IDENTIFICATION OF THERAPEUTIC INDEX ED50 AND LD50 USING DIGOXIN AND NaCL IN WHITE RATS (Rattus Norvegicus)

Maulina Putri Mantulangi[1)], Suryaningsi Umaili[2)], and Rini Daud Supu[3)]
[1,2,3)] University of Bina Mandiri Gorontalo
E-mail: putrymantulangi15@gmail.com

ABSTRACT

This research aims to obtain an experimental picture to obtain DE50 and DL50 and understand the concept of therapeutic index and dose response of drugs for white rats (Rattus Novergicus).

The research method used is a laboratory experimental method using 4 experimental animals, male white rats inith weight 200 g. Meanwhile, the drug being tested for its therapeutic index is digoxin. In addition to drugs, physiological NaCl was also used as a negative control.

The results showed that menunjukkan administration of drug doses to experimental animals, namely white rats (Rattus novergicus), LD50 and ED50 were not obtained because the data were insufficient and after the study, in rat I experienced tremors at minute 11 after being given 5 mL of digoxin solution. Furthermore, the second rat experienced tremor at 9 minutes with 10 mL of digoxin solution. Then the III rats were given 15 mL of digoxin solution and experienced tremors at 4 minutes. Then the IV rats were given 5 mL of physiological NaCl. After that, it was counted at 8 minutes, IV rats experienced tremors.

Keywords: lethal Dose (LD), effective dose (ED), digoxin, NaCL

INTRODUCTION

Pharmacy is a profession related to health sciences and chemistry, especially about drugs. In pharmacy we learn about pharmacology. Pharmacology is the study of the relationship between drugs and living things. From this understanding we can conclude that pharmacology has a special relationship with pharmacy, namely how to make, formulate, store and provide drugs. In making medicine, we should also learn about toxicology. Toxicology is the study of the adverse effects or effects of drug poisoning.

Pharmacology and toxicology is the science that deals with the basic principles of drug action. Drugs are substances used to diagnose, reduce pain, and treat and prevent disease. However, drugs do not only have a therapeutic effect but also have a toxic effect. The therapeutic effect of the drug and the toxic effect of the drug are the result of the interaction of the drug with molecules in the patient's body. Most drugs act by incorporating specific macromolecules by altering the biophysical and biochemical activity of macromolecules. In this therapeutic effect and toxic effect is closely related to the therapeutic index and response to dose [11].

Drugs are usually given in regular or average doses, which are suitable for most patients. However, for other patients, this dose is too large to cause a toxic effect or too small to be ineffective. Most drugs are converted in the liver, sometimes in the kidneys and others. If the liver function is not good then the drug is usually changed in the liver unchanged or only partially changed. This causes the effect of the drug to last longer and the drug to be more toxic.

The response to a low drug usually increases in direct proportion to the dose. However, with increasing dose the response decreased. Eventually, a dose is reached that no longer improves response. In an ideal system or in vitro system, the relationship between drug concentration and drug effect is described by a hyperbolic curve.

The therapeutic index, also known as the therapeutic ratio, is a quantitative measurement of the relative safety of a drug. While the response to a low dose of drug usually increases directly proportional to the dose. However, with increasing dose the response decreased. In the end, a dose is reached that can no longer increase the response [4].

In pharmacology, the basics of drug action are described in two phases, namely the pharmacokinetic phase and the pharmaco dynamic phase. In drug therapy, drugs that enter the body through various routes of administration generally undergo absorption, distribution, and binding to get to the site of action (receptors) and cause effects, then with or without biotransformation (metabolism) and then excreted from the body [5]. This process is known as a pharmacokinetic process. Pharmacodynamics, describes the interaction of drugs with drug receptors; This phase plays a role in the biologic effects of drugs on the body [1].

Dosage and patient response are closely related to the relative pharmacological potency and maximal efficacy of the drug in relation to the expected therapeutic effect. The dose response is strongly influenced by:

1. Dosage given
2. Decrease or increase in blood pressure
3. Heart condition
4. Metabolic rate and excretion [6].

Each individual's drug response is different. Idiosyncratic responses are usually caused by genetic differences in drug metabolism or by immunologic mechanisms, including allergy. Four general mechanisms affect the ability to respond to a drug:

1. Changes in drug concentration,
2. Variation and concentration of an endogenous receptor ligand,
3. Changes in the number or function of receptors,
4. Changes in the respondastal component of the receptor (Katzung, Betram, 2001).

Drug dose-percent responsive relationship, to cause a drug effect with a certain intensity in the population, one dose range is needed. If the frequency distribution of responsive individuals (within 10%) in the dose range (in the dose log) is made, a normal distribution curve will be obtained. Drug dose-response relationship: 1) drug potency: the potency of a drug is influenced by absorption, distribution, biotransformation, metabolism, excretion. The ability to bind to the receptor and effector system or the size of the drug dose required to produce a response; 2) maximum efficacy: the maximum effect of the drug is expressed as maximum efficacy (efficacy) or simply called efficacy.

Efficacy depends on the ability of the drug to produce its effect after interacting with the receptor. Efficacy can be limited by the occurrence of unwanted effects, so the dose should be limited. Which means that the maximum effect is not achieved. Each drug has a different efficacy [3].

To express the acute toxicity of a drug, the LD50 is generally used, which is a dose that can kill 50% of a group of experimental animals. Likewise, as a measure of the effective dose (therapeutic dose) commonly used as a measure is ED50, which is the dose that gives a certain effect in 50% of a group of experimental animals. LD50 is determined by giving the drug in varying doses (stages) to a group of experimental animals. Each animal was given a single dose [9]. After a certain

period of time (eg 24 hours) some of the experimental animals died, and this presentation is applied in a graph showing the relationship between dose (in abscissa) and percentage of dead animals (in ordinates) [12].

Hypnotics and Sedatives

Sedative hypnotics are a class of central nervous system (CNS) depressants that are relatively non-selective, ranging from mild i.e. causing drowsiness, slumber, to severe i.e. loss of consciousness, anesthesia, coma and death, depending on the dose. At therapeutic doses of sedative drugs suppress activity, reduce response to emotional stimuli and calm. Hypnotic drugs cause drowsiness and facilitate sleep and maintain sleep that resembles physiological sleep. Hypnotic and sedative drugs are usually benzodiazepine derivatives. Several sedative-hypnotic drugs from the benzodiazepine group are also used for other identification, namely as muscle relaxants, antiepileptic, antianxiety and as an anesthetic inducer.

Absorption

The amount of drug that can be absorbed by the body, expressed by the bioavailability of the drug. The high value of drug bioavailability depends on many factors, which determine how drug molecules cross the gastrointestinal tract barrier and successfully enter the blood vessels and are transported to their receptors.

1. These factors include:
   a. method of preparation and dosage form,
   b. molecular size,
   c. Solubility of molecules in lipids: the more easily soluble in lipids, the higher the bioavailability,
   d. water and lipid solubility: soluble in both, very good bioavailability; which is soluble only in water, its bioavailability is low because the molecules are easily dissociated,
   e. active transport,
   f. interaction with food,
   g. stability in the gut,
   h. gastric emptying,
   i. metabolism in the intestines and in the liver,
   j. The patient's own individual factors and the pathological condition of the patient [1].
2. Some of the important factors are discussed below:
   a. Drugs must cross cell barriers in various tissues (transport across membranes, and a small portion may pass through intercellular spaces or across capillary endothelium),
   b. cell membrane,
   c. Means of drug transport across membranes (semipermeable) [7].

How to give medicine

1. How to administer drugs orally, this method is most commonly used because it is easy, safe and inexpensive. However, for drugs given orally, there are three factors that affect bioavailability, the drug factor itself (lipid, water or both soluble):
   a. Patient factors (pathological state of digestive organs and metabolism)
   b. Interaction in absorption in the gastrointestinal tract. (interaction with food) as an independent task [1].
2. How to administer drugs by injection?
   a. The advantages of parenteral administration of drugs compared to oral, namely the effects occur more quickly and regularly, can be given to patients who are uncooperative, unconscious or vomiting and are very useful in an emergency.
   b. The weakness of the method of administering drugs by injection is that it requires an aseptic method, causes pain, the possibility of transmission of the disease by injection, cannot be done alone by the patient, and is not economical [1].
3. How to administer drugs through the lungs, this method is called the

inhalation method, only for drugs in the form of gases or volatile liquids, such as general anesthetics and drugs in aerosol form. Absorption through lung epithelium and airway mucosa [1].

Distribution

Drug distribution occurs through two phases based on its distribution, namely:

1. Distribution of the first phase: ie to organs that are very well perfused (heart, liver, kidneys and brain) occurs immediately after further absorption.
2. Distribution of the second phase: the organs are not very well perfused (viscera muscle, skin and not fat) [1].

Drugs that are fat soluble will cross the cell membrane and are distributed into cells, drugs that are not fat soluble are difficult to penetrate the cell membrane so that their distribution is limited, especially in the extracellular fluid. Distribution is limited by drug binding to plasma proteins and only free drug can diffuse into cells and reach equilibrium [14].

Pharmacodynamics

The branch of science that studies the biochemical and physiological effects of drugs and their mechanism of action is called pharmacodynamics [14]. The mechanism of action of the drug is:

1. Drugs can change the speed of the body's physiological (physiological) activities,
2. Drugs do not cause a new function, but only modulate an existing function (this does not apply to gene therapy) [1].

The purpose of studying the mechanism of action of drugs is to:

1. Researching the main effects of drugs,
2. Knowing the interaction of drugs with cells,
3. Drug interactions with biopolymers [1].

All drug molecules that enter the body, most likely bind to tissue constituents or biopolymers such as proteins, fats, nucleic acids, mucopolysaccharides, bio transformation enzymes and receptors. Drug interactions can be:

1. Atypical interactions, atypical interactions are interactions that do not result in long lasting effects and do not cause changes in the molecular structure of the drug or biopolymer. This interaction is reversible and does not produce a biological response. For example: drug interactions that only change the physico-chemical environment of body structures (tissue proteins, nucleic acids, mucopolysaccharides, water and fats), for example: general anesthetics change the structure of water in the brain; Osmotic diuretics change the osmotic pressure in the kidneys.
2. Typical interactions, typical interactions are interactions that cause changes in the macromolecular structure of the receptor so that stimulation of changes in normal physiological functions can be observed as a biological response. Interactions with receptors and interactions with biotransforming enzymes, are typical interactions [2].

Therapeutic index

The therapeutic index is a measure of drug safety because a large value indicates that there is a wide margin between effective and toxic doses [1]. The therapeutic index is determined by measuring the frequency of the desired response and the toxic response at various drug doses. The therapeutic index of a drug is the ratio of the dose that produces the tolerance to the dose that produces an effective response [10].

The therapeutic index is the ratio between the toxic dose and the effective dose or describes the relative safety of the drug under normal use. Estimated as LD50 against ED50. Due to different effects may need different doses. The term LD50 is often used in toxicology, namely the dose that will kill 50% of the experimental population.

The therapeutic index of a drug is expressed in the following statement:

Therapeutic index = or$\frac{\text{TD50}}{ED50}\frac{CD50}{ED50}$

The ideal drug produces a therapeutic effect in all patients without causing toxic effects in any patient, therefore TD1 1. A drug measure, drugs that have a high therapeutic index are safer than drugs that have a lower therapeutic index. TD50: Dose that is toxic to 50% of animals receiving the dose, death is the last toxicity:

1. Determination of the therapeutic index, the therapeutic index is determined by measuring the frequency of desired response and toxic response to various drugs.
2. Quantitative aspects of drug elimination through the kidneys.
3. Effective ratio, decrease in the plasma concentration of the drug from the arterial venous blood of the kidney,
4. Expression Speed, elimination of a drug usually follows a kinetic order and the plasma concentration of the drug decreases exponentially with time. This point is used to determine the half-life of the drug.
5. Volume of distribution and half-life of the drug, the half-life of a drug is inversely related to clearance and is directly proportional to the volume of distribution.
6. Clinical conditions that increase the half-life of a drug are important in predicting the patient's possible length of the drug's half-life [13].

There is Various DL50 calculation methods commonly used include the Miller-Tainer method using special graph paper, namely logarithmic-probit paper which has a logarithmic scale as the abscissa and a probit scale as an ordinate. On the paper a graph is made between the percent mortality against the logarithm of the dose.

The reed-muench method is based on the cumulative value of the number of living and dead animals. It is assumed that animals that die at a given dose will die with a larger dose, and animals that live will live with a smaller dose. And the Kirber method. The principle uses the interval average of the number of deaths in each animal group and the difference in the dose interval is the same.

The therapeutic index only applies to one effect, so drugs that have several therapeutic effects also have several therapeutic indices. Example: Aspirin has analgesic and antirheumatic effects. The therapeutic index or the safety limit of aspirin as an analgesic is greater than the therapeutic index as an antirheumatic because the antirheumatic therapy dose is greater than the analgesic dose [2].

Although the comparison of therapeutic dose and toxic dose is very useful for a drug, such data are difficult to obtain from clinical research. (It is difficult to find respondents who are willing to do clinical trials). Therefore, drug selectivity is expressed indirectly, which is calculated from the data: 1) the pattern and incidence of side effects caused by drugs in therapeutic doses, and 2) the percentage of patients who stop drugs or reduce drug doses due to side effects.

It must be remembered that the description or statement that the drug is safe for most sufferers, does not guarantee safety for every patient because there is always the possibility of a distorted response. For example: penicillin can be declared safe for most patients but can cause death for people who are allergic to the drug.

Individual responses to drugs vary widely, which can be in the form of: 1) hyperactivity (very low doses can have an effect); 2) hyporeactive (to get the effect, requires very high doses); 3) hypersensitivity (people allergic to certain drugs); 4) tolerance (to get the effect of drugs that have been consumed before, require higher doses); 5) resistance (drug effect is reduced due to genetic formation); 6) idiocycracy (odd drug effects, which are

allergic reactions to drugs or due to genetic differences).

The effect of a drug compound depends on the number of doses given. If the dose given is below the threshold (subliminal drug), there will be no effect. The response depends on the natural effect being measured. Increasing the dose may increase the effect at that intensity. As with antipyretic or hypotensive drugs, the level of use can be determined, in the sense that the range of body temperature and blood pressure can be measured.

The dose-effect relationship may vary depending on the sensitivity of the individual taking the drug. The dose-frequency relationship resulted from differences in individual sensitivity as a formula shown in a log of normal distribution. If the cumulative frequency (total number of animals that respond to a given dose) is plotted in logarithms it will be a sigmoid curve. The point bend in the curve is in the dose state of one-half of the dose group that has responded. The dose range includes a dose-frequency relationship reflecting individual variations in sensitivity to a drug.

Evaluation of the dose-effect relationship in a group of human subjects can be found because there are differences in sensitivity in different individuals. In order to determine the biological variation, measurements have been carried out on a representative sample and averaged. This will allow the therapeutic dose to be appropriate in most patients [8].

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1. The pattern and incidence of drug-induced side effects in therapeutic doses, and
2. Percentage of patients who stop the drug or reduce the dose of the drug due to side effects

It must be remembered that the description or statement that the drug is safe for most sufferers, does not guarantee safety for every patient because there is always the possibility of a distorted response. For example: penicillin can be declared safe for most patients but can cause death for patients who are allergic to the drug [1]

Individual responses to drugs vary widely, which can be

1. Hyperactivity (very low doses can have an effect),
2. Hyporeactive(to get the effect, requires a very high dose),
3. Hypersensitivity (people allergic to certain drugs),
4. Tolerance (to get the effect of drugs that have been consumed before, require higher doses),
5. Resistance (drug effect is reduced due to genetic formation) [1].

Factors Affecting Drug Action

1. Weight
2. Age
3. Gender
4. Condition
5. Genetics
6. How to give medicine [6].

The ideal drug treats all patients without causing toxic effects in any patient and is therefore PD 1. A drug measure, drugs with a higher therapeutic index are safer than drugs with a lower therapeutic

index. TD50: dose that is toxic to 50% of the animals receiving the dose, death is the final toxicity. The effect of a drug compound depends on the number of doses given. If the dose given is below the threshold, no effect will be obtained. The response depends on the natural effect being measured. increasing the dose may increase the effect at that intensity. Such as antipyretic or hypotensive drugs can be determined the level of use. In the sense that the area of body temperature and blood pressure can be measured, the dose-effect relationship point varies depending on the sensitivity of the individual who is using the drug. The dose-frequency relationship resulted from differences in individual sensitivity as a formula shown in a log of normal distribution.

Material Description

Digoxin

Digoxin is an inotropic agent mainly used to treat congestive heart failure (CHF) and atrial fibrillation, the core agent is partially adsorbed and after adsorption, a large fraction is cleared by the kidneys.

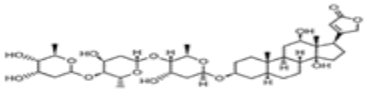
Digoxin is a drug used to treat heart failure. Digoxin (cardiac glycoside) is useful for strengthening weak cardiac contractions, thereby strengthening the pump function of the heart. Digoxin is found in the leaves of the plants Digitalis purpurea and D. Lanata as aglucans and glycosides [15].

Description of digoxin (FI 1979).

chemical name : DYHIDROXY METHYLOXAN

Molecular formula : C41H64O14

Structural formula :



Molecular weight: 780,938 g/ml

Description : Crystalline, clear to white or white crystal powder, Odorless.

Solubility : Practically insoluble in water, and in ether, easily soluble in pyridine, sparingly soluble in dilute ethanol and chloroform.

Animal Description

Classification of White Rats (*Rattus Novergicus)*

Kingdom : *Animalia*
phylum : *chordates*
Class : *Mammals*
Order : *Rodentia*
Sub order : *Odontoceti*
Family : *Muridae*
Genus : Rattus
Species : *Rattus Novergicus* (Natawidjaya, 1983).

Morphology of White Rat (Rattus Novergicus)

Rats have 4 legs, 4 fingers, white fur, red eyes, have a length of up to 70-100 mm and even more. The weight of an adult rat ranges from 200-300.

RESEARCH METHODS

This research was conducted at the Laboratory of Bina Mandiri University, Gorontalo. The method used is the experimental labortorik using 4 experimental animals male white rats. first mmake a solution of digoxin 4.5 g in 1000 mL of water. Then the rats were given the marks of rat I, rat II, rat III, and rat IV. After that, each rat was given a solution of digoxin drug orally as much as5 mL, 10 mL, and 15 mL and IV rats were given 5 mL of physiological NaCl orally. Then record any changes that occur

Tools and materials

The tools used are 1 mL and 5 mL syringes, lumping and pestle, balance scales, beakers, cannula, and stopwatch. The materials to be used are digoxin, physiological NaCl, aquadest, and 4 male white rats.

RESEARCH RESULTS

The research was conducted at the Bina Mandiri University Laboratory, Gorontalo. The method used is the experimental labortorik using 4 experimental animals male white rats. first mMake a solution of digoxin 4.5 g in 1000 mL of water. The goal is because Digoxin is one of the drugs used to treat heart failure.In rat I, 5 mL of digoxin solution was given. At the 11th minute, rat I experienced tremors. Furthermore, rat II was given 10 mL of digoxin solution.

After that, the changes were calculated using a stopwatch. At the 9th minute, rat II had tremors. Again, rat III was given 15 mL of digoxin solution. After that, the change is calculated using a stopwatch. At minute 4, rat III experienced tremor. Then the rat to IV was given 5 mL of physiological NaCl. After counting every change that occurred. At minute 8, rat IV experienced tremor. This is due to the administration of doses that are too small or the administration of drugs that are not appropriate.

Table 1. Observation Results

Animal	Dosage (mL)		Time	The reaction that occurs	Dead animal
	Digoxin	NaCl			
Rat 1	5 mL	-	11 minutes	Tremor	-
Rat II	10 mL	-	9 minutes	Tremor	-
rat III	15 mL	-	4 minutes	Tremor	-
IV rats	-	5 mL	8 minutes	Tremor	-

Source: Microbiology Practicum Report

DISCUSSION

The therapeutic index is the ratio between the toxic dose and the effective dose or describes the relative safety of a drug under normal use. It is estimated as the ratio of LD50 (Lethal dose in 50% of cases) to ED50 (Effective dose in 50% of cases). While the therapeutic window is the range of plasma concentrations of a drug that will produce a response or the distance between MEC and MTC. To find out the therapeutic index of a drug by giving different dose levels/dose in test animals [14].

This trial of drug dose response and therapeutic index aims to obtain (LD50) and (ED50) and understand the concept of therapeutic index in experimental animals, namely rats weighing 200 g. Meanwhile, the drug being tested for its therapeutic index is digoxin. In addition to drugs, physiological NaCl was also used as a negative control.

The drug is given orally. As the name implies, oral administration of drugs is by mouth, either in the form of tablets, capsules, syrups, and other dosage forms. but before that, the drug will be refined as shown in Figure 1 below:

Figure 1. Powder making

After the digoxin drug is mashed, the solution is made as shown in Figure 2 below:

Figure 2. Solution preparation

In detail, the steps for making the solution will be explained as follows, the first thing to do is make a solution of 4.5 g of digoxin in 1000 mL of water. Then put it in a 5 mL dispenser. After that, it was given to experimental animals orally using a cannula. The dose given to each experimental animal was increased.

The first thing that was done in this study was to mark each rat. Rats were marked using a marker on the tail according to the numbering on the test

animal. After all the mice were numbered, the next step was to know the weight of the test mice by weighing them one by one on the balance to see the effect that worked on each dose, then a time span was given.

In this study, rats were used as test animals, because they are relatively easy to use, relatively large in size and relatively inexpensive. This animal has a blood circulation system that is almost the same as humans and does not have the ability to vomit because it has a valve in the stomach point. In addition, rats are tame animals, easy to manage and feeding and drinking are very easy.

The drug that will be tested on rats in this study is the drug digoxin. Digoxin is a cardiac glycoside drug that works by affecting several types of minerals that are important in the work of the heart, namely sodium and potassium.

The administration of digoxin to rats through the oral route, namely by inserting it directly into the mouth. Oral administration is the easiest way to do, this method is also more comfortable because it is not as invasive as if the drug is injected.

The dose itself was administered to each rat with increasing variation in size. Dosage with increasing size variation is needed to find out at which dose the desired effectiveness occurs, so that later LD50 and ED50 can be known. Both will show the index of drug therapy.

The calculation of the therapeutic index is intended to estimate the safety of the drug, the greater the therapeutic index the safer the use of the drug because the range between LD50 and ed50 is quite far, if the therapeutic index is small, the range between LD50 and ED50 is close so that the dose given must be appropriate if it can cause toxicity and even death.

The therapeutic index is a parameter of drug safety, so if we want to know the safety level of a drug, we must first know the therapeutic area. The therapeutic area is the distance between LD50 and ED50, also called the safety distance. The therapeutic area is also useful as an indication for the safety of drugs with a small therapeutic area, it is easy to cause poisoning if the normal dose is exceeded

The first rat was given 5 mL of digoxin solution. After that, the change was calculated using a stopwatch. At the 11th minute, rat I had tremors. Furthermore, the second rat was given 10 mL of digoxin solution. After that, the change was calculated using a stopwatch. 9, rat II had tremors. Again, rat III was given 15 mL of digoxin solution. After that, the changes were calculated using a stopwatch. At minute 4, rat III had tremor. Then the rat IV was given 5 mL of physiological NaCl. it was calculated every change that occurred. At the 8th minute IV rats had tremors.

In this experiment, no experimental animals died. Mice remained active and only had tremors. This is due to the administration of a dose that is too small or the administration of the drug is not appropriate. Oral administration of drugs is quite difficult because not all of the drug solution enters the mouth. This is because the mouse's small mouth

CONCLUSION

Based on the experimental results of administering drug doses to experimental animals, namely mice, LD50 and ED50 was not obtained due to insufficient data.

The therapeutic index is the ratio between the dose that caused death in 50% of the experimental animals used (LD50) divided by the dose that produced the effect studied in 50% of the experimental animals used (ED50). The principle of therapeutic index, the greater the index of drug therapy, the greater the therapeutic effect.

In this study, it is recommended for further researchers to be more careful in handling experimental animals and do the method according to the instructions in handling mice so that there is less risk of being bitten by mice and to be more careful

in carrying out practicum to minimize dead experimental animals.

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