PRACTICAL WORK GUIDANCE : PHARMACOLOGY

PHARMACOLOGY LABORATORY

MEDICAL FACULTY

BRAWIJAYA UNIVERSITY

MALANG

2015

PRACTICAL WORK GUIDANCE OF PHARMACOLOGY

Whether using the classical method or the Problem Based Learning (PBL) method, the teaching of pharmacology given to medical students is incomplete without conducting a pharmacological practical work.

This practical work is not aimed to increase the practical skill of students – the purpose is to help students obtain a direct explanation from observing the effects and responses of drug in various levels of organisms: organs, tissues and even towards cell cultures.

The participants would actively give a predetermined treatment to the organs or tissues, observe and note the changes or responses that occur, report it in groups and in class, and, more importantly, conduct an analysis and discuss any results found deviating from the existing theories. For that purpose, and also as a guide in completing the practical work and the report, this module is made.

As for the practical work itself, participants are expected to prepare and learn beforehand the pharmacological effects of certain drug used in the forthcoming practical work, as it would ensure that the practical work runs smoothly. Participants should work earnestly, seriously, with discipline and peacefully, so the practical work would give benefit to the study of pharmacology.

Hopefully this guidebook would give benefit. We are thankful for any criticism and suggestions towards the improvement of the contents and materials in this practical work.

Practical Work 1: CONVULSION AND ANTICONVULSANT DRUGS

INTRODUCTION

A seizure which occurs periodically and unpredictably is a symptom of epilepsy. It is usually followed by convulsion. Besides epileptic seizures, there are also non-epileptic seizures, which can occur on normal brain given an electroshock treatment or certain chemical compounds.

Principally, seizures are caused by the decrease of inhibitory or the increase of excitatory synaptic activity. Neurotransmitter mediating most of synapses transmission in the brain is GABA and glutamate. Several researches show that the administration of GABA receptor antagonist to experimental animals cause the occurrence of seizures, in the other hand the administration of glutamate receptor antagonist at the experimental animal which is first given an electroshock treatment or penthylene tetrazole can repress the occurrence of seizures.

Based on this case, an anticonvulsant medicine which works by repressing depolarization, causing the activity of excitatory synaptic decreases, and the drug which acts as an GABA receptor agonist, increasing the inhibitory synaptic, was developed.

OBJECTIVES

1. Observe the convulsion effect caused by pentylentetrazol (metrazole) and strychnine simulation.
2. Observe the anticonvulsant effects of luminal and dilantin.

METHODS

1. Experimental animals:
   The experimental animal used is a mature mouse weighing ± 30 g. Each group is given 4 mice.
2. Methods and dosage of drug administration:
   The drug is injected intraperitonially (i.p) by grabbing the dorsal neck of mouse. When injecting the drugs, the head of mouse should be turned down in order to avoid the needle of syringe wounding the mice intestine. The volume of the drug given (cc) should be less than 2 cc to avoid the mouse diaphragm pushing. The time of injection should be noted to observe the reaction of the drug (the appearance of a convulsion).
   Mark the mouse with markers colored according to the drugs used to avoid false marking
   - Strychnine, a convulsion drug, with a dosage of 3 mg/kg BW i.p. and metrazole with a dosage of 70 mg/kg BW i.p.
   - Dilantin, an anticonvulsant drug, with a dosage of 100 mg/kg BW i.p. and luminal with a dosage of 50 mg/kg BW i.p.
   - Anticonvulsants are administered before convulsions, and then waited until the anticonvulsant drugs start to work.
   - For
     - Luminal wait until 20 minutes convulsant
     - Dilantin, wait until 30 minutes convulsant

3. Experimental design:
   Each mouse is only used once. The division and group tasks are as following:
   Group I-IV each group gets 4 mice:
   1st mouse strychnine
   2nd mouse metrazole
   3rd mouse dilantin + strychnine
   4th mouse dilantin + metrazole

Group V-VIII each group gets 4 mice

1st mouse strychnine

2nd mouse metrazole

3rd mouse luminal + strychnine

4th mouse luminal + metrazole

OBSERVATION

After the convulsant is given to the mice, observe and take note:

- the appearance of convulsions (minutes)
- the symptoms before convulsion (scratching)
- the length of convulsion (minutes)
- The type of convulsion:
    - tonic
    - clonic
    - symmetric
    - asymmetric
    - coordinated
    - uncoordinated
- The occurrence of a repeated convulsion (the frequency of repeated convulsion after the first convulsion)
- The occurrence of death: if death follows an episode of a convulsion, note how long it has passed after administration of the drugs (minutes)

DISCUSSION

1. Explain the mechanism of strychnine and metrazole as convulsants
2. Explain the mechanism of dilantin and luminal in repressing convulsion caused by strychnine and metrazole.
3. Explain the mechanism of action of tetanus toxin in causing a convulsion.
4. Name and explain the mechanism of action of anticonvulsant agents that can overcome tetanus convulsion.
5. What is the meaning of febrile convulsion and discuss its non pharmacologic and pharmacologic therapy.

Practical Work 2: AUTACOID – ANALGESIC ANTIINFLAMMATION

INTRODUCTION

Autacoids are biological factors which act like local hormones, have a brief duration, act near the site of synthesis, and are not blood borne. Autacoids are primarily characterized by the effect they have upon smooth muscle. With respect to vascular smooth muscle, there are both vasoconstrictor and vasodilator autacoids. **Inflammation** is a response of a tissue to injury. It is characterized by increased blood flow to the tissue causing increased temperature, redness, swelling, and pain.

OBJECTIVES

1. To observe the inflammation and antiinflammation effects on the plantar (hind paw) of rat.
2. To understand the mechanism of inflammation and antiinflammation drug.

INSTRUMENT

1. Analgesimeter (Rendall Salitto Test)
2. Restricted cage
3. Spuit

MATERIAL

1. Animal model: Rat (*Rattus norvegicus*)
2. Yeast / Carrageenan 5%
3. Antiinflammation drug (Novalgin)

METHODS

1. Place the animal model in restricted cage.
2. Rat I: check the pain response of plantar (hind paw) of rat (control rat) using Rendall Salitto Test.
   Record the pain response based on the reflex and activity of the rat.
   Rat I: inject antiinflammation drug (Novalgin) 0.2 mL intramuscular. Wait for 5 minutes.
   Record the pain response based on the reflex and activity of the rat.
3. Rat II: inject the plantar (hind paw) of rat with 5% yeast 0.2 mL. Wait for 30 minutes.
   Record the pain response based on the reflex and activity of the rat.
   Rat II: inject antiinflammation drug (Novalgin) 0.2 mL intramuscular. Wait for 5 minutes.
   Record the pain response based on the reflex and activity of the rat.
4. Compare the pain threshold of those different treatments.

ASSIGNMENTS

1. What effect did you observe after antiinflammation drug was administered to the rat I?
2. What effect did you observe after 5% yeast and then antiinflammation drug was administered to the rat II?
3. Explain the mechanism of inflammation.
4. How is the mechanism of Novalgin (NSAID) as antiinflammation drug?

Practical Work 3: THE EFFECT OF LOCAL ANESTHETIC DRUGS ON RABBIT'S EYE

INTRODUCTION

A physician is often required to perform minor surgeries in ophthalmology, such as the extraction of a corpus alienum from the cornea, abscess (hordeolum) incision on the palpebra and maybe an enucleation of the eye ball because of some serious trauma on the eyes. For this, a physician should choose local anesthetic and should know their mode of application. Choosing a proper local anesthetic for a procedure involves considering the principles of drug selection: the objectives of therapy, consideration of the benefit from a pharmacokinetic-pharmacodynamic sides, safety (accounting the side effects), precision (the presence of contraindication and drug interaction) and cost.

Adrenaline or epinephrine is often added to the local anesthetic drug with the purpose of increased anesthetic effect.

OBJECTIVES

Compare local anesthetic pharmacologic effect with or without adrenaline given topically on the eye's mucosa.

PHARMACOLOGY OF LOCAL ANESTHETIC

A local anesthetic works by blocking the conduction of impulse in the axon particularly in the sensory nerves. Development of local anesthetics is aimed to discover drugs that do not irritate the tissue where the drug is given, drugs that have minimal systemic effects (side effects), fast onset, and long duration. Types of local anesthetic recognized today are noted in this table:

Groups and examples of local anesthetic drug	Potency	Duration
(Procaine = 1)		
Ester		
Cocaine	2	Medium
Procaine (novocaine)	1	Short
Tetracaine (pontocaine)	16	Long
Benzocaine (Only topical use)		
Amide		
Lidocaine (xylocaine)	4	Medium
Mepivacaine	2	Medium
Bupivacaine	16	Long
Etidocaine	16	Long
Pilocaine	3	Medium

The ester group is hydrolyzed in the plasma by plasma cholinesterase, while the amide group is hydrolyzed in the liver with the help of a microsome enzyme. In a patient with liver disease, a decrease in metabolism rate would occur, causing a drug's duration to increase 3 to 4 fold. If the dosage isn't adjusted to that condition, the risk of a systemic effect would also become bigger.

Local anesthetics are weak bases (read the Henderson-Hasselbach equation to discover changes of pH towards the changes in the ionic form). Its active form is the cationic form, has a receptor inside the nerve cells and works by blocking the sodium channel, disabling the occurrence of a impulse conduction. The principle that a drug would have to be lipid soluble to be able to go through a membrane also applies here. Thus, the more lipid soluble an

anesthetic, the more potent it is. This can be seen in the potency of is lidocaine, procaine, and mepivacaine, which are all water soluble, compared to tetracaine, etidocaine, and bupivicaine, which are more lipid soluble than the groups mentioned before. The magnitude of impulse conduction blockage is also determined by the nerve fibers. A type B (preganglionic autonomic nerve) and type C (dorsal nerves for pain, sympathetic postganglionic nerves) are more sensitive compared to type A alpha, beta, gamma, and delta.
The side effects and toxicity worth noting are:

1. Central Nervous System
   A toxic dosage would cause symptoms of CNS depression. In a sub lethal or lethal dose, death is caused by central breathing system depression or convulsion.
2. Neurotoxicity in the Peripheral Nerves
   This occurs in a high concentration administration of local anesthetic. The symptoms include sensory and motor function paralysis.
3. Cardiovascular system
   A functional disorder occurs in the heart which is caused by a blockage of its conduction system. The blood vessels would vasodilate, except by cocaine, which would increase the secretion of noradrenaline and cause vasoconstriction.
4. Methemoglobin forming
   Pilocaine with a dosage more than 10 mg/kg BW would cause an excessive formation of alpha-toladine, which would transform hemoglobin into methemoglobin.
   The symptoms of methemoglobinemia would manifest if the concentration is greater than 3-5 mg/dl.
5. Allergic Reaction
   This is more commonly found caused by the ester group because the metabolite from it is p-aminobenzic which in some individuals incites a hypersensitivity reaction.

METHODS

1. Animal lab: Rabbit
2. Drug: Lidocaine and lidocaine + adrenaline (pehacaine)
3. Experiment Method:
   - Cut the eyelashes of the rabbit used as short as possible.
   - Observe the palpebra reflex (by rubbing a cotton ball), conjunctive blood vessels, and the presence of irritation on both eyes of the rabbit (as a control group).
   - Drip the right eye with 2 drops of lidocaine and the left eye with 2 drops of lidocaine + adrenaline.
   - Observe with step number 2 every 5 minutes up until 45 minutes.

RESULTS

Time (minutes)	Palpebra Reflex		Conjunctive Blood Vessels		Irritation	
	Right	**Left**	**Right**	**Left**	**Right**	**Left**
Control						
5'						
10'						
15'						
20'						
25'						
30'						
35'						
40'						
45'						

DISCUSSION

1. Name the indicators of local anesthesia in the practice.
2. Is there any difference of the effects of local anesthesia (onset and duration) between the local anesthetic with and without adrenaline? Explain.
3. How is the mechanism of the local anesthetic?
4. Why is the administration of local anesthetic in the presence of an inflammation not effective?
5. Name local anesthetics and its uses in ophthalmologic surgery.
6. Explain the benefits and the loss of the administration of adrenaline accompanying a local anesthetic drug.

PRAKTIKUM DIURETIK

PENDAHULUAN

Diuretic adalah obat yang digunakan untuk meningkatkan laju pembentukan diuretic urine dan ekskresi natrium. Diuretic digunakan untuk menyesuaikan volume dan/ komposisi cairan tubuh pada berbagai situasi klinis seperti hipertensi,gagal jantung, sindroma nefrotik dan sirosis.

TUJUAN

1. Membandingkan onset kerja diuretic yang diberikan secara peroral maupun injeksi
2. Membandingkan durasi kerja diuretic yang diberikan secara peroral maupun injeksi

ALAT DAN BAHAN

1. Tikus dewasa dengan BB $\pm$ 200 gram (10 ekor terdiri dari 2 kontrol dan 8 ekor perlakuan)
2. Metabolic cage 10 buah
3. Furosemid sediaan tablet dan ampul
4. Spuit 1 cc
5. Kapas alcohol
6. Sonde
7. Gelas ukur

METODE

1. Binatang percobaan
   Mahasiswa dibagi dalam 4 kelompok masing-masing mendapat 2 ekor tikus. Dan 2 ekor tikus dipergunakan sebagai control.
2. Cara pemberian obat dan dosis
   Obat disuntikkan secara intra muscular di tungkai belakang dengan jarum mengarah ke ekor. Pemberian peroral dilakukan melalui sonde.
   - Dosis peroral 2 mg/ 100 gram BB tikus
   - Dosis injeksi intra muscular/ intravena 1 mg/ 100 gram BB tikus.
3. Rancangan percobaan
   Tiap tikus hanya dipakai satu kali. Tiap kelompok memberi perlakuan furosemid per oral untuk 1 ekor tikus dan 1 ekor tikus lainnya diberikan furosemid melalui injeksi intramuscular/ intravena. Sedangkan 2 ekor tikus control tidak diberikan perlakuan apapun.

PENGAMATAN

Setelah tikus diberi perlakuan, catatlah produksi urine tikus tersebut tiap 15 menit. Sampai menit ke-60.

	Produksi urine tiap 15 menit (dalam mL)				
	Menit-0	Menit 15	Menit 30	Menit 45	Menit 60
Tikus control					
Tikus perlakuan per oral					
Tikus perlakuan per injeksi					

DISKUSI

1. Bagaimanakah mekanisme kerja furosemid sebagai diuretik?
2. Adakah perbedaan onset kerja furosemid yang diberikan secara peroral dibandingkan dengan injeksi?
3. Mengapa dosis pemberian peroral lebih besar daripada dosis pemberian injeksi?