | 42.0                                   |

Source: Feldmann and Maibach, 1967.
**Oral administration**

Oral administration is the easiest way of administration, which is very comfortable for almost all of the patients. The anatomy and physiology of the GI tract can briefly be described as follows. The GI tract is the combination of the stomach and the small and large intestine (Figure 5A). The gastric wall and the wall of the small intestine show a different histology (Figure 5B and 5C). The small intestine is generally the most important for drug absorption. The total absorptive area of the small intestine, composed largely of microvilli (Figure 5C), has been calculated to be about 200 m². The corresponding estimate for the stomach is only 1 m². Villi are the most striking feature of the mucosa. They cover the entire surface of the mucosa and give it a characteristic velvety appearance. The mucosa houses a dynamic, self-renewing population of epithelial cells, including goblet cells, which secrete mucus, endocrine cells, which produce hormones and peptides, panted cells, which secrete a large amount of protein-rich materials, undifferentiated cells for renewal of the intestinal mucosa, and absorptive cells, which take nutrients from the lumen and transport them into blood, fulfilling the basic function of the digestive system. The muscularis of the small intestine consists of two layers of smooth muscle. The outer, thinner layer contains longitudinally arranged fibers. The inner, thicker layer contains circularly arranged muscular fibers (Tortora and Grabowski, 1996).

**Rectal administration**

The rectum is a potential site of administration when oral administration is not possible or not easy (e.g., for drugs destroyed by gastric acidity or by enzymes in the intestinal wall and microflora, as well as in cases of nausea and vomiting). The rate and extent of rectal drug absorption are often lower than with oral absorption. In addition, the composition of the rectal formulation appears to be an important factor in the absorption process by determining the pattern of drug release. For a number of drugs, the extent of rectal absorption has been reported to exceed oral values, which may reflect partial avoidance of hepatic first-pass metabolism after rectal delivery. Indeed, part of the rectal blood supply bypasses the hepatic portal circulation and dumps directly into the inferior vena cava. The rectum has a low water content and hydrophilic molecules cannot be dissolved. The rectum is a suitable site of administration for lipophilic drugs.

**Subcutaneous and intramuscular administration**

In contrast to the small intestine, and, indeed, to the entire gastrointestinal tract, absorption of most drugs in solution from muscle and subcutaneous tissue is perfusion rate–limited; increases in blood flow hasten absorption. This dependence of absorption on perfusion may be explained by the nature of the barrier (the capillary wall) between the site of injection (interstitial fluid) and blood. This capillary wall, a much more loosely knit structure than the epithelial lining of the GI tract, offers little impedance to the movement of drugs into blood, even for polar ionized drugs. For example, gentamycin, a water-soluble, ionized, polar base of molecular weight 477, has greater difficulty penetrating the GI mucosa, but is rapidly and completely absorbed from an intramuscular site. This low impedance by the capillary wall in muscle and subcutaneous tissue applies, however, to drugs of molecular size up to approximately 5,000.

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**Figure 5.** Gastrointestinal (GI) tract. (A) Anatomy: GI tract (scheme): 1. Esophagus. 2. Stomach. 3. Small intestine: duodenum. 4. Small intestine: jejunum. 5. Small intestine: ileum. 6. Large intestine: colon. 7. Large intestine: appendix. 8. Rectum. 9. Anus. (B) Histology: gastric wall (human sample) (H&E, magnification X40): 1. Mucosa. 2. Submucosa. 3. Muscularis. (C) Histology: jejunum wall (human sample) (H&E, magnification X40): 1. Mucosa. 2. Submucosa. 3. Muscularis. 4. Villi. H&E.
**Eye as site of administration**

The eye is the organ for visual perception of the environment. In order to form a picture, a perceptive and a refractive system is needed. The perceptive system is located in the rear part of the eye, while the refractive system is mainly located in the anterior part.

Tears constantly wash the eye to remove foreign objects, and the lids and eyelashes also help protect it in front. The eyeball has three coats: the cornea is on the front of the eyeball and continuous with the tough, white sclera; the choroid is the middle layer, containing blood vessels; and the retina is the inner layer, containing the rods and cones that are sensitive to light. Behind the cornea and in front of the lens is the circular, pigmented iris that surrounds the pupil; the iris works like the diaphragm of a camera, adjusting the size of the pupil according to different light conditions. Figure 6 gives an overview on the architecture of the eye.

The outmost tissue layer of the eye is the tunica externa oculi and it mainly consists of connective tissue, called “sclera.” The sclera has a white appearance and is made of collagen. The front part of this outer shell is the cornea, which is incremented to the sclera in an hourglass-like fashion. The cornea is clearly distinguishable from the sclera and has different properties, of which the optical (refractive) and barrier function are the most important ones.

The conjunctiva is a thin, vascularized membrane that lines the inner surface of the eyelids and covers the anterior part of the sclera (Newell, 1986). It helps lubricate the eye by producing mucus and tears (although in a smaller amount than the lacrimal gland). It also contributes to immune surveillance and helps to prevent the entrance of pathogens into the eye.

For the treatment of the eye, drugs are applied as solution in water or oil, as suspension, or cream. A significant part of the dose applied can be eliminated by tears. Hydrophilic drugs are absorbed better than lipophilic drugs.

**Nose as site of administration**

The nose serves as the only means of bringing warm, humidified air into the lungs. It is the primary organ for filtering out particles in inspired air, and it also serves to provide first-line immunologic defense by bringing inspired air in contact with mucus-coated membranes that contain immunoglobulin A (IgA). Inspired air is brought high into the nasal cavity (Figure 7) to come in contact with the olfactory nerves, thereby providing the sense of smell, which is intimately associated with the taste sensation (Drettner, 1979).

The external part of the nose is composed of skin, muscles, bones, and cartilage. The overlying skin of the nose may also be divided into vertical thirds. The skin of the upper third is fairly thick, but tapers into a thinner mid-dorsal region. The inferior third regains the thickness of the upper third owing to the more sebaceous nature of the skin in the nasal tip. The dorsal skin is usually the thinnest of the three sections of the nose. The difference in the skin thickness must be appreciated during dorsal reduction. The nasal muscles are encountered deep in the skin and comprise four principal groups: the elevators, the depressors, the compressor, and the dilators. The elevators include the procerus and levator labii superioris alaeque nasi. The depressors are the alar nasalis and depressor septi nasi. The compressor of the nose is the transverse nasalis, whereas the dilators are the dilator naris anterior and posterior. The muscles are interconnected by an aponeurosis termed the nasal superficial musculoaponeurotic system. A harmonious coordination between these muscles is needed when snuffing or when use of intranasally administered drugs.

**Figure 6.** Eye. (A) Anatomy: eye (scheme). 1. Lens. 2. Aqueous humor. 3. Vitreous humor. 4. Iris. 5. Cornea. 6. Conjunctiva. 7. Sclera. 8. Retina. 9. Optic nerve. (B) Histology: eye (animal sample): 1. Lens. 2. Aqueous humor. 3. Vitreous humor. 4. Iris. 5. Cornea. 6. Conjunctiva: a. bulbar conjunctiva; b. palpebral conjunctiva. 7. Inferior conjunctival fornix. 8. Superior conjunctival fornix. 9. Inferior eyelid with annexes (muscles, tarsus, sebaceous and tarsal glands, and cils). 10. Superior eyelid with annexes. (C) Histology: conjunctiva (human sample) (H&E, magnification X40): 1. Epithelium. 2. Basal membrane. 3. Lamina propria: lymphoid layer.
The internal nasal lining consists of, anteriorly, squamous epithelium in the vestibule (Wexler and Davidson, 2004) and, posteriorly, a respiratory mucosa (Watelet and van Cauwenbergue, 1999) (Figure 7).

Like an administration via eye or absorption through the mouth, mucosa drug absorption via nose mucosa bypasses the first-pass effect.

The intranasal administration is discussed extensively below.

**Absorption**

**General considerations**

Absorption is defined as the process by which unchanged drug proceeds from the site of administration (e.g., GI tract, nasal cavity, or conjunctiva) to the site of measurement (i.e., systemic blood circulation) within the body (Rowland and Tozer, 1995c). During absorption, there are several possible reasons for drug loss or for absorption delay, which may contribute to variable drug response and, occasionally, to therapeutic failure.

For drugs developed to act locally (e.g., local anesthetics, nasal decongestants, or nasal/ocular antiallergic agents), movement of drug from the application site to the systemic circulation influences time of onset, intensity, and duration of effects.

The most useful pharmacokinetic parameter to characterize the absorption process is the determination of the bioavailability, symbolized by “F”.

The bioavailability characterizes the extent of drug input to the systemic circulation and can be considered as the fraction, or percentage, of the administered dose reaching the systemic circulation without being modified (e.g., by gut and liver enzymes). When supplemental data obtained after intravenous (i.v.) administration are available, the absolute bioavailability is calculated as the total area under the curve (AUC) following extravascular administration divided by the AUC after i.v. bolus, appropriately correcting for dose.

For example, the absolute bioavailability of an intranasal drug (e.g., intranasal corticosteroids) is defined as the ratio of the AUC after intranasal administration to that after i.v. administration, as shown in the following equation:

\[
F_{a.u} = \left( \frac{AUC_{e.v}}{AUC_{i.v}} \right) \left( \frac{Dose_{i.v}}{Dose_{e.v}} \right)
\]

with \( AUC_{e.v} \) = the AUC after extravascular administration

with \( AUC_{i.v} \) = the AUC after i.v. administration

with \( Dose_{i.v} \) = the i.v. dose

with \( Dose_{e.v} \) = the extravascular dose

When there are no i.v. data available, relative bioavailability is determined by comparing different dosage forms, different routes of administration, or different conditions (e.g., diet, disease state).

In the following sections, the absorption processes after oral, ocular, and nasal administration will be discussed, with emphasis on drugs used in the treatment of allergic diseases, such as allergic rhinitis.

**Absorption after oral administration**

After oral administration, a drug encounters several barriers and sites of loss in its movement during GI
absorption. The first limiting step is the dissolution from the solid dosage form. An incomplete dissolution may result in a poor absorption reflected by a low oral bioavailability. Once dissolved, the drug must resist to the possible degradation inside the GI lumen, but also to the metabolism by enzymes located in the gut wall. Once having arrived in the portal vein, the drug can be extracted from the blood stream by the liver, cleared by metabolism, and finally secreted into the bile. The loss as the drug passes, for the first time, through sites of elimination, such as the GI membranes and the liver, is known as the first-pass effect.

Among the second-generation H₁ antihistamines used for the oral treatment of the allergic disease (e.g., the allergic rhinitis, chronic urticaria), the first marketed compounds (i.e., terfenadine, loratadine, and astemizole, but not cetirizine) were poorly bioavailable due to a high hepatic first-pass effect, which resulted in a high interindividual variability in their PK. Due to the potential for drug-drug interactions through metabolic inhibition for most of these first marketed second-generation H₁-antihistamines, levocetirizine, desloratadine, and fexofenadine were developed to minimize these drug-drug interactions and to obtain a higher oral bioavailability with a linear, predictable pharmacokinetics. Levocetirizine is highly bioavailable, with at least 85% of the oral dose being absorbed. Although the absolute bioavailability is unknown for cetirizine and desloratadine, the absorption is estimated to be at least 70 and 40%, respectively. Fexofenadine, the active metabolite of terfenadine, has a moderate bioavailability of about 30% (Molimard et al., 2004). The rate of absorption characterized by the time to reach the peak plasma level (T_max) is an important factor, which drives the time of onset. After oral drug delivery, it is important for H₁-antihistamine to be rapidly absorbed in order to assure rapidity of action. The T_max is shortest for cetirizine (1 hour) and levocetirizine (0.9 hours) and longest for desloratadine (3 hours or more). The T_max of fexofenadine is intermediate, in the range of 1–3 hours. Even if the T_max is an important factor for the rapidity of action, T_max is not necessarily reflecting the time to reach a plasma concentration sufficient for an effective blockade of H₁-receptors. For example, maximal and complete wheal inhibition by levocetirizine occurred at 2 hours, against 4 hours for fexofenadine in the same experimental conditions (Grant et al., 2002). This example shows the importance of the determination of the PK-PD relationship in drug development in order to help the clinicians in the right use of drug for the patient.

Absorption after ocular administration

Topical antiallergic drug, delivered as eyedrops, into the lower conjunctival cul-de-sac (Figure 6), is the most common method of drug treatment in ocular allergic diseases (e.g., allergic conjunctivitis).

Although the external eye structures are readily accessible, the biological barriers limit ocular drug absorption.

The corneal and conjunctival epithelial barriers cover the ocular surface. The blood-aqueous barrier, composed of the uveal capillary endothelia and ciliary epithelia, limits the access of compounds from the systemic circulation to the anterior chamber, whereas the blood-retina barrier limits the drug diffusion from the systemic blood to the retina and vice versa (Del Amo and Urtti, 2008). The barrier has two components: outer and inner blood-retina barriers that are formed by the retinal pigment epithelium (RPE) and the tight retinal capillary walls, respectively. After topical administration of eye drops in the conjunctiva, only less than 5% of the dose is absorbed into the eye itself (Urtti, 2006). The dose is mostly absorbed to the systemic blood circulation via the conjunctival and nasal blood vessels. For example, at least 70% of the timolol dose is systemically absorbed within 5 minutes (Urtti, 1990, 2006). Ocular absorption is limited by the corneal epithelium, and it is only modestly increased by prolonged ocular contact. Owing to the extensive conjunctival systemic absorption, the maximal attainable ocular absorption is only about 10% of the dose (Urtti, 1990).

Factors influencing the ocular absorption of drugs administered in the conjunctival cul-de-sac

Physicochemical drug properties. Drugs penetrate across the corneal epithelium via the transcellular or paracellular pathway. Lipophilic drugs prefer the transcellular route. Hydrophilic drugs penetrate primarily through the paracellular pathway, which involves passive or altered diffusion through intercellular spaces (Borchardt et al., 1990). For most topically applied drugs, passive diffusion along their concentration gradient, either transcellularly or paracellularly, is the main permeation mechanism across the cornea.

Physicochemical drug properties, such as lipophilicity (Schoenwald and Huang, 1983), solubility, molecular size and shape (Grass and Robinson, 1988; Liaw et al., 1992; Huang et al., 1989), charge (Rojanasakul et al., 1992; Liaw et al., 1992), and degree of ionization (Sieg and Robinson, 1977; Brechue and Maren, 1993) affect the route and rate of permeation in the cornea. A parabolic (Chang et al., 1987; Ahmed et al., 1987; Chien et al., 1991) and a sigmoidal (Wang et al., 1991) relationship have been found to describe the influence of lipophilicity on corneal drug permeability. The optimum octanol/buffer pH 7.4 distribution coefficient for corneal absorption is in the range of 100–1,000 (Schoenwald and Ward, 1978; Schoenwald and Huang, 1983). The rate-limiting barrier for ocular penetration of
highly hydrophilic drugs is the lipophilic corneal epithelium, while for highly lipophilic drugs, partitioning from the epithelium to the hydrophilic stroma is rate limiting and, for the most part, determines corneal permeability. More specifically, the rate-limiting barrier is located at the surface of the epithelium for moderately lipophilic β-blockers, while the whole corneal epithelium is the barrier for hydrophilic compounds.

Permeation of an ionizable drug (i.e., weak acids and weak bases) depends on the chemical equilibrium between the ionized and unionized drug in the eyedrop and eventually in the lacrimal fluid (Friedriech et al., 1993). The unionized species usually penetrates the lipid membranes more easily than the ionized form.

In the case of ionized molecules, not only the degree of ionization, but also the charge of the molecule affects their corneal penetration (Liaw et al., 1992). The corneal epithelium is negatively charged above its isoelectric point (3.2) (Rojanasakul and Robinson, 1989). Consequently, hydrophilic charged cationic compounds permeate more easily through the cornea than anionic species. At the physiological pH of 7.4, the ratio of transport numbers for positive over negative ions was 1.63. Below this pH, the cornea is selective to negatively charged molecules. However, this pH is too acidic and irritating for clinical use. Thus in practice, a charge discriminating effect of the corneal epithelium decreases the absorption of negatively charged drugs.

**Drug elimination from lacrimal fluid.** As mentioned above, after the instillation of an eyedrop, typically less than 5% of an applied dose reaches the intraocular tissues. This is explained by the relatively impermeable corneal barrier and rapid drainage of the instilled solution. Lee and Robinson (1979) have shown that drugs are mainly eliminated from the precorneal lacrimal fluid by solution drainage, lacrimation, and nonproductive absorption to the conjunctiva of the eye. These factors and the corneal barrier limit the penetration of the topically administered drug into the eye. Only a small percentage of the applied dose is delivered into intraocular tissues, while the major part (50–100%) of the dose is absorbed systemically (Urtti, 2006; Lee et al., 1993). The normal commercial eye dropper delivers a drop volume of 25–56 µL (average, 39) (Lederer and Harold, 1986). When an eyedrop is instilled, the human conjunctival cul-de-sac may momentarily contain a 30-µL volume, but the instilled solution is rapidly removed by spillage from the conjunctival sac or loss through the puncta to the lacrimal drainage system until the tears return to their normal volume (7 µL). The initial first-order drainage rate of eyedrops from an ocular surface is typically 0.5–0.7/min in rabbits and 1.5/min in humans (Zaki et al., 1989; Chrai et al., 1973). This rate decreases with viscosity (Ludwig and Van Ooteghem, 1989; Meseguer et al., 1993) and increases with larger eyedrop volumes (Chrai et al., 1973). If the volume of an eyedrop is decreased to 5–10 µL and the applied dose is kept constant by increasing the concentration, the ocular bioavailability can be substantially increased and systemic absorption decreased (Urtti and Salminen, 1993). It has been shown, using a mass transport theorem, that, maximally, a 4-fold improvement in ocular bioavailability may be achieved for topically applied drugs with a low corneal permeability, if the applied volume is decreased sufficiently (Keister et al., 1991). Compared to the initial drainage rate, the normal rate of tear turnover is much slower, approximately 0.16/min (1.2 µL/min) in humans (Sugrue, 1989) and 0.07/min (0.5 µL/min) in rabbits (Chrai et al., 1973). Thus, the normal tear turnover has a minor role in the removal of an instilled solution from the ocular surface. Ocular administration of irritating drugs or vehicles increases the drug loss from the precorneal area to a further extent due to induced lacrimation. An important route of drug loss from the lacrimal fluid is systemic absorption through the conjunctiva of the eye. Conjunctival permeability coefficients for many compounds, such as β-blockers (Wang et al., 1991) and timolol prodrugs (Chien et al., 1991), are higher than their corneal permeabilities. In addition, the surface area of the conjunctiva (16–18 cm²) is larger than that of the cornea (1 cm²) (Watsky et al., 1988). Due to the relative leakiness of the membrane, rich blood flow, and large surface area, conjunctival uptake of a topically applied drug from the tear fluids is typically an order of magnitude greater than corneal uptake (Urtti et al., 1985). Because of systemic drug absorption following conjunctival uptake, even substantial prolongations of the residence times of the vehicle in the conjunctival sac may not always result in significant improvements in ocular drug absorption (Gurny et al., 1987).

**Topical ocular application of antihistamines in allergic conjunctivitis.** Management of allergic conjunctivitis may be approached by the oral administration of antihistamines as used to treat the allergic rhinitis. However, oral antihistamines do not reliably relieve the ocular symptoms, especially in a short time. The treatment of ocular symptoms with topical medications delivered as eyedrops provides symptomatic relief without, or with decreased, systemic side effects.

Topical medications act directly at the site of application (the eyes), but ocular application has also been shown to relieve nasal allergy symptoms (Crampton, 2002) due to the drainage of the medication through the nasolacrimal duct to the nose. The absorption through the nasal mucosa is also responsible for producing possible systemic side effects.
The topical application of antihistamines provides a faster onset and greater relief, when compared to their systemic administration. For example, the onset of therapeuetic effect for levocabastine (Heykants et al., 1995) after topical application was very fast, within 15 minutes after the first application of a levocabastine eyedrop (Walker et al., 1988). After ocular delivery, the systemic bioavailability of levocabastine eyedrops was ranging from 30 to 60%.

Besides the rapid onset, the topical application appears to be safe for pediatric patients.

### Absorption after intranasal administration

#### The nasal cavity

The nasal cavity (Figure 7) has an important protective function in that it filters, warms, and humidifies the inhaled air before it reaches the lower airways. Any inhaled particles or microorganisms are trapped by the hairs in the nasal vestibule or by the mucus layer covering the respiratory area of the nasal cavity. Due to the mucociliary clearance mechanism, the mucus layer will gradually carry such particulates to the back of the throat, down the esophagus, and further into the GI tract. Further, the nasal mucosa has a metabolic capacity that will help convert endogenous materials into compounds that are more easily eliminated (Watelet and Van Cauwenberge, 1999; Van Cauwenberge et al., 2004).

A midline septum divides the human nasal cavity into two nonconnected parts. Each part consists of three regions: first, the vestibule consisting of the region just inside the nostrils with an area of about 0.6 cm² second, the olfactory region situated in the roof of the nasal cavity (Figure 7) and only covering about 10% of the total nasal cavity (150 cm²); and third, the respiratory region, which constitutes the remaining region. The respiratory region contains the three nasal turbinates (*conchae*), the superior, the middle, and the inferior, which project from the lateral wall of each half of the nasal cavity. The presence of these turbinates creates a turbulent airflow through the nasal passages, which ensures a better contact between the inhaled air and the mucosal surface (Arora et al., 2002).

The nasal vestibule is covered with stratified squamous epithelium, which gradually changes posteriorly into a pseudostratified columnar epithelium. The respiratory epithelial cells are covered by microvilli, and the major part of these cells is also covered with cilia. These cilia, which are long (4–6 µm), thin projections, are mobile and beat with a frequency of 1,000 strokes per minute. The beat of each cilium consists of a rapid movement, where the cilium reaches forward movement, and where the cilium is stretched and the tip of the cilium reaches into the mucus layer and carries this forward, followed by a slow return beat, where the cilium is bent and moves into the sol layer that lies beneath the mucus layer. In this way, the mucus layer is propelled in a direction from the anterior toward the posterior part of the nasal cavity. The mucus-flow rate is in the order of 5 mm/min, and hence, the mucus layer is renewed every 15–20 minutes (Illum, 2003).

#### Factors influencing nasal absorption

Lipophilic drugs are generally well absorbed from the nasal cavity, with the PK profiles often identical to those obtained after an i.v. injection and bioavailabilities approaching 100%. However, despite the large surface area of the nasal cavity (about 150 cm²) and the extensive blood supply, the permeability of the nasal mucosa is normally low for polar molecules, for which the bioavailabilities are generally around 10% (Costantino et al., 2007).

Another factor of importance for low membrane transport is the general rapid clearance of the administered formulation from the nasal cavity due to the mucociliary clearance mechanism. This is especially the case for drugs that are not easily absorbed across the nasal membrane. It has been shown that for both liquid and powder formulations, that are not mucoadhesive, the half-life of clearance is of the order of 15–20 minutes. It has further been suggested that the deposition of the formulation in the anterior part of the nasal cavity can decrease clearance and promote absorption, as compared to deposition further back in the nasal cavity. The type of delivery device for nasal administration has also been shown to influence the potential for systemic bioavailability. Pressured metered-dose inhalers (pMDIs), aqueous pump sprays and a powder inhaler have been used to topically administer nasal corticosteroids. The aerosol generated from the pMDI has a high velocity and is highly directional, resulting in a narrow proximal deposition in the nasal cavity. Comparatively, the aerosol from an aqueous pump spray displays a large droplet size with a more dispersed pattern of deposition. The nasal distribution pattern with a powder inhaler lies somewhere between the other two devices. For the intranasal formulation of budesonide, it was shown a significantly higher absorption level with the aqueous pump spray, compared with the pMDI and powder formulations. Due to the chlorofluorocarbone (CFC) propellant, the pMDIs are no longer used for nasal administration, and aqueous pump spray is now the recommended standard delivery device in the treatment of allergic rhinitis. In a recent study, it has been shown that the bioavailability of fluticasone propionate nasal drop formulation (0.06%) was approximately 8 times lower than that of the nasal spray (0.51%), which may be explained by the findings that nasal drops are cleared more quickly from...
the nose than nasal sprays. Another important factor is the administered volume of formulation. Indeed, the optimal formulation volume for nasal administration is 25–200 µL per nostril. Larger volumes will drain out of the nose. The most practical volume is 100 µL per nostril (Behl et al., 1998).

Another contributing factor to nasal absorption is the possibility of enzymatic metabolism in the nasal mucosa. Along with oxidative enzymes of the P450 system, several other enzymes exist in the nasal secretions (e.g., lactate dehydrogenase, oxidoreductases, hydrolases, acid phosphatase, and esterases). Oxidative phase 1 enzymes of the P450 system as well as of other systems and conjugative phase 2 enzymes are present in the nasal epithelium (Illum, 2003) (see below).

### Intranasal antihistamines and corticosteroids in allergic rhinitis

Intranasal antihistamines and intranasal corticosteroids represent major therapeutic options as first-line medications in the management of allergic rhinitis because of the prominent role of histamine as a mediator of rhinitis and the underlying nature of allergen-induced inflammation, which is glucocorticoid-responsive. Further, topical intranasal therapy allows site-directed treatment with a reduced risk of systemic effects because of the low bioavailability of intranasal antihistamines and intranasal corticosteroids from this site (Salib and Howarth, 2003).

#### Intranasal Antihistamines

The intranasal route of administration delivers drug directly to the target organ, thereby minimizing the potential for the systemic adverse effects that may be evident with oral administration. When administered at standard recommended therapeutic dosage, the intranasal antihistamines do not cause significant sedation or impairment of psychomotor function, effects that would be evident when these agents are administered orally at a therapeutically relevant dosage.

**Levocabastine.** Available as a 0.5-mg/mL microsuspension nasal spray, the recommended nasal dosage for levocabastine is 0.1 mg into each nostril twice-daily. It has a rapid onset of action (10–15 minutes) and is effective for up to 12 hours. Levocabastine is absorbed following intranasal administration, with a systemic bioavailability of 60–80% after a single-dose nasal administration with peak plasma concentration reached after 1–4 hours. The C_{max} value of 0.78 µg/L was reached 2.9 hours following a single nasal application of 0.1 mg (Heykants et al., 1995; Dechant and Goa, 1991).

**Azelastine.** Azelastine is a second-generation H₁-receptor antagonist, but caused sedation when administered orally and was thus developed for topical application to the nose. Topical administration via the intranasal route confines the effect largely to the nose and reduces the likelihood of adverse effects due to systemic absorption. Standard dosage of topical azelastine is 0.14 mg into each nostril twice-daily. The onset of action is within 30 minutes after a single intranasal administration. Azelastine is effective for up to 12 hours. The estimated systemic exposure to the intranasal drug was 6- to 8-fold lower than with oral azelastine (Riethmuller-Winzen et al., 1994). A systemic bioavailability of 40% has been shown following the intranasal administration of azelastine (Weliky et al., 1990).

#### Intranasal corticosteroids

Intranasal corticosteroids are currently recognized as the most potent, effective topical medication available for the treatment of moderate to severe seasonal or perennial allergic rhinitis. The rational for using intranasal corticosteroids in the treatment of allergic rhinitis was that high drug concentrations could be achieved at receptor sites in the nasal mucosa, with only a minimal risk of systemic adverse effects (i.e., hypothalamic-pituitary-adrenal axis suppression) (Salib and Howarth, 2003).

Beclomethasone dipropionate was the first topical corticosteroid introduced as a nasal spray for the treatment of allergic rhinitis. Thereafter, several other intranasal corticosteroids have been developed, such as budesonide (Stanaland, 2004), flunisolide, fluticasone propionate/furoate (Kaiser et al., 2007), mometasone furoate (Zitt et al., 2007; Hochlaus, 2008), and triamcinolone (Lumry, 1999).

The ideal intranasal corticosteroid has a high potency and a high affinity for the local glucocorticoid receptors and a low systemic toxicity at therapeutic doses, leading to a favorable benefit-risk ratio.

Mometasone furoate nasal spray typifies a satisfying intranasal corticosteroid. This compound has an important first-pass effect associated with the lowest systemic bioavailability (0.46%), compared to the other available intranasal corticosteroids (Table 3). The low systemic bioavailability of mometasone furoate could be explained by its rapid hepatic metabolism and inherently low aqueous solubility, resulting in a negligible amount of drug crossing the nasal mucosa and entering the bloodstream. This low systemic bioavailability contributes to obtain high local concentrations in the nasal mucosa after nasal administration (van Drunen et al., 2005). The use of lipophilic mometasone furoate in a suspension-based nasal delivery system favors a long nasal residence time due to sustained dissolution of drug particles, and once the particles are dissolved, facilitate absorption into nasal tissue.

Fluticasone propionate has PK characteristics similar to those of mometasone furoate, although the unbound fraction is higher.

Table 3 summarizes the distinguishing factors of newer intranasal corticosteroids used in the treatment of allergic rhinitis.
### Distribution

**What is distribution?**

Distribution is a process during which a drug moves to and from the blood and tissues or organs within the body. It eventually also plays an important role in determining the pharmacologic response to a drug (Lin, 2006), although a high volume of distribution does not necessarily correlate with pharmacological activity (Tayab and Hochhaus, 2007).

In essence, four types of distribution patterns can occur (Figure 8; Tillement, 1995): 1) drugs that remain largely within the vascular system; this pattern can be seen with drugs that are very tightly bound to plasma proteins; 2) drugs that equally distribute throughout the body water, a pattern observed for some water-soluble compounds with a low molecular weight; 3) drugs that specifically concentrate in one organ or tissue, which does not necessarily have to be the target organ or tissue: and 4) drugs that exhibit a nonuniform distribution pattern, typically a combination of the former three distribution patterns.

### Volume of distribution

The volume of distribution is a primary PK parameter that relates a drug’s plasma concentration to the amount of drug in the body. It can provide an indication on the type of distribution pattern. At steady state, the volume of distribution corresponds to the equivalent plasma volume in which a drug is distributed into the body (Poulin and Theil, 2002). This volume would correspond to the sum of plasma volume \( V_p \) with the sum of each tissue volumes \( V_T \) multiplied by the corresponding tissue:plasma partition coefficients \( K_{T:P} \) (Sawada et al. 1984; Poulin and Theil, 2002):

\[
V_d = V_p + \sum V_T \cdot K_{T:P}
\]

Based on mass balance considerations, the volume of distribution can also be expressed in terms of apparent volume of tissue and, given that distribution equilibrium is achieved when the unbound concentrations in plasma and tissues are equal, the volume of distribution can be described as follows:

\[
V_d = V_p + V_{TW} \left( f_u / f_uT \right),
\]

where \( V_p \) is the plasma volume, \( V_{TW} \) is the aqueous volume outside the plasma into which the drug distributes, \( f_u \) is the fraction of unbound drug in plasma, and \( f_uT \) is the fraction unbound in tissue (Rowland and Tozer, 1995a). The reference volumes where drug distribution can take place are: 1) plasma volume (3 L in a standard 70-kg human subject, approximately 4% of body weight); 2) extracellular space (15 L, approximately 20% of body weight); and 3) total body water (42 L, approximately 60% of body weight).

When the volume of distribution is equal or higher than the total body water, then the drug is distributed into tissues. A volume of distribution smaller than the total amount of body water indicates that the drug is retained in plasma or in the extracellular water and probably is bound to plasma proteins.

### Factors affecting drug distribution

Several factors determine the ability of a drug to distribute into organs and tissues.

The molecular size of drug will determine its ability to move from the plasma compartment into the extracellular fluid compartment, whereas the exchange from this latter compartment into the cells will be determined by the lipophilic character of the drug. Movement through cell membranes can occur by passive diffusion, active transport, facilitated diffusion, and endocytosis. Passive diffusion does not require energy. Weak acids and bases need to be in their nonionized form in order to move via passive diffusion. Active transport, on the other hand, requires energy and a transport protein. These transport proteins can

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**Table 3.** Clinically distinguishing factors of newer intranasal corticosteroids (Hochhaus, 2008; Zitt et al., 2007; Stanaland, 2004; Lumry, 1999).

| Intranasal corticosteroids | Bioavailability (%) | Unbound fraction (%) | Onset of action | Daily dose (µg) |
|---------------------------|--------------------|----------------------|----------------|----------------|
| Beclomethasone            | 44                 | —                    | Within 3 days  | 336–400        |
| Budesonide                | 31                 | 0.12                 | From 24 hours to a few days | 128 |
| Flunisolide               | 40–50              | 0.20                 | 4–7 days       | 200            |
| Triamcinolone             | 46                 | 0.29                 | From <24 hours to maximum of 7 days | 220 |
| Fluticasone propionate    | 0.42–0.51          | 0.10                 | From 12 hours to several days | 200 |
| Mometasone furoate        | 0.46               | 0.002–0.01           | 36 hours       | 200            |

**Figure 8.** Schematic representation of the various drug-distribution patterns (adapted from Bourne, 2008c)
carry a drug against a gradient (see below). The latter two mechanisms are used by only a very few drugs. In case of facilitated diffusion (e.g., sugar, aminoacids, and some drugs, such as penicillin, furosemide, and morphine), a protein exists in the cell membrane that binds the compound and facilitates its movement down its concentration gradient, which, similarly to passive diffusion, does not require energy. However, since a transporter is involved, the process may be saturable. Endocytosis is the incorporation in the cell of small droplets or particles containing the compound. Its role in the delivery and/or distribution of drugs is not known.

The blood perfusion through the organ or tissue also plays an important role; tissues with a high blood perfusion will exhibit a faster drug uptake than tissues with a low perfusion rate. Brain, kidney, liver, and heart are the organs with the highest perfusion rates.

Body composition also affects the distribution of a drug. Fat-soluble drugs, for example, may be stored in larger amounts in obese people, when compared to thin people. The elderly also may tend to store fat-soluble drugs in larger amounts as the proportion of body fat increases with aging.

Binding to plasma and tissue proteins as well as the presence of and affinity for influx and efflux transporters influence the distribution of a drug into the body (Lin, 2006). The most common plasma and extracellular protein to which drugs bind is albumin. Within the different compartments, drugs can exist in bound or unbound form. However, only the unbound drug can cross the cellular barriers between compartments and can induce a pharmacological or a toxic effect. An example of such a cellular barrier between two different compartments is the blood-brain barrier (BBB). It is a dynamic interface between the blood and the brain, which actually determines whether or not a drug can enter the central nervous system. This can either be by passive diffusion or through carrier- or receptor-mediated transport (Lin 2006; de Boer et al. 2003). The integrity of the barrier, as well as the homeostasis within the brain, is ensured by many transport systems, including P-glycoprotein (P-gp) (de Boer et al. 2003), which is expressed at the luminal membranes of endothelial cells of the BBB (Cordon-Cardo et al. 1989). In general, drugs with an extensive plasma-protein binding tend to have a lower volume of distribution as they stay mainly in the blood compartment. Plasma-protein binding can also be a source of variability within and among patients (Rowland and Tozer, 1995a) due to physiopathological status and/or genetic variability (see review article Gender and Interindividual variability in Pharmacokinetics, and review article Pharmacokinetics in special populations of this Special issue). Binding to tissue proteins can, but does not necessarily have to, be related to the pharmacological action of the drug.

**H<sub>1</sub>-antihistamines**

For having a pharmacological effect, H<sub>1</sub>-antihistamines do not require an extensive tissue distribution (Tillement, 1995). A small volume of distribution can even be considered advantageous, not only in terms of efficacy, but also in relation to their safety profile (Tillement, 1995; Baltes et al., 2001). Indeed, a link has been suggested between the frequencies of adverse effects and the corresponding volume of distribution in this class of drugs (Tillement, 1995). Since antihistamines are usually not used for the treatment of life-threatening disorders, but are mainly prescribed for chronic use, it is important to consider this benefit-risk ratio.

First-generation antihistamines, including hydroxyzine and diphenhydramine, are quite lipophilic. They can thus easily cross the BBB, and once in the brain, they can interact with central histamine receptors and, as such, give rise to sedative side effects (Ibiapina et al., 2008; Schran et al, 1996; Ünal and Hafiz, 2005; Willisie, 2002). Moreover, these first-generation drugs are often less selective for H<sub>1</sub>-receptors (Snyder and Snowman, 1987; Tillement, 1995).

Second-generation antihistamines, on the other hand, are less lipophilic and do not easily penetrate the BBB (Sendur and Uslu, 2005). Their passive membrane permeability is, on average, 1.8-fold lower than those of first-generation antihistamines (Polli et al. 2003). In addition, these second-generation antihistamines often are a substrate for the P-gp efflux protein, which is expressed at the BBB (de Boer et al., 2003) and actively pumps the drug out of the brain. Fexofenadine, the active metabolite of terfenadine, for example, is a P-gp substrate (Tannergren et al. 2003a, 2003b) and is devoid of any sedative effects, even at supratherapeutic doses (Tashiro et al., 2004). Carebastine, a first-pass active carboxylic acid metabolite of ebastine, is also a P-gp substrate, in contrast to the parent compound (Tamai et al., 2000). Other antihistamine-related adverse effects, which rarely occur and are mostly seen following an overdose, are cardiac or liver toxicity, and these are probably due to either accumulation of the drug in these respective tissues (Simons and Simons, 1991b; Davies et al., 1989) or is metabolism related (Hey et al., 1996) (see below).

As said previously, H<sub>1</sub>-antihistamines ideally have a low distribution volume (Tillement, 1995). This actually means that it should be less than the volume of exchangeable water in the body (i.e., 0.6 L/kg) (Tillement et al., 1984). Cetirizine and its active enantiomer, levocetirizine, are probably the only antihistaminic drugs, which fulfill this criterion: They have the lowest volumes
of distribution (i.e., 0.4–0.5 L/kg) (Whomsley and Strolin Benedetti, 2005; Strolin Benedetti et al., 2001; Baltes et al., 2001). Antihistaminic drugs with a high volume of distribution and thus an extensive tissue distribution are ebastine (>100 L/kg), loratadine (119 L/kg), and desloratadine (49 L/kg) (Whomsley and Strolin Benedetti, 2005). Fexofenadine (Wang et al., 2002), mizolastine (Simons, 1999; Mesnil et al., 1997; Lebrun-Vignes et al., 2001), and levocabastine (Heykants et al., 1995) have lower distribution volumes of ca. 6, ca. 1–1.4, and 1.14 L/kg, respectively (Table 4).

\( \text{V}_{dss} \) (determined after an i.v. dose of 0.2 mg).

\( \text{F} \), volume of distribution during the terminal phase/

bioavailability; \( \text{PPB} \), plasma-protein binding.

\( \text{V}_{dss} \) (determined after an i.v. dose of 0.2 mg).

Table 4. Volume of distribution and plasma-protein binding of selected H1-antihistamines (see text for references).

| H1-antihistamines | VA/F (L/kg) | PPB (%) |
|-------------------|------------|---------|
| Cetirizine        | 0.5        | 88-90   |
| Levocetirizine    | 0.4        | 91      |
| Mizolastine       | 1.0–1.4    | 98      |
| Levocabastine     | 1.14        | 55      |
| Fexofenadine      | 5.4–5.8    | 60–70   |
| Loratadine        | 119        | 97–99   |
| Desloratadine     | 49         | 82–87   |
| Ebastine          | >100       | NA (very extensive metabolism) |

\( \text{Vdss} \), volume of distribution during the terminal phase/bioavailability; \( \text{PPB} \), plasma-protein binding.

\( \text{Vdss} \) (determined after an i.v. dose of 0.2 mg).

Table 5. Volume of distribution and plasma-protein binding of inhaled and intranasal corticosteroids (based on Rohatagi et al., 2004; Winkler et al., 2004).

| Corticosteroid          | \( \text{Vdss} \) (L) | PPB (%) |
|-------------------------|----------------------|---------|
| Mometasone furoate      | —                    | 98–99   |
| Fluticasone propionate  | 318–859              | 90      |
| Beclomethasone dipropionate | 20              | 87      |
| Beclomethasone monopropionate | 424          | —       |
| Ciclesonide             | 207                  | >99     |
| Desciclesonide          | 897                  | >99     |
| Budesonide              | 183–301              | 88      |
| Loteqrednol etabonate   | 37\*                 | 90      |
| Triamcinolone acetamide | 103                  | 71      |
| Flunisolide             | 61–96                | 80      |

\( \text{PPB} \), plasma-protein binding.

\( \text{Vdss} \) (determined after an i.v. dose of 0.2 mg).

\( \text{PPB} \), plasma-protein binding.

\( \text{Vdss} \) (determined after an i.v. dose of 0.2 mg).

\( \text{PPB} \), plasma-protein binding.

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\( \text{PPB} \), plasma-protein binding.

\( \text{Vdss} \) (determined after an i.v. dose of 0.2 mg).

\( \text{PPB} \), plasma-protein binding.
of the drug into other tissues, hence increasing the potential for unwanted systemic side effects (Zitt, 2005). In terms of lipophilicity, corticosteroids can be ranked in decreasing order as follows: ciclesonide > des-ciclesonide > fluticasone propionate > beclomethasone monopropionate > beclomethasone dipropionate > budenoside > triamcinolone acetonide > flunisolide (Zitt, 2005; Derendorf and Meltzer, 2008). Corticosteroids, such as fluticasone propionate, ciclesonide, and des-ciclesonide (the hydrolysis product of ciclesonide), have a very large volume of distribution (>300 L) and are thus extensively distributed and bound to tissues (Kaliner, 2006; Winkler et al., 2004). This increased volume of distribution also implies a longer elimination half-life of the drug, which is not in agreement with one of the properties of an “ideal intranasal” corticosteroid, namely a rapid systemic elimination (Derendorf and Meltzer, 2008). Pulmonary concentrations for ciclesonide and des-ciclesonide are greater than those of fluticasone propionate. Upon activation in the lungs, des-ciclesonide reversibly conjugates to intracellular lipids (Nave et al., 2005), which hampers its diffusion out of the lung, and consequently, less active drug is present in the systemic circulation (Kaliner, 2006; Zitt, 2005). Once des-ciclesonide is diffused into the systemic circulation, its volume of distribution is very high. However, about 99% of the drug is bound to plasma proteins and thus only 1% could theoretically cause systemic adverse effects (Kaliner, 2006; Winkler et al., 2004; Nave et al., 2004; Zitt, 2005), whereas the free fraction for fluticasone propionate is 10 times higher (Nave et al., 2004; Zitt, 2005).

Typical values of volume of distribution for inhaled and intranasal corticosteroids are listed in Table 5.

Corticosteroids do not easily cross the BBB. This is related to their affinity to P-gp and BCRP (breast cancer resistance protein) transporter proteins (Cooray et al., 2006). Budesonide and prednisone are known P-gp substrates (Dilger et al., 2004). Beclomethasone dipropionate, mometasone furoate, and des-ciclesonide were shown to inhibit the efflux in BCRP-expressing MCF7/MR breast cancer cells and in P-gp-expressing SW620/R colon carcinoma cells, all at concentrations ranging from 0.1 to 10 µM (Cooray et al., 2006). The observation that these three compounds also increased the sensitivity of P-gp-expressing cells to the toxic effects of doxorubicin and stimulated ATPase-activity associated with BCRP expressed in bacterial membrane vesicles, suggest that they might be substrates for both BCRP and P-gp transporters as well (Cooray et al., 2006). P-gp transporters are also present in the lungs, where they are expressed at the apical side of ciliated epithelial cells and on the apical surface of serous cells of bronchial glands (Lechapt-Zalcman et al., 1997; van der Deen et al., 2005). However, the function of P-gp in the lungs remains, at present, unknown (van der Deen et al., 2005). Nevertheless, the fact that some corticosteroids display a certain affinity to drug-transporter proteins suggests the likely existence of drug-drug interactions (for drug-drug interaction, review article Drug Interactions of this Special issue) or pharmacogenomic differences among patients at the transporter level (Tayab and Hochhaus, 2007) (see section Transporters of this review article and review article Drug Interaction of this Special issue).

Leukotriene inhibitors

Leukotriene inhibitors can be divided into antagonists of leukotriene receptors, such as zafirlukast and montelukast or inhibitors of leukotriene synthesis (e.g., zileuton). These drugs are strongly bound to plasma proteins with free fractions of 0.01 for montelukast and zafirlukast and 0.07 for zileuton. Data on their distribution are rather scarce. An apparent volume of distribution (Vd/F) of 0.157 L/kg has been reported for montelukast in a clinical study in dyspeptic children (Friesen et al., 2004).

Metabolism

Introduction

Small-molecule drugs are generally xenobiotics (i.e., molecules foreign to man's normal biochemistry), although there are notable exceptions (e.g., some natural steroids and hormones, which are used as drugs). Ideally, a drug should reach its pharmacological target and be eliminated once its effect is no longer required. Some drugs are eliminated from the human body intact (without any structural changes due to the metabolism); however, most drugs need to be rendered water soluble through chemical modification to facilitate their excretion in the urine or bile. These modification processes are called drug metabolism. Drug metabolism can be seen as a detoxification function that the human body possesses to defend itself from environmental hostility (Nadendla, 2004). During the process of drug development, it is important to check that the pharmacokinetics of a potential drug are appropriate for its intended action (e.g., a potential drug may be rapidly eliminated, resulting in subtherapeutic levels, or its elimination may be too slow, resulting in accumulation and unwanted effects). If not modified chemically, some drugs would persist in the tissues indefinitely (e.g., lipophilic drugs in adipose tissues). Metabolism normally results in the termination of the drug’s intended action, although, in some cases, the metabolites may also be pharmacologically active. For prodrugs, the active metabolite is essentially responsible for the pharmacological activity of a
drug. The active form may be poorly absorbed or subject to rapid first-pass elimination. Once administered, the prodrug is metabolized in vivo into an active metabolite. The use of prodrugs can enhance the absorption of drugs and can protect the active moiety from rapid first-pass metabolism, providing more sustained levels of the active principle. The study of drug metabolism, therefore, serves primarily two purposes: to elucidate the function and fate of the drug and to manipulate the metabolic process of a potential drug.

The liver is considered the major “metabolic clearing house” for both endogenous chemicals (e.g., cholesterol, steroid hormones, fatty acids, and proteins) and xenobiotics (Gonzales and Tukey, 2005). The small intestine has the ability to metabolize drugs by numerous pathways and is responsible for the initial metabolic processing of many orally administered drugs prior to transport via the portal circulation to the liver (Figure 9). While a portion of active drug escapes this first-pass metabolism in the GI tract and liver, subsequent recirculation through the liver results in continued metabolism of the parent drug until it is completely eliminated (Gonzales and Tukey, 2005). Drugs that are poorly metabolized remain in the body longer, with longer elimination half-lives than drugs that are rapidly metabolized, if they are not eliminated through other mechanisms. Other organs that contain significant xenobiotic-metabolizing enzymes include the nasal mucosa and the lungs, which play important roles in the first-pass metabolism of antiallergic drugs that are administered through nasal sprays, aerosols, or puffs. Transcripts for nine drug-metabolizing enzymes (ALDH6, ALDH7, CYP1B1, CYP2E1, CYP2F1, CYP4B1, FMO1, GSTP1, and UGT2A1; see below for further information) have been demonstrated in human nasal mucosa by Zhang et al. (2005). These findings, combined with previous detection of other enzymes in human nasal mucosa (CYP2A6, CYP2A13, CYP2B6, CYP2C, CYP2J2, CYP3A, NADPH-cytochrome P450 reductase, microsomal epoxide hydrolase, GSTA, GSTP1, and UGT2A1; see below for further information) (Ding and Dahl, 2003; Ding and Kaminsky, 2003), provide strong support for the idea that human fetal, as well as adult nasal, mucosa plays an active role in the biotransformation of numerous xenobiotics. These tissues are also the first line of contact with hazardous airborne chemicals. Kidneys may also play an important role in the metabolism of xenobiotics, and blood can also play a significant role in the metabolism of some drugs and prodrugs.

Drug-metabolizing enzymes have been grouped into phase I reactions, in which enzymes carry out oxidation, reduction, or hydrolytic reactions, and phase II reactions, which involve the introduction of a hydrophilic endogenous species to the drug molecule (Gonzales and Tukey, 2005). The phase I enzymes lead to the introduction of functional groups, resulting in a modification of the drug, for example, an –OH, -COOH, -SH, -O-, or NH2 group. The phase 2 reactions may occur directly on the parent compounds that contain appropriate structural motifs or on functional groups added or exposed by phase I oxidation (Zamek-Gliszczynski et al., 2006).

Sulfation, glucuronidation, and glutathione conjugation represent the three most prevalent classes of phase II metabolism. These conjugation reactions impart a greater water solubility and increased molecular weight to the metabolite, in addition to adding a negative charge to the molecule. Consequently, the membrane permeability of many phase II conjugates is poor, and carrier-mediated transport is required for eventual excretion. Phase 2 reactions are generally cytosolic, with the exception of glucuronidation, which is microsomal. Conjugation reactions also generally terminate the biological activity of the drug, although there are exceptions (e.g., many glucuronide conjugates of

**Figure 9.** Liver. (A) Anatomy: liver and annexes (scheme): 1. Liver. 2. Gallbladder. 3. Bile duct. 4. Stomach. 5. Pancreas. 6. Small intestine (duodenum). 7. Inferior vena cava. 8. Aorta. 9. Vena portae. (B) Histology: liver (human sample) (H&E, magnification X100): 1. Sinusoids. 2. Lobule boundary. Portal area: 3. Bile duct. 4. Hepatic artery. 5. Portal vein. (C) Histology: liver (human sample) (H&E, magnification X400): 1. Sinusoids. 2. Sheet of hepatocytes. 3. Central vein. H&E.
opiates have a significant biological activity). The catalytic rates of phase 2 reactions are generally significantly faster than the rates of the CYPs (Gonzales and Tukey, 2005). Therefore, the initial (phase 1) oxidation reaction is normally rate limiting.

**Phase I reactions**

**Oxidation**

The most common phase I transformations are oxidative reactions that involve cytochrome P450 (CYP) enzymes. The CYP isozymes are a superfamily of heme-containing mono-oxygenases comprising over 50 members that catalyze the oxidative metabolism of many structurally diverse drugs and chemicals. These enzymes are located in the lipophilic membranes of the smooth endoplasmic reticulum of the liver and other tissues. When isolated, the smooth endoplasmic reticulum reforms into vesicles called microsomes, which are extensively used in the *in vitro* study of drug metabolism and drug interactions. The CYP families 1–4 are mainly involved in xenobiotic metabolism, while other CYP families are mainly involved in the metabolism of endogenous substrates. CYPs are responsible for the phase I metabolism of most clinically used drugs and are also responsible for the metabolic activation of many chemical carcinogens and toxins. These enzymes catalyze the following reactions: aromatic hydroxylation; aliphatic hydroxylation; N-, O-, and S-dealkylation; N-hydroxylation; N-oxidation; sulfoxidation; deamination; and dehalogenation. The main CYP isoforms responsible for the metabolism of xenobiotics are CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4, and CYP3A5. Interestingly, some second-generation antihistamines have been shown to be substrates for CYP2J2 and CYP4F12, isoforms with a significant extrahepatic expression (Whomsley and Strolin Benedetti, 2005). Zeldin et al. (1996) have demonstrated an abundant pulmonary CYP2J2 protein expression in human lung. Using immunochemical analysis, these researchers demonstrated that CYP2J2 proteins appear to be primarily expressed in ciliated epithelial cells lining the airway, but also in nonciliated airway epithelial cells, bronchial and pulmonary vascular smooth muscle cells, pulmonary vascular endothelium, and alveolar macrophages, whereas a minor expression was noted in alveolar epithelial cells (Figure 10). CYP2J2 has recently been shown to be an important enzyme in the metabolism of epoxygenase-derived eicosanoids that play important functional roles in pulmonary physiology and may contribute to the pathogenesis of asthma. Polonikov et al. (2007) have shown that promoter polymorphism G-50T of a human CYP2J2 epoxygenase gene is associated with common susceptibility to asthma. Finally, Thum et al. (2006) found that in bronchial biopsies of smokers, CYP2J2 was repressed. Five of the human CYPs (1A2, 2C9, 2C19, 2D6, and 3A4) are involved in about 95% of the CYP-mediated metabolism of drugs representing about 75% of drug metabolism. CYP3A4 is the most commonly implicated isoform in drug metabolism, being responsible, at least in part, for the metabolism of approximately 50–60% of clinical drugs used today.

**Non-CYP oxidative metabolism**

Although the majority of oxidative metabolic reactions are mediated by the CYP superfamily of enzymes, non-CYP-mediated oxidative reactions can additionally play an important role in the metabolism of xenobiotics (Strolin Benedetti et al., 2006). There are numerous non-CYP oxidative enzymes that are capable of directly oxidizing xenobiotics or of further oxidizing metabolites formed by CYP isoforms. Like CYPs, other oxidative enzymes

![Figure 10. Respiratory tract. (A) Anatomy: respiratory tract (scheme): 1. Nasal cavity. 2. Mouth. 3. Pharynx. 4. Larynx. 5. Trachea. 6. Lung. 7. Bronchi. 8. Bronchioli. 9. Alveoli. (B) Histology: lung parenchyma (human sample) (H&E, magnification X40): 1. Bronchi. 2. Bronchioli. 3. Alveolar sac. 4. Alveoli. 5. Capillaries. (C) Histology: Alveoli (scheme and human sample) (H&E, magnification X400): 1. Alveoli. 2. Capillaries. H&E.](image-url)
involved in the metabolism of drugs or other xenobiotics can also produce therapeutically active metabolites, reactive/toxic metabolites, modulate the efficacy of therapeutically active drugs, or contribute to detoxification. The (major) oxidative enzymes other than CYPs involved in the metabolism of drugs and other xenobiotics are the flavin-containing mono-oxigenases (FMOs), the molybdenum hydroxylases [aldehyde oxidase (AO) and xanthine oxidase (XO)], prostaglandin synthase (PGHS), the lipoxygenases (LOs), the amine oxidases [monoamine oxidase (MAO), polyamine oxidase (PAO), diamine oxidase (DAO), and semicarbazide-sensitive amine oxidase (SSAO)] (see also the review article Drug Interactions of this special issue), the alcohol dehydrogenases (ADH), and aldehyde dehydrogenases (ALDH). According to the drug involved (e.g., hydroxyzine or felbamate), the same enzyme (ADH) can form either a therapeutically active metabolite (cetirizine) or a toxic metabolite (atropaldehyde).

Reduction
Although oxidative processes constitute the majority of phase I metabolic pathways for drugs, reductive reactions have also been identified and may be of pharmacological importance, particularly if they generate active or toxic metabolites. Reduction of anthracines (daunorubicin, doxorubicin, and idarubicin) in humans by the cytoplasmic aldo-ketoreductases and the microsomal cytochrome P-450 reductase produces active metabolites (e.g., idarubicinol, the 13-dihydroderivative of idarubicin, identified as the major urinary metabolite of idarubicin, has antitumor activity similar to that of idarubicin in animal models) (Strolin Benedetti et al., 1991). Reduction is also important in the metabolism of fenofibrate, a hypolipidaemic agent structurally related to clofibrate (Weil et al., 1987).

Many of the products of oxidative metabolism are substrates for reductive reactions. This can result in redox cycling, with the balance of cofactors and oxygen concentrations ultimately deciding the position of the equilibrium (Kappus et al., 1986).

Many of the enzymes involved in reductive reactions are the same as those involved in oxidative reactions: e.g. cytochrome P450, cytochrome P450 reductase, ADH, ALDH, and molybdenum hydroxylases.

Hydrolysis
Hydrolysis is also observed for a wide variety of drugs. Examples of enzymes involved in hydrolysis are epoxide hydrolases, esterases, amidases, and proteases. Two forms of epoxide hydrolase carry out hydrolysis of epoxides, which are organic three-membered oxygen compounds arising from the oxidative metabolism of xenobiotic and endogenous compounds via chemical and enzymatic oxidation by CYPs (Fretland and Omiecinski, 2000). Epoxides are highly reactive electrophiles that can bind to cellular nucleophiles found in protein, RNA, and DNA, resulting in cell toxicity and transformation. The soluble epoxide hydrolase (sEH) is expressed in the cytosol while the microsomal epoxide hydrolase (mEH) is localized to the membrane of the endoplasmic reticulum. Microsomal epoxide hydrolase (mEH), which has a broad substrate specificity, seems to be responsible for the detoxification of most epoxides, while the soluble epoxide hydrolase enzyme seems to preferentially act on lipid epoxides (Arand et al., 2003). Epoxide hydrolases generally convert epoxides to dihydrodiols. The antiepileptic drug, carbamazepine, is a prodrug that is converted to its pharmacologically active derivative, carbamazepine-10,11-epoxide by CYP3A4. This metabolite is hydrolyzed to a trans-dihydrodiol by mEH, resulting in the inactivation of the drug. Soluble epoxide hydrolase was originally found in the liver and was thought to be involved primarily in the metabolism of xenobiotic compounds; it is now known to be expressed in a variety of other organs and tissues, including vascular smooth muscle, where it can modulate the activity of endogenous epoxides, including the epoxyeicosatrienoic acids (EETs).

EETs are produced by the metabolism of arachidonic acid (AA) by cytochrome P450 (CYP) epoxygenase enzymes. Several CYP isoforms exhibit epoxygenase activity, including the 1A, 2B, 2C, 2D, 2E, 2J, and 4A sub-families. Most of these are found predominantly in the liver. However, several of these are expressed in other tissues, where they metabolize endogenous substrates, including AA. The CYP2J2 (a member of the 2J subfamily) has recently emerged as a potential source of EETs in extrahepatic tissues, as it is prominently expressed in the heart, lung, kidney, pancreas, small intestine, and vascular smooth muscle. Modulation of EET bioavailability generally occurs either by metabolic breakdown of these compounds by sEH or by reincorporation of these compounds into the phospholipid membrane (Larsen et al., 2006). AS EETs may modulate many aspects of vascular physiology, and as they also possess anti-inflammatory properties, a promising approach for enhancing long-term EET bioavailability appears to be the pharmacological inhibition of EET metabolism by sEH. The development of specific sEH inhibitors has been the focus of intense investigation, although the potential benefits of sEH inhibitors are accompanied by important limitations, and future studies will be necessary to understand the global effects of sEH inhibition (Larsen et al., 2006).

Phase II reactions
Glucuronidation
Uridine 5’-diphosphate-glucuronosyltransferases (UGTs) are one of the major classes of conjugative enzymes.
responsible for phase 2 reactions in the metabolism of many drugs. These enzymes catalyze the conjugation of glucuronic acid from the cofactor UDP-glucuronic acid to a nucleophilic substrate to form glucuronides. Any atom with sufficient nucleophilicity is capable of being glucuronidated. There are four types of glucuronides (O-glucuronide, N-glucuronide, S-glucuronide, and C-glucuronide). Functional groups known to be conjugated include alcoholic and phenolic hydroxyl groups, amines, carboxyl, sulfhydryl, and carbonyl moieties (e.g., α,β-unsaturated ketone). There are 19 human genes that encode the UGT proteins divided into three subfamilies of UGTs in humans: UGT1A, 2B, and 2A. The tissue distribution of UGT isoforms is similar to that of CYPs; however, their subcellular localization within the endoplasmic reticulum (ER) differs. While the CYPs are oriented in the ER such that the majority of the protein, including the active site, is cytosolic, the UGTs exist in the ER in a conformation such that the majority of the protein is luminal, which results in a phenomenon of latency for the glucuronidation reaction (Fisher et al., 2001).

Sulfation

The sulfotransferases (SULTs) are cytosolic enzymes catalyzing the sulfation of numerous xenobiotics, drugs, and endogenous compounds. Sulfation results from the transfer of a sulfate group from 3'-phosphoadenosine 5'-phosphosulfate (PAPS) to the hydroxyl groups of aromatic and aliphatic compounds. In humans, 12 SULT enzymes have been identified and classified into the SULT1A, SULT2, and SULT4 families (Lindsay et al., 2008). SULTs are implicated in the conjugation of many drugs (e.g., the oestrogenic component of the oral contraceptive pill, ethinylestradiol, is metabolized, to a large extent, by SULT1E1) (Schrag et al. 2004). Sulfotransferases and glucuronosyltransferases often have overlapping substrate specificities.

Glutathione conjugation

The glutathione-S-transferases (GSTs) are dimeric enzymes that catalyze the conjugation of glutathione (GSH) to reactive electrophiles generated in molecules (i.e., xenobiotics and/or endogenous compounds) during metabolic processes. This serves to protect cellular macromolecules from interactions with electrophilic heteroatoms (-O, -N, and -S) and, in turn, protects the cellular environment from damage. For this conjugation reaction, reduced GSH is necessary as a cosubstrate. GSH exists in the cell as oxidized (GSSG) or reduced (GSH), and the ratio of GSH/GSSG is critical in maintaining a cellular environment in the reduced state (Gonzales and Tukey, 2005). GSH is found in virtually all mammalian cells, and, as the concentration is as high as 1–10 mM (Saito et al., 1984), many drugs and xenobiotics can react nonenzymatically with GSH. However, the GSTs have been found to occupy up to 10% of the total cellular protein concentration and this assures the efficient conjugation of GSH to reactive electrophiles (Gonzales and Tukey, 2005). Cytosolic GST forms have more importance in the metabolism of drugs and xenobiotics, whereas the microsomal GSTs are important in the endogenous metabolism of leukotrienes and prostaglandins (Townsend and Tew, 2003).

N-Acetylation

The cytosolic N-acetyltransferases (NATs) catalyze the transfer of an acetyl group from acetyl CoA to the terminal nitrogen of the aromatic amine or hydrazine group. Unlike most phase II reactions, the addition of the acetyl group can generate a metabolite that is less water soluble, because the potentially ionizable amine is neutralized by the covalent addition of the acetyl group. These enzymes have also been implicated in the metabolic activation of chemicals into DNA-binding electrophiles and have been postulated to be causative agents in the development of certain forms of human cancer. NATs were the first enzymes to be identified as being polymorphic, as demonstrated in the inactivation of the antitubercular drug, isoniazid. Two polymorphic isoforms (NAT1 and NAT2) have been described in humans (Boukouvala and Fakis, 2005) (see also the review article Pharmacokinetics in special populations of this special issue). Both enzymes are capable of N-acetylation, O-acetylation, and N, O-acetylation (Brockton et al., 2001). Many allelic variants of NAT1 and NAT2 have been characterized, and many studies have demonstrated an association between the NAT phenotype/genotype and drug toxicities (Windmill et al., 2000).

Methylation

The transfer of methyl groups from S-adenosyl-L-methionine to methyl acceptor substrates is one of the most common reactions in nature. The methyl groups are transferred to a sulfur-nucleophile or nitrogen-nucleophile or oxygen-nucleophile (Strolin Benedetti et al., 2007). The conjugation reactions are carried out by S-methyltransferases, N-methyltransferases, and O-methyltransferases, the latter including catechol-O-methyltransferases (COMT). As far as we know, humans express three N-methyltransferases, one catechol-O-methyltransferase (COMT), one phenol-O-methyltransferase (POMT), one thiopurine S-methyltransferase (TPMT), and one thiol methyltransferase (TMT) (Gonzales and Tukey, 2005).

Factors influencing drug metabolism

Besides the intrinsic clearance of the enzyme-mediated reaction, the rate of biotransformation of a drug depends
on many factors. The extent of plasma-protein binding is an important determinant as only the free drug is available for metabolism. The availability of cofactors can also influence the rate of metabolism. The rate of drug metabolism is also dependent on the entry of the drug into the cell across the cellular membrane, and this may be controlled by the expression of uptake and efflux transporters on the cell membrane. In the past decade, important new insights have been made relating to the regulatory mechanisms governing the expression of drug-metabolizing enzymes and transporters by ligand-activated nuclear receptors. There is evidence to demonstrate that the pregnane X receptor (PXR), farnesoid X receptor (FXR), small heterodimer partner (SHP), hepatocyte nuclear factor-4α (HNF-4α), liver X receptor (LXR), glucocorticoid receptor (GR), constitutive androstane receptor (CAR), vitamin D receptor (VDR), and aryl hydrocarbon receptor (AhR) form a battery of nuclear receptors that regulate the expression of enzymes responsible for both phase I and II reactions (Urquhart et al., 2007). The regulation of CYP isoforms, in particular, has been extensively studied, and most of the drug-metabolizing isoforms, with the exception of CYP2D6, are prone to induction by agents that interact with the nuclear receptors. The expression of CYP enzymes may, on the other hand, be decreased during infection, an effect suspected to be mediated by increased cytokine levels (Abdel-Razzak et al., 1993). In general, most non-CYP oxidative enzymes appear not to be inducible (e.g., MAOs) or much less inducible than the CYP system, while many phase II reactions, glucuronidation in particular, may be highly inducible (Strolin Benedetti et al., 2006).

Although the second-generation antihistamine terfenadine is now withdrawn from the market of antiallergic drugs, the mechanistic studies into the delineation of its metabolic pathways following the reports of sudden death in patients taking terfenadine in combination with CYP inhibitors were instrumental in the development of regulatory guidelines on drug-metabolism studies (Whomsley and Strolin Benedetti, 2005). In the process of drug development, it is now a regulatory requirement to identify the major routes of drug metabolism mainly by use of in vitro assays with cellular and subcellular preparations of human liver. These studies, aiming not only at the identification of the chemical structure of the metabolites, but also at the identification of the enzymes involved, are important in the identification of subgroups of patients who may be at risk from the adverse effects of a drug [e.g., due to polymorphism of drug-metabolizing enzymes, drug-drug interactions (cfr the review article Drug Interactions of this special issue) or hepatic insufficiency, or patients who may be prone to subtherapeutic levels of a drug due to rapid elimination].

**Metabolism of drugs used for the treatment of allergy**

Loratadine, although possessing antihistaminic activity, can be considered to be essentially a prodrug as its circulating levels are too low to exert a meaningful effect. Loratadine is metabolized to desloratadine principally by CYP2D6 and CYP3A4 (Yumibe et al., 1996). Desloratadine is extensively metabolized to a metabolite frequently reported to be active, 3-OH desloratadine, although no data apparently have been published on its activity, and the enzymes responsible for its formation have not yet been identified. This metabolite is subsequently glucuronidated by UGT1A1, UGT1A3, and UGT2B (Ghosal et al., 2004). Desloratadine is subject to polymorphic metabolism, and 7% of the general population are slow metabolizers, with higher prevalence estimates in African Americans (Prenner et al., 2006). Cetirizine is metabolized, to some extent, by CYP3A4 and other unidentified CYP isoforms; however, it is not heavily dependent on metabolic clearance for its elimination, with over 60% being excreted unchanged in the urine following an oral dose (Whomsley and Strolin Benedetti, 2005). The elimination of cetirizine is stereoselective, with dextrocetirizine being more rapidly eliminated than levocetirizine. There is evidence that this may be due to differences in both the metabolism and urinary excretion of the enantiomers. Levocetirizine is even less dependent on metabolism than cetirizine for its elimination, with less than 15% being cleared by nonrenal mechanisms (Whomsley and Strolin Benedetti, 2005). Mizolastine is metabolized mainly by glucuronidation (70%), but also by oxidation through CYP3A4, CYP1A2, and CYP2A6 (Chaufour et al., 1999).

The absolute bioavailability of ebastine is low, as it is subject to extensive metabolism by CYP3A4, CYP2J2, and CYP4F12 (Hashizume et al., 2002). Like loratadine, ebastine acts essentially as a prodrug, with the main pharmacological activity being mediated through its metabolite, carebastine. Interestingly, this active metabolite is formed by CYP2J2, a CYP isoform also implicated in the metabolism of astemizole and terfenadine, which is structurally very similar to ebastine (Whomsley and Strolin Benedetti, 2005).

The major route of metabolism of corticosteroids (e.g., budesonide) occurs via CYP3A4 (Mortimer et al., 2006). As most corticosteroids are recognized to be metabolized mainly by CYP3A4 in the liver, low constitutive levels of the enzyme might lead to an excessive response to corticosteroids and could result in adverse events. Budesonide is also conjugated by cytosolic SULTs. The major route of fluticasone furoate metabolism in humans is the loss of the S-fluoromethyl carbothioate moiety. The loss of this moiety is also the significant route of metabolism of fluticasone propionate, with the biotransformation being suggested to be catalyzed...
by cytochrome P450 3A4 (Hughes et al., 2008; Pearce et al., 2006) (see also the review article Therapeutic Management of Allergic Diseases of this Special Issue).

In vitro studies using human liver microsomes indicate that CYP3A4 and 2C9 are involved in the metabolism of the leukotriene receptor antagonist, montelukast (Chiba et al., 1997).

Excretion

General considerations and concept of clearance

Once a drug has reached the general circulation and has been distributed, the body activates several mechanisms in order to eliminate the drug. The pathway of elimination may involve either biotransformation into metabolites (active or inactive, not discussed in this section) and/or excretion by some organs. Excretion is the process whereby drugs and/or their metabolites are removed from the body and displaced to the external environment. Kidneys and liver are generally considered as the major excretory organs, while the contribution to excretion of other routes (e.g., exhaled breath, saliva, sweat, tears, breast milk, hair, nails, etc.) is small. Pulmonary excretion via exhaled breath remains limited except for gaseous and volatile substances. Salivary excretion cannot be considered an efficient method for drug elimination, as the drug excreted in the saliva will usually be swallowed and reabsorbed. Finally, excretion via breast milk, although not essential for the mother, is a possible source of unwanted exposure in nursing infants. Substances, both basic and lipid soluble (since milk contains 3–4% lipids), are excreted into milk by simple diffusion. Basic substances can be concentrated in milk, since milk is more acidic (pH, ~6.5) than blood plasma.

Because chemicals must pass through membranes in order to leave the body, the same chemical and physical properties that governed passage across membranes applies to excretory organs as well. The exception is the lung, where polar (hydrophilic or water-soluble) substances have a definite advantage over lipid-soluble toxicants as regards elimination from the body.

The pharmacokinetic parameter, which reflects and quantifies the capacity of the body to eliminate the drug after it has reached the general/systemic circulation, is called the clearance. The clearance of a drug may be defined more generally as the volume of blood or plasma completely and irreversibly cleared of the compound per unit time (expressed as a flow, in mL/min). As a drug generally undergoes several elimination processes, the total clearance corresponds to the sum of all individual clearances (renal, metabolic, biliary, etc.). The clearance of a compound by an organ is dependent on the blood flow through this organ and extraction ratio of the drug by the same organ, as shown in the following equation:

\[ CL_{\text{organ}} = \frac{\text{Rate of Elimination}}{\text{Plasma Concentration}}, \text{or } CL_{\text{organ}} = Q \times E, \]

where \( Q \) is the blood flow of the organ (i.e., the rate at which blood is presented to the eliminating organ); and \( E \) is the extraction ratio (i.e., the fraction of the drug in the blood extracted by that organ on each passage through the clearing organ, or extraction ratio, or intrinsic ability of the eliminating organ to clear the drug). This value ranges between 0 (no extraction) and 1 (complete extraction).

The total clearance or body clearance or systemic clearance (\( CL \)) is also equal to the ratio of the dose, injected intravenously, over the blood or plasma extent of exposure (i.e., the area under the curve, AUC, of the blood or plasma concentrations), as shown in the following equation:

\[ CL = \frac{\text{Intravenous Dose}}{\text{Intravenous AUC}}, \text{or after oral administration,} \]

\[ CL = F \times \frac{\text{Oral Dose}}{\text{Oral AUC}}, \]

where \( F \) is the oral bioavailability (see section Absorption of this review article).

Renal/Urinary excretion

In the early 20th century, Marshal and Vickers (Marshal and Vickers, 1923) and Schmith et al. (Schmith et al., 1938) were the first to describe the renal elimination of drugs with the observation that the phenol red administered as a collyrium was able to colorate urines.

The kidney plays an important role in the elimination of numerous hydrophilic xenobiotics, including drugs, toxins, and endogenous compounds (Figure 11). The renal excretion of drug is mainly controlled by three processes occurring in the nephron, the functional unit of the kidney (Figure 12): 1) glomerular filtration in Bowman’s capsule, 2) tubular secretion, and 3) tubular reabsorption.

Factors affecting renal excretion of drugs include kidney function, protein binding, urine pH, and urine flow. Renal excretion of drugs is quantitatively described by the renal clearance value for the drug, which is the net result of passive glomerular filtration, active secretion, and passive and active reabsorption. Transport systems for organic anions and cations are primarily involved in the active phenomenon (i.e., secretion and reabsorption) of drugs excretion in the renal tubules. As a consequence, renal clearance (\( CL_{\text{r}} \)) is also affected by interaction and/or transport of drugs via these diverse pathways.
secretory and absorptive transporters (Inui et al., 2000), as shown in the following equation:

\[ CL_R = \frac{\text{Glomerular filtration rate} + \text{Secretion rate}}{-\text{Reabsorption rate}} \]

\[ \text{Plasma Concentration} \]

**Renal glomerular filtration**

Approximately 25% of cardiac output goes to the kidneys (i.e., \( Q_{\text{kidney}} = 19.1 \text{mL/min/kg} \); Labaune, 1989). About 10% of the blood, which enters the glomerulus, is filtered through pores in the glomerular endothelium. The glomerular filtration rate (GFR) varies from individual to individual, but, in healthy subjects, the normal range is 110–130 mL/min (i.e., 1.8 mL/min/kg). In the glomerulus, all molecules of low molecular weight are filtered out of the blood, unless they are tightly bound to large molecules, such as plasma protein, or have been incorporated into red blood cells. Studies have shown that the molecular size limit is close to that of albumin, whose molecular weight is ~60,000 D. The presence of albumin in the urine indicates that the glomerulus filtering system is damaged, letting large molecules pass through (Labaune, 1989; Draggan and Monosson, 2007). For unbound drug (\( fu \)) of low molecular weight, filtration into the proximal tubule always occurs. Thus, when \( CL_R = fu \times GFR \), it is likely that the drug is only filtered. It is also possible that secretion and reabsorption balance and cancel each other out but are still occurring (Li et al., 2006).

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**Figure 11.** Kidney. (A) Anatomy: kidney and urinary tract (scheme): 1. Kidney. 2. Adrenal gland. 3. Aorta. 4. Inferior vena cava. 5. Renal vein. 6. Renal artery. 7. Ureter. 8. Bladder. 9. Urethra. (B) Anatomy: kidney (scheme): 1. Renal vein. 2. Renal artery. 3. Renal pyramid. 4. Minor calyx. 5. Major calyx. 6. Renal papilla. 7. Renal lobe. 8. Renal capsule. 9. Renal columns. 10. Hilus. 11. Ureter. Renal parenchyma: a. cortex; b. medulla. (C) Histology: kidney (human sample): I. Scheme: 1. artery; 2. vein; 3. tubule; 4. renal corpus. II. Histology (human sample): renal corpuscle (H&E, magnification X400): 1. renal corpuscle; 2. glomerulus; 3. Bowman’s capsule. III. Histology (human sample): renal parenchyma (H&E, magnification X100): 1. cortex; 2. medulla. H&E.

**Figure 12.** Structure of the nephron (adapted from Boomer, 2008).
Renal tubular secretion

After filtration, the remaining plasma (as blood) is shunted around the tubule in the arterioles adjacent to the proximal tubule where drugs may actively be secreted from the arterial into the proximal tubule (or reabsorbed in the opposite direction). Active tubular secretion in the proximal tubule is important in the elimination of drugs, even if the drugs are protein bound. Actually, only free drug is transported (Bow et al., 2006), and consequently, this process is affected by changes in free fraction. Nevertheless, the protein-drug complex rapidly dissociates in cases where secretion is very rapid and, as a consequence, virtually all the drug can be removed by a single pass through the kidney ($E_{\text{renal}}$ close to 1). When active secretion occurs without any reabsorption or when reabsorption is quantitatively less important than secretion, $CL_R$ is then equal or superior to $fu^*GFR$ (Bourne 2008a).

Secretion of drugs and their metabolites from blood to luminal fluid in the nephron is a protein-mediated process (i.e., requiring a carrier or transporter) that normally involves either the direct or indirect expenditure of energy. Active uptake of organic anions and cations across the basolateral membrane (BLM), and their extrusion into the urine across the brush-border membrane (BBM), mainly takes place in the renal proximal tubule cells and is facilitated via a range of polyspecific tubular transporters. This secretory process can also be saturated. When saturation is reached following a rise in the plasma or blood concentration of the drug, renal clearance is reduced. Competition for renal excretory transporters (drugs actively secreted compete with each other, also called competitive inhibition) may cause drugs to accumulate in the body leading to toxicity, which is a potential hazard of concomitant drug administration (El-Sheikh et al., 2008) (see the review article Drug Interaction of this special issue).

Anions (and weak acids) and cations (and weak bases) are handled by separate transport mechanisms. The phase II conjugates of drugs (with glycine, sulfate, or glucuronic acid) are also anionic compounds, and therefore, renal organic anion transporters are important determinants of their elimination. The functional characteristics, such as substrate-specificity and transport mechanisms, and the localization of drugs transporters will be the subject of the next transporters-dedicated section (see below).

Renal tubular reabsorption

After glomerular filtration, the drug filtered into the proximal renal tubule may be actively or passively reabsorbed into arterioles. In the case where net active/passive reabsorption is inferred, $CL_R$ becomes, then, equal or inferior to $fu^*GFR$. Some exogenous compounds, including glucose, vitamins, or drugs, are actively reabsorbed across the tubular BBM. This appears to be mediated by transporters (see below). The drugs filtered in urine can also be passively reabsorbed into the blood vessels. This occurs in the renal distal tubules, only as a consequence of the concentration gradient produced in this part of the nephron. Water is removed from the urinary filtrate at the level of proximal renal tubule, into the blood vessels adjacent to the loop of Henle, and from the urine as it proceeds down the renal tubule. Reabsorption of the water into the blood vessels makes urine more concentrated and, therefore, generates a concentration gradient favorable for drug reabsorption.

Diffusion rate is directly proportional to the concentration gradient, but passive reabsorption is also influenced by the physicochemical properties of the drugs, mainly the lipophilicity, size, and degree of ionization (Bourne 2008a). Since the kidney tubular membrane is lipoidal, nonionized and lipid-soluble drugs are extensively reabsorbed into plasma (or blood), while polar compounds as well as ionized acids or bases are less likely to be reabsorbed across the kidney tubule membrane and are excreted via urine. Octanol (i.e., octanol/buffer pH 7.4 distribution coefficient or logD$_{7.4}$) represents a very good mimic of the kidney tubule membrane and thus provides a reliable model to investigate the impact on lipophilicity on renal excretion. Compounds with logD$_{7.4}$ values below 0 (i.e., hydrophilic compounds) are poorly reabsorbed and thus undergo considerable renal clearance, while compounds with logD$_{7.4}$ values above 0 (i.e., lipophilic compounds) undergo near complete reabsorption and tend to reintegrate the metabolic pathways, in order to render them more hydrophilic (van De Waterbeemd et al., 2001). Due to the impact of drug ionization in tubular reabsorption, urine pH plays an important role in passive reabsorption, as does urine volume. Urine pH, which varies widely from 4.5 to 8.0, may be influenced by diet (e.g., meat can cause more acidic urine), exercise, disease, or drugs, and tends to be lower during the day than at night (Bourne 2008a). Changing the pH of the urine would result in changing the reabsorption characteristics of weakly acidic or basic drugs (by consideration of drug pKa according to the Henderson-Hesselbach equation). Urine alkalization favors the ionized form of weak acids and promotes excretion. Alternatively, acidification promotes the renal clearance of weak bases.

Transporters involved in renal active secretion and active reabsorption

It is well described that drugs are actively secreted into renal tubules, and/or reabsorbed, via transporters, and that several molecules may interact at this level. The first clinical application of this concept appeared soon after World War II with penicillin. During the war, this antibiotic was widely used and physicians had difficulties with
maintaining efficient blood concentrations of the drug because of its high-speed renal elimination. In 1951, probenecid was developed as a competitive inhibitor of penicillin renal secretion and allowed physicians to slacken the antibiotic renal elimination and thus to maintain adequate drug concentrations for a longer time (Beyer et al., 1951). In 1988, the racing cyclist, Pedro Delgado, was proved to use probenecid in order to inhibit the renal secretion, and thus, the urinary detection of steroids. More recently, probenecid has been used as a nephroprotective therapy against cidofovir renal tubular toxicity, since it had been shown to inhibit the entry of cidofovir into the tubular cell (Lacy et al., 1998; Launay-Vacher et al., 2006).

The process of secreting organic anions and cations through the proximal tubular cells is achieved via transcellular transport, involving the uptake of the drugs and/or their metabolites from the blood into the cells across the basolateral membrane, followed by the transport across the BBM into urine (Tsuji, 2002). Tubular reabsorption of some compounds (e.g., peptide-like drugs, such as beta-lactam antibiotics) across the BBMs appears to be also mediated by transporters (Inui et al., 2000). These polyspecific transporters, which accept compounds with different sizes and molecular structures, exhibit large variations in affinity and turnover for compounds (Koepsell et al., 2007). Their types and localization could provide information regarding renal mechanisms underlying the handling of drugs, as well as transporter-mediated drug interactions (see the review article Drug Interactions of this special issue).

In human kidneys, the first step of secretion involves the basolateral uptake of drugs, while the second step is the release across the luminal or brush border apical membrane of cells. There are many renal tubular multispecific drug transporters, localized either at the basolateral membranes and/or at the BBM (Figure 13). Multispecific drug transporters belong to several families: organic anion and cation transporters families (OATs and OCTs, mainly expressed at the basolateral membrane and mediating cellular uptake, such as OAT1-3, OCT1-3; OAT4 is located in the BBM), organic anion-transporting polypeptide family (OATPs), type I sodium-phosphate transporters (NTPs, localized at the apical membrane of proximal tubular cells), ATP-dependent organic ion transporters, such as MDR1/P-glycoprotein (P-gp) family, and the multidrug-resistant associated proteins (MRPs) (Tsuji, 2002). MDR1/P-glycoprotein, which pumps various hydrophobic xenobiotics from tubular cells into the lumen, is expressed in the BBM, whereas MRPs, which mediate primary active luminal secretion, are located in the basolateral membrane (Tsuji, 2002). Some peptide-like drugs (e.g., beta-lactam antibiotics) are known to be reabsorbed from the lumen into the cells of renal tubules through oligopeptidic transporters, the peptide transporter (PEPT) family, on the brush border apical membranes. PEPT1 and PEPT2 mediate the luminal uptake of peptide drugs (Inui et al. 2000). Further, the recently identified multidrug and toxin extrusion transporters (MATEs) have been shown to either transport or interact with several drugs. The mammalian MATE-type transporters, which might be energetically coupled with a plasma-membrane-type vacuolar H⁺-ATPase or Na⁺-H⁺ antiporter, is mainly expressed in the kidney and is considered to be responsible for the final step of urinary excretion of cationic drugs (Terada et al., 2006; Ohta et al., 2006; Matsushima et al., 2008b; Musuda et al., 2006).

Finally, there are several transport proteins on the basolateral and apical membranes of tubular cells in

Figure 13. Schematic models of multispecific drug transporters in renal proximal tubule (adapted from Launay-Vacher et al., 2006).
human kidneys that have not been fully characterized and whose role in the secretion/reabsorption of organic anions and cations remains to be determined (see below) (Robertson and Rankin, 2006).

**Biliary excretion**

The liver owns the capacity to eliminate endogenous and exogenous compounds not only because of the presence of a number of enzymatic systems, but also because it has a secretory system, leading to the formation of the bile, which transports endogenous as well as exogenous substances, such as drugs and their metabolites. Therefore, hepatic clearance is the sum of two complementary processes occurring in the liver, the first relating to metabolic activity, and the second corresponding to biliary excretion. In humans, the liver secretes 0.25–1.0 L (average, 800 mL per day) of bile each day. The bile generated by the liver is secreted into the hepatic duct, which divides into two branches: the cystic duct which joins the gallbladder, and the common bile duct whereby a fraction of bile is continuously secreted into the duodenum (Figure 14).

Bile is drained into the gallbladder to fill it to its maximum capacity (40–70 mL) in approximately 6 hours. The expulsion of bile from the gallbladder into the small intestine is intermittent and incomplete (Ghibellini et al., 2006b). Drugs or metabolites excreted in the bile can be either irreversibly excreted into feces or reabsorbed into hepatic portal vein, which is called enterohepatic recycling (discussed below).

Biliary clearance is determined as:

$$CL_{bile} = \frac{\text{Bile flow} \times \text{Bile Concentration}}{\text{Plasma Concentration}}.$$  

Of notice, biliary excretion eliminates substances from the body only to the extent that enterohepatic recycling is incomplete (i.e., when some of the secreted drug is not reabsorbed from the intestine). When the bile concentration of the drug is the same as the plasma concentration, the biliary clearance is equal to the bile flow and, therefore, is poor. A drug has a high biliary clearance when its bile concentration is much higher than its plasma concentration. Because these drugs are transported across the biliary epithelium against a concentration gradient, at least part of the biliary excretion requires active secretory transport. This secretory transport can be saturated, when the upper limit is reached due to high plasma drug concentration. Also, substances with similar physicochemical properties may compete with each other for excretion.

In humans, the most commonly used approach for the determination of biliary excretion as a route for drug elimination is the quantification of the drug of interest in feces. Often, these studies are performed with a radiolabeled compound in order to increase the selectivity and the recovery of drug and unknown metabolites, resulting in good mass balance. Several drawbacks are associated with the quantification of drugs in feces. Most important, this method cannot distinguish biliary excretion from intestinal secretion processes. Further, for orally administered drugs, this method does not distinguish between unabsorbed drug (remaining exclusively in the lumen of the GI tract) and that absorbed and subsequently excreted by the liver and/or secreted by the gut back into the intestinal lumen. Therefore, the percentage of the dose in feces does not necessarily reflect the amount excreted into bile. Moreover, relatively unstable drugs, or compounds that are transformed into unstable metabolites, may not be recovered in feces due to the long exposure to, and degradation by, the intestinal contents and colonic flora. Finally, drugs subjected to enterohepatic or enteroenteric recycling, such as glucuronide

![Figure 14. Macroanatomy of the hepatobiliary system (adapted from Ghibellini et al. 2006a).](image-url)
conjugates, can be reabsorbed in the colon upon intestinal degradation by beta-glucuronidases of the microbial flora and will not be recovered in the feces (Ghibellini et al., 2006a).

Characteristics of biliary excreted drugs
Factors affecting biliary excretion include drug characteristics (i.e., chemical structure, polarity, and molecular size), transport across sinusoidal plasma membranes, and canaliculi membranes (Roberts et al., 2002). Anions, cations, and nonionized molecules containing both polar and lipophilic groups are excreted into the bile, provided that the molecular weight is greater than 500–600 in humans (Ghibellini et al., 2006a). Lower molecular weight compounds are reabsorbed before being excreted from the bile duct and are generally excreted only in negligible amounts in bile. Conjugation, particularly with glucuronic acid, facilitates biliary excretion because these metabolites are often of sufficient molecular weight (i.e., molecular weight +177 for glucuronides) (Boomer, 2008; Bourne, 2008b).

While the majority of small, lipophilic compounds flow through the space of Disse and enter the hepatocyte via the basolateral membrane by simple passive diffusion, more polar and bulky molecules require transport systems to cross the sinusoidal membrane (Figure 15). Once inside the hepatocyte, compounds can be transported into bile either unchanged or as more hydrophilic metabolites after phase I and/or phase II biotransformation, or can be excreted into blood by basolateral transport proteins. The presence of several transport proteins on the canalicular domain of hepatocytes results in the excretion of solutes into the bile canaliculi, which are dilated intercellular spaces.

Enterohepatic recycling
Drugs and drug conjugates entering the gut (i.e., small intestine) through bile often undergo some degree of reabsorption along the GI tract. The fraction reabsorbed in the hepatic portal vein may subsequently be metabolized, excreted in urine, or returned to the bile. This process is called enterohepatic circulation or enterohepatic recycling (Rollins and Klaassen, 1979).

This occurs particularly with small, less polar drugs. Glucuronide conjugates of drugs may also be hydrolyzed by enzymes in the intestinal microflora (e.g., beta-glucuronidase) to liberate the parent lipid-soluble drug, which is then reabsorbed. Enterohepatic recycling is also a very important physiological process for bile salt homeostasis. In humans, the intestinal reabsorption of bile salts is very efficient and more than 95% of the bile salt pool is reabsorbed in the distal ileum (Kullak-Ublich et al., 2004).

Some studies have attempted to interrupt the enterohepatic recycling of toxicants (such as pesticides and heavy metals) through the oral administration of nonabsorbable, nonspecific adsorbents, such as cholestyramine. The results, generally a decrease in drug half-life, have been surprising, in that they suggest that many more drugs undergo enterohepatic recycling than previously suspected. The contribution of biliary excretion to the overall disposition and pharmacological effect of some drugs has been underestimated due to the lack of methodologies to accurately quantify the amount of drug subjected to enterohepatic recirculation and a poor understanding of the processes involved in hepatic excretion and reabsorption of drugs from the canalicular space, bile ducts, and intestine (Ghibellini et al., 2006a).

Transporters involved in drug biliary excretion
Several types of transporters located on the liver-plasma membranes are involved in drug biliary excretion. Drugs are taken up in hepatocytes from blood by active transporters located at the basolateral or sinusoidal membrane and then are subjected to metabolic conversion and/or biliary excretion. Drugs and their conjugated metabolites are excreted from hepatocytes in bile by primary active transporters on the bile canalicular membrane. In particular, primary active transport mechanisms have been shown to be responsible for the biliary excretion of anticancer drugs, endogenous bile acids, and organic anions, including glutathione and glucuronic acid conjugates. Primary active excretion into bile means the positive removal of xenobiotics from the body, and this elimination process is now designated as "phase III" (Ishikawa, 1992) in the detoxification mechanisms for xenobiotics, in addition to phases I and II.

Several kinds of transporters have been identified to play an important role in biliary excretion (Figure 16). Among those, nonexhaustively, there are ATP-binding cassette (ABC) transporters, multidrug resistance–associated protein 2 (MRP2), P-glycoprotein/multidrug resistance 1 (P-gp/MDR1), breast cancer resistance
protein (BCRP), organic anion–transporting polypeptide (OATP) family of proteins, organic anion and cation transporters (OATs and OCTs), bile salt export pump (BSEP), and MRP3 and MRP4 (Matsushima et al., 2008a; Funk, 2008). Some of them are located on the sinusoidal membrane and are either involved in hepatic uptake, as is the case for OCT1, OATP-C, OATP-B, OATP8, and OAT2, or involved in hepatic secretion, such as, for example, MRP1, MRP3, and MRP4. Transporters involved in the liver excretion into bile are located on canalicular membrane of the hepatocyte, such as ABC transporters, MRP2, P-gp/MDR1, BCRP, and BSEP, which function as cellular exporters (Zhang et al., 2006; Funk, 2008). Of notice, OCT and P-gp/MDR1 preferentially accept hydrophobic cationic or neutral compounds, whereas MRP2 and BCRP are more responsible for the biliary excretion of a wide variety of organic anions, including glutathione, glucuronide conjugates, and sulfate conjugates (Matsushima et al., 2008a, 2008c). Although BSEP was believed to accept only bile salts, recent studies indicate that BSEP transports also some drugs (Lecureur et al., 2000). On the other hand, it has become clear that MRP3 (ABCC3) and MRP4 (ABCC4) are important transporters in sinusoidal efflux (Borst et al., 2007). MRP3 can transport a wide variety of organic anions, such as glucuronides, glutathione conjugates, and bile acids. The hMRP3 and hMRP4 transporters may also be involved in the hepatic distribution of drugs because both are expressed in human liver under physiological conditions (see below) (Rius et al., 2003).

**Excretion of some antiallergic drugs**

**H<sub>1</sub>-Antihistamines**

Second-generation, relatively nonsedating histamine H<sub>1</sub>-receptor antagonists (H<sub>1</sub>-RAs) are extensively used worldwide for the symptomatic treatment of allergic rhinoconjunctivitis and chronic idiopathic urticaria. Overall, there is a paucity of information on the pharmacokinetics of H<sub>1</sub>-RA during lactation (Simons and Simons, 1991a) or excretion by ways other than urine or bile. In this section, only urinary and biliary excretion are considered and compared to the extent of metabolization.

Most H<sub>1</sub>-RAs are metabolized by the hepatic cytochrome P450 system: terfenadine, astemizole, loratadine, azelastine, and ebastine and have one or more active metabolites which are present in serum in higher concentrations than the respective parent compound (Simons and Simons, 1991a). Mizolastine and desloratadine are also extensively metabolized. The metabolic pathway of mizolastine (which is the glucuronidation of the parent compound) accounts for approximately 65% of the administered dose. After the oral administration of [14C] mizolastine, 84–95% of the radioactive dose is recovered in feces and 8–15% in urine, with less than 0.5% excreted as unchanged parent drug in urine (Simons, 1999). For desloratadine, the AUC of the parent compound after the administration of [14C] desloratadine represents only 8% of the plasma AUC of total radioactivity, and the main metabolite is 3-hydroxydesloratadine (reported as active, even if it
would appear to exhibit less H₁-receptor antagonism than desloratadine, and published comparative data on the subject do not appear to be available). Globally, 41 and 47% of the radioactivity are recovered in urine and feces, respectively (Molimard et al., 2004), with several metabolites present in urine (in particular, 3-hydroxylesloratadine and its glucuronide).

Cetirizine, an active metabolite of the first-generation H₁-receptor antagonist, hydroxyzine, subsequently developed as a drug, is not metabolised to any great extent in vivo, and is mainly eliminated via renal excretion. For [¹⁴C] levocetirizine, the active enantiomer of cetirizine also developed as a drug, excretion by feces is a minor route (13% of total radioactivity), and the radioactivity excreted in urine (85% of total radioactivity) is mainly represented by the unchanged drug (Molimard et al., 2004). As a consequence of its large urinary excretion, the half-life of cetirizine (and levocetirizine) is prolonged in patients with impaired renal function (i.e., with mild, moderate, and severe renal insufficiency), compared with age-matched individuals with normal renal function (see the review article Pharmacokinetics in special populations of this special issue). This translates into total body clearance and renal clearance of the drug, which are both significantly lower in patients with renal insufficiency (Matzke et al., 1987). The renal excretion of cetirizine and levocetirizine occurs both by glomerular filtration and active tubular secretion (Strolin Benedetti et al., 2001, 2008), and this has been extensively discussed in the review article Pharmacokinetics in special populations of this special issue.

Fexofenadine, the active metabolite of terfenadine, is also very poorly metabolized in vivo (approximately 5% of the total oral dose). After the oral administration of [¹⁴C] fexofenadine to healthy volunteers, 80% of the dose was recovered in feces and 12% in urine, in unchanged form (Lippert et al., 1995). In rats, after the oral administration of [¹⁴C] fexofenadine, the radioactivity was recovered in the urine (2.5%), bile (28%), and feces (69%) of rats whose bile duct had been cannulated, and thus, its oral absorption is, at most, 30%, and biliary excretion likely plays a major role in its elimination (Tahara et al., 2005). Because the absolute oral bioavailability of fexofenadine is reported to be 33%, it follows that two thirds of the bioavailable fexofenadine in humans is excreted into bile. Therefore, hepatic transport of fexofenadine is one of the determinants for its systemic clearance (Matsushima et al., 2008a).

As observed for cetirizine, levocabastine undergoes only minimal hepatic metabolism (i.e., ester glucuronidation to generate an inactive acylglucuronide derivative) and is predominantly cleared by the kidneys. Renal excretion of the unchanged drug is the primary route of elimination of levocabastine, accounting for approximately 70% of an orally administered dose, while about 20% are excreted unchanged in feces. As oral bioavailability of levocabastine is very high, it is likely that some enterohepatic recycling occurs (Heykants et al., 1993). The acylglucuronide metabolite is both urinary and biliary excreted. The amount of acylglucuronide found in urine accounts for about 10% of the oral dose of levocabastine. The biliary-excreted acylglucuronide metabolite is hydrolyzed in the gut, then undergoes some degree of reabsorption along the gastrointestinal tract. Finally, the concentration of levocabastine secreted in breast milk is very low (with a milk/plasma concentration ratio estimated to be 0.7) (Heykants et al., 1995).

**Corticosteroids**

A number of intranasal corticosteroids are available for the treatment of allergic rhinoconjunctivitis, including beclomethasone dipropionate, budesonide, flunisolide, fluticasone propionate and fluroate, mometasone fuorate, and triancinolone acetonide. With the exception of beclomethasone dipropionate, all of these drugs are quickly metabolized to less-active metabolites and have minimal systemic absorption (Stanaland, 2004; Hochhaus, 2008).

**Antileukotrienes**

Leukotriene receptor antagonists are used for improving allergic rhinoconjunctivitis symptoms but were found to be no more effective than H₁-antihistamines and less effective than intranasal corticosteroids (Herman, 2007).

After an oral dose of [¹⁴C] montelukast to 6 healthy subjects, 86% of the radioactivity was excreted in the feces and <0.2% in the urine over a period of 120 hours. Plasma analysis showed that the AUC for total radioactivity was slightly higher than that for unchanged montelukast, reflecting the presence of low levels of metabolites in the systemic circulation. Radioactivity in the feces could be largely due to biliary and intestinal excretion of metabolites and the parent compound, as well as to nonabsorbed dose. It is likely that montelukast in healthy subjects was well absorbed and underwent hepatic/gut metabolism and biliary excretion (Balani et al., 1997). Zafirlukast, another antileukotriene drug, is also mainly eliminated in the feces, while urinary excretion accounts for <10% of an orally administered dose (Dekhuisen and Koopmans, 2002). In animals (e.g., mice, rats, dogs), it was shown that the primary route of
excretion for \[^{14}C\] zafirlukast-derived radioactivity is in bile and, subsequently, feces, with <3% excreted in urine (Savidge et al., 1998).

**Transporters**

**Introduction**

Transporters are a class of membrane proteins that translocate endogenous compounds (such as bile acids, lipids, sugars, amino acids, steroids, hormones, and electrolytes) and xenobiotics (such as drugs or toxins) across biological membranes to maintain cellular and physiologic solute concentrations and fluid balance (i.e., homeostasis) as well as to detoxify any potentially harmful foreign substances (Xia et al., 2007). Transporters function together with drug-metabolizing enzymes to eliminate xenobiotics and their metabolites. In addition, transporters may profoundly influence tissue distribution and serve as protective barriers to particular organs and cell types. Consistent with their critical roles in regulating the transport of chemicals across the plasma membrane, recent estimates indicate that approximately 2,000 genes (i.e., 6% of the human genome) may encode for membrane transport proteins (Ballatori, 2005). Membrane transporters may function through facilitated (i.e., equilibrative, not requiring energy) or active (i.e., requiring energy) processes (Giacomini and Sugiyma, 2006). Drug transporters can be generally classified into the (ATP)-binding cassette (ABC) transporter superfamily and the solute carrier (SLC) family of proteins, which interact dynamically to mediate the accumulation and translocation of drugs or endogenous substrates into a cell (Xia et al., 2008). Certain transporters exhibit both influx and efflux properties, according to their cellular localization (Ho and Kim, 2005). The expression of transporters may be increased in the presence of enzyme inducers, such as St.John’s wort and rifampin, and their activity modulated in the presence of inhibitors or by genetic polymorphisms that influence the function of the protein.

The influence of individual human transport proteins in the pharmacokinetics of xenobiotics has been investigated mainly in vitro. A recent review has provided a comprehensive summary of these methods together with their merits and limitations (Xia et al., 2007). The methods used include cell- and membrane-based assays. Cell-based assays include those investigating cytotoxicity, drug accumulation or efflux in cell-lines preferentially expressing specific transport proteins, or in cell lines or oocytes transfected with human uptake or efflux proteins (Xia et al., 2007). Currently, cell types used include Caco-2, Madin-Darby canine kidney (MDCK), LLC-PK1, and certain primary cultured cells, such as primary cultured brain endothelial, conjunctiva epithelial, and alveolar epithelial cells.

**ATP-binding cassette proteins**

A total of 49 ABC transporters have been identified, forming one of the largest protein families encoded in the human genome (Zhou et al., 2008). P-glycoprotein (P-gp) [also known as multidrug resistance 1 (MDR1) protein] in the ABCB subfamily is one of the major ABC transporters to confer resistance in tumor cells and to efflux xenobiotics out of normal tissues. P-gp is member 1 of the ABC subfamily B class; hence, the nomenclature is ABCB1. P-gp is present on the apical membrane and transports drugs out of cells into luminal areas, such as the intestine, bile, or urinary collecting duct. Transport usually occurs against a concentration gradient and requires energy, the source of which is intracellular production of ATP that occurs when drugs bind to this protein.

There are 13 proteins in subfamily ABCC, of which nine are multidrug resistance–associated proteins (MRPs) and designated MRP1 through MRP9 (Toyoda et al., 2008). These proteins share structural and functional similarities and function as energy-dependent efflux transporters with differing tissue-specific expression patterns and substrate specificities (Borst and Elferink, 2002). The MRPs have been shown to confer multidrug resistance in tumor cells and their substrates include doxorubicin, vincristine, etoposide, tenoposide, camptaphacin, methotrexate, glutathione (GSH) conjugates, glucuronide conjugates, and sulfate conjugates (Toyoda et al., 2008). Many members of the MRP family require GSH for transport, although the extent of transport enhancement varies among the transporters. In some cases, it appears that GSH is involved in cotransport, whereas in others, in allosteric stimulation (Toyoda et al., 2008).

Breast cancer resistance protein (BCRP, ABCG2) has been shown to play an important role in the disposition of drugs (Ayrton and Morgan, 2008). BCRP is widely expressed in many tissue barriers, including the epithelium of the small intestine and liver canalicual membrane, the blood-brain, blood-placenta, and the blood-testis barriers. Its high expression levels in placental trophoblast cells suggest that BRCR plays an important role in protecting the fetus from toxic xenobiotics/metabolites (Koshiba et al., 2008). Substrates for BCRP include fluorourinolone antibiotics, grepafloxacin, ulixoflacin, ciprofloxacin, topotecan, and methotrexate (Ayrton and Morgan, 2008; Koshiba et al., 2008).

**Solute carrier family of proteins**

The solute carrier family of transporters is the largest superfamily of transporters, with about 225 members.
Solute carrier families 15A, 21A, 22A, and 47 are those primarily involved with the transport of drugs and xenobiotics (Beringer and Slaughter, 2005).

**Solute carrier family 15: peptide cotransporters**

The peptide cotransporters (PEPT1, PEPT2) are integral membrane proteins responsible for the cellular uptake of di- and tripeptides. They are hydrogen-dependent transporters, which also transport peptidomimetic compounds, such as valganciclovir, midodrine, cephalosporins, and penicillins. PEPT1 is a low-affinity, high-capacity transporter and is mainly expressed in the small intestine, which participates in the oral absorption of drugs. PEPT2 is a high-affinity, low-capacity transporter with a broader distribution in the organism, including the kidney, where it contributes to the proximal tubular reabsorption of drugs (Rubio-Aliaga and Daniel, 2008).

**Solute carrier family 21A: organic anion transporter superfamily**

Organic anion-transporting polypeptides (in humans, called OATPs) are part of a superfamily of sodium-independent transport systems, with 11 members identified in man (Hagenbuch and Gui, 2008). Four of these (OATP1A2, OATP1B1, OATP1B3, and OATP2B1) have been well characterized and are thought to play a significant role in the disposition of xenobiotics.

OATP1A2 is expressed in the brain, at the BBB, in the liver in cholangiocytes but not hepatocytes, at the BBM in the distal nephron, and in the apical membrane of enterocytes (Hagenbuch and Gui, 2008). OATP1B1 and OATP1B3 are considered to be liver-specific transporters. OATP2B1 is present in hepatocytes, in blood vessels in the heart, at the BBB, and at the BBM of the small intestine (Hagenbuch and Gui, 2008).

Compounds known to be transported by OATPs include steroid hormones and their conjugates, bile salts, thyroid hormones, and many amphipathic drugs.

**Solute carrier family 22A: organic anion and cation transporters**

There are currently five known members of the organic anion transporters (OAT) family in humans: OAT1–OAT5 (Srimaroeng et al., 2008). These transporters play an important role in the urinary excretion of anionic drugs and zwitterions. The most abundantly expressed OATs in the human kidney are OAT1, OAT3, and OAT4. OAT1 and OAT3 are expressed at the basolateral membrane, while OAT4 is expressed at the apical membrane of renal proximal tubule (Babu et al., 2002; Ekaratanawong et al., 2004). OAT1 and OAT3 transport their substrates from the circulation into the cell, whereas OAT4 is implicated in reabsorption from the lumen of the proximal tubule. The mechanism for these three transporters is organic anion exchange. Drugs transported by OATs include antibiotics, adenosine, and cidofovir, and prostaglandins (Srimaroeng et al., 2008).

Organic cation transporters (OCTs) are responsible for the cellular translocation of endogenous or exogenous compounds that, at physiological pH, are positively charged. OCTs are polyspecific transporters with a broad substrate specificity. Drugs shown to be substrates of OCTs include propanidid, cimetidine, metformin, pilocarpine, quinidine, vecuronium, and cardiac glycosides. Three members of this family have been identified and shown to be important for the transport of cations and zwitterions in humans. OCT1 is expressed in many tissues, including the liver, intestine, and proximal tubule in the kidney. OCT2 is primarily expressed in the kidney and dopamine-rich areas of the brain (Beringer and Slaughter, 2005). OCT3 has a widespread tissue distribution and is involved with uptake of drugs into the heart and transport of drugs across the placenta. OCTs work in concert with other transporters to support vectorial movement of cations across cells. In polarized cells of the kidney and liver, the first step for organic cation secretion is their absorption from the basolateral side into the cells. This process in human kidney is mainly mediated by OCT2, while in the liver it is performed by OCT1 (Clarimbioli, 2008).

**Solute carrier family 47: multidrug and toxin extrusion family**

Recently, human orthologs of the multidrug and toxin extrusion (MATE) family have been isolated and demonstrated to function as cation antiporters. The MATE family was assigned as the SLC47 family (SLC47A1: MATE1; SLC47A2: MATE2 and MATE2-K) by the HUGO Gene Nomenclature Committee in 2007 (Terada and Inui, 2008). MATE1 is predominantly expressed in the liver and kidneys and is localized in the BBM and bile canaliculi. MATE2, an isoform of MATE1, is predominantly expressed in the kidneys and is expressed as a few variants, including MATE2K, a truncated variant. MATEs function as electroneutral H+/OCs exchangers and have similar substrate specificities. It has been concluded that they are the long searched for renal and hepatic OCs export responsible for the final step of excretion of OCs (Moriyama et al., 2008).

**Tissue/Cellular distribution of transporters**

**Small intestine**

In the small intestine, a number of transporters critical for absorption of dietary constituents and drugs
are expressed on the BBM of enterocytes (Ho and Kim, 2005). Among these proteins, P-gp and MRP2 actively extrude drugs back into the intestinal lumen, effectively limiting the extent (i.e., bioavailability) of substrate drug absorption, while PEPT1 and OATP1A2 can increase absorption of drugs with otherwise poor physicochemical properties for penetrating the cell membrane.

Liver
The function of the liver as the primary organ involved in the removal of drugs from the body is accomplished by the concerted action of transporters and enzymes. The efficient extraction of large or ionized drugs from the blood into the hepatocyte across the sinusoidal (basolateral) membrane and transport across the biliary canicular membrane and excretion into the bile is largely mediated by transporters (Beringer and Slaughter, 2005). The principal transporters involved with uptake include the OAT and OCT families, as well as the OATP family, while the principal transporters involved with canicular transport include P-gp and MRP2. Drugs excreted into the bile may be reabsorbed from the intestine intact as metabolites or parent drug (following cleavage of conjugates by intestinal enzymes) or may be eliminated in the feces.

Kidney
As described previously, the primary transporters involved with uptake into the proximal tubular cells across the basolateral membrane include OAT1/3 and OCT2. Efflux across the BBM is mediated by transporters, including OATP1A2, MRP2/4, OCT2, OCTN1/2, MATE1/2, and MDR1, while OAT4, PEPT1/2, and MRP1/2/5/6, respectively, may participate in reabsorption (Beringer and Slaughter, 2005; Moriyama et al., 2008). The net drug secretion represents the coordinate function of uptake and efflux transporters and reabsorption of drug from the urine.

Nasal mucosa and respiratory tract
The nasal mucosa and respiratory tract may be an important point of entry for some antiallergic drugs delivered by intranasal administration or inhalation; therefore, transporters expressed in these tissues may influence the pharmacokinetics of drugs administered in this way. Drugs can be absorbed in the nasal mucosa and throughout the conducting airway from the trachea down to the bronchioles and, ultimately, in the distal lung across the alveolar epithelium (Shen and Lee, 2002). Recently, both OCT1 and OCT2 have been localized in nasal mucosa and may provide a means for systemic absorption for cationic drugs (Chemuturi and Donovan, 2007). The high-affinity transporter, PEPT2, has been found in the respiratory tract and is expressed in the bronchial epithelium and in alveolar type II pneumocytes in human airways (Paul et al., 2005). It is possible, therefore, that this may represent a target for delivery of peptidomimetic drugs and prodrugs.

Central nervous system
The central nervous system (CNS) is separated from the systemic circulation by two barriers, the BBB and the blood-cerebrospinal fluid barrier (BCSFB), which form an effective barrier to the free diffusion of hydrophilic compounds into the brain. The BBB and BCSFB exhibit very low paracellular permeability, and targeted transporter expression is critical to the maintenance of barrier function (Ho and Kim, 2005). Whereas expression of certain uptake transporters facilitates CNS entry of essential endogenous substances, such as glucose, amino acids, and nucleosides, a large number of drugs have lower brain permeability than would be predicted from a determination of their lipid solubility due to their efflux by transporters that are present in the BBB and BCSB. While this may be beneficial in the case of drugs whose therapeutic target is outside the CNS, it is problematic for those drugs whose effect is directed at the CNS.

P-gp was the first of these ABC transporters to be described in the CNS, followed by the multidrug resistance–associated proteins (MRP) and, more recently, breast cancer resistance protein (BCRP) and several members of the OATP-family, and they may play a significant role in drug efflux at the BBB and BCSF barrier (Lösch and Potschka, 2005).

Transporters and antiallergic drugs
Most investigations on the interaction of antiallergic drugs with transporters have focused on the second-generation H1-antihistamines. Fexofenadine is, by far, the most thoroughly investigated antihistamine with regard to its transport characteristics. Fexofenadine is barely metabolized, and its absorption, distribution, and elimination are largely governed by transporters. Fexofenadine is a zwitterion whose absolute oral bioavailability has not been determined, although it has recently been estimated to be approximately 30% (Chen, 2007). It is thought that the efflux of fexofenadine in the small intestine by P-gp limits its absorption. Fexofenadine is known to be a substrate of the P-gp efflux pump (Cvetkovic et al., 1999). This has been demonstrated in vitro and is supported by clinical reports showing that the P-gp inhibitor, itraconazole, increases fexofenadine exposure and the amount excreted unchanged in the urine by approximately 3-fold (Shimizu et al., 2006). Also the recent data on the effect of quercetin on the pharmacokinetics of fexofenadine.
in healthy volunteers indicate that fexofenadine is a P-gp substrate (Kim et al., 2009).

The higher than expected absorption of fexofenadine, despite its physicochemical characteristics and efflux by P-gp, is, in part, due to its uptake in the intestine by OATP1A2 (Glaeser et al., 2007). The reduced absorption of fexofenadine in the presence of grapefruit and other fruit juices is due to inhibition of its intestinal uptake by OATP1A2 (see also the review article Drug Interactions of this special issue). It is likely that the extent of fexofenadine absorption is determined by the interplay between OATP1A2 and P-gp in the intestine (Glaeser et al., 2007). The low penetration of certain second-generation H1-antihistamines into the brain, compared to that of the first-generation H1-antihistamines, has been explained by their affinity for P-gp and other efflux transporters; however, in rats, fexofenadine has been shown to have low potential to cross the BBB independent of its affinity for P-gp (Obradovic et al., 2008). Fexofenadine is eliminated in the bile and the urine. There are conflicting reports on the transporter(s) responsible for the hepatic uptake of fexofenadine. One group claims that the uptake is mediated by OATP1B1 and OATP1B3 (Matsushima et al., 2008a), while another reported that fexofenadine hepatic uptake is likely mediated by OCT1 and not by OATP transporters (Glaeser et al., 2007). The efflux transporters involved in the biliary excretion of fexofenadine appear to include Pgp, MRP2, and BSEP but not BCRP or MRP4 (Matsushima et al., 2008a). Fexofenadine is also a substrate of MRP3, a transporter located on the sinusoidal membrane of hepatocytes. The urinary excretion of fexofenadine is thought to be mediated, at least in part, via OAT3 (Tahara et al., 2006) for uptake from the blood and via MATE1 for its luminal efflux into the urine (Matsushima et al., 2008b).

Cetirizine is, like fexofenadine, a zwitterion; however, unlike fexofenadine, its permeability characteristics in Caco-2 cells suggest that it is well absorbed. The enantiomer, levocetirizine (eutomer), has similar permeability to cetirizine in vitro, with the fraction absorbed following oral administration being at least 85% (Whomsley and Strolin Benedetti, 2005). Although levocetirizine is a substrate of OATP1A2, its permeability characteristics in Caco-2 cells suggest that this transporter does not play an important role in determining the extent of absorption. Cetirizine (and levocetirizine) are substrates of P-gp; however, like fexofenadine, it would appear that the poor brain penetration of cetirizine is not solely dependent on efflux by P-gp (Whomsley and Strolin Benedetti, 2005). The transporters responsible for the uptake of cetirizine and levocetirizine in the kidney have not been identified, although it would appear that OAT1, OAT3, or OCT1 are not capable of transporting levocetirizine (Schwarz et al., 2006). Levocetirizine is also a substrate for OAT4, which may be involved in reabsorption in the proximal tubule (Schwarz et al., 2006).

The other antihistamines have been studied nearly exclusively as substrates of human P-gp. Loratadine is an unambiguous P-gp substrate, as it has been investigated not only in the MDR1-MDCK monolayer efflux, but also in the ATPase and MDR1-MDCK calcein assay, with positive results in all three (Polli et al. 2001; Wang et al. 2001). Acrivastine, bepotastine, desloratadine, ebastine/carebastine, epinastine, and olopatadine would all appear to be substrates of P-gp, although their relative affinities and kinetic constants for transport by this transport protein have not been determined (Mahar Doan et al., 2004; Ohashi et al., 2006; Ishiguro et al., 2004; Wang et al., 2001; Tamai et al., 2000; Mimura et al., 2008).

Budesonide and prednisone have been shown to be substrates of P-gp in LLC-PK1 cells stably transfected with human MDR1 cDNA (Dilger et al., 2004). Drug secretion via P-gp into gut lumen might play an important role in the pharmacokinetics and pharmacodynamics of these corticosteroids in the treatment of diseases, such as Crohn’s disease. Budesonide has also been shown to have the potential to influence P-gp expression in vitro (Maier et al., 2007).

The physicochemical properties of montelukast suggest that it may require assistance from a transport protein to be absorbed. A recent article suggests that OATP2B1 influences the pharmacokinetics and pharmacodynamics of montelukast, and that polymorphic expression of the transporter is associated with significantly reduced plasma montelukast concentrations in patients (Mougey et al., 2009). There is also a report of an interaction of montelukast with MRPl 4 in vitro (Rius et al., 2008).

**Dose and time dependency**

**Autoinduction**

Some drugs can induce their own metabolism by increasing the rate of synthesis of the enzyme(s) involved. Autoinduction has a number of consequences (e.g., it limits one’s ability to use information from a single dose to predict kinetics after repeated doses or continuous administration). Dose dependence is expected in the degree of autoinduction for drugs that undergo this behavior.

The antiepileptic drug, carbamazepine, undergoes autoinduction: This is evidenced by the declining plasma concentration, assessed on days 7, 8, 15, 16, 22, and 23 of an oral multiple-dose regimen of 6 mg/kg taken by a subject once-daily in the morning for 22 consecutive days (Rowland and Tozer, 1995b). Autoinduction in humans
has been reported also for the antimycobacterial agents, rifampicin and rifabutin (Strolin Benedetti et al., 1990). In Table 6, the observed (i.e., experimental) and theoretical plasma levels of unchanged rifabutin 24 hours after the single daily dose (450 mg), as well as 24 hours after the third, the fifth, the seventh, the ninth, and the tenth dose of the repeated treatment, are presented. From the fifth dose on, the experimental values were significantly lower than the theoretical ones.

Among the drugs used to treat allergy, no autoinduction has been reported, to date.

**Autoinhibition**

Some drugs can inhibit, either reversibly or irreversibly, the enzyme(s) involved in their own metabolism. The irreversible inhibition is also called mechanism-based inactivation.

Autoinhibition can be observed since the first dose (e.g., lack of linearity in plasma concentrations producing concentrations higher than expected from the single doses administered). If the inhibition is irreversible or there is a substantial accumulation of the drug, the autoinhibition can increase after repeated administration.

The antidepressant, paroxetine, undergoes autoinhibition (Venkatakrishnan and Obach, 2005). This drug

![Figure 17. Metabolism of paroxetine.](image-url)
is metabolized by CYP2D6 (Figure 17), and there is evidence for mechanism-based inactivation of this isozyme by paroxetine. Paroxetine possesses a methylenedioxyphenyl substituent and is metabolized to a catechol metabolite. This finding supports the mechanism of the paroxetine inactivation of CYP2D6 as occurring via the formation of a carbene-heme metabolite intermediate complex (Bertelsen et al., 2003; Johnson, 2008). A classical approach to verify autoinhibition is to compare the observed concentration profiles after repeated administration (e.g., after 14 doses) with the profile expected by linear accumulation, which is obtained by the profile of the single dose by using the method of superposition: If the observed plasma concentrations are higher than expected, autoinhibition has occurred. To predict the nonlinear accumulation kinetics of paroxetine resulting from autoinhibition of clearance, it is necessary to know not only the inhibitor concentration and the \( K_I \) value (for the reversible inhibition), but also other parameters relating to mechanism-based inhibition [e.g., the inactivation rate constant \( k_{inact} \), the enzyme-inhibitor dissociation constant \( K_I \), and the rate constant for degradation (turnover)] of the enzyme (CYP2D6) in vivo in humans (Venkatakrishnan and Obach, 2005).

Among the drugs used to treat allergy, no autoinhibition has been reported, to date. The oxidative metabolism of zafirlukast occurs through CYP2C9 and CYP3A4. CYP3A4-mediated zafirlukast metabolism results in a reactive metabolite that can inactivate the isozyme CYP3A4-mediated zafirlukast metabolism (Figure 17), and there is evidence for mechanism-based inactivation by other enzyme systems; therefore, compensation for the inhibition of CYP2C9 and 3A4 by other enzymes will probably occur.

**Chronopharmacokinetics**

**General concepts**

Chronopharmacokinetics can be defined as time-dependent kinetics (i.e., kinetics dependent on when the drug is administered in the day or in which day of the month or in which season of the year).

There are many reasons for this behavior. For example, diurnal variations in renal function, urine pH, \( \alpha_1 \)-acid glycoprotein concentration, gastrointestinal physiology (i.e., food and drink), and cardiac output all occur. In particular, several functions of the cardiovascular system in humans vary markedly with the time of day.

A number of drugs that act on the cardiovascular system exhibit circadian variations in their pharmacokinetics. In the case of some \( \beta \)-adrenergic receptor antagonists, differences have been shown between clinical effects during the day and during the night. An example of diurnal changes in drug absorption and disposition is represented by verapamil (Hla et al., 1992): The highest plasma concentrations are observed when the drug is administered at 8 a.m. (mean \( C_{max} \) 59.4 µg/mL) and the lowest when it is administered at 8 p.m. (mean \( C_{max} \) 25.6 µg/mL) (Table 7).

Food is a major cause of diurnal variations, and the study of the effect of food on drug pharmacokinetics is now regularly carried out.

Many endogenous substances (hormones, in particular, e.g., cortisol, thyroid hormones, and prolactin) (Rohatagi et al., 2003a; Strolin Benedetti et al., 1984) are known to undergo cyclic changes in concentration in plasma and tissue with time. The amplitude of the change in concentration varies among substances. The period of the cycle is often diurnal (approximately 24 hours), although there may be both shorter and longer cycles upon which the daily one is superimposed. The menstrual cycle and seasonal variations in the concentrations of some endogenous substances are examples of cycles with a long period. Drug pharmacokinetics (and response) may, therefore, change with time as a consequence of the changes with time of endogenous substances (e.g., the same enzyme system can be involved in the metabolism of a drug and of an endogenous substance).

Appropriate timing in administration has been frequently considered in cancer chemotherapy (e.g., cisplatin, 5-fluorouracil, and doxorubicin) (Canal et al., 1991; Etienne-Grimaldi et al., 2008). Migraine is also a disorder that exhibits periodicity in its symptoms, and...
so, chronotherapy may be beneficial in treating the problem (Poondru et al., 2000).

Drugs used in the treatment of allergic diseases

Even when the pharmacokinetics of a drug do not change substantially according to the time of administration, it may be important to administer it at a given time if the symptoms of the illness vary during the day. For a drug to be used in allergic rhinitis, it is important to know the diurnal rhythm of the rhinitis symptoms. On the basis of the main symptomatology, patients with rhinitis can be subdivided into "sneezers and runners" and "blockers." According to Van Cauwenberge and Van Hoecke (2005), sneezers and runners are worse during the day and improve at night, whereas blockers are constant by day and night, but may be worse at night. According to Storms (2004), nasal congestion is thought to be the leading symptom responsible for rhinitis-related sleep problems. The severity of nasal congestion follows a circadian rhythm, being worst at night and in the early morning (Storms, 2004).

For some antihistamines, recommendations are given to administer them in the morning (ebastine) (Storms, 1996) or in the evening (mequitazine) (Storms, 2004), with the recommendation being based in both cases on improved efficacy and without parallel information on pharmacokinetics. In a recent paper by Haye et al. (2005), no statistically significant difference in efficacy was seen whether desloratadine was given in the morning or in the evening. The same appears to be the case for cetirizine (UCB data on file). Also, in these studies, there are no pharmacokinetic data available.

Leucotriene receptor antagonists are indicated for evening administration: These drugs significantly improve nighttime rhinitis symptoms (Storms, 2004). The evaluation of the diurnal variation in the pharmacokinetics of zileuton, an inhibitor of the synthesis of leukotrienes, in healthy volunteers has been carried out by Awni et al. (1997). A single dose of zileuton (600 mg) was administered to the same subjects either at 7 a.m. or at 11 p.m., but the differences observed in the pharmacokinetic parameters were not statistically significant. Intranasal corticosteroids administered in the morning have demonstrated efficacy in improving nighttime symptoms; however, it is unknown whether evening administration would improve their effects on nocturnal rhinitis symptoms (Storms, 2004). Limited chronopharmacokinetic information has been published for dexamethasone administered orally (Lamiable et al., 1991) (Figure 18). After the evening administration, the $T_{\text{max}}$ is longer and the $C_{\text{max}}$ is lower, compared with the morning administration (mean $C_{\text{max}}/T_{\text{max}}$ 5.3 and 15.8 at 11 p.m. and 8 a.m., respectively).

Studies in which both plasma concentrations of the antiallergic drug and improvement of the symptoms are measured following the administration of the antiallergic drug at different times of the day would be necessary to interpret the results.

Enantiomers

Many drugs are marketed as racemates, that is, equimolar mixtures of molecules with the same molecular formula (isomers) that differ only in the arrangement of their atoms in space (stereoisomers) and are related as two nonsuperimposable mirror images (enantiomers).

Under “ordinary conditions” defined as an achiral (i.e., symmetrical) environment, in which only achiral reagents are used, no difference whatsoever can be discerned between a pair of nonsuperimposable mirror images, but the biological environment is chiral, the macromolecules can distinguish between a pair of enantiomeric drugs, and consequently, pharmacokinetics and pharmacodynamics are enantioselective. This means that absorption, disposition, and activity of enantiomers may be, and in general are, different. Names of chiral compounds whose absolute configuration (i.e., the actual orientation of the atoms in space has been determined) is known are differentiated by prefixes R (for rectus, Latin for right) and S (for sinister, Latin for left).

When considering a particular biological activity, the more active isomer is termed the eutomer, the less active the distomer.

Natural products are synthesized in a chiral environment; consequently, they are generally found as a single stereoisomer. Pharmaceuticals of natural origin are optically active (e.g., levomorphone from Papaver somniferum and dextrodigitoxin from Digitalis purpurea). Contrary to the natural products, synthetics are usually obtained as isomeric mixtures, such as racemates.

Examples of racemates among the antiallergic drugs are cetirizine, fexofenadine, and also terfenadine (withdrawn from the market). The continuously increasing demand for more selective drugs, which are more targeted in their action and
show less side effects, is providing an important stimulus to pharmaceutical companies to market these products as pure enantiomers (Caldwell, 2001). Consequently, there is an increasing demand for methods for the industrial-scale synthesis of optically active compounds.

As the authorities realized the differences in activity, binding, or toxicity of enantiomers in racemic mixtures and their potential for adverse reactions in particular patients or groups of patients (e.g., the elderly), they began to emphasize the importance of data on the individual enantiomers. Failure to use a stereospecific chemical assay to measure the individual enantiomers can lead to problems when attempting to correlate plasma concentration with response following administration of the racemate. In a word, when important differences in activity and toxicity of enantiomers of a racemic drug are detected, it is essential to measure plasma concentrations of the individual enantiomers to define the “therapeutic window.”

Examples of marketed enantiomers among the antiallergic drugs are levocabastine (Leonov and Bielory, 2007), levocetirizine (Tillement et al., 2003) (see Figure 19), and montelukast (Bielory and Leonov, 2008). Both levocabastine and levocetirizine have been found to be much more potent than their dextrocounterparts, whereas in the case of epinastine no significant difference between L- and D-enantiomer in the antihistaminic effects and CNS depression effects has been found (Tasaka et al., 1991).

Fexofenadine is a racemic mixture, with its enantiomers having equal potencies, but different pharmacokinetics.

**Stereoselective ADME**

Since a racemate is not a single compound, but a 50:50 mixture of two enantiomeric drugs, the application of a stereospecific analysis to the study of its clinical pharmacokinetics (e.g., to measure plasma concentrations) is not only recommendable, but mandatory for the interpretation of the data. After a single oral dose of fexofenadine (60 mg) in healthy volunteers, plasma concentrations of the R-enantiomer were almost 2-fold higher than those of the S-enantiomer (ratio AUC R/AUC S = 1.7–1.8) (Miura et al., 2007; Tateishi et al., 2008). According to these researchers, the stereoselective pharmacokinetics of fexofenadine are due to P-gp-mediated transport. Also, after a single oral dose of cetirizine (20 mg) in healthy volunteers, the plasma concentrations of the enantiomers were different (ratio AUC levocetirizine/AUC dextrocetirizine = 2) (Baltes et al., 2001). Stereoselectivity in drug disposition seems to be the rule rather than the exception, and, depending on whether the active or less active enantiomer is preferentially affected, there may be amplification or attenuation of in vivo, as compared to the in vitro, drug potency.

**Absorption**

Most drugs diffuse passively through biological membranes, although absorption involves diffusion through chiral barriers. Enantiomers do not differ in their lipid/water partition coefficient; consequently, stereoselectivity is not expected. When an active process (transporter-mediated) is involved, the two enantiomers may differ in their absorption characteristics. An example is fexofenadine, a substrate of P-gp and of OATP, whose enantiomers not only have different AUC when fexofenadine is administered alone, but have their pharmacokinetics affected differently by the coadministration of itraconazole, a P-gp inhibitor (Tateishi et al., 2008). Itraconazole administration decreased the ratio AUC R/AUC S of fexofenadine enantiomers from 1.84 to 1.43, which implies that the inhibitory effect of P-gp-mediated transport by itraconazole is insufficient to eliminate the stereoselectivity of fexofenadine enantiomers.

Stereoselective absorption arises also when enantiomers differ in their ability to constrict or dilate blood vessels at sites of administration (e.g., enantiomers of local anesthetics have different effects on local blood flow, which, in turn, account for differences in rates of systemic absorption) (Aps and Reynolds, 1978). Finally, the aqueous solubilities and crystal forms of racemates can differ from those of the individual enantiomers, and this may give rise to corresponding differences in dissolution rates at sites of administration. Racemic talidomide, for example, is 5–9 times less soluble than the separate enantiomers in many solvents (Hague and Smith, 1988).

**Distribution**

The extent of distribution of drugs is determined by plasma and tissue protein binding and partition
The latter is a physical property, identical for the two enantiomers and, therefore, not enantioselective. The extent of binding of enantiomers may be different, and enantioselectivity expressed at the recognition interaction by macromolecules present in tissues and plasma can be an important determinant of the pharmacokinetics of enantiomers. The discrimination of enantiomers by proteins is not surprising. Proteins consisting of chiral amino-acid units are macromolecules possessing chiral secondary structure by forming asymmetric cavities or helical sequences, thus providing chiral environments for small bioactive molecules. Consequently, the fitting is unequal. The percentages of binding of levocetirizine and dextrocetirizine to human plasma proteins, as well as the volume of distribution of the two enantiomers, are different (Strolin Benedetti et al., 2008).

**Metabolism**

Much of the stereoselectivity observed in pharmacokinetics is due to metabolism. Both phase I and II metabolic reactions may be stereoselective. We can observe selectivity in the biotransformation of chiral drugs (substrate stereoselectivity), in the production of chiral metabolites from prochiral drugs (product stereoselectivity), or in both instances (substrate/product stereoselectivity). When hydroxyzine, a racemic drug, is administered and the plasma pharmacokinetics of the parent compound and of an important metabolite, cetirizine, are investigated, it would be important to measure the respective amounts of levo- and dextrohydroxyzine and of levo- and dextrocetirizine by a chiral assay, as the affinity of the two enantiomers for the $H_1$-histamine receptors is quite different, both for hydroxyzine and for cetirizine (Gillard et al., 2002).

An interesting example of stereoselective metabolism is the metabolic chiral inversion of 2-aryl propionic acids. These drugs constitute an important group of nonsteroidal anti-inflammatory agents (NSAIDs). They contain a chiral center and are generally administered as racemates, but the anti-inflammatory activity resides in the dextroenantiomers [(S)-isomers]. In vivo, some of these drugs undergo a metabolic inversion of the chiral center with a unidirectional reaction that transforms the R(-)-enantiomer to its S(+)antipode (Hutt and Caldwell, 1983). The in vivo transformation of the inactive to the active enantiomer should, in principle, be welcome, but if one examines what is known about the mechanism of chiral inversion, the situation is not as simple and desirable as might have appeared. The first example of this reaction is that of ibuprofen, reported first in 1975, and since then, a considerable number of profens have been shown to undergo this chiral inversion. However, the enantiomeric inversion observed in man for some of these compounds (fenoprofen, ibuprofen, and benoxaprofen) cannot be assumed to be a general phenomenon, as some do not possess this property (e.g., flurbiprofen and indoprofen) (Jamali, 1988). A plausible reaction mechanism for the chiral inversion has been proposed by Nakamura et al. (1981). This involves the stereoselective conversion of the R-enantiomer of the acid to its acyl CoA thioester, a process that results in the activation of the proton in position $\alpha$ with respect to both the phenyl ring and the thioester group. The acyl CoA may then undergo one of three separate fates, as follows (see Figure 20): 1) hydrolysis with retention of configuration to yield the original R-2-arylpropionate; 2) racemization of the chiral center, since the activated $\alpha$ proton allows the formation of an equilibrium between the acyl CoA thioester and the corresponding hemithioacetal. The acyl CoA racemate so produced will then hydrolyze to give a mixture of the R- and S-enantiomers of the parent profen; and 3) acyl transfer of the profen moiety into a hybrid triglyceride, resulting in the retention of the profen in adipose tissue. Indeed, the biosynthesis of naturally occurring triglycerides occurs in the same way, with the fatty acid acyl-CoA required as a precursor.

![Figure 20](image-url)  
**Figure 20.** Mechanism of the chiral inversion and hybrid triglyceride formation from “profen” drugs.
It is interesting to note that ketoprofen, which, together with ibuprofen and fenoprofen, was shown by Fears et al. (1978) to undergo incorporation into triglycerides, has since then been reported to undergo metabolic chiral inversion.

There are approaches that allow chiral drug candidates to be screened for configurational stability. However, as the chiral inversion has been related to toxicity or can produce a modification of the activity of a drug, the authorities request that the search of a possible chiral inversion be done in vivo, when developing an enantiomer. There was no evidence of the chiral inversion of levocetirizine in humans, this result being consistent with that obtained in preclinical studies. Indeed, the plasma and urinary concentrations of dextrocetirizine were under the limit of detection after the administration of levocetirizine (Strolin Benedetti et al., 2001; Baltes et al., 2001). It has also been shown that there is no apparent bioinversion of montelukast to its S-configuration when studied in humans (Liu et al., 1997).

Excretion

Renal excretion. Renal drug clearance is the net result of passive glomerular filtration, active secretion, passive and active reabsorption, and renal drug metabolism.

Stereoselectivity in glomerular filtration and passive reabsorption is apparent, secondary to any difference in the plasma protein binding of the isomers. The other processes may be expected to be stereoselective.

The stereoselective renal clearance of metoprolol (75 mL/min for dextroisomer and 70 mL/min for levosomer) (Lennard et al., 1983) is probably due to the difference in the unbound fraction of the enantiomers (Lee and Williams, 1990). Active renal tubular secretion, not excluding active reabsorption and renal drug metabolism, is believed to be responsible for the stereoselective renal clearance of pindolol (Hsyu and Giacomini, 1985), chloroquine (Ofori-Adjei et al., 1986), and disopyramide (Lima et al., 1985; Giacomini et al., 1986; Le Corre et al., 1988). Selectivity has been found also for the diastereoisomers, quinidine and quinine, the unbound renal clearance of the former being 4 times greater (Notterman et al., 1986).

As mentioned previously, dextrocetirizine differentiates from levocetirizine in a number of pharmacological and pharmacokinetic parameters, which can be summarized as follows: the affinity for H1-receptors, the plasma-elimination half-life, the volume of distribution, the CLR and nonrenal (metabolic) clearance, and the percent of dose eliminated unchanged in urine (Strolin Benedetti et al., 2008) (see Table 8).

The higher CLR of dextrocetirizine is partly due to the higher unbound concentration available for glomerular filtration. However, as this accounts for <30% of the total renal clearance for both dextrocetirizine and levocetirizine, the net tubular secretion appears to be the major determinant in the difference in renal elimination between the two enantiomers. As tubular secretion is higher for dextrocetirizine, the distomer is likely to have a higher uptake and/or efflux by renal transporters underlying the secretion pathway than levocetirizine, the eutomer. For the cetirizine enantiomers, it is unknown if the stereoselective tubular secretion is due to differences in affinity for drug transporters or to differences in the free drug available for secretion. It has been shown that the uptake of some drugs by renal transporters is dependent on the free drug concentrations (Bow et al., 2006). The stereoselective tubular secretion of the two cetirizine enantiomers, as well as the possible consequences for drug interactions, should be further investigated.

A stereoselective renal tubular secretion has been reported also for fexofenadine (Robbins et al., 1998). Biliary excretion. Both active and passive transport mechanisms are involved in the excretion of drugs and their metabolites into the bile. Stereoselective excretion into the bile has been reported for ketoprofen enantiomers in rats (Foster and Jamali, 1988) and in cholecystomized patients (Foster et al., 1988).

### Table 8. Plasma and urinary pharmacokinetic parameters of levocetirizine and dextrocetirizine in healthy volunteers (n=24) after administration of 20 mg of cetirizine dihydrochloride (mean ± SD).

| Pharmacokinetic parameter | Levocetirizine | Dextrocetirizine |
|---------------------------|---------------|-----------------|
| AUC (ng.h/mL)             | 4,091±646.2   | 1,909.5±392.92  |
| Cmax (ng/mL)              | 508.92±100.61 | 290.13±62.67    |
| t1/2 (hours)              | 0.80±0.29     | 0.83±0.33       |
| tmax (hours)              | 7.80±1.96     | 5.52±1.85       |
| CL/F (mL/min)             | 41.68±6.30    | 90.60±17.16     |
| V/F (L)                   | 27.89±7.64    | 40.35±9.04      |
| Ae (µg)                   | 7,259.8±1,284.6 | 6,361.6±1,330.2 |
| Fe (%)a                   | 72.6±12.9     | 63.6±13.30      |
| CLa (mL/min)              | 311.98±8.32   | 61.43±17.66     |

*aPercentage of the corresponding enantiomer administered.

New approaches in pharmacokinetics: microdosing

During the process of drug development, the first administration to a human subject requires a long list of prerequisites: For instance, 2-week toxicity studies are required in a rodent and in a nonrodent species. Also, the initial dose to be used in a standard dose-escalation study is based on interspecies scaling which, in some cases, may be faulty. For several years, there have been some projects in the United States and Europe to improve the process of drug development. In particular,
a procedure for testing microdoses of an experimental
drug in human subjects has been proposed, requiring a
limited preclinical toxicity evaluation.

The prerequisites to carry out a microdosing study are
different in Europe and in the United States. The EMEA
position paper (European Medicines Agency, 2004) rec-
ommends single- and repeated-dose studies in a single
species, but a single-dose study can be accepted pro-
vided the species could be justified, for example, based
on in vitro metabolism data. The (initial) toxicology
study may cover 14 days, with an interim sacrifice on
day 2, and should include toxicological observations,
gross necropsy, haematology, clinical chemistry, and
histopathology. The dose administered in the safety
assessment is typically 1,000 times the intended micro-
dose to be used in humans. The U.S. Food and Drug
Administration (FDA) (FDA Guidance for Industry,
2006; Wilding, 2005) proposes similar requirements,
except the safety margin is 100 instead of 1,000.

What is microdosing? What does it work for?
The microdosing procedure is the administration of
a drug to human subjects, using a very low amount of
dose. The microdose is defined as a 100th of the phar-
macological dose (or predicted pharmacological dose)
or a maximum of 100 µg. The very low dose supposes the
absence of induction of any toxic effects. Microdosing
is a technique for studying the behavior of compounds
in vivo through the administration of doses so low they
are unlikely to produce whole-body effects, but high
enough to allow the pharmacokinetics to be studied.

Microdosing can result in a basic plasma
concentration-time profile, some absorption profiling,
and limited metabolic profiling. There is some poten-
tial to get an idea of the interindividual variability of
pharmacokinetics.

The method, prerequisites, and limitations
In microdosing, a single dose of trace-enriched 14C-
labeled drug is given to a group of 4–6 human volunteers,
preferably by using a crossover design (in which one
dose is given intravenously and the other, after a suitable
wash-out period, by the proposed route of administra-
tion, usually oral). The typical dose is between 1 and
100 µg. Within 3–5 plasma half-lives, blood and urine
samples are collected.

Considering that the apparent volume of distribution
in human subjects can be as high as hundreds of liters,
a dose in micrograms may result in a plasma concentra-
tion of the drug of the order of picograms per milliliter
or even less. Therefore, a highly sensitive analytical
method is mandatory (European Medicines Agency,
2004; European Union Microdosing AMS Partnership
Program, 2006). Examples of the analytical method are
positron emission tomography (PET), accelerator mass
spectrometry (AMS), or LC/MS/MS (Lappin and Garner,
2003).

AMS is an ultrasensitive technology for the analysis
of bioanalytical samples, using an isotope ratio method.
The drug is isotopically enriched, for example, with 14C.
Because of its high sensitivity, only low amounts of 14C
are necessary in keeping the levels of radioactivity lower
than those required for regulatory approval. For exam-
ple, in the classical microdosing study, the amount of
radioactivity administered does not exceed 100 nCi, cor-
responding to the naturally occurring 14C radioactivity in
the body. AMS quantification can detect concentrations
of hundreds of femtogram per milliliter (fg/mL) (1 fg
is equal to 10⁻¹⁵ g). The detection limit is less than one
attogram (ag) (1 ag is 10⁻¹⁸ g).

The prerequisites to carry out a microdosing study
are different in Europe and in the United States, as pre-
viously discussed.

Why to use microdosing?
Basic plasma-concentration profiles of a drug and their
metabolites can easily be characterized by using the
microdosing approach. Based on these data, the selec-
tion of better candidate drugs can be started. The micro-
dosing method requires a lighter package of toxicology
and safety data, compared to classical phase I studies.
Another advantage of the technique is the possibility to
assess the absolute bioavailability of the drug.

Finally, microdosing can reduce the direct study costs
(Wilding and Bell, 2005).

Examples of microdosing
The Consortium for Resourcing and Evaluating AMS
Microdosing (CREAM) did an evaluation on the method
for the prediction of pharmacokinetics at the therapeu-
tic dose. The study was based on five drugs. Each com-
pound was selected to represent a situation in which
prediction of human pharmacokinetics from nonclini-
cal data might be considered problematic, as follows:

- Midazolam—selective substrate for CYP3A4
- Diazepam—CYP2C19 elimination with low
clearance
- ZK 253—extremely low bioavailability
- Warfarin—CYP2C9 substrate
- Erythromycin—substrate for 3A4 and efflux trans-
porter P-glycoprotein

Warfarin has a low clearance but a high binding to plasma
proteins. The terminal half-life after the administration
of a therapeutic dose is 1.5–2.0 days, while the microdosing experiment demonstrated a terminal half-life of about 11 days. The volume of distribution is 17.9 L following administration of a therapeutic dose, while the microdosing experiment estimated a volume of 67.3 L. For the other four drugs, the prediction of the pharmacokinetic profile and corresponding pharmacokinetic parameters were within the accepted range of 2 in comparing the extrapolated data with the reference of data after administration of a therapeutic dose. One arm of the CREAM study used the orally administration of an unlabeled therapeutic dose together with a microdose of 14C-labeled erythromycin to estimate the absolute bioavailability. All results of the study are published by the members of the CREAM consortium (Lappin et al., 2006; Lappin and Garner 2006).

Another example is the EUMAPP (European Union Microdosing AMS Partnership Program) initiative, where seven drugs were selected for the microdosing design, taking into account their uncertainty in quantitative prediction of the pharmacokinetics based on in vivo and in vitro preclinical data. Preliminary results are suggesting that microdosing correctly characterized the shape of the concentration-time profile at the therapeutic dose (http://www.xceleron.com/metadot/index.pl?id=2754 &isa=DBRow&op=Show&dbview_id=2596).

Current status: pros and cons

Microdosing is a tool in the drug selections’ toolbox and has to be intelligently applied in the context of the specific drug development. To date, microdosing has offered promising results and can be considered as an additional tool to facilitate decisions. The requirement of 14C-labeled drug could be seen as an apparent disadvantage, but with the highly sensitive AMS method, and in combination with standard methods, basic information can be obtained on the nature of drug transport, the extent of bioavailability, and metabolism.

The extrapolation of the PK profiles evaluated by using a microdose study up to therapeutic dose levels are accurate for compounds characterized by linear PK. Discrepancies may be important for drugs with high clearance where capacity-limited metabolism may occur. Also, compounds with low water solubility may not be characterized by constant bioavailability. Large discrepancies may be evident also in the case of biologicals, which are often characterized by nonlinear pharmacokinetics. In all these cases, the extrapolation from the results of a microdose study may be very difficult.

More data on a wider range of compounds (e.g., substrates of PgP or other transporters) are needed to help further clarify the uses and limitations of the microdosing procedure.

Conclusions

Although the basic concepts of pharmacokinetics and metabolism have been described in this article, special attention has been payed to those aspects, which can be relevant for the drugs used in the treatment of allergic diseases.

Therefore, together with the oral route of administration, other routes, such as the ocular, nasal, and cutaneous/transdermal ones have been particularly discussed, as well as the corresponding absorption of the drugs administered in those ways.

The significance of absolute bioavailability is not the same when, for a drug, a route of administration, such as the nasal one, has been chosen.

The volume of distribution value and the degree of binding to plasma and tissue proteins may have a different relevance if an antiallergic drug is an antihistamine or a corticosteroid.

Information on drug-metabolizing enzymes and transporters other than the hepatic and the renal ones have tentatively been provided (e.g., enzymes and transporters present in CNS, nasal mucosa, and respiratory tract). It appears clearly that further research is necessary in this field (e.g., the importance of the P-450 epoxygenase pathway of metabolism of arachidonic acid in the pathogenesis of allergic/nonallergic asthma or the role of the transporters present in the CNS and in the kidneys to control the sedative effects of antiallergic drugs and to avoid drug-drug interactions.

As there are antiallergic drugs marketed as enantiomers (e.g., levocetirizine, levocabastine, and montelukast), the stereoselective ADME has been extensively discussed.

Finally, it has been considered useful to mention new approaches in PK, such as microdosing, and discuss its predictivity, even if specific applications to antiallergic drugs have not yet been described.

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