Practical Pharmacology

Barbiturates

Thiopental and Dose Calculations

2021-2022

Calculate Your Dose

- **The dose** is the amount of drug taken at any one time.
  - Weight of drug (e.g. 250 mg)
  - Volume of drug solution (e.g. 10 mL, 2 drops)
  - The number of dosage forms (e.g. 1 capsule, 1 suppository)
  - Other quantity (e.g. 2 puffs)
- **The dosage regimen** is the frequency at which the drug doses are given. Ex. 2.5 mL twice a day, one tablet three times a day…
- Accurate dosing is critical for the proper utilization of all pharmaceuticals.
- First you need to know what **volume** you want to inject into the animal with each treatment being administered, then you need to know **how much** drug should be in that given volume

Calculate Your Dose

- To calculate the correct dose of drug you need to know:
  - **The concentration of the drug**
  - **The weight of the animal**
  - **The recommended dose rate of the drug for each specific animal model**
- **Concentration of the drug:**
  - **mg/ml**: Manufacturers usually provide concentrations of their product in milligrams (mg) of drug per (ml) of solvent
  - **% : grams per 100 ml**. ex: 10% solution of xylazine is 100 mg/ml
  - **IU/ml**: International Units per ml of, like some of the fat soluble vitamins
  - **Powders**: The mg of active drug in the vial. For example, Telazol (tiletamine and zolazepam) comes in powdered form with 500mg per vial:
    - If you add 5ml of sterile water for injection to the vial thus providing 5ml of 100mg/ml drug
    - If you add 2.5ml of sterile water for injection, will make 2.5ml of a 200mg/ml solution.

Calculate Your Dose

- **Weight of the animal:**
  - It is always best to use a scale and get an accurate weight.
  - If you cannot weigh the animal prior to injection, you need to be experienced in estimating the weight.
- **Dose rate of the drug:**
  - Always look up the drug dose for the species you are working with - it often varies.

Calculate Your Dose

✓ Practice:

❖ **For most applications the following formula is applicable:**

$$(C1)(V1) = (C2)(V2)$$

- Ex. You have 20 ml of a 10 mg/ml solution and you want to make 15 ml of a 2.5 mg/ml solution. Set up the math as follows:

  C1 = 10 mg/ml C2 = 2.5 mg/ml V1 = unknown V2 = 15 ml

  (10 mg/ml) (V1) = (2.5 mg/ml) (15 ml)

  V1 = 3.75 ml

  So you dilute 3.75 ml of C1 to a final volume of 15 ml therefore you need to add:

  15 - 3.75 =11.25 ml of diluent.

Calculate Your Dose

✓ Practice:

- How to administer xylazine at a dose rate of 10mg/kg to a 300 g rat? You are using 2% xylazine.

  The proper dose for a 300g rat is: 10x0.3kg= 3 mg of xylazine.

  2% xylazine is 20 mg/ml
  3/20=0.15 ml of 2% xylazine

Calculate Your Dose

✓ Practice:

- Let's say you want to treat 15 rats with 75 mg/kg of a compound at the rate of 0.1 ml/20 gm of body weight and you want to prepare enough drug to dose 4 days, weight of each rat is 100 gm.
  - Number of animals= 15
  - Dose to each animal= 75 mg/kg **OR** 7.5 mg/100gm
  - Volume injected to each rat= 0.1ml/20 gm **OR** o.5 ml/100 gm
  - Thus, each 7.5 mg compound should be dissolved in 0.5 ml vehicle (for one rat)
  - (7.5mg/0.5 ml) x 15 = 112.5 mg/7.5ml (for 15 rats in each day)
  - (112.5mg/7.5ml) x 4 = 450 mg/ 30 ml (to 15 rats for 4 days)
  - **SO**, we weight 450 mg compound and dissolve it in 30 ml vehicle, and inject each rat with 0.5 ml of the solution for 4 days.

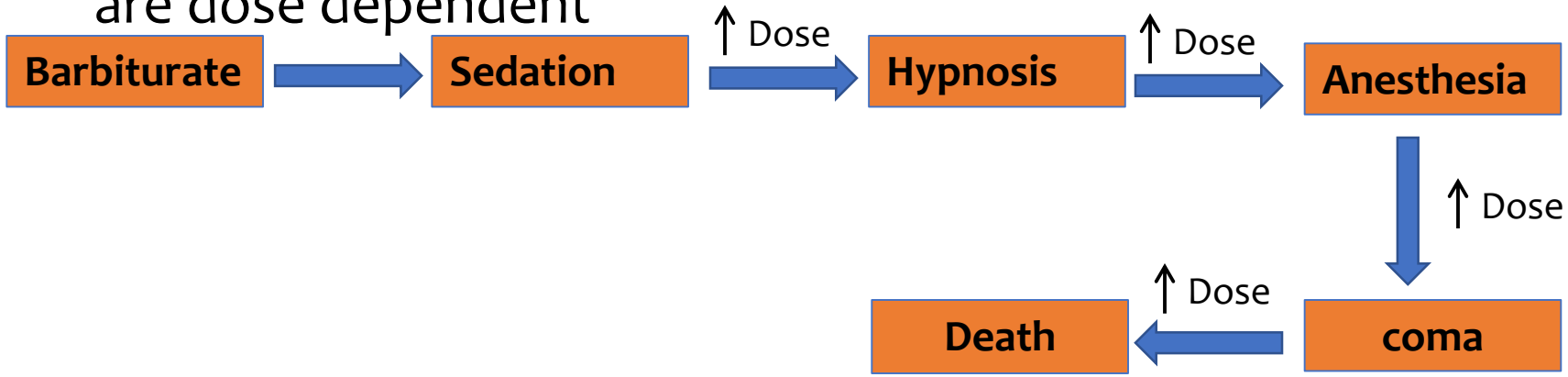
- Steps may be done at random !!

Barbiturates

- Class of drugs that act as central nervous system depressants, and can therefore produce a wide spectrum of effects, from mild sedation to total anesthesia.
  - Long acting bartiturates, ex. **Phenobarbital**. which has **6-8 hr** (duration of action), **4-5 days** elimination half-life.
  - Short acting barbiturates, ex. **Butobarbital** and **Pentobarbital**. 2-4 hrs.(duration of action)
  - Ultra short acting barbiturates, ex. **Thiopental**. (Duration of action **20-25 min**, **h1/2=8-10 hrs**.)
- They are also effective as **anxiolytics**, **hypnotics**, and **anticonvulsants**.
- Barbiturates also have analgesic effects, however these effects are somewhat weak, preventing barbiturates from being used in surgery in the absence of other analgesics.
- They have addiction potential, both physical and psychological.

Barbiturates

- CNS depressant effects of barbiturates: are dose dependent



- Used in anesthesia, insomnia and the treatment of epilepsy and seizure disorders.
- Respiratory Effects are dose-dependent:
  - At hypnotic doses, little effects.
  - At high doses, depress respiratory center.
  - Death due to respiratory failure.

Barbiturates

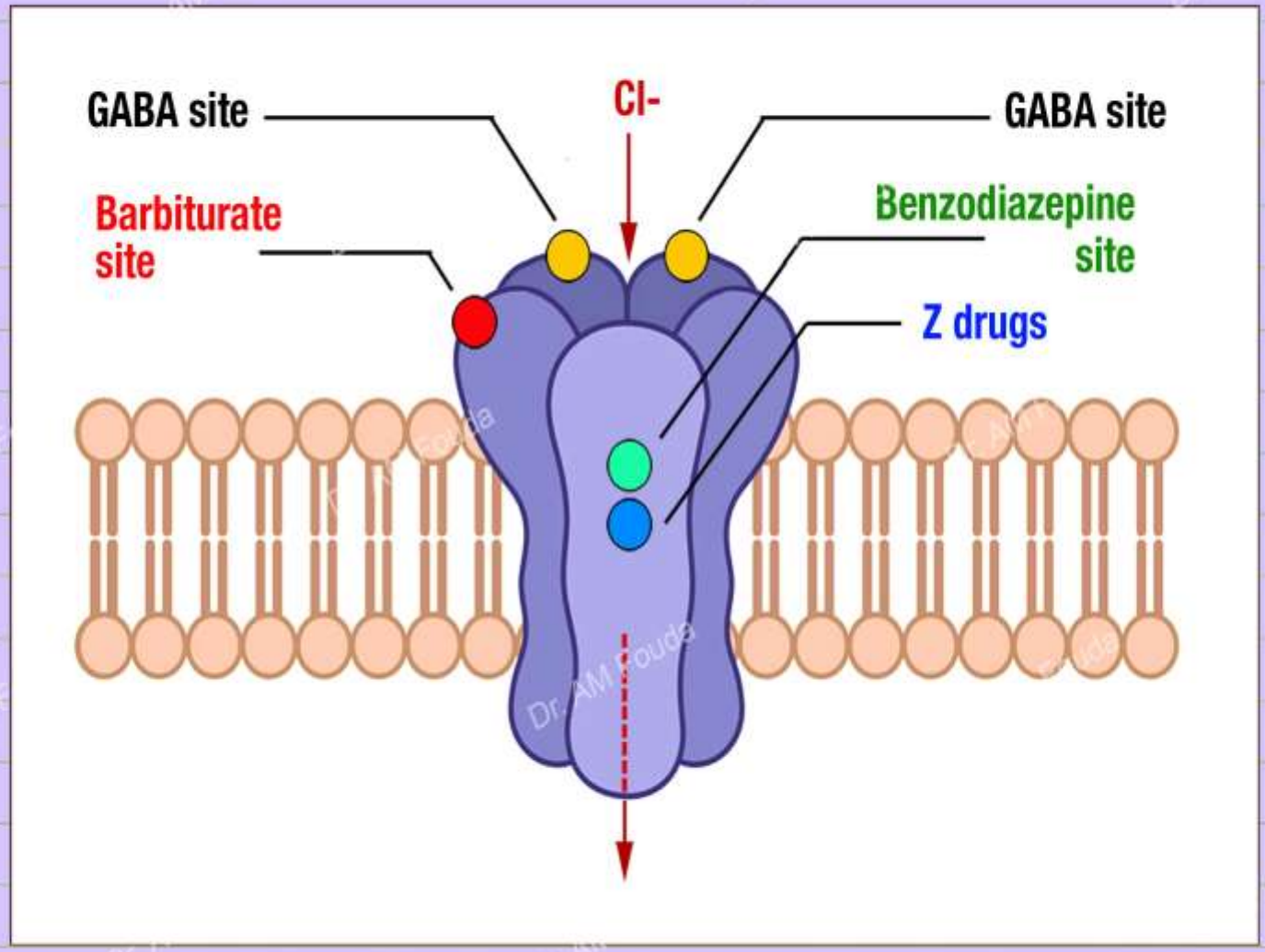
- Barbiturates have now largely been replaced by benzodiazepines in routine medical practice, mainly because benzodiazepines are significantly less dangerous in overdose.

  - Narrow therapeutic index
  - Suppress REM sleep,
    - **barbiturate shortened the time taken to sleep and lengthened the delay to rapid eye movement (R.E.M.) sleep**)
  - Quick tolerance
  - High potential for dependence and abuse (addiction).
  - Potent inducer for liver metabolizing enzymes and drug interactions.

Mechanism of action

(γ-aminobutyric acid)

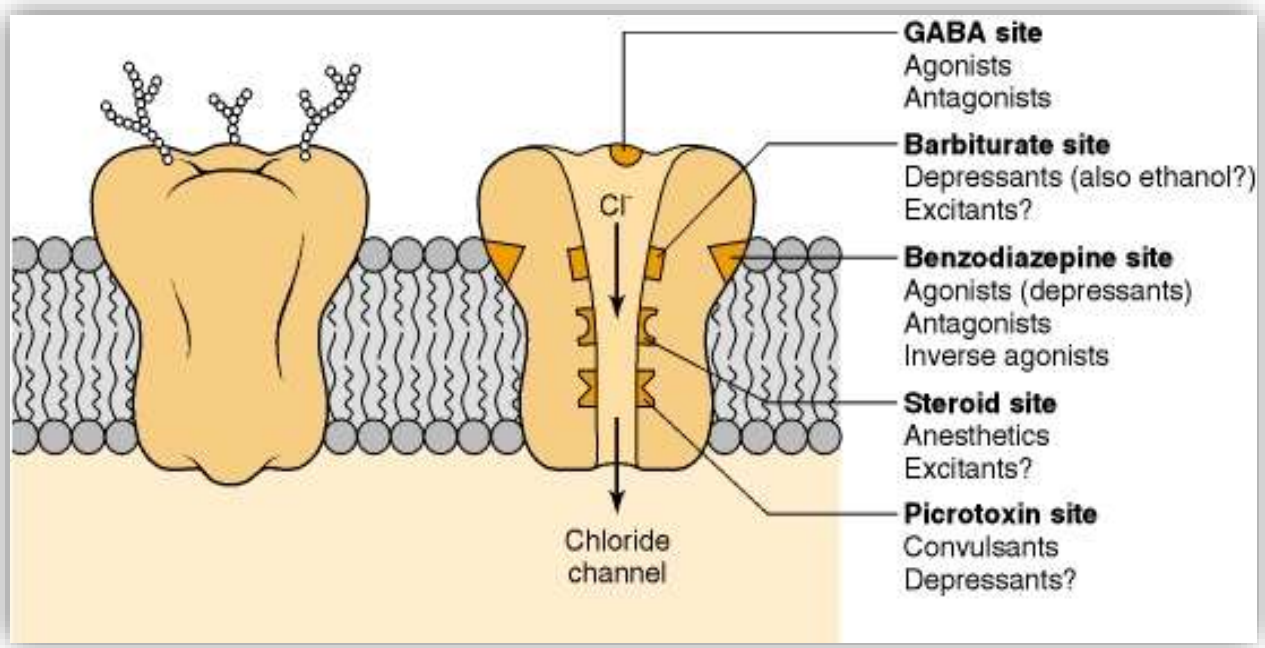
GABA-A Receptor



Barbiturates

Mechanism of action

- **GABA Modulators**:
- Enhance binding of inhibitory NT GABA with its receptors GABA-A binding site.
- they cause allosteric modulation of GABA-action on GABA-A receptor, prolonged the duration of the GABA-gated Cl-channel opening (influx of Cl) → hyperpolarization →CNS depression.



At higher concentration:

- ✓ Barbiturate directly increase Cl- conductance (GABA mimetic action; contrast BZDs which have only GABA facilitatory action).
- ✓ Block the AMPA receptor (a subtype of glutamate receptor), Leading to decrease the activity of excitatory glutamate neurotransmitter and depress the neuronal depolarization effect.
- ✓ They inhibit the Ca^{+2}-dependent release of neurotransmitters.
- ✓ The action of barbiturates is non-selective i.e. increase dose of barbiturates ⟶ generalized CNS inhibition.

Barbiturates

Pharmacokinetics:

- High lipid solubility → Cross blood brain barrier, rapid onset.
- Redistribution to other tissues outside the CNS → Short duration of action.
- Liver: All metabolized into inactive metabolites except (**Phenobarbital excreted unchanged**)
- Renal excretion.
- Alkalization of urine in case of toxicity.

Adverse effects:

- Drowsiness, disorientation, respiratory depression.
- Tolerance: Decreased responsiveness to the drug upon repeated administration.
- Dependence: both psychological and physiological.
- Withdrawal is much more sever than that associated with opiates and can result in death.
- Peripherally: hypotension due to myocardial depression and depression of VMC.

➢Potent inducer for liver metabolizing enzymes.

- Chronic use of barbiturates will cause upregulation, or induction, of the microsomal enzymes (CYPs) ,increasing the metabolism of endogenous substrates and other drugs metabolized by these enzymes. This can lead to patients requiring larger dosages of medication to achieve therapeutic effect and/or increased clearance.
- This enzyme induction also causes **barbiturate tolerance** due to increased barbiturate metabolism.

Experimental protocol

- Thiopental 1 gm vial
- Thiopental , 40mg/kg I.P in mice.
- 6 mice receive **I.P**

➢ **Measured Parameters:**

✓ General Activity

✓ Characteristics of Breathing

✓ Onset of Sleep (mins)

✓ Duration of Sleep (mins)

➢ Barbiturates are hypnotic drugs:

- **Onset of action** is the time required to loss the righting reflex.
- **Duration of action** in mice can be measured by the ‘sleeping time’ (i.e. the time from the loss of righting reflex to recovery of reflex).

Experimental protocol

- **The loss of righting reflex (LORR) assay** is used to evaluate sedative/hypnotic effects.
- **Righting reflex:** the ability to assume an optimal position when there has been a departure from it
- The onset time of sleep was noted for all animals. After induction of sleep, mice were placed in the inverted position and when sedation was over, the mice came to normal posture and time was noted
- Record:
  - LORR was recorded as the time at which the animal was unable to turn itself ***(onset of action)***
  - The time to regain the righting reflex ***(duration of action,DOA)***

Judgment of sleep and wake

Loss of righting reflex (sleep)

Recovery of righting reflex (wake)

Experimental protocol

Report:

- Name & aim of experiment
- Methods: species/drug/dose/ROA
- Results: onset of action/duration of action/ analyze data (t-test)
- Discussion: interpretation of the results

Experimental protocol

Report:

- Name & aim of experiment:
  - You need to write the name of experiment and the aim of the work, for example:
    - ***We aimed to investigate and evaluate the sedative/hypnotic effects. Thiopental administered IP in Mice.***

Experimental protocol

Report:

- Methods:
  - Mention the animal used, the drug injected with the doses. Describe the work. For example:
  - ***six mice were injected with thiopental 40 mg/kg IP. The LORR and duration of sleep were recorded…….etc.***

Experimental protocol

Report:

- Discussion: mention and discuss your results, for example:
  - ***From the results obtained, we noted that onset of action was faster in IP than SC route. This is due to…..etc.***

Experimental protocol

Report:

- Results:
  - Compare Onset and duration for between IP and SC routes using t-test (***two groups, use excel, p<0.05 is considered significant difference***).

Animal	I.P. LORR time	SC LORR time	I.P. sleep time	SC sleep time
Mouse 1				
Mouse 2				
Mouse 3				
Mouse 4				

Thank You!

Any questions?