The Myanmar Health Sciences Research Journal, Vol. 15, No. 1-3, 2003

Neutralisation of biological activities of Russell's viper (*Daboia russelii siamensis*) venoms from different localities of Myanmar by monospecific antivenom

Tun Pe, Aye Aye Myint & Kyi May Htwe

Venom Research Laboratory
Department of Medical Research (Lower Myanmar)

Neutralisation of biological properties of Russell's viper venoms from 5 divisions of Myanmar namely Mandalay, Magwe (Upper Myanmar), Yangon, Bago, Ayerawaddy (Lower Myanmar) by a monospecific antivenom was studied by WHO recommended methods of neutralisation in rodent. In general, 2 to 8 times more antivenom is required to neutralise different biological activities of the venoms of Upper Myanmar compared to the Lower. Forty times more antivenom is needed to neutralise procoagulant activity of the venom of Taungdwingyi (Magwe Division) compared to that of Daedaye (Ayerawaddy Division). The antivenom is equally effective in neutralising procoagulant activity of the venom of Wundwin (Mandalay division). Variable neutralisation of the properties of venom by the antivenom among venoms of Lower Myanmar is probably due to intra divisional variation in venom properties. The observation calls for use of widely pooled venoms in raising antivenom for common use.

INTRODUCTION

Russell's viper (*Daboia russelii siamensis*) bite occurs in rice growing divisions of Myanmar. Monospecific antivenom manufactured by Myanmar Pharmaceutical Factory (MPF) is used for treating Russell's viper bite cases throughout Myanmar. The source of venom used for raising antivenom is mainly derived from venoms collected from Russell's vipers caught in Yangon, Bago and Ayerrawaddy divisions (Lower Myanmar) which tend to vary from year to year. However, recent observations of geographical variation of Russell's viper venom [1-5], variation of venom neutralising efficacy of a batch of mono specific antivenom against venoms from 3 different localities of Myanmar [6] and variable performance of antivenom in correcting coagulation defect in Russell's viper bite cases of different localities of Myanmar [7-10] prompted us to do this study.

MATERIALS AND METHODS

Characterisation of the venoms

Russell's vipers caught from five divisions of Myanmar namely Mandalay, Magwe (Upper Myanmar), Yangon, Bago, Ayerawaddy (Lower Myanmar) were housed separately and kept in the snake firm of MPF. Individual snake was milked, lyophilised and pooled according to locality and length into young (<90cm) and adult (>90 cm). Pooled venom of adult vipers of Mandalay Division (Wundwin n=2), Magwe (Taungdwingyi n=4), Yangon (Kungyankone n=25), Bago (Tharawaddy n=14) and Ayerawaddy (Daedaye n=7) were stored in dark at 41°C. Characterisation of the biological properties of the venoms such as lethal (LD_{50}iv), coagulant (MCD), defibrinogenating (MDD), necrotic (MND), haemorrhagic (MHD) and capillary permeability increasing (MCPID) was determined according to WHO

recommended methods [11]. Lyophilised antivenom was reconstituted, aliquoted and stored frozen at -20°C until tested.

Venom-antivenom neutralisation test

Monospecific antivenom, batch no. DN 86608B exp. 4/92 was used for neutralisation test in rodent according to WHO standard tests of neutralising activity [11]. For venom-antivenom neutralisation test, $5LD_{50}$iv, 10MCD, 3MHD, 3MND, 5MDD and 100 MCPID doses were used. Briefly, a fixed amount of venom is mixed with variable dilutions of antivenom, mixed, incubated at 37°C for 30 min and then injected into the laboratory animals. The median effective dose (ED_{50}) is taken as the amount of antivenom required to neutralise 50% of the biological activity of venom [11].

RESULTS

Venom doses used for neutralisation of the antivenom are shown in Table 1.

Results of venom neutralising dose of the antivenom with venoms from 5 different divisions of Myanmar are shown in Table 2. It is observed that in general 2 to 8 times more antivenom is needed to neutralise different biological properties of the venoms of Upper Myanmar (Magwe and Mandalay) compared to Lower Myanmar (Tharawaddy, Kungyankone and Daedaye). Forty times more antivenom is needed to neutralise procoagulant activity of the venom of Taungdwingyi (Magwe) compared to the Daedaye (Ayerawaddy). However, it is equally effective in neutralising procoagulant activity of the venom of Wundwin (Mandalay). It was observed that two to four times more antivenom is needed to neutralise lethal and defibrinogenating and 2-4 times less for necrotic and capillary permeability increasing activities of the venom of Kungyankone (Yangon) in comparing venom neutralising efficacy of the antivenom among the venoms of Kungyankone (Yangon), Tharawaddy (Bago) and Daedaye (Ayera-waddy) (Lower Myanmar).

DISCUSSION

The study highlights that more antivenom are needed to neutralise biological activities of the venoms of Upper Myanmar (Taung-dwingyi and Wundwin) compared to Lower Myanmar (Tharawaddy, Kungyankone and Daedaye). It has been reported that more antivenom were needed to neutralise

Table 1. Venom doses used for venom-antivenom neutralisation test

Source of venom	$5LD_{50}$iv µg/mouse	10MCD µg/ml	3MHD µg/rat	3MND µg/rat	5MDD µg/mouse	100MCPID µg
Mandalay Division Wundwin	16.9	31.62	135.2	112.5	15.0	0.295
Magwe Division Taungdwingyi	19.9	50.12	92.7	97.1	6.0	0.832
Bago Division Tharawaddy	23.4	25.12	126.5	119.4	12.0	0.724
Yangon Division Kungyankone	30.2	31.25	79.0	70.3	25.5	0.115
Ayerawaddy Division Daedaye	14.3	1.35	84.5	83.6	8.9	0.892

LD_{50}.iv=Median lethal dose
MCD = Minimum coagulant dose
MHD = Minimum haemorrhagic dose
MND = Minimum necrotic dose
MDD = Minimum defibrinogenating dose
MCPID= Minimum capillary permeability increasing dose

Table 2. Amount of antivenom required for neutralising biological properties of venoms from different localities of Myanmar

Source of venom	5LD $_{50}$iv µl	10MCD µl	3 MHD µl	3 MND µl	5 MDD µl	100 MCPID µl
Magwe Division Taungdwingyi	10	2.5	20	20	1.25	20
Mandalay Division Wundwin	10	0.078	20	20	1.25	20
Yangon Division Kungyankone	5.0	0.0625	0.625	2.5	2.5	1.25
Bago Division Tharawaddy	2.5	0.0625	2.5	2.5	0.625	2.5
Ayerawaddy Division Daedaye	2.5	0.0625	2.5	2.5	0.625	2.5

biological activities of Taungdwingyi venom compared to Daedaye [12]. Since venoms used for raising antivenom for common use is mainly derived from pooled venoms of lower Myanmar, a better neutralisation with the venoms from Lower Myanmar was observed in this study. The fect that antivenom raised with local venom is good for use in that locality was observed by Phillips et al (1988) [13] where Indian Haffkine antivenom failed to correct coagulation defect produced by bites of Ceylonese Russell's viper (*V. russelii puchella*) and up to 530ml of the antivenom was proved to be necessary for restoring haemostatic defect. Jayanthi and Gowda (1988) [14] indicated that there was lack of protection afforded in Russell's viper bite victims from southern India when given Haffkine antivenom. All observations called for use of antivenom raised with local venoms, or local antivenom raised with venoms from Upper or Lower Myanmar, if possible, or common antivenom raised with widely pooled venoms from different localities since there is geographical variation in composition of venoms [15].

REFERENCES

1. Tun-Pe, Nu-Nu-Lwin, Aye-Aye-Myint and Khin-Aung-Cho. Geographical variation of biological properties of Russell's viper venom: A preliminary report. *Myanmar Health Sciences Research Journal* 1993; 5: 107-112.
2. Aye-Aye-Myint, Tun-Pe, Kyi-May-Htwe and Khin-Aung-Cho. Variation in biological properties of venoms from different localities of Ayerawaddy division. *Myanmar Health Sciences Research Journal* 1993; 5: 121-124.
3. Aye-Aye-Myint, Tun-Pe, Kyi-May-Htwe, Khin-Aung-Cho and Theingie. Geographical variation of biological properties of Russell's viper (*Daboia russelii siamensis*) venom: Yangon division. *Myanmar Health Sciences Research Journal* 1994; 6: 87-89.
4. Aye-Aye-Myint, Tun-Pe, Kyi-May-Htwe, Khin-Aung-Cho and Theingie. Geographical variation of biological properties of Russell's viper venom: Bago division. *Myanmar Health Sciences Research Journal* 1995; 7: 92-94.
5. Sann-Mya, Tun-Pe, Aye-Aye-Myint, Kyi-May-Htwe, Khin-Aung- Cho and Theingie. Geographical variation of biological properties of Russell's viper venom: Magway division. *Myanmar Medical Journal* 1994; 39: 7-9.
6. Tun Pe, Sann Mya, Aye Aye Myint, Kyi May Htwe, Khin Aung Cho and Theingie. Biological properties of Russell's viper venom of upper and lower Myanmar, *Myanmar Medical Journal* 1994; 39: 1-6.
7. Myint-Lwin, Warrell, D.A., Phillips, R.E.,Tin-Nu-Swe, Tun-Pe, and Maung-Maung-Lay (1985). Bites by Russell's viper (*V. russelii siamensis*) in Burma: haemostatic, vascular and renal disturbances and response to treatment. *Lancet* 1985; ii: 1259-1264.
8. Sann-Mya, Tun-Pe, Aye-Aye-Myint, Nu-Nu-Aung, Khin-Aye- Kyu and Tin-Oo. Russell's viper bites in Taungdwingyi hospital: clinical features and response to antivenom. *Myanmar Health Sciences Research Journal* 1998; 10: 26-30.
9. Kyaw-Than, Tun-Pe, Aye-Aye-Myint, and Nu-Nu-Aung. Clinical features and response to antivenom of Russell's viper bite cases of

Danuphyu, Ayerawaddy division. *Myanmar Health Sciences Research Journal* 1997; 9: 93-96.

10. Tun-Pe, Aye-Aye-Myint, Sann-Mya, Khin-Aye-Kyu, Kyaw-Than, Aung-Myint, Tint-Lwin, Myint-Soe and Min-Than. A study of Russell's viper (*Daboia russelii siamensis*) bites from six township hospitals. Myanmar Health Research Congress 2000b abstract, p74.
11. Theakston, R.D.G. and Reid, H.A. The development of simple standard assay procedures for characterisation of snake venoms. *Bull. W.H.O* 1983; 61: 949-956
12. Tun-Pe, Sann-Mya, Aye-Aye-Myint, Kyi-May-Htwe, Khin-Aung-Cho and Theingie. Biological properties of Russell's viper venom of upper and lower *Myanmar*. *Myanmar Medical Journal* 1994; 39: 1-6.
13. Phillips, R.E., Theakston, R.D.G., Warrell, D.A., Galigedara, Y., Abeysekera, DTDJ. Dissanayaka, P., Hutton, R.A., and Aloysius, D.J. Paralysis, rabdomyolysis and haemolysis caused by bites of Russell's viper (*Vipera russelii puchella*) in Srilanka: failure of Indian (Haffkine) antivenom. *Quartly. J. Med* 1988 (New series, 68); 257: 691-716.
14. Jayanthi, G.P. and Gowda, T.V. Geographical variation in India in the composition and lethality potency of Russell's viper (*Vipera russelii*) venom. *Toxicon* 1988, 26: 257-264
15. Tun-Pe, Aye-Aye-Myint, Kyi-May-Htwe, Khin-Aung-Cho, and Theingie. Geographical variation of biological properties of Russell's viper (*Daboia russelii siamensis*) venom. Myanmar Health Research Congress abstract 2000a, p.75.