International Journal of Veterinary Science and Medicine (2016) 4, 1–4

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International Journal of Veterinary Science and Medicine

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The new algorithm for calculation of median lethal dose (LD_{50}) and effective dose fifty (ED_{50}) of Micrarus fulvius venom and anti-venom in mice

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Received 11 June 2016; accepted 8 September 2016
Available online 29 November 2016

KEYWORDS

Effective dose;
Median lethal dose;
Snake venom;
Snake anti venom;
Mice;
Micrarus fulvius

Abstract One million people throughout the world are bitten yearly by poisonous snakes. Of this, one-tenth died and three-tenth suffer some forms of disabilities. In view of this, anti-snake venoms are currently being developed against viper and colubrid snake venoms using mice. Therefore, a new algorithm for calculation of median lethal dose (LD_{50}) and effective dose fifty (ED_{50}) was developed for Micrarus fulvius venom and antivenom respectively. This paper compared the formula of effective dose fifty (ED_{50}) developed by Spearman and Karber with ideal median lethal dose ($IMLD_{50}$) formula developed by Saganuwan with a view to bringing out their difference and similarity in calculation of ED_{50} that could be used to develop a new median lethal dose formula for calculation of Micrarus fulvius venom in mice. The findings revealed that ED_{50} value (477 mg/kg) from Spearman and Karber's formula ($ED_{50} = \log ED_{50} = \log X_{100} - \frac{\log FD}{n}(\Sigma t - n/2)$ is comparatively similar with ideal median lethal dose value (428.75 mg/kg) from Saganuwan's formula ($MLD_{50} + MSD_{50}/2$). The new LD_{50} formula ($LD_{50} = \left(\frac{ED_{50}}{3}\right) \times Wm \times 10^{-4}$) yielded value (0.29 mg/kg) of comparative significance with reported value (0.32 mg/kg). When ED_{50} is equal to $2LD_{50}$, the denominator of $\frac{ED_{50}}{3}$ becomes 2. In conclusion, the new formula would yield low doses of snake anti-venoms with reduced possibility of hypersensitivity reaction.

1. Introduction

Snakes are represented on earth today by some 3150 species [1]. Of these 2700 species known as Caenophidia or "advanced snakes" with fangs, and venom glands [2]. Venomous snakes are responsible for an estimated 75,000 human deaths annually [3]. In the United Sates approximately 45,000 snake bites occur each year, of which about 8000 are by 20 species of venomous snakes. Deaths do not exceed 10–12 per year [4]. Of hospitalized snakebite victims, 0.5% of bites were inflicted by coral snakes, 7.3% by cottonmouths, 28.6% by copper heads, 29.8% by unidentified snakes and 33% by rattlesnake [5] with diamondbacks causing the most fatalities. More than 95% of bites occur between April and October and 77% occur during day time [6]. Snake venom metalloproteinases are responsible for major local symptoms in snakebite causing

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Peer review under responsibility of Faculty of Veterinary Medicine, Cairo University.

http://dx.doi.org/10.1016/j.ijvsm.2016.09.001

haemorrhage, oedema, hypotension, hypovolemia, inflammation and necrosis [7]. Because of considerable ophidic snake bites, advances have been achieved in the production of new antivenoms using new processes [8], Coral snake envenomation could be handled using medication [9]. Specific treatments with antivenoms continue to be the chosen method as it deactivates the venom [10]. Aguilar et al. [11] prepared snake antivenom against Micranis fulvius in chicken (Gallis domesticus) with median effective dose (ED_{50} = 477 mg/kg). Because of hypersensitivity reactions that do result from snake antivenom treatment, a new algorithm has been developed for calculation of median lethal dose (LD_{50}) and effective dose fifty (ED_{50}) for snake venom and antiveonom respectively.

2. Materials and methods

Reed and Muench [12] introduced arithmetical method for determination of median lethal dose (LD_{50}) in 1938 which was modified by Saganuwan [13]. The possible modifications involved calculating percent of test animals both that died and survived at all the test dose levels. The average of a dose that caused 50% death and another dose that caused 50% survival gave a relatively ideal LD_{50}. However, Aguilar et al. [11] estimated effective dose fifty (ED_{50}) of coral snake antivenom according to the method of Spearman and Karber [14] using mice of 18–20 g. Various antivenom concentrations of 17.2, 8.6, 4.3, 21.5 and 5.3 mg per mouse weighing 20 g were used for calculation of Ideal Median Lethal Dose ($IMLD_{50}$) proven to be comparatively similar to the Effective Dose Fifty (ED_{50}) calculated by Aguilar et al. [11] using Spearman and Karber's formula. ED_{50} was also used to develop a new formula for calculation of median lethal dose (LD_{50}) of snake venom in mice.

$$\therefore \mathrm{ED}_50 = \log \mathrm{ED}_{50} = \log X_{100} - \frac{\log \mathrm{FD}}{n}(\Sigma t - n/2)$$

$$\text{The ideal Median lethal Dose} = \frac{\mathrm{MLD}_{50} + \mathrm{MSD}_{50}}{2}$$

2.1. Definition of terms

ED_{50} = the 50% effective dose; $\log X_{100}$ = log dose giving 100% survival and having 100% survival for all higher doses; $\log \mathrm{FD}$ = the log dilution factor; N = number of mice used at each dose level; Σ = the sum of mice surviving at every dose level, the ED_{50} is the effective dose of (lgy) that will protect 50% of the mice population when injected with $3LD_{50s}$. Median lethal dose (MLD_{50}) is the dose that kills 50% of test mice whereas median survival dose (MSD_{50}) is the dose survival by 50% of test mice.

2.2. Ideal median lethal dose ($IMLD_{50}$) of snake venom is equal to effective dose fifty (ED_{50}) of snake antivenom in mice

The calculation done by Aguilar et al. [11] for determination of ED_{50} is confirmed using Ideal Median Lethal Dose (MLD_{50}) formula of Saganuwan [13] proving that Spearman and Karber's formula gives ED_{50} that approximates Ideal Median Lethal Dose (LD_{50}).

2.3. Hypothesis

$$\begin{aligned}\mathrm{ED}_{50} = \log \mathrm{ED}_{50} &= \log X_{100} - \frac{\log \mathrm{FD}}{n}(\sum t - n/2)\\ &= \frac{\mathrm{MLD}_{50} + \mathrm{MSD}_{50}}{2}\\ &= \text{ideal Median Lethal Dose} = (\mathrm{IMLD}_{50})\end{aligned}$$

2.4. Median Lethal Dose of Snake Venom Deduced from Effective Dose Fifty (ED_{50}) and Ideal Median Lethal Dose ($IMLD_{50}$)

Since ED_{50} is the effective dose of 1gy that will protect 50% of the mouse population when injected with $3LD_{50}$, the LD_{50} of venom in the present context should be determined as follows:

$ED_{50} = 3LD_{50}$

$LD_{50} = \frac{ED_{50}}{3}$ (this cannot give correct LD_{50} value)

i. But there is need to know the weight of individual mouse in gramme (Wm) in relation to that of human in kilogramme (1000 g) since antivenom is developed for human use.
ii. Also safety factor of 1/10 is considered for mouse as compared to snake

$$\therefore \mathrm{LD}_{50} = \left(\frac{\mathrm{ED}_{50}}{3}\right) \times \frac{\mathrm{Wm}}{1000} \times \frac{1}{10}$$

$$\mathrm{LD}_{50} = \left(\frac{\mathrm{ED}_{50}}{3}\right) \times \mathrm{Wm}$$

$$\mathrm{LD}_{50} = \left(\frac{\mathrm{ED}_{50}}{3}\right) \times \mathrm{Wm} \times 10^{-4}\ \mathrm{mg/kg}$$

3. Results

Proof: Ideal Median Lethal Dose ($IMLD_{50}$) of Snake Venom is Equal to Effective Dose (ED_{50}) of Snake Antivenom in Mice

$\frac{50.0-25.0}{62.5-25.0} = \frac{25.0}{37.5} = 0.666$	$\frac{50.0-37.5}{75.0-37.5} = \frac{12.0}{37.5} = 0.333$
Dose log dose	$\frac{21.5}{8.6} = 2.5$
21.5 1.3324	log 2.5 = 0.3979
8.6 $\underline{0.9344}$	0.333 × 0.3979
$= \underline{0.398}$	0.1325007
$\therefore$ 0.666 × 0.3989 = 0.265068	
Antilog of 0.9344 + 0.265068	Antilog of 0.9344 × 0.1325007
= 1.199468	= 0.12380
MLD_{50} = 15.82 mg/mouse	= 1.32
	MSD_{50} = 1.33 mg/mouse
Therefore, IMLD = $\frac{MLD_{50}+MSD_{50}}{2} = \frac{15.82+1.33}{2} = \frac{17.15}{2}$	
= 8.75 mg/mouse	

Proof: New Median Lethal Dose of Snake Venom Deduced from Effective Dose Fifty (ED_{50}) and Ideal Median Lethal Dose ($IMLD_{50}$)

The ED_{50} is the effective dose of Igy that will protect 50% of the mouse population when injected with $3LD_{50}$ (Table 1).

Table 1 Effective dose fifty (ED_{50}) of yielded antibodies (Igy coral snake antivenom neutralizing lethal toxic activity of coral snake venoms) using Saganuwan method [12].

Total protein of antivenom (mg/20 g mouse)	Log dose	Cumulative							
		Dead	Survived	Dead	Survived	Total	Mortality rate	% Mortality	% Survival
17.2	1.2355	0	8	0	8	8	$\frac{0}{8}$	0.0	100
8.6	0.9344	4	4	4	12	16	$\frac{4}{16}$	25.0	75
4.3	0.6334	8	0	12	12	24	$\frac{12}{24}$	50.0	50.0
21.5	1.3324	8	0	20	12	32	$\frac{20}{32}$	62.5	37.5
5.3	0.7242	8	0	28	12	40	$\frac{28}{40}$	70.0	30

Average weighed mouse is 20 g

$\therefore$ 8.575 mg $\rightarrow$ 20 g

x $\rightarrow$ 1000 g

$x = \frac{1000 \times 8.575}{20} = 428.75 \text{mg/kg} = \text{IMLD}_{50}$

But the ED_{50} reported by Aguilar et al. [1] is 477 mg/kg

But $IMLD_{50}$ = 451.3 mg/kg

$\therefore$

LD_{50} of the venom $= \frac{ED_{50}}{3} \times Wm \times 10^{-4} = \frac{IMLD_{50}}{3} \times Wm \times 10^{-4}$

$= \frac{428.75}{3} \times 20 \times 10^{-4}$

LD_{50} = 0.29 mg/kg

$IMLD_{50} \simeq ED_{50}$

$\therefore$ $IMLD_{50}$ can be used to calculate ED_{50}

4. Discussion

The $IMLD_{50}$ (428.75 mg/kg) obtained in our present investigation is close to the ED_{50} value (477 mg/kg) reported by Aguilar et al. [11] indicating that $IMLD_{50}$ can be used to calculate ED_{50} of snake antivenom. The two values are within the acceptable ED_{50} ranges of other antivenoms tested on different snake venoms [15]. Normally antivenoms are achieved by immunizing horses with increasing doses of venom to obtain a high-quality antibody titer [16]. But since the value of our $IMLD_{50}$ is little lower than that of Aguilar et al., it may connote that at lower level of ED_{50}, low side effects including anaphylaxis may exist. The elevated concentrations of proteins, which are not antibodies, existing in many antivenoms produce most of these side effects [17]. Immunoglobulin (Igy) produce antibody against M. isozomus [11] and Scolapadra gigaitea toxin [18]. WHO [14] patented Sodium Silicate Complex (SSC) which comprises Trimeric Sodium Silicate (Na_2SiO_3) and Sodium Silicate Pentahydrate (Na_2SiO_3)·$5H_2O$ using a relatively ideal median lethal dose formula developed by Saganuwan [13] confirming the reliability, predictive validity and precision of the formula. SSC has antivenomous activity against Crotalus atrox, Agkistrodon contortrix contortrix and Agkistrodon piscivorus leucostoma venoms [14] of our estimated LD_{50} (0.29 mg/kg) using the new developed formula agrees with the report of Aguilar et al. [11] indicating that the LD_{50} of Micrarus fulvius in mice is 0.32 ± 0.12 mg/kg body weight in mice.

Snake venoms are known to be the most complex of all natural venoms. They contain more than one hundred toxic and non-toxic biological molecules [19]. The cell toxicity assay has been adapted as an alternative to assess toxicity in animals [20]. In the 1960s and 1970s, mice became the universally accepted test animals and comparative toxicity was expressed as LD_{50}. Since ideal median lethal dose is approximately equal to effective safety dose fifty (ED_{50}) of antivenom and now LD_{50} formula is deduced from ED_{50}, the two formulas can be of great value in toxinology of snake venom and antivenom.

5. Conclusion

In line with the principles of replacement, reduction and refinement, ideal median lethal dose and new developed LD_{50} formula can be of great use for assessment of snake antivenom and snake venom respectively. In conclusion, the new formula would yield low doses of snake anti-venoms with reduced possibility of hypersensitivity reaction.

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