International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

TOXICITY STUDIES IN PRECLINICAL DEVELO-PMENT OF A NCE - FOCUSS ON CAPTOPRIL.

Fathima Jazeela M A[1], Sharun Balan[2], Shama Shreen[3], Shaima Noora[4].

Department of pharmacology, Malik Deenar College of Pharmacy, Seethangoli, Bela, Kasaragod, Kerala, 671321.

INTRODUCTION :

Toxicity testing is critical in the evaluation of novel medications before they are used on humans. The goal of toxicity testing is to identify any potential hazardous consequences that a test chemical may have, not merely to determine how safe it is. The main goals of toxicity testing are to determine how test compounds affect lab animals and whether they have any direct hazardous effects on humans. Secondly, high doses of test substances are given to lab animals in order to assess any potential risks to humans who receive much lower levels.[1]

DIFFERENT EXPERIMENTAL ANIMALS IN TOXICITY STUDIES

Rat

Albino rat is one of the common laboratory animal suitable for experimental purpose because of its small size and greater sensitivity to most drugs.it is also the most standardised of all laboratory animals.

2. Mice

Mice are employed widely in acute toxicity studies. They are also used for the assay of insulin and analgesic, the later specially against chemically induced pain.

3. Guinea Pig

Guinea pigs are employed for the evaluation of bronchodilator compound against experimentally induced asthma (histamine or acetylcholine aerosols). They are widely used in immunology, particularly in the studies of delayed hypersensitivity.

4. Rabbit

rabbits are very docile animals employed for variety of studies. Rabbits are employed for the pyrogen testing in intravenous fluid. Insulin and other antidiabetic drug, curare and sex hormones are tested I n rabbit.[2]

REGULATORY AGENCIES

1. ICH (international council for harmonization)
2. OECD (Organisation For Economic Corporation Development)
3. FDA (food and drug administration)
4. WHO (world health organisation)

ETHICAL CONSIDERATION

1. Ensuring The Safety of animal
2. Well, Being of animal
3. Rights Of Participants
4. Maintaining Transparency

FACILITIES AND DOCUMENTATION AND STATISTICS REQUIRED

1. Animal Care
2. Laboratory Equipment
3. Secure Storage
4. Waste Disposal
5. Quality Assurance
6. Documents Should Be Well Mainatained.

ACUTE TOXICITY STUDIES IN ANIMALS

Acute toxicity studies in animals are designed to evaluate the adverse effects that occur within a short period (usually 24 hours to 14 days) after a single dose or multiple doses of a chemical, drug, or substance. These studies help determine the *lethal dose (LD_{50})*, *target organs*, and potential symptoms of toxicity, which are crucial for assessing the safety of chemicals and pharmaceuticals before human exposure.

Purpose of Acute Toxicity Studies

- Determine **LD_{50}** (the dose lethal to 50% of the test animals).
- Identify **clinical signs** of toxicity (e.g., convulsions, salivation, lethargy).
- Assess **target organs** affected by the substance.
- Provide information for:
    - **Classification and labelling** (e.g., under GHS).
    - **Dose selection** for subacute/sub chronic studies.
    - **Risk assessment** for human exposure.

Acute toxicity test methods measure the adverse effects that occur within a short time after administration of a single dose of a test substrate. This testing is performed principally in rodence and is usually done early in the development of a new chemical or product to provide information of its potential toxicity.[6] This information may also be extrapolated for use in the diagnosis and treatment of toxic reaction in humans.

The results from acute toxicity tests can provide information for comparison of toxicity and dose-response among members of chemical classes and help in the selection of candidate materials for further work. They are further used to standardize certain biological products such as vaccines.[3]

PARAMETERS OBSERVED

1. Body temperature
2. Pulse rate
3. Respiratory rate
4. Arterial blood pressure
5. Volume of urine produced
6. Volume of tear produced[8]

CALCULATION OF LD50

The LD50 is a standard measure used in toxicology to determine the median lethal dose of a substance. It is typically expressed in milligrams of substance per kilogram of body weight (mg/kg). For instance, if an LD50 value is 200 mg/kg, it means that 200 milligrams of the substance per kilogram of body weight is sufficient to kill half the population used in the test.[4]

Subacute Toxicity Studies in Animals

Subacute toxicity studies (also called repeated dose 28-day studies) are used to evaluate the toxic effects of a substance after repeated exposure over a short period, typically 28 days. These studies help identify target organs, dose-response relationships, and No-Observed-Adverse-Effect Levels (NOAELs).[10]The animals used in subacute toxicity are bigger rodents such as rats. These animals are manipulated probably on a daily basis (according to designed protocol) and will have to resist test duration. For example, daily oral administration of test substance with an intubation needle or even IP (which are common exposure routes) for about 28 days may be stressful for mice [46–48]. Hence they are not very suitable.17 Even when the test product has to be mixed with feed or water for animals to freely consume orally or a product is meant for inhalation route, the use of mice should be strongly justified.Rodents (usually rats) preferred for oral and inhalation studies; rabbits for dermal studies; non rodents (Usually dogs) recommended as a second species for oral tests.[5]

DETAIL OF OBSERVATION

Sub acute and chronic toxicity studies are designed to characterize the toxic effects of drugs upon repeated daily administration for periods of time ranging from 2 weeks to 1 year and to determine no-toxic-effect dosage levels for short to long-term repeated dosing.[6]

RECORDING AND ANALYSIS OF DATA

Data Recording

Accurate and detailed recording of all observations and data is crucial for a thorough analysis. This includes:

 Individual animal records: Detailed daily observations of clinical signs, including their onset, duration, and severity.

 Body weight: Measured and recorded at regular intervals (e.g., daily or every few days).

 Food and water consumption: Measured and recorded at regular intervals per cage. If animals are group-housed, individual consumption may not be possible.[7]

Data Analysis

The collected data should be analysed using appropriate statistical methods to

determine if there are any statistically significant differences between the treated groups and the control group. Key aspects of data analysis include:

 Descriptive statistics: Calculation of means, standard deviations, and ranges for all quantitative parameters.

 Inferential statistics: Use of appropriate statistical tests (e.g., ANOVA, t-tests, chi-square tests) to compare treatment groups with the control group.

The choice of test depends on the type of data and the experimental design.[8]

1. Purpose of Animal Toxicity Studies in NCE Development

When developing a new chemical entity (NCE), animal studies are performed to:

 Assess safety and toxicological profile.

 Identify target organs of toxicity.

 Determine no observed adverse effect level (NOAEL).

 Understand mechanism of action and off-target effects.

 Evaluate potential drug-drug interactions.

Hypertension

Hypertension commonly known as **high blood pressure**, is a chronic medical condition that affects millions of individuals worldwide. Often called the **"silent killer"**, it frequently presents with no symptoms while quietly damaging blood vessels and vital organs over time. As a leading risk factor for heart disease, stroke, kidney failure, and other serious conditions, understanding hypertension is crucial for effective prevention, early diagnosis, and appropriate management.

Purpose of Antihypertensive Drug Studies in Animals

- Animal studies are essential in understanding:
- The mechanisms of hypertension
- The efficacy of antihypertensive drugs
- Potential side effects
- Organ protection (heart, kidney, brain)

These studies help develop and refine drugs before human clinical trials.

Key Mechanisms in Hypertension Pathophysiology

1. Endothelial Dysfunction

A hallmark of hypertension is impaired endothelial function, characterized by:

- Reduced nitric oxide (NO) bioavailability.
- Increased production of reactive oxygen species (ROS).
- Elevated levels of endothelin-1 and angiotensin II.
- Inflammatory responses.

These factors collectively contribute to vascular stiffness, increased peripheral resistance, and target organ damage.

2. Vascular Re-Modelling

Chronic hypertension leads to structural changes in blood vessels, including:

- Inward eutrophic remodelling: Reduction in lumen diameter without changes in wall thickness, observed in models like spontaneously hypertensive rats (SHR) and angiotensin II infusion.
- Hypertrophic re-modelling: Thickening of the vessel wall, noted in renovascular hypertension and salt-sensitive models.
- Rarefaction: Loss of microvascular density, contributing to increased peripheral resistance.

3. Sympathetic Nervous System Activation

Elevated sympathetic tone leads to:

- Increased heart rate and vascular resistance.
- Enhanced renin release from the kidneys
- Elevated blood pressure

This activation is often observed in genetic and pharmacological hypertension models.

4. Renin-Angiotensin-Aldosterone System (RAAS) Dysregulation

Overactivation of RAAS results in:

- Vasoconstriction
- Sodium and water retention
- Increased blood pressure

This pathway is central in models like DOCA-salt and angiotensin II infusion.

Purpose of Toxicity Studies for Antihypertensive NCEs

1. **Identify toxic effects and target organs** (e.g., heart, kidneys, CNS).
2. Determine **NOAEL** (No Observed Adverse Effect Level) and **LOAEL** (Lowest Observed Adverse Effect Level).
3. Assess **dose-response relationships**.
4. Evaluate **species-specific responses**.
5. Support **regulatory filings** (IND, CTD Module 4).
6. Predict potential **human adverse effects**.

Blood Pressure Measurement Methods in Animals:

1.Indirect (Non-Invasive) Methods

- Tail-Cuff Method: Commonly used in rodents, this method involves wrapping a cuff around the tail and inflating it to occlude blood flow, then slowly deflating to detect the return of blood flow. While non-invasive, it can be influenced by animal stress and is less accurate for continuous monitoring.
- Doppler Ultrasonography: Utilizes an ultrasonic probe to detect blood flow velocity, allowing for the estimation of systolic BP. It's widely used in veterinary practice for companion animals.
- Oculometry: Measures oscillations in the arterial wall as the cuff deflates. This method is automated and suitable for high-throughput studies, though it may be less accurate in small or stressed animals.

2. Direct (Invasive) Methods: Catheterization: Involves surgically implanting a catheter connected to a pressure transducer to measure BP directly within the artery. This method provides continuous, real-time data and is considered the gold standard for BP measurement in animals.

- Radiotelemetry: Implants a small device under the skin that transmits BP data wirelessly. It allows for continuous, unrestrained monitoring of BP in conscious animals, making it ideal for long-term studies. [9]

Common Antihypertensive Drugs in Animal Research

- **ACE Inhibitors**

Example-Enalapril, Captopril, Ramipril
Mechanism of Action-Inhibit angiotensin-converting enzyme (ACE), reducing Ang II and increasing bradykinin.
Common animal models-SHR, 2K1C, DOCA-salt rats.
Observation-↓ BP, ↓ cardiac hypertrophy, ↑ renal protection, ↓ inflammation.

- **ARBs (Angiotensin II Receptor Blockers)**

Example -Losartan, Valsartan .
Mechanism of Action -Block AT1 receptors → vasodilation and ↓ aldosterone.
Animal models --SHR, diabetic nephropathy rats.
Observation-↓ BP, ↓ proteinuria, ↓ cardiac and renal fibrosis.

- **Calcium Channel Blockers**

Example -Amlodipine, Nifedipine.
Mechanism Of Action -Block L-type calcium channels → vasodilation.
Animal used -SHR, stroke-prone SHR.
Observation - ↓ BP, ↓ vascular resistance, ↑ cerebral blood flow.

- **Beta-Blockers**

Example - Propranolol, Atenolol.
Mechanism of action -Block β-adrenergic receptors → ↓ heart rate and contractility.
Animal used -SHR, DOCA-salt rats.
Observation -↓ BP, ↓ cardiac remodelling, ↓ sympathetic activity

- **Diuretics**

Example -Hydrochlorothiazide, Furosemide
Mechanism of Action -Increase sodium and water excretion → ↓ plasma volume
Animal used - SHR, salt-sensitive rats.

- **ACE INHIBITOR**

CAPTOPRIL

Captopril, an angiotensin-converting enzyme (ACE) inhibitor, is commonly used to treat hypertension and heart failure in humans. In the context of animal toxicity studies during the development of a new chemical entity (NCE), captopril may be studied in several ways:

- **How Captopril Affects Toxicity Studies in Animals**

As a Comparator or Reference Drug

- In studies of other antihypertensives or cardiovascular drugs, captopril may be used as a positive control or comparator.
- This allows researchers to compare the new compound's safety and efficacy against a known ACE inhibitor.

B. Toxicity Profile of Captopril in Animals

Animal studies have shown the following toxicity effects at high doses or prolonged exposure:

- Species -Rats & Mice.
  Observable effects -Weight loss, renal tubular degeneration, anaemia.
  Notes - Dose-dependent.
- Species -Dogs.
  Observable effects - Hypotension, renal toxicity, GI disturbances.
  Notes - Seen at high doses.

C. Mechanism of Toxic Effects

- ✓ Hypotension: From vasodilation due to ACE inhibition.
- ✓ Renal toxicity: ACE inhibitors reduce glomerular filtration pressure, which can impair kidney function, especially in compromised animals.
- ✓ Electrolyte imbalance: Alterations in sodium and potassium levels.

Considerations When Using Captopril in NCE Studies.

- ❖ Species differences: Metabolism and sensitivity to ACE inhibition vary between species.
- ❖ Dose selection: High doses may not translate directly to human toxicology outcomes.
- ❖ Combination toxicity: If the NCE is tested in combination with captopril, synergistic or antagonistic toxic effects may occur.
- ❖ Ethical compliance: Must follow GLP (Good Laboratory Practice) and OECD guidelines for toxicity testing.[10]

Conclusion of Animal Studies on Antihypertensive Drugs

Animal studies play a fundamental role in evaluating the efficacy, safety, and mechanism of action of antihypertensive drugs prior to human clinical trials. A variety of established animal models(e.g., spontaneously hypertensive rats [SHR], renal artery-ligatrats DOCA-salt hypertensive rats, Dahl salt-sensitive rats, and transgenic models) have been utilized to assess these compounds.

Overall Conclusion

The animal toxicity studies of NCE [Insert Compound Code] demonstrate an acceptable safety profile across multiple species and durations of exposure. The NOAELs identified support a sufficient safety margin for proposed clinical dosing. Observed toxicological effects were generally mild, reversible, and consistent with exaggerated pharmacological activity or non-specific systemic stress at high doses. Based on these findings, the NCE is considered suitable for progression to human clinical trials (Phase I), pending final regulatory review. Additional studies (e.g., carcinogenicity, reproductive toxicology) may be required based on the intended clinical indication and duration of use. Animal toxicity studies are a critical component of the non-clinical safety assessment of a new chemical entity (NCE). These studies provide essential information on the potential adverse effects of a compound before it is tested in humans. Below is a structured, detailed conclusion typically found at the end of animal toxicity studies for an NCE:

REFERANCE

1. Avanappu Srinivasa Rao,BhagyaLakshmi(2014)Textbookpharmacologicalscreening methods and toxicology,pharmaMed press, page no.265-6.1Atul kambra,p.p.Singh uppal(2014)Textbook pharmacology and toxicology,s.vikas and company(medical publishers)India (pv books),page no.3

2. M n Ghosh, fundamentals of experimental pharmacology ,Fourth edition Mbbsphd (lond) Retired prof of pharmacology and director, Jawaharlal institute of postgraduate medical education and research, Hilton and company109,collegestreet ,Chennai-7000197

3.Olson, H., Betton, G., Robinson, D., Thomas, K., Monro, A., Kolaja, G., et al. (2000).Concordance of the toxicity of Pharmaceuticals in Humans and in animals. Regulatory Toxicology and Pharmacology, 2000(32), 56–67.

4. Avanapu Srinivasa Rao, Namburi Bhagya Lakshmi(2014) Textbookpharmacologicalscreening methods and toxicology ,Pharma Med Press, Page no.277-6.5.1

5. Ahmed, M., 2008. Isolation, characterization and possible biocontrol application ofBdellovibrionaceae (BD) isolated from New Zealand Sources. PhD Thesis, Massey University, New Zealand

6.Duncan, I. J. H. & D. Fraser, 1997. Under standing. In: Animal Welfare, eds M. A. Appleby& B. O. Hughes, Wal long Fund, CABI publication, UK.

7.Kothari, N., S. Kothari, Y. J. Choi, A. Dey, D. E. Briles, D. K. Rhee & R. Carbis, 2015. A bivalent conjugate vaccine containing PSPA families 1 and 2 has the potential to protect against a wide range of Streptococcus pneumonia strains and Salmonella ty phi. Vaccine, 33, 783–788.

8. Saga Nuwan, S. A., 2011. A modified arith metical method of Reed and Munch for determination of a relatively ideal median le thal dose (LD50). African Journal of Pharmacy and Pharmacology, 5, 1543 1546.

9. Forni, C., 2014. Pollutants toxicity towards aquatic macrophytes. Journal of Clinical Toxicology, 4, 71. Deschamps, F. J., 2014. A new challenge: Assessment of metal prosthesis intoxicant ton. Journal of Clinical Toxicology, 4, 72.

10. Van Der Laan, J.-W., 2014. Current topics on nonclinical safety assessment of human pharmaceuticals from a European perspec tive. The Journal of Toxicological Sci ences, 39, 89. Robinson, S. & K. Chapman, 2009. Are acute toxicity studies required to support over dose for new medicines? Regulatory Toxi cology & Pharmacology, 55, 110.