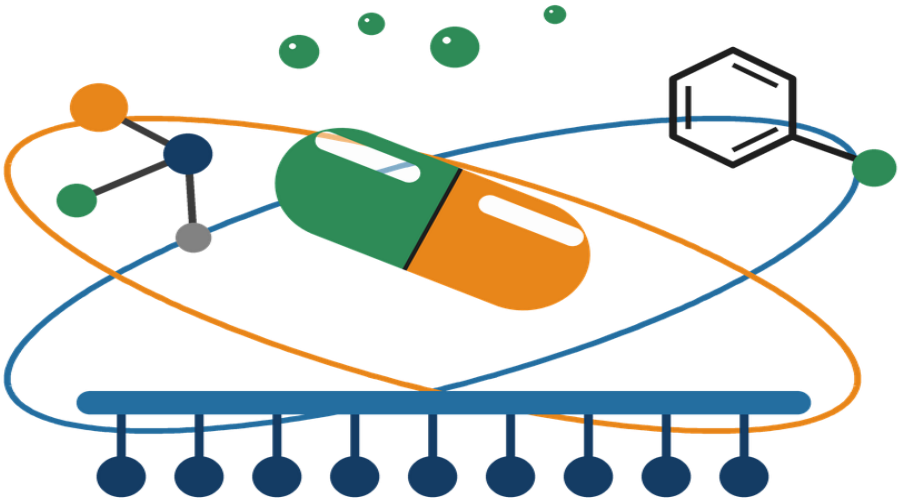
University of El Oued
Faculty of Nature and Life Sciences
Department of Cellular and Molecular Biology

CELLULAR PHARMACOLOGY

For First-Year Master's Students – Toxicology



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List of Tables

Table 1. Summary of pharmacological targets by cellular localization 6
Table 2.1. Levels of exploration in pharmacology: goals, advantages and limitations..... 15
Table 2.2. The four phases of clinical development 20
Table 2.3. Types of blinding in clinical trials 21
Table 3. Summary of the main pharmacokinetic parameter..... 34

List of Figures

Figure 1.1. Core sequence of cellular drug action and signal transduction 2
Figure 1.2. Distribution of targets based on the number of medicines 7
Figure 1.3. Saturation binding curve: 9
Figure 1.4. Displacement (competition) curve: 10
Figure 1.5. Dose (concentration)–effect relationship of an agonist. 11
Figure 1.6. Log dose–effect relationships of four agonists (A–D). 11
Figure 1.7. Inhibition of the agonist effect by increasing concentrations of an antagonist 12
Figure 1.8. Competitive antagonist 13
Figure 1.9. Non-competitive antagonist 14
Figure 1.10. Therapeutic index of drug A (narrow) and drug B (wide). 14
Figure 3.1. Overview of the four ADME processes. 22
Figure 3.2. Main mechanisms of transport across a biological membrane. 23
Figure 3.3. Plasma concentration–time curves after IV and oral administration. 25
Figure 3.4. Concentration–time profiles of two drugs after a single dose.. 26
Figure 3.5. Hepatic first-pass effect. 27
Figure 3.6. Phases I and II of drug metabolism. 29
Figure 3.7. Exponential decline of the plasma concentration: it is halved every half-life. 33
Figure 5.1. Major Vaccine Types, Mechanisms of Action, and Immune Response 42
Figure 5.2: RNA Interference and Antisense Technologies 43
Figure 6. Origins and Sources of Active Pharmaceutical Substances 46
Figure 7. Pharmacological of antibiotics 49
Figure 8. Pharmacological uses of anti-infective drugs. 53
Figure 9. Pharmacological uses of anti-inflammatory drugs. 57
Figure 10. Pharmacological uses of hormones. 64
Figure 11. Anticancer agents and immunomodulators. 69
Figure 12. Vitamins as pharmacological agents. 70

Table of Contents

Definitions and scope 1
Mechanism of action of drugs 2
Chapter 1: Main pharmacological targets (Drug receptors) 4
1.1 Classes of pharmacological targets 4
1.2 Pharmacodynamics (dose–concentration/receptor/effect relationships) 7
Chapter 2: Methods of Exploration in Pharmacology 15
2.1 Levels of exploration 15
2.2 Pre-clinical evaluation – experimental toxicology 16
2.3 Clinical trials 19
Chapter 3: Fate of the Drug in the Body (Pharmacokinetics) 22
3.1 Absorption 22
3.2 Distribution 27
3.3 Metabolism (biotransformation) 28
3.4 Excretion 31
Chapter 4: Sources of Variability in the Effect of Drugs 35
Chapter 5: Monoclonal Antibodies, Vaccines, RNA Interference, and Therapeutic Proteins 39
5.1 Biologics versus small molecules 39
5.2 Monoclonal antibodies (mAbs) 39
5.3 Vaccines 40
5.4 RNA interference and antisense technologies 42
5.5 Other therapeutic proteins 44
Chapter 6 : Pharmacology of Active Substances: Origin, Pharmaceutical Forms, Mechanisms of Action 44
6.1 Origin of active pharmaceutical substances 45
6.2 Pharmaceutical forms (dosage forms) 46
6.3 From chemical structure to mechanism of action 47
6.4 Formulation, route of administration, and the concept of bioequivalence 48
Chapter 7: Antibiotics 49
Chapter 8 : Anti-infectives 53

Chapter 9 : Anti-inflammatories 57
Chapter 10 : Hormones 61
Chapter 11 : Anticancer Agents and Immunomodulators 66
Chapter 12 : Vitamins 70
References 74

1. Definitions and scope

Pharmacology is the science of the interaction of chemical substances with living systems to produce a biological effect. It is traditionally divided into pharmacodynamics (what the drug does to the body—mechanisms of action, receptor and target interactions, dose–response relationships) and pharmacokinetics (what the body does to the drug—absorption, distribution, metabolism and excretion). Related but different disciplines are pharmacy (the practical science of formulating, dispensing and managing drugs), pharmacognosy (the study of drugs derived from natural sources), toxicology (the study of the adverse effects of chemical substances, which overlaps considerably with pharmacology, as the reference texts for this module show), pharmacogenetics/pharmacogenomics (the study of genetic influences on drug response), and clinical pharmacology (the application of pharmacological principles to the care of individual patients, including choice, dosage and monitoring of drugs).

Cellular pharmacology focuses on how chemical agents interact with specific molecular targets inside or on the surface of cells to alter cellular function and physiological outcomes. At its core, the process relies on three sequential stages: Reception, Transduction, and Response.

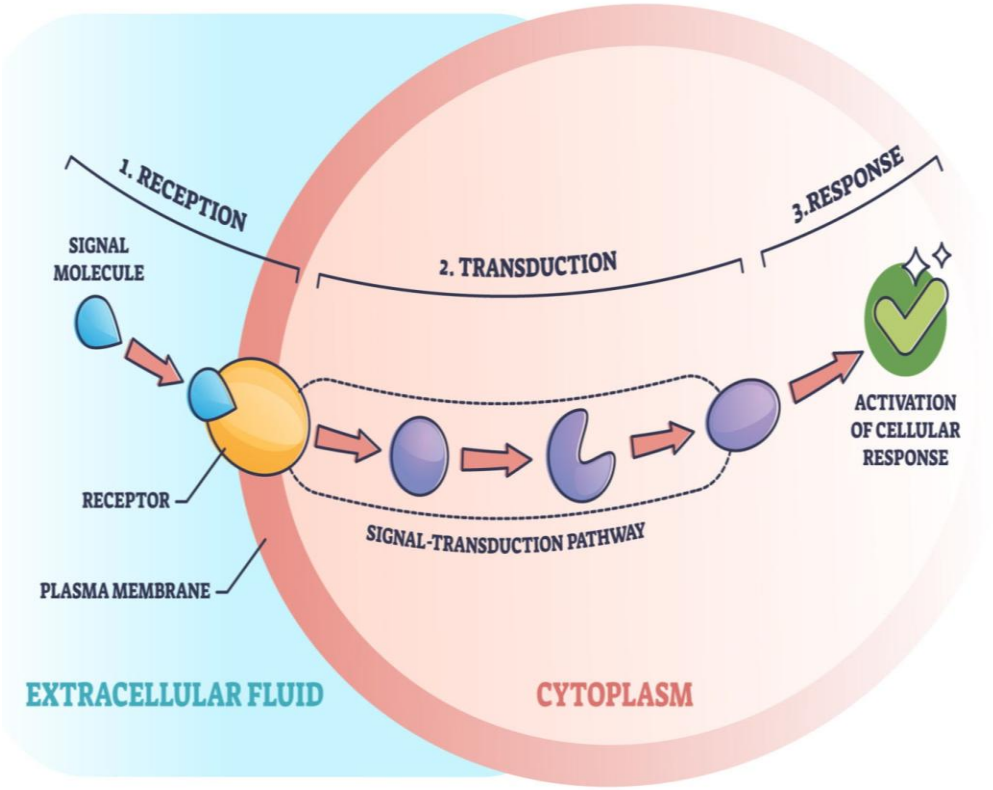


Figure 1.1. Core sequence of cellular drug action and signal transduction

2. Mechanism of action of drugs

1. Substitution (replacement of a substance necessary for the body): replaces an essential endogenous substance that is deficient due to synthesis defects, insufficient dietary intake, or physiological decline.
   - ✓ *Insulin* in Type 1 Diabetes (pancreatic beta-cell destruction).
   - ✓ *Vitamin D3* (cholecalciferol) for rickets.
   - ✓ *Vitamin B12* (cyanocobalamin) for pernicious (Biermer's) anemia due to lack of intrinsic factor.
2. Interaction with the metabolism of an endogenous substance. Targeting enzymatic pathways to alter the rate of synthesis or breakdown of functional endogenous molecules.

- ✓ *ACE Inhibitors* (e.g., ramipril, lisinopril) block the conversion of Angiotensin I to the potent vasoconstrictor Angiotensin II, lowering blood pressure and reducing cardiac remodeling.
- ✓ *Statins* (e.g., atorvastatin) competitively inhibit HMG-CoA reductase, the rate-limiting enzyme in hepatic cholesterol synthesis, leading to up-regulation of LDL receptors.

3. Interaction with targets of endogenous substances: Binding to cell-surface or intracellular receptors to either mimic (agonist) or block (antagonist) natural signaling molecules.
   - ✓ *β2 agonists* (e.g., salbutamol) cause bronchodilation in asthma.
   - ✓ *H2 antagonists* (e.g., famotidine) decrease gastric acid secretion by parietal cells.
4. Interaction with membrane channels or trans-membrane ion transport systems: Modulating cross-membrane ion flux to alter membrane potential, cellular excitability, or chemical gradients.
   - ✓ *Local Anesthetics* (e.g., lidocaine/xylocaine) and *Class I Anti-arrhythmics* block Na+ influx, stopping action potential propagation.
   - ✓ *Cardiac Glycosides* (e.g., digoxin) inhibit Na+ K+ ATPase, increasing intracellular Ca2+ via the Na+ Ca2+ exchanger to boost myocardial inotropy.
5. Interaction with bacteria/viruses, parasites/fungi: Interactions with pathogens: destruction of bacterial cell walls, fungal membranes, or viral/parasitic enzymes
   - ✓ *beta-lactams* (penicillins, cephalosporins) inhibit transpeptidase enzymes, preventing bacterial peptidoglycan cross-linking leading to osmotic lysis.
   - ✓ *Chloroquine / Quinine* prevent the polymerization of toxic free heme into non-toxic hemozoin inside the *Plasmodium falciparum* food vacuole, killing the parasite.

Chapter 1: Main pharmacological targets (Drug receptors)

1.1 Classes of pharmacological targets

Pharmaceutical targets are classified according to their cellular localisation:

- ✓ Plasma membrane: very rich in potential targets (receptors, channels, transporters)
- ✓ Cytoplasm: enzymes, intracellular organelles (membranes and channels)
- ✓ Nucleus: nuclear receptors (e.g., steroid hormones) and DNA or DNA-processing enzymes (e.g., anticancer agents)

1.1.1. G protein-coupled receptors (GPCRs)

GPCRs represent the largest family of cell-surface receptors. Structurally, they consist of a single polypeptide chain with seven transmembrane alpha-helices (7TM domain), an extracellular N-terminus, and an intracellular C-terminus.

- Activation Mechanism: Ligand binding induces a conformational change in the 7TM domain, facilitating the exchange of GDP for GTP on the alpha-subunit of the heterotrimeric G-protein (Gαβγ).
- Signaling Pathways:
  - Gαs: activates adenylate cyclase → increases cAMP → activates protein kinase A (PKA).
  - Gαi: inhibits adenylate cyclase → decreases cAMP production.
  - Gαq/11: activates phospholipase C-β (PLC-β) → cleaves PIP2 into inositol trisphosphate (IP3) and diacylglycerol (DAG). IP3 triggers Ca2+ release from the endoplasmic reticulum, while DAG activates protein kinase C (PKC).
- Desensitization: Mediated by G-protein Coupled Receptor Kinases (GRKs) phosphorylating the C-terminus, leading to beta-arrestin recruitment, uncoupling from G-proteins, and subsequent clathrin-mediated endocytosis.

1.1.2. Enzyme-Linked Receptors

Usually monomeric with a single transmembrane helix

Ligands: extracellular peptides/proteins (e.g., insulin, growth factors)

The intracellular chain of the receptor protein (receptor) includes an enzymatic site with guanylate cyclase, tyrosine kinase, serine/threonine kinase or tyrosine phosphatase activity.

1.1.3. Ion Channels

Ion channels allow the regulated passage of ions across cellular membranes, down their electrochemical gradients. They are categorized based on their gating mechanisms:

- Ligand-Gated Ion Channels (Ionotropic Receptors): Multi-subunit complexes that open directly upon agonist binding. Examples include the Nicotinic Acetylcholine Receptor (Na+/K+ permeable), GABA_A Receptor (Cl- permeable, causing hyperpolarization), and NMDA Glutamate Receptors (Ca2+ permeable).
- Voltage-Gated Ion Channels: Regulated by changes in membrane potential. Structurally feature a voltage-sensing domain (typically containing positively charged arginine/lysine residues). Crucial in excitable tissues (Na, K and Ca channels).

1.1.4. Nuclear receptors: intracellular receptors for lipophilic ligands that act as ligand-regulated transcription factors. Class I (steroid) receptors (e.g., glucocorticoid, oestrogen) are located in the cytoplasm bound to chaperone proteins (HSP90); after ligand binding they translocate to the nucleus and bind DNA response elements. Class II receptors (e.g., thyroid hormone receptors, PPARs) are already in the nucleus bound to DNA response elements and often act as repressors until ligand binding converts them into transcriptional activators.

1.1.5. Receptors of various structures

Receptors of the immunoglobulin superfamily and cytokine receptors (coupled to JAK kinases)

Table 1. Summary of pharmacological targets by cellular localization

Localization	Target Subtype	Speed of Signal / Action	Representative Drug Example
Plasma Membrane	GPCR	Seconds	Salbutamol (β2 agonist)
Plasma Membrane	Ion Channel	Milliseconds	Lidocaine (NaV blocker)
Cytoplasm	Soluble Enzyme	Minutes to Hours	Methotrexate (DHFR inhibitor)
Cytoplasm	Organelle Channel	Seconds to Minutes	Dantrolene (RyR blocker)
Nucleus	Nuclear Receptor	Hours to Days	Dexamethasone (Glucocorticoid)
Nucleus	DNA / Topoisomerase	Hours to Days (Cell Cycle)	Doxorubicin (Intercalator/Topo II)

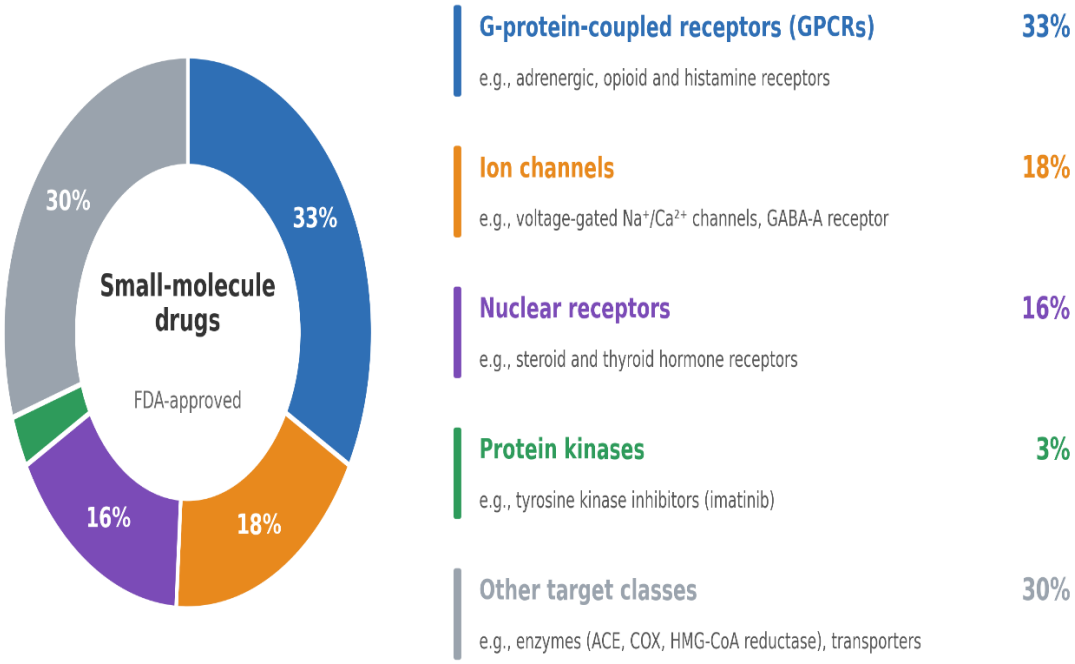


Figure 1.2. Distribution of targets based on the number of medicines

1.2 Pharmacodynamics (dose–concentration/receptor/effect relationships)

- A ligand, when it binds to a receptor, can activate it (induction of the effect) or, on the contrary, block it.
- When it binds by stimulating it, it is called an agonist, when it attaches by blocking it, it is called an antagonist.
- The binding of a ligand to its receptor is by definition saturable, since it is limited by the number of receptors on which it can bind.
- The binding of an agonist to a receptor induces a specific effect of this binding. The study of this action has two aspects: binding to the receptor on the one hand and the induction of the effect itself on the other hand. The interaction of a ligand (and therefore of a drug) with its receptor can therefore be analysed

on the one hand in terms of binding characteristics on the receptor (ligand-receptor relationship) and on the other hand in terms of dose-response (or concentration-effect) relationship.

1.2.1 Study of ligand-receptor relationships (binding methods)

The main objective in practice of these ligand-receptor relationship studies is the determination of the binding capacity, called affinity, of the ligand for its receptor. It is characterized by the concentration of the ligand occupying 50% of the receptors (dissociation constant: *Kd*) on a membrane preparation. The determination of the *Kd* will make it possible to know with what affinity a ligand will bind to a type of receptor. The comparison of the different affinities of a ligand for different receptors will thus make it possible to predict the pharmacological profile of a new ligand and to choose the ligand whose affinity profile best matches the desired objective. This is the basis for the selection of newly synthesized substances and pharmacological screening.

The dissociation constant *Kd* can be determined by two types of methods for studying the binding of the ligand to its receptor: the so-called saturation methods and the so-called displacement methods.

They are both based on the fact that the binding of the ligand to its receptor follows the law of mass action.

a. Saturation methods

They are used when the radioactive labeling of the ligand to be studied is available. An increasing amount of radioactive ligand (L) is added to a membrane preparation.

The objective here is to directly determine the affinity (Kd)

The principle: A fixed concentration of receptors is exposed to increasing concentrations of labeled ligand (usually radioactive or fluorescent). What is measured: A distinction is made between total binding and non-specific binding (the one that sticks everywhere except at the active site). By subtraction, the specific bond is obtained.

Result: We obtain a hyperbolic curve. Kd is the concentration of ligand required to occupy 50% of the receptors.

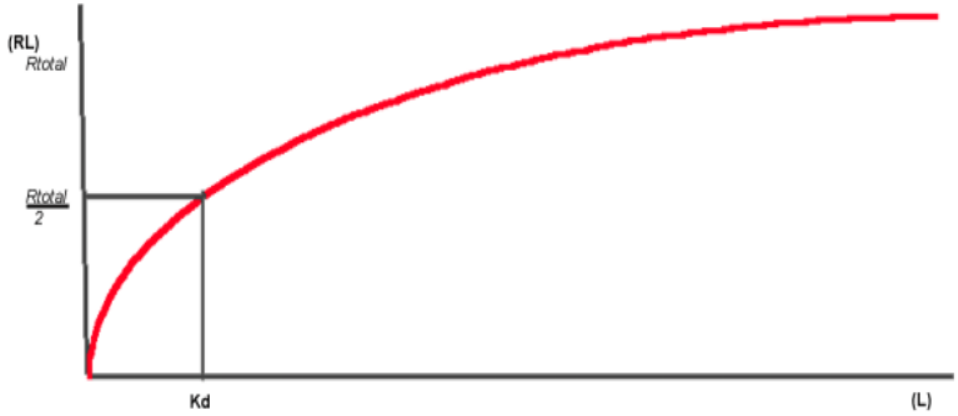


Figure 1.3. Saturation binding curve

b. Displacement (competition) methods

It is used much more frequently than the previous one because most of the time there is no labeling of the new ligand to be studied. Fixed amount of membrane, Fixed L* Ligand, Added Ligand I.

These methods are often preferred in industrial screening, because they allow the study of unlabeled molecules (simpler and less expensive).

The principle: A fixed concentration of receptor and a fixed concentration of "tracer" (a known and labelled ligand) are used. Increasing concentrations of the new compound to be tested (the "competitor") are then added.

What is measured: The ability of the new compound to eject the tracer from its binding site.

Result: We obtain a decreasing sigmoid curve. The IC50 (the concentration that displaces 50% of the tracer) is measured. It is converted into an inhibition constant with the Cheng–Prusoff equation: Ki = IC50 / (1 + [L*]/Kd), where [L*] is the concentration of tracer and Kd its dissociation constant.

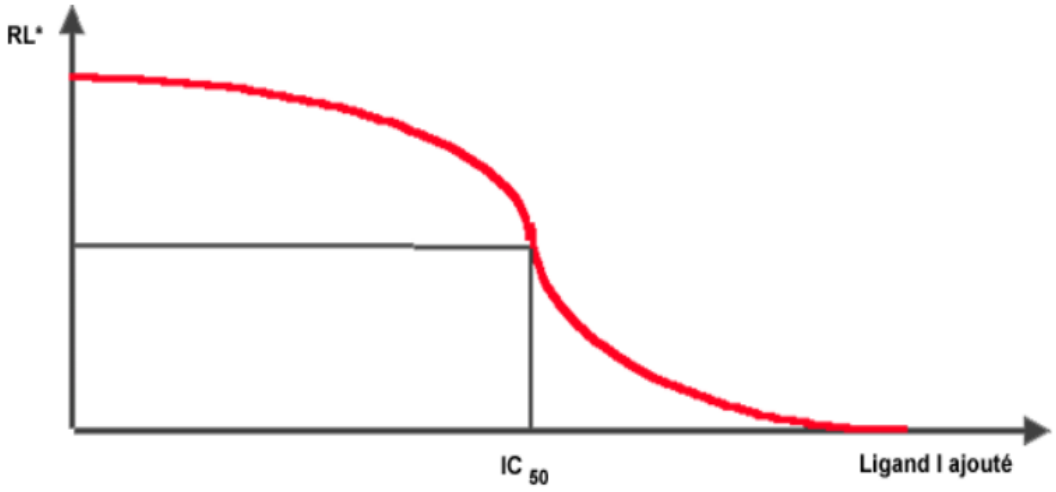


Figure 1.4. Displacement (competition) curve

1.2.2 Study of the dose (or concentration)–effect relationship

Effect of agonists

- Dose-effect relationship: it is hyperbolic in nature, (or sigmoid when concentrations or doses are expressed in logarithmic coordinates).
- When the doses (or concentrations at the site of action) are infinite, the maximum effect is achieved.
- The dose (or concentration) that induces half of the maximum effect is called the ED50 or EC50.
- The mathematical model that characterizes the dose-concentration-effect (E) relationship is thus of the type: $E = (\alpha Emax \times D) / (D + ED50)$ (Figure 1.5).
- The effectiveness of a substance is characterized by the Emax it induces.
- The α coefficient characterizes the intrinsic activity of the agonist, i.e., its ability to cause the coupling of the receptor to its effector. When α is equal to 1, the intrinsic activity is maximum, we speak of a full agonist. When α is less than 1, it is called a partial agonist (a ligand with $\alpha = 0$ is a pure antagonist).

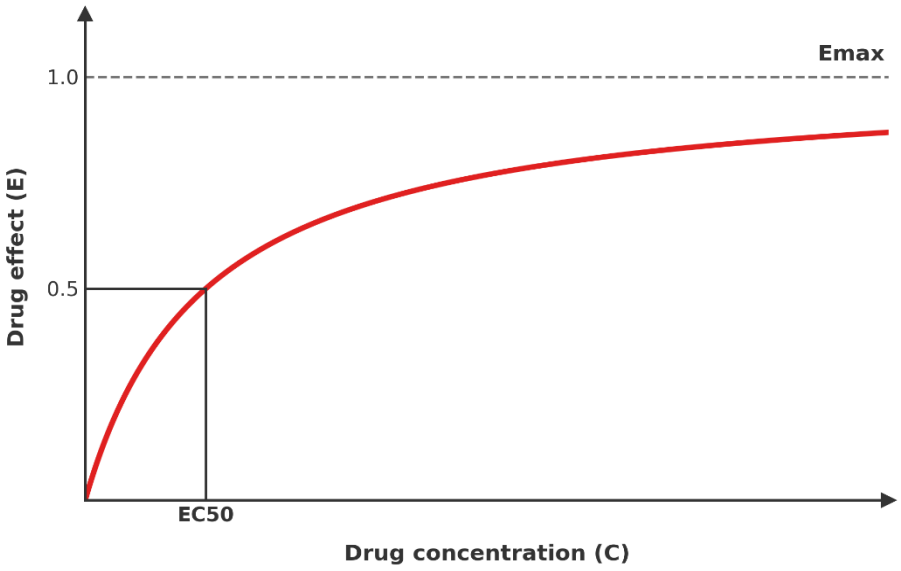


Figure 1.5. Dose (concentration)–effect relationship of an agonist.

- The greater the maximum effect, the more effective the substance will be. Thus, in Figure 1.6, drug B is less effective than drug A.
- The ED50 or EC50 characterizes the *potency* of a substance. The lower the EC50, the greater the potency of the substance. Thus, in Figure 1.6, substances C and D are less potent than substances A and B.

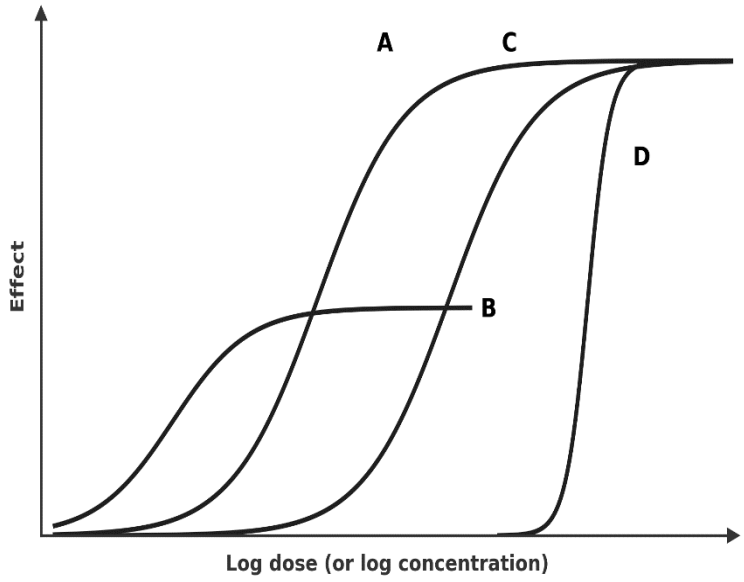


Figure 1.6. Log dose–effect relationships of four agonists (A–D).

a. Agonist Comparison

✓ Comparison of EC50 and Emax for a type of effect observed on a given preparation;

✓ Comparison of *Kd* (or *Ki*): depends on the type of receptor.

b. Characterization of agonists

- An agonist will have the same *Kd* for the same receptor regardless of the tissue.
- The binding of a ligand on the same receptor but on different tissues may induce differences because the receptor-effector coupling may be different.
- For the same receptor, the EC50 of an agonist for the corresponding effect is less than or equal to the *Kd* of the agonist for the receptor (amplification of the response).
- An agonist has different *Kd* to different receptors and different EC50s for the corresponding effects.
- An agonist may produce different effects depending on the dose (or concentration), because its affinity varies from one receptor to another. Thus, beneficial effects may be observed within a given dose range, while undesirable effects appear at other doses or concentrations. The selectivity of action of an agonist (or of an antagonist) therefore depends on its affinity.

Effect of antagonists

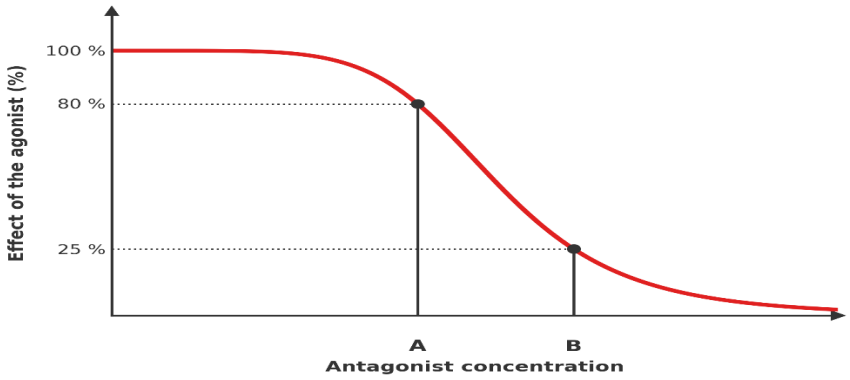


Figure 1.7. Inhibition of the agonist effect by increasing concentrations of an antagonist.

shows the progressive inhibition of the agonist effect as the antagonist concentration increases: at point A (low antagonist concentration) the agonist still produces about 80% of its maximal effect, whereas at point B (higher concentration) only about 25% of the effect remains.

An antagonist is a substance that blocks the receptor by binding either at the site of action of the endogenous substance (competitive antagonist) or at a different site (non-competitive antagonist). A competitive antagonist shifts the dose–effect curve of the agonist in parallel to the right without changing Emax, because its effect can be overcome by increasing the agonist dose (Figure 1.8). A non-competitive antagonist lowers the maximal effect (Emax) and cannot be overcome by increasing the agonist dose (Figure 1.9).

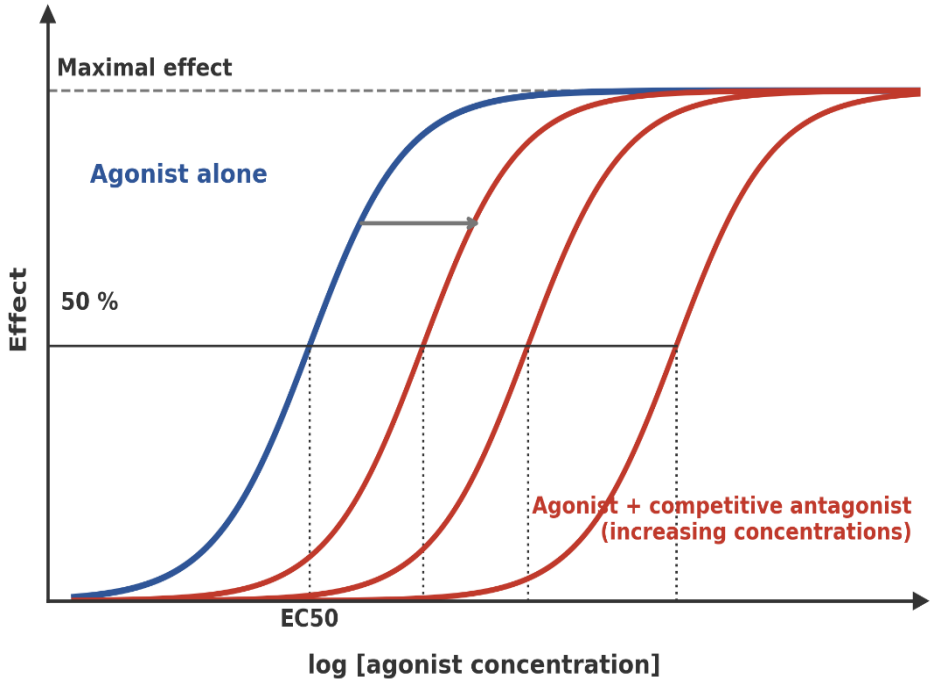


Figure 1.8. Competitive antagonist

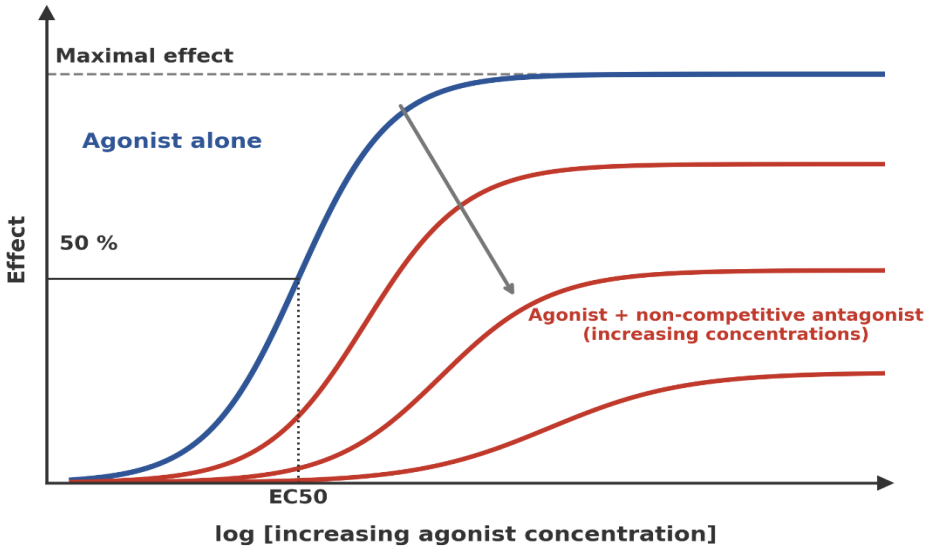


Figure 1.9. Non-competitive antagonist

1.2.3 Therapeutic index

The ratio between the toxic dose and the therapeutic dose of a drug

Therapeutic Index (TI) = Toxic Dose (TD 50) / Therapeutic Dose (ED 50)

- Wide therapeutic index (e.g., TI = 10): relatively safe drug (example B)
- Narrow therapeutic index (e.g., TI = 2): high-risk drug (example A)

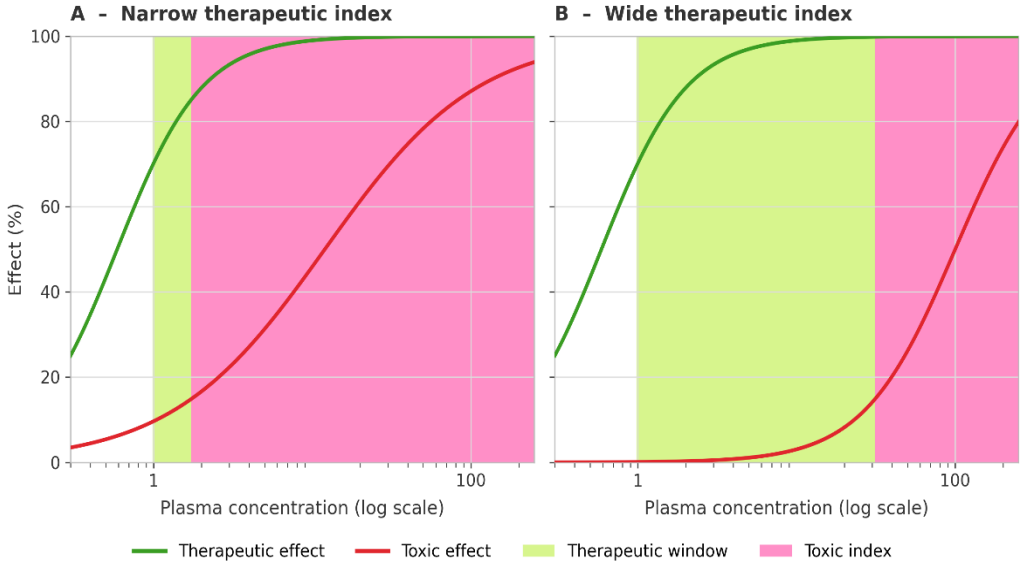


Figure 1.10. Therapeutic index of drug A (narrow) and drug B (wide).

Chapter 2: Methods of Exploration in Pharmacology

Methods of exploration in pharmacology span multiple levels of biological organization—from single molecules to whole organisms. These methods are used to discover new drug candidates, elucidate mechanisms of action, evaluate toxicity, and quantify pharmacokinetics and pharmacodynamics.

2.1 Levels of exploration

Table 2.1. Levels of exploration in pharmacology: goals, advantages and limitations

Exploration Level	Primary Goal	Key Advantage	Main Limitation
In Silico	Target screening, virtual optimization	Ultra-high throughput, low cost	Predictions require empirical validation
In Vitro	Receptor kinetics, biochemical mechanisms	High throughput, precise molecular control	Lacks systemic organ crosstalk & metabolism
Ex Vivo	Tissue-level functional responses	Preserves native microarchitecture	Limited tissue viability duration
In Vivo	Systemic efficacy, toxicity, PK/PD	Captures full physiological response	Complex variables, lower throughput, ethical limits

1. *In Silico* Methods (Computational Pharmacology)

In silico approaches utilize computational modeling and bioinformatics to predict drug-target interactions and properties prior to wet-lab testing. Typical tools include molecular docking, virtual screening, QSAR models and ADMET prediction.

2. *In Vitro* Methods (Cellular & Molecular Level)

These methods allow for high-throughput, controlled experimentation on isolated biological components. Examples: receptor-binding and enzyme assays, cell-based assays (cell cultures, reporter genes) and patch-clamp electrophysiology.

3. *Ex Vivo* Methods (Isolated Tissues/Organs)

Ex Vivo exploration uses living functional tissues or organs isolated from animals, preserving native cellular architecture and local physiology without systemic metabolic interference. Classic examples: isolated organ baths (aortic rings, guinea-pig ileum) and the Langendorff perfused heart.

4. *In Vivo* Methods (Whole Animal / Systems Pharmacology)

In vivo experiments evaluate drug behavior within a complete, living system to account for systemic metabolism, neural feedback, and multi-organ interaction. Animal models of disease (e.g., hypertension, diabetes, pain, inflammation) are used to assess efficacy, pharmacokinetics and toxicity.

2.2 Pre-clinical evaluation – experimental toxicology

1. Mandatory Prerequisite Trials (Before Clinical Phases)

A. Acute Toxicity (Single Administration)

- Principle: A single, typically high dose is administered to the subjects.
- Objectives: Qualitative evaluation (symptom monitoring) and quantitative assessment: determining the Minimum Lethal Dose (MLD) and the Lethal Dose 50 (LD50) (the dose that kills 50% of the tested population).
- Protocol: Utilizes at least 6 increasing doses across 6 groups (10-20 animals per group), 2 administration routes (including IV), and 2 mammalian species (one rodent, one non-rodent). Animals are observed for a minimum of 14 days, followed by macroscopic and histopathological autopsies.
- LD50 Calculation: Calculated using mathematical methods (Karber and Behrens) or graphical methods like Miller and Tainter (Trevan's Curve).
- Therapeutic Index (TI): TI = LD50 / ED50. A wide TI (e.g., penicillins)

indicates a highly secure profile, while a narrow TI (e.g., theophylline) signals high toxicity risk.

B. Subacute Toxicity (Short-Term Repeated Administration)

- Objective: Detect cumulative toxic effects, functional alterations, or anatomopathological damage to target organs.
- Protocol: Tested on 2 species (rats and dogs). Consists of 1 control group + 3 test groups (a high dose causing toxicity but no death, a low dose causing no effects, and an intermediate dose). Duration ranges from 2 weeks to 6 months, depending on the intended duration of human use.
- Key Outcome: Determination of the NOAEL (No Observed Adverse Effect Level).

C. Mutagenicity Trials

- Objective: Screen for alterations to genetic material (gene mutations, chromosomal aberrations, or DNA damage). It serves as a rapid screening tool prior to long-term carcinogenicity testing.
- Standard Reference Tests:
  - *In vitro* (Gene Mutations): AMES Test using *Salmonella typhimurium* (detecting reverse mutations from $His^{-} \rightarrow His^{+}$).
  - *In vitro* (Mammalian): MLA Test (Mouse Lymphoma Assay) targeting the Thymidine Kinase gene.
  - *In vivo*: Testing on Drosophila.
  - *Chromosomal Aberrations*: *In vitro* karyotyping (cells blocked in metaphase using colchicine) and the *in vivo* Micronucleus Test on bone marrow erythrocytes in mice.
  - *DNA Damage*: Comet Assay (SCGE) via electrophoresis to visualize DNA strand breaks.

2. Post-requisite Trials (Conducted Parallel to or After Clinical Initiation)

A. Chronic Toxicity (Long-Term Repeated Administration)

- Objective: Define the nature of toxicity during prolonged exposure and extrapolate the safe daily dose for humans.
- Protocol: Similar setup to subacute testing but over a significantly longer duration: 2 years in rats, and 7 or more years in dogs/monkeys.

B. Carcinogenicity Testing

- Objective: Identify whether a molecule can induce tumors or increase their baseline incidence.
- Indications: Required if structural analogues are known carcinogens, if long-term chronic toxicity tests raise suspicions, if mutagenicity tests are positive, or if the drug is intended for prolonged human use.
- Protocol: Daily administration starting right after weaning for the majority of the animal's lifespan (24 months for mice/hamsters, 30 months for rats). Uses groups of 50 animals per sex. Doses include the MTD (Maximum Tolerated Dose) alongside fractions (1/2 or 1/4 of MTD). Histopathological evaluation is mandatory for final diagnosis.

C. Reproductive Toxicity (Reprotoxicity)

Evaluates impacts on fertility, gestation, and offspring across three distinct segments:

- Segment I: Fertility and Early Embryonic Development: Males are treated for 4–10 weeks and females for 2 weeks prior to mating to assess gamete quality, libido, and embryo implantation.
- Segment II: Embryo-fetal Development (Teratogenicity): Pregnant females are treated strictly during the period of embryonic organogenesis. Females are sacrificed 2 days before natural delivery to count uterine resorption sites (in utero deaths), visible malformations, and fetal weight.
- Segment III: Pre- and Post-natal Development: Treatment spans from the end of gestation through the lactation period to evaluate parturition, nursing

behavior, and the physical/mental development of the F1 generation.

2.3 Clinical trials

Clinical trials are the process required for testing new drugs on human subjects.

1. Foundations and Methodology

- Evidence-Based Medicine (EBM): Establishes a clear hierarchy of clinical evidence from strongest to weakest: Systematic reviews / meta-analyses → Randomized Controlled Trials (RCTs) → Cohort studies → Case-control studies → Cross-sectional studies → Case series.
- Justification: Clinical trials are the only reliable way to gather objective proof of efficacy, preventing medical errors based purely on theoretical pathophysiological reasoning (e.g., class I antiarrhythmic drugs given after myocardial infarction, which the CAST trial showed to increase mortality, with tens of thousands of deaths estimated in the USA).
- Group Assignment: Achieved through randomization (random drawing) to ensure initial comparability between groups, eliminating bias from the patient, physician, or treatment selection.

2. The 4 Phases of Clinical Development

Pre-clinical trials are mandatory before any human testing can begin. Clinical trials are typically conducted in a hospital setting and progress through four distinct phases:

Table 2.2. The four phases of clinical development

Phase	Population	Primary Objectives	Characteristics / Duration
Phase I	Healthy volunteers (10 to 50)	Assess safety, absence of toxicity, Maximum Tolerated Dose (MTD), and pharmacokinetics.	Open-label, non-comparative trial.
Phase II	A few hundred patients	Determine optimal dosing (dose-effect), observe tolerance, and identify side effects.	Open-label, lasts from ~3 months to < 2 years.
Phase III	A few thousand patients	Demonstrate efficacy (superiority, equivalence, or non-inferiority) and monitor safety.	Comparative, randomized, double-blind when feasible, often multicenter. Required before Marketing Authorization (AMM).
Phase IV	General population	Monitor long-term safety (pharmacovigilance), assess cost-effectiveness, and explore new indications.	Post-marketing (post-AMM). Can result in drug withdrawal if severe risks appear.

A controlled trial is a trial that compares the effectiveness of the test substance to that of a placebo or a known active substance: some of the participants take a placebo or another active substance and constitute a "control" group.

Table 2.3. Types of blinding in clinical trials

Type	**Description**
Open-label or unblinded trial	All parties know what treatment the participant receives
Single blind or simple blind	Only the participants do not know what treatment they are receiving
Double-blind	The participant and the clinical team/data collectors do not know what treatment the participant is receiving
Triple-blind	The participant, clinical team/data collectors, and outcome assessors/data analysts do not know which treatment the participant is receiving

3. Data Analysis and Biases

- Intention-to-Treat (ITT) Analysis: Includes all randomized patients in the final analysis, regardless of whether they completed or strictly followed the protocol. This preserves the initial comparability established by randomization.
- Per-Protocol (PP) Analysis: Evaluates only the patients who completed the treatment exactly as prescribed (carries a high risk of bias).
- Bias Control: Selection bias is managed by randomization; performance and detection biases are managed by double-blinding; attrition bias is managed by ITT analysis.

4. Ethical and Regulatory Frameworks

- Trials must strictly comply with international declarations (Nuremberg, Helsinki, Tokyo).
- In France (Huriet–Sérusclat Law of 1988, replaced by the Jardé Law of 2012

and EU Regulation 536/2014): requires clear identification of the sponsor/investigator, a favorable opinion from a Protection Committee (CPP), and authorization from the competent authority (ANSM) before starting.

- Patient Rights: Full information disclosure, informed consent, reasonable risk-to-benefit ratio, and mandatory insurance coverage.

Chapter 3: Fate of the Drug in the Body (Pharmacokinetics)

Pharmacokinetics describes what the body does to a drug. It can be summarised in four processes: Absorption, Distribution, Metabolism and Excretion (ADME). Metabolism and excretion together make up drug elimination. These processes determine the time course of the drug concentration in the body (in plasma and, through it, at the site of action) and, therefore, the time course of the effect.

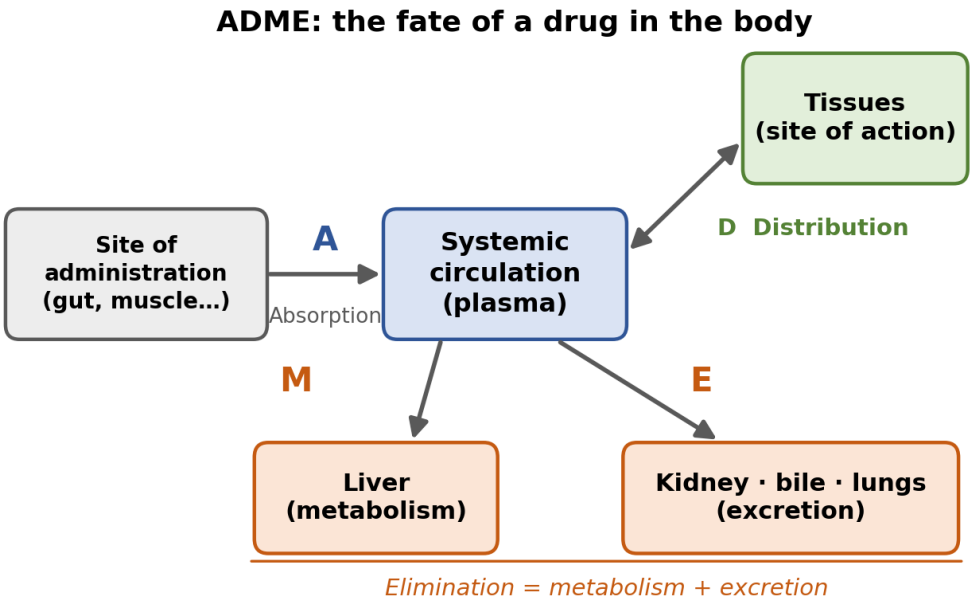


Figure 3.1. Overview of the four ADME processes.

3.1 Absorption

Absorption is the process by which the unchanged drug passes from its site of administration into the general (systemic) circulation. The route of administration influences this first phase: the intravenous (IV) route is the reference route because,

by definition, the entire administered dose reaches the general circulation, unlike other routes (oral, for example).

To be absorbed, the drug must cross a barrier separating it from the general circulation (the intestinal epithelium in the case of oral administration, for example). Of the various mechanisms involved, two are the most important:

- Passive diffusion: down the concentration gradient / no energy consumption / non-specific / no competition / not saturable ⇒ governed by Fick's law (the rate is proportional to the concentration gradient and is favoured by lipophilicity and by the non-ionised form of the drug).
- Active transport: against the concentration gradient / requires energy / specific / saturable / subject to competition between substrates.

Facilitated diffusion (carrier-mediated, along the gradient, without energy) also exists. Conversely, efflux transporters such as P-glycoprotein limit absorption by pumping the drug back into the intestinal lumen.

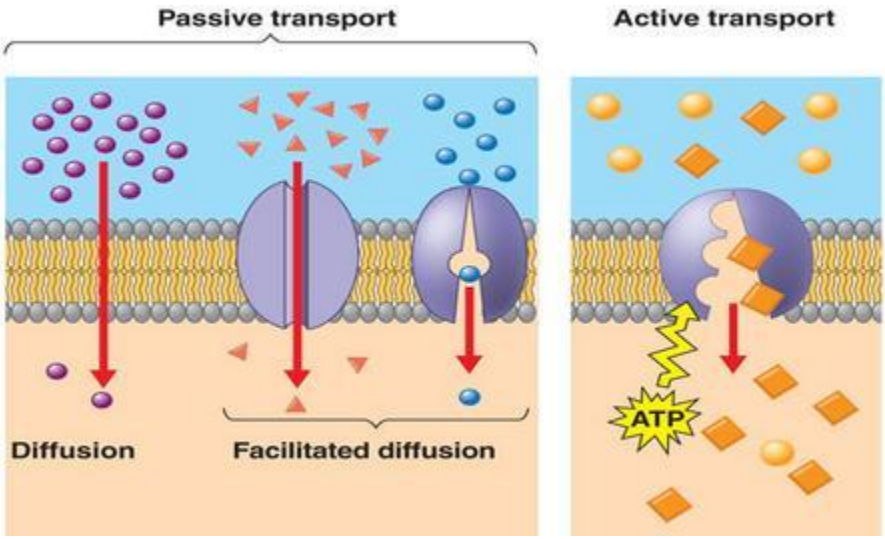


Figure 3.2. Main mechanisms of transport across a biological membrane.

Absorption also depends on drug-related factors (solubility and dissolution rate, lipophilicity, ionisation according to pKa and local pH, molecular size,

pharmaceutical form) and on physiological factors (gastric emptying and intestinal motility, surface area and blood flow at the site of absorption, presence of food).

Assessing absorption: bioavailability

Bioavailability is defined as the fraction of the administered dose that reaches the general circulation unchanged and the rate at which it does so.

Absolute bioavailability: an extravascular form (oral, for example) is compared with the reference form, which is the same drug administered intravenously, since by definition the entire dose then reaches the general circulation.

$$\mathbf{F = (AUC_{po} \times Dose_{iv}) / (AUC_{iv} \times Dose_{po})} \quad \textbf{(absolute bioavailability)}$$

Relative bioavailability: the reference form is administered by a route other than the intravenous route. This reference form may be administered by the same route as the form being tested, but corresponds either to a different pharmaceutical form (aqueous solution, suspension, etc.) or to a different formulation of a product that has been marketed for a long time (as with generics).

In general, the bioavailability factor (F) is quantified by comparing the areas under the plasma concentration–time curves (AUC) obtained after administration of each form separately. These areas are proportional to the amount of drug that has reached the general circulation (see figure below).

$F_{rel} = (AUC_{test} \times Dose_{ref}) / (AUC_{ref} \times Dose_{test})$ (relative bioavailability)

When the same dose is given by both routes, the first formula simplifies to $F = AUC_{po} / AUC_{iv}$.

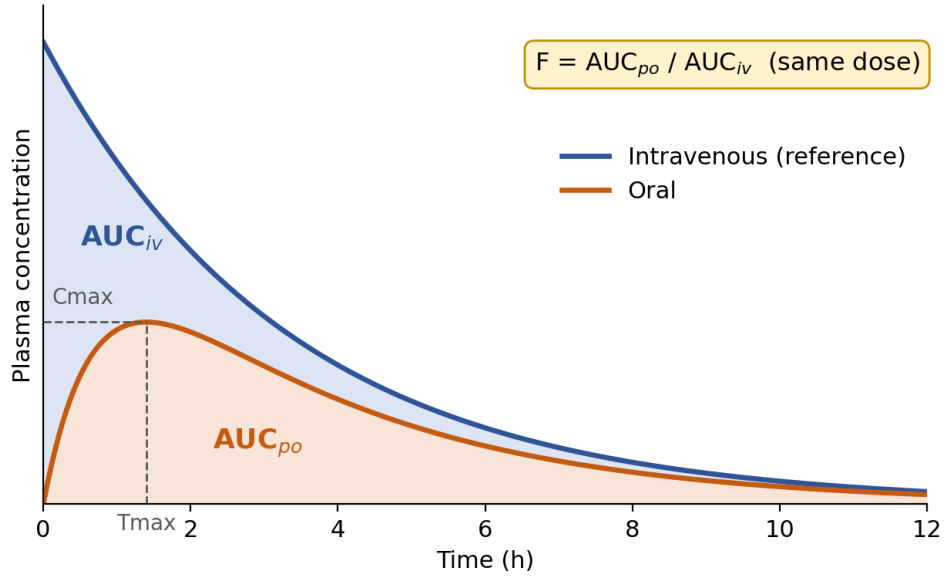


Figure 3.3. Plasma concentration–time curves after IV and oral administration

The rate of absorption

is assessed by the absorption rate constant (Ka) or, more simply, by the maximum concentration (Cmax) and the time to reach this maximum (Tmax).

Like the amount absorbed, the rate of absorption is an important parameter for the onset of action of an active substance. It is predominant for drugs intended for rapid action (an analgesic, for example) given as a single dose or for short-term use. For chronic treatments, where a constant level of drug exposure is sought, the concept of Tmax is less critical.

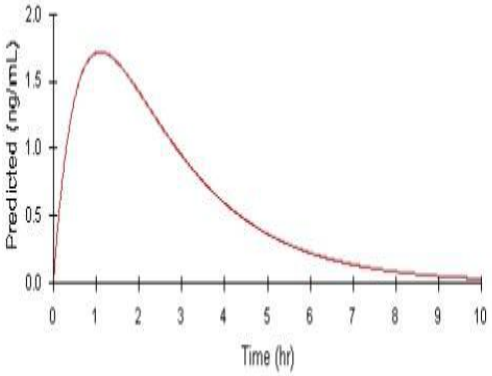
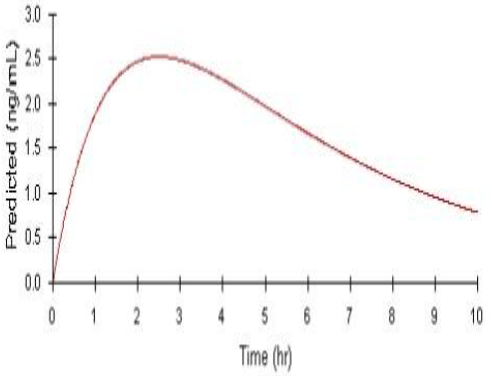
<table>
<tr><td>
</td><td>
</td></tr>
<tr><td>Drug A</td><td>Drug B</td></tr>
<tr><td colspan="2">Tmax: shorter for A than for B (1 h versus 2.5 h)
Cmax: higher for B than for A (2.5 ng/mL versus 1.7 ng/mL)</td></tr>
</table>

Figure 3.4. Concentration–time profiles of two drugs after a single dose.

True intestinal absorption (the amount of active substance that crosses the intestinal wall and enters the portal circulation) is difficult to measure directly, since the portal circulation is not readily accessible. The amount available at the systemic level is therefore estimated indirectly from drug concentrations in plasma sampled at a peripheral site, i.e. after passage through the liver.

The amount of drug reaching the general (systemic) circulation depends on the amount absorbed (and therefore on the administered dose), but also on other pre-systemic (first-pass) elimination processes:

- Degradation within the intestinal lumen (gastric acid, digestive enzymes, gut bacteria),
- Metabolism within the enterocytes (see 3.3 Metabolism) and efflux back into the lumen (e.g. P-glycoprotein),
- Substantial hepatic uptake on first pass. When a drug has a high affinity for hepatocytes and hepatic enzymes (high hepatic extraction), part of the absorbed dose is taken up during its first passage through the liver, i.e. before

it even reaches the general circulation. The amount of drug found in the systemic circulation is then reduced. This is known as the hepatic first-pass effect.

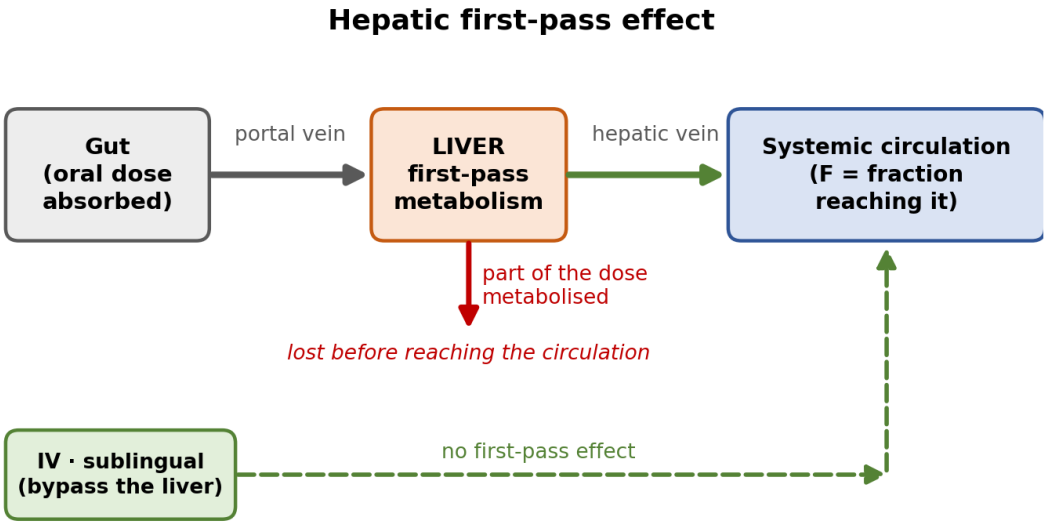


Figure 3.5. Hepatic first-pass effect

Oral bioavailability can therefore be written as $F = F_{abs} \times F_{gut} \times F_{hep}$ (the fraction absorbed, the fraction escaping gut-wall metabolism and the fraction escaping hepatic extraction). Propranolol and morphine are examples of drugs with a marked first-pass effect; glyceryl trinitrate is given sublingually for the same reason.

3.2 Distribution

Distribution is the process by which a drug leaves the general (systemic) circulation, reversibly, and moves into the interstitial fluid and then into the cells of the various tissues. The key determinants are:

- Plasma protein binding — many drugs bind reversibly to albumin (mainly acidic drugs) or α_1-acid glycoprotein (mainly basic drugs). Only the unbound (free) fraction is pharmacologically active and available for distribution, metabolism and excretion. Displacement interactions (one drug displacing another from protein-binding sites) are clinically significant mainly for drugs

that are highly protein-bound, have a low volume of distribution and have a narrow therapeutic index (e.g. warfarin).

- Tissue perfusion and permeability — highly perfused organs (brain, heart, liver, kidney) equilibrate rapidly; poorly perfused tissues (fat, bone) equilibrate slowly. Specialised barriers, notably the blood–brain barrier (tight endothelial junctions plus efflux transporters) and the placental barrier, restrict access of many drugs.
- Volume of distribution (Vd) — a proportionality constant relating the total amount of drug in the body to the plasma concentration (see below).

Vd = Amount of drug in the body / Plasma concentration

After an IV bolus, Vd = Dose / C_0, where C_0 is the plasma concentration extrapolated to time zero.

Vd is a theoretical (apparent) volume, not a physical space. A low Vd (close to the plasma volume, ≈ 0.04 L/kg, or the extracellular volume, ≈ 0.2 L/kg) indicates a drug that stays mainly in the vascular compartment (e.g. warfarin, ≈ 0.1–0.2 L/kg). A very high Vd (far exceeding total body water, ≈ 0.6 L/kg) indicates extensive tissue binding or sequestration (e.g. chloroquine, digoxin, > 4–5 L/kg). Vd is also used to calculate a loading dose (target concentration × Vd).

Factors favouring extensive tissue distribution (high Vd)

- Low plasma protein binding
- High affinity for tissue proteins
- High lipid solubility (lipophilicity)
- Good tissue perfusion

3.3 Metabolism (biotransformation)

Metabolism refers to the enzymatic transformation of a drug into one or more other compounds (metabolites), which may be pharmacologically active, inactive or, occasionally, toxic. In general, it converts lipophilic drugs into more polar, more readily excreted compounds. Many tissues can carry out this transformation (gut wall,

lung, kidney, skin, plasma, etc.), but the main site of biotransformation is the liver, specifically the microsomal enzymes of the hepatocytes. This is explained by the liver's high content of drug-metabolising enzymes and its abundant blood supply: as a "clearing" organ, it receives about 1.5 litres of blood per minute (approximately 1.2 L via the portal vein and 0.3 L via the hepatic artery).

Hepatocytes contain a large number of enzymes involved in drug transformation, particularly for oxidation–reduction reactions, hydroxylations and the oxidative cleavage of N–C and O–C bonds. The key component of this enzymatic system is cytochrome P450 (CYP), a superfamily of haem-containing monooxygenases comprising numerous isoenzymes. CYP3A4, CYP2D6, CYP2C9, CYP2C19 and CYP1A2 together account for the metabolism of the majority of marketed drugs.

Metabolism is schematically divided into two phases according to the type of transformation involved: Phase I and Phase II reactions. They often occur in sequence, but not necessarily: a drug that already carries a suitable functional group may undergo Phase II directly.

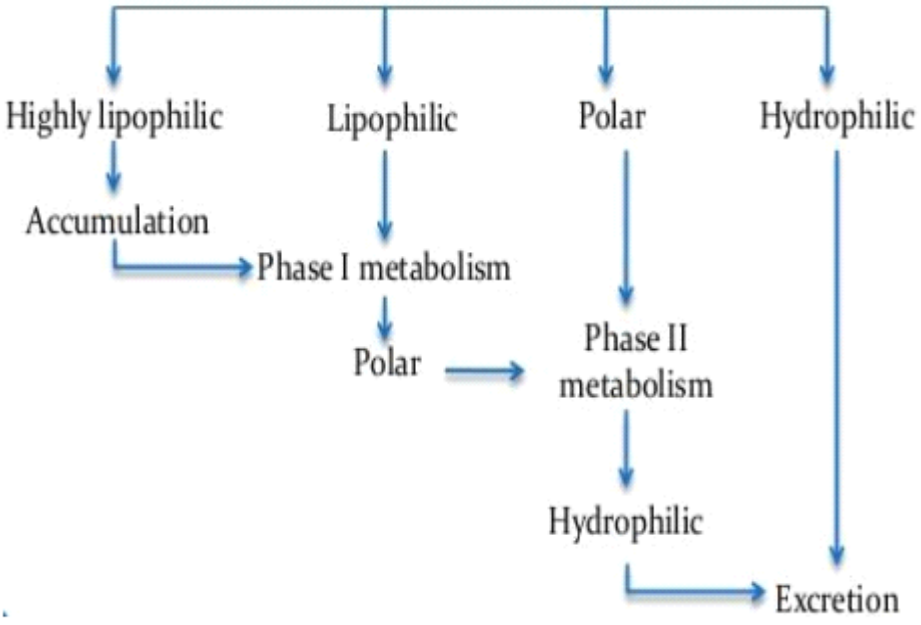


Figure 3.6. Phases I and II of drug metabolism.

Phase I reactions

Phase I reactions (oxidation, reduction and hydrolysis) introduce or unmask a polar functional group, most often a hydroxyl (–OH), amine ($–NH_2$) or carboxyl (–COOH) group, usually via the CYP system. Phase I metabolites can be inactive, active (contributing to or prolonging the effect) or, occasionally, toxic (reactive intermediates, e.g. NAPQI formed from paracetamol).

- Oxidation reactions are mostly localised in hepatic microsomes. They consume NADPH (reduced nicotinamide adenine dinucleotide phosphate) and molecular oxygen, and proceed via cytochrome P450 enzymes.
- Reduction reactions are much less frequent and less well studied. Reduction does not occur exclusively in the liver; it also takes place in the intestine, mediated by the bacterial flora.
- Hydrolysis is a common metabolic pathway that occurs in the liver, in various tissues and even in plasma. The enzymes involved (esterases, amidases) are usually non-specific. Ester bonds are generally cleaved very rapidly, whereas amide bonds are cleaved more slowly (e.g. procaine, an ester, is rapidly hydrolysed by plasma esterases, whereas lidocaine, an amide, is metabolised much more slowly).

Phase II reactions

Phase II reactions (conjugation) attach an endogenous polar moiety to the functional group generated by Phase I (or already present on the drug): glucuronic acid, sulfate, glycine, glutathione, or acetyl or methyl groups. Conjugation usually inactivates the drug and greatly increases its water solubility, favouring renal or biliary excretion. There are exceptions: morphine-6-glucuronide, for example, remains pharmacologically active.

Glucuronidation: conjugation with glucuronic acid is the most common form of conjugation. It is catalysed by the UDP-glucuronosyltransferase (glucuronyltransferase) enzyme system and involves molecules bearing a hydroxyl,

carboxyl or amine group. Glucuronides are highly water-soluble, which explains why they are easily eliminated in urine and bile. In some cases, the resulting ester glucuronides are unstable and, after hydrolysis in urine or plasma, regenerate the parent molecule.

Prodrugs, enzyme induction and inhibition

- Prodrugs are inactive as administered and require metabolic activation to exert their effect (e.g. clopidogrel, activated mainly by CYP2C19; codeine, O-demethylated to morphine by CYP2D6) — a mechanism with direct pharmacogenetic consequences (Chapter 4).
- Enzyme inducers (e.g. rifampicin, carbamazepine, phenytoin) increase the synthesis of CYP enzymes, which accelerates the clearance of co-administered substrates and may reduce their effect; induction develops over days.
- Enzyme inhibitors (e.g. ketoconazole, ritonavir, clarithromycin, erythromycin; also grapefruit juice for CYP3A4) reduce enzyme activity, which slows the clearance of co-administered substrates and may lead to toxicity; inhibition usually acts rapidly.

3.4 Excretion

Excretion removes unchanged drug and metabolites from the body, chiefly via the kidney and, for some drugs, via bile/faeces or the lungs (volatile agents).

- Renal excretion combines glomerular filtration (of the unbound fraction), active tubular secretion (via organic anion and organic cation transporters, which can be saturated or competitively inhibited — e.g. probenecid delaying penicillin excretion) and passive tubular reabsorption (favoured by lipophilicity and by the non-ionised form of the drug, which depends on urine pH for weak acids and bases; this is exploited therapeutically in the alkalinisation of urine to enhance salicylate excretion in overdose).
- Biliary/faecal excretion is important for drugs with a high molecular weight or that undergo glucuronide conjugation. Some glucuronides are hydrolysed by

gut flora and the released drug is reabsorbed (enterohepatic recirculation), prolonging the drug's half-life.

- Renal impairment reduces the clearance of renally eliminated drugs and their active metabolites and is a major source of pharmacokinetic variability (Chapter 4), requiring dose adjustment guided by creatinine clearance or estimated glomerular filtration rate (eGFR).

3.4.1 Clearance

Clearance (CL) is the volume of plasma completely cleared of the drug per unit of time (e.g. mL/min or L/h). It expresses the efficiency of the eliminating organs:

CL = Rate of elimination / Plasma concentration

Total (systemic) clearance is the sum of the clearances of the individual organs ($CL = CL_{renal} + CL_{hepatic} + CL_{other}$). Clearance determines the maintenance dose rate needed to reach a target steady-state concentration (Css): Dose rate = CL × Css / F.

3.4.2 Drug half-life

A widely used summary parameter for expressing the elimination of a drug from the body is the half-life. The $t_{1/2}$ corresponds to the time required for the plasma concentration to drop to half its value, regardless of the starting level of that concentration (this holds for drugs with first-order, i.e. linear, kinetics). Another way of expressing the same concept is to view $t_{1/2}$ as the time it takes for the total amount of drug in the body to decrease by half, regardless of the dose.

Half-life depends on both the volume of distribution and the clearance:

$$\mathbf{t_{1/2} = 0.693 \times Vd / CL = 0.693 / k_{el}}$$

Half-life therefore increases when Vd increases or when CL decreases (in renal or hepatic impairment, for example). It is generally calculated from plasma concentrations measured during the elimination phase.

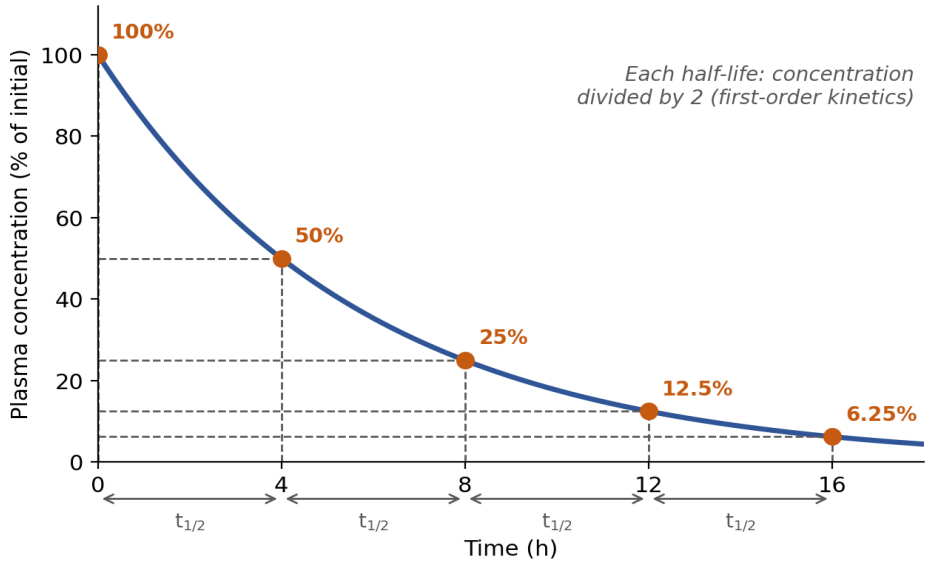


Figure 3.7. Exponential decline of the plasma concentration: it is halved every half-life.

In practice, $t_{1/2}$ plays a key role in determining the dosing schedule. It allows estimation of:

- The time needed to reach steady state during repeated dosing: approximately 4–5 × $t_{1/2}$ (about 94–97 % of the steady-state level), independently of the dose and of the dosing interval. In the same way, a drug is almost completely eliminated about 5 half-lives after the last dose.
- The fluctuation in concentrations between doses.

When a drug is administered chronically, the concentration profile depends on the ratio between $t_{1/2}$ and the dosing interval: the shorter the interval relative to $t_{1/2}$, the greater the accumulation and the smaller the fluctuation between peak and trough; if the interval is much longer than $t_{1/2}$, the drug is almost completely eliminated between doses and accumulates little.

Table 3. Summary of the main pharmacokinetic parameters

Parameter	Meaning	Key relationship
Bioavailability (F)	Fraction of the administered dose reaching the systemic circulation	$F = (AUC_{po} \times Dose_{iv}) / (AUC_{iv} \times Dose_{po})$
Cmax and Tmax	Peak concentration and time to reach it (rate of absorption)	Read from the concentration–time curve
Volume of distribution (Vd)	Apparent volume relating the amount of drug in the body to the plasma concentration	$Vd = Dose / C_0$ (IV bolus)
Clearance (CL)	Volume of plasma cleared of drug per unit of time	CL = Rate of elimination / C
Half-life ($t_{1/2}$)	Time for the concentration to fall by half	$t_{1/2} = 0.693 \times Vd / CL$
Time to steady state	Time to reach a stable level during repeated dosing	$\approx 4–5 \times t_{1/2}$

Chapter 4: Sources of Variability in the Effect of Drugs

The pharmacological effects of a drug may vary from one individual to another or even within the same individual, for reasons related to changes in pharmacokinetics (altering the concentration achieved at a given dose), pharmacodynamics (altering the response produced at a given concentration). These changes arise from genetic, physiological, pathological, and environmental sources, often interacting with one another.

4.1 Genetic variability — pharmacogenetics

Genetic polymorphisms in drug-metabolising enzymes, transporters, and targets produce discrete, often clinically actionable, subpopulations of drug response:

- CYP2D6 polymorphisms define poor, intermediate, extensive (normal), and ultrarapid metabolisers, affecting drugs such as codeine (ultrarapid metabolisers risk life-threatening morphine toxicity; poor metabolisers get no analgesia), tamoxifen, and many antidepressants and antipsychotics.
- CYP2C19 polymorphisms affect activation of the prodrug clopidogrel — poor metabolisers have reduced antiplatelet effect and higher cardiovascular risk.
- CYP2C9 and VKORC1 polymorphisms explain much of the interindividual variability in warfarin dose requirement.
- Thiopurine S-methyltransferase (TPMT) and NUDT15 deficiency predispose to severe, potentially fatal myelosuppression with standard doses of thiopurines (azathioprine, 6-mercaptopurine).
- HLA allotypes predict severe hypersensitivity reactions: HLA-B*57:01 and abacavir hypersensitivity; HLA-B*15:02 and carbamazepine-induced Stevens–Johnson syndrome in patients of Han Chinese and Southeast Asian ancestry.
- G6PD deficiency, an X-linked enzymopathy, predisposes to haemolysis with oxidant drugs (primaquine, dapsone, sulfonamides).

4.2 Physiological variability

- Age — neonates have immature hepatic metabolism and renal function, and different body-water/fat composition, requiring markedly different dosing from adults; the elderly typically show reduced hepatic blood flow and CYP activity, reduced renal clearance, reduced lean body mass and total body water (altering Vd), reduced plasma albumin, and increased pharmacodynamic sensitivity to CNS-active drugs (sedatives, anticholinergics).
- Sex — differences in body composition, gastric emptying, CYP3A4 activity (generally higher in women), and, historically, under-representation of women in early-phase trials, have produced sex differences in both pharmacokinetics and adverse-event rates (e.g. QT prolongation, zolpidem dosing).
- Pregnancy — increased plasma volume, glomerular filtration rate, and hepatic enzyme activity generally increase clearance of many drugs, alongside placental transfer risks to the fetus (teratogenicity, categorised historically by FDA pregnancy categories and now by narrative risk summaries).
- Body weight and composition — obesity alters Vd disproportionately for lipophilic versus hydrophilic drugs, complicating weight-based dosing.

4.3 Pathophysiological variability

- Hepatic impairment reduces first-pass metabolism (raising oral bioavailability of high-extraction drugs), reduces plasma protein synthesis (raising the free fraction of highly protein-bound drugs), and reduces overall metabolic clearance; dosing is adjusted using clinical scoring systems such as Child–Pugh.
- Renal impairment reduces clearance of renally eliminated drugs and active metabolites (Chapter 3); dose adjustment is guided by creatinine clearance/eGFR, and some drugs are contraindicated outright in severe renal failure (e.g. metformin, at very low eGFR, because of lactic acidosis risk).

- Cardiac failure reduces hepatic and renal perfusion, indirectly reducing clearance and altering absorption (via gut oedema).
- Inflammation and critical illness alter plasma protein levels (acute-phase reactants), fluid distribution (capillary leak, altering Vd), and CYP/transporter expression (cytokine-mediated downregulation), all contributing to markedly altered pharmacokinetics in intensive-care patients.

4.4 Drug–drug and drug–food interactions

Interactions are pharmacokinetic (one drug alters the absorption, distribution, metabolism, or excretion of another — enzyme induction/inhibition being the dominant mechanism) or pharmacodynamic (additive, synergistic, or antagonistic effects at the same or convergent targets, independent of concentration — e.g. combining two QT-prolonging drugs, or an ACE inhibitor with a potassium-sparing diuretic). Food can alter absorption (grapefruit juice inhibits intestinal CYP3A4 and P-glycoprotein, raising exposure to several drugs including some statins and calcium-channel blockers; vitamin-K-rich foods antagonise warfarin) and, for some drugs, must be co-administered to enhance absorption (e.g. lipophilic drugs with a fatty meal).

4.5 Environmental and behavioural variability

Smoking induces CYP1A2 (affecting theophylline, clozapine, olanzapine clearance); chronic alcohol use induces CYP2E1 while acute intoxication can inhibit hepatic metabolism; adherence (compliance) to the prescribed regimen is itself a major, and frequently underestimated, source of “apparent” variability in clinical response, and circadian rhythm affects both pharmacokinetics (chronopharmacokinetics) and the intensity of some pharmacodynamic effects (chronopharmacodynamics), the rationale for chronotherapy in conditions such as asthma and rheumatoid arthritis.

4.6 Pharmacodynamic variability

Pharmacodynamic variability concerns differences in the response obtained at a given concentration. It may be linked to individual differences in receptor number, affinity

or signalling (phenomena of desensitization or hypersensitization), to adaptation to repeated exposure (tolerance, tachyphylaxis, dependence), to disease-related changes in target sensitivity, and to homeostatic reflexes that counteract the effect of the drug (e.g., reflex tachycardia with vasodilators).

4.7 Managing variability in practice

Three complementary strategies are used to manage predictable variability: weight- or body-surface-area-based dosing (especially in paediatrics and oncology), therapeutic drug monitoring for narrow-therapeutic-index drugs, and pre-emptive pharmacogenetic testing where validated (e.g. before starting abacavir, or dosing clopidogrel/warfarin/thiopurines in specific populations). Regulatory drug labels increasingly include specific dosing recommendations stratified by genotype, organ function, and age.

Chapter 5: Monoclonal Antibodies, Vaccines, RNA Interference, and Therapeutic Proteins

5.1 Biologics versus small molecules

The drug classes considered so far are mostly small molecules (typically <900 Da) that diffuse across membranes and are metabolised by CYP enzymes. Biologics — monoclonal antibodies, recombinant proteins, vaccines, and nucleic-acid therapeutics — are large, structurally complex molecules produced in living systems (bacteria, yeast, mammalian cell culture), administered parenterally because they are destroyed by gastrointestinal digestion, and eliminated predominantly by proteolytic catabolism (often via the reticuloendothelial system and target-mediated drug disposition) rather than by hepatic CYP metabolism or renal filtration.

5.2 Monoclonal antibodies (mAbs)

A monoclonal antibody is a homogeneous immunoglobulin, usually IgG, engineered to bind a single epitope on a specific target with high affinity and specificity.

Nomenclature reflects origin: murine mAbs (suffix *-omab*, now largely obsolete owing to immunogenicity), chimeric mAbs (*-ximab*, human constant regions with murine variable regions, e.g. rituximab, infliximab), humanised mAbs (*-zumab*, murine complementarity-determining regions grafted onto a human framework, e.g. trastuzumab, omalizumab), and fully human mAbs (*-umab*, produced via phage display or transgenic mice, e.g. adalimumab, nivolumab).

Mechanisms of action fall into several categories: neutralisation of a soluble ligand (e.g. bevacizumab neutralising VEGF), blockade of a cell-surface receptor (e.g. cetuximab blocking EGFR), agonism of a receptor, immune-mediated cytotoxicity against the cell to which the antibody binds (antibody-dependent cellular cytotoxicity and complement-dependent cytotoxicity, exploited by anti-CD20 mAbs such as rituximab in B-cell malignancies), and immune checkpoint blockade, which releases an endogenous brake on T-cell activity rather than directly killing tumour

cells (anti-PD-1: nivolumab, pembrolizumab; anti-CTLA-4: ipilimumab; anti-PD-L1: atezolizumab — Chapter 11).

Antibody-drug conjugates (ADCs) combine a mAb (for targeting) with a cytotoxic payload (for killing), linked by a chemical linker designed to release the payload selectively inside the target cell — e.g. trastuzumab emtansine in HER2-positive breast cancer.

Pharmacokinetic peculiarities: long half-lives (days to weeks, owing to FcRn-mediated recycling that protects IgG from lysosomal degradation), non-linear (target-mediated) clearance at low concentrations that saturates at higher concentrations, immunogenicity (formation of anti-drug antibodies that can accelerate clearance or neutralise efficacy), and infusion-related reactions.

5.3 Vaccines

Vaccines are biological preparations that induce active, adaptive immunity against a specific pathogen without causing the disease itself, working by presenting antigen to the immune system so that memory B and T cells are generated for rapid response on subsequent exposure.

- Live attenuated vaccines (measles, mumps, rubella, yellow fever, oral polio) use a weakened form of the pathogen, closely mimic natural infection, and generally produce the most durable immunity, but are contraindicated in immunocompromised patients and pregnancy.
- Inactivated (killed) vaccines (inactivated polio, hepatitis A, most influenza vaccines) cannot replicate and are safer in immunocompromised patients, but generally produce a weaker, shorter-lived response, often requiring adjuvants and booster doses.
- Subunit, recombinant, and polysaccharide/conjugate vaccines use a purified antigenic component (hepatitis B surface antigen; pneumococcal and meningococcal conjugate vaccines, which link a polysaccharide to a carrier

protein to convert a T-cell-independent response in infants into a T-cell-dependent one with immune memory).

- Toxoid vaccines (tetanus, diphtheria) use a chemically or heat-inactivated bacterial toxin that retains immunogenicity but not toxicity.
- mRNA vaccines (a technology that reached large-scale clinical use with the COVID-19 pandemic) deliver lipid-nanoparticle-encapsulated messenger RNA encoding a pathogen antigen; host cells transiently translate the mRNA into antigen, which is then presented to the immune system, combining rapid design/manufacture with a strong immune response without a separately added adjuvant.
- Adjuvants (aluminium salts, oil-in-water emulsions, TLR agonists) enhance the immune response to co-administered antigen, particularly important for inactivated and subunit vaccines.

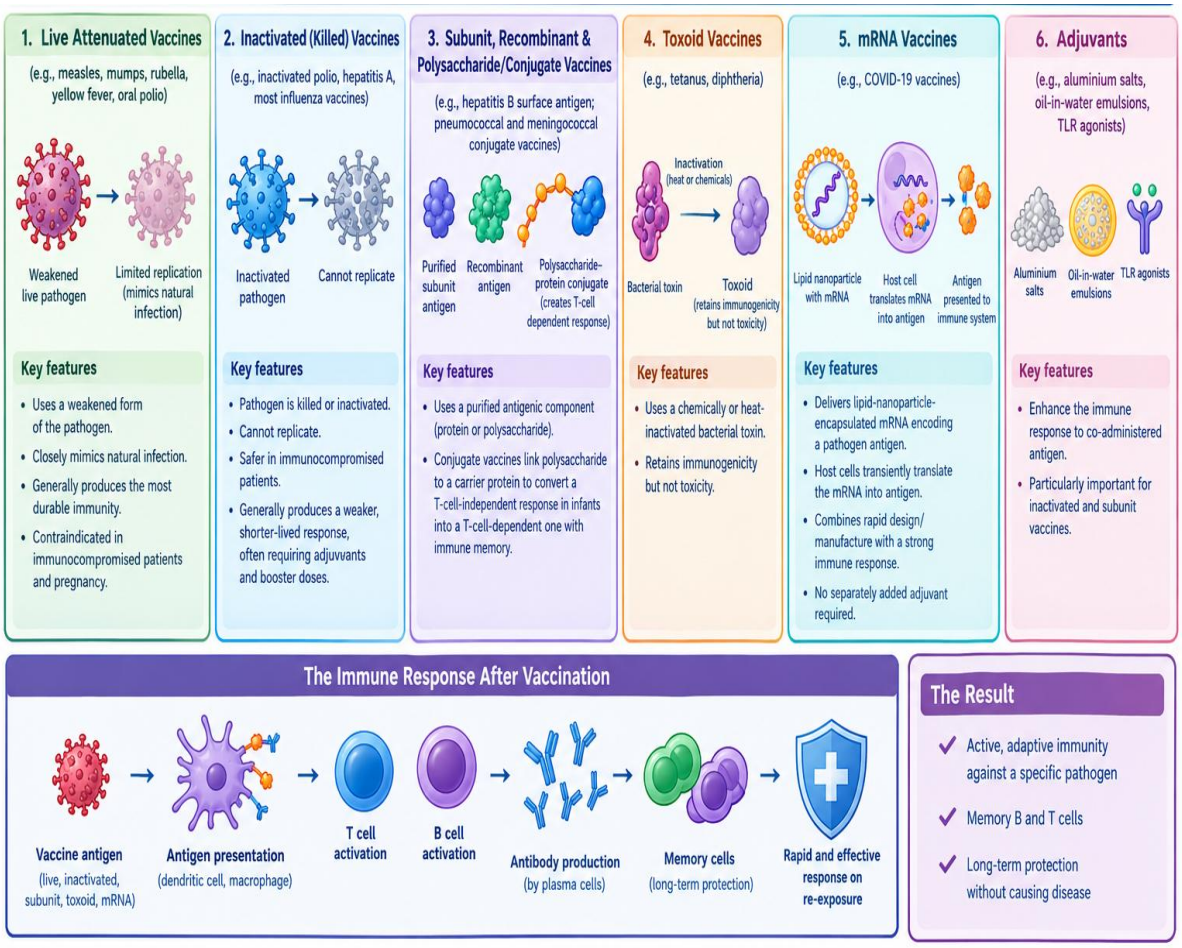


Figure 5.1. Major Vaccine Types, Mechanisms of Action, and Immune Response

5.4 RNA interference and antisense technologies

RNA-based therapeutics act upstream of protein synthesis, silencing or modulating gene expression rather than blocking a protein once it is made.

- Small interfering RNA (siRNA) exploits the endogenous RNA-interference pathway: a synthetic double-stranded RNA is loaded into the RNA-induced silencing complex (RISC), which uses the antisense strand to guide sequence-specific cleavage of complementary messenger RNA, silencing expression of the target gene. Patisiran (hereditary transthyretin amyloidosis) and inclisiran

(a long-acting siRNA against PCSK9 mRNA for hypercholesterolaemia, dosed twice yearly) are approved examples.

- Antisense oligonucleotides (ASOs) are single-stranded, chemically modified nucleic acids complementary to a target mRNA; depending on chemistry, they trigger RNase-H-mediated mRNA degradation or sterically block splicing/translation without degradation (e.g. nusinersen, which modifies SMN2 pre-mRNA splicing in spinal muscular atrophy).
- Delivery is the central pharmaceutical challenge for nucleic-acid therapeutics: naked RNA is rapidly degraded by nucleases and does not cross cell membranes efficiently, so delivery relies on chemical modification (2′-O-methyl, phosphorothioate backbones) and/or conjugation or encapsulation (GalNAc conjugation for hepatocyte-targeted delivery; lipid nanoparticles for broader tissue/mRNA vaccine delivery).

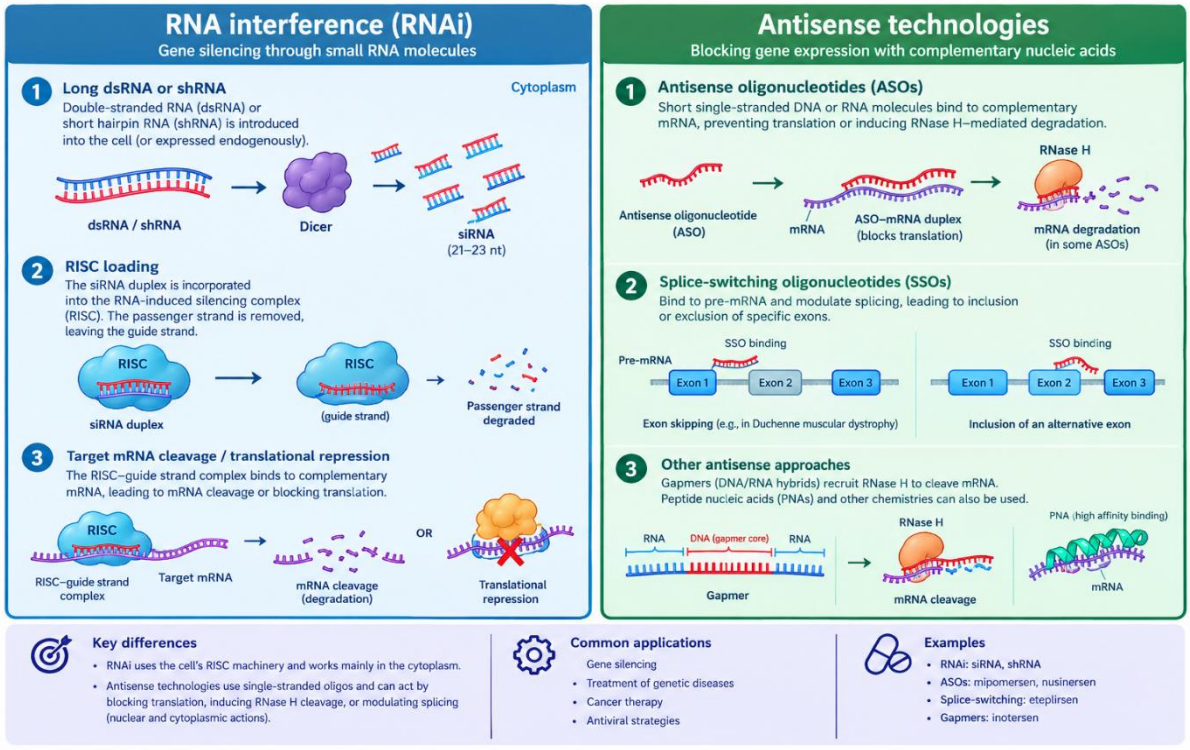


Figure 5.2. RNA interference and antisense technologies: mechanisms of gene silencing and therapeutic applications

5.5 Other therapeutic proteins

- Recombinant hormones and enzymes: recombinant human insulin and insulin analogues, recombinant growth hormone.
- Fusion proteins: etanercept, a fusion of the TNF receptor extracellular domain with an IgG Fc region, neutralises soluble TNF-alpha in rheumatoid arthritis.
- Cytokines and interferons: interferon-alpha and interferon-beta used in viral hepatitis and multiple sclerosis respectively; recombinant interleukin-2 and, more recently, engineered cytokines in oncology.
- Cell and gene therapies, a rapidly expanding category adjacent to this chapter, include chimeric antigen receptor (CAR) T cells.

Chapter 6: Pharmacology of Active Substances: Origin, Pharmaceutical Forms, Mechanisms of Action

6.1 Origin of active pharmaceutical substances

Drugs in clinical use derive from several distinct origins, each with characteristic advantages and constraints:

- Natural products — plants (morphine and codeine from *Papaver somniferum*, digoxin from *Digitalis lanata*, paclitaxel from *Taxus brevifolia*, artemisinin from *Artemisia annua*), microorganisms (penicillin from *Penicillium* moulds, most other antibiotics from soil actinomycetes, statins originally isolated from fungi), animals (insulin, historically bovine/porcine; heparin from porcine intestinal mucosa; several venom-derived peptides, e.g. exenatide, derived from Gila monster venom), and minerals (lithium salts).
- Semi-synthetic derivatives — chemical modification of a natural scaffold to improve potency, stability, oral bioavailability, or resistance to degradation (e.g. semi-synthetic penicillins and cephalosporins derived from the natural beta-lactam nucleus).
- Fully synthetic (chemical) drugs — designed and manufactured de novo through medicinal chemistry, typically the product of a structure-activity-relationship optimisation campaign around a lead compound (most NSAIDs, most antihypertensives).
- Biotechnological (biological) products — proteins, antibodies, and nucleic acids produced in living systems, as detailed in Chapter 5.

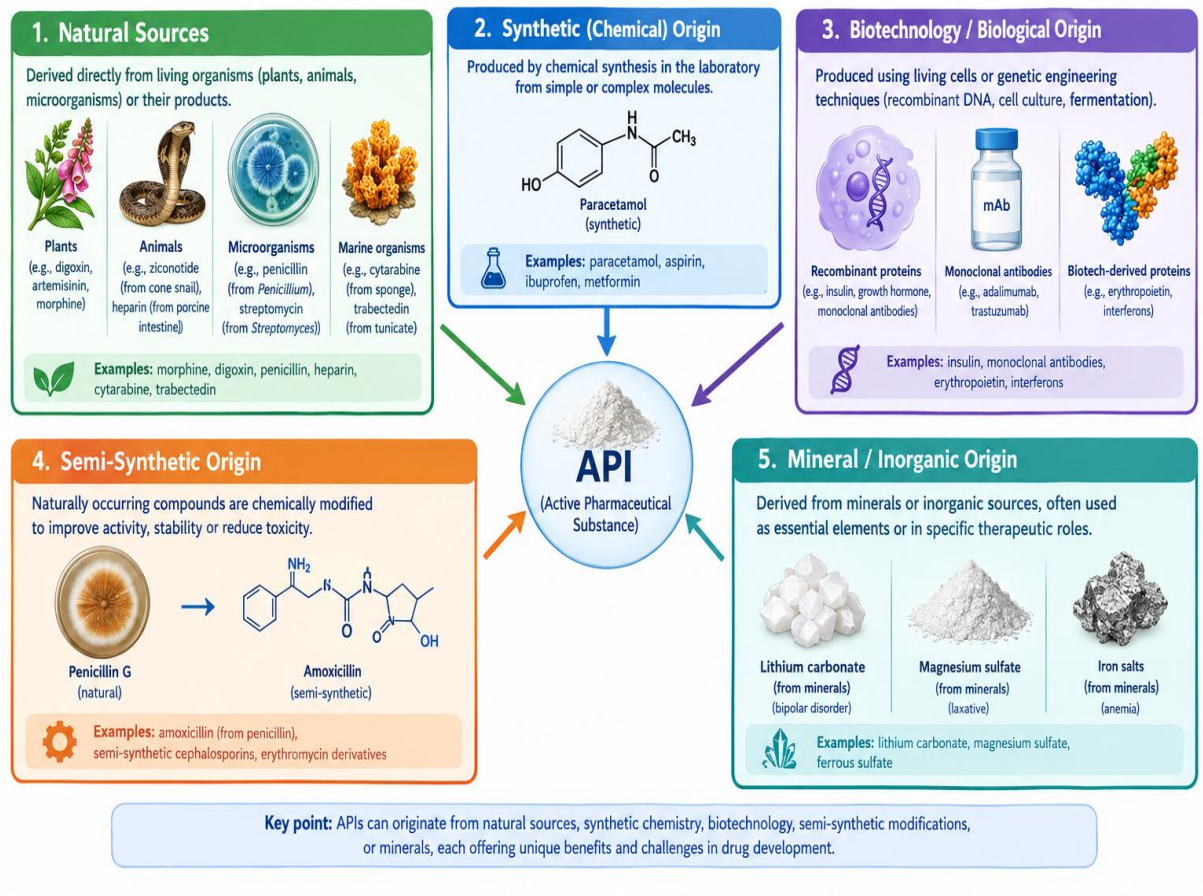


Figure 6. Origins and Sources of Active Pharmaceutical Substances

6.2 Pharmaceutical forms (dosage forms)

The active substance is rarely administered alone; it is formulated into a dosage form that governs its release, absorption, and stability.

- Solid oral forms: tablets (compressed, film-coated, enteric-coated to protect an acid-labile drug from gastric acid or the stomach from an irritant drug, and modified-release tablets designed for sustained release), capsules (hard gelatin capsules containing powder or pellets; soft gelatin capsules for liquid or oily fills), and granules/sachets.
- Liquid oral forms: solutions, syrups, suspensions (for drugs poorly soluble in water), and emulsions.

- Parenteral forms: solutions or suspensions for intravenous, intramuscular, or subcutaneous injection, requiring sterility, pyrogen-free preparation, and, for IV use, isotonicity.
- Topical and transdermal forms: creams, ointments, gels (local action), and transdermal patches (systemic action via sustained percutaneous absorption, e.g. fentanyl, nicotine, and hormone-replacement patches).
- Inhaled forms: pressurized metered-dose inhalers, dry-powder inhalers, and nebulizer solutions, delivering drug directly to the respiratory tract for local action (bronchodilators, inhaled corticosteroids) or, less commonly, for rapid systemic absorption.
- Rectal and vaginal forms: suppositories and pessaries, useful when oral administration is not possible or to reduce first-pass metabolism.
- Modified-release systems: formulations engineered to control the rate and/or site of drug release — sustained/extended-release matrices, delayed-release (enteric) coatings, and osmotic pump systems — to reduce dosing frequency, smooth the plasma concentration profile, and improve adherence.

6.3 From chemical structure to mechanism of action

The therapeutic effect of an active substance is the macroscopic, integrated consequence of a molecular interaction with one or more of the target classes described in Chapter 1. A systematic pharmacological description of any active substance addresses, in order: its chemical/pharmacological class, its molecular target(s) and mechanism of action, its principal pharmacodynamic effects (therapeutic and adverse), its pharmacokinetic profile (absorption, distribution, metabolism, excretion, half-life), its indications, its contraindications, and its clinically significant interactions. This template is applied systematically to the therapeutic classes covered in Chapters 7–12.

6.4 Formulation, route of administration, and the concept of bioequivalence

Two products containing the same active substance in the same dose are not necessarily therapeutically interchangeable: the dosage form, excipients, and manufacturing process can alter the rate and extent of absorption. Bioequivalence between a generic and a reference (originator) product is established by demonstrating that the 90% confidence interval for the ratio of key pharmacokinetic parameters (Cmax, AUC) falls within a predefined range (conventionally 80–125%) in a crossover study in healthy volunteers — the regulatory basis on which generic substitution is permitted.

Chapter 7: Antibiotics

7.1 General principles of antibacterial chemotherapy

An ideal antibiotic exploits selective toxicity: it interferes with a structure or process present in bacteria but absent, or sufficiently different, in human cells (the bacterial cell wall, the bacterial ribosome's 70S architecture, bacterial-specific enzymes). Antibiotics are classified as bactericidal (kill bacteria, generally preferred in severe infections and in immunocompromised patients) or bacteriostatic (inhibit growth, relying on the host immune system to clear the pathogen), and their activity is described by the minimum inhibitory concentration (MIC), the lowest concentration preventing visible growth, which underpins susceptibility testing and dosing strategy (time-dependent versus concentration-dependent killing).

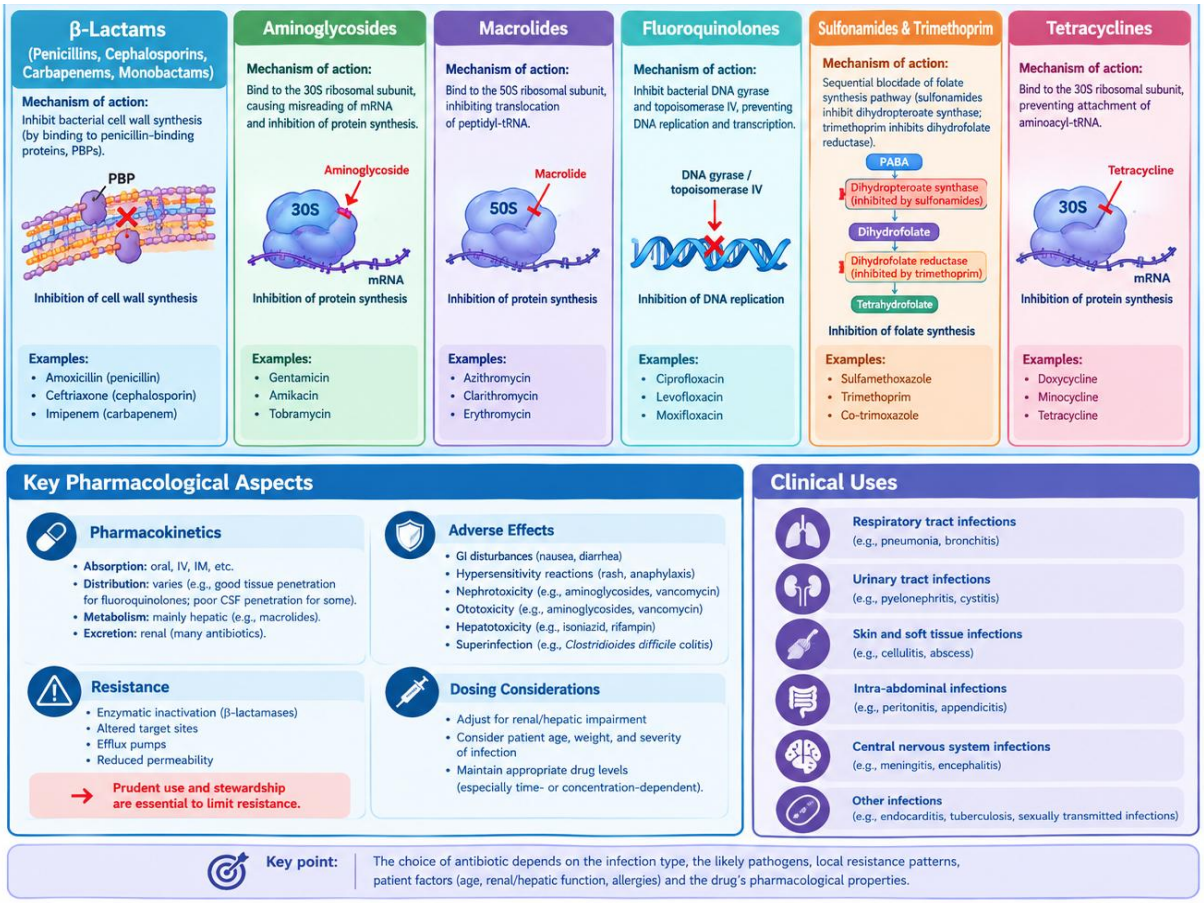


Figure 7. Pharmacological of antibiotics

7.2 Inhibitors of cell-wall synthesis

- Beta-lactams (penicillins, cephalosporins, carbapenems, monobactams) share a beta-lactam ring that mimics the terminal D-Ala-D-Ala of the peptidoglycan precursor, covalently inhibiting penicillin-binding proteins (transpeptidases) and blocking the final cross-linking step of peptidoglycan synthesis; because bacterial cells are hypertonic relative to their environment, the weakened wall leads to lysis. Resistance arises chiefly through beta-lactamase production (hydrolysing the beta-lactam ring, countered by combining a beta-lactam with a beta-lactamase inhibitor such as clavulanic acid) and through altered penicillin-binding proteins (as in methicillin-resistant *Staphylococcus aureus*).
- Glycopeptides (vancomycin, teicoplanin) bind the D-Ala-D-Ala terminus of peptidoglycan precursors directly, sterically blocking transpeptidation and transglycosylation; reserved largely for Gram-positive infections, including MRSA, because their large size prevents penetration of the Gram-negative outer membrane.

7.3 Inhibitors of protein synthesis

- Aminoglycosides (gentamicin, amikacin) bind the bacterial 30S ribosomal subunit, causing misreading of mRNA and inhibiting translocation; bactericidal, concentration-dependent, with a narrow therapeutic index (nephrotoxicity, ototoxicity) requiring therapeutic drug monitoring.
- Tetracyclines (doxycycline, minocycline) bind the 30S subunit and block aminoacyl-tRNA attachment; bacteriostatic, broad-spectrum, but contraindicated in pregnancy and young children (dental staining, bone growth effects).
- Macrolides (erythromycin, azithromycin, clarithromycin) bind the 50S subunit and block translocation; useful in atypical respiratory pathogens and as an alternative in penicillin allergy, with clinically important CYP3A4 inhibition (erythromycin, clarithromycin) and QT-prolongation risk.

- Lincosamides (clindamycin) and oxazolidinones (linezolid, active against resistant Gram-positive organisms including VRE, but with monoamine-oxidase-inhibitor-like interactions) also target the 50S subunit.
- Chloramphenicol inhibits peptidyl transferase on the 50S subunit; broad-spectrum but restricted by rare, dose-independent aplastic anaemia.

7.4 Inhibitors of nucleic acid synthesis

- Fluoroquinolones (ciprofloxacin, levofloxacin, moxifloxacin) inhibit bacterial DNA gyrase (topoisomerase II) and topoisomerase IV, preventing DNA supercoiling and replication; bactericidal, broad-spectrum, but associated with tendinopathy, QT prolongation, and increasing resistance that has narrowed their first-line role.
- Rifamycins (rifampicin) inhibit bacterial DNA-dependent RNA polymerase; central to antituberculous therapy, but a potent inducer of hepatic CYP enzymes, producing numerous drug interactions.
- Metronidazole, activated by reduction in anaerobic organisms to a cytotoxic radical that damages DNA, is active against anaerobic bacteria and protozoa.

7.5 Inhibitors of folate synthesis

Sulfonamides competitively inhibit dihydropteroate synthase and trimethoprim inhibits dihydrofolate reductase, blocking sequential steps of bacterial folate synthesis; combined (co-trimoxazole) for synergistic, sequential blockade. Bacteria, unlike human cells, must synthesise folate de novo (they cannot take up dietary folate), which underlies the selective toxicity of this class.

7.6 Inhibitors of membrane integrity

Polymyxins (colistin) act as cationic detergents that disrupt the bacterial outer and inner membrane, reserved for multidrug-resistant Gram-negative infections owing to significant nephrotoxicity. Daptomycin inserts into the Gram-positive cell membrane in a calcium-dependent manner, causing depolarisation and cell death.

7.7 Mechanisms of antibiotic resistance

Bacteria acquire resistance through four principal mechanisms: enzymatic inactivation of the drug (beta-lactamases, aminoglycoside-modifying enzymes), target modification (altered penicillin-binding proteins, altered ribosomal binding sites, altered DNA gyrase), reduced permeability/increased efflux (porin loss in Gram-negative outer membranes, efflux pump overexpression), and bypass pathways (alternative metabolic routes that circumvent the blocked step). Resistance genes spread both vertically (mutation and selection) and horizontally (plasmids, transposons, transformation, conjugation), the latter accounting for the rapid, often multidrug, spread of resistance seen clinically.

7.8 Principles of rational antibiotic use

Appropriate use requires matching the spectrum of activity to the likely or confirmed pathogen, dosing according to whether killing is time-dependent (beta-lactams, requiring the time above MIC to be maximised) or concentration-dependent (aminoglycosides, fluoroquinolones, requiring a high peak-to-MIC ratio), adjusting for site of infection (e.g. penetration across the blood–brain barrier in meningitis, or into abscesses), and antimicrobial stewardship to slow the emergence of resistance.

Chapter 8: Anti-infectives

This chapter covers antimicrobial classes directed against pathogens other than bacteria: viruses, fungi, and parasites (protozoa and helminths), each presenting a distinct pharmacological challenge because, unlike bacteria, these pathogens are eukaryotic (fungi, parasites) or obligate intracellular replicators using host-cell machinery (viruses) — narrowing the margin of selective toxicity.

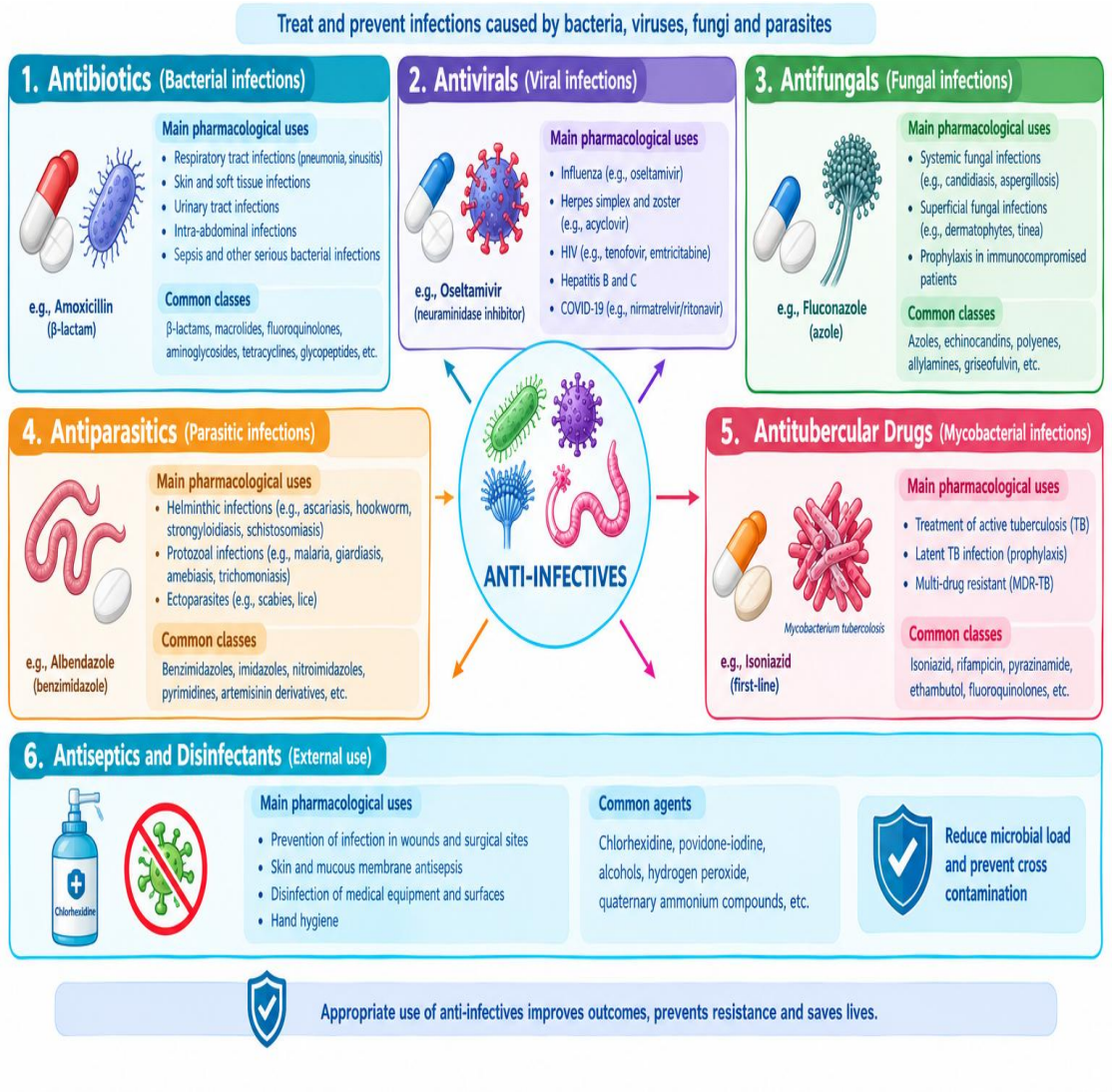


Figure 8. Pharmacological uses of anti-infective drugs

8.1 Antivirals

- Nucleoside/nucleotide analogues are activated by phosphorylation (often by a viral-specific kinase, enhancing selectivity) to compete with natural nucleotides for incorporation into viral nucleic acid, causing chain termination or lethal mutagenesis: aciclovir (herpesviruses, activated selectively by viral thymidine kinase), tenofovir and other nucleos(t)ide reverse transcriptase inhibitors (HIV, hepatitis B), and sofosbuvir (hepatitis C NS5B polymerase inhibitor).
- Non-nucleoside reverse transcriptase inhibitors (efavirenz, rilpivirine) bind an allosteric site on HIV reverse transcriptase.
- Protease inhibitors (ritonavir, lopinavir, and HCV protease inhibitors) block the viral protease responsible for cleaving polyprotein precursors into mature, functional viral proteins.
- Integrase strand-transfer inhibitors (dolutegravir, raltegravir) block insertion of the HIV pro-viral DNA into the host genome.
- Neuraminidase inhibitors (oseltamivir) block release of newly formed influenza virions from infected cells.
- Combination antiretroviral therapy (typically two nucleoside reverse transcriptase inhibitors plus an integrase inhibitor or a non-nucleoside reverse transcriptase inhibitor) is standard for HIV, both to maximise virological suppression and to raise the genetic barrier to resistance, since a virus would need to acquire multiple simultaneous mutations to escape all agents at once.
- Direct-acting antivirals for hepatitis C, combining agents against the NS3/4A protease, NS5A, and NS5B polymerase, achieve sustained virological response (functional cure) in the great majority of patients within 8–12 weeks — a major recent therapeutic success.

8.2 Antifungals

- Polyenes (amphotericin B) bind ergosterol in the fungal cell membrane, forming pores that disrupt ion gradients; broad-spectrum and fungicidal, but nephrotoxic, reflecting imperfect selectivity for fungal ergosterol over the structurally similar human membrane sterol, cholesterol.
- Azoles (fluconazole, itraconazole, voriconazole) inhibit fungal lanosterol 14-alpha-demethylase (a cytochrome P450 enzyme, CYP51), blocking ergosterol synthesis; the shared CYP mechanism with human drug-metabolising enzymes underlies extensive azole drug–drug interactions via inhibition of human CYP3A4 and other isoforms.
- Echinocandins (caspofungin, micafungin) inhibit 1,3-beta-D-glucan synthase, blocking synthesis of a fungal cell-wall polysaccharide that has no human counterpart, giving a favourable selectivity and safety profile.
- Allylamines (terbinafine) inhibit squalene epoxidase, an earlier step in ergosterol synthesis, used mainly topically or orally for dermatophyte infections.

8.3 Antiparasitics

- Antimalarials: chloroquine concentrates in the parasite digestive vacuole and interferes with detoxification of haem released during haemoglobin digestion; artemisinin derivatives generate reactive oxygen species and alkylate parasite proteins after activation by intraparasitic haem/iron, and are always used in artemisinin-based combination therapy to protect against resistance; mefloquine and other quinoline derivatives act at least partly through similar haem-related mechanisms. Chemoprophylaxis and case management strategies are informed by the local pattern of chloroquine and artemisinin resistance.
- Antiprotozoals: metronidazole (giardiasis, amoebiasis, trichomoniasis, via the mechanism described in Chapter 7), and antitrypanosomal/antileishmanial

agents with historically narrow therapeutic indices, reflecting the difficulty of achieving selective toxicity against a eukaryotic pathogen.

- Antihelminthics: benzimidazoles (albendazole, mebendazole) bind parasite beta-tubulin selectively, disrupting microtubule-dependent glucose uptake; ivermectin binds glutamate-gated chloride channels found in invertebrate but not vertebrate neurons, causing paralysis of the parasite; praziquantel increases calcium influx in schistosome tegument, causing paralysis and tegumental damage exposing the parasite to host immune attack.

8.4 Resistance across anti-infective classes

As with antibiotics (Chapter 7), resistance to antivirals, antifungals, and antiparasitics arises through target mutation (reducing drug binding, e.g. HIV reverse transcriptase mutations, *Plasmodium* chloroquine-resistance transporter mutations), reduced drug activation (e.g. loss of viral thymidine kinase reducing aciclovir activation), efflux pump upregulation, and, for some fungi, biofilm formation. Combination therapy (antiretrovirals, artemisinin-based combinations, some antituberculous regimens) is a deliberate strategy to raise the genetic barrier to resistance by requiring simultaneous mutations against multiple independent targets.

Chapter 9: Anti-inflammatories

9.1 The inflammatory cascade as a set of drug targets

Inflammation is initiated by tissue injury or infection, involving release of arachidonic-acid-derived autacoids (prostaglandins, thromboxane, leukotrienes), cytokines (TNF-alpha, IL-1, IL-6), histamine, and bradykinin, and the recruitment of leukocytes. Anti-inflammatory drugs act at different points of this cascade, giving rise to two major pharmacological classes: non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids, alongside disease-modifying and biological agents that overlap with Chapter 11.

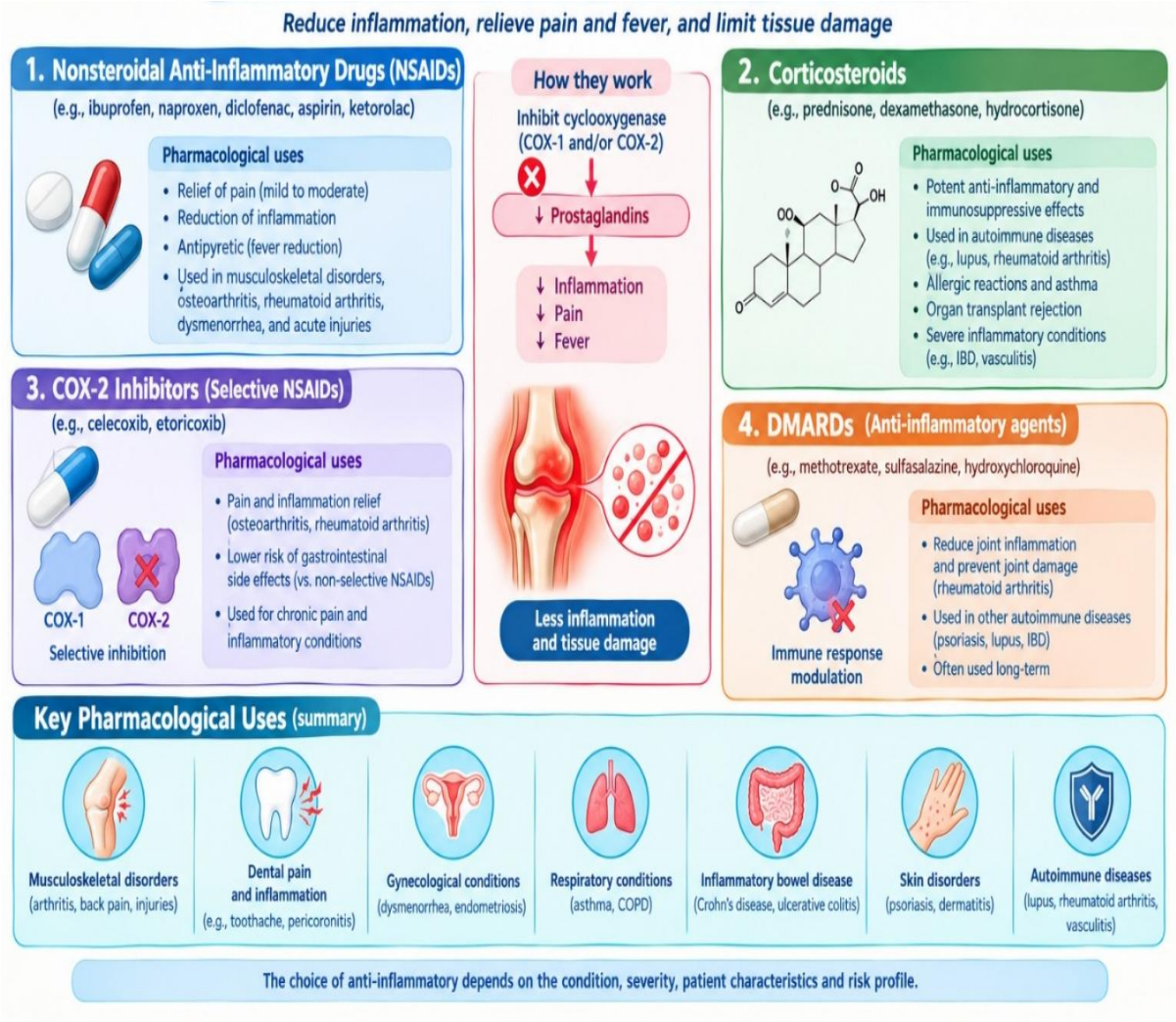


Figure 9. Pharmacological uses of anti-inflammatory drugs

9.2 Non-steroidal anti-inflammatory drugs (NSAIDs)

Mechanism: NSAIDs inhibit cyclo-oxygenase (COX), the enzyme that converts arachidonic acid to prostaglandin H2, the common precursor of prostaglandins and thromboxane A2. Two isoforms are pharmacologically relevant: COX-1, constitutively expressed, responsible for "housekeeping" prostaglandins that protect gastric mucosa, support renal blood flow, and enable platelet thromboxane A2 production; and COX-2, largely inducible at sites of inflammation (though also constitutively expressed in kidney and elsewhere), responsible for most of the prostaglandin production driving pain, fever, and inflammation.

- Non-selective NSAIDs (ibuprofen, naproxen, diclofenac) inhibit both isoforms, explaining both their efficacy and their characteristic adverse-effect profile: gastric mucosal damage and peptic ulceration (loss of COX-1-dependent mucosal protection), renal impairment (loss of prostaglandin-dependent afferent arteriolar vasodilation, particularly in volume-depleted or elderly patients), and, at low dose, an antiplatelet effect via thromboxane A2 inhibition.
- Aspirin is pharmacologically distinct: it irreversibly acetylates COX-1 (and COX-2), and because platelets cannot synthesise new enzyme (they are anucleate), a single low dose produces an antiplatelet effect lasting the lifespan of the platelet (~7–10 days) — the basis of low-dose aspirin in cardiovascular prevention, at doses well below those needed for analgesic/anti-inflammatory effect.
- Selective COX-2 inhibitors (coxibs) (celecoxib) were designed to spare COX-1-dependent gastric protection while retaining anti-inflammatory efficacy, reducing gastrointestinal toxicity; however, by also reducing COX-2-dependent vascular prostacyclin without affecting platelet thromboxane, they shift the thromboxane/prostacyclin balance towards thrombosis, and are

associated with increased cardiovascular risk, which curtailed their use after the withdrawal of rofecoxib.

- Paracetamol (acetaminophen), though often grouped with NSAIDs clinically, has weak peripheral COX inhibition and a poorly defined central mechanism (possibly involving central COX inhibition and the endocannabinoid system); it has analgesic and antipyretic but negligible peripheral anti-inflammatory or antiplatelet activity, and lacks the gastric/renal toxicity of NSAIDs, but carries a distinct risk of dose-dependent, glutathione-depletion-mediated hepatotoxicity in overdose.

9.3 Corticosteroids (glucocorticoids)

Mechanism: Glucocorticoids diffuse across the cell membrane and bind the cytoplasmic glucocorticoid receptor, a nuclear receptor (Chapter 1); the activated complex translocates to the nucleus and modulates gene transcription — transactivating anti-inflammatory genes (e.g. annexin-1/lipocortin-1, which inhibits phospholipase A2 and thereby reduces the availability of arachidonic acid, upstream of both the COX and lipoxygenase pathways) and transrepressing pro-inflammatory genes (via inhibition of transcription factors NF-κB and AP-1, reducing transcription of cytokines, chemokines, and adhesion molecules). This genomic mechanism is broader and slower in onset than NSAID action but far more comprehensively suppresses the inflammatory and immune response.

Clinical consequences of the breadth of action: efficacy across a very wide range of inflammatory and autoimmune conditions (asthma, rheumatoid arthritis, inflammatory bowel disease, transplant rejection prophylaxis) is matched by a correspondingly wide range of adverse effects on prolonged systemic use — hypothalamic-pituitary-adrenal axis suppression (requiring gradual tapering rather than abrupt withdrawal after prolonged use), hyperglycaemia, osteoporosis, myopathy, impaired wound healing, susceptibility to infection, cushingoid features, and psychiatric effects. Topical, inhaled, and intra-articular routes are used

specifically to concentrate anti-inflammatory effect at the target site while minimizing systemic exposure.

9.4 Antihistamines

H1-receptor antagonists competitively block histamine at the H1 receptor, reducing the vasodilation, increased vascular permeability, and pruritus of allergic and inflammatory reactions. First-generation agents (chlorphenamine, diphenhydramine) cross the blood–brain barrier and cause sedation and anticholinergic effects; second-generation agents (cetirizine, loratadine, fexofenadine) are engineered to be substrates of P-glycoprotein efflux at the blood–brain barrier, greatly reducing CNS penetration and sedation while retaining peripheral H1 antagonism.

9.5 Disease-modifying and biological anti-inflammatory agents

Conventional synthetic disease-modifying antirheumatic drugs (methotrexate, sulfasalazine, hydroxychloroquine) and biological agents targeting specific cytokines (anti-TNF agents: infliximab, adalimumab, etanercept; anti-IL-6: tocilizumab; anti-IL-17, anti-IL-23 agents in psoriasis) act further upstream than NSAIDs or, in some cases, more selectively than corticosteroids, aiming to arrest the underlying autoimmune/inflammatory process.

Chapter 10: Hormones

10.1 General principles of hormone pharmacology

Hormonal pharmacology exploits, replaces, or blocks the endocrine axes that regulate metabolism, growth, reproduction, and homeostasis. Because most endogenous hormones are subject to negative feedback regulation (a hypothalamic-pituitary-target-gland axis, or direct feedback of a hormone on its own secretion), exogenous hormone administration characteristically suppresses endogenous production — a principle with direct clinical consequences, from adrenal suppression by corticosteroids (Chapter 9) to ovarian suppression by combined hormonal contraceptives.

10.2 Insulin and glucose-lowering hormones

Insulin, acting through the insulin receptor tyrosine kinase (Chapter 1), promotes cellular glucose uptake (chiefly via GLUT4 translocation in muscle and adipose tissue), glycogen synthesis, lipogenesis, and protein synthesis, while suppressing hepatic gluconeogenesis and lipolysis. Recombinant human insulin and structurally modified insulin analogues — rapid-acting (insulin lispro, aspart, glulisine, engineered to dissociate from hexamers faster after subcutaneous injection) and long-acting (insulin glargine, degludec, engineered to precipitate or self-associate at the injection site or bind albumin, prolonging absorption) — allow flexible reproduction of physiological basal-bolus insulin profiles. GLP-1 receptor agonists (liraglutide, semaglutide) potentiate glucose-dependent insulin secretion, suppress glucagon, slow gastric emptying, and act centrally to reduce appetite.

10.3 Thyroid hormones and antithyroid drugs

Levothyroxine (T4), converted peripherally to the more active triiodothyronine (T3) by deiodinase enzymes, is used as replacement therapy in hypothyroidism, acting via nuclear thyroid hormone receptors to regulate basal metabolic rate. Antithyroid drugs (carbimazole, methimazole, propylthiouracil) inhibit thyroid peroxidase, the enzyme

responsible for iodination and coupling of thyroglobulin tyrosine residues, thereby blocking new hormone synthesis in hyperthyroidism (Graves' disease); propylthiouracil additionally inhibits peripheral T4-to-T3 conversion.

10.4 Adrenal corticosteroids beyond anti-inflammatory use

Beyond their anti-inflammatory application (Chapter 9), glucocorticoids are used as physiological replacement in adrenal insufficiency (hydrocortisone, mimicking the normal circadian cortisol rhythm), and mineralocorticoids (fludrocortisone) replace aldosterone deficiency, acting on the mineralocorticoid receptor in the renal distal tubule and collecting duct to promote sodium reabsorption and potassium/hydrogen excretion.

10.5 Sex steroids

- Oestrogens and progestogens are used in combined hormonal contraception (suppressing the hypothalamic-pituitary-ovarian axis, chiefly by negative feedback inhibition of gonadotropin-releasing hormone and consequently of luteinising-hormone-driven ovulation) and in menopausal hormone therapy.
- Selective oestrogen receptor modulators (SERMs), such as tamoxifen, act as oestrogen-receptor antagonists in some tissues (breast) while retaining partial agonist activity in others (endometrium, bone) — a tissue-selective pharmacology exploited in oestrogen-receptor-positive breast cancer treatment (Chapter 11).
- Anti-androgens (spironolactone, at high dose; finasteride, a 5-alpha-reductase inhibitor blocking conversion of testosterone to the more potent dihydrotestosterone) are used in hirsutism, benign prostatic hyperplasia, and androgenetic alopecia.
- Androgen-deprivation therapy in prostate cancer (GnRH agonists/antagonists, and direct androgen-receptor antagonists such as bicalutamide) is developed further in Chapter 11.

10.6 Pituitary and hypothalamic hormones

Gonadotropin-releasing hormone (GnRH) illustrates an important pharmacological principle: continuous (non-pulsatile) administration of a GnRH agonist (leuprolide, goserelin) paradoxically *suppresses* gonadotropin secretion after an initial "flare," by causing receptor desensitisation and downregulation, whereas the endogenous hormone is normally secreted in pulses and stimulates gonadotropin release — continuous exposure abolishes the pulsatility that pituitary GnRH receptors require to maintain responsiveness. GnRH agonists are therefore used for medical castration in prostate cancer and in endometriosis, while newer GnRH antagonists (degarelix) suppress gonadotropins immediately, without an initial flare.

10.7 Adrenal medullary and other hormone-related agents

Catecholamines (adrenaline, noradrenaline) and their synthetic analogues act at adrenergic receptors (Chapter 1) rather than through classical endocrine feedback loops, but are considered alongside hormone pharmacology because of their origin as adrenal medullary hormones; their pharmacology (alpha- and beta-adrenergic receptor subtype selectivity) underlies uses ranging from anaphylaxis and cardiac arrest (adrenaline) to shock (noradrenaline) and asthma/tocolysis (selective beta-2 agonists).

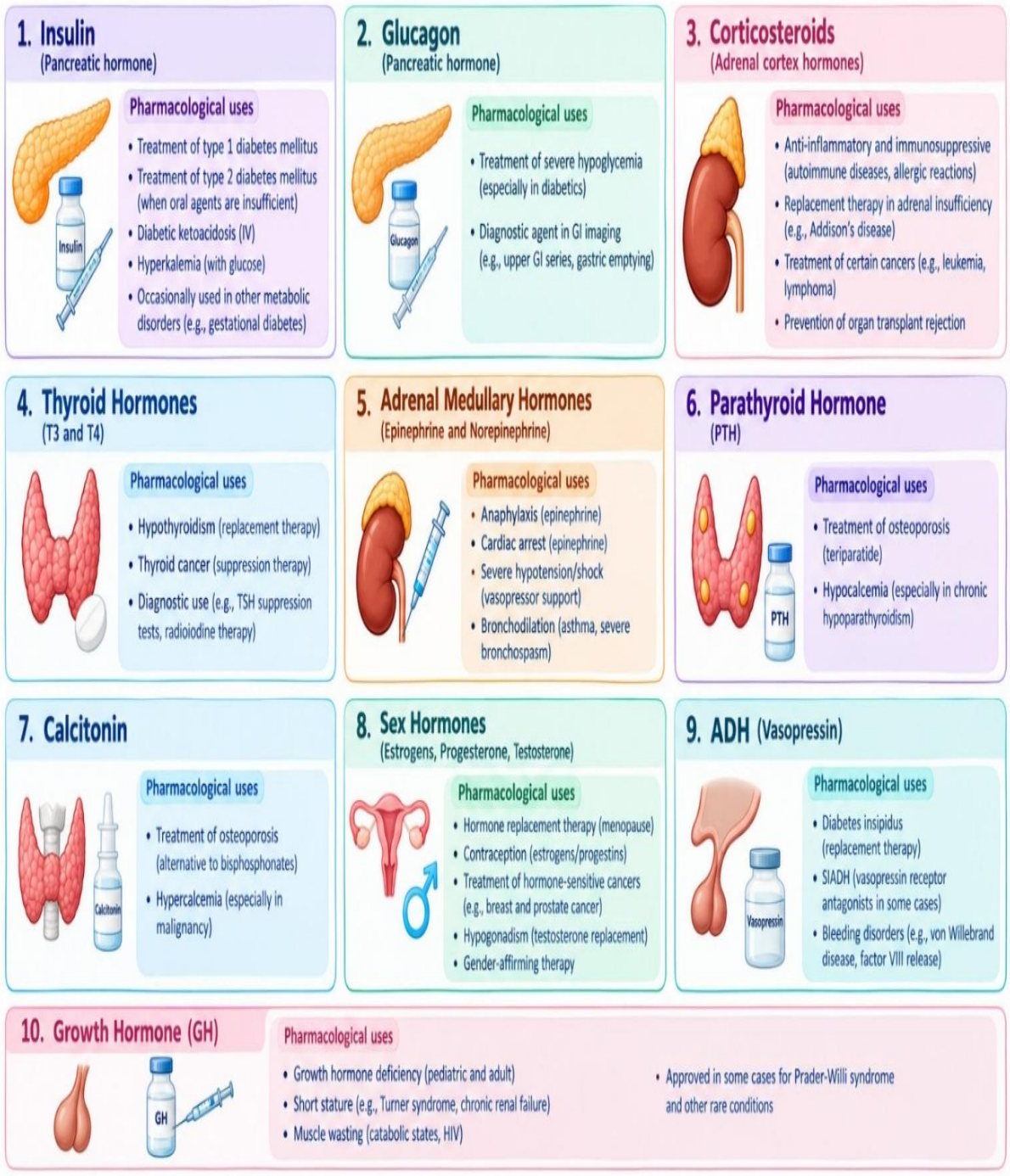


Figure 10. Pharmacological uses of hormones

Tutorial exercise

A patient with adrenal insufficiency is prescribed hydrocortisone in divided doses, with the larger dose given in the morning and a smaller dose in the afternoon, rather than a single equal daily dose.

(a) What physiological principle is this dosing schedule attempting to reproduce?

(b) Why might simply giving a single large morning dose instead risk inadequate cortisol activity later in the day?

Suggested solution:

(a) The regimen attempts to reproduce the normal circadian rhythm of endogenous cortisol secretion, which peaks shortly after waking and declines through the day to a trough around midnight — a pattern driven by the hypothalamic-pituitary-adrenal axis under central circadian control, which this patient can no longer generate endogenously.

(b) Hydrocortisone has a relatively short plasma half-life, so a single large morning dose would produce supraphysiological cortisol levels in the morning followed by a period of relative cortisol deficiency later in the day and overnight, as the dose is eliminated well before the next physiological trough would normally occur — divided dosing better sustains adequate, if imperfect, cortisol exposure across the full 24-hour cycle.

Chapter 11: Anticancer Agents and Immunomodulators

11.1 General principles of cancer pharmacology

Cancer chemotherapy exploits the fact that malignant cells, though derived from and resembling normal host cells, divide more rapidly and are enriched for specific molecular vulnerabilities. Because the margin of selectivity between tumor and normal proliferating tissue (bone marrow, gut epithelium, hair follicles) is narrower than in antimicrobial chemotherapy, dose-limiting toxicity is a defining feature of this drug class, and modern oncology increasingly favors agents that exploit tumor-specific molecular abnormalities (targeted therapy) or the immune system (immunotherapy) over cytotoxic agents that act indiscriminately on all dividing cells.

11.2 Cytotoxic (conventional) chemotherapy

- Alkylating agents (cyclophosphamide, cisplatin) form covalent cross-links within or between DNA strands, preventing strand separation during replication and transcription and triggering apoptosis; cell-cycle-non-specific, so active against both dividing and quiescent tumor cells.
- Antimetabolites (methotrexate, a dihydrofolate reductase inhibitor blocking thymidylate synthesis; 5-fluorouracil, a thymidylate synthase inhibitor after intracellular activation; cytarabine, a nucleoside analogue incorporated into DNA) mimic normal metabolic substrates and block nucleotide synthesis or incorporate into DNA/RNA to halt replication; generally, S-phase-specific.
- Antitumor antibiotics (doxorubicin) intercalate into DNA and inhibit topoisomerase II, preventing resealing of DNA strand breaks generated during replication, causing double-strand breaks and apoptosis; doxorubicin’s clinically important, cumulative-dose-dependent cardiotoxicity (via topoisomerase-II-beta-mediated mitochondrial injury and free-radical generation) limits lifetime exposure.

- Topoisomerase I inhibitors (irinotecan) trap the topoisomerase-I–DNA cleavage complex, converting a normally transient single-strand break into a replication-fork-collision-induced double-strand break.
- Mitotic spindle poisons: vinca alkaloids (vincristine) bind tubulin and prevent microtubule polymerization; taxanes (paclitaxel) bind tubulin and stabilize microtubules against depolymerization — both mechanisms arrest cells in mitosis and trigger apoptosis, and both produce dose-limiting peripheral neuropathy.

11.3 Targeted therapy

- Small-molecule tyrosine kinase inhibitors occupy the ATP-binding site of a constitutively activated or overexpressed kinase driving tumor growth: imatinib inhibits the BCR-ABL fusion kinase in chronic myeloid leukemia (a paradigm-defining example of molecularly targeted therapy); EGFR inhibitors (erlotinib, osimertinib) target activating EGFR mutations in non-small-cell lung cancer; BRAF inhibitors (vemurafenib) target the V600E-mutant kinase in melanoma.
- Monoclonal antibodies against tumor antigens or growth-factor pathways (Chapter 5): trastuzumab against HER2 in breast cancer; bevacizumab against VEGF, an anti-angiogenic strategy that starves the tumor of its blood supply rather than killing cells directly.
- PARP inhibitors (olaparib) exploit synthetic lethality: in tumors with a pre-existing defect in homologous-recombination DNA repair (e.g. BRCA1/2 mutations), inhibition of the base-excision-repair enzyme PARP leaves the cell unable to repair DNA damage by any pathway, selectively killing the tumor cell while sparing repair-competent normal cells.
- Proteasome inhibitors (bortezomib) block degradation of pro-apoptotic regulatory proteins, particularly exploited in multiple myeloma, where plasma

cells are highly dependent on proteasome function to manage the stress of high immunoglobulin production.

11.4 Cancer immunotherapy

- Immune checkpoint inhibitors (Chapter 5) block inhibitory receptors that tumors exploit to evade immune attack: anti-PD-1/PD-L1 antibodies restore T-cell cytotoxic activity against tumor cells expressing PD-L1; anti-CTLA-4 antibodies act earlier, at the level of T-cell priming in lymph nodes. Because these agents release a physiological brake on the immune system rather than acting on the tumor directly, their characteristic toxicities are immune-related adverse events (colitis, pneumonitis, endocrinopathies) resulting from loss of self-tolerance.
- CAR T-cell therapy (Chapter 5) has produced durable remissions in relapsed/refractory B-cell malignancies, at the cost of characteristic toxicities — cytokine release syndrome and neurotoxicity — arising from massive, rapid T-cell activation.

11.5 Immunomodulators beyond oncology

- Calcineurin inhibitors (ciclosporin, tacrolimus) bind intracellular immunophilins (cyclophilin, FKBP respectively); the resulting complex inhibits calcineurin, a phosphatase required to activate the transcription factor NFAT, blocking IL-2 gene transcription and T-cell activation — central to prophylaxis of transplant rejection.
- mTOR inhibitors (sirolimus) block a downstream kinase integrating growth-factor and nutrient signalling, inhibiting T- and B-cell proliferation, used in transplantation and, increasingly, in oncology.
- Antiproliferative immunosuppressants (mycophenolate mofetil, azathioprine) inhibit purine synthesis, preferentially affecting lymphocytes, which are more dependent than most cell types on de novo (rather than salvage-pathway) purine synthesis.

- JAK inhibitors (tofacitinib, baricitinib) block Janus kinases downstream of cytokine receptors, interrupting cytokine signal transduction broadly, used in rheumatoid arthritis, inflammatory bowel disease, and myeloproliferative neoplasms.

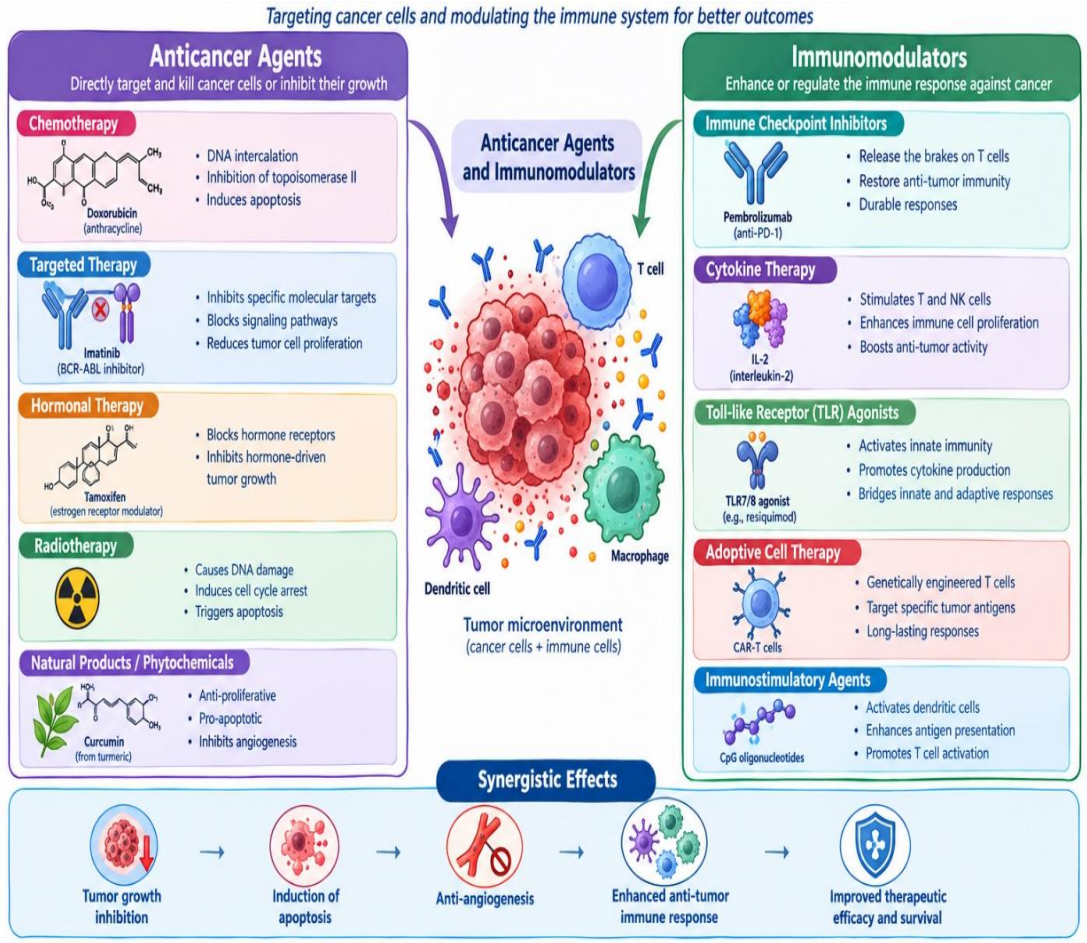


Figure 11. Anticancer agents and immunomodulators

Chapter 12: Vitamins

12.1 Vitamins as pharmacological agents

Vitamins are organic micronutrients required in small amounts for normal metabolism, that the body cannot synthesize (or cannot synthesize in sufficient quantity) and that must therefore be obtained from the diet or, pharmacologically, from supplementation. They become drugs (rather than nutrients) when administered to prevent or treat a defined deficiency state, or, for some vitamins, when used at pharmacological (supraphysiological) doses to exploit an effect distinct from correction of deficiency.

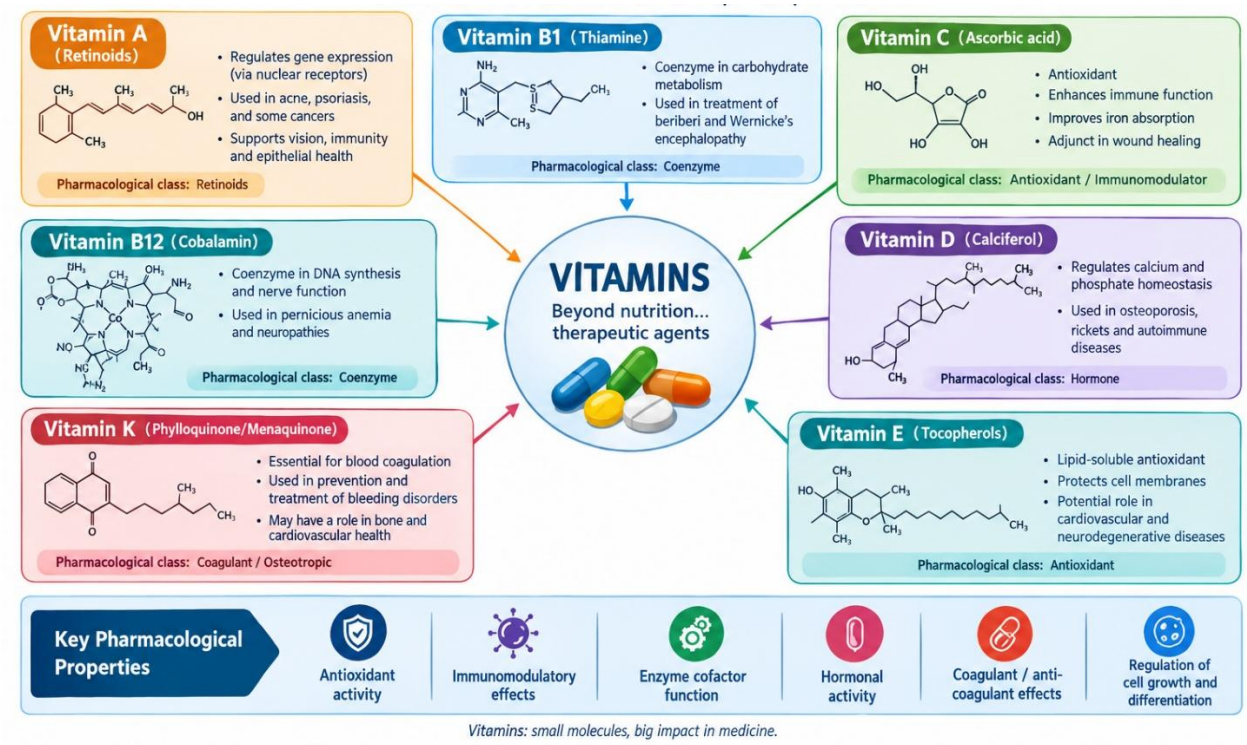


Figure 12. Vitamins as pharmacological agents

12.2 Fat-soluble vitamins

- Vitamin A (retinol and retinoids) is required for visual pigment (rhodopsin) regeneration, epithelial differentiation, and, via nuclear retinoic acid and retinoid X receptors, gene transcription. Deficiency causes night blindness and xerophthalmia; pharmacological retinoids (isotretinoin, all-trans retinoic acid)

are used in severe acne and, strikingly, as differentiation therapy in acute promyelocytic leukaemia, where all-trans retinoic acid overcomes a differentiation block caused by the PML-RARA fusion protein. Retinoids are potent teratogens, and isotretinoin use requires strict pregnancy-prevention measures.

- Vitamin D functions as a prohormone: cholecalciferol (from skin synthesis or diet) is hydroxylated in the liver to 25-hydroxyvitamin D and then in the kidney to the active metabolite 1,25-dihydroxyvitamin D (calcitriol), which binds a nuclear vitamin D receptor to increase intestinal calcium and phosphate absorption and regulate bone mineralisation. Deficiency causes rickets in children and osteomalacia in adults; active vitamin D analogues (calcitriol, alfacalcidol) bypass impaired renal hydroxylation in chronic kidney disease.
- Vitamin E (tocopherols) is a lipid-soluble chain-breaking antioxidant that protects polyunsaturated fatty acids in cell membranes from peroxidation; clinical deficiency is rare, seen mainly in fat-malabsorption states, and causes a peripheral neuropathy and ataxia.
- Vitamin K is an essential cofactor for hepatic gamma-glutamyl carboxylase, which carboxylates glutamate residues on clotting factors II, VII, IX, and X (and on proteins C and S), enabling their calcium-dependent binding to phospholipid membranes during coagulation. Warfarin acts by inhibiting VKORC1, the enzyme that regenerates reduced vitamin K required for this carboxylation cycle — the pharmacological basis of warfarin anticoagulation, and the reason vitamin K administration reverses warfarin's effect.

12.3 Water-soluble vitamins

- Vitamin B1 (thiamine) is a cofactor for pyruvate dehydrogenase, alpha-ketoglutarate dehydrogenase, and transketolase, all central to carbohydrate metabolism; deficiency causes beriberi and, in the context of alcohol use disorder, Wernicke encephalopathy, a medical emergency requiring parenteral

thiamine before glucose administration (to avoid precipitating or worsening Wernicke encephalopathy by consuming residual thiamine reserves in glucose metabolism).

- Vitamin B2 (riboflavin) and B3 (niacin) are precursors of the flavin (FAD, FMN) and nicotinamide (NAD, NADP) coenzymes central to cellular oxidation-reduction reactions; pharmacological-dose niacin also lowers LDL-cholesterol and triglycerides and raises HDL-cholesterol (via inhibition of hepatocyte diacylglycerol acyltransferase and adipocyte lipolysis), though its cardiovascular outcome benefit beyond statin therapy has not been established in outcome trials, and use is limited by flushing (a prostaglandin-D2-mediated effect that can be attenuated by aspirin pre-treatment).
- Vitamin B6 (pyridoxine) is a cofactor for transamination and decarboxylation reactions, including neurotransmitter synthesis; co-administered with isoniazid to prevent isoniazid-induced peripheral neuropathy (isoniazid depletes pyridoxine by forming an inactive hydrazone).
- Vitamin B9 (folate/folic acid) is required for one-carbon transfer reactions in nucleotide synthesis; deficiency causes megaloblastic anaemia and, in pregnancy, is associated with neural-tube defects, the rationale for periconceptional folic-acid supplementation. Folate metabolism is the pharmacological target of methotrexate (Chapter 11) and trimethoprim (Chapter 7), and folinic acid (leucovorin), a reduced folate that bypasses dihydrofolate reductase, is used as “rescue” after high-dose methotrexate.
- Vitamin B12 (cobalamin) is required, together with folate, for DNA synthesis (as a cofactor for methionine synthase) and, independently, for myelin maintenance (as a cofactor for methylmalonyl-CoA mutase); deficiency, often due to impaired absorption (pernicious anaemia, from loss of intrinsic factor; or long-term metformin or proton-pump-inhibitor use), causes megaloblastic anaemia and a distinct, progressive neurological syndrome (subacute

combined degeneration of the spinal cord) that folate supplementation alone does not prevent and may even mask the deficiency by correcting the haematological but not the neurological manifestation.

- Vitamin C (ascorbic acid) is a cofactor for prolyl and lysyl hydroxylases required for collagen cross-linking, and functions as a water-soluble antioxidant; deficiency causes scurvy, characterised by impaired collagen synthesis (poor wound healing, gum disease, perifollicular haemorrhage).

12.4 Pharmacological principles specific to vitamin therapy

Vitamin pharmacology illustrates several principles that generalize across the course: the distinction between replacement (physiological) dosing, which simply corrects a deficiency, and pharmacological (supraphysiological) dosing, which exploits an effect unrelated to deficiency correction (retinoids in leukaemia, niacin in dyslipidaemia); the risk of toxicity from fat-soluble vitamin accumulation (vitamin A and D toxicity, because excess is stored rather than excreted, unlike water-soluble vitamins which are generally cleared renally once tissue stores and binding capacity are saturated); and the importance of route and sequencing in emergency correction of deficiency (parenteral thiamine before glucose in suspected Wernicke encephalopathy).

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