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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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 $X_{100}$ – 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$_{50}$ = ED$_{50}$/2) yielded value (0.29 mg/kg) of comparative significance. When ED$_{50}$ is equal to 2LD$_{50}$, the denominator of ED$_{50}$ 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
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 Micrurus fulvius in chicken (Gallis domesticus) with median effective dose (ED50 = 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 (LD50) and effective dose fifty (ED50) for snake venom and antivenom respectively.

2. Materials and methods

Reed and Muench [12] introduced arithmetical method for determination of median lethal dose (LD50) 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 LD50. However, Aguilar et al. [11] estimated effective dose fifty (ED50) 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 (IMLD50) proven to be comparatively similar to the Effective Dose Fifty (ED50) calculated by Aguilar et al. [11] using Spearman and Karber’s formula. ED50 was also used to develop a new formula for calculation of median lethal dose (LD50) of snake venom in mice.

\[ \text{ED}_{50} = \log \text{ED}_{50} = \log X_{100} - \frac{\log \text{FD}}{n} (\Sigma t - n/2) \]

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

2.1. Definition of terms

ED50 = the 50% effective dose; \( \log X_{100} = \log \text{dose giving 100% survival and having 100% survival for all higher doses; } \frac{\text{log FD}}{n} = \text{log dilution factor; } N = \text{number of mice used at each dose level; } \Sigma = \text{the sum of mice surviving at every dose level, the ED50 is the effective dose of (Igy) that will protect 50% of the mice population when injected with 3LD50. Median lethal dose (MLD50) is the dose that kills 50% of test mice whereas median survival dose (MSD50) is the dose survival by 50% of test mice.} \]

2.2. Ideal median lethal dose (IMLD50) of snake venom is equal to effective dose fifty (ED50) of snake antivenom in mice

The calculation done by Aguilar et al. [11] for determination of ED50 is confirmed using Ideal Median Lethal Dose (MLD50) formula of Saganuwan [13] proving that Spearman and Karber’s formula gives ED50 that approximates Ideal Median Lethal Dose (LD50).

2.3. Hypothesis

\[ \text{ED}_{50} = \log \text{ED}_{50} = \log \frac{\text{log FD}}{n} (\Sigma t - n/2) \]

\[ = \frac{\text{MLD}_{50} + \text{MSD}_{50}}{2} \]

= ideal Median Lethal Dose = (IMLD50)

2.4. Median Lethal Dose of Snake Venom Deduced from Effective Dose Fifty (ED50) and Ideal Median Lethal Dose (IMLD50)

Since ED50 is the effective dose of Igy that will protect 50% of the mouse population when injected with 3LD50, the LD50 of venom in the present context should be determined as follows:

\[ \text{LD}_{50} = \frac{\text{ED}_{50}}{3} \] (this cannot give correct LD50 value)

\[ \text{i. But there is need to know the weight of individual mouse in gramme (Wm) in relation to that of human in kilogramme (1000 g) since antivenom is developed for human use.} \]

\[ \text{ii. Also safety factor of 1/10 is considered for mouse as compared to snake} \]

\[ \therefore \text{LD}_{50} = \frac{\text{ED}_{50}}{3} \times \frac{\text{Wm}}{1000} \times \frac{1}{10} \]

\[ \text{LD}_{50} = \frac{\text{ED}_{50}}{3} \times \text{Wm} \]

\[ \text{LD}_{50} = \frac{\text{ED}_{50}}{3} \times \text{Wm} \times 10^{-4} \text{ mg/kg} \]

3. Results

Proof: Ideal Median Lethal Dose (IMLD50) of Snake Venom is Equal to Effective Dose (ED50) of Snake Antivenom in Mice

| 0.0–25g | 0.0666 | 0.6664 | 0.0333 |
|---------|-------|-------|-------|
| Dose log dose | 0.3980 | 0.132507 |
| Antidote of 0.9344 × 0.265068 | 0.0333 × 0.3979 |
| = 1.199468 | 0.12380 |
| MLD50 = 15.82 mg/mouse | 1.32 |
| MSD50 = 1.33 mg/mouse | 8.75 mg/mouse |
| Therefore, IMLD = \frac{MLD_{50} + MSD_{50}}{2} = \frac{15.82 + 1.33}{2} = 8.75 } |

Proof: New Median Lethal Dose of Snake Venom Deduced from Effective Dose Fifty (ED50) and Ideal Median Lethal Dose (IMLD50)

The ED50 is the effective dose of Igy that will protect 50% of the mouse population when injected with 3LD50 (Table 1).
Table 1  Effective dose fifty (ED50) 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    | 0             | 0.0        | 100       |
| 8.6                                       | 0.9344   |            | 4    | 4        | 12   | 16       | 4    | 25.0          | 75         |           |
| 4.3                                       | 0.6334   |            | 8    | 0        | 12   | 24       | 12   | 50.0          | 50.0       |           |
| 21.5                                      | 1.3324   |            | 8    | 0        | 20   | 12       | 32   | 62.5          | 37.5       |           |
| 5.3                                       | 0.7242   |            | 8    | 0        | 28   | 12       | 40   | 70.0          | 30         |           |

Average weighed mouse is 20 g
\[ x = \frac{1000 \times 0.575}{20} = 428.75 \text{mg/kg} = \text{IMLD}_{50} \]
But the ED50 reported by Aguilar et al. [1] is 477 mg/kg
But IMLD50 = 451.3 mg/kg
\[ \text{LD}_{50} \text{ of the venom} = \frac{\text{ED50} \times \text{Wm} \times 10^{-4}}{\text{IMLD}_{50} \times \text{Wm} \times 10^{-4}} = \frac{428.75 \times 20 \times 10^{-4}}{451.3 \times 20 \times 10^{-4}} \text{LD}_{50} = 0.29 \text{mg/kg} \]
\[ \text{IMLD}_{50} \simeq \text{ED}_{50} \]
\[ \therefore \text{IMLD}_{50} \text{ can be used to calculate ED}_{50} \]

4. Discussion

The IMLD50 (428.75 mg/kg) obtained in our present investigation is close to the ED50 value (477 mg/kg) reported by Aguilar et al. [1] indicating that IMLD50 can be used to calculate ED50 of snake antivenom. The two values are within the acceptable ED50 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 IMLD50 is little lower than that of Aguilar et al., it may connote that at lower level of ED50, low side effects including anaphylaxis may exist. The elevated concentrations of proteins, which are not antibodies, existing in many antivenoms produce non-toxic biological molecules [19]. The cell toxicity assay would yield low doses of snake anti-venoms with reduced possibility of hypersensitivity reaction.

5. Conclusion

In line with the principles of replacement, reduction and refinement, ideal median lethal dose and new developed LD50 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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