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Median Lethal Dose of Some Local Anaesthetic Preparations(ทดสอบการเป็นพิษอย่างเฉียบพลันของยาชาเฉพาะที่)

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Original Article

Median Lethal Dose of Some Local Anaesthetic Preparations

Abstract

The median lethal dose (LD_{50}) of local anaesthetics in mice was determined by the method of Reed & Muench. No statistical difference ($P < 0.05$) was found in comparing the (LD_{50}) of the four commonly-used local anaesthetic preparations; they were lidocaine 2%, lidocaine 2% with adrenaline 1:80,000, lidocaine 2% with noradrenaline 1:50,000 and prilocaine 3% with felypressin 0.03 IU. It is suggested therefore that, in mice, the (LD_{50}) of these local anaesthetics are comparable. However, lidocaine 2% with noradrenaline 1:50,000 had a wider therapeutic index, therefore it could be the safest local anaesthetic among the other three drugs.

Key words : *Local Anaesthetics, Acute Toxicity Test (LD_{50}), Therapeutic Index.*

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Introduction

Local anaesthetics have been widely used by dental surgeons to abolish pain from a painful procedures e.g. tooth extractions. Without local or general analgesia, no operation could be performed. A painless dental surgery today is a result of the development of local anaesthetics beginning from Niemann's observations in 1860 on the anaesthetizing effect of cocaine on the sensitivity of the tongue. Later, Koller's application of the substance as an analgesic in ophthalmic surgery in 1884, and followed by Halsted's successful attempt at a mandibular block injection on himself in 1885 because of his toothache. Afterward, clinical local analgesia therefore started in dentistry, and was seen practised all over the world, but frequent accidents and deaths threatened to discredit the new discovery.[1],[2],[3] The purpose of this investigation is to determine the therapeutic index and to compare the local anaesthetic toxicity (LD_{50}) of the commercially-prepared local anaesthetics in mice.

Materials and Methods

A. Drugs

The following drugs were obtained commercially and used without further modification: lidocaine hydrochloride 2%; lidocaine 2% with adrenaline 1:80,000; lidocaine hydrochloride with noradrenaline 1:50,000; prilocaine 3% with felypressin 0.03 I.U.

B. Animals

In bred mice weighing 20-25 gm of either sex were used in this experiment. They were supplied by the animal house of Department of Physiology, Faculty of Medicine (Siriraj Hospital), Mahidol University. Twenty-four hours before experiment, the animals were fasted and were selected randomly for the experiment.

C. Calculation of (LD_{50})

The acute toxicity test (LD_{50}) was determined according to the method of Reed & Muench.[5]

D. Principles of Reed and Muench's Method

1. The number of animals for experiment must be constant such as 6 animals per dose.

2. The increased dose have to be at equal interval.

3. Experiment in a constant room temperature at about 82-87° F.

4. The animal should come from the same stock.

E. Procedure

Mice of either sex were divided into a group of six. In each group of animals was injected intraperitoneally with the same dose in ml of local anaesthetics under investigation. The numbers of death and alive were then recorded within 3-hour period. The other group of six were then injected intraperitoneally with the increase or decrease of the equal to zero. The (LD_{50}) was then calculated by the use of the following equation.[6]

$$(LD_{50}) = \log X + (\log Y) \left[\frac{50 - x}{y - x}\right]$$

X = dose which killed the animal just below 50%

log Y = log of interval of doses

x = percent mortality just below 50%

y = percent mortality just above 50%

$$\text{Percent mortality} = \frac{\text{Total number of deaths} \times 100}{\text{Total number of survivors} + \text{Total number of deaths}}$$

Estimation of standard error of the median dose as obtained by the Reed & Muench method.

$$\text{Standard error of } (LD_{50}) = \sqrt{\frac{0.79\,hR}{n}}$$

h = interval between dose

R = the interquartile range from the cumulative percentages

n = the number of animals per dose

Statistics

The values are expressed as mean ± s.e.m.* For testing the statistical significance of difference between two means, unpaired t-test was used.

Results

The LD_{50} of the tested local anaesthetics are shown in table 1. There was no statistical difference among the LD_{50} of these local anaesthetics ($P > 0.05$)

(Table 1). Their therapeutic indexes are shown in Table 2. These results showed that the therapeutic index of lodocaine hydrochloride 2% with noradrenaline 1:50,000 was greater than that of prilocaine 3% with felypressin 0.03% I.U., lidocaine hydrochloride 2% and Lidocaine hydrochloride 2% with adrenaline 1:80,000 (Table 2).

Anaesthetics	(LD_{50}) (ml)/22.5 gm mice	n
Lidocaine hydrochloride 2%	0.155 ± 0.020	6
Lidocaine hydrochloride 2% with adrenaline 1:80,000	0.156 ± 0.023	6
Lidocaine hydrochloride 2% with noradrenaline 1:50,000	0.164 ± 0.018	6
Prilocaine 3% with felypressin 0.03 I.U.	0.168 ± 0.015	6

*s.e.m. = standard error of mean

Table 1. LD_{50} expressed in ml of local anaesthetics with standard errors.

Anaesthetics	LD_{50} (mg) 22.5 gm mice	LD_{50} (mg/kg)	Maximum tolerated dose**		Therapeutic Index*
			mg	mg/kg	
Lidocaine hydrochloride 2%	3.104	137.9	2.00	88.8	1.55
Lidocaine hydrochloride 2% with adrenaline 1:80,000	3.126	138.8	2.00	88.8	1.56
Lidocaine hydrochloride 2% with noradrenaline 1:50,000	3.288	145.9	1.50	66.6	2.19
Prilocaine 3% with felypressin 0.03 I.U.	5.031	223.4	3.75	166.5	1.34

* Therapeutic index was calculated from LD_{50} (mg) divided by maximum tolerated dose (mg).[7]

** Maximum tolerated dose is the dose of the drugs that the animals can survive 100% after intraperitoneal injection.

Table 2. LD_{50} (mg), maximum tolerated dose (mg) and therapeutic index of local anaesthetics.

Discussion

It has been reported that the acute toxicity of prilocaine hydrochloride was found to be 60% of that of lidocaine by intravenous 0.25% or intraperitoneal 2% or subcutaneous administration 4%.[8] Results of the present study revealed that lidocaine hydrochloride 2% alone or with adrenaline 1:80,000 or with noradrenaline 1:50,000 and prilacaine hydrochloride 3% with felypressin 0.03 I.U. had the same acute toxic effect in mice by intraperitoneal injection. It is well known that adrenaline, noradrenaline and felypressin cause vasoconstriction which prolongs the duration of local anaesthetics and decreases toxicity of local anaesthetics by delaying the absorption of the local anaesthetics into systemic circulation.[9] Intraperitoneal injection of local anaesthetics for the acute toxicity test in mice in these experiments showed that there was no difference between local anaesthetics with and without vasoconstrictors. This may be due to the insensitivity of the blood vessels of the mice to those concentrations of vasoconstrictors. Therefore, the toxicity of the mice induced by local anaesthetics in the present study was about the same. Interestingly, lidocaine hydrochloride 2% with noradrenaline 1:50,000 had greater therapeutic index than other three of local anaesthetics under investigation. This indicated that lidocaine with noradrenaline 1:50,000 may be safer than those local anaesthetics because it has a wider margin of safety, the larger amount of drug required to produce toxic effect in mice.

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บทวิทยาการ

ทดสอบการเป็นพิษอย่างเฉียบพลันของยาชาเฉพาะที่

บทคัดย่อ

ผลการศึกษาการเป็นพิษอย่างเฉียบพลันในหนูถีบจักรโดยวิธีของ Reed & Muench พบว่าไม่มีความแตกต่างกันทางสถิติระหว่างปริมาณของยาชาที่ทำให้สัตว์ตาย 50% โดยใช้ยาชา Lidocaine 2%, Lidocaine 2% ผสม adrenaline 1:80,000, Lidocaine 2% ผสม noradrenaline 1:50,000 และ Prilocaine 3% มี felypression 0.03 I.U. ผสมอยู่ การเป็นพิษอย่างเฉียบพลันของยาชาเหล่านี้ในหนูถีบจักรเหมือนกัน อย่างไรก็ตาม เมื่อเปรียบเทียบ therapeutic index ของยาชาเหล่านี้ พบว่า Lidocaine 2% ผสม noradrenaline 1:50,000 มี therapeutic index กว้างกว่า แสดงว่าเป็นยาชาที่ปลอดภัย คือขนาดของยาที่ทำให้สัตว์ทดลองตาย 50% และขนาดของยาสูงสุดที่สัตว์สามารถทนได้ มีขนาดห่างกันมากกว่ายาชาตัวอื่น

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