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Cross neutralization of cobra (*N. kaouthia*) antivenom manufactured by Myanmar Pharmaceutical factory with spitting cobra (*N. mandalayensis*) venom

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In 1998 a new species of spitting cobra (*N. mandalayensis*) was identified in mid-land, Myanmar by J.B. Slowinski. Myanmar Pharmaceutical Factory manufactures mono specific cobra (*Naja kaouthia*) antivenom for treating cobra bite cases. Venom ophthalmia following spitting of spitting cobra has been reported earlier. The objective of the study is to determine possibility of use of cobra antivenom in treating spitting cobra bite cases. In order to elucidate this fact, cross protective study of lethal effect of spitting cobra venom by cobra antivenom was assessed in rodents. Immunodiffusion was carried out between cobra antivenom and spitting cobra venom. It was found that 120µl of cobra antivenom will neutralise 3 LD_{50} i.v. spitting cobra venom. The cobra antivenom with cobra and spitting cobra venoms recognizes a similar number of precipitin bands. The study highlighted that cobra antivenom could be used to treat spitting cobra bite cases.

INTRODUCTION

Snakebite is endemic in Myanmar. Russell's viper is responsible for 90% of the bites and followed by cobra (*Naja kaouthia*) 8% [1]. Cobra has a wide distribution in whole Myanmar except Chin, Kachin, Kayah and Shan states [1]. Venom ophthalmia following spitting of venom into eyes by spitting cobra (*Naja siamensis*) in mid-land was reported in 1997 [2], but its bite has not been reported. In later year, the reported spitting cobra was found out to be a new species of cobra and named as *Naja mandalayensis* by J.B. Slowinski [3]. Myanmar Pharmaceutical factory manufactures monospecific antivenom for treating cobra bites. Since there is no specific antivenom for spitting cobra bite, it is highly desirable to know that whether the cobra antivenom could be used in treating spitting cobra bite cases. The study attempts to verify the assumption.

MATERIALS AND METHODS

Spitting cobras (*Naja mandalayensis*) (n=15) (length>100cm) caught from Mandalay Division (Thazi Township) were milked and lyophilized. Cobra (*Naja kaouthia*) venom and enzyme refined lyophilized monospecific cobra (*Naja kaouthia*) antivenom were bought from Myanmar Pharmaceutical Factory. Agarose gel (FMC Bio Products (USA), Coomassic brillant blue R -250(Nakari chemicals Ltd), acetic acid, glacial (E Merck) and methanol (Wako pure chemical) were used.

METHODS

Immunodiffusion

Antigen antibody reaction was performed in 1.5% agarose gel plate using Ouchterlony method [4]. Cobra antivenom in the centre well was allowed to react with the cobra (*N. kaouthia*) (1mg/ml) and the spitting cobra (*N Mandalayensis*) (1mg/ml) venoms in surrounding wells at room temperature for 24hr. The plate was washed with physiological saline, stained with 0.025% w/v Coomassie brillant blue R-250 in methanol: water: acetic acid (50:45:5)

mixture, de-stained in water: acetic acid: methanol (87:8:5) mixture and dried.

Determination of median lethal dose (LD_{50iv})

Determination of median lethal dose (LD_{50iv}) of venom was carried out according to WHO recommended method [5].

In brief, it was determined by intravenous injection of 0.2ml of the variable concentrations of venom in physiological saline into tail vein of 18-20g male ICR mice. Six mice per dose were used. The LD_{50iv} of the venom was calculated by probit analysis [6] of death occurring within 24 h following injection of the venom.

Neutralisation of lethal activity of the venom by antivenom

Neutralisation of lethal activity of venom by antivenom was performed according to WHO recommended standard test of neutralising activity [5].

Briefly a fixed amount of venom was mixed with a variable dilution of antivenom, incubated at 37°C for 30 min. and injected intravenously into the laboratory animals. Deaths were recorded following 24 h after injection. The result was expressed as median effective neutralising dose (ED_{50iv}): the minimum amount of antivenom that will save 50% of the test animals in 24h after injection.

RESULTS

Determination of lethal activity of venom

LD_{50iv} of the cobra (*Naja kaouthia*) venom was 4.95 (3.54-6.73)µg/mouse (95% confidence limit) [7] and that of spitting cobra (*Naja mandalayensis*) was 56.1 (49.09-66.5) µg/ mouse.The results were means of three experiments.

Immunodiffusion

Two distinct precipitin bands were observed following reaction between the cobra antivenom and the cobra venom and three distinct cross-reacting precipitin bands between the former and the spitting cobra venom (Figure).

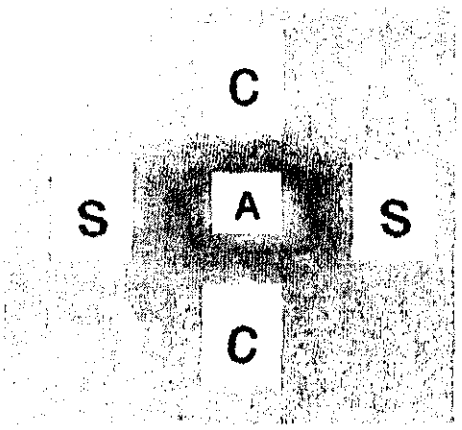
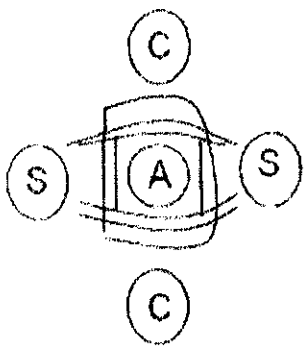


Fig. Precipitin reactions between spitting cobra, cobra venoms and cobra antivenom .



Diagramatic presentation of precipitin reactions
S= Spitting cobra (*N. mandalayensis*)
C= Cobra (*N. kaouthia*)
A= Cobra antivenom (*N. kaouthia*)

Neutralisation of lethal activity of the spitting cobra venom by the cobra antivenom

120 (127.7-130.6) µl (95% confidence limit) of the cobra antivenom was required to neutralise 3 LD_{50iv} of the spitting cobra venom whereas it required 11.07(2.87-21.37) µl to neutralise 3LD_{50iv} of the cobra (*Naja kaouthia*) venom.

DISCUSSION

Although venom ophthalmia following spitting of venom into eyes by spitting cobra (*Naja siamensis*) was reported in 1997 [2], its bite has not been reported. Since 700 spitting cobras caught in mid-land (Thazi

Township) were brought for sales to Myanmar Pharmaceutical Factory, Yangon in the reporting year [2], it is possible that its bite may have mistaken for cobra bite and treated accordingly with cobra antivenom. Detail study of the cobra bite cases from mid-land may reveal incidence of spitting cobra bite. The reported spitting cobras were found to belong to a new species of cobra and named by JB Slowinski as *Naja mandalayensis* [3].

The LD_{50iv} of Myanmar cobra (*Naja kaouthia*) is 4.95 µg/mouse and spitting cobra (*Naja mandalayensis*) 56.1µg/mouse whereas that of Thai cobra (*Naja kaouthia*) is 6.61µg/mouse and spitting cobra (*Naja siamensis*) 21.4µg/mouse [8]. Results indicate that spitting cobra venoms are less lethal than that of the cobras.

In cross neutralisation study, 10 times more Myanmar cobra antivenom is needed to neutralise the lethal effect of the spitting cobra (*Naja mandalayensis*) venom whereas about 3 times more Thai cobra antivenom is required to neutralise lethal effect of spitting cobra (*Naja siamensis*) venom [8].

The study highlights the cross reaction between spitting cobra (*Naja mandalayen sis*) venom and cobra (*Naja kaouthia*) anti-venom and cross protection of the lethal activity of spitting cobra venom by the cobra antivenom. The results are in consistent with those reported by Minton [9] about the presence of genus specificity in cobra antivenoms. This is the first report of cross protection provided by the cobra antivenom against the lethal activity of spitting cobra (*Naja mandalayensis*) venom.

It suggests that in the absence of mono specific antivenom for spitting cobra, cobra (*Naja kaouthia*) antivenom could be used for treating spitting cobra bite however, more antivenom will be needed to neutralise the lethal effect of the venom.

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