Name of Unit	Preclinical Screening Models for CVS Activity and others
Subject /Course name	Experimental Pharmacology
Subject/Course ID	BP810ET
Class: B.Pharm. Semester	VIII
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Learning Outcome of Module 04

LO	Learning Outcome	Course Outcome Code
LO 1	To remember and understand the preclinical screening models used for cardiovascular system (CVS) drugs.	BP810.4
LO 2	To understand and explain the experimental models used for antihypertensives, diuretics, antiarrhythmic, antidyslipidemic, antiaggregatory, coagulants, and anticoagulants.	BP810.4
LO 3	To apply screening methods to evaluate drugs such as antiulcer, antidiabetic, anticancer, and antiasthmatic agents.	BP810.5
LO 4	To analyze and evaluate the pharmacological activity of different drugs using appropriate experimental models.	BP810.5

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Topic
• Preclinical Screening Models for CVS Activity Antihypertensives Diuretics Antiarrhythmic Antidyslepidemic anti aggregatory coagulants and anticoagulants. • Preclinical Screening Models for Other Important Drugs Antiulcer Antidiabetic Anticancer Antiasthmatics.

PRECLINICAL SCREENING MODELS FOR CVS ACTIVITY

HYPERTENSION MODELS

Hypertension is the most common cardiovascular disease and is a major public health issue in developed as well as developing countries. Although it is common and readily detectable, it can often lead to lethal complications if left untreated. Because of its high incidence and morbidity, various classes of drugs and regimens have been advocated for the control of hypertension. Despite the large armamentaria of drugs being available for the treatment of hypertension, the last two decades have witnessed the introduction of a number of new antihypertensive drugs. Recent research during this period has also added considerably to our knowledge of the mechanisms involved in the pathogenesis of hypertension. Human essential hypertension is a complex, multifactorial, quantitative trait under polygenic control. In order to understand the pathogenesis and to study the treatment and prevention of a disease, it is useful to develop animal models. Various models of experimental hypertension have been primarily developed to obtain information on the etiopathogenesis of hypertension. These models are also used in the pharmacological screening of potential antihypertensive agents. In the past, hypertensive animal models have been used infrequently for testing antihypertensive potential of drugs. As new molecules are being synthesized in a large number, the use of animal models is increasing for testing these molecules. New animal models of hypertension are being developed as new insights in to the pathogenesis of hypertension are revealed.

The animal models of hypertension share many features which are common to human hypertension. Many of these models have been developed by utilizing the etiological factors that are presumed to be responsible for human hypertension such as excessive salt intake, hyperactivity of renin angiotensin-aldosterone system (RAAS) and

genetic factors. Since regulation of blood pressure (BP) is multifactorial, the effectiveness of an antihypertensive agent in one model does not necessarily mean that the mechanism of action of a given agent in a given model is related to the pathogenesis of elevated blood pressure.

An ideal animal model of hypertension should fulfill the following criteria:

- It should be feasible in small animals.
- It should be simple to perform and uniformly reproducible.
- It should be able to predict the potential antihypertensive properties of an agent.
- It should consume minimal quantities of compounds.
- It should be comparable to some form of human hypertension.

Animals used:

Spontaneous hypertensive rat (SHR), the genetic strain of hypertensive rat, is the animal of choice for screening antihypertensive agents. SHR is the cornerstone of medical research in experimental hypertension1. Rabbits, monkeys, pigs and mice are also used to produce experimental hypertension2. The various types of animal models of hypertension being used are:

Category	Animal Models / Tests
Renal Hypertension Models	Goldblatt hypertension (2-kidney 1-clip), Goldblatt hypertension (1-kidney 1-clip), Renal artery constriction model
Genetic Hypertension Models	Spontaneously Hypertensive Rats (SHR), Dahl salt-sensitive rats
Mineralocorticoid-Induced Hypertension Models	DOCA-salt induced hypertension
Salt-Induced Hypertension Models	High-salt diet model, Dahl salt-sensitive rat model
Neurogenic Hypertension Models	Stress-induced hypertension, CNS stimulation model
Endocrine / Hormone-Induced Hypertension Models	Angiotensin II-induced hypertension, Catecholamine-induced hypertension
Pharmacological Screening Models	Pithed rat model, Blood pressure measurement in anesthetized rats/dogs
Secondary Hypertension Models	Renal failure-induced hypertension, Diabetes-associated hypertension

1. Goldblatt Renal Hypertension Model (Renal Artery Constriction)

Principle

The Goldblatt model is based on the concept that **reduction in renal blood flow stimulates the renin–angiotensin–aldosterone system (RAAS)**. Partial constriction of the renal artery causes decreased perfusion of the kidney, which stimulates renin release. This results in increased production of angiotensin II and aldosterone, leading to vasoconstriction, sodium retention, and persistent elevation of blood pressure. This model closely resembles renovascular hypertension in humans.

Procedure

Adult rats weighing about 200–300 g are used for the experiment. The animals are anesthetized with suitable anesthetic agents. A midline abdominal incision is made to expose the kidneys. The renal artery of one kidney is carefully isolated and partially constricted by placing a silver clip with a defined internal diameter around the artery. In the **two-kidney one-clip (2K1C)** model the second kidney remains intact, whereas in the **one-kidney one-clip (1K1C)** model the contralateral kidney is removed surgically. The incision is sutured and animals are allowed to recover. Over a period of **2–4 weeks**, persistent hypertension develops due to activation of RAAS. After the development of hypertension, test antihypertensive drugs are administered orally or parenterally to evaluate their effect.

Evaluation

Blood pressure is measured using **tail-cuff plethysmography or direct arterial cannulation**. Mean arterial pressure and systolic blood pressure are recorded before and after administration of test drugs. A significant reduction in blood pressure compared with hypertensive control animals indicates antihypertensive activity.

2. Spontaneously Hypertensive Rat (SHR) Model

Principle

The spontaneously hypertensive rat is a **genetically hypertensive animal strain** that develops high blood pressure without any external intervention. The hypertension observed in these rats closely resembles **essential hypertension in humans**, including progressive elevation of blood pressure,

vascular remodeling, and cardiac hypertrophy. Therefore, this model is widely used to evaluate long-term effects of antihypertensive drugs.

Procedure

Young spontaneously hypertensive rats aged about **10–12 weeks** are selected for the study. The animals are housed under standard laboratory conditions with free access to food and water. Baseline systolic blood pressure is recorded using the tail-cuff method. Test antihypertensive drugs are administered orally or intraperitoneally for several days or weeks depending on the experimental design. During the treatment period, blood pressure measurements are recorded at regular intervals.

Evaluation

The antihypertensive effect of the test compound is evaluated by comparing **systolic and diastolic blood pressure values** before and after treatment. Heart rate and body weight may also be monitored. A significant reduction in blood pressure compared with untreated control animals indicates the effectiveness of the test drug.

3. DOCA-Salt Induced Hypertension Model

Principle

This model is based on the ability of **deoxycorticosterone acetate (DOCA)**, a mineralocorticoid, to produce hypertension when administered along with a high-salt diet. DOCA promotes **sodium and water retention**, resulting in expansion of blood volume and increased cardiac output. This ultimately leads to sustained hypertension.

Procedure

Adult rats are anesthetized and subjected to **unilateral nephrectomy**, usually removal of the left kidney. After recovery, animals receive DOCA injections subcutaneously at a dose of approximately **20–30 mg/kg twice weekly**. In addition, the animals are given **1% sodium chloride solution as drinking water** to enhance sodium retention. This treatment is continued for **3–4 weeks**, during which blood pressure gradually increases. Once hypertension is established, test antihypertensive drugs are administered to evaluate their effects.

Evaluation

Blood pressure is measured using a tail-cuff plethysmograph or direct arterial catheterization. Changes in **systolic blood pressure, mean arterial pressure, body weight, and heart weight** are recorded. A reduction in blood pressure after drug administration indicates antihypertensive activity.

4. Angiotensin II-Induced Hypertension Model

Principle

Angiotensin II is a potent **vasoconstrictor hormone** involved in the regulation of blood pressure. Continuous administration of angiotensin II produces a rapid increase in peripheral vascular resistance and blood pressure. This model is particularly useful for evaluating drugs that interfere with the **renin–angiotensin system**, such as ACE inhibitors and angiotensin receptor blockers.

Procedure

Rats are anesthetized and prepared for blood pressure measurement by cannulating the carotid artery. Angiotensin II is administered either by **intravenous infusion or subcutaneous osmotic pump** to produce a sustained increase in blood pressure. Once hypertension is induced, the test antihypertensive compound is administered. Blood pressure is continuously monitored during the experiment.

Evaluation

Mean arterial pressure is recorded before and after administration of the test drug. The **degree of inhibition of angiotensin II-induced elevation in blood pressure** is calculated. A significant reduction indicates antihypertensive activity.

5. Pithed Rat Model

Principle

In this model, the **central nervous system is destroyed by pithing**, eliminating reflex cardiovascular responses. This allows researchers to study the **direct peripheral effects of drugs on blood vessels and heart** without interference from central regulatory mechanisms.

Procedure

Rats are anesthetized and pithed by inserting a steel rod through the spinal canal to destroy the spinal

cord. The animals are then artificially ventilated. The carotid artery is cannulated for recording blood pressure and the jugular vein is cannulated for drug administration. After stabilization, test antihypertensive drugs are injected intravenously, and changes in blood pressure are monitored.

Evaluation

Direct measurement of **arterial blood pressure and heart rate** is carried out using a pressure transducer. The antihypertensive effect is determined by the **decrease in blood pressure produced by the test drug**.

6. Salt-Induced Hypertension Model (Dahl Salt-Sensitive Rats)

Principle

Certain rat strains, known as **Dahl salt-sensitive rats**, develop hypertension when exposed to a high-salt diet. The mechanism involves impaired sodium excretion, leading to fluid retention and increased blood pressure.

Procedure

Dahl salt-sensitive rats are maintained on a **high-salt diet containing about 8% sodium chloride**. Over a period of **3–4 weeks**, these animals gradually develop hypertension. During the hypertensive phase, test antihypertensive drugs are administered to determine their therapeutic potential.

Evaluation

Blood pressure is measured using tail-cuff plethysmography or direct arterial catheterization. The effectiveness of the test drug is evaluated by comparing the **blood pressure values of treated animals with hypertensive control animals**.

DIABETES MODELS

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from defects in insulin secretion, insulin action, or both. It is associated with disturbances in carbohydrate, lipid, and protein metabolism and can lead to severe complications such as nephropathy, neuropathy, retinopathy, and cardiovascular diseases. Because of the increasing global prevalence of diabetes, there is a continuous need to discover and develop new therapeutic agents for its management.

In drug discovery research, animal models play a crucial role in understanding the pathophysiology of diabetes and evaluating the pharmacological activity of potential antidiabetic compounds. Experimental models help simulate various forms of human diabetes, including Type 1 diabetes, Type 2 diabetes, and secondary diabetes. These models enable researchers to study mechanisms of insulin deficiency, insulin resistance, β-cell dysfunction, and metabolic abnormalities.

According to Vogel, diabetic animal models can be produced by chemical induction, genetic manipulation, surgical methods, dietary modifications, or hormonal treatments. Chemicals such as streptozotocin and alloxan selectively destroy pancreatic β-cells, producing insulin deficiency similar to Type 1 diabetes. Genetic models, such as spontaneously diabetic animals, mimic inherited forms of the disease. Diet-induced models, particularly those involving high-fat or high-sugar diets, are used to study insulin resistance and metabolic syndrome resembling Type 2 diabetes.

These models are extensively used to evaluate hypoglycemic agents, insulin sensitizers, insulin secretagogues, and other antidiabetic therapies. Parameters such as blood glucose levels, glucose tolerance, insulin levels, lipid profile, and pancreatic histology are commonly measured to assess the effectiveness of test compounds.

Category	Animal Models / Tests
In Vivo Models	Saline-loaded rat diuresis test
	Lipschitz test in rats
	Metabolic cage method for urine collection
	Water-loaded rat model
	DOCA-induced sodium retention model
In Vitro Models	Isolated kidney tubule transport studies
	Ion transport studies in renal epithelial cells
Ex Vivo Models	Isolated perfused kidney model
	Kidney slice technique

1. Alloxan-Induced Diabetes Model

Principle

The alloxan-induced diabetes model is based on the selective toxic effect of **alloxan** on the insulin-producing **β-cells of the pancreatic islets of Langerhans**. Alloxan causes degeneration and necrosis of β-cells by generating reactive oxygen species, which results in decreased insulin secretion and persistent hyperglycemia. This model resembles **Type-1 diabetes mellitus** and is commonly used to evaluate hypoglycemic agents and insulin preparations.

Procedure

Healthy adult rats or mice are fasted overnight before the experiment. Diabetes is induced by a single intraperitoneal or intravenous injection of alloxan monohydrate at an appropriate dose, usually between 120–150 mg/kg in rats. Because alloxan may cause sudden hypoglycemia due to acute insulin release, animals are provided with glucose solution after administration to prevent fatal hypoglycemia. After about 48–72 hours, blood glucose levels rise significantly due to destruction of pancreatic β-cells. Animals showing fasting blood glucose levels above a defined value (commonly around 200 mg/dL) are considered diabetic and selected for the experiment. The test antidiabetic compounds are then administered orally or intraperitoneally for a specified period.

Evaluation

The antidiabetic activity of the test compound is evaluated by measuring **fasting blood glucose levels** at regular intervals. Other parameters such as **glucose tolerance, body weight changes, serum insulin levels, lipid profile, and glycogen content in liver and muscle** may also be determined. Histopathological examination of pancreatic tissue is sometimes performed to observe β-cell damage or regeneration. A significant reduction in blood glucose compared with diabetic control animals indicates antidiabetic activity.

2. Streptozotocin-Induced Diabetes Model

Principle

This model is based on the diabetogenic effect of **streptozotocin**, a compound that selectively damages pancreatic β-cells. Streptozotocin enters β-cells through the glucose transporter and causes DNA

alkylation, oxidative stress, and cell death. As a result, insulin production decreases markedly, leading to hyperglycemia. This model closely resembles **Type-1 diabetes**, although lower doses combined with high-fat diets can mimic **Type-2 diabetes**.

Procedure

Experimental animals such as rats or mice are fasted overnight before treatment. Streptozotocin is dissolved in freshly prepared citrate buffer and injected intraperitoneally or intravenously at a dose generally ranging from 40–65 mg/kg depending on the species and experimental design. After administration, animals may be given glucose solution for several hours to prevent early hypoglycemic shock. Within 48–72 hours, animals develop hyperglycemia. Blood glucose levels are measured, and animals with glucose levels above a predetermined threshold are considered diabetic. The test antidiabetic drugs are then administered for several days or weeks to study their effect.

Evaluation

Evaluation includes measurement of **fasting blood glucose levels**, **oral glucose tolerance**, and **serum insulin concentration**. Additional parameters such as **lipid profile, glycated hemoglobin, and body weight** may also be assessed. The reduction of hyperglycemia in treated animals compared with diabetic controls indicates the effectiveness of the test drug.

3. Genetic Models of Diabetes

Principle

Genetic models are based on animals that develop diabetes due to **inherited genetic defects affecting insulin secretion or insulin action**. These animals spontaneously develop hyperglycemia and metabolic abnormalities similar to human diabetes, especially **Type-2 diabetes mellitus**.

Procedure

Commonly used genetic diabetic animals include obese and diabetic mouse strains or rats that naturally develop the disease. These animals are maintained under standard laboratory conditions with free access to food and water. Blood glucose levels are monitored periodically to confirm the development of

diabetes. Once hyperglycemia is established, potential antidiabetic drugs are administered over a defined treatment period.

Evaluation

Evaluation involves monitoring **fasting blood glucose levels, glucose tolerance tests, plasma insulin concentration, body weight, and lipid levels**. Improvement in these metabolic parameters after treatment indicates the antidiabetic potential of the test compound.

4. Diet-Induced Diabetes Model

Principle

Diet-induced diabetes is produced by feeding animals a **high-fat or high-sugar diet**, which leads to obesity, insulin resistance, and impaired glucose tolerance. This condition resembles **Type-2 diabetes and metabolic syndrome** observed in humans.

Procedure

Experimental animals such as rats or mice are fed a diet rich in fat or carbohydrates for several weeks. This dietary regimen gradually induces weight gain, insulin resistance, and elevated blood glucose levels. Once diabetes develops, the animals are divided into control and treatment groups. The test compounds are administered for a specific duration to evaluate their effect on glucose metabolism.

Evaluation

The antidiabetic activity is evaluated by measuring **fasting blood glucose, glucose tolerance, insulin levels, body weight, and lipid profile**. Improvement in glucose metabolism and reduction in hyperglycemia indicate the effectiveness of the test drug.

5. Oral Glucose Tolerance Test (OGTT)

Principle

The oral glucose tolerance test is used to assess the ability of the body to **utilize and regulate glucose after an oral glucose load**. It is particularly useful for evaluating drugs that improve glucose tolerance and insulin sensitivity.

Procedure

Animals are fasted overnight before the experiment. The test drug is administered orally or intraperitoneally. After a predetermined time, a glucose solution is given orally at a specific dose. Blood samples are collected from the tail vein at different time intervals such as 0, 30, 60, and 120 minutes to determine blood glucose levels.

Evaluation

The results are evaluated by plotting **blood glucose levels against time** to obtain a glucose tolerance curve. A decrease in the peak blood glucose level or faster return to normal glucose levels indicates improved glucose tolerance and potential antidiabetic activity.

ARRYTHMIC MODELS

Cardiac arrhythmias are disorders of the heart rhythm characterized by abnormal electrical activity of the heart, which may result in irregular, excessively fast (tachycardia), or slow (bradycardia) heartbeats. These disturbances arise due to abnormalities in impulse generation, impulse conduction, or both. Arrhythmias may occur in various pathological conditions such as myocardial ischemia, electrolyte imbalance, drug toxicity, or structural heart disease. Severe arrhythmias can impair cardiac output and may lead to life-threatening complications including ventricular fibrillation and sudden cardiac death.

In the process of drug discovery and development, experimental animal models play an essential role in studying the mechanisms of arrhythmia and in screening potential antiarrhythmic agents. These models help researchers understand how drugs influence cardiac electrophysiology, ion channels, and conduction pathways. Animal models of arrhythmia are designed to reproduce abnormal cardiac rhythms similar to those observed in humans, allowing the evaluation of therapeutic interventions.

According to Vogel, arrhythmias can be experimentally induced in animals by chemical agents, electrical stimulation, myocardial ischemia, or surgical manipulation. Chemicals such as aconitine, calcium chloride, ouabain, and barium chloride are commonly used to produce arrhythmias by altering ionic balance and excitability of cardiac cells. Ischemia-induced arrhythmia models simulate arrhythmias occurring during myocardial infarction, while electrical stimulation models allow controlled induction of abnormal rhythms. In addition, isolated heart preparations and cardiac tissue studies are used to investigate the direct effects of drugs on cardiac electrical activity.

These experimental models are valuable tools for evaluating the antiarrhythmic potential of new compounds, understanding their mechanisms of action, and assessing their safety. Parameters such as electrocardiographic changes, heart rate, incidence of ventricular tachycardia or fibrillation, and survival rate are commonly measured to determine the effectiveness of test drugs. Thus, animal models of arrhythmia play a crucial role in the development of safer and more effective antiarrhythmic therapies.

Category	Animal Models / Tests
Chemically Induced Arrhythmia Models	Aconitine-induced arrhythmia
	Calcium chloride-induced arrhythmia
	Barium chloride-induced arrhythmia
	Ouabain-induced arrhythmia
	Digitalis-induced arrhythmia
Ischemia-Induced Arrhythmia Models	Coronary artery ligation-induced arrhythmia
	Myocardial ischemia and reperfusion arrhythmia
Electrically Induced Arrhythmia Models	Electrical stimulation-induced ventricular arrhythmia
Surgically Induced Arrhythmia Models	Atrial flutter or fibrillation induced by surgical manipulation
In Vitro Cardiac Models	Isolated perfused heart (Langendorff preparation)
Ex Vivo Cardiac Tissue Models	Isolated atrial or ventricular muscle preparations

1. Aconitine-Induced Arrhythmia Model

Principle

The aconitine-induced arrhythmia model is based on the ability of **aconitine**, an alkaloid obtained from the plant *Aconitum*, to produce cardiac arrhythmias by affecting sodium ion channels in myocardial cells. Aconitine keeps sodium channels persistently activated, which leads to prolonged depolarization and increased excitability of cardiac tissue. This results in abnormal electrical activity in the heart such as ventricular tachycardia and fibrillation. Antiarrhythmic drugs that block sodium channels or stabilize the cardiac membrane can suppress aconitine-induced arrhythmias.

Procedure

Experimental animals such as rats, guinea pigs, or dogs are anesthetized, and electrocardiogram (ECG) electrodes are attached for continuous monitoring of cardiac activity. Aconitine is administered intravenously in gradually increasing doses until arrhythmias appear. The development of abnormal cardiac rhythms such as premature ventricular contractions, ventricular tachycardia, or fibrillation is observed. Once arrhythmia is induced, the test antiarrhythmic drug is administered intravenously or orally. The ECG is continuously recorded to monitor changes in cardiac rhythm after administration of the test compound.

Evaluation

The antiarrhythmic activity is evaluated by observing **changes in ECG patterns, heart rate, and the onset of arrhythmia**. The dose of aconitine required to produce arrhythmia and the time taken for arrhythmia development are recorded. A test drug is considered effective if it **delays the onset of arrhythmia, reduces the severity of abnormal rhythms, or prevents ventricular fibrillation** compared with control animals.

2. Calcium Chloride-Induced Arrhythmia Model

Principle

This model is based on the ability of **excess calcium ions** to disturb normal cardiac electrical activity. High levels of calcium increase myocardial excitability and contractility, which can result in abnormal

cardiac rhythms such as ventricular tachycardia and fibrillation. Drugs that block calcium channels or stabilize cardiac membranes can prevent or reduce these arrhythmias.

Procedure

Laboratory animals such as mice or rats are used in this experiment. The animals are anesthetized and placed under ECG monitoring. Calcium chloride solution is injected intravenously at a specific dose to induce arrhythmias. Shortly after administration, animals develop abnormal heart rhythms due to increased intracellular calcium levels. Test antiarrhythmic drugs are administered before or after calcium chloride injection to evaluate their protective effects.

Evaluation

The antiarrhythmic effect is assessed by recording **ECG changes, heart rate, and survival rate of animals**. The incidence and severity of arrhythmias are compared between treated and untreated groups. A significant reduction in arrhythmia occurrence or improvement in survival rate indicates antiarrhythmic activity.

3. Barium Chloride-Induced Arrhythmia Model

Principle

Barium chloride produces arrhythmia by **blocking potassium channels in cardiac muscle cells**. This interference with potassium ion movement alters the normal repolarization process of cardiac cells, leading to abnormal electrical activity and arrhythmias. Antiarrhythmic drugs that stabilize membrane potential or modify ion channel activity can inhibit these effects.

Procedure

Experimental animals such as rats are anesthetized and connected to ECG recording equipment. Barium chloride is administered intravenously or intraperitoneally at a specific dose to induce arrhythmia. Within a short time after administration, animals develop abnormal cardiac rhythms such as ventricular tachycardia or fibrillation. The test compound is administered either before or after the arrhythmogenic agent, and ECG recordings are monitored throughout the experiment.

Evaluation

The effectiveness of the test drug is evaluated by observing **changes in ECG pattern, reduction in the number of arrhythmic episodes, and delay in the onset of arrhythmia**. A drug that significantly reduces arrhythmia incidence or severity is considered to possess antiarrhythmic properties.

4. Ouabain (Digitalis)-Induced Arrhythmia Model

Principle

Ouabain is a cardiac glycoside that inhibits the **sodium-potassium ATPase pump** in cardiac cells. This inhibition leads to accumulation of intracellular calcium, which enhances cardiac contractility but may also cause arrhythmias at higher doses. This model is used to study drugs that can prevent arrhythmias caused by digitalis toxicity.

Procedure

Animals such as dogs or guinea pigs are anesthetized and instrumented for ECG recording. Ouabain is administered intravenously in incremental doses until arrhythmias such as ventricular extrasystoles or ventricular fibrillation appear. The test antiarrhythmic drug is administered during or before ouabain infusion. ECG recordings are taken continuously to observe changes in cardiac rhythm.

Evaluation

Evaluation involves recording **ECG parameters, onset time of arrhythmia, and the dose of ouabain required to produce arrhythmia**. Drugs that delay the onset of arrhythmia or prevent ventricular fibrillation are considered effective antiarrhythmic agents.

5. Coronary Artery Ligation-Induced Arrhythmia Model

Principle

This model is based on the fact that **myocardial ischemia** caused by obstruction of coronary blood flow can lead to severe arrhythmias. Reduced oxygen supply to heart tissue alters the electrical properties of cardiac cells and results in ventricular tachycardia or fibrillation. Antiarrhythmic drugs that improve cardiac electrophysiology or reduce ischemic damage can suppress these arrhythmias.

Procedure

Experimental animals such as rats or dogs are anesthetized and subjected to thoracotomy to expose the heart. The **coronary artery is ligated** using a surgical thread to block blood flow to a portion of the myocardium. Shortly after ligation, ischemia develops and arrhythmias appear. ECG recordings are taken throughout the experiment. The test antiarrhythmic drug is administered before or after ligation to evaluate its protective effect.

Evaluation

The effectiveness of the drug is determined by monitoring **ECG changes, frequency of ventricular tachycardia or fibrillation, duration of arrhythmia, and survival rate of animals**. A reduction in the number or severity of arrhythmias compared with control animals indicates antiarrhythmic activity.

6. Electrical Stimulation-Induced Arrhythmia Model

Principle

Arrhythmias can be produced by applying electrical stimuli directly to the myocardium. Excessive electrical stimulation disturbs normal impulse generation and conduction, leading to abnormal cardiac rhythms.

Procedure

Animals are anesthetized and the heart is exposed surgically. Electrodes are placed on the myocardium, and electrical impulses are delivered using a stimulator. These impulses induce arrhythmias such as ventricular tachycardia or fibrillation. Test drugs are administered before electrical stimulation to assess their protective effect.

Evaluation

The antiarrhythmic effect is evaluated by measuring **threshold voltage required to produce arrhythmia, duration of abnormal rhythm, and ECG changes**.

7. Isolated Perfused Heart (Langendorff) Model

Principle

The isolated perfused heart preparation allows the study of cardiac electrophysiology in a **controlled**

environment without neural influences. This method is used to investigate direct effects of drugs on cardiac tissue.

Procedure

The heart of a rat or guinea pig is rapidly excised and mounted on a **Langendorff apparatus**. The heart is perfused with oxygenated physiological solution through the aorta. Electrical activity and heart contractions are recorded. Arrhythmias can be induced by drugs or electrical stimulation, and the effect of test antiarrhythmic compounds is studied.

Evaluation

Parameters such as **heart rate, conduction velocity, ECG changes, and incidence of arrhythmia** are recorded. Drugs that normalize cardiac rhythm or reduce arrhythmia are considered effective.

ANTIHYPERLIPIDEMIC MODELS

Dyslipidemia is a metabolic disorder characterized by **abnormal levels of lipids in the blood**, including elevated total cholesterol, triglycerides, and low-density lipoproteins (LDL), along with decreased levels of high-density lipoproteins (HDL). These abnormalities in lipid metabolism are considered major risk factors for the development of **atherosclerosis, coronary artery disease, and other cardiovascular disorders**. The increasing prevalence of dyslipidemia worldwide has created a significant need for the development of effective lipid-lowering drugs.

In the process of drug discovery, experimental animal models play an important role in understanding the **pathophysiology of lipid metabolism disorders and in screening potential antidyslipidemic agents**. These models help researchers investigate how drugs influence lipid synthesis, absorption, transport, and metabolism in the body. They also allow evaluation of the effects of test compounds on plasma lipid levels and the development of atherosclerotic lesions.

According to Vogel, hyperlipidemia in animals can be produced through **dietary manipulation, chemical induction, genetic factors, or metabolic disorders such as diabetes**. Diet-induced models involve feeding animals high-fat or high-cholesterol diets to mimic human hyperlipidemia. Chemical agents such as Triton WR-1339 or poloxamer can also produce rapid increases in plasma lipid levels by interfering with lipid metabolism. In addition, genetic models and atherosclerosis models are used to study long-term lipid abnormalities and vascular changes.

These experimental models are widely used to evaluate the lipid-lowering potential of new compounds by measuring **serum cholesterol, triglycerides, LDL, HDL levels, and the extent of lipid deposition in tissues**. Thus, animal models of dyslipidemia provide a valuable experimental tool for the **development and evaluation of new antidyslipidemic therapies** aimed at preventing cardiovascular diseases.

Category	Animal Models / Tests
Diet-Induced Hyperlipidemia Models	High-fat diet-induced hyperlipidemia
	High-cholesterol diet-induced hyperlipidemia
	Triton WR-1339 induced hyperlipidemia
Chemical-Induced Hyperlipidemia Models	Poloxamer-407 induced hyperlipidemia
	Alloxan-induced diabetic hyperlipidemia
Genetic Models	Genetically obese or hyperlipidemic animals
Atherosclerosis Models	Cholesterol-fed rabbit model of atherosclerosis
	Diet-induced atherosclerosis in rodents
In Vitro Models	Lipoprotein lipase activity assay
	Cholesterol synthesis inhibition assay
Ex Vivo Models	Liver lipid metabolism studies
	Isolated tissue lipid metabolism studies

1. Triton WR-1339 Induced Hyperlipidemia Model

Principle

The Triton WR-1339 induced hyperlipidemia model is based on the ability of **Triton WR-1339**, a non-ionic detergent, to block the clearance of lipoproteins from the bloodstream. Triton inhibits the activity of **lipoprotein lipase**, the enzyme responsible for the breakdown of triglyceride-rich lipoproteins. As a result, there is a rapid accumulation of cholesterol and triglycerides in plasma, producing acute hyperlipidemia. Drugs that lower plasma lipid levels can reverse this effect and therefore demonstrate antidyslipidemic activity.

Procedure

Experimental animals such as rats or mice are fasted overnight before the experiment. Triton WR-1339 is administered by intravenous or intraperitoneal injection at an appropriate dose to induce hyperlipidemia. Within a few hours after administration, plasma lipid levels rise significantly. Test antidyslipidemic drugs are administered either before or after Triton injection depending on the experimental design. Blood samples are collected at different time intervals for analysis of lipid levels.

Evaluation

The effectiveness of the test drug is evaluated by measuring **serum total cholesterol, triglycerides, LDL cholesterol, and HDL cholesterol**. These values are compared with those of untreated hyperlipidemic control animals. A significant reduction in cholesterol and triglyceride levels indicates antidyslipidemic activity.

2. High-Fat Diet Induced Hyperlipidemia Model

Principle

This model is based on the fact that excessive consumption of dietary fat leads to abnormal lipid metabolism and accumulation of lipids in blood and tissues. Feeding animals a **high-fat diet** increases plasma cholesterol and triglyceride levels and may also lead to obesity and insulin resistance. This model resembles the hyperlipidemia associated with dietary habits in humans.

Procedure

Experimental animals such as rats are divided into control and experimental groups. The experimental animals are fed a **diet rich in fat and cholesterol** for several weeks, usually between four and eight weeks.

This diet gradually elevates blood lipid levels. Once hyperlipidemia is established, test drugs are administered daily for a specified period while the animals continue to receive the high-fat diet.

Evaluation

Blood samples are collected periodically to determine **serum cholesterol, triglycerides, LDL, and HDL levels**. Body weight and food intake may also be recorded. A significant reduction in lipid levels in treated animals compared with untreated hyperlipidemic animals indicates the lipid-lowering effect of the test drug.

3. Poloxamer-407 Induced Hyperlipidemia Model

Principle

Poloxamer-407 is a synthetic compound that induces hyperlipidemia by **inhibiting lipoprotein lipase and hepatic lipase**, enzymes responsible for lipid metabolism. This results in accumulation of triglycerides and cholesterol in plasma. The model is useful for studying drugs that influence lipid synthesis or clearance.

Procedure

Animals such as mice or rats are fasted overnight and then injected intraperitoneally with **poloxamer-407 solution** at a specific dose. Within a short period, there is a marked elevation in plasma lipid levels. Test compounds are administered either before or after induction of hyperlipidemia, and blood samples are collected at predetermined intervals.

Evaluation

Evaluation is carried out by measuring **plasma cholesterol and triglyceride concentrations**. The lipid values obtained from treated animals are compared with those of hyperlipidemic controls. A reduction in lipid levels indicates potential antidyslipidemic activity.

4. Cholesterol-Fed Rabbit Model (Atherosclerosis Model)

Principle

Rabbits are highly sensitive to dietary cholesterol. Feeding rabbits a **cholesterol-rich diet** leads to accumulation of cholesterol in plasma and deposition of lipids in arterial walls, resulting in atherosclerotic lesions. This model is useful for evaluating drugs that reduce lipid levels and prevent atherosclerosis.

Procedure

Rabbits are divided into groups and fed a diet containing **high levels of cholesterol** for several weeks. During this period, serum cholesterol levels increase significantly, and lipid deposition occurs in blood vessels. Test antidyslipidemic drugs are administered orally during the feeding period. Blood samples are collected periodically to monitor lipid levels.

Evaluation

Evaluation includes measurement of **serum cholesterol, triglycerides, and lipoprotein levels**. After completion of the experiment, animals may be sacrificed and the **aorta is examined for atherosclerotic plaque formation**. A reduction in serum lipid levels and decreased plaque formation indicates antidyslipidemic activity.

5. Diabetic Hyperlipidemia Model

Principle

Diabetes mellitus is often associated with abnormal lipid metabolism, resulting in increased levels of cholesterol and triglycerides. Induction of diabetes in animals leads to secondary hyperlipidemia, which can be used to evaluate the lipid-lowering effects of drugs.

Procedure

Diabetes is induced in experimental animals using diabetogenic agents such as **alloxan or streptozotocin**. After induction, animals develop both hyperglycemia and elevated lipid levels. Test drugs are administered for several days or weeks. Blood samples are collected during the treatment period.

Evaluation

The lipid-lowering effect of the drug is assessed by measuring **serum cholesterol, triglycerides, LDL, and HDL levels**. Improvement in lipid profile along with reduction in blood glucose indicates effective antidyslipidemic activity.

6. Lipoprotein Lipase Activity Model

Principle

Lipoprotein lipase (LPL) is an important enzyme responsible for the hydrolysis of triglycerides present in circulating lipoproteins. Inhibition or reduced activity of this enzyme leads to accumulation of triglycerides in plasma, causing hyperlipidemia. The lipoprotein lipase activity model is used to evaluate the ability of drugs to **enhance lipid metabolism by stimulating LPL activity**.

Procedure

Experimental animals such as rats are treated with the test compound for a specific period. Blood or tissue samples, particularly from adipose tissue or muscle, are collected after treatment. These samples are processed in the laboratory to determine the activity of lipoprotein lipase using biochemical assays. The enzyme activity is measured by evaluating the hydrolysis of triglyceride substrates under controlled experimental conditions.

Evaluation

The antidyslipidemic effect is determined by comparing **lipoprotein lipase activity in treated animals with that of control animals**. An increase in enzyme activity indicates improved lipid metabolism and suggests that the test compound has lipid-lowering potential.

7. Cholesterol Biosynthesis Inhibition Model

Principle

Cholesterol in the body is synthesized mainly in the liver through a series of enzymatic reactions. Inhibition of enzymes involved in cholesterol synthesis, particularly **HMG-CoA reductase**, leads to a

decrease in cholesterol production. This model is used to evaluate compounds that inhibit cholesterol biosynthesis.

Procedure

Experimental animals are treated with the test compound for a specified duration. Liver tissues are then isolated and processed to study cholesterol synthesis. Radiolabeled precursors such as acetate may be used in experimental systems to measure the rate of cholesterol formation in liver cells or microsomal preparations.

Evaluation

The rate of cholesterol synthesis in treated samples is compared with that of untreated controls. A significant reduction in cholesterol production indicates that the test compound has the ability to **inhibit cholesterol biosynthesis**, suggesting antidyslipidemic activity.

8. Isolated Liver Perfusion Model

Principle

The liver plays a central role in lipid metabolism, including cholesterol synthesis, lipoprotein production, and triglyceride metabolism. The isolated liver perfusion model allows the study of **hepatic lipid metabolism in a controlled experimental environment** without the influence of other organs.

Procedure

In this method, the liver of an experimental animal such as a rat is surgically removed and connected to a perfusion apparatus. The organ is perfused with oxygenated physiological solution that maintains normal metabolic activity. Test drugs are added to the perfusion medium, and changes in lipid metabolism are observed over time.

Evaluation

Samples of perfusion fluid are collected periodically to measure **cholesterol, triglycerides, and lipoprotein levels** released by the liver. Reduction in lipid synthesis or secretion after drug treatment indicates antidyslipidemic activity.

9. Liver Slice Technique

Principle

The liver slice technique is used to study metabolic processes occurring in liver tissue. Thin slices of liver maintain enzymatic activity and metabolic functions for a limited time when incubated in appropriate solutions. This method helps in evaluating the direct effect of drugs on **hepatic lipid metabolism**.

Procedure

The liver is removed from an experimental animal and cut into thin slices using specialized instruments. These slices are placed in incubation medium containing oxygen and nutrients. Test compounds are added to the medium, and the slices are incubated under controlled conditions for a specific period.

Evaluation

After incubation, the medium and tissue samples are analyzed to determine **lipid content, cholesterol synthesis, and triglyceride levels**. A decrease in lipid formation or secretion in the presence of the test drug indicates potential antidyslipidemic activity.

10. Isolated Tissue Lipid Metabolism Model

Principle

Different tissues such as liver, adipose tissue, and muscle are involved in lipid metabolism. Studying these tissues in isolation helps determine the **direct effect of drugs on lipid synthesis, breakdown, and storage**.

Procedure

Tissues are isolated from experimental animals and incubated in suitable physiological solutions containing nutrients and oxygen. Test compounds are added to the incubation medium, and metabolic activity is observed under controlled laboratory conditions.

Evaluation

Biochemical analysis is performed to measure **changes in lipid synthesis, triglyceride breakdown, and fatty acid metabolism**. Improvement in lipid metabolism compared with control samples indicates the antidyslipidemic potential of the test compound.

ANTIAGGREGATORY MODELS

Platelet aggregation plays a crucial role in the process of hemostasis and thrombus formation. Under physiological conditions, platelets adhere to damaged vascular endothelium and aggregate to form a platelet plug that prevents excessive bleeding. However, excessive or uncontrolled platelet aggregation can lead to the formation of pathological thrombi, which are responsible for serious cardiovascular disorders such as myocardial infarction, stroke, and peripheral vascular diseases. Therefore, the development of drugs that inhibit platelet aggregation is an important strategy in the prevention and treatment of thrombotic disorders.

For the discovery and evaluation of antiplatelet agents, several experimental models have been developed to study platelet function and thrombus formation under laboratory conditions. These screening methods allow researchers to assess the ability of new compounds to inhibit platelet activation, aggregation, and thrombus formation. In Vogel's pharmacological assay methods, anti-aggregatory drugs are evaluated using a variety of **in vitro, ex vivo, and in vivo experimental models**. In vitro models involve the study of platelet aggregation in platelet-rich plasma or whole blood using different agonists such as ADP, collagen, thrombin, and arachidonic acid. Ex vivo models measure platelet function in blood samples obtained from animals after administration of the test compound. In vivo models evaluate the ability of drugs to prevent thrombus formation in living animals by inducing thrombosis through mechanical, chemical, or electrical methods

Sr. No.	Category of Model	Screening Models
1	**In Vitro Models**	Platelet aggregation in platelet-rich plasma (PRP)
		Whole blood platelet aggregation test
		Turbidimetric platelet aggregation assay
		Impedance platelet aggregation assay
		ADP-induced platelet aggregation
		Collagen-induced platelet aggregation
		Arachidonic acid-induced platelet aggregation
		Thrombin-induced platelet aggregation
2	**Ex Vivo Models**	Ex vivo platelet aggregation in platelet-rich plasma after drug administration

1. Platelet Aggregation in Platelet-Rich Plasma (PRP)

Principle:

This model is based on the ability of platelets to aggregate when stimulated by agonists such as ADP, collagen, thrombin, or arachidonic acid. When platelet aggregation occurs, platelet-rich plasma becomes clearer because platelets clump together and settle, allowing more light to pass through. Antiplatelet drugs inhibit this aggregation and therefore reduce the increase in light transmission.

Procedure:

Blood is collected from animals or human volunteers using anticoagulants such as sodium citrate. The blood is centrifuged at low speed to obtain platelet-rich plasma (PRP). PRP is placed in a cuvette of an aggregometer and maintained at 37 °C with continuous stirring. A platelet aggregation-inducing agent such as ADP, collagen, or arachidonic acid is then added to the PRP in the presence or absence of the test drug. Aggregation of platelets causes a change in optical density, which is recorded by the aggregometer.

Evaluation:

The degree of platelet aggregation is determined by measuring the increase in light transmission through the PRP sample. The inhibitory effect of the test compound is expressed as the percentage inhibition of aggregation compared with the control sample without the drug.

2. Whole Blood Platelet Aggregation Test

Principle:

Unlike PRP assays, this model evaluates platelet aggregation in whole blood, where platelets interact with red blood cells and leukocytes. Platelet aggregation causes an increase in electrical impedance between two electrodes placed in the blood sample. Antiplatelet drugs inhibit aggregation and therefore reduce the change in impedance.

Procedure:

Fresh anticoagulated whole blood is placed in a measuring chamber containing two electrodes. The sample is kept at 37 °C and stirred continuously. An aggregation-inducing agent such as ADP or collagen is added to stimulate platelet aggregation. The test compound is either added directly to the sample or administered to animals before blood collection. Platelet aggregation leads to deposition of platelet aggregates on the electrodes, causing an increase in electrical impedance that is recorded by the instrument.

Evaluation:

The increase in electrical impedance over time reflects platelet aggregation. The antiplatelet activity of the test drug is determined by comparing the impedance change in treated samples with that in control samples.

3. Turbidimetric Platelet Aggregation Assay

Principle:

This classical method, also called light transmission aggregometry, is based on the change in turbidity of platelet-rich plasma during platelet aggregation. Aggregated platelets form clumps, making the plasma clearer and allowing more light to pass through.

Procedure:

Platelet-rich plasma is prepared from citrated blood by centrifugation. The PRP is placed in an aggregometer cuvette and stirred at 37 °C. A platelet agonist such as ADP, collagen, thrombin, or arachidonic acid is added. In the presence of the test compound, platelet aggregation may be inhibited. Changes in light transmission through the PRP are continuously recorded.

Evaluation:

The degree of platelet aggregation is determined by the percentage increase in light transmission relative to platelet-poor plasma. The inhibitory effect of the test compound is calculated as the percentage reduction in aggregation compared with the control.

4. Impedance Platelet Aggregation Assay

Principle:

This method measures platelet aggregation by detecting the increase in electrical resistance (impedance) between electrodes immersed in whole blood. As platelets aggregate and adhere to the electrode surfaces, electrical resistance increases.

Procedure:

Anticoagulated whole blood is placed in a measuring chamber containing electrodes. The sample is maintained at physiological temperature and continuously stirred. Platelet aggregation is induced by adding agonists such as ADP or collagen. The test compound is added either before or together with the agonist. Aggregation of platelets on the electrodes leads to an increase in impedance, which is recorded electronically.

Evaluation:

The increase in impedance is proportional to platelet aggregation. Antiplatelet activity is assessed by comparing impedance changes between treated and untreated samples.

5. ADP-Induced Platelet Aggregation

Principle:

ADP is a physiological platelet agonist that activates platelet receptors and triggers aggregation. Antiplatelet drugs inhibit this ADP-mediated platelet activation and aggregation.

Procedure:

Platelet-rich plasma is prepared from citrated blood and placed in an aggregometer. ADP is added to induce platelet aggregation. The test compound is added before ADP administration. Aggregation of platelets is recorded using turbidimetric or impedance methods.

Evaluation:

The extent of aggregation induced by ADP is measured as an increase in light transmission or electrical impedance. Reduction in aggregation in the presence of the test drug indicates antiplatelet activity.

6. Collagen-Induced Platelet Aggregation

Principle:

Collagen is exposed at sites of vascular injury and acts as a potent stimulus for platelet activation and aggregation. Drugs that inhibit platelet aggregation reduce the response to collagen.

Procedure:

Platelet-rich plasma is placed in an aggregometer cuvette at 37 °C with constant stirring. Collagen is added to induce platelet aggregation. The test compound is added before collagen stimulation. Changes in optical density due to platelet aggregation are recorded.

Evaluation:

The aggregation response to collagen is measured by the increase in light transmission. Antiplatelet activity is expressed as the percentage inhibition of collagen-induced aggregation compared with control samples.

7. Arachidonic Acid-Induced Platelet Aggregation

Principle:

Arachidonic acid is converted by cyclooxygenase in platelets to thromboxane A_2, a potent platelet

aggregation promoter. Drugs such as aspirin inhibit cyclooxygenase and therefore block arachidonic acid-induced platelet aggregation.

Procedure:

Platelet-rich plasma is obtained from anticoagulated blood and placed in an aggregometer. The test compound is added to the PRP followed by arachidonic acid. Platelet aggregation is recorded as changes in light transmission.

Evaluation:

The degree of aggregation induced by arachidonic acid is measured and compared with the control. A reduction in aggregation indicates inhibition of the cyclooxygenase pathway and antiplatelet activity.

8. Thrombin-Induced Platelet Aggregation

Principle:

Thrombin is a strong platelet activator that stimulates platelet aggregation through activation of protease-activated receptors. Antiplatelet drugs can inhibit thrombin-induced aggregation.

Procedure:

Platelet-rich plasma is placed in an aggregometer at 37 °C. The test drug is added before thrombin administration. Thrombin is then added to stimulate platelet aggregation, and the aggregation process is recorded.

Evaluation:

Aggregation is measured by changes in light transmission or impedance. The antiplatelet activity of the compound is expressed as the percentage inhibition of thrombin-induced aggregation compared with untreated control samples.

9. Arteriovenous Shunt Thrombosis Model

Principle:

This in vivo model evaluates the ability of drugs to inhibit thrombus formation in an artificial

arteriovenous shunt inserted between an artery and a vein. Platelet aggregation and thrombus formation occur in the shunt due to blood flow and contact with foreign surfaces.

Procedure:

Rats or other laboratory animals are anesthetized, and an arteriovenous shunt containing a thrombogenic material such as silk thread is inserted between the carotid artery and jugular vein. The test compound is administered before the experiment. Blood flows through the shunt for a fixed period, allowing thrombus formation on the thread.

Evaluation:

After the experiment, the thread is removed and the thrombus formed on it is weighed. A reduction in thrombus weight in treated animals compared with control animals indicates antiplatelet or antithrombotic activity.

10. Ferric Chloride-Induced Arterial Thrombosis

Principle:

Ferric chloride damages the vascular endothelium and exposes subendothelial collagen, leading to platelet adhesion, aggregation, and thrombus formation. Antiplatelet drugs inhibit this thrombus formation.

Procedure:

Animals such as rats or mice are anesthetized, and the carotid artery is exposed surgically. A filter paper soaked in ferric chloride solution is applied to the artery for a short period to induce endothelial injury and thrombosis. The test drug is administered before induction of thrombosis.

Evaluation:

Thrombus formation is assessed by measuring the time required for occlusion of the artery or by measuring thrombus weight. Prolongation of occlusion time or reduction in thrombus formation indicates antiplatelet activity.

11. Collagen or ADP-Induced Pulmonary Thrombosis

Principle:

Intravenous administration of collagen or ADP induces platelet aggregation in the pulmonary circulation, leading to thrombus formation and respiratory distress in animals. Antiplatelet drugs inhibit this aggregation.

Procedure:

Mice are treated with the test compound and then injected intravenously with collagen or ADP. These agents induce platelet aggregation in the lungs, leading to pulmonary thrombosis and sometimes death.

Evaluation:

The protective effect of the test drug is evaluated by measuring survival rate, reduction in mortality, or decrease in platelet aggregation compared with control animals.

12. Electrically Induced Carotid Artery Thrombosis

Principle:

Electrical stimulation of an artery damages the vascular endothelium and triggers platelet aggregation and thrombus formation. Antiplatelet drugs inhibit thrombus formation under these conditions.

Procedure:

Animals are anesthetized and the carotid artery is exposed. A small electrode is placed on the artery and an electric current is applied to induce endothelial injury. The test drug is administered before electrical stimulation.

Evaluation:

The time required for thrombotic occlusion of the artery is measured using blood flow monitoring devices. Prolongation of occlusion time indicates antithrombotic activity.

13. Photochemical-Induced Thrombosis Model

Principle:

In this model, a photosensitive dye such as rose bengal is injected into the bloodstream and activated by

light at a specific wavelength. This produces free radicals that damage the endothelium and induce platelet aggregation and thrombosis.

Procedure:

Animals are injected with a photosensitive dye and the targeted blood vessel is illuminated with a laser or strong light source. The test compound is administered before the procedure. Light activation of the dye causes endothelial injury and thrombus formation.

Evaluation:

The time to vascular occlusion or the size of the thrombus is measured. Drugs with antiplatelet activity prolong the occlusion time or reduce thrombus formation.

COAGULANTS/ ANTICOAGULANTS MODEL

the process of blood coagulation is an essential physiological mechanism that prevents excessive blood loss following vascular injury. It involves a complex cascade of enzymatic reactions leading to the conversion of fibrinogen into fibrin, which forms a stable blood clot. The coagulation process is regulated by a balance between procoagulant and anticoagulant factors within the circulatory system. Disturbances in this balance may result in pathological conditions such as thrombosis, hemorrhage, and disseminated intravascular coagulation.

Coagulant drugs are used therapeutically to promote hemostasis in conditions involving excessive bleeding, whereas anticoagulant agents are widely used in the prevention and treatment of thromboembolic disorders such as deep vein thrombosis, pulmonary embolism, myocardial infarction, and stroke. Because of the clinical importance of these agents, reliable experimental methods are required for the discovery and pharmacological evaluation of compounds that influence blood coagulation and thrombosis.

In the pharmacological assay methods described in Vogel's book, several experimental models are employed to investigate the effects of drugs on the coagulation system. These models include **in vitro, ex vivo, and in vivo techniques** designed to evaluate different aspects of the coagulation cascade, platelet function, and thrombus formation. In vitro assays measure clotting parameters such as prothrombin time, activated partial thromboplastin time, thrombin time, and plasma recalcification time

using plasma or whole blood samples. Ex vivo models involve the administration of test compounds to animals followed by analysis of blood coagulation parameters. In vivo models assess the formation or inhibition of thrombus directly in experimental animals under controlled conditions.

These screening methods provide valuable information regarding the **mechanism of action, potency, and therapeutic potential of coagulant and anticoagulant agents**, and they play an important role in the development of new drugs for the management of bleeding and thrombotic disorders.

Sr. No.	Category	Screening Models / Tests
1	**In Vitro Models**	Whole blood clotting time
		Plasma recalcification time
		Prothrombin time (PT) test
		Activated partial thromboplastin time (aPTT)
		Thrombin time (TT)
		Fibrinogen determination assay
		Factor Xa inhibition assay
		Platelet clot retraction test
2	**Ex Vivo Models**	Measurement of PT, aPTT, or clotting time after administration of test drug in animals
		Ex vivo thrombin time determination
		Ex vivo plasma recalcification time
3	**In Vivo Models**	Tail bleeding time in mice or rats
		Venous thrombosis model
		Arteriovenous shunt thrombosis model
		Stasis-induced venous thrombosis
		Ferric chloride–induced arterial thrombosis

Sr. No.	Category	Screening Models / Tests
		Laser-induced thrombosis model
		Disseminated intravascular coagulation (DIC) model

1. Whole Blood Clotting Time

Principle:

This test measures the time required for whole blood to clot under standardized conditions. Blood coagulation occurs through activation of clotting factors leading to conversion of fibrinogen into fibrin. Drugs that promote coagulation shorten clotting time, while anticoagulant agents prolong the time required for clot formation.

Procedure:

Fresh blood is collected from experimental animals or human volunteers without anticoagulants and placed in clean glass tubes maintained at 37°C. The tubes are tilted gently at regular intervals until the blood no longer flows when the tube is inverted. Test compounds may be mixed with the blood sample before incubation or administered to animals prior to blood collection.

Evaluation:

The clotting time is recorded as the time interval between blood collection and the formation of a stable clot. The effect of the test compound is determined by comparing clotting time in treated samples with control samples.

2. Plasma Recalcification Time

Principle:

Plasma recalcification time evaluates the intrinsic coagulation pathway. When calcium ions are added to citrated plasma, coagulation is initiated. The presence of anticoagulant substances prolongs the recalcification time.

Procedure:

Blood is collected using sodium citrate as an anticoagulant to obtain plasma. The plasma is separated by centrifugation and incubated at 37°C. Calcium chloride solution is added to initiate coagulation. The test compound is added to the plasma before the addition of calcium ions.

Evaluation:

The time required for clot formation after the addition of calcium chloride is measured. A prolonged clotting time indicates anticoagulant activity of the test compound.

3. Prothrombin Time (PT) Test

Principle:

Prothrombin time measures the activity of the extrinsic pathway of blood coagulation. The test evaluates clotting factors such as prothrombin, factor V, factor VII, and fibrinogen. Anticoagulant drugs affecting this pathway prolong the prothrombin time.

Procedure:

Citrated plasma is obtained from blood samples by centrifugation. The plasma is incubated at 37°C and thromboplastin reagent containing tissue factor and calcium ions is added. The test compound may be added to the plasma before the reagent.

Evaluation:

The time taken for clot formation after addition of thromboplastin reagent is measured using a coagulometer. A prolonged prothrombin time indicates inhibition of the extrinsic coagulation pathway.

4. Activated Partial Thromboplastin Time (aPTT)

Principle:

This test measures the intrinsic pathway of blood coagulation and evaluates factors such as XII, XI, IX, VIII, X, V, and prothrombin. Anticoagulant agents affecting intrinsic pathway components prolong the activated partial thromboplastin time.

Procedure:

Platelet-poor plasma is prepared from citrated blood. The plasma is incubated with a partial thromboplastin reagent and an activator such as kaolin or silica. After incubation, calcium chloride is added to initiate clotting. The test compound may be added before the reaction begins.

Evaluation:

The clotting time is recorded using a coagulometer. Prolongation of aPTT compared with control samples indicates anticoagulant activity.

5. Thrombin Time (TT)

Principle:

Thrombin time measures the conversion of fibrinogen into fibrin after the addition of thrombin. Drugs that interfere with fibrin formation prolong thrombin time.

Procedure:

Platelet-poor plasma is prepared from citrated blood. The plasma is incubated at 37°C and a standardized thrombin solution is added. The test compound may be mixed with plasma before thrombin addition.

Evaluation:

The time taken for fibrin clot formation is recorded. Prolongation of thrombin time indicates inhibition of fibrin formation or thrombin activity.

6. Fibrinogen Determination Assay

Principle:

Fibrinogen is a key plasma protein involved in clot formation. The assay measures fibrinogen concentration in plasma to evaluate the effect of drugs on coagulation.

Procedure:

Plasma samples are treated with thrombin to convert fibrinogen into fibrin. The resulting clot is measured either gravimetrically or by using optical methods to determine fibrinogen concentration.

Evaluation:

Changes in fibrinogen levels compared with control samples indicate the effect of the test compound on fibrin formation and coagulation processes.

7. Factor Xa Inhibition Assay

Principle:

Factor Xa plays a central role in the coagulation cascade by converting prothrombin to thrombin. Inhibition of factor Xa prevents thrombin formation and reduces clotting.

Procedure:

A purified factor Xa enzyme system is used with a chromogenic substrate. The test compound is incubated with factor Xa before adding the substrate. If the compound inhibits factor Xa activity, cleavage of the substrate is reduced.

Evaluation:

The rate of substrate cleavage is measured spectrophotometrically. Reduced enzyme activity compared with control samples indicates factor Xa inhibitory activity.

8. Platelet Clot Retraction Test

Principle:

Clot retraction occurs when platelets contract and pull fibrin strands together, strengthening the clot. Drugs affecting platelet function can alter clot retraction.

Procedure:

Whole blood or platelet-rich plasma is allowed to clot in a glass tube. The clot gradually shrinks as platelets contract. Test compounds may be added before clot formation.

Evaluation:

The degree of clot retraction is observed visually or measured by determining the volume of serum released from the clot. Reduced clot retraction indicates impaired platelet function.

In Vivo Models

9. Tail Bleeding Time in Mice or Rats

Principle:

Bleeding time measures the ability of blood vessels and platelets to stop bleeding after vascular injury. Anticoagulant or antiplatelet drugs prolong bleeding time.

Procedure:

Animals are anesthetized and a small portion of the tail tip is cut using a sharp blade. The tail is immediately immersed in isotonic saline maintained at 37°C. The test compound is administered before the experiment.

Evaluation:

The time required for bleeding to stop completely is recorded. Prolongation of bleeding time compared with control animals indicates anticoagulant or antiplatelet activity.

10. Venous Thrombosis Model

Principle:

Venous thrombosis models simulate thrombus formation in veins under conditions of reduced blood flow or endothelial damage. Anticoagulant drugs prevent or reduce thrombus formation.

Procedure:

In anesthetized animals, a vein such as the jugular vein is partially ligated to reduce blood flow. A thrombogenic stimulus may be applied to induce clot formation. Test compounds are administered before the procedure.

Evaluation:

After a specified time, the vein is removed and the thrombus is weighed or measured. Reduced thrombus formation indicates anticoagulant activity.

11. Arteriovenous Shunt Thrombosis Model

Principle:

This model evaluates thrombus formation under continuous blood flow conditions. Contact of blood with artificial surfaces promotes platelet aggregation and clot formation.

Procedure:

An arteriovenous shunt containing a thrombogenic material is surgically inserted between an artery and a vein in anesthetized animals. Blood flows through the shunt for a defined period. Test compounds are administered prior to shunt insertion.

Evaluation:

The thrombus formed on the thrombogenic material is removed and weighed. Reduction in thrombus weight indicates antithrombotic or anticoagulant activity.

12. Ferric Chloride-Induced Arterial Thrombosis

Principle:

Ferric chloride damages vascular endothelium, exposing subendothelial structures that promote platelet adhesion and thrombus formation.

Procedure:

The carotid artery of anesthetized animals is exposed surgically. Filter paper soaked with ferric chloride solution is applied to the artery for a short time to induce thrombosis. Test compounds are administered before induction.

Evaluation:

The time required for complete occlusion of the artery is measured using blood flow monitoring. Prolonged occlusion time indicates anticoagulant activity.

13. Disseminated Intravascular Coagulation (DIC) Model

Principle:

DIC is characterized by widespread activation of coagulation leading to formation of microthrombi and consumption of clotting factors. The model evaluates drugs that prevent pathological coagulation.

Procedure:

DIC is induced in animals by intravenous administration of agents such as endotoxin or thrombin. Test compounds are administered before or during induction of DIC.

Evaluation:

Parameters such as platelet count, fibrinogen levels, and clotting times are measured. Drugs that prevent the development of DIC normalize these parameters

ANTIULCER MODELS

Peptic ulcer is one of the most common gastrointestinal disorders and is characterized by the formation of lesions in the mucosal lining of the stomach or duodenum. Ulcer formation occurs when there is an imbalance between aggressive factors such as gastric acid, pepsin, Helicobacter infection, alcohol, drugs, and stress, and the defensive mechanisms of the gastric mucosa including mucus secretion, bicarbonate production, mucosal blood flow, and prostaglandin synthesis. When the protective mechanisms are weakened or the aggressive factors become dominant, damage to the gastric or duodenal mucosa occurs, leading to ulceration.

The development of anti-ulcer drugs aims to either reduce gastric acid secretion or enhance the defensive mechanisms of the gastric mucosa. Drugs such as proton pump inhibitors, H_2-receptor antagonists, antacids, and cytoprotective agents are widely used in the treatment of peptic ulcer disease. For the discovery and evaluation of new anti-ulcer compounds, several experimental models have been developed that simulate ulcer formation under different pathological conditions.

Anti-ulcer drugs are evaluated using a variety of **experimental animal models** that reproduce ulceration by different mechanisms such as hypersecretion of gastric acid, reduction of mucosal defense, stress, drug-induced mucosal damage, and chemical injury. These models include pylorus ligation-induced ulcers, stress-induced ulcers, ethanol-induced ulcers, drug-induced ulcers, and chronic ulcer models. Such experimental methods allow researchers to study both the **protective and healing effects of potential anti-ulcer agents**, as well as their mechanisms of action. These screening models therefore play an important role in the development and pharmacological evaluation of new therapeutic agents for the treatment of gastric and duodenal ulcers.

Sr. No.	Category	Screening Models
1	**In Vivo Models**	Pylorus ligation-induced gastric ulcer (Shay rat model)
		Stress-induced gastric ulcer
		Ethanol-induced gastric ulcer
		Aspirin-induced gastric ulcer
		Indomethacin-induced gastric ulcer
		Histamine-induced gastric ulcer
		Serotonin-induced gastric ulcer
		Acetic acid-induced chronic gastric ulcer
		Cysteamine-induced duodenal ulcer
		Reserpine-induced gastric ulcer
		Cold restraint stress-induced ulcer
2	**In Vitro / Ex Vivo Models**	Gastric acid secretion in isolated stomach preparation
		Measurement of gastric mucus secretion
		Gastric mucosal cell protection assay
		Measurement of prostaglandin synthesis in gastric mucosa

1. Pylorus Ligation-Induced Gastric Ulcer (Shay Rat Model)

Principle:

This model is based on the accumulation of gastric acid and pepsin in the stomach after ligation of the pyloric end. Continuous secretion of gastric juice without emptying leads to autodigestion of the gastric mucosa and ulcer formation. Anti-ulcer drugs reduce gastric secretion or increase mucosal protection and therefore prevent ulcer formation.

Procedure:

Rats are fasted for about 24 hours but allowed free access to water. Under light anesthesia, the abdomen is opened and the pyloric end of the stomach is carefully ligated without damaging blood supply. The abdomen is then sutured and the animals are allowed to recover. The test drug is administered before or after pylorus ligation depending on the experimental design. After about 4–6 hours, the animals are sacrificed and the stomach is removed. Gastric contents are collected to measure volume, acidity, and pH, and the stomach is opened along the greater curvature to observe ulcer lesions.

Evaluation:

The severity of ulcers is assessed by calculating the **ulcer index** based on number and severity of lesions. Gastric juice parameters such as volume, free acidity, total acidity, and pH are also measured. Reduction in ulcer index and gastric secretion compared with control animals indicates anti-ulcer activity.

2. Stress-Induced Gastric Ulcer

Principle:

Stress conditions such as immobilization or cold exposure cause increased gastric acid secretion, reduced mucosal blood flow, and increased gastric motility, leading to ulcer formation. Anti-ulcer drugs protect the gastric mucosa against stress-induced damage.

Procedure:

Experimental animals such as rats are fasted before the experiment. The animals are subjected to stress conditions such as immobilization, cold exposure, or water immersion stress for several hours. The test drug is administered before exposure to stress. After the stress period, animals are sacrificed and the stomach is removed for examination.

Evaluation:

The gastric mucosa is examined for hemorrhagic lesions and ulcers. The ulcer index is calculated by measuring the number and severity of lesions. A decrease in ulcer index compared with control animals indicates protective activity of the test drug.

3. Ethanol-Induced Gastric Ulcer

Principle:

Ethanol causes direct damage to the gastric mucosal barrier by increasing lipid peroxidation, decreasing mucus production, and causing vascular injury. Drugs with cytoprotective or antioxidant properties reduce ethanol-induced gastric lesions.

Procedure:

Rats are fasted for about 24 hours before the experiment. The test compound is administered orally before giving absolute ethanol or ethanol solution. Ethanol rapidly induces gastric mucosal lesions. After about one hour, animals are sacrificed and the stomach is removed and opened along the greater curvature.

Evaluation:

The gastric mucosa is examined for ulcer lesions and hemorrhagic streaks. The ulcer index is calculated based on lesion size and number. Reduction in lesion severity indicates anti-ulcer activity.

4. Aspirin-Induced Gastric Ulcer

Principle:

Aspirin inhibits cyclooxygenase enzyme leading to decreased prostaglandin synthesis in the gastric mucosa. Prostaglandins play an important role in maintaining mucosal integrity, mucus secretion, and blood flow. Their reduction leads to ulcer formation.

Procedure:

Experimental animals are fasted before the experiment. Aspirin is administered orally to induce gastric

ulcers. The test compound is given before aspirin administration. After several hours, animals are sacrificed and the stomach is removed for examination.

Evaluation:

The number and severity of gastric lesions are observed and the ulcer index is calculated. A decrease in ulcer index indicates protective effect of the test compound.

5. Indomethacin-Induced Gastric Ulcer

Principle:

Indomethacin, a non-steroidal anti-inflammatory drug, causes gastric ulceration by inhibiting prostaglandin synthesis and increasing gastric acid secretion. Drugs that protect gastric mucosa or enhance prostaglandin production reduce ulcer formation.

Procedure:

Animals are fasted before the experiment. Indomethacin is administered to induce gastric ulcers. The test compound is given before or along with indomethacin. After a fixed time period, animals are sacrificed and the stomach is removed and opened.

Evaluation:

The gastric mucosa is examined for ulcers and the ulcer index is calculated. Reduction in ulcer severity compared with control animals indicates anti-ulcer activity.

6. Histamine-Induced Gastric Ulcer

Principle:

Histamine stimulates gastric acid secretion through H_2 receptors present in gastric parietal cells. Excess acid secretion damages gastric mucosa and leads to ulcer formation. Drugs that block H_2 receptors or reduce acid secretion inhibit histamine-induced ulcers.

Procedure:

Experimental animals are fasted prior to the experiment. Histamine is administered to induce gastric

acid secretion and ulcer formation. The test compound is given before histamine administration. After a specific period, animals are sacrificed and the stomach is removed.

Evaluation:

Ulcer lesions are observed and the ulcer index is calculated. The effectiveness of the drug is determined by comparing ulcer scores between treated and control animals.

7. Serotonin-Induced Gastric Ulcer

Principle:

Serotonin causes gastric mucosal damage by inducing vasoconstriction and reducing mucosal blood flow. This leads to ischemia and ulcer formation. Anti-ulcer drugs prevent mucosal damage and improve blood flow.

Procedure:

Rats are fasted before the experiment. Serotonin is injected to induce gastric ulcers. The test compound is administered before serotonin treatment. After a few hours, animals are sacrificed and the stomach is examined.

Evaluation:

The gastric mucosa is inspected for hemorrhagic lesions and ulcers. Ulcer index is calculated and reduction in lesion severity indicates anti-ulcer activity.

8. Acetic Acid-Induced Chronic Gastric Ulcer

Principle:

Application of acetic acid to the gastric wall produces deep chronic ulcers that resemble human peptic ulcers. This model is useful for evaluating drugs that promote ulcer healing.

Procedure:

Under anesthesia, the stomach of rats is exposed surgically. A small amount of acetic acid is applied to the serosal surface of the stomach. The abdomen is sutured and animals are allowed to recover. The test drug is administered daily for several days.

Evaluation:

After the treatment period, animals are sacrificed and the stomach is examined for ulcer size and healing. Reduction in ulcer area compared with control animals indicates healing activity of the test compound.

9. Cysteamine-Induced Duodenal Ulcer

Principle:

Cysteamine induces ulcers mainly in the duodenum by increasing gastric acid secretion and reducing mucosal protection. This model mimics duodenal ulcer conditions in humans.

Procedure:

Rats are fasted before the experiment. Cysteamine is administered to induce duodenal ulcers. The test compound is given before or after cysteamine administration. Animals are sacrificed after a specified period and the duodenum is examined.

Evaluation:

Ulcer lesions in the duodenum are observed and the ulcer index is calculated. A decrease in lesion severity indicates anti-ulcer activity.

10. Reserpine-Induced Gastric Ulcer

Principle:

Reserpine increases gastric acid secretion and reduces protective mucus in the stomach, leading to ulcer formation. Drugs that reduce acid secretion or enhance mucosal protection prevent ulcer formation.

Procedure:

Animals are fasted prior to the experiment. Reserpine is administered to induce gastric ulcers. The test compound is given before reserpine treatment. After several hours, animals are sacrificed and the stomach is examined.

Evaluation:

Ulcer lesions are recorded and the ulcer index is calculated. Reduction in ulcer index indicates anti-ulcer activity.

11. Cold-Restraint Stress-Induced Ulcer

Principle:

Exposure to cold temperature combined with restraint stress leads to increased gastric acid secretion and reduced mucosal blood flow, resulting in ulcer formation.

Procedure:

Rats are fasted before the experiment. The animals are immobilized and kept at low temperature for several hours to induce stress. The test compound is administered before the stress exposure.

Evaluation:

After the experiment, the stomach is removed and examined for ulcer lesions. The ulcer index is calculated and compared with control animals.

ANTICANCER MODELS

Cancer is a complex disease characterized by uncontrolled proliferation of abnormal cells that can invade surrounding tissues and spread to distant organs through the process of metastasis. The development of malignant tumors involves multiple genetic and biochemical changes that affect cell growth, differentiation, and apoptosis. Because of the increasing incidence and high mortality associated with cancer worldwide, the discovery and development of effective anticancer agents is a major focus in pharmaceutical research.

The evaluation of potential anticancer drugs requires reliable experimental systems that can reproduce different aspects of tumor growth and progression. In Vogel's pharmacological assay methods, a variety of **in vitro and in vivo screening models** are used to study the effects of new compounds on tumor cells. In vitro assays involve cultured tumor cell lines and are primarily used to evaluate cytotoxicity,

inhibition of cell proliferation, and induction of apoptosis. These methods allow rapid and controlled testing of large numbers of compounds.

In addition to cell culture studies, **in vivo tumor models in experimental animals** are widely used to evaluate the antitumor activity of drugs under physiological conditions. These models include transplantable tumors such as leukemia and solid tumor models, human tumor xenografts in immunodeficient mice, chemically induced cancer models, and genetically engineered animal models. Such experimental approaches help to assess not only the antitumor efficacy of compounds but also their effects on tumor growth, metastasis, and survival of the host.

Therefore, anticancer screening models described in Vogel's book provide essential tools for understanding the biological activity of new drug candidates and play an important role in the **preclinical evaluation and development of novel anticancer therapies**.

Sr. No.	Category of Model	Screening Models
1	**In Vitro Models**	Tumor cell culture cytotoxicity assays
		Cell viability assays (MTT, SRB assay)
		Clonogenic assay
		DNA synthesis inhibition assay
		Apoptosis detection assays
2	**In Vivo Tumor Transplant Models**	Ehrlich Ascites Carcinoma (EAC) model
		Sarcoma-180 tumor model
		L1210 lymphoid leukemia model
		P388 lymphocytic leukemia model
		B16 melanoma model
		Lewis lung carcinoma model
3	**Xenograft Tumor Models**	Human tumor xenografts in nude mice
		Human breast cancer xenograft
		Human colon cancer xenograft

Sr. No.	Category of Model	Screening Models
		Human lung cancer xenograft
4	**Chemically Induced Cancer Models**	DMBA-induced skin cancer
		DMBA-induced breast cancer
		Nitrosamine-induced gastric cancer
		Aflatoxin-induced liver cancer
5	**Genetically Engineered Animal Models**	Transgenic mice models of cancer
		Knockout mice for tumor suppressor genes
		Oncogene-induced tumor models

1. Tumor Cell Culture Cytotoxicity Assay

Principle:

This in vitro model evaluates the cytotoxic effect of test compounds on cultured cancer cells. Anticancer drugs inhibit cell proliferation or induce cell death in tumor cells. The decrease in viable cell number reflects the cytotoxic activity of the test compound.

Procedure:

Cancer cell lines are cultured in suitable growth medium under controlled conditions of temperature and carbon dioxide. The cells are seeded into culture plates and allowed to attach and grow. Different concentrations of the test compound are added to the culture medium and the cells are incubated for a specified period. After incubation, cell viability is measured using appropriate assays such as dye exclusion or metabolic activity assays.

Evaluation:

The number of viable cells in treated cultures is compared with untreated control cultures. Reduction in cell viability indicates cytotoxic activity. The inhibitory concentration (IC_{50}) of the compound can be calculated to determine its potency.

2. Cell Viability Assays (MTT / SRB Assay)

Principle:

These assays measure cell survival based on metabolic activity or protein content of viable cells. Living cells convert tetrazolium salts such as MTT into colored formazan products, whereas dead cells cannot perform this conversion.

Procedure:

Cancer cells are cultured in microtiter plates and treated with various concentrations of the test compound. After incubation, MTT reagent is added to each well and incubated further to allow formation of formazan crystals. The crystals are dissolved in an appropriate solvent and the absorbance is measured using a spectrophotometer.

Evaluation:

The absorbance values correspond to the number of viable cells. The percentage of cell growth inhibition is calculated relative to control wells. A decrease in absorbance indicates cytotoxic activity of the compound.

3. Clonogenic Assay

Principle:

The clonogenic assay evaluates the ability of a single cancer cell to grow and form a colony. Anticancer drugs inhibit cell reproductive capacity, preventing colony formation.

Procedure:

Cancer cells are seeded at low density in culture plates and exposed to the test compound for a defined period. After treatment, the cells are washed and allowed to grow for several days until colonies are formed. The colonies are then fixed and stained.

Evaluation:

The number of colonies formed in treated cultures is compared with untreated controls. A reduction in colony formation indicates inhibition of cell proliferation by the test compound.

4. DNA Synthesis Inhibition Assay

Principle:

Cancer cells proliferate rapidly by synthesizing DNA. Anticancer drugs often inhibit DNA replication. Measurement of DNA synthesis provides information about the antiproliferative effect of test compounds.

Procedure:

Cultured tumor cells are incubated with the test compound and a labeled nucleotide precursor such as tritiated thymidine. The labeled thymidine is incorporated into newly synthesized DNA. After incubation, the cells are harvested and the radioactivity incorporated into DNA is measured.

Evaluation:

The amount of labeled thymidine incorporated into DNA is compared between treated and control samples. A reduction in DNA synthesis indicates inhibitory activity of the compound on cell proliferation.

5. Apoptosis Detection Assays

Principle:

Many anticancer agents induce programmed cell death (apoptosis) in tumor cells. Detection of apoptotic cells helps determine the mechanism of action of test compounds.

Procedure:

Tumor cells are cultured and treated with the test compound. After incubation, apoptotic cells are detected using techniques such as DNA fragmentation assays, flow cytometry, or staining methods that identify apoptotic markers.

Evaluation:

The proportion of apoptotic cells is determined and compared with control samples. An increase in apoptosis indicates that the test compound induces programmed cell death in cancer cells.

In Vivo Tumor Transplant Models

6. Ehrlich Ascites Carcinoma (EAC) Model

Principle:

This model uses rapidly growing Ehrlich ascites tumor cells implanted into mice. Anticancer drugs inhibit tumor cell proliferation and increase survival of tumor-bearing animals.

Procedure:

Ehrlich ascites carcinoma cells are collected from donor mice and injected intraperitoneally into experimental mice. The test compound is administered after tumor implantation for several days.

Evaluation:

Parameters such as tumor cell count, ascitic fluid volume, body weight, and survival time are measured. A reduction in tumor growth and increased survival time indicate anticancer activity.

7. Sarcoma-180 Tumor Model

Principle:

Sarcoma-180 is a transplantable tumor used to evaluate anticancer drugs. The model measures inhibition of tumor growth in treated animals.

Procedure:

Sarcoma-180 tumor cells are implanted subcutaneously or intraperitoneally in mice. After tumor implantation, animals are treated with the test compound for a specific duration.

Evaluation:

Tumor size or weight is measured at the end of the experiment. The percentage inhibition of tumor growth compared with control animals is calculated.

8. L1210 Lymphoid Leukemia Model

Principle:

This model uses leukemia cells that proliferate rapidly in mice. Anticancer agents inhibit the growth of leukemia cells and prolong survival of animals.

Procedure:

L1210 leukemia cells are injected intraperitoneally into mice. The test compound is administered according to a treatment schedule. Animals are observed for signs of tumor progression.

Evaluation:

The main parameter measured is survival time of animals. Increased life span of treated animals compared with controls indicates antileukemic activity.

9. P388 Lymphocytic Leukemia Model

Principle:

P388 leukemia is a widely used model for evaluating antitumor drugs. Compounds with cytotoxic activity reduce proliferation of leukemia cells.

Procedure:

P388 leukemia cells are injected into mice. After tumor implantation, the test compound is administered daily for a defined treatment period.

Evaluation:

The therapeutic effect is evaluated by measuring survival time and calculating the percentage increase in life span compared with control animals.

10. B16 Melanoma Model

Principle:

B16 melanoma cells produce aggressive tumors in mice. Anticancer drugs inhibit tumor growth and metastasis in this model.

Procedure:

B16 melanoma cells are injected subcutaneously into mice to produce solid tumors. The test compound is administered after tumor development.

Evaluation:

Tumor size and weight are measured at the end of the experiment. Reduction in tumor growth indicates anticancer activity.

11. Lewis Lung Carcinoma Model

Principle:

This model is used to study tumor growth and metastasis in lung carcinoma. Anticancer drugs inhibit primary tumor growth and metastatic spread.

Procedure:

Lewis lung carcinoma cells are implanted subcutaneously into mice. Test compounds are administered according to a treatment schedule.

Evaluation:

Tumor size, weight, and number of metastatic nodules in lungs are determined. Reduction in these parameters indicates anticancer activity.

12. Human Tumor Xenograft Model (Nude Mice)

Principle:

This model involves transplantation of human tumor tissues or cancer cell lines into immunodeficient animals such as nude mice that lack functional T-cells. Since these animals cannot reject foreign tissues, human tumors can grow in them. Anticancer drugs inhibit the growth of transplanted human tumors.

Procedure:

Human tumor cells obtained from cultured cell lines or tumor tissues are implanted subcutaneously into nude mice. The animals are monitored until tumors reach measurable size. The test compound is then administered for a defined treatment period. Tumor growth is measured periodically using calipers.

Evaluation:

Tumor volume or weight is measured and compared between treated and control groups. Reduction in tumor size or delay in tumor growth indicates anticancer activity of the test compound.

Chemically Induced Cancer Models

13. DMBA-Induced Skin Cancer Model

Principle:

7,12-Dimethylbenz[a]anthracene (DMBA) is a potent carcinogen that induces skin tumors in experimental animals. Anticancer drugs reduce the number or size of tumors produced by this carcinogen.

Procedure:

The dorsal skin of mice is shaved and treated with DMBA solution. Repeated application leads to development of skin tumors. The test compound is administered either before or during tumor induction.

Evaluation:

The number of tumors, tumor size, and time required for tumor development are recorded. Reduction in tumor incidence compared with control animals indicates anticancer activity.

14. DMBA-Induced Breast Cancer Model

Principle:

DMBA induces mammary tumors in female rats by causing mutations in mammary gland cells. This model is useful for evaluating drugs against hormone-dependent breast cancer.

Procedure:

Female rats are administered DMBA orally or by injection to induce mammary tumors. Animals are observed for development of palpable tumors. The test compound is administered after tumor induction or during tumor development.

Evaluation:

Tumor incidence, number of tumors per animal, and tumor weight are measured. Reduction in tumor growth compared with control animals indicates anticancer effect.

15. Nitrosamine-Induced Gastric Cancer Model

Principle:

Nitrosamines are carcinogenic compounds that induce tumors in the gastrointestinal tract by causing DNA damage. Anticancer drugs reduce tumor formation induced by these chemicals.

Procedure:

Experimental animals are treated with nitrosamine compounds for a specific period to induce gastric tumors. The test compound is administered before or during carcinogen exposure.

Evaluation:

After the experimental period, animals are sacrificed and the stomach is examined for tumor lesions. Tumor number and size are recorded. Reduction in tumor incidence indicates anticancer activity.

16. Aflatoxin-Induced Liver Cancer Model

Principle:

Aflatoxin B_1 is a potent hepatocarcinogen that causes liver tumors by inducing mutations in liver cells. Drugs that prevent carcinogenesis reduce tumor formation.

Procedure:

Animals are treated with aflatoxin for a defined period to induce liver cancer. The test compound is administered simultaneously or after carcinogen exposure.

Evaluation:

The liver is examined for tumor nodules and histopathological changes. Reduction in tumor formation indicates protective or anticancer activity.

Genetically Engineered Animal Models

17. Transgenic Mouse Cancer Model

Principle:

In transgenic models, animals are genetically modified to express oncogenes that lead to spontaneous tumor development. These models closely resemble human cancer conditions.

Procedure:

Transgenic mice carrying cancer-related genes are used. The animals spontaneously develop tumors during their lifespan. Test compounds are administered to evaluate their ability to prevent or reduce tumor growth.

Evaluation:

Tumor incidence, tumor size, and survival rate of animals are measured. Drugs that reduce tumor formation or delay tumor development show anticancer potential.

18. Knockout Mouse Model (Tumor Suppressor Gene Deletion)

Principle:

In this model, specific tumor suppressor genes are deleted or inactivated. Loss of these genes leads to increased susceptibility to cancer development. Anticancer drugs are evaluated for their ability to inhibit tumor progression.

Procedure:

Knockout mice lacking specific tumor suppressor genes are used in experiments. Test compounds are administered to animals during tumor development.

Evaluation:

Tumor growth, number of tumors, and survival rate are measured. Reduction in tumor progression indicates therapeutic potential of the compound.

ANTIASTHMATIC MODELS

Bronchial asthma is a chronic inflammatory disease of the airways characterized by reversible airway obstruction, bronchial hyperresponsiveness, and excessive mucus secretion. The disease is associated with narrowing of the airways caused by contraction of bronchial smooth muscles, inflammation of the airway mucosa, and release of inflammatory mediators such as histamine, leukotrienes, prostaglandins, and cytokines from mast cells and other inflammatory cells. These pathological changes lead to symptoms such as wheezing, coughing, shortness of breath, and chest tightness.

The development of antiasthmatic drugs aims to relieve bronchoconstriction, reduce airway inflammation, and prevent allergic reactions that contribute to asthma attacks. Common therapeutic agents include bronchodilators, antihistamines, leukotriene antagonists, mast cell stabilizers, and anti-inflammatory drugs. For the discovery and evaluation of new antiasthmatic agents, reliable experimental models are required to reproduce the pathophysiological features of asthma under controlled laboratory conditions.

In the pharmacological assays described in Vogel's book, several **in vivo, in vitro, and ex vivo experimental models** are used to study bronchial constriction, airway inflammation, and allergic responses. In vivo models such as histamine-induced bronchospasm, allergen-induced asthma, and chemical-induced asthma simulate bronchial obstruction and hypersensitivity in experimental animals. In vitro models involve isolated airway tissues or cell preparations to evaluate bronchodilator activity and inhibition of mediator release. Ex vivo methods are used to measure airway resistance, inflammatory cells, and mediators in lung tissues after treatment with test compounds.

These screening methods provide valuable information regarding the **bronchodilatory, anti-inflammatory, and antiallergic properties of potential drug candidates** and play an important role in the preclinical development of new therapeutic agents for the treatment and management of bronchial asthma.

Sr. No.	Category of Model	Screening Models
1	**In Vivo Models**	Histamine-induced bronchospasm in guinea pigs
		Acetylcholine-induced bronchospasm
		Ovalbumin-induced allergic asthma model
		Passive cutaneous anaphylaxis

Sr. No.	Category of Model	Screening Models
		Active systemic anaphylaxis
		Antigen-induced bronchoconstriction
		Platelet-activating factor (PAF)-induced bronchospasm
		Leukotriene-induced bronchoconstriction
		Prostaglandin-induced bronchospasm
		Toluene diisocyanate (TDI)-induced asthma
2	In Vitro Models	Isolated guinea pig tracheal chain preparation
		Isolated bronchial smooth muscle preparation
		Mast cell degranulation assay
		Histamine release assay
		Leukotriene synthesis inhibition assay
3	Ex Vivo Models	Measurement of airway resistance after drug treatment
		Bronchoalveolar lavage fluid (BALF) analysis
		Measurement of inflammatory mediators in lung tissue

1. Histamine-Induced Bronchospasm in Guinea Pigs

Principle:

Histamine is a potent bronchoconstrictor that causes contraction of bronchial smooth muscles leading to airway obstruction. In guinea pigs, inhalation of histamine produces bronchospasm and respiratory distress similar to asthma. Antiasthmatic drugs prevent or delay histamine-induced bronchoconstriction.

Procedure:

Guinea pigs are placed in a chamber and exposed to an aerosol of histamine solution. Exposure to histamine produces dyspnea and bronchospasm in the animals. The test compound is administered before histamine exposure either orally, intraperitoneally, or by inhalation. The animals are then exposed again to histamine aerosol.

Evaluation:

The time taken for the onset of pre-convulsive dyspnea (difficulty in breathing) is recorded.

Antiasthmatic drugs increase the latency period before the onset of bronchospasm. A longer time to dyspnea compared with control animals indicates bronchodilatory activity.

2. Acetylcholine-Induced Bronchospasm

Principle:

Acetylcholine causes contraction of bronchial smooth muscles through stimulation of muscarinic receptors, resulting in bronchoconstriction. Drugs that possess bronchodilator or anticholinergic activity inhibit acetylcholine-induced bronchospasm.

Procedure:

Guinea pigs are exposed to aerosolized acetylcholine solution in a chamber. This produces bronchospasm and respiratory distress. The test compound is administered prior to acetylcholine exposure.

Evaluation:

The onset time of bronchospasm and severity of respiratory distress are recorded. Drugs that delay or reduce bronchospasm demonstrate antiasthmatic activity.

3. Ovalbumin-Induced Allergic Asthma Model

Principle:

Ovalbumin acts as an allergen that induces an immune response leading to airway inflammation, bronchoconstriction, and mucus secretion similar to allergic asthma in humans.

Procedure:

Animals such as mice or guinea pigs are sensitized with ovalbumin by injection along with an adjuvant. After sensitization, the animals are challenged with inhaled or injected ovalbumin to produce allergic asthma symptoms. The test compound is administered before allergen challenge.

Evaluation:

Parameters such as airway resistance, inflammatory cell infiltration, and mucus production in lung

tissues are measured. Reduction in airway inflammation and bronchoconstriction indicates antiasthmatic activity.

4. Passive Cutaneous Anaphylaxis

Principle:

This model evaluates the ability of drugs to inhibit allergic reactions mediated by antibodies and mast cell degranulation. Antiasthmatic drugs that stabilize mast cells prevent the release of histamine and other mediators.

Procedure:

Serum containing antibodies against a specific antigen is injected intradermally into animals such as rats. After a sensitization period, the animals are challenged with the antigen along with a dye injected intravenously. The test compound is administered before antigen challenge.

Evaluation:

Leakage of dye at the injection site indicates increased vascular permeability due to mediator release. Drugs that reduce dye leakage demonstrate antiallergic or mast cell stabilizing activity.

5. Active Systemic Anaphylaxis

Principle:

Active systemic anaphylaxis is a severe allergic reaction caused by antigen-antibody interaction leading to release of inflammatory mediators such as histamine, leukotrienes, and prostaglandins.

Procedure:

Animals are sensitized with an antigen such as ovalbumin. After the sensitization period, the animals are challenged with the same antigen to induce systemic anaphylaxis. The test compound is administered before antigen challenge.

Evaluation:

Severity of symptoms such as bronchospasm, respiratory distress, and mortality is observed. Reduction in symptoms or increased survival rate indicates antiasthmatic or antiallergic activity.

6. Antigen-Induced Bronchoconstriction

Principle:

In sensitized animals, exposure to a specific antigen causes bronchial smooth muscle contraction and airway obstruction due to release of inflammatory mediators.

Procedure:

Animals are sensitized with an antigen such as ovalbumin. After sensitization, the animals are challenged with the antigen via inhalation. The test compound is administered before antigen challenge.

Evaluation:

Airway resistance, lung function, and bronchoconstriction are measured using respiratory monitoring equipment. Drugs that reduce airway resistance indicate antiasthmatic activity.

7. Platelet-Activating Factor (PAF)-Induced Bronchospasm

Principle:

PAF is an inflammatory mediator that causes bronchoconstriction and airway inflammation. Drugs that inhibit PAF activity reduce bronchospasm.

Procedure:

Experimental animals are exposed to PAF through inhalation or injection. The test compound is administered before exposure.

Evaluation:

Respiratory distress, airway resistance, and inflammatory response are measured. A reduction in these parameters indicates antiasthmatic activity.

8. Leukotriene-Induced Bronchoconstriction

Principle:

Leukotrienes are potent mediators of bronchoconstriction and inflammation in asthma. Drugs that block leukotriene receptors or inhibit their synthesis reduce bronchospasm.

Procedure:

Animals are exposed to leukotrienes that induce bronchoconstriction. The test compound is administered prior to leukotriene exposure.

Evaluation:

Airway resistance and respiratory symptoms are measured. Reduction in bronchoconstriction indicates effectiveness of the test compound.

9. Prostaglandin-Induced Bronchospasm

Principle:

Certain prostaglandins can cause bronchial smooth muscle contraction and airway inflammation. Drugs that inhibit prostaglandin action can prevent bronchospasm.

Procedure:

Prostaglandins are administered to experimental animals to induce bronchospasm. The test compound is given before prostaglandin administration.

Evaluation:

The degree of bronchoconstriction and respiratory distress is recorded. Reduction in bronchospasm indicates bronchodilator activity.

10. Toluene Diisocyanate (TDI)-Induced Asthma Model

Principle:

TDI is a chemical irritant that induces airway inflammation and hypersensitivity similar to occupational asthma.

Procedure:

Animals are exposed to TDI vapors repeatedly to induce asthma-like symptoms. The test compound is administered during the sensitization or challenge phase.

Evaluation:

Airway inflammation, mucus secretion, and bronchial hyperresponsiveness are measured. Reduction in these symptoms indicates antiasthmatic activity.

In Vitro Models

11. Isolated Guinea Pig Tracheal Chain Preparation

Principle:

This model studies contraction and relaxation of airway smooth muscle in response to bronchoconstrictors and bronchodilators.

Procedure:

The trachea of guinea pigs is removed and cut into rings which are connected to form a chain. The preparation is suspended in an organ bath containing physiological solution. Bronchoconstrictors such as histamine are added followed by the test compound.

Evaluation:

Changes in smooth muscle contraction are recorded using a recording device. Relaxation of tracheal muscle indicates bronchodilator activity.

12. Mast Cell Degranulation Assay

Principle:

Mast cells release histamine and other mediators during allergic reactions. Drugs that stabilize mast cells prevent mediator release and reduce asthma symptoms.

Procedure:

Mast cells are isolated from animal tissues and exposed to allergens in the presence or absence of the test compound.

Evaluation:

The percentage of degranulated mast cells is counted under a microscope. Reduction in mast cell degranulation indicates antiallergic activity.

13. Histamine Release Assay

Principle:

Allergic reactions cause release of histamine from mast cells. Antiasthmatic drugs inhibit histamine release.

Procedure:

Isolated mast cells or lung tissues are incubated with allergens in the presence of the test compound.

Evaluation:

The amount of histamine released into the medium is measured using biochemical methods. Decreased histamine release indicates antiasthmatic activity.

14. Isolated Bronchial Smooth Muscle Preparation

Principle:

Bronchial smooth muscle contraction plays a major role in airway obstruction during asthma. This model studies the direct effect of test compounds on bronchial smooth muscle contraction and relaxation.

Procedure:

Bronchial tissues are isolated from experimental animals such as guinea pigs and mounted in an organ bath containing physiological solution maintained at 37°C. The tissue is connected to a force transducer to record muscle contraction. Bronchoconstrictor agents such as histamine or acetylcholine are added to induce contraction. The test compound is then added to observe its bronchodilator effect.

Evaluation:

Changes in muscle tension are recorded using a physiograph. Reduction in contraction or relaxation of bronchial smooth muscle indicates bronchodilator activity.

15. Leukotriene Synthesis Inhibition Assay

Principle:

Leukotrienes are inflammatory mediators involved in bronchoconstriction, mucus secretion, and airway inflammation in asthma. Drugs that inhibit leukotriene synthesis can reduce asthma symptoms.

Procedure:

Inflammatory cells such as leukocytes are isolated from animal blood or tissues and stimulated to produce leukotrienes using chemical agents. The test compound is added to the system before stimulation.

Evaluation:

The amount of leukotriene produced is measured using biochemical or immunological methods. A decrease in leukotriene production compared with control samples indicates inhibitory activity.

Ex Vivo Models

16. Measurement of Airway Resistance After Drug Treatment

Principle:

Asthma is characterized by increased airway resistance due to bronchoconstriction and inflammation. Drugs that reduce airway resistance demonstrate antiasthmatic activity.

Procedure:

Animals such as guinea pigs are treated with the test compound and then exposed to bronchoconstrictor agents such as histamine or allergens. Airway resistance is measured using respiratory monitoring instruments.

Evaluation:

The degree of airway resistance before and after treatment is compared. A decrease in airway resistance indicates bronchodilator or antiasthmatic activity.

17. Bronchoalveolar Lavage Fluid (BALF) Analysis

Principle:

Inflammation in asthma leads to infiltration of inflammatory cells such as eosinophils, neutrophils, and lymphocytes into the lungs. BALF analysis allows measurement of these inflammatory cells and mediators.

Procedure:

Animals are sensitized with allergens to induce asthma-like inflammation. After treatment with the test compound, bronchoalveolar lavage is performed by flushing the lungs with saline solution. The recovered fluid is collected for analysis.

Evaluation:

The number of inflammatory cells and levels of inflammatory mediators in the BALF are measured. A decrease in inflammatory cell count indicates anti-inflammatory and antiasthmatic activity.

18. Measurement of Inflammatory Mediators in Lung Tissue

Principle:

Asthma involves release of inflammatory mediators such as histamine, leukotrienes, cytokines, and prostaglandins in lung tissues. Drugs that inhibit the release of these mediators reduce airway inflammation.

Procedure:

Animals are sensitized with allergens and treated with the test compound. After the experimental period, lung tissues are collected and homogenized. Biochemical assays are performed to measure inflammatory mediators.

Evaluation:

Levels of mediators such as histamine, leukotrienes, and cytokines are compared between treated and control groups. Reduced mediator levels indicate antiasthmatic activity.

ANTIDIURETIC MODEL

The regulation of body water balance is an essential physiological function controlled mainly by the kidneys and the hormone vasopressin (antidiuretic hormone, ADH). Antidiuretic mechanisms reduce urine formation by increasing the reabsorption of water in the renal tubules, thereby maintaining fluid balance, blood volume, and osmotic pressure of body fluids. Disturbances in this regulatory system may lead to excessive loss of water through urine, as observed in conditions such as diabetes insipidus.

Antidiuretic drugs are substances that decrease urine output by promoting water reabsorption in the kidneys. These agents are mainly used in the treatment of disorders associated with excessive diuresis. In pharmacological research, experimental models are required to evaluate the effectiveness and mechanism of action of such compounds. Therefore, several screening procedures have been developed to study the antidiuretic activity of new substances.

In Vogel's pharmacological assays, different experimental approaches are employed to evaluate antidiuretic effects. These include models based on water-loaded animals, vasopressin-deficient conditions, and measurements of urine volume and osmolarity after administration of test compounds. Such models help to determine whether a compound can reduce urine output, increase water reabsorption, or enhance the action of endogenous antidiuretic hormone.

These experimental methods provide valuable information about the **efficacy, mechanism of action, and pharmacological profile of potential antidiuretic agents** and are important tools in the preclinical evaluation and development of drugs affecting renal water balance.

Sr. No.	Category of Model	Screening Models
1	**In Vivo Models**	Lipschitz test in rats (saline load method)
		Water-loaded diuresis in rats
		Osmotic diuresis model
		Potassium-sparing diuretic test
		Mercurial diuretic test
		Loop diuretic activity test
		Thiazide diuretic activity test
2	**Ex Vivo Models**	Measurement of electrolyte excretion in urine (Na^+, K^+, Cl^-)

Sr. No.	Category of Model	Screening Models
		Measurement of urine volume and osmolarity
		Renal clearance studies
3	In Vitro Models	Isolated kidney tubule transport studies
		Ion transport inhibition assays
		Enzyme inhibition assays related to renal transport mechanisms

1. Lipschitz Test (Saline Load Method)

Principle:

The Lipschitz test is one of the most widely used methods for evaluating diuretic activity. Diuretics increase urine output by promoting the excretion of water and electrolytes such as sodium and chloride from the kidneys. In this method, saline loading produces a uniform state of hydration in experimental animals, and the ability of test compounds to enhance urine output and electrolyte excretion is assessed in comparison with standard diuretics.

Procedure:

Albino rats are fasted overnight but allowed free access to water. The animals are divided into groups such as control, standard, and test groups. All animals receive a fixed volume of isotonic saline solution to ensure equal fluid load. The test drug is administered orally to the test group, while a standard diuretic drug is given to the standard group. The animals are then placed in metabolic cages for urine collection over a specified period, usually 5–24 hours.

Evaluation:

The volume of urine produced is measured, and electrolyte concentrations such as sodium, potassium, and chloride in the urine are determined using biochemical methods. Increased urine volume and electrolyte excretion compared with control animals indicate diuretic activity. The diuretic index and natriuretic activity may also be calculated.

2. Water-Loaded Diuresis in Rats

Principle:

Administration of excess water increases urine production due to suppression of antidiuretic hormone activity. Diuretic drugs enhance the elimination of this excess water through increased urine formation.

Procedure:

Experimental rats are deprived of food overnight but allowed access to water. The animals receive a known volume of water orally to induce water diuresis. The test compound is administered to the test group, while the control group receives only water or vehicle.

Evaluation:

Urine is collected at regular intervals, and the total urine volume is measured. A significant increase in urine output in treated animals compared with control animals indicates diuretic activity.

3. Osmotic Diuresis Model

Principle:

Osmotic diuretics increase urine output by increasing osmotic pressure in the renal tubules, which prevents water reabsorption. This model evaluates the ability of compounds to produce diuresis through osmotic mechanisms.

Procedure:

Animals are administered osmotic agents such as mannitol to induce diuresis. The test compound is administered before or along with the osmotic agent. Animals are placed in metabolic cages for urine collection.

Evaluation:

Urine volume and osmolarity are measured. Drugs that enhance osmotic diuresis produce increased urine volume and decreased water reabsorption in the renal tubules.

4. Potassium-Sparing Diuretic Test

Principle:

Potassium-sparing diuretics increase sodium and water excretion while preventing potassium loss. This model is used to determine whether a test compound exhibits potassium-sparing activity.

Procedure:

Experimental animals are treated with the test compound and placed in metabolic cages for urine collection. Electrolyte concentrations in the urine are measured after a specified period.

Evaluation:

Increased sodium excretion with minimal potassium loss compared with control animals indicates potassium-sparing diuretic activity.

5. Mercurial Diuretic Test

Principle:

Mercurial diuretics act by inhibiting sodium reabsorption in renal tubules. This model evaluates compounds with similar mechanisms of action.

Procedure:

Animals receive mercurial compounds or test drugs that may possess similar diuretic activity. Urine is collected for a fixed period following drug administration.

Evaluation:

Urine volume and sodium excretion are measured. Increased sodium excretion indicates diuretic activity similar to mercurial diuretics.

6. Loop Diuretic Activity Test

Principle:

Loop diuretics act on the ascending limb of the loop of Henle by inhibiting sodium, potassium, and chloride reabsorption. This leads to increased urine output and electrolyte excretion.

Procedure:

Animals receive the test compound, and urine is collected over a specific period. Electrolyte concentrations in urine are analyzed to determine the drug's effect on ion transport in the kidney.

Evaluation:

A marked increase in sodium, potassium, and chloride excretion along with increased urine volume indicates loop diuretic activity.

7. Thiazide Diuretic Activity Test

Principle:

Thiazide diuretics inhibit sodium and chloride reabsorption in the distal convoluted tubules of the kidney. This results in moderate diuresis and increased electrolyte excretion.

Procedure:

Experimental animals are administered the test compound and placed in metabolic cages for urine collection. Electrolyte concentrations are measured in the urine.

Evaluation:

Increased sodium and chloride excretion with moderate diuresis indicates thiazide-like diuretic activity.

Ex Vivo Models

8. Measurement of Electrolyte Excretion in Urine

Principle:

Diuretic drugs affect the renal excretion of electrolytes such as sodium, potassium, and chloride. Measuring these electrolytes helps determine the mechanism of diuretic action.

Procedure:

Urine samples collected from treated animals are analyzed using biochemical methods such as flame photometry or ion-selective electrodes.

Evaluation:

Changes in electrolyte levels compared with control animals indicate the effect of the drug on renal tubular transport.

9. Renal Clearance Studies

Principle:

Renal clearance studies measure the rate at which substances are removed from the blood by the kidneys. Diuretics alter renal clearance of electrolytes and water.

Procedure:

Animals are administered test compounds, and blood and urine samples are collected. The clearance of electrolytes and other substances is calculated using standard physiological formulas.

Evaluation:

An increase in renal clearance indicates enhanced excretion of substances by the kidneys and demonstrates diuretic activity.

In Vitro Models

10. Isolated Kidney Tubule Transport Studies

Principle:

Renal tubules play a crucial role in reabsorption and secretion of ions and water. Studying isolated kidney tubules helps determine the direct effect of drugs on tubular transport mechanisms.

Procedure:

Kidney tubules are isolated from experimental animals and incubated in physiological solutions. The test compound is added to the system to observe its effect on ion transport.

Evaluation:

Changes in ion transport across the tubular membrane are measured. Inhibition of sodium reabsorption indicates diuretic activity.

IMPORTANT QUESTIONS

Very Short Questions (2 marks each)

1. What is the primary action of antihypertensive drugs?
2. Name one commonly used diuretic in preclinical studies.
3. Define antiarrhythmic drug.
4. What is an antidyslipidemic drug used for?
5. Give an example of an anticoagulant.
6. What is the role of antiaggregatory drugs?
7. Mention one preclinical screening model for antiulcer drugs.
8. Name a commonly used antidiabetic drug in preclinical studies.
9. What is the primary objective of anticancer drugs?
10. Define antiasthmatic drug.

Short Questions (5 marks each)

1. Describe the mechanism of action of antihypertensive drugs in preclinical screening models.
2. Explain the preclinical screening process for diuretics.
3. Discuss the importance of antiarrhythmic drugs in cardiovascular research.
4. Summarize the methods used to evaluate antidyslipidemic drugs in preclinical studies.
5. Illustrate the procedure for testing anticoagulants in animal models.
6. Compare the screening models for coagulants and anticoagulants.
7. Explain the preclinical screening methods for antiulcer drugs.
8. Describe the animal models used for evaluating antidiabetic drugs.
9. Discuss the role of animal models in anticancer drug screening.
10. Explain the significance of preclinical screening for antiasthmatic drugs.

Long Questions (10 marks each)

1. Analyze the preclinical screening models for antihypertensive drugs, including their mechanisms and evaluation criteria.
2. Evaluate the various preclinical screening models for diuretics, focusing on methodology and expected outcomes.

3. Critically assess the screening models for antiarrhythmic drugs and their implications for cardiovascular research.
4. Discuss the comprehensive process of preclinical screening for antidyslipidemic drugs.
5. Compare and contrast the screening models for coagulants and anticoagulants, highlighting their significance in research.
6. Describe the preclinical screening models for antiulcer drugs, including preparation, methodology, and evaluation.
7. Evaluate the advancements in preclinical screening models for antidiabetic drugs and their impact on diabetes research.
8. Discuss the challenges and methodologies involved in the preclinical screening of anticancer drugs.
9. Analyze the screening models for antiasthmatic drugs and their relevance in respiratory research.
10. Discuss the ethical considerations and challenges in the preclinical screening of cardiovascular and other important drugs.