BP304T · SEMESTER III

BP304T General Pharmacology

Unit IV — Drug Discovery & Recent Trends

Drug Discovery and Preclinical Evaluation
Human-Relevant Screening Techniques
Recent Trends in Pharmacology

Pills & Python
Pharmacy • Data • AI • Healthcare

CHAPTER 14 — Drug Discovery and Preclinical Evaluation of New Drugs

(Unit IV: drug discovery overview; evaluation of new drugs; preclinical evaluation)

14.1 Learning Objectives

After studying this chapter, the student will be able to:

1. Define drug discovery, preclinical studies, and screening.
2. Describe the step-by-step journey of a new drug from idea to clinical trials.
3. List the main sources of new drug leads.
4. Explain how preclinical studies prove safety and effectiveness before human testing.
5. Name the essential animal experiments done before a drug enters clinical trials.

14.2 Definitions

- **Drug discovery:** The complete process of finding a new chemical compound (a "lead") that can become a useful medicine for a disease.
- **Lead compound (or lead):** A chemical that shows useful action against a disease target and is worth developing further.
- **Target:** The molecule in the body (usually a receptor or an enzyme) that the drug is designed to act on.
- **Preclinical studies:** All laboratory and animal experiments done BEFORE a drug is first tested in humans, to check safety and likely effectiveness.
- **Screening:** Testing many compounds quickly to find the few that have the desired action.
- **Hit:** A compound that shows positive action in an initial screen (weaker evidence than a "lead").
- **IND (Investigational New Drug) application:** The formal request submitted to drug regulators to get permission to start testing a new drug in humans.

14.3 Terminology

Term	Simple meaning
Lead optimization	Improving a lead's structure so it works better and is safer
SAR (Structure–Activity Relationship)	How changes in a chemical's structure change its action
Pharmacophore	The smallest part of a molecule needed for its action
HTS (High-Throughput Screening)	Testing thousands of compounds very fast with machines
ADME	Absorption, Distribution, Metabolism, Excretion (what the body does to the drug)
PK (Pharmacokinetics)	How the drug moves through the body over time
PD (Pharmacodynamics)	What the drug does to the body
Bioavailability	Fraction of the dose that actually reaches the blood
Therapeutic index	Rough measure of safety margin (toxic dose ÷ effective dose)
Pilot-scale synthesis	Making the drug in kilogram amounts for trials
GLP (Good Laboratory Practice)	Rules for running reliable, honest lab studies
GMP (Good Manufacturing Practice)	Rules for manufacturing clean, consistent medicines
GCP (Good Clinical Practice)	Rules for running safe, ethical human trials

14.4 Explanation — The Drug Discovery Journey

14.4.1 What is drug discovery?

Technical concept: Drug discovery is the multidisciplinary process of identifying a biological target involved in a disease and finding chemical compounds that modify it safely and effectively. **Simple explanation:** Think of a disease as a broken lock inside the body. Drug discovery is the long hunt for the right key (the drug) that fits the lock (the target) and fixes the problem — without breaking other locks nearby. **Example:** The "lock" is the enzyme HMG-CoA reductase, which makes cholesterol. The "key" was lovastatin, discovered in fungi, which blocks this enzyme and lowers blood cholesterol.

14.4.2 Sources of new drug leads

1. **Natural products:** Plants, microbes, marine life — e.g., penicillin from a fungus, digoxin from foxglove leaves, vincristine from periwinkle.
2. **Existing drugs (drug repurposing):** Old drugs tested for new diseases — cheaper and faster.
3. **Combinatorial chemistry libraries:** Thousands of chemicals made automatically and stored for screening.
4. **Computer-designed compounds (rational design):** Designing molecules on a computer to fit a known target shape.
5. **Serendipity (lucky accidents):** E.g., the antibiotic penicillin and the antidiabetic sulfonylureas were found by accident.

14.4.3 The stages of drug development (the complete pipeline)

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STEP 1: Target identification
  → Find a protein/enzyme/receptor linked to the disease.
STEP 2: Target validation
  → Prove that acting on this target really helps (animal models, gene studies).
STEP 3: Lead discovery (screening)
  → Test thousands of compounds → find "hits" → pick best "leads".
STEP 4: Lead optimization
  → Chemists improve the lead: stronger, more selective, fewer side effects.
STEP 5: Preclinical studies (animals + labs)
  → Safety, toxicity, ADME tests in animals (mice, rats, dogs).
STEP 6: IND application
  → If preclinical data looks safe, ask regulators for human-trial permission.
STEP 7: Clinical trials (Phase I-IV)
  → Tested in humans: safety (Phase I), effectiveness (Phase II-III), monitoring (Phase IV).
STEP 8: Marketing approval & post-marketing surveillance
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Analogy: Developing a drug is like developing a new student for an exam team — first you identify the talent pool (target), run trials among thousands of candidates (screening), train the best ones (lead optimization), test them in practice matches (preclinical/animal), then select them for the final tournament (clinical trials).

14.4.4 Preclinical evaluation — what exactly is tested in animals?

Preclinical studies must answer THREE questions:

1. **Is it safe?** (toxicity studies — single dose, repeated dose)
2. **Does it work?** (pharmacology — does it cure the disease in animal models?)
3. **What does the body do to it?** (ADME/PK — absorption, half-life, metabolism)

Key preclinical tests:

Test	What it checks
Acute toxicity	What happens after ONE dose; finds lethal dose (LD50)
Sub-acute / sub-chronic toxicity	What happens after repeated doses for weeks
Chronic toxicity + carcinogenicity	Months of dosing; does it cause cancer?
Pharmacokinetics (ADME)	How the drug is absorbed, distributed, broken down, removed
Safety pharmacology	Effect on heart, brain, lungs, kidneys (vital organs)
Reproductive toxicity	Effect on fertility, pregnancy, baby development
Genotoxicity	Does it damage DNA/mutations?
Formulation studies	Can it be made into a stable tablet/capsule/injection?

(Note: Units in Chapter 18 go deeper into toxicity tests.)

14.4.5 Who decides the rules?

- **India:** CDSCO (Central Drugs Standard Control Organisation) under the Drugs and Cosmetics Act — must approve human trials.
- **USA:** FDA (Food and Drug Administration) — approves IND and final marketing.
- **Europe:** EMA (European Medicines Agency).
- **International standards:** ICH guidelines (harmonized global rules), OECD test guidelines for toxicity tests, GLP for labs, GMP for manufacturing.

14.5 Classification / Comparison

Types of screening:

Type	Meaning	Example
In vitro screening	Tests done in glass/plates on cells or tissues (no whole animal)	Testing on cultured liver cells
In vivo screening	Tests done on a whole living animal	Testing blood pressure fall in rats
In silico screening	Tests done by computer simulation	Docking a molecule into an enzyme on screen
Ex vivo screening	Test on a tissue removed from the body, kept alive briefly	Isolated frog heart

14.6 Diagrams (draw these)

1. **Drug development pipeline diagram:** A left-to-right timeline arrow: Target ID → Target validation → Lead discovery → Lead optimization → Preclinical → IND → Clinical Phase I → II → III → Approval → Phase IV (post-marketing). Label approximate effort/cost rising at each step; most failures happen early.
2. **Funnel diagram:** A wide funnel at top (10,000 compounds screened) narrowing to: 250 leads → 10 preclinical candidates → 1–2 reach market. Title: "The drug discovery funnel — why drug development is expensive."
3. **Decision flow for a lead compound:** Lead → [Strong action on target? No → reject; Yes →] → [Safe in animals? No → modify or reject; Yes →] → [Good ADME? No → optimize; Yes →] → IND application → human trials.

14.7 Infographic Concept Box — "From Idea to Injection"

One-page visual: a winding road with 8 milestone flags (idea → target → lead → animal tests → human trials → approval). Icons: microscope (target), test tubes (screening), rat (preclinical), person (clinical), pill bottle (approval). Red stamp "Average time: 10–15 years". Bottom strip: "Only ~1 in 10,000 screened compounds reaches the market."

14.8 Examples

- **Natural lead → blockbuster:** Lovastatin (from the fungus *Aspergillus terreus*) → the statin family for cholesterol.
- **Serendipity:** Alexander Fleming noticed penicillin mould killing bacteria — accidental discovery.
- **Repurposing:** Sildenafil was developed for heart problems; became famous for erectile dysfunction.
- **Rational design:** Imatinib (Gleevec) designed on computer to block the BCR-ABL protein in chronic myeloid leukaemia.

14.9 Analogies

- Drug discovery funnel → filtering thousands of job applicants down to one hire.
- Target validation → checking the address before posting an important letter.
- Preclinical testing → a dress rehearsal before the main stage performance (clinical trials).

14.10 Pharmacy Relevance

A pharmacist must know how drugs are born, because new medicines arrive with limited data — the pharmacist counsels patients on proper use and watches for new side effects (pharmacovigilance). Pharmacists also sit on ethics committees that approve clinical trials, and work in clinical research as trial coordinators.

14.11 India Context

India is a major hub for clinical trials and generic drug manufacturing ("pharmacy of the world"). The CDSCO (New Drugs and Clinical Trials Rules) governs trial permissions. Indian companies and institutes (e.g., CSIR labs, NIPER) participate in drug discovery, especially from natural products like Ayurvedic plants. Ethical trial conduct and patient consent are strictly required by Indian law.

14.12 Key Points

1. Drug discovery = finding a lead → preclinical = proving it safe in animals → clinical = testing in humans.
2. Hits → leads → optimized leads → preclinical candidate.
3. Preclinical must answer: safe? effective? what does the body do to it?
4. Only ~1 in 10,000 screened compounds becomes a marketed drug; the process takes roughly 10–15 years.

14.13 Common Confusions

- **Hit vs lead:** A hit only shows early promise; a lead is a confirmed, developable compound.
- **Preclinical vs clinical:** Preclinical = animals/lab; clinical = humans. Confusing them is a common exam mistake.
- **Screening vs evaluation:** Screening finds candidates fast; evaluation proves them thoroughly.

14.14 Common Mistakes

1. Saying animal testing is "optional" — regulators REQUIRE preclinical safety data before human trials.
2. Calling a computer-designed molecule "already a drug" — it still needs years of testing.
3. Mixing up IND (permission for human trials) with final marketing approval.

14.15 Mnemonic

"T-L-O-P-I-C-A" — Target, Lead, Optimize, Preclinical, IND, Clinical, Approval. (Or: "**T**en **L**ong **O**rdeals **P**recede **I**ntroduction to **C**linics, then **A**pproval.")

14.16 Exam Summary

- Drug discovery stages: target ID → validation → lead discovery → optimization → preclinical → IND → clinical trials → approval.
- Lead sources: natural products, repurposing, combinatorial libraries, rational design, serendipity.
- Preclinical tests cover safety (acute/sub-chronic/chronic toxicity), efficacy (animal disease models), and ADME.
- Regulators: CDSCO (India), FDA (USA), EMA (Europe); standards: ICH, GLP, GMP, GCP, OECD.

14.17 One-Minute Revision

Target → lead → optimize → animal safety tests → human trials → approval. ~1/10,000 compounds succeed. Preclinical answers: safe? works? ADME? Regulators need this data before any human testing.

14.18 Exam Questions

Short notes: (a) Sources of drug leads. (b) Stages of drug discovery. (c) Role of preclinical studies. (d) GLP, GMP, GCP. **Long answer:** Describe the complete drug discovery and development process from target identification to marketing approval, including the essential preclinical studies. **MCQs:**

1. The first stage of drug development is: (a) Clinical trial (b) Target identification (c) Marketing (d) Lead optimization — Ans: (b)
2. A "lead compound" is: (a) The final marketed drug (b) A compound with confirmed useful action worth developing (c) A failed chemical (d) A placebo — Ans: (b)
3. IND application is submitted for: (a) Animal testing (b) Permission to start human trials (c) Export license (d) Patent filing — Ans: (b)
4. Which is NOT a preclinical study? (a) Acute toxicity (b) Phase III trial (c) ADME (d) Genotoxicity — Ans: (b)
5. In India, clinical trials are approved by: (a) WHO (b) CDSCO (c) ICMR (d) NMC — Ans: (b)

Viva: Q: Why are animal studies required before human trials? A: To detect toxicity and prove likely safety/effectiveness; regulators require it. Q: What is a "hit" vs "lead"? A: Hit = early positive signal; lead = confirmed developable compound. **Application-based:** A company wants to test a new Ayurvedic plant extract for diabetes. List the first three steps they must take before human trials. (Target identification → screening for active lead → preclinical safety/toxicity studies.)

14.19 References

1. Brunton LL, Hilal-Dandan R, Knollmann BC, editors. Goodman & Gilman's The Pharmacological Basis of Therapeutics. McGraw-Hill.
2. Rang HP, Dale MM, Ritter JM, Flower RJ, Henderson G. Rang and Dale's Pharmacology. Elsevier.
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CHAPTER 15 — Human-Relevant Screening Techniques: In Vitro, Ex Vivo and In Silico

(Unit IV: reconstructed human epidermis; organ-on-chip; skin irritancy by reconstructed corneal epithelium; skin corrosivity by Direct Peptide Reactivity Assay; advantages/disadvantages of in vitro and in silico; pharmacological screening and evaluation)

15.1 Learning Objectives

After studying this chapter, the student will be able to:

1. Define in vitro, ex vivo, in silico, and human-relevant screening.
2. Describe reconstructed human epidermis, organ-on-chip, and reconstructed corneal epithelium tests.
3. Explain the Direct Peptide Reactivity Assay for skin corrosivity.
4. List advantages and disadvantages of in vitro and in silico methods.
5. Compare traditional animal screening with modern human-relevant methods.

15.2 Definitions

- **Human-relevant screening:** Testing methods that use human cells, human tissues, or computer models of human biology instead of (or before) animal experiments, so results are closer to what happens in people.
- **In vitro:** Literally "in glass" — experiments done in laboratory vessels (test tubes, plates) using cells or tissues, not a whole living animal.
- **Ex vivo:** "Out of the living" — experiments on tissues or organs taken from a body and kept alive for a short time outside it.
- **In silico:** "In silicon" (computer chips) — experiments done by computer simulation, using software models instead of living material.
- **Reconstructed human epidermis (RhE):** A lab-grown sheet of human skin cells (keratinocytes) that forms a skin-like outer layer, used to test irritation and corrosion without animals.
- **Organ-on-chip:** A tiny transparent device containing living human cells arranged to mimic a human organ (lung, liver, heart), with fluids flowing through micro-channels like blood.
- **Reconstructed human corneal epithelium:** Lab-grown human eye-surface cells used to test whether a chemical irritates the eye.
- **Direct Peptide Reactivity Assay (DPRA):** A chemistry-based test that measures whether a chemical reacts with small protein pieces (peptides) — reactivity predicts skin sensitization/allergy risk.
- **Skin irritancy:** Reversible damage (redness, swelling) caused by a chemical touching the skin.
- **Skin corrosivity:** Irreversible, deeper destruction of skin tissue by a chemical (burn-like damage).
- **The 3Rs:** Replace, Reduce, Refine — the ethical principle guiding modern animal testing (replace animals where possible, reduce numbers, refine procedures to minimize suffering).

15.3 Terminology

Term	Simple meaning
Keratinocyte	The main cell type of the outer skin layer
Viability	Whether cells are alive and healthy
Cytotoxicity	Ability of a chemical to kill cells
Microfluidics	Handling tiny amounts of liquid in microscopic channels
Molecular docking	Computer simulation showing how a drug molecule fits into a target protein
QSAR (Quantitative SAR)	Math models linking a chemical's structure to its activity
Validation	Official proof that a new test method works reliably (accepted by OECD)
OECD Test Guideline	An internationally accepted standard method for a safety test
Positive control	A known-active substance run alongside to prove the test is working
False negative	A dangerous chemical wrongly judged "safe" (the worst error)
False positive	A safe chemical wrongly judged "dangerous"

15.4 Explanation

15.4.1 Why human-relevant methods? The problem with animals alone

Technical concept: Animal biology differs from human biology (different enzymes, receptors, metabolism), so some drugs that are safe in animals fail in humans, and vice versa. Human-relevant methods close this "translation gap." **Simple explanation:** A rat is not a small human. Testing only on rats is like judging a Hindi movie by showing it to people who only speak French — you will miss a lot. Human cells and computer models of human biology give answers closer to real patients. **Example:** Some painkillers work well in mice but poorly in humans because the pain receptors differ slightly. Testing on human nerve cells in a dish can catch this earlier.

15.4.2 Reconstructed human epidermis (RhE) — skin testing without animals

Technical concept: Human skin cells (keratinocytes) are grown in the lab on a membrane at the air–liquid interface, where they form a layered, skin-like outer surface. Test chemicals are applied on top; damage is measured by cell viability (living cells convert a dye; dead cells cannot). **Simple explanation:** Scientists grow a small patch of artificial human skin in a dish. They put the test chemical on it. If the "skin" cells die, the chemical is irritating or corrosive — no rabbit needed. **Diagram to draw:** A cross-section: (top) applied chemical droplet → layered keratinocytes → membrane support → nutrient medium below. Arrow to a second panel showing "measure viability with dye → less colour = more damage." **Used for:** Skin irritation and skin corrosion testing (replacing the old rabbit skin test).

15.4.3 Organ-on-chip — a tiny human organ on a slide

Technical concept: Living human cells are placed in micro-channels on a chip; nutrient fluid flows through like blood. Different chambers can mimic lung (breathing motion), liver (drug breakdown), heart (beating cells), gut, or kidney. **Simple explanation:** Imagine a computer chip, but instead of wires it has tiny canals with living human cells and liquid flowing like blood. It behaves like a mini-liver or mini-lung, so you can watch how a drug is broken down or whether it harms the organ — in real time, under a microscope. **Example:** A "liver-on-chip" can show how fast liver cells break down a new drug, predicting how long the drug lasts in the human body. **Diagram to draw:** A rectangle chip with two parallel channels separated by a porous membrane; top channel = cells of the organ; bottom channel = flowing fluid; arrows showing flow; labels for inlet/outlet.

15.4.4 Skin irritancy by reconstructed corneal epithelium (eye irritation test)

Technical concept: Human corneal (eye-surface) cells are grown into a 3D tissue resembling the eye's outer layer. The test chemical is applied; damage is judged by cell viability. It replaces the old rabbit eye test (Draize test). **Simple explanation:** Instead of putting chemicals in a rabbit's eye (the old, cruel Draize test), scientists grow a tiny model of the human eye surface in the lab and test the chemical on it. Less harm to the model = safer for human eyes. **Used for:** Eye irritation classification of cosmetics, cleaners, industrial chemicals.

15.4.5 Skin corrosivity by Direct Peptide Reactivity Assay (DPRA)

Technical concept: Skin allergy (sensitization) often starts when a chemical binds to skin proteins. DPRA is a pure chemistry test — no cells at all: the chemical is mixed with synthetic peptides (small protein pieces); if the chemical reacts with them and depletes them (measured by chromatography), it is flagged as a potential skin sensitizer/corrosive agent. **Simple explanation:** Allergic skin reactions begin when a chemical sticks to skin proteins. DPRA simply mixes the chemical with tiny protein pieces in a tube and checks: did they stick together? Sticking = warning sign of skin allergy risk. **Diagram to draw:** Test tube 1: chemical + peptides → measure remaining free peptides. Bar chart: high remaining peptides = low risk; low remaining = high risk (chemical reacted). **Key point:** DPRA is part of a battery of non-animal tests used together to judge skin sensitization.

15.4.6 In vitro — advantages and disadvantages

Advantages	Disadvantages
No or fewer animals needed (ethical, follows 3Rs)	A dish of cells is simpler than a whole body — misses whole-body effects
Faster and cheaper than animal studies	Cells in a dish may behave differently than in the body
Uses HUMAN cells → more human-relevant	No blood circulation, immune system, or organ interactions (unless chip models)
Easy to control conditions precisely	Difficult to study long-term (chronic) effects
Good for screening thousands of compounds quickly	A negative result still usually needs animal confirmation for regulators

15.4.7 In silico — advantages and disadvantages

Advantages	Disadvantages
Extremely fast and cheap — screen millions of compounds	Only as good as the data and model behind it ("garbage in, garbage out")
Zero animals, zero chemicals, zero waste	Cannot fully replace real biology — predictions must be confirmed in the lab
Can predict ADME, toxicity, and drug–target fit before synthesis	Complex biology (immune reactions, rare side effects) is hard to model
Helps design better molecules (fewer dead ends)	Regulators accept it as supporting evidence, not as the only proof
Useful for QSAR, molecular docking, predicting metabolism	Requires skilled scientists and good software

Analogy: In silico is like a flight simulator for pilots — excellent for training and practice, cheap and safe, but the pilot must still fly a real plane before carrying passengers. Similarly, computer predictions must be confirmed by real lab tests.

15.4.8 Pharmacological screening and evaluation — putting it together

Modern screening follows a **tiered (step-by-step) strategy**:

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Step 1: IN SILICO — computer screening of lakhs of compounds → shortlist hundreds
Step 2: IN VITRO — test shortlist on cells/tissues → shortlist dozens
Step 3: EX VIVO — test on isolated tissues (e.g., isolated heart, gut strip)
Step 4: IN VIVO (animals) — only the best candidates, minimum animals (3Rs)
Step 5: Human-relevant confirmatory tests (organ-on-chip, RhE) alongside
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Key takeaway: Each tier filters out failures cheaply, so expensive animal tests are used only for the most promising compounds.

15.5 Classification

Screening methods by system:

1. **Whole-animal (in vivo):** e.g., blood pressure in anaesthetized rats, pain tests in mice.
2. **Isolated tissue (ex vivo):** e.g., isolated guinea-pig ileum for gut drugs, isolated frog/rat heart.
3. **Cell-based (in vitro):** e.g., cultured liver cells for metabolism, RhE for skin.
4. **Cell-free (in vitro chemistry):** e.g., DPRA, enzyme inhibition assays.
5. **Computer-based (in silico):** e.g., docking, QSAR, ADME prediction.

15.6 Diagrams (draw these)

1. **3Rs triangle:** Three corners labelled Replace, Reduce, Refine with one-line meanings.
2. **Tiered screening funnel:** Wide top (in silico, lakhs of compounds) narrowing through in vitro → ex vivo → in vivo (few candidates).
3. **Organ-on-chip schematic:** As described in 15.4.3.
4. **DPRA concept:** Chemical + peptide → reacted complex; bar chart of remaining free peptide vs risk.

15.7 Infographic Concept Box — "Testing Without Tears"

One-page visual: left side shows the OLD way (rabbit with a red cross over it, labelled "old animal-only testing"), right side shows the NEW way with four icons — petri dish with skin cells (RhE), microchip with cells (organ-on-chip), eye model (corneal epithelium), computer (in silico). Banner: "3Rs: Replace • Reduce • Refine". Bottom line: "Human cells + smart computers = safer medicines, fewer animals."

15.8 Examples

- **RhE:** A new fairness cream is tested on reconstructed human epidermis before any animal test — if cells die, the formula is rejected.
- **Organ-on-chip:** A new TB drug candidate is run through a liver-on-chip to check whether liver cells break it down too fast.
- **DPRA:** A hair-dye chemical is mixed with peptides; heavy peptide depletion flags it as a likely skin sensitizer.
- **In silico:** Before making 500 new molecules, chemists dock them into the target enzyme on computer and synthesize only the best-fitting 20.

15.9 Analogies

- Tiered screening → sieving flour: coarse sieve first (computer), then finer sieves (cells), keeping only the finest powder (best candidates).
- Organ-on-chip → a miniature aquarium that behaves like a real pond ecosystem.
- DPRA → checking whether two magnets stick together in a box, instead of testing on a person's skin.

15.10 Pharmacy Relevance

Pharmacists explain to patients why new medicines take years to arrive — safety testing is the reason. Pharmacists in industry work in drug safety departments running these screens. Knowledge of in vitro/in silico methods helps pharmacists critically read "new drug" news and advise on cosmetics and herbal products tested by modern methods.

15.11 India Context

India's CDSCO accepts OECD-validated non-animal methods, and Indian pharma companies increasingly use in silico and cell-based screening to cut costs. Indian institutes (CSIR-CDRI Lucknow, NIPER) have organ-on-chip and computational drug-design facilities. Reducing animal use also aligns with CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) rules that govern animal houses in India.

15.12 Key Points

1. Human-relevant methods = human cells/tissues + computer models; they reduce the animal-to-human translation gap.
2. RhE tests skin irritation/corrosion; reconstructed corneal epithelium tests eye irritation; DPRA is a chemistry test for skin sensitization risk.
3. Organ-on-chip mimics human organs with flowing fluids.
4. In vitro: fast, cheap, human cells — but simpler than a whole body. In silico: fastest and cheapest — but predictions need lab confirmation.
5. Modern strategy is tiered: in silico → in vitro → ex vivo → in vivo (minimum animals), guided by the 3Rs.

15.13 Common Confusions

- **In vitro vs ex vivo vs in silico:** In vitro = cells in a dish; ex vivo = tissue taken from a body; in silico = computer only.
- **Irritation vs corrosion vs sensitization:** Irritation = reversible redness; corrosion = irreversible tissue destruction; sensitization = allergy that develops after repeat contact.
- **RhE vs corneal epithelium:** RhE models SKIN; corneal epithelium models the EYE — different tests, different purposes.

15.14 Common Mistakes

1. Saying "in silico can fully replace animal testing" — regulators still require animal safety data; computers support but do not replace.
2. Calling DPRA a "cell test" — it is a cell-free chemistry test (peptides in a tube).
3. Thinking organ-on-chip is a "computer model" — it uses REAL living cells on a chip.

15.15 Mnemonic

"3Rs save lives twice" — Replace, Reduce, Refine: methods that save animal lives AND give results closer to human lives. For the four named techniques: **"RECO-D"** — Reconstructed Epidermis, Corneal epithelium, Organ-on-chip, DPRA.

15.16 Exam Summary

- Human-relevant screening bridges the animal–human gap using human cells and computer models.
- RhE (skin), corneal epithelium (eye), organ-on-chip (organ functions), DPRA (peptide reactivity for sensitization).
- In vitro pros: ethical, fast, human cells; cons: simplified, needs confirmation. In silico pros: fastest/cheapest; cons: model-dependent, needs lab proof.
- Tiered screening + 3Rs is the modern standard.

15.17 One-Minute Revision

Human cells in dishes (RhE for skin, corneal cells for eye), organs on chips, peptide chemistry (DPRA), and computer models (in silico) now screen drugs before/without animals. Pros: faster, cheaper, ethical, human-relevant. Cons: simplified biology, predictions need proof. Always tiered: computer → cells → tissues → minimal animals. 3Rs: Replace, Reduce, Refine.

15.18 Exam Questions

Short notes: (a) Reconstructed human epidermis. (b) Organ-on-chip. (c) DPRA. (d) 3Rs principle. (e) Advantages and disadvantages of in silico screening. **Long answer:** Describe the human-relevant alternatives to animal testing in drug evaluation. Discuss the advantages and disadvantages of in vitro and in silico methods. **MCQs:**

1. RhE is used to test: (a) Liver function (b) Skin irritation/corrosion (c) Blood clotting (d) Kidney damage — Ans: (b)
2. DPRA measures: (a) Cell death (b) Chemical reactivity with peptides (c) Blood pressure (d) DNA damage — Ans: (b)
3. "In silico" means: (a) In glass vessels (b) In a living animal (c) By computer simulation (d) In isolated tissue — Ans: (c)
4. The 3Rs are: (a) Read, Write, Remember (b) Replace, Reduce, Refine (c) Rat, Rabbit, Rodent (d) Risk, Reward, Review — Ans: (b)
5. Reconstructed corneal epithelium replaces which old test? (a) Rabbit eye (Draize) test (b) Blood sugar test (c) Skin patch test (d) Liver biopsy — Ans: (a)

Viva: Q: Why can't in silico fully replace animal testing today? A: Models are simplifications; complex whole-body effects and rare toxicities need living systems; regulators require animal data. Q: Is DPRA a cell-based test? A: No — cell-free chemistry test using synthetic peptides. **Application-based:** A cosmetic company wants to launch a new face wash. Suggest a testing plan using at least two human-relevant methods and justify the order. (In silico ingredient check → RhE skin irritation test → corneal epithelium eye test; animals avoided per 3Rs.)

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CHAPTER 16 — Recent Trends in Pharmacology

(Unit IV: chronopharmacology — biological clock, rhythms, hormones, diseases/drugs affected, chronokinetics, chronotherapy, future scope; pharmacogenomics, gene therapy, biosimilars, precision medicine — introduction, principles, applications, scope)

16.1 Learning Objectives

After studying this chapter, the student will be able to:

1. Define chronopharmacology, biological rhythms, and chronotherapy.
2. Name the types of biological rhythms with examples.
3. Explain how circadian rhythm affects hormones, diseases, and drug action.
4. Define chronokinetics and state the importance of chronotherapy.
5. Define pharmacogenomics, gene therapy, biosimilars, and precision medicine, and give applications of each.

16.2 Definitions

- **Chronopharmacology:** The study of how the TIMING of drug administration (time of day/night) changes the drug's effects — because the body itself runs on rhythms.
- **Biological clock:** The body's internal timekeeping system (mainly in the brain's suprachiasmatic nucleus) that runs ~24-hour cycles of sleep, hormones, temperature, and metabolism.
- **Circadian rhythm:** A biological rhythm repeating roughly every 24 hours (e.g., sleep–wake cycle).
- **Chronokinetics:** How the body's handling of a drug (absorption, metabolism) changes with the time of day.
- **Chronotherapy:** Giving medicines at the RIGHT TIME of day to get maximum benefit and minimum side effects.
- **Pharmacogenomics:** The study of how a person's GENES affect their response to drugs (who benefits, who gets side effects, what dose is right).
- **Gene therapy:** Treating disease by delivering working genes (or fixing faulty genes) inside the patient's cells.
- **Biosimilar:** A medicine that is highly similar (not identical) to an already-approved biological medicine (the "reference" product), with no meaningful clinical difference.
- **Precision medicine (personalized medicine):** Choosing treatment based on the individual patient's genes, lifestyle, and disease characteristics — "the right drug, right dose, right patient, right time."
- **Biological (biologic):** A medicine made from living cells (e.g., insulin, monoclonal antibodies, vaccines) — large, complex molecules.

16.3 Terminology

Term	Simple meaning
Ultradian rhythm	Rhythm shorter than a day (e.g., heartbeat, sleep stages ~90 min)
Infradian rhythm	Rhythm longer than a day (e.g., menstrual cycle ~28 days)
Circannual rhythm	Yearly rhythm (e.g., seasonal mood changes)
Zeitgeber	An external cue that sets the clock (mainly light; "time-giver")
Melatonin	The "night hormone" — rises in darkness, promotes sleep
Cortisol	The "morning hormone" — peaks early morning, prepares the body for the day
Polymorphism	A common variation in a gene between people
Vector	The carrier (often a harmless virus) used to deliver genes in gene therapy
Immunogenicity	Tendency of a biologic to trigger an immune reaction
Companion diagnostic	A lab test that checks a patient's gene before prescribing a matched drug
Interchangeable	A biosimilar judged safe to substitute for the reference product

16.4 Explanation

16.4.1 The biological clock — your body has a timetable

Technical concept: The suprachiasmatic nucleus (SCN) in the brain acts as the master clock, synchronized mainly by light. It drives 24-hour rhythms in hormone release, body temperature, blood pressure, and liver enzyme activity. **Simple explanation:** Your body is like a railway station with a fixed timetable — certain "trains" (hormones, body functions) always arrive at certain times. Cortisol (energy hormone) peaks in the early morning; melatonin (sleep hormone) rises at night; body temperature is lowest around 4 a.m. **Diagram to draw:** A 24-hour clock face: mark 6 a.m. (cortisol peak, blood pressure rises), 12 noon (peak alertness), 10 p.m. (melatonin rises), 2–4 a.m. (lowest temperature, deepest sleep, asthma attacks common).

16.4.2 Types of biological rhythms

Type	Period	Examples
Ultradian	< 24 hours	Heartbeat, breathing, 90-min sleep cycles, hourly hormone pulses
Circadian	~24 hours	Sleep–wake, cortisol/melatonin cycles, body temperature
Infradian	> 24 hours	Menstrual cycle (~28 days)
Circannual	~1 year	Seasonal depression, seasonal allergies

16.4.3 Hormones, diseases, and drugs affected by circadian rhythm

Hormones: Cortisol (morning peak), melatonin (night rise), growth hormone (released in deep sleep), insulin sensitivity (better in morning). **Diseases with day–night patterns:**

- **Asthma:** attacks worst in late night/early morning (airways narrowest ~4 a.m.).
- **Heart attack & stroke:** most common in early morning (blood pressure and clotting peak).
- **Arthritis pain:** worst in the morning (stiffness after night).
- **Allergic rhinitis:** sneezing worst in the morning.
- **Stomach acid:** peaks at night (ulcer pain wakes patients).

Drugs whose timing matters:

- **Statins (cholesterol drugs):** cholesterol is made mostly at night → short-acting statins work best taken in the EVENING.
- **Asthma inhalers:** evening dosing protects against night-time attacks.
- **Blood pressure drugs:** morning dosing matches the morning blood-pressure surge.
- **Anti-ulcer drugs:** evening dose controls night-time acid.

16.4.4 Chronokinetics and chronotherapy

Chronokinetics: The same dose of a drug can be absorbed faster, broken down slower, or removed quicker depending on the time of day — because gut movement, liver enzymes, and kidney function all follow rhythms. **Chronotherapy:** Deliberately timing doses to match the body's rhythm — e.g., giving an asthma drug in the evening so its peak covers the dangerous early-morning hours. **Importance:** Better effect, lower dose needed, fewer side effects, better patient compliance. **Future scope:** "Smart" pills and pumps that release drugs at programmed times; hospital dosing schedules redesigned around rhythms; chronotherapy combined with precision medicine (right drug + right gene + right time). **Analogy:** Watering plants — you water in the cool morning/evening, not at burning noon, because timing decides how much the plant actually absorbs. Same drug, different time = different result.

16.4.5 Pharmacogenomics — genes decide drug response

Technical concept: Small gene variations (polymorphisms) change drug-metabolizing enzymes, drug targets, or transporters, so the same dose can be perfect for one patient, useless for another, or toxic for a third. **Simple explanation:** Just as people have different shoe sizes, people have different "drug sizes" written in their genes. A standard dose is like giving everyone size-8 shoes — it fits most, but some need size 6 and some size 10. **Example:** The liver enzyme CYP2D6 breaks down many drugs. "Poor metabolizers" (slow gene version) get drug build-up and side effects from normal doses; "ultra-rapid metabolizers" clear the drug too fast and get no benefit. **Applications:** Choosing the right dose, predicting side effects, companion diagnostics (test the gene first, then prescribe), avoiding trial-and-error prescribing. **Diagram to draw:** Three patients → same tablet → three outcomes: poor metabolizer (drug accumulates → toxicity), normal (good effect), ultra-rapid (drug cleared → no effect).

16.4.6 Gene therapy — fixing the faulty instruction

Technical concept: A working copy of a gene is delivered into the patient's cells using a vector (often a harmless modified virus), so the cells start making the missing or correct protein. **Simple explanation:** If a disease is caused by a broken "instruction page" (gene) in the body's manual, gene therapy pastes in a correct replacement page so the body can read it and make the right protein. **Example:** In haemophilia (missing clotting factor), gene therapy delivers the clotting-factor gene to liver cells so the patient starts producing it. **General principles:** (1) Identify the faulty gene; (2) package a correct copy in a vector; (3) deliver to target cells; (4) confirm the protein is made and the disease improves. **Cautious note:** Still expensive and developing; long-term safety is under study; usually reserved for serious genetic diseases with no good alternatives.

16.4.7 Biosimilars — "almost twins" of biologic drugs

Technical concept: Biologics are made in living cells, so exact copies are impossible; a biosimilar is proven through comparison studies to be highly similar to the reference biologic, with no clinically meaningful difference in safety or effectiveness. They are NOT the same as generic drugs (generics are exact chemical copies of small-molecule drugs). **Simple explanation:** A generic drug is like a photocopy — exactly the same. A biosimilar is like a hand-painted copy of a famous painting by the same skilled artist — looks and works the same, but made through a living process that can never be pixel-perfect. Regulators check it very carefully before approval. **Example:** Biosimilar versions of insulin and of monoclonal antibodies used in cancer and arthritis cost less than the originals. **Applications/scope:** Lower treatment cost, wider access to expensive biologics, growing market in India and worldwide.

16.4.8 Precision medicine — treatment tailor-made for the patient

Technical concept: Combines the patient's genetic profile, biomarkers, lifestyle, and disease subtype to select the therapy most likely to work — moving away from "one drug fits all." **Simple explanation:** Instead of giving every fever patient the same tablet, precision medicine first asks: what exactly is causing YOUR fever, and what do YOUR genes say about which drug suits you? Then it picks the best match. **Example:** In some cancers, the tumour is tested for specific gene mutations, and only patients with that mutation receive the matching targeted drug (companion diagnostic). **Scope:** Cancer treatment, rare genetic diseases, psychiatry, cardiology — expected to grow as gene testing becomes cheaper.

16.5 Classification / Comparison

Biosimilar vs generic:

Feature	Generic drug	Biosimilar
Type of original	Small chemical molecule	Large biologic from living cells
Copy exactness	Identical chemical copy	Highly similar, not identical
Approval basis	Bioequivalence study	Extensive comparison (lab, animal, clinical)
Example	Generic paracetamol	Biosimilar insulin

The "omics" family (don't confuse):

- Pharmacogenomics → genes vs drug response
- Pharmacogenetics → similar, usually single-gene focus (older term)
- Proteomics → all proteins; Metabolomics → all small metabolites

16.6 Diagrams (draw these)

1. **24-hour body clock** (as in 16.4.1).
2. **Chronotherapy timing chart:** Disease (asthma, hypertension, ulcer, high cholesterol) → worst time → best dosing time.
3. **Pharmacogenomics outcomes** (as in 16.4.5).
4. **Gene therapy steps:** Faulty gene → vector with correct gene → delivery → protein made → disease improves.

16.7 Infographic Concept Box — "Right Drug, Right Gene, Right Time"

One-page visual split into two halves. Left half: a clock with drug tablets at 8 p.m. labelled "Chronotherapy — timing matters" and disease icons (lungs at 4 a.m., heart at 6 a.m.). Right half: a DNA helix with three patient silhouettes getting different tablet sizes labelled "Pharmacogenomics + Precision medicine — dose by gene". Bottom strip: gene therapy (fixed DNA) and biosimilars (two similar vials, lower price tag).

16.8 Examples

- Statin at night (cholesterol made at night).
- Evening inhaled steroid for night-time asthma.
- CYP2D6 testing before codeine (poor metabolizers get no pain relief; ultra-rapid metabolizers risk toxicity).
- Biosimilar insulin at lower cost for diabetes.
- Tumour gene test before targeted cancer therapy (precision medicine).
- Gene therapy for haemophilia (clotting factor gene delivered to liver).

16.9 Analogies

- Biological clock → railway timetable; chronotherapy → catching the right train.
- Pharmacogenomics → shoe sizes for drugs.
- Biosimilar vs generic → hand-painted copy vs photocopy.
- Gene therapy → replacing a torn page in an instruction manual.

16.10 Pharmacy Relevance

Pharmacists counsel patients WHEN to take medicines (morning/evening, before food) — chronotherapy knowledge makes this scientific. Pharmacists interpret pharmacogenomic test reports, watch for biosimilar substitution rules, and educate patients that biosimilars are safe, effective alternatives — not "duplicate/fake" drugs.

16.11 India Context

India's large generic industry is now a growing biosimilar hub ("Similar Biologics" guidelines by CDSCO/DBT). Pharmacogenomic testing is becoming available in Indian metro hospitals. Gene therapy research is at an early stage in India with high costs; ethical guidelines govern its use. Timing-based dosing advice is already common in Indian practice (e.g., statins at night).

16.12 Key Points

1. Chronopharmacology = time-of-day effects on drugs; chronotherapy = dosing at the best time.
2. Rhythms: ultradian (<24 h), circadian (~24 h), infradian (>24 h), circannual (yearly).
3. Asthma, heart attacks, arthritis, acidity all follow daily patterns — timing of drugs matters.
4. Pharmacogenomics = genes decide drug response (e.g., CYP2D6 metabolizer types).
5. Gene therapy delivers working genes via vectors; biosimilars are highly similar (not identical) copies of biologics; precision medicine matches treatment to the individual.

16.13 Common Confusions

- **Chronokinetics vs chronotherapy vs chronopharmacology:** Kinetics = how drug handling varies with time; therapy = dosing at the right time; pharmacology = the whole science of time effects.
- **Biosimilar vs generic:** Generics are identical chemical copies; biosimilars are similar but not identical (biologics can't be copied exactly).
- **Pharmacogenomics vs gene therapy:** Pharmacogenomics STUDIES gene–drug links; gene therapy TREATS by delivering genes.

16.14 Common Mistakes

1. Saying "take all medicines in the morning" — timing must match the disease rhythm.
2. Calling a biosimilar a "generic" — wrong category, wrong approval pathway.
3. Thinking gene therapy = "changing the patient's DNA permanently in all cells" — most current therapies target specific tissues, and germline (inheritable) editing is not the standard approach.

16.15 Mnemonic

"CLOCK" for chronotherapy examples — **C**holesterol (statins at night), **L**ungs (asthma drugs in evening), **O** (blood pressure — morning), **C** (acidity — night), **K**eep timing in mind! For trends: **"PG-BGP"** — **P**harmaco**g**enomics, **B**iosimilars, **G**ene therapy, **P**recision medicine.

16.16 Exam Summary

- Biological clock (SCN, light as zeitgeber); rhythms: ultradian, circadian, infradian, circannual.
- Cortisol (morning), melatonin (night); diseases: asthma (4 a.m.), MI/stroke (morning), arthritis (morning), acidity (night).
- Chronokinetics (time-varying ADME) → chronotherapy (right-time dosing); future: timed-release systems.
- Pharmacogenomics: gene variants (e.g., CYP2D6) → dose/toxicity differences; companion diagnostics.
- Gene therapy: vector-delivered working genes. Biosimilars: highly similar biologics, cheaper. Precision medicine: right drug/dose/patient/time.

16.17 One-Minute Revision

Body runs on a 24-hour clock; drugs work differently at different times — dose asthma drugs in the evening, statins at night, BP drugs in the morning. Genes decide your "drug size" (pharmacogenomics). Broken genes can be replaced (gene therapy). Expensive biologics get cheaper near-twins (biosimilars). All together = precision medicine: right drug, right dose, right patient, right time.

16.18 Exam Questions

Short notes: (a) Biological clock and types of rhythms. (b) Chronotherapy with two examples. (c) Pharmacogenomics. (d) Biosimilars vs generics. (e) Gene therapy — principles. **Long answer:** Explain chronopharmacology: biological clock, types of rhythms, and how circadian rhythm affects diseases and drug therapy. Add a note on chronotherapy and its future scope. / Write notes on pharmacogenomics, gene therapy, biosimilars, and precision medicine with applications. **MCQs:**

1. The master biological clock is located in the: (a) Liver (b) Suprachiasmatic nucleus (c) Heart (d) Kidney — Ans: (b)
2. Short-acting statins are best taken: (a) Morning (b) Afternoon (c) Evening/night (d) Anytime — Ans: (c)
3. Asthma attacks are worst at: (a) Noon (b) Late night/early morning (c) Evening (d) After lunch — Ans: (b)
4. A biosimilar is: (a) An identical chemical copy (b) Highly similar to a reference biologic, not identical (c) A placebo (d) A herbal drug — Ans: (b)
5. CYP2D6 poor metabolizers given a normal dose may show: (a) No effect at all (b) Drug accumulation and toxicity (c) Faster cure (d) Immunity — Ans: (b)

Viva: Q: Define chronotherapy with one example. A: Dosing at the best time of day — e.g., statins at night. Q: How is a biosimilar different from a generic? A: Generic = identical small molecule; biosimilar = highly similar (not identical) biologic needing extensive comparison. **Application-based:** A patient on codeine gets no pain relief while another on the same dose feels drowsy and nauseous. Explain using pharmacogenomics. (CYP2D6 ultra-rapid vs poor/intermediate metabolism — codeine needs conversion to morphine; gene variants change conversion.)

16.19 References

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