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Pharmacology and Drug Targets – The Basis of Therapeutics

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Summary

This chapter covers (i) basics of pharmacology such as dosage forms, routes of administration, delivery to the target site, (ii) pharmacodynamics (PD), and (iii) pharmacokinetics (PK) and provides (iv) examples of its use in drug discovery and development. In particular, basic principles of drug–target receptor interactions, agonistic and antagonistic mechanisms of action (MoA), liberation/absorption/distribution/metabolism/elimination (LADME) phases of PK, dosage forms, PK/PD modelling, drug–drug interactions (DDIs), drug transporters, aspects of clinical pharmacology, therapeutic window/index calculations, and illustrative examples of how these tools are utilized in drug discovery and development are provided.

Tools

- In vitro systems
- In vivo systems
- Genetics
- Screening
- Modelling

Regulatory framework

- ICH

(Continued)

Principles of Biomedical Sciences and Industry: Translating Ideas into Treatments, First Edition.
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Risks

- Limited understanding of pathophysiology
- Target validation/reproducibility
- Lack of appropriate models

Success factors

- Translating pre-clinical compounds into drugs for patients

2.1 Introduction

After human health was believed to be determined solely by God and fate for long times, the theory of humourism (also known as the four humours) developed in antiquity under the influence of important Greek savants as Hippocrates and Galen dominated Western medicine until modern times. In his works, **Galen** wrote numerous texts on the composition and manufacture of medicines and gave his name to the Galenic formulation of medicines. In the sixteenth century, the famous savant **Paracelsus** also included chemical substances in drug therapy. Under his influence, the pharmacy laboratory also gained more importance. During the course of the industrialization in the nineteenth century, pharmaceutical industry evolved from pharmacies specialized on the manufacturing of pharmaceuticals and the tar and paint industry. At that time, the prevailing teaching of humoral pathology was replaced by the research of the physician Rudolf Virchow and based on it the so-called cellular pathology and formed the fundament for science-based medicine.

Pharmacology (greek for '*pharmacon*': drug and '*-logos*': doctrine/knowledge of) explores interactions between compounds and organism and describes it in a quantitative and qualitative manner (Tozer and Rowland 2016; Simmons 2011). The term **pharmacon** describes the active element of a drug and is non-judgemental; e.g. it contains no statement if the substance is harmful (poison) or healing (drug).

The interaction between drug and organism can be classified in three phases:

1. **Pharmaceutical phase**
   The pharmaceutical phase includes administration and drug release. This is decisively determined by the type of administration (e.g. oral, intravenous (iv), intramuscular, subcutaneous, inhalative, epicutaneous, sublingual, and rectal) and the galenics (composition and manufacture of drugs). Together with so-called adjuvants, the drug becomes a dosage form. Galenics can be used, for example, to influence the duration of action, the concentration of the active substance in the blood and sometimes even the site of action.
2. **Pharmacokinetics (PK)**
   Pharmacokinetics deals with the concentrations of a drug in the body and is affected by all processes to which a drug is exposed to in the body. Pharmacokinetics includes absorption, distribution, metabolism and excretion of a compound (ADME).

3. **Pharmacodynamics (PD)**
   Pharmacodynamics describes the effect of the drug on the organism. Here, binding to a target (e.g. receptors, ion channels, enzymes, and bacterial metabolism) and the resulting biological effect play a major role. The pharmacological effect of a substance can be divided into desirable and undesirable effects (side effects).

Pharmacodynamics: Effect of the Drug on the Organism. The pharmacological effect of a substance can be divided into desirable (potency) and undesirable effects (side effects).

Pharmacokinetics: Effect of the Organism on the Drug. Concentrations of a drug in the body, affected by all processes to which a drug is exposed to, including absorption, distribution, metabolism, and excretion of a compound (ADME).

The pharmacology differs between individuals – for instance higher weight subjects often show lower concentrations. Diseases may need different concentration levels for efficacy but might also influence the pharmaceutical phase and the PK. There are many factors that influence the interaction between drug and organism (Figure 2.1) – and all these aspects influence the dose and regimen (i.e. frequency) that provide an optimal benefit–risk relationship for the individual patient.

Studying the pharmacology of a compound during drug development is essential to learn about the influencing factors on the pharmacokinetics and pharmacodynamics and, if necessary, provide information in the drug label on adjusting the dose or regimen accordingly (Rosenbaum 2016).

In this chapter, we describe general concepts mainly related to small molecules (low molecular weight compounds) and monoclonal antibodies. Of note, there exist newer therapeutic approaches, like cell and gene therapies (e.g. CAR-T therapies or

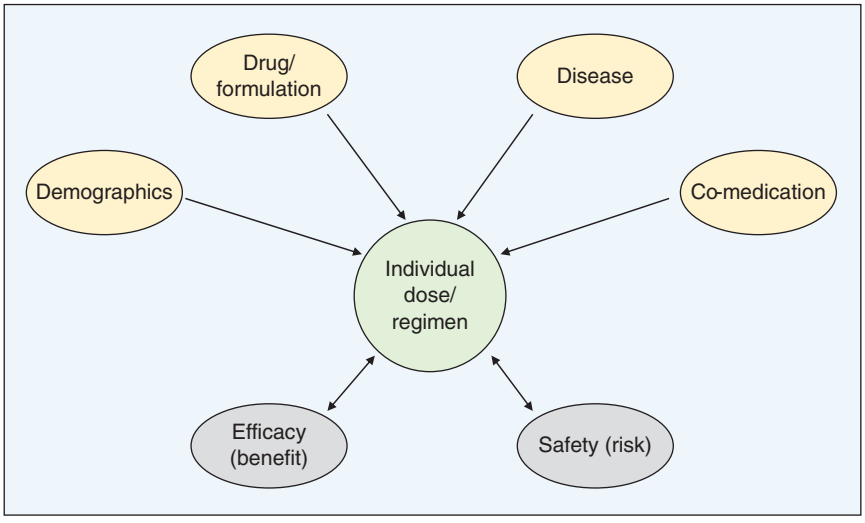


Figure 2.1 Factors influencing the interaction between a drug and an organism. Source: Book editors based on Hartl, Fink, and Beer-Hammer.

adeno-associated virus vector therapies) which can show different dynamics over time and are not covered here even though the basic concepts of pharmacology still hold.

2.2 Pharmacodynamics

Pharmacodynamics is the science of biochemical and physiological drug effects on animal or human organisms as well as microorganisms and parasites.

Pharmacodynamics:

- Type of action (profile and quality)
- Mechanisms of action (MoA)
- Place of action
- Potency
- Efficacy

Specific substances interact with defined endogenous target molecules that are structurally proteins such as receptors, transporters, and enzymes, DNA, RNA, or lipids. They already act in low dosages or concentrations, and their effect depends on the chemical structure and thus on the shape, size, and stereochemical arrangement of the molecule. The specific effect also means that a drug affects as selectively as possible on the target structures.

Two main classes of molecules used as therapeutics:

a) **Small molecules**
   - low molecular weight compounds
   - chemically produced
   - defined by exact chemical structure
   - usually oral drugs

b) **Large molecules/biomolecules**
   - recombinant proteins or monoclonal antibodies
   - produced in genetically modified cells
   - usually intravenous (IV) or subcutaneously (SC) administered drugs

Small molecules are mostly less specific in their action than biomolecules or monoclonal antibodies; they can bind to receptors other than the target (off-target binding) which can result in unwanted side effects (off-target toxicity, which is compound specific). Strong inhibition of a pathway by on-target binding can also induce adverse effects (on-target toxicity, which is target-specific), for instance, immunosuppressive drugs often lead to higher infection risks. At the molecular pharmacological level,

specificity implies that the drug binds to its target with sufficient affinity and has the ability to enhance or inhibit its function as a result of this binding.

Unspecific substances are characterized by the fact that they do not react specifically with endogenous compounds and that they do not change their effect if the chemical modification is not too profound.

Most pharmacological effects can be attributed to a few MoA:

- Interaction with membrane receptors (stimulation or inhibition)
- Opening or blockade of voltage-dependent or ligand-controlled ion channels
- Regulation of gene transcription by binding to intracellular receptors
- Influence of transmembrane or intracellular transporters
- Inhibition or activation of enzymes

2.3 Receptors and Ion Channels

Pharmacological receptors are characterized as intracellular or membrane receptors, which upon binding of an endogenous or exogenous ligand to a specific binding site elicit direct or indirect effects (E). With this definition, the basic equation is ligand (L)–receptor (R) interaction: $L + R \leftrightarrow [LR] \rightarrow\rightarrow E$. Thus, a (pharmacological) receptor has a dual role: (i) signal recognition through interaction with the ligand and formation of the ligand–receptor complex and (ii) direct or indirect triggering of an effect. Receptors can be found intracellularly or bound to membranes. Intracellular receptors, which act as transcription factors, include the receptors of (i) steroid hormones, (ii) retinoids, and (iii) thyroid hormones. The membrane receptors can be divided into (i) G-protein-coupled receptors (GPCRs), (ii) ion channels (voltage- and ligand-controlled), and (iii) receptor protein kinases (enzyme-associated receptors).

2.3.1 G-protein-Coupled Receptors (GPCRs)

With 800 genes, GPCRs are not only the largest group within the family of membrane receptors in the human genome, but also the group with the highest diversity. Various extracellular stimuli induce intracellular signalling cascades via the intracellularly coupled Guanine-nucleotide-binding proteins (G-protein). This receptor group includes numerous neurotransmitter receptors that are particularly important for drug therapy, such as adenosine-, adrenergic, ATP- (P2Y-), dopamine-, $GABA_B$-, metabotropic glutamate-, histamine-, muscarinergic, opioid- and serotonin (except $5\text{-}HT_3$) receptors. In GPCRs, signal transmission takes place via the G-protein. Activation of GPCRs by an agonist results in dissociation of the heterotrimeric G protein into the Gα- and Gβγ-subunit after exchange of GDP bound to the α-subunit for guanosine triphosphate (GTP). According to the various functions of GPCRs, there is a multitude of different G proteins, e.g. cyclase stimulating (G_s proteins), cyclase inhibiting (G_i proteins) or phospholipase C-activating G proteins (G_q proteins).

2.3.2 Ion Channels

Ion channels are in third place after the GPCRs and protein kinases and play important roles in a variety of biological processes, e.g. formation of action potentials, cardiac, skeletal and smooth muscle constrictions, epithelial transport, T-cell activation or insulin secretion. Ion channels are integrated in the cell membrane and consist of several subunits. These subunits form a channel pore that is opened or closed by conformational changes. Due to their selective permeability for distinct ions, they are named sodium-, potassium-, calcium-, and chloride channel. The inflow and outflow of the respective ions is controlled by the concentration gradient between extracellular and intracellular space and by the membrane potential. The extent of the ion flow depends on the number of open channels, the duration of opening and the permeability of the corresponding ions of the so-called conductivity. There are two kinds of receptors distinguished:

- Ligand-controlled ion channels or ionotropic receptors: (e.g. ATP- (P2X-), $GABA_A$-, glutamate- (NMDA- and AMPA-), glycine-, 5-HT_3- and nicotine receptors, and K^+ (ATP-sensitive, Ca^{2+}/calmodulin-activated, G_i-protein-regulated 'GIRK'), where opening and closing of the channel is controlled by extracellular binding of a ligand. For example, binding of acetylcholine or nicotine to the α-subunit of the nicotinic receptor leads to the opening of the channel, thus releasing an action potential through the influx of sodium ions.
- Voltage-controlled channels**:** (e.g. Na^+-, Ca^{2+}- [L-type, N-type, T-type, P/Q-type] and K^+- [Kγ, hERG-, KCNQ-, Kir-] channels) that are opened or closed by membrane depolarization or hyperpolarization. For example, the influx of Na^+ ions into a myocardial cell allows rapid membrane depolarization, which is necessary to open L-type Ca^{2+} channels. The incoming calcium ions now lead to Ca^{2+} release from the endoplasmic reticulum and allow the initiation of the contraction of cardiomyocytes. K^+ channels, which are also activated by depolarization, repolarize the cell membrane and allow previously inactivated Na^+- and Ca^{2+} channels to revert to the activatable state by conformational change and thus be available again for subsequent excitation.

2.3.3 Enzyme-Associated Receptors

This group of receptors belong (i) receptors with tyrosine kinase activity, (ii) receptors with associated tyrosine kinases, (iii) receptors with guanylyl cyclase-activity, (iv) receptor serine/threonine kinases as well as (v) tumour necrosis factor (TNF) receptors, which mediate apoptosis.

Tyrosine kinase receptors are characterized to have a ligand binding site extracellularly and a domain with the property of a tyrosine kinase on the cytosolic protein part and thus exert both the function of a receptor and that of an enzyme. Mitogen-activated protein kinases (MAP kinases) are involved in further **signal transduction**. Since they regulate a variety of cellular activities such as gene expression, mitosis, differentiation, and apoptosis/necrosis, they are of great importance for the whole organism. Notably, their proliferative effect is critical for

signal transduction of most oncogenes. MAP kinases are divided into four groups: extracellular signal-regulated kinases (ERK), c-Jun N-terminal kinases (JNK), p38 kinases, and as a special ERK form ERK5. The signalling cascade of ERK is mainly stimulated by growth factors, JNK and p38 kinases are active in the presence of stress stimuli such as cytokine release, UV radiation, heat or osmotic shock. ERK5, on the other hand, is activated by both growth factors and stress stimuli. Receptors with tyrosine kinase activity include the receptors of insulin, insulin-like growth factor (IGF-1), as well as various other growth factors (e.g. vascular endothelial cell factor [VEGF], epidermal growth factor [EGF], fibroblast growth factor [FGF], and platelet-derived growth factor [PDGF]). Insulin and IGF-1 each consist of two α- and β-subunits, which are linked by disulphide bridges. The other growth factors, however, are monomeric proteins that dimerize only after ligand binding. The dimerization leads to autophosphorylation of tyrosine residues in the cytosolic part of the receptor. This generates docking sites for signalling proteins that bind to the phosphorylated residues of the receptor. In this way, receptor tyrosine kinases are coupled to the Ras signalling cascade, which controls cell growth and proliferation.

Receptors with associated tyrosine kinases, like the growth factor receptors, are monomeric membrane proteins with a transmembraneous region, which in turn dimerize after ligand binding, but the receptor group does not possess its own tyrosine kinase domain. This group of receptors includes numerous cytokine receptors as well as growth hormone, prolactin, and erythropoietin receptors. Upon activation and dimerization, just another kinase (JAK) proteins dock and phosphorylate tyrosine residues of the receptor. As a result, signal transducers and activators of transcription (STAT) proteins associate with the phosphorylated receptor domains. The associated STAT proteins are then also phosphorylated by JAK kinases. Finally, the phosphorylated STAT proteins dimerize, are translocated into the nucleus and activate transcription of specific genes.

Receptors with guanylyl cyclase activity (membrane-bound guanylyl cyclase) in particular comprise the receptors for natriuretic peptides and the intestinal hormone guanylin. These monomeric transmembrane proteins, like the receptors with tyrosine kinase activity, have an extracellular binding site for the activating ligand and an intracellular enzyme domain. When a ligand binds to receptors with guanylyl cyclase activity, their guanylyl cyclase domain is activated. As a consequence, GTP is formed into cyclic guanosine monophosphate (cGMP) which, as second messenger, triggers further reactions, e.g. the relaxation of smooth muscle cells or the secretion of chloride in the intestinal lumen.

Receptor serine/threonine kinases include the receptors of transforming growth factor-β (TGF-β), of which two types, TGF-β-R-I and TGF-β-R-II, exist. Also, the cytokine bone morphogenetic protein 2 (BMP2) unfolds its action by means of such a receptor type. TGF-β is a local cytokine which acts via TGF-β receptors and is associated with healing processes but also fibrosis of tissue, e.g. diabetic nephropathy, renal and pulmonary fibrosis, as well as cardiac remodelling after myocardial infarction. Angiotensin converting enzyme (ACE) inhibitors reduce the release of TGF-β. The transduction mechanism of TGF-β receptors is the following: initially, TGF-β binds to TGF-β-R-II and then forms a heterodimer together with

TGF-β-R-I. In the next step, a transphosphorylation of TGF-R-II to TGF-R-I occurs, which triggers the actual signal transduction. The activated receptor complex then triggers gene expression via so-called Smad proteins, which migrate into the nucleus in an active form.

TNF receptors, also called death receptors, include 29 different receptor subtypes. They are integrated into the membrane of most cells. Important representatives are the TNF receptor 1 and FAS. The binding of TNF to its receptor results in homotrimerization and recruitment of an adapter protein (e.g. TRADD, TRAF, and RIP) to the so-called 'death domains' of the three subunits. The nature of the associated adapter protein determines which signalling pathways (e.g. apoptosis and inflammation) are induced by the stimulation of the TNF receptor. In the case of programmed cell death, apoptosis, the resulting complex activates the caspase cascade, which leads to inactivation of enzymes and degradation of structural proteins as well as fragmentation of genomic DNA.

2.3.4 Non-receptor-Mediated Effects

Beside of receptor-mediated effects, there are also drug effects mediated by **transporters** or **enzymes**. The transport of small organic molecules or ions through the cell membrane often occurs with the help of transport molecules when the molecules to be transferred are too polar to overcome the membrane alone. In addition to the transporters for neurotransmitters in terminal nerve endings (neurotransmitter-specific transporters, e.g. for norepinephrine, serotonin, or GABA), which serve to resume the secreted transmitter in the presynaptic neuron, and the transporters for electrolytes (e.g. $Na^+/K^+/2Cl^-$ – and Na^+/Cl^- – symporter), which predominantly occur in epithelia with secretory function (among others in renal tubules, bronchial epithelium, and intestinal mucosa), there are also transporters for glucose and amino acids.

In particular, the **transporters** for neurotransmitters and electrolytes represent targets for important drugs such as antidepressants, diuretics, cardiac glycosides, and proton pump inhibitors. Antidepressants inhibit the active (re-)transport of norepinephrine and/or serotonin. Diuretics are to be characterized as selective electrolyte transport inhibitors: Furosemide-type loop diuretics block the $Na^+/K^+/2Cl^+$ and thiazides the Na^+/Cl^- symporter. Cardiac glycosides inhibit the outward transport of sodium ions from the intracellular space into the extracellular space as well as the inward transport (extracellular/intracellular) of potassium ions by blocking the sodium–potassium pump (Na^+/K^+ ATPase). The proton pump inhibitors used as antiulcer drugs suppress the production of hydrochloric acid in the stomach by inhibiting the proton potassium pump (H^+/K^+ ATPase).

Numerous effects of drugs are due to the inhibition or (less common) of the activation of **enzymes**. Similar to the pharmacon receptor interaction, it first comes to the formation of a drug–enzyme complex and thereby, depending on the nature of the drug, to enzyme inhibition or enzyme activation. At constant enzyme concentration, the reaction rate depends on the substrate concentration. The more the substrate there is, the more the enzymes can be occupied and cause the reaction.

Substrate saturation: From a certain substrate concentration, all enzymes are present in enzyme–substrate complexes, and thus the maximum velocity $\boldsymbol{V}_{\mathbf{max}}$ for this amount of enzyme is reached.

Substrate excess: If more substrate molecules are present than can be bound by the enzymes, the reaction rate is no longer increased.

The substrate concentration $\boldsymbol{K}_{\mathbf{m}}$, which is half of the maximum reaction rate, is called **Michaelis constant ($\boldsymbol{K}_{\mathbf{m}}$)**. It is a measure of the affinity of an enzyme for its substrate, i.e. the smaller the K_{m}, the higher the enzyme–substrate affinity, because the less substrate it takes to occupy half of all binding sites of the enzymes. The value of the maximum velocity ($\boldsymbol{V}_{\mathbf{max}}$) depends on the enzyme concentration. The more the enzymes are present, the more the enzyme–substrate complexes can be formed, which is why a higher maximum speed can be achieved. Also, the half-maximal velocity is larger and is still reached at the substrate concentration K_{m} (the affinity is independent of the enzyme concentration).

Drug-induced **enzyme inhibition** may be competitive or non-competitive. Competitive inhibition occurs when the drug reversibly competes with the substrate for its binding site. In non-competitive inhibition, the drug reacts irreversibly with the active site or suppresses the formation of the substrate–enzyme complex following reaction and not the binding of the substrate to the enzyme. Important examples of enzyme blocking drugs are the monoamine oxidase inhibitors, non-steroidal anti-inflammatory drugs (NSAID) as inhibitors of cyclooxygenase, anticoagulants as xanthine oxidase inhibitors, indirect parasympathicomimetics as choline esterase blocker, hydroxy-methyl-glutaryl-coenzyme-A reductase inhibitors (HMG-CoA reductase inhibitors, CSE inhibitors, and statins), phosphodiesterase inhibitors, ACE inhibitors, and tyrosine kinase inhibitors. Many anti-infective agents also exert their effect by selective enzyme inhibition in microorganisms, e.g. penicillins and other beta-lactam antibiotics by inhibiting transpeptidases, gyrase inhibitors by interacting with DNA gyrase, azole antifungals by blocking lanosterol dimethylase or antiviral HIV by interacting with viral polymerases or proteases.

Enzyme activation is usually effected by second messengers such as cAMP, cGMP, or Ca^{2+}. Nitrates catalyse the soluble guanylyl cyclase via nitric oxide (NO). The effect of some coagulation factors on blood clotting is based on the fact that they convert inactive into active proteases. Fibrinolytic convert plasminogen to plasmin, also a protease.

2.4 Receptor Agonism and Antagonism

As physiological ligands drugs can interact as exogenous ligands with the receptor. The prerequisite for this is a drug (D)–receptor (R) complex. This is dependent on the affinity of the drug to the receptor. The higher the affinity, the higher the tendency that the drug forms a complex with the receptor. Hereby, the receptor can exist in two conformations, in an inactive (R) and in an active (R*) state. Both conformations

are in dynamic equilibrium, which in the absence of an endogenous or exogenous ligand is usually shifted to the inactive side. Receptors, which are active even without a ligand, are called constitutively active receptors.

Agonist: Substances that both bind to and stimulate the receptor

Inverse agonists: Substances which bind to a constitutively active receptor, shift their equilibrium to the inactive state and reduce the proportion of constitutively active receptors even more than at basal levels.

Antagonist: Substances which reduce or completely abolish a receptor-mediated effect

Substances that both bind to and stimulate the receptor are **agonists** (Figure 2.2). Substances which reduce or completely abolish a receptor-mediated effect are **antagonists**.

Substances which bind to a constitutively active receptor shift their equilibrium to the inactive state and reduce the proportion of constitutively active receptors even more than at basal levels are called **inverse agonists**.

Agonists possess affinity as well as intrinsic activity. The intrinsic activity is usually indicated as relative intrinsic activity α. This is proportional to the quotient of the effect E_A triggered by the agonist and the maximum possible effect E_m in the biological system. The maximum relative intrinsic activity is evident as $\alpha = E_A/E_m$. Agonists with an intrinsic activity of 1 are named as **full agonists**. Agonists with an intrinsic activity >0 and <1 are named **partial agonists**. Partial agonists are

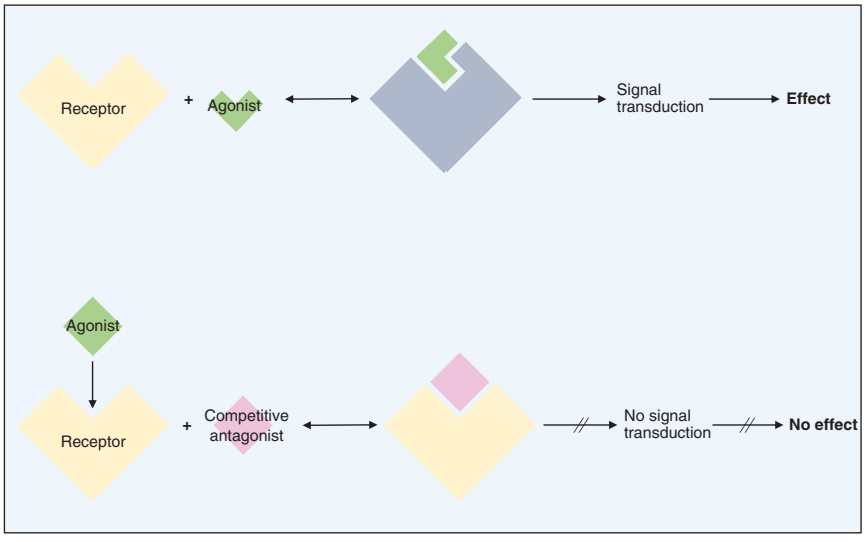


Figure 2.2 Illustration of drug–receptor intercations leading to activation or inhibition of signal transduction and an effect. Source: Book editors based on Hartl, Fink, and Beer-Hammer.

dualistic, i.e. they have both agonistic and antagonistic properties. In the presence of concentrations of a full agonist that produce a greater effect than the intrinsic activity of the partial agonist, it attenuates the action of the full agonist (partial antagonist activity). At low concentrations or absence of a full agonist, on the other hand, a partial agonist is agonistic.

Receptor antagonists can be divided into the following types:

- Competitive
- Non-competitive
- Functional
- Chemical

Competitive antagonists are able, in the same way as agonists, to attach to receptors to which they have affinity. In contrast to agonists, however, they are not able to trigger an effect, and they have no intrinsic activity. Since the agonist and competitive antagonist compete for the same receptor, increasing the concentration of one substance can displace the other from the receptor. An essential feature of the competitive antagonist is the parallel glazing of the dose–response curve of the agonist to the right. The degree of parallel shift of the agonistic curve on the abscissa is a measure of the affinity of the antagonist to the receptor. Typical examples of competitive antagonists are α- and β-adrenoreceptor blockers, angiotensin 2 receptor blockers, serotonin receptor antagonists, and antiandrogens.

Non-competitive antagonists may attenuate the effect of an agonist in several ways. Thus, the drug cannot attack at the receptor site with which the agonist interacts, but at another, **allosteric,** site of the receptor protein. The inhibitory effect is due to the fact that it negatively changes the conditions for binding of the agonist at its binding site. Other possibilities of non-competitive inhibition are that the processes occurring after the formation of the agonist–receptor complex are influenced. In all cases, the concentration–response curve of the agonist is altered by the non-competitive antagonist as follows: the respective effects induced by the agonist are attenuated depending on the concentration of the antagonist, that is, the slope of the curve and the maximum effect (V_{max}) decrease. Finally, at high concentrations of the antagonist, the effect of the agonist is completely blocked. Although receptor occupancy by the agonist can be accomplished in full, the influence of the non-competitive antagonist – unlike the competitive antagonists – cannot be offset by high concentrations of the agonist. The law of mass action does not apply here. Typical non-competitive antagonists are ketamine at the NMDA receptor and palonosetron at the 5-HT_3 receptor.

A **functional antagonist** is said to act as an agonist by counteracting the effect of the second agonist attacking other receptors by an opposite effect. An example of this is the antagonism between cholinergic and histaminergic substances and α-adrenergic substances on the bronchial musculature.

Chemical antagonists are substances that react chemically with an active ingredient and thereby deactivate it – independently of the receptor. This type of antagonism is particularly important in the treatment of overdoses and intoxications (e.g. abolition of heparin action by protamine sulfate, prevention of poisoning

with barium chloride by administration of sodium sulphate, detoxifying effect of various chelating agents in heavy metal poisoning). The essential result of a chemical antagonism is the lowering of the active ingredient concentrations in the biophase.

2.5 Implications for Drug Development

Pharmacology provides the basis for pre-clinical and clinical drug development (Rosenbaum 2016; FDA Pharmaceutical Science and Clinical Pharmacology Advisory Committee 2020). Prior to the general use of a drug in patients, two points have to be demonstrated: its (i) effectiveness and the (ii) concentration (dose) at which the desired effect occurs. The concentration–effect relationship determined in the organ bath or in animal experiments cannot be transferred to humans without further testing. Therefore, for each drug in selected patients, the relationship between effect (e.g. increase in blood pressure) and orally administered dose is determined. This is called a **dose–response relationship**. From this, important drug constants can be determined. The **ED50** is the dose at which half of the maximum effect is reached or in which 50% of the test objects show the expected effect. Despite the ED50 being the parameter which is most often referred to, in early drug development it is the ED90 (dose at which 90% of the effect is reached) that is considered to more closely reflect the target dose.

With this knowledge, therapy with a new drug can still not be started on the patient: as an essential parameter, there is still a statement to be made at which doses the drug triggers unwanted effects. First, animal experiments are used. In addition to acute toxicity, the chronic toxicity of a substance is an essential parameter. The aim of the acute toxicity studies is to determine the toxic effect of a substance with a single application of relatively high doses. Often the dose is estimated at which 50% of the animals die (LD50, lethal dose for 50% of the animals). In contrast, the aim of the chronic toxicity study, repeated application of relatively low doses of an active substance, is to exert side effects on various characteristics such as body mass, haematological and clinical-chemical parameters, organ masses and the histology of various tissues. In addition, before a clinical trial, the reproductive toxicity, i.e. teratogenicity studies, mutagenicity studies (e.g. Ames test) and carcinogenicity studies are performed.

The **therapeutic range** of a substance is a measure of the certainty between therapeutic and toxic effects: the more harmless the drug is, the greater its therapeutic range. Usually, this is expressed in terms of the therapeutic index (TI) (or quotient) as the ratio of LD50 to ED50: TI = LD50/ED50. However, an absolutely reliable statement about the therapeutic safety is only possible from the entire course of the dose–response and the dose–lethality curve and not by the determination of a certain quotient. For registration, the clinical safety findings are given a higher weight than pre-clinical assessments. At submission for market authorization, the dose to be selected is the one with the best benefit–risk assessment, which includes a synoptic and holistic assessment of all clinical pharmacology, efficacy, and safety studies.

2.6 Pharmacokinetics

Pharmacokinetics covers all processes that an active substance is exposed to the body, starting with the administration and liberation of an active substance through its absorption, distribution, and metabolism to excretion (Tozer and Rowland 2016; Rosenbaum 2016). Simplified, this is also referred to as the **LADME scheme (Liberation, Absorption, Distribution, Metabolism, and Excretion)**.

2.6.1 Liberation and Administration

On its way to the site of action, the drug has to overcome physical, chemical or biological barriers that significantly influence its efficacy profile. For example, the dosage form and the site of administration (Table 2.1) already play a decisive role.

The most common form of drug administration is oral. It must be borne in mind that the drug usually has to pass first through the gastrointestinal tract before it can enter the large bloodstream and thus reach the site of action. This is where the biggest disadvantage of oral administration becomes apparent: the so-called **first pass effect** (**FPE,** Figure 2.3). During passage through the gastrointestinal tract,

Table 2.1 Routes of administration.

Enteral (Administration through the gastrointestinal tract)	
Enteral	Directly into the intestine
Oral/peroral (p.o. per os)	Through the mouth
Rectal	Through the rectum
Sublingual (s.l.)	Under the tongue
Parenteral (any route that is not enteral)	
Epidural/peridural	Injection into the epidural space
Intraarterial	Into the artery
Intraarticular/joint injection	Into a joint
Intracardiac	Into the heart
Intracerebral	Into the cerebrum
Intramuscular (i.m.)	Into a muscle
Intraocular	Into the eye
Intraperitoneal	Into the peritoneum
Intrathecal	Into the spinal canal
Intrauterine	Into the uterus
Intravaginal	Into the vagina
Intravenous (i.v.)	Into a vein
Nasal	Through the nose
Subcutaneous (s.c.)	Under the skin
Transdermal	Diffusion through the skin

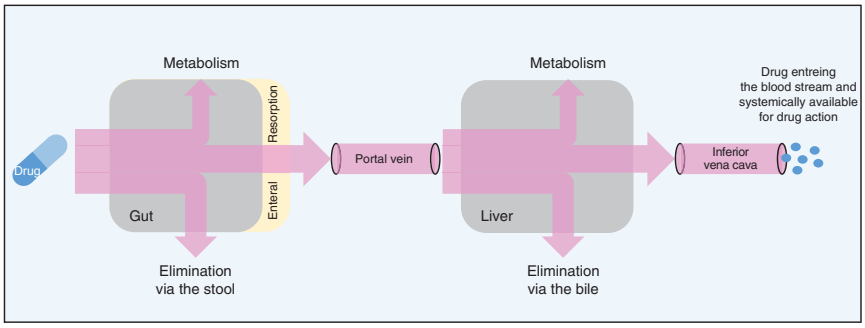


Figure 2.3 Processes involved before an orally adminstered drug becomes available in the systemically circulation. Source: Book editors based on Hartl, Fink, and Beer-Hammer.

the portal vein and the liver into the inferior vena cava (the so-called portal vein system), the first metabolization and partial elimination of the active substance takes place, depending on the active substance. This happens in particular through the **cytochrome P450 (CYP) enzymes** located in the intestinal wall and liver. Intravenous administration is much more direct as it bypasses gastrointestinal passage.

Depending on the target structure, very different types of administrations are possible. For example, the alveoli in the lungs are best reached by inhalation, and in the case of skin diseases, a dermal administration is usually chosen. With a rectal administration (into the rectum), the active ingredient can bypass the portal vein system from the last section of the rectum directly into the inferior vena cava. In some cases, however, the FPE is also used. In asthma therapy, for example, there are inhalative drugs which are almost completely inactivated by the FPE in the case of systemic resorption. This enables a local effect in the lung without significant systemic side effects. The same principle can also be used in dermatology with topical applied creams. Another possibility to make use of the FPE is the production of drugs, which are only activated by the FPE. This type of active ingredient is called a prodrug. Examples are codeine, metamizole, and clopidogrel. Since some drugs or fruits inhibit or induce the activity of the CYP enzymes, this can lead to serious interactions with other drugs via the accelerated or slowed metabolism. Intravenous or intramuscular administration can shorten the route to the site of action and thus significantly reduce the FPE. For certain indications, local administration is also possible, for example, the inhalative administration of glucocorticoids in asthma or dermal administration in dermatology. The subsequent release of the active substance from the dosage form depends on the corresponding galenics. Here, for example, the release profile can be influenced by certain tablet coatings or the processing into appropriate matrices. For example, it is possible to coat a pill with an enteric coating (e.g. proton pump inhibitors) in order to achieve a release only in certain sections of the intestine or to achieve a delayed release of the active substance (e.g. calcium channel blocker). Similarly, to the FPE of small molecules, antibodies as large protein structures are to a large extent degraded by proteases in the stomach and intestine. They thus lose their structure and function when

orally administered. This makes it necessary that they need to be administered intravenously or subcutaneously rather than orally.

2.6.2 Absorption

After liberation, the active ingredient must first be absorbed into the bloodstream before it can reach its site of action. Normally, biological membranes have to be crossed. The ability of a substance to pass through this lipid bilayer largely depends on its molecular composition. In contrast to polar molecules, for small lipophilic nonpolar molecules, it is possible to overcome the lipid bilayer of the cell membrane by diffusion. The speed of diffusion then depends on the diffusion gradient and the degree of ionization of the molecule. Various transport mechanisms are available for all molecules that are too large and or hydrophilic for diffusion. These include carrier-mediated transport, various transport proteins and uptake by endocytosis. After subcutaneous or intramuscular administration, the compound stays locally at the injection site, which can act as a depot from which the molecules are slowly released into the bloodstream. Antibodies and other large proteins are absorbed mainly via the lymphatic system into the bloodstream.

An important pharmacokinetic parameter for the evaluation of a drug is its **bioavailability (BA or F)**. This refers to the proportion of a drug that reaches the central bloodstream after absorption. The BA is usually highest with intravenous administration and is determined by the type of administration, the extent of the FPE or degradation and the chemical properties of the drug. If biologically inactive substances are only metabolized into an effective form in the organism, one speaks of so-called **prodrugs**. Prodrugs are used, for example, to increase the resorption rate (e.g. enalapril), to increase the half-life (e.g. haloperidol decanoate), to overcome the blood–brain barrier (BBB) (e.g. levodopa), to improve the taste (e.g. chloramphenicol) or to reduce systemic side effects (e.g. Ciclesonide).

2.6.3 Distribution

After successful absorption, the agent now is distributed in the organism via the bloodstream. It floods most rapidly into the organs with the strongest blood supply, such as the kidneys, the heart, and the liver. The storage capacity of the respective distribution areas plays a decisive role in the accumulation of the agent. In body fat, for example, it is very high for lipophilic substances. Since the body fat, however, has only little perfusion, the agent floods only slowly and under certain circumstances a large part of the agent already has been metabolized, so that often only a lower maximum concentration of the active substance can be achieved here. In addition to the accumulation capacity of the individual organ, the strength of the perfusion is also important when the concentration of the agent is being eliminated. The transfer of the agent from the blood to the target tissue depends on various factors. Thus, in addition to the already mentioned strength of the perfusion of the organs, the permeability of the membranes, the physical–chemical properties of the substance and the extent of plasma protein binding (PPB) also play a role. A high PPB reduces its

availability to bind to the target receptors. The most important plasma protein in this context is albumin, which particularly binds acidic agents. Basic agents preferentially bind to α_1 glycoprotein. But also the other way around, an active substance bound to proteins in the cell cannot easily leave the cell again. For CNS active drugs, overcoming the BBB poses a particular challenge. This highly selective filter mechanism provides the central nervous system with special protection against harmful substances and pathogens but also complicates the absorption of pharmaceuticals. In addition to the ability of some very small lipophilic molecules to overcome the BBB by diffusion, a number of selected transport systems are also available. The organism can be divided into different compartments. The extracellular space comprises the plasma water, the interstitial space and the transcellular fluid. The intracellular space is composed of the intracellular fluid and the solid cell components. An important parameter in pharmacology is the **plasma concentration** of a drug. This is relatively easy to measure and normally correlates closely with the pharmacological effect of a drug. For the use of pharmaceuticals in paediatrics, it is important to note that the proportion of total body water is significantly higher than in adults. In advanced age, the proportion of total body water further decreases.

If the applied amount of drug (A) is related to its plasma concentration (C), the apparent **volume of distribution (V)** is obtained: $V = A/C$. In this calculation, a uniform distribution space is assumed. However, since the drug is not normally distributed homogeneously throughout the entire body, this is the fictitious volume in which the drug would have to be distributed to explain the measured plasma concentration. However, this formula provides a prediction of the proportion of the drug not present in the plasma. If the volume of distribution is very high, this might be an indication that the drug accumulates in lower compartments (e.g. in fatty tissue). In this case, it should be noted that, for example, the fatty tissue has a depot function for certain pharmaceuticals and therefore a prolonged effect must be expected. An increased volume of distribution can also indicate binding to (on- or off-target) receptors outside the plasma. The volume of distribution can be used to calculate the **loading dose** (LD) of a drug that is required to achieve a plasma concentration within the therapeutic range.

Antibodies or larger therapeutic proteins cannot enter the cells; thus their distribution is limited to the extracellular space (~17 l). Due to their size, it takes a few days until the distribution from the plasma and rapidly accessible tissue (~3 l) into the tissues is at equilibrium.

2.6.4 Metabolism/Biotransformation

In the course of biotransformation, the body's own degradation products and non-usable foreign substances are converted by enzymes in order to render them more polar and thus easier to excrete (see Figure 2.4). This is necessary because lipophilic substances are largely reabsorbed in the kidney after glomerular filtration. At the same time, lipophilic molecules run the risk of accumulating in fatty tissue and remaining in the body. Most of the enzymes involved in biotransformation are located in the endoplasmic reticulum of the liver, but also in the kidneys and gut.

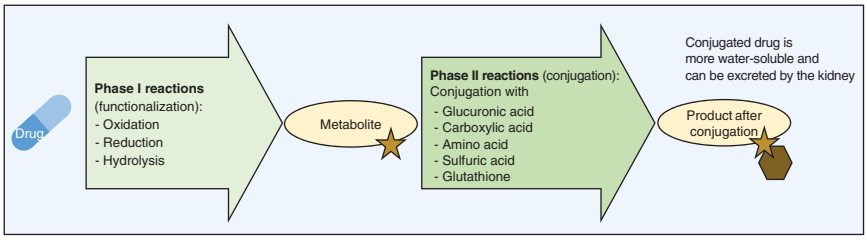


Figure 2.4 Reactions involved in the degradation and elimination of drugs. Source: Book editors based on Hartl, Fink, and Beer-Hammer.

Thus, hepatic biotransformation represents the most important part of metabolism. The chemical modification might also change the effect of the substances. Some are inactivated and some are activated. Therefore, biotransformation cannot per se be described as detoxification since some harmless substances are transformed into harmful metabolites in this way. Example: Conversion of methanol via formaldehyde to formic acid, which leads to metabolic acidosis.

2.6.5 Phase I Reaction

In preparation for phase II reaction, phase I reaction functional groups are introduced or unmasked. This functionalization takes place in the form of oxidation, reduction or hydrolysis.

2.6.5.1 Oxidation

The oxidation in phase I is performed by monooxygenases (transferring one oxygen atom), peroxidases (catalysing the reduction of peroxide), and others. The most important group in this context is cytochrome P-450-containing monooxygenases (CYP enzymes). These contain hemp proteins of the cytochrome P-450 type, which owes its name to the fact that it forms a reduced complex with carbon monoxide, with an absorption maximum of 450 nm. Over 90% of CYP enzymes are found in the liver and pose an important part of biotransformation. However, the CYP enzymes located in the intestine are also of great importance for the FPE. In the reaction catalysed by CYP enzymes, one oxygen atom is transferred from molecular oxygen to the substrate (oxidation), while the other oxygen atom is reduced to water. The required reduction equivalent (electron) is transferred from nicotinamide adenine dinucleotide phosphate (NADPH) to the CYP enzyme by the flavine enzyme NADPH cytochrome P 450 reductase. In summary, the general reaction is as follows: $P\text{-}H + O_2 + NADPH + H^+ \rightarrow P\text{-}OH + H_2O + NADP^+$ (P-H: substrate). Since we have an oxidation of the substrate on the one hand and the reduction of the remaining oxygen atom to water on the other hand, it also is called mixed-function monooxygenase.

The human organism contains a multitude of different CYP enzymes, which are divided into 18 families (e.g. CYP3), 43 subfamilies (e.g. CYP3A), and even more isoforms (e.g. CYP3A4) according to their amino acid sequence. Families 1–3 are of

particular importance for the metabolism of drugs. The most important CYP enzyme for the metabolism of drugs is CYP3A4.

Due to genetic polymorphisms and induction of the expression level, the amount of individual CYP enzymes considerably varies between individuals. Due to the occurring polymorphisms, there are so-called 'poor metabolizer' (PM; very weak metabolism), 'intermediate metabolizer' (IM; medium weak metabolism) and for CYP2D6 even 'ultra-rapid metabolizer' (UM). Especially in the case of drugs metabolized via CYP2C9, CYP2C19, and CYP2D6 with a small therapeutic window, the type of metabolism should therefore be known. Examples are vitamin K antagonists (CYP2C9), which increase the risk of severe bleeding for poor and intermediate metabolizers, and codeine (CYP2D6), which is rapidly converted to morphine from ultra-rapid metabolizer. Therefore, codeine cough syrups are contraindicated for children under 12 years of age due to the increased risk of respiratory depression. The induction or inhibition of the expression level of various CYP enzymes is responsible for numerous interactions in drug therapy. The CYP enzymes CYP1A2, CYP2B6, and CYP3A4 are of great importance in this context. The induction of a metabolizing CYP enzyme can lead to a faster degradation of a drug and, associated with this, to reduced or non-existent efficacy (for example, taking certain antibiotics and St. John's wort induces the faster degradation of hormonal contraceptives). On the other hand, the inhibition of a CYP enzyme can lead to the accumulation and increased side effects of a drug (for example, the risk of rhabdomyolysis increases under statin therapy if CYP inhibitors such as ketoconazole or macrolide antibiotics are taken at the same time). Overall, however, it should be noted that in the case of induction or inhibition of individual CYP enzymes, metabolism may also be maintained by other metabolic pathways. Other enzymes responsible for oxidation include peroxidases and flavin-containing monooxygenases, which are mainly responsible for the conversion of amines but also sulphurous compounds.

2.6.5.2 Reduction and Hydrolysis

Compared to oxidation, reduction, hydrolysis, and decarboxylation only play a minor role in biotransformation. Reduction plays a role especially in the degradation of azo and nitro compounds as well as halogenated hydrocarbons. Esters, amides, and epoxides can be prepared for the phase II reaction by hydrolysis.

2.6.5.3 Phase II Reaction

In phase II reactions, small endogenous molecules such as glucuronic acid, sulfuric acid, carboxylic acids, amino acids or glutathione are transferred to the substrate by transferases. Therefore, phase II reactions are also called conjugation reactions. In contrast to phase I reactions, which often produce active metabolites, phase II reactions usually produce biologically inactive products. The introduction of acid groups (exception: conjugation with acetic acid and methylation) considerably increases the hydrophilicity of the substrate through salt formation which than can subsequently be excreted renal or biliary.

The most important reaction of phase II metabolism represents glucuronidation. The transfer of active glucuronic acid mainly takes place by UDP-glucuronosyltransferase (UGT), which occurs similar to CYP enzymes in different isoforms with different substrate specificity and expression types. Also, UGT shows a distinct interindividual variability due to genetic polymorphisms or induction. One example for the effect of UGT polymorphism is the metabolism of irinotecan. In patients with UGT1A1*28 polymorphism, glucuronidation of the active metabolite of irinotecan (SN-38G) is decreased, leading to increased concentrations of SN-38G and thus to increased occurrence of neutropenia under irinotecan therapy.

During acetylation, carboxylic acids are activated with the aid of coenzyme A and subsequently transferred to the amino group of the substrate by means of acetyltransferase. The most important acetyltransferases for biotransformation are cytosolic *N*-acetyltransferases (NAT1 and NAT2). A variety of genetic variations has also been described for these enzymes. Of particular importance in this context is a polymorphism of NAT2, which distinguishes about half of the European population into so-called fast or slow acetylators. Under tuberculosis therapy with isoniazid, hepatotoxicity and the risk of isoniazide-induced polyneuropathy increase in slow acetylators. Unlike most other phase II reactions, the hydrophilicity of the substrate may decrease during acetylation. Thus, for example, crystalluria can develop during therapy with sulfonamides due to the poor solubility of acetylated sulfonamides in the acid pH of urine.

2.6.5.4 Phase III Reaction

The transport of the substrate out of the cell following the biotransformation sometimes is termed phase III reaction. Transporters involved are, for example, ATP-binding cassette transporters (ABC transporters).

2.6.6 Excretion

The excretion of drugs and their metabolites takes place mainly via the kidney (renal), the bile (biliary) or via the intestinal mucosa (intestinal). In rare cases, excretion can also take place via the lungs (pulmonary, for example, in inhalation narcotics) or via the skin and its appendages. The way in which a substance is excreted primarily depends on its physicochemical properties and the size of the molecule.

2.6.6.1 Renal Elimination

The largest part of the elimination is contributed by the kidneys, through which especially small, polar, and water-soluble molecules can be excreted. The extent and speed of renal elimination is determined by the relationship between glomerular filtration, tubular reabsorption, and tubular secretion.

2.6.6.1.1 Glomerular Filtration

In the glomerulus, all smaller molecules are filtered into the primary urine solely according to their size, regardless of their solubility properties (lipophilic or

hydrophilic). Since proteins are too large for glomerular filtration, substances bound to plasma proteins are not filtered. Thus, the glomerular filtration of a substance is determined not only by the renal blood flow but also by the extent of PPB. The so-called glomerular filtration rate (GFR) is an important parameter for the assessment of renal function. It indicates the volume filtered per time unit by the glomeruli of the kidneys. In healthy adults, the GFR is approx. 125 ml/min, which means that 180 l are filtered into the glomeruli per day. However, since a large part of the primary filtered volume subsequently is reabsorbed, the renal excretion rate is on average only 1.5 l/day. Since only a few substances are found which are completely filtered renally without being subsequently secreted and at least partially reabsorbed, it is difficult to determine the GFR directly. However, if this is the case (inulin, creatinine, etc.), GFR can be equated with renal clearance. This refers to the amount of a substance eliminated per unit of time from the plasma by the kidneys. Under these conditions, the GFR can therefore be determined with the aid of the plasma concentration of a substance. Usually the serum-creatinine concentration is determined and the GFR is calculated using different formulas (e.g. Cockroft-Gault, MDRD or CKD-EPI). It is important to remember that all these formulas only allow an approximate estimation of the GFR and that different formula are best suited for different situations.

2.6.6.1.2 Tubular Reabsorption

The following reabsorption of water leads to the concentration of urine. Based on the resulting concentration gradient, lipophilic substances also are reabsorbed by passive diffusion. The reabsorption of a substance depends on its solubility properties, its pK_a value and the pH value of the urine. For example, bases are less reabsorbed at lower urine pH values and are therefore excreted to a greater extent, acids at higher urine pH values are excreted to a lesser extent because they are then increasingly present in their charged (more hydrophilic) form. The excretion rate of a substance can therefore be influenced by alkalization or acidification of the urine, which can be used, for example, in intoxications.

2.6.6.1.3 Tubular Secretion

In contrast to tubular reabsorption, tubular secretion takes place via active transport processes against the concentration gradient of the substances in the urine. The substances are first taken up into the tubule epithelial cell via a basolateral transporter and then secreted into the urine via further transport proteins. Tubular secretion in particular concerns organic cations and anions. These are first secreted into the tubule epithelial cell by means of solute carriers (SLC) transporters (organic cations via OCT: organic cation transporter and organic anions via OAT: organic anion transporter) and thereafter into the urine via ABC transporters or another SLC transporter. It should be borne in mind that the transporters involved here only have a limited capacity and simultaneously administered drugs therefore inhibit each other in their transport, which may lead to delayed elimination and associated toxic effects.

2.6.6.2 Biliary Elimination

After resorption via the gastrointestinal tract, the drugs enter the liver via the portal vein, where they are largely metabolized during biotransformation. Then they are released into the bile, from where they return to the intestinal lumen. There, a large part of the bile (about 95%) is reabsorbed and again transported to the liver via the portal vein (enterohepatic circulation). In contrast to renal elimination, larger molecules (>500 Da) are excreted via the bile. The transport of the metabolites from the liver into the bile ducts usually is carried out via ABC transporters.

2.6.6.3 Intestinal Elimination

With oral administration, a drug is predominantly metabolized in the gastrointestinal tract or it is transported directly from the enterocyte back into the intestinal lumen without biotransformation and excreted via the stool (FPE). Both CYP enzymes expressed in the intestine (CYP3A4) as well as ABC transport proteins play an important role in this process.

2.6.6.4 Pulmonary Elimination

Pulmonary elimination mainly plays a role in inhaled narcotics and occurs as passive diffusion along the concentration or pressure gradient between blood and breathing air.

2.7 Quantitative Pharmacokinetics

2.7.1 Dosing Regimen

One of the most important questions in drug development is to find the dosing regimen that shows the best benefit–risk ratio for the majority of patients. Small molecules are often given as tablets which means that every patient receives the same dose (i.e. 'flat dosing'). As the concentration depends on the 'distribution volume' of the patient, this means that most often lighter patients have higher plasma concentrations. For children, special formulations have to be developed that allow dosing per body weight, which is also normal for many injectables (i.e. 'per-kg dosing').

Besides the dose administered, one has to determine the optimal regimen, i.e. the frequency at which a compound is taken. For small molecules, this is often once, twice or thrice daily as the compounds are rapidly metabolized and/or cleared from the body. In contrast, monoclonal antibodies can stay in the body much longer and thus they are given every two, four or eight weeks or even less frequent.

2.7.2 Clearance (CL)

The clearance of a compound determines how fast the compound is either metabolized (cleared in the liver) or excreted (vie the kidney) or both – or internalized and degraded (as antibodies are), i.e. it describes how fast the compound is not available anymore in the body in its active form. The clearance is the most important

pharmacokinetic parameter. Two metrices are related to clearance: area under the concentration curve (AUC) and half-life.

Often these clearance mechanisms are so abundant or powerful that they are independent of the current drug concentration ('linear clearance'), but sometimes these processes have physical limitations, e.g. due to limited amount of enzymes or internalization by target receptors which leads to concentration-dependent ('non-linear') and thus time-dependent total clearance.

2.7.3 Exposure as Area Under the Curve

One of the main measures for drug exposure is the area under the concentration curve (AUC), which is derived directly from the raw data, given that the drug concentrations are measured at sufficient time points to provide an accurate approximation.

For linear and time-independent clearance, the area under the concentration curve follows a simple equation, as in this case AUC is only dependent on dose, bioavailability (F), and clearance (CL). It also holds that $AUC_{0\text{-inf}}$ after a single dose is equal to $AUCss_{0\text{-tau}}$ at steady state after multiple doses. $AUC_{0\text{-inf}}$ = Dose * F/CL.

The AUC is often correlated with wanted and unwanted effects; therefore, it is one of the central assessments for assessing bio-equivalence or to derive the safety margin to the NOAEL in animals.

2.7.4 Bioavailability

In general, as described above, intravenous infusions provide the maximum exposure (AUC), and thus the **absolute bioavailability** of another route of administration is given as proportion F to the AUC after iv administration. Note, that high bioavailability most often is associated with less between-patient variability in the AUC.

If no experiments with iv administration have been done, one can still compare for instance two oral formulations and provide the **relative bioavailability** of one with respect to the second formulation.

2.7.5 C_{max}, C_{trough}, and Peak-to-Trough-Ratio

Besides the AUC which reflects the overall ('average') exposure, the main other metrics relate to the extremes of the pharmacokinetic profile over time: maximal observed concentration (C_{max}) concerning the safety and pre-dose concentration (C_{trough}) concerning the efficacy of the drug. A typical anchor for early dose selection is for instance the dose that reaches 90% target inhibition at C_{trough}.

In general, it is optimal to have a small peak-to-trough ratio, i.e. C_{max}/C_{trough}, as this indicates that the compound has – given a consistent efficacy during the dosing interval (being above a target C_{trough}) – the lowest possible safety events (due to the low C_{max}). As more frequent dosing reduces the peak-to-trough ratio, twice-daily dosing has most often a better benefit–risk profile than once daily dosing, but due to convenience and often a large enough safety margin once daily dosing is preferentially targeted, if feasible.

2.7.6 Volume of Distribution

For an intravenous dose, the concentration right after the dose is determined by the 'immediate volume of distribution' (for monoclonal antibodies ~3 l). Despite the volume having an influence on all concentrations measured, it has no influence on the AUC of the PK, which is not trivial to accept.

When the compound is immediately binding to membrane-bound receptors, also the immediate volume of distribution can be larger than physiologically reasonable volumes. As most compounds distribute into tissues (interstitial and maybe also intracellular fluids), the steady-state distribution volume is larger than the immediate one. The relative proportion of this change provides some hint on the relevance of tissue distribution for the compound. Depending on the target site, this could indicate a need for higher or lower doses.

2.7.7 Half-Life

Given that the compound shows linear clearance, the pharmacokinetics can be described by multi-exponential curves for drug distribution and clearance (CL). Often absorption and distribution are faster than CL and then the terminal phase of the pharmacokinetic curve is related to CL.

As the clearance is eliminating the same proportion of compound each time unit, one can derive a standardized value for this: the terminal half-life, i.e. the time it takes for the concentration to half its value (this law of proportions is very common in physiology and leads to a straight line when using a log-scale for the *y*-axis.)

When the PK curve is not a single exponential, it is not a given that the last part of the curve represents the clearance process. On one hand, the absorption could be slower (i.e. 'flip-flop phenomenon'); on the other hand, there could be a binding process, where the unbinding from the target is slower than the clearance and then determines the last part of the curve.

2.7.8 Accumulation and Loading Dose

Based on the linear clearance properties: six elimination half-lives after a dose most of the compound is gone from the body: = 1.6% of the compound remain. If one doses earlier than that, the concentrations increase with each dose and accumulate. When dosing at an interval equal to the half-life of the drug then at steady state the $C_{\text{trough}} = \text{Dose}/V$ because giving the next dose $C_{\max} = 2 * \text{Dose}/V$, which then halves during the dosing interval (equal to the half-life) to C_{trough} again. The shorter the interval between doses, the more accumulation one observes, and the lower the peak-to-trough ratio.

For warfarin (which reduces platelet aggregation after stroke), this comes with advantages and disadvantages. Due to the balance between efficacy (reduced aggregation) and safety (no aggregation leading to bleeding) and the small therapeutic index, it is very positive that warfarin has a long half-life (~40 hours, [range of 20–60 hours]) as this reduces the peak-to-trough ratio after accumulation. However,

due to the high variability in half-life (threefold) and in its efficacy, the dose must be individualized for each patient. Due to the long half-life, it takes on average a week to reach steady-state, so the dose adjustments cannot be done too quickly (without a model-based approach).

In order to reach steady-state of PK and thus efficacy faster for compounds with substantial accumulation, it is common to apply one or several 'loading doses'. This helps to immediately reach higher exposure levels and not to have to wait for the slower accumulation process. This is often done with monoclonal antibodies due to their long half-life of weeks – and thus PK steady-state only after months (without loading doses).

2.7.9 Induction and Maintenance Dose

There is no coherent definition of the terms loading, induction, and maintenance doses. A possible distinction is that the loading dose is mainly to reach PK steady-state faster, whereas an induction and maintenance dosing regimen yields much higher concentrations during the induction than in the maintenance phase. There are at least two rationales for induction regimen: (i) speed of onset of efficacy could be increased (e.g. due to faster distribution to the target tissue) and (ii) when dosing is started during active disease, forcing the system to remission could need higher concentrations than maintaining inactive disease once remission is reached.

2.8 Pharmacokinetic Models

There are two basic kinds of 'models':

- Non-compartmental analysis (NCA), and
- Compartmental analysis (Physiologically based PK [PBPK], Population PK [PopPK]).

The NCA method uses the raw data and uses 'curve fitting' only for estimating the 'terminal half-life'. It is basically built on the assumptions of 'linear, time-independent clearance' without influence on the PK of drug binding to the target (or off-target). The moment these assumptions are violated, the numbers estimated by NCA will appear to be dose- and regimen-dependent (e.g. decrease of volume of distribution with increasing dose).

Compartmental analyses use the concept of different body compartments (plasma, blood cells, liver, kidney, muscles, brain, skeleton, lungs, skin, etc.). This allows inclusion of distribution of compound from the plasma over time into the different tissues. Compartmental analyses are often of advantage as they allow extrapolation to dosing regimen that have not been evaluated (yet). They can include dose non-linear bioavailability, concentration-dependent clearance, target-mediated drug disposition, etc. This flexibility comes with the constraint that these analyses are difficult to automate and to fully standardize.

PBPK models use the physicochemical properties of the drugs (i.e. small molecules) and the knowledge on active and passive processes during absorption, distribution, and elimination to describe the concentration–time curve and possible dose-dependent bioavailability especially of oral administration.

PopPK models are more descriptive and less explanatory models that describe between-patient variability and observed kinetics with a limited amount of compartments (1, 2, or 3 – as the noise in the data does numerically not allow for estimation of more than three exponential curves – i.e. one per compartment). This limits the pharmacological learnings but allows simple and fast prediction of future studies with alternative dosing regimen.

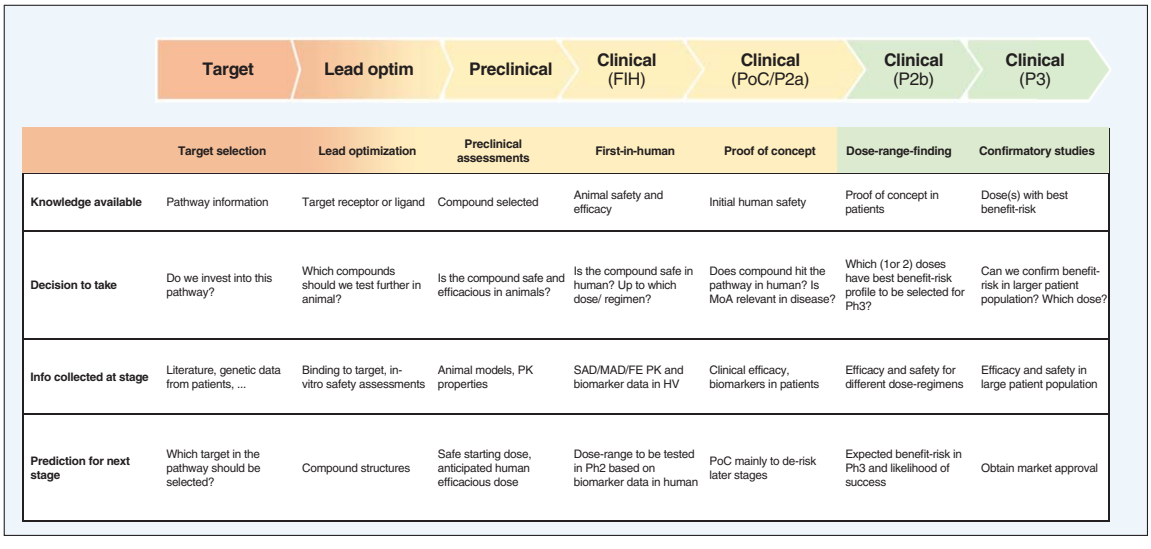
2.9 Implications for Drug Development

Pharmacology plays a key role throughout the entire drug development process (Tozer and Rowland 2016; Rosenbaum 2016) (Figure 2.5): to predict efficacy and drug concentrations in human based on animal studies, to select dosing regimens for dose range finding (DRF) studies, if formulations are changed from capsules to tablets, or even after marketing when generics or biosimilars have to show bio-equivalence to the originator compounds. At each stage of drug development, the doses or dosing regimens for the following stage have to be selected based on the knowledge collected so far. Each of these decisions is directly based on pharmacological concepts and the causal relationship between observed (systemic) exposure, biomarker activity, and expected wanted and unwanted effects. Understanding which factors (e.g. food, disease, age, gender, ethnic, renal or liver impairment, genetics) influence the exposure, efficacy, and safety of the drug is essential to decide about dose adjustments in the label or in the clinical setting for special populations (paediatrics, elderly, etc.).

2.9.1 PK/PD Modelling

'Modelling' in the drug development context mostly refers to pharmacokinetic–pharmacodynamic (PKPD) modelling and simulation (for an in-depth description, see (Bonate 2011). PKPD modelling is implemented in the majority of nowadays drug development programmes. In some instances, no 'modelling' needs to be done, for instance when the (biomarker) response reacts with little delay to the currently observed concentration. Then, a simple plot for the response versus the concentration (on log-scale) is sufficient to observe the concentrations leading to 50% or 90% of response.

When the response is delayed with respect to the concentration kinetics, then plotting can only be reasonably done using PD steady-state data of different dose levels. A model-based approach using a 'turnover model', however, can integrate the whole time course for each individual and thus provides a more precise estimate on the concentration–response relationship. Moreover, it allows to make predictions on speed of onset, need for loading doses after drug holidays, and time to recovery after cessation of treatment (for instance as preparation for a surgical operation).



	Target selection	Lead optimization	Preclinical assessments	First-in-human	Proof of concept	Dose-range-finding	Confirmatory studies
Knowledge available	Pathway information	Target receptor or ligand	Compound selected	Animal safety and efficacy	Initial human safety	Proof of concept in patients	Dose(s) with best benefit-risk
Decision to take	Do we invest into this pathway?	Which compounds should we test further in animal?	Is the compound safe and efficacious in animals?	Is the compound safe in human? Up to which dose/ regimen?	Does compound hit the pathway in human? Is MoA relevant in disease?	Which (1or 2) doses have best benefit-risk profile to be selected for Ph3?	Can we confirm benefit-risk in larger patient population? Which dose?
Info collected at stage	Literature, genetic data from patients, ...	Binding to target, in-vitro safety assessments	Animal models, PK properties	SAD/MAD/FE PK and biomarker data in HV	Clinical efficacy, biomarkers in patients	Efficacy and safety for different dose-regimens	Efficacy and safety in large patient population
Prediction for next stage	Which target in the pathway should be selected?	Compound structures	Safe starting dose, anticipated human efficacious dose	Dose-range to be tested in Ph2 based on biomarker data in human	PoC mainly to de-risk later stages	Expected benefit-risk in Ph3 and likelihood of success	Obtain market approval

Figure 2.5 Pharmacological considerations along the pharmaceutical value chain. Source: Book editors based on Hartl, Fink, and Beer-Hammer.

In some instances, these PKPD models can grow in complexity, e.g. when there is a cascade of transitions needed before the effect can be observed. Similarly, when there seems to be a tolerance phenomenon: the body adjusts (most often increases) the production of the target due to an inhibitory drug effect and, when the drug is removed, the biomarker (and clinical) response can show a rebound, i.e. an overshoot beyond the baseline response (transient worsening, i.e. worse outcome than at the beginning).

One big advantage of a Population PKPD model is that variabilities in PK as well as in the concentration–response relationship are assessed simultaneously and possible explanatory variables (like baseline demographics) on their contribution to that variability can be investigated. If the influence is large, dose adjustment could be warranted. Even if the variability cannot be explained by demographics or other assessments, the goal is to show good efficacy in 80–90% of patients and not just 50% (the median or mean response). Therefore, taking variability into account avoids under-dosing and thus failing clinical studies.

2.9.2 Characteristics of Pharmacokinetics in Age, Disease and Others

The variability of PK can be caused by different aspects, which can be tested with PopPK models. Some countries also would like to see ethnic sensitivity analysis, i.e. to get an understanding if their population should actually receive a standard dose or maybe a slightly higher/lower one. Typical other aspects to investigate are body weight, age and renal or liver impairment, or disease characteristics that can change the metabolism of the drug or drug–drug interactions (DDIs) with co-medications. Based on pharmacological knowledge, a number of these influencing factors are expected but the actual quantification of the strength of influence is often delayed to phase III where large patient populations are exposed to the drug and thus sufficient data are collected for precise enough estimates.

The influence of body weight could be considered as an exception as 'allometric scaling' has shown substantial benefits in scaling PK from animal to man but also between humans of different shape – or from adults to paediatrics. The volume parameters are scaled linearly with body weight, and the clearance parameters with an exponent of 0.75. This provides a very good basis for extrapolations and is also recommended by health authorities. In specific cases, the exponents could differ – or for very young children (<2 years of age) the developmental changes in liver enzymes might become more important than simple scaling by size.

2.9.3 Dose Finding/Determination of Dosing Regimens

In drug development, one speaks of phase II being the phase for DRF studies. However, as the Figure 2.1 indicates, at each stage one has to select dosing regimens for the next stage based on newly collected data. Therefore, the whole drug development process has dose finding at its core (for an in-depth description, see (Bonate 2011; Sheiner 1989). Depending on the disease severity of the patients, the safety margin, and the unmet medical need, the evidence needed for having selected the

'optimal' dose varies. In non-lethal diseases, health authorities want to see evidence that a lower dose than the chosen one would be less efficacious, this means that the selected one is the 'minimal efficacious dose'. The same is true for dosing regimen, for instance for injections into the eye the injection volume is so limited that in general the maximum volume is given and the regimen, i.e. the interval between doses is varied. In this specific case, one could also opt for regimen individualization.

2.9.4 Individualized Dosing and Therapeutic Drug Monitoring

Different reasons lead to the need or substantial patient benefit of dose–regimen individualization. Very often (like for warfarin), it is related to a narrow safety margin (therapeutic window) and a too high between-patient variability. In order to find a safe but efficacious dose for each patient, the dose can be adjusted based on any kind of assessment that is easy enough to do and relevant enough. This could be PK trough concentrations (some transplant drugs), biomarker assessments (warfarin) or clinical endpoints (if ethical). Similarly, doses of antibodies (e.g. in the eye) can be given on an as-needed basis (pro re nata: PRN) or with a 'Treat and Extend' individualized regimen. Overall, individualized dosing needs reasonably low within-patient variability of the outcome assessment given constant dosing – otherwise, the dose adjustment is difficult (or erratic). It also needs a constant disease activity or not too rapidly declining health. Finally, the outcome measure should be consistent and reflective of the efficacy of the drug.

2.10 Conclusions

Pharmacology as a discipline is universally involved in the process of drug development, ranging from pre-clinical studies to post-marketing. Regulatory agencies demand safety information (pre-market safety reviews) for new drug approvals, and substantial evidence of efficacy. The latest tendencies show a stronger push for 'precision medicine' but also further 'individualization' of treatment dose and regimen. This means the need for enough pharmacological understanding of the influencing factors that indicate how to adjust the dose–regimen for each individual, given co-morbidities, co-medication, disease status, and demographics.

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