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MPL103T Pharmacological and Toxicological Screening Methods I, M.Pharm Semester 1

Unit 4: Preclinical Screening: Cardiovascular, Metabolic, Anticancer and Hepatoprotective Pharmacology

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4.1 Screening of antihypertensive drugs

Hypertension screening needs animals whose blood pressure is genuinely raised, plus acute preparations that reveal mechanism.

Model	How hypertension is produced	What it screens best
Spontaneously hypertensive rat (SHR)	Genetic, pressure rises with age	Chronic oral therapy of all classes; the standard chronic model
Renal hypertension (Goldblatt)	Renal artery clipping (two kidney one clip, or one kidney one clip) raises renin dependent pressure	Renin angiotensin system drugs: ACE inhibitors, angiotensin receptor blockers
DOCA salt model	Mineralocorticoid plus salt and uninephrectomy, volume dependent, low renin	Diuretics and agents working in low renin states
L-NAME model	Chronic nitric oxide synthase inhibition	Endothelium targeted and vasodilator agents
Acute anaesthetised preparations	Direct arterial recording in rat, cat or dog	Immediate mechanism: ganglion blockade, alpha blockade, adrenaline reversal

Endpoints are systolic and diastolic pressure and heart rate in conscious animals (tail cuff or, better, radiotelemetry) over days to weeks of dosing. Captopril or losartan are standard comparators in renin dependent models, amlodipine and hydrochlorothiazide across the battery.

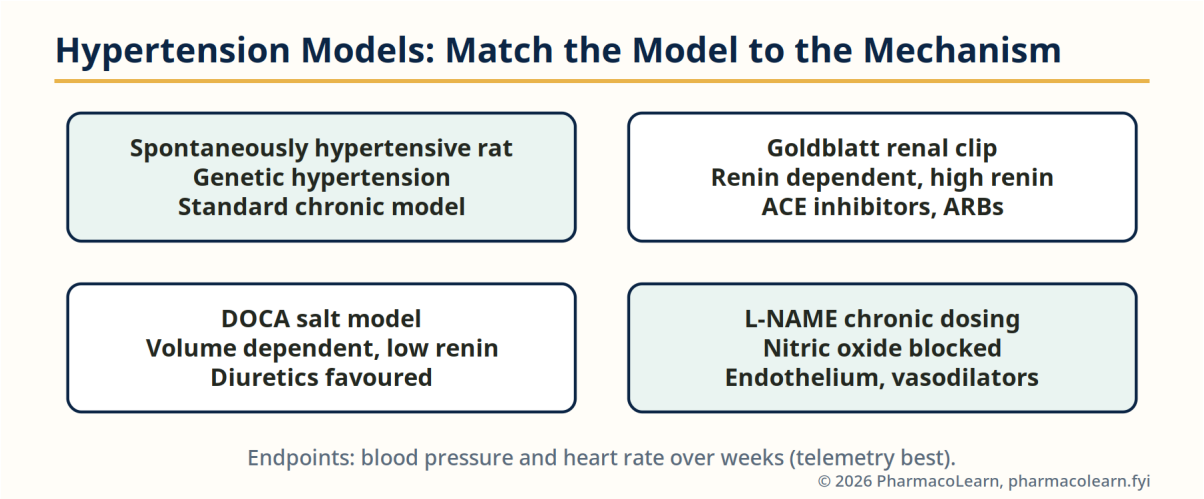

Figure: Four hypertension models, four different driving mechanisms. The model chosen decides which drug classes can show their effect.

4.2 Screening of antiarrhythmic drugs

Arrhythmias are induced by chemistry, electricity and ischaemia, and each inducer reads a different drug class.

- **Chemically induced:** aconitine (opens sodium channels; screens sodium channel blockers), ouabain or digoxin toxicity arrhythmias, adrenaline or isoprenaline arrhythmias in sensitised (halothane anaesthetised) animals, calcium chloride and chloroform adrenaline models, and barium chloride arrhythmias.
- **Electrically induced:** programmed stimulation and fibrillation threshold in isolated hearts (Langendorff) and in vivo.
- **Ischaemia induced:** coronary ligation in rats and dogs produces occlusion and reperfusion arrhythmias, the closest model of the clinical event.
- Isolated atrial and Purkinje fibre preparations classify the mechanism Vaughan Williams style (sodium, potassium, calcium channel or beta blockade) before whole animal work.

Lidocaine, amiodarone and verapamil serve as standards according to the model chosen.

4.3 Screening of antianginal drugs

Angina is a supply demand imbalance, so screening measures coronary flow and cardiac work. Isolated Langendorff hearts and working heart preparations quantify coronary flow, rate and contractility under controlled load. In vivo, isoprenaline induced myocardial necrosis and tachycardia in rats models demand ischaemia, and coronary ligation or ameroid constriction models supply failure; endpoints include ECG ST segment changes, infarct size (triphenyltetrazolium staining) and cardiac enzymes. Vasospastic components are read on isolated coronary artery rings contracted with ergonovine or potassium. Glyceryl trinitrate, propranolol and amlodipine are the standing standards for the nitrate, beta blocker and calcium channel mechanisms respectively.

4.4 Screening of antiatherosclerotic agents and diuretics

Antiatherosclerotic screening builds the lesion first: rabbits and, increasingly, transgenic mice (apoE knockout, LDL receptor knockout) are fed cholesterol rich diets for weeks, and serum lipids, aortic plaque area (en face staining) and arterial histology are the endpoints. The cholesterol fed rabbit made this field and remains the clearest lesion model.

Diuretic screening is one of the oldest quantitative screens in pharmacology. In the **Lipschitz test**, rats are given a standard water and salt load, urine is collected over 5 to 24 hours in metabolic cages, and volume plus sodium, potassium and chloride excretion are compared with control and with furosemide or hydrochlorothiazide standards. The sodium to potassium ratio separates potassium wasting loop and thiazide agents from potassium sparing ones; urine pH and bicarbonate excretion reveal carbonic anhydrase inhibition (acetazolamide). Isolated perfused kidney and clearance studies refine mechanism.

4.5 Screening of antidiabetic drugs

Diabetes models divide into insulin deficient and insulin resistant types, and the screen must match the claimed mechanism.

Model	Induction	Suits
Streptozotocin (STZ) rat or mouse	Beta cell toxin, insulin deficient diabetes	Insulin, insulin secretagogues in residual function models
Alloxan model	Beta cell toxin (older, less selective)	Classical oral hypoglycaemic screening
Neonatal STZ, high fat diet plus low dose STZ	Partial deficiency with resistance	Type 2 agents broadly

Model	Induction	Suits
db/db mouse, ob/ob mouse, Zucker fatty rat	Genetic obesity and insulin resistance	Insulin sensitisers, metformin, thiazolidinediones
Glucose tolerance tests (oral and intraperitoneal)	Glucose challenge in normal or diabetic animals	Any agent, first line screen

Endpoints are fasting and post challenge glucose, HbA1c in chronic studies, insulin levels and tolerance tests. Alpha glucosidase inhibitors are screened in vitro against the enzyme and by sucrose loading tests in vivo (acarbose standard); DPP-4 inhibitors by enzyme assay plus glucose tolerance. Glibenclamide is the classical sulfonylurea standard and metformin the biguanide standard.

4.6 Screening of antidyslipidemic agents

Lipid screening begins in normal and diet loaded rats: a cholesterol and fat rich diet (often with cholic acid) raises serum cholesterol over days to weeks, and the test agent's effect on total cholesterol, LDL, HDL, triglycerides and the atherogenic index is compared with atorvastatin as the standing standard. The Triton WR-1339 model, in which a detergent blocks lipoprotein clearance and produces a sharp acute hyperlipidaemia, separates inhibitors of lipid synthesis from enhancers of clearance within hours. Transgenic apoE and LDL receptor knockout mice (section 4.4) carry the work into true atherosclerosis endpoints.

4.7 Screening of anticancer agents

Anticancer screening is the largest screening enterprise in pharmacology and runs in tiers.

1. **In vitro tier:** panels of human tumour cell lines (the NCI 60 panel is the reference set) measure growth inhibition (GI50) across cancers of many organs; a distinctive pattern of sensitivity hints at mechanism. Normal cell lines run alongside to estimate selectivity.
2. **Transplantable animal tumours:** rapidly growing syngeneic tumours in mice (Ehrlich ascites carcinoma, Sarcoma 180, Dalton's lymphoma, B16 melanoma, L1210 and P388 leukaemias) give the first in vivo read; endpoints are tumour weight and volume, and survival time.
3. **Human tumour xenografts:** human cancer cells grown in nude or SCID mice (which cannot reject them, section 1.5) test the agent against actual human tumour biology.
4. **Chemically induced and transgenic models:** agents such as DMBA and azoxymethane induce mammary and colon tumours for chemoprevention and slower growing tumour studies; oncogene transgenic mice model specific cancers.

Cyclophosphamide and 5-fluorouracil are among the standard comparators, chosen by tumour type. The toxicological shadow of this section is deliberate: anticancer screening always reports the therapeutic index, because these agents are given close to toxic doses.

4.8 Hepatoprotective screening methods

The liver is injured in a controlled way and protection is measured by enzymes, bilirubin and histology.

- **Carbon tetrachloride (CCl4):** converted by hepatic cytochrome P450 to a free radical that destroys hepatocytes; the classical acute hepatotoxicity model.
- **Paracetamol overdose:** glutathione depletion by the toxic metabolite NAPQI produces centrilobular necrosis; also the model where N-acetylcysteine is the mechanistic standard antidote.
- **Thioacetamide and D-galactosamine:** produce necrosis and a hepatitis like picture respectively; galactosamine injury is endotoxin sensitised and models viral like hepatitis.

- **Anti-tubercular drug (isoniazid plus rifampicin) and alcohol models** give clinically flavoured chronic injury.

Endpoints are serum SGOT (AST), SGPT (ALT), alkaline phosphatase and bilirubin, liver weight, and graded histopathology of sections. Silymarin, the milk thistle flavonolignan mixture, is the almost universal standard hepatoprotective comparator in this literature.

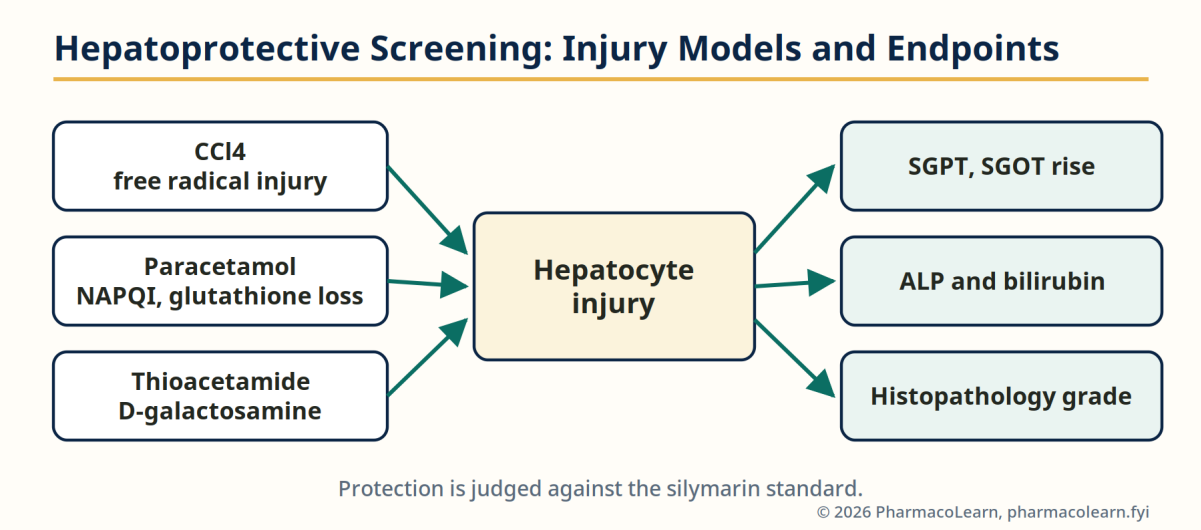

Figure: Hepatoprotective screening logic: controlled injury on the left, measurable liver endpoints on the right, silymarin as the standard.

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