Introduction to Experimental Pharmacology course

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History of Pharmacology

- **2500 years ago - 17th century**
  ancient Greece used willow leaves

- **17th century - 18th century**
  salicylic acid was found in the bark of a willow tree

- **1899** Bayer marketed aspirin (acetyl-salicylic acid)

- **1970s** the mechanism is known: inhibition of prostaglandin synthesis via a cyclo-oxygenase (COX) enzyme
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History of Pharmacology

Francois Magendie (1783 - 1855)

Claude Bernard was doing experiment (1842).
History of Pharmacology

Rudolf Buchheim (1820-1879)
Founder of experimental pharmacology

Oswald Schmiedeberg (1838-1921)
Founder of modern pharmacology
Aim

- to develop understanding and skills in the process of scientific research in experimental pharmacology/pharmacology.
Outline

- Basic knowledge of pharmacological experimental design
- Basic techniques of pharmacological experiments
- Video: basic skills for experimental pharmacology
- Practice for the basic skills for experimental pharmacology
Introduction to experimental pharmacology course

Basic knowledge of pharmacological experimental design
Three elements in study design

- Subjects
- Treatments (primary, secondary, and third grade)
- Efficacy
Three elements in study design

- Treatments (primary, secondary, and third grade)
Three elements in study design

- Subjects
- Treatments (primary, secondary, and third grade)
- Efficacy
BP

X drug  α antagonist  X drug
Three principles in study design

- **Randomization**: to avoid subjective bias in researchers

- **Replication**: how many times was the experiment repeated under the same conditions, and how many samples were used in the experiment.

- **Control**: self-control design; block-control design/case-control design
X drug \[\alpha\] antagonist X drug

BP
Design of sample size

i. Empirical methods for determination of sample size:

<table>
<thead>
<tr>
<th>Animals</th>
<th>Quantitative data</th>
<th>Qualitative data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse, rat, frog, fish</td>
<td>$\geq 10$</td>
<td>$\geq 30$</td>
</tr>
<tr>
<td>Rabbit, guinea pig</td>
<td>$\geq 6$</td>
<td>$\geq 20$</td>
</tr>
<tr>
<td>Dog, cat, monkey, sheep</td>
<td>5-10</td>
<td></td>
</tr>
</tbody>
</table>

ii. Statistical methods: Discussed in lectures on statistics.
Design of drug dosage

- Based on pilot experiment or references
- Use wide dose interval to determine effective dose range
- Use regular dose interval when observe dose-effect relationship
- Use dose equivalent to compare potency of drugs: LD50, ED50, EC50, etc

(dose-response curve)
Example for design of drug dosage

<table>
<thead>
<tr>
<th>µg/ml</th>
<th>0.001</th>
<th>0.01</th>
<th>0.1</th>
<th>1</th>
<th>10</th>
<th>100</th>
<th>1000</th>
<th>10000</th>
</tr>
</thead>
</table>

- 1 µg/ml
- 3 µg/ml
- 10 µg/ml
- 30 µg/ml
- 100 µg/ml
- 300 µg/ml

![Graph showing % of control OD490 vs log C(µg/ml)]
Design of drug treatment regime

- Pretreatment and post-treatment
- Regular drug treatment
- Determination of the first drug treatment

(time-course curve)
Record of the experiment

- Experimenters, date, room temperature
- Experimental methods, instruments, apparatus, animals (species, sex, weight and other conditions), groups, drugs and chemicals or solutions used
- Processes
- Results (any raw data, traces, photos)
Examples for experimental record

<table>
<thead>
<tr>
<th>Treatment 1 (dose)</th>
<th>Treatment 2 (dose)</th>
<th>Blood pressure (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Adrenaline (       )</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Noradrenaline (    )</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Isoprenaline (     )</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Phentolamine (     )</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Adrenaline (       )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Noradrenaline (    )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isoprenaline (     )</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Examples for experimental record
<table>
<thead>
<tr>
<th>Rat</th>
<th>Mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marker</td>
<td>Brain</td>
</tr>
</tbody>
</table>

三号兔子血清1：1000，未加Ag  
二抗为1：5000（荧光），60微克/孔
080305  

三号兔子血清1：1000，加入的Ag为1：10  
二抗为1：5000（荧光），60微克/孔
080305
Writing an experimental report

- Subject/title
- Objective
- Materials and methods
- Result
- Discussion
- Conclusion
Sample of an experimental report
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Basic techniques of pharmacological experiments
Animal uses in Pharmacology

- Pharmacodynamics:
  - Efficacy
  - Drug safety: side effects or toxicity of medicinal candidates
  - Exploration of pathogenesis and mechanisms of drug action

- Pharmacokinetics: metabolism
Animals used in Pharmacology

- Mammals
  - Rodents: e.g. mouse, rat, rabbit, guinea pig, ferret, chinchilla.
  - Other mammals: e.g. cat, dog, monkey.

- Others
  - Toad
  - Insects: e.g. fruit fly
Ethics in animal use

- Minimize the animal’s suffering
- Minimize the animal number used
- Ethic committee approved protocols
Introduction to experimental pharmacology course

Animal Holding and Drug Administration
Ways for drug administration

- Oral or per os (through the mouth, gavage)
- Intraperitoneal: i.p.
- Intravenous: i.v.
- Intramuscular: i.m.
- Subcutaneous: s.c.
Mouse

- Holding the tail tip for simple catch and transfer.
- Techniques for drug administration, such as i.p., per o.s. / gavage
Mouse

use one hand

use both hands
Mouse

i.p. drug administration for the mouse
0.1-0.2 ml/10 g body weight
Mouse

- ensisternum
- symphysis ossium
- i.p. position
- pubis
Mouse

i.v. drug administration for the mouse (0.05~0.1 ml/10g body weight)
Gavage drug administration for the mouse (0.1~0.2 ml/10g body weight)
Holding the middle part of the tail for simple catch of rats.

Grabbing the skin of the neck and the back or holding the animal from the back when catching rats with heavy weight.
Rat

Holding the rat
i.p. drug administration for the rat (1)  
0.1-0.2 ml/100 g body weight
i.p. drug administration for the rat (2)
0.1-0.2 ml/100 g body weight
Rat

Gavage drug administration for the rat (0.3~0.5 ml/100g body weight)
Guinea pig

- Holding the guinea pig with both hands
Guinea pig

- Gavage drug administration:
  - rubber tubing
  - a piece of wood stick with a hole
  - a cup of water to test air bubbles
- i.v. drug administration for guinea pig: usually through cephalic vein.
Rabbit

- Grabbing the skin of the neck and the back with one hand, supporting the haunch with the other hand from below.
Intravenous injection through marginal ear vein in the rabbit
Other mammals: Dog, Cat and Monkey

Beagle
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Determination of the gender of the animal (rodent)

U: urethra
V: vagina
A: anus
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Animal mark
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Animal Anesthesia
General anesthesia

Gas inhalation: e.g. halothane, isofluorane, ether.

Systemic anesthetics: e.g. pentobarbital, chloral hydrate, chloralose, urethane, ketamine.
<table>
<thead>
<tr>
<th>Anesthetics</th>
<th>Urethane</th>
<th>pentobarbital</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Concentration(%)</strong></td>
<td>20⁺ 25</td>
<td>1⁺ 4</td>
</tr>
<tr>
<td><strong>Dose unit</strong></td>
<td>g/ kg</td>
<td>mg/ kg</td>
</tr>
<tr>
<td><strong>Mouse</strong></td>
<td>1⁺ 1.5 ip</td>
<td>45⁺ 50 (ip)</td>
</tr>
<tr>
<td><strong>Rat</strong></td>
<td>1⁺ 1.5 ip</td>
<td>45⁺ 50 (ip)</td>
</tr>
<tr>
<td><strong>Guinea pig</strong></td>
<td>1⁺ 1.5 ip</td>
<td>45⁺ 50 (ip)</td>
</tr>
<tr>
<td><strong>Rabbit</strong></td>
<td>1⁺ 1.2 (ip)</td>
<td>20⁺ 25 (iv)</td>
</tr>
<tr>
<td><strong>Cat</strong></td>
<td>1⁺ 1.5 (ip)</td>
<td>30⁺ 40 (ip)</td>
</tr>
<tr>
<td><strong>Dog</strong></td>
<td></td>
<td>25⁺ 30 (iv)</td>
</tr>
<tr>
<td><strong>Anesthesia duration</strong></td>
<td>2⁺ 4hr</td>
<td>2⁺ 4hr</td>
</tr>
</tbody>
</table>
Animal euthanization

- Dislocation of cervical vertebra
- CO₂ euthanization
- Decapitation
- Air embolism
- Destroying the brain
- Exsanguinations
- Over-dose of anesthetics