Toxicon, Vol. 17, pp. 661–664.

0041-0101/79/1101-0661$02.00/0

THE LETHALITY IN MICE OF DANGEROUS AUSTRALIAN AND OTHER SNAKE VENOM

A. J. BROAD, S. K. SUTHERLAND and A. R. COULTER

Department of Immunology Research, Commonwealth Serum Laboratories, Parkville, Australia, 3052

(*Accepted for publication* 15 *May* 1979)

COVACEVICH and WOMBEY (1976) showed that the small-scaled snake (*Parademansia microlepidotus*) was taxonomically distinct and geographically removed from the taipan (*Oxyuranus scutellatus*). BROAD *et al.* (in press) demonstrated by two-directional polyacrylamide gel electrophoresis that venom from the two snakes had characteristically distinct electrophoretograms. Toxicity studies with venom from *P. microlepidotus* showed it was considerably more toxic than any other Australian snake venom (SUTHERLAND *et al.*, 1978; BROAD *et al.*, in press). It was decided therefore to determine the exact toxicity status of *P. microlepidotus* venom relative to other toxic terrestrial snake venoms. A literature survey could not locate a comprehensive and statistically sound study of the toxicities of the major Australian elapid venoms. The work of BAXTER and GALLICHIO (1976) on tiger snake (*Notechis scutatus*) venom was reproducible. Unfortunately, most other studies merely reinforced the views expressed by RUSSELL (1966) that little uniformity exists between laboratories when parameters for toxicity studies are being selected. An exhaustive toxicity study was therefore commenced on 25 snake venoms including venoms from some foreign snakes. The whole comparative study was conducted in one laboratory with rigorously controlled conditions.

Desiccated Australian snake venoms were obtained from Mr. Eric Worrell of Gosford, Australia and Mr. Charles Tanner of Cooktown, Australia. Some of the rarer venoms which had been stored for up to 40 years were obtained from the Commonwealth Serum Laboratories central venom collection. The older venoms were checked for lethality, if they were found to possess the same lethality as venom of more recent origin, it was considered valid to conduct studies on the older large pools. Foreign venoms were either obtained from the Commonwealth Serum Laboratories collection or from snakes kept by Mr. Worrell.

LD_{50} determinations were made using stock venom solutions of 5 mg/ml prepared in 0·85% saline and stored at —20°C. Portions of the stock solutions were thawed and diluted with 0·85% saline or 0·1% bovine serum albumin (Commonwealth Serum Laboratories) in 0·85% saline prior to each toxicity determination. Five dose levels were generally used at a dilution interval of 1:1·25. Commonwealth Serum Laboratories white Swiss mice of either sex in the weight range 18–21 g were used in the assay. Four mice were used at each dose level. Each dose was given subcutaneously (s.c.) into the flank in a volume of 0·2 ml. Results were read after 48 hr. About 5000 mice were required for the study. The LD_{50} values of the venoms were calculated using the Spearman–Kärber method (FINNEY, 1964).

TABLE 1. SNAKE VENOM LD_{50} DETERMINATIONS IN MICE (18–21 g) BY SUBCUTANEOUS INJECTION

Snake (common name in parenthesis)	LD_{50} mg/kg (95% confidence (limits)) Saline	0·1% bovine serum albumin in saline
Parademansia microlepidotus (Small-scaled snake)	0·025 (0·020–0·029)	0·010 (0·007–0·014)
Pseudonaja textilis (Brown snake)	0·053 (0·041–0·065)	0·041 (0·033–0·051)
Oxyuranus scutellatus (Taipan)	0·099 (0·081–0·123)	0·064 (0·052–0·078)
Notechis scutatus (Tiger snake)	0·118 (0·095–0·146)	0·118 (0·088–0·157)
Notechis ater niger (Reevesby Island Tiger snake)	0·131 (0·107–0·163)	0·099 (0·083–0·120)
Enhydrina schistosa (Beaked sea snake)	0·164 (0·149–0·185)	0·173 (0·144–0·210)
Notechis ater occidentalis (Western Australian Tiger snake)	0·194 (0·161–0·234)	0·124 (0·102–0·152)
Notechis ater serventyi (Chappell Island Tiger snake)	0·338 (0·278–0·414)	0·271 (0·220–0·335)
Acanthophis antarcticus (Death adder)	0·400 (0·336–0·472)	0·338 (0·278–0·413)
Pseudonaja nuchalis (Gwardar)	0·473 (0·393–0·570)	0·338 (0·271–0·423)
Austrelaps superba, Australia (Australian Copperhead)	0·560 (0·448–0·700)	0·500 (0·415–0·605)
Naja naja (Indian Cobra)	0·565 (0·450–0·705)	0·500 (0·415–0·605)
Pseudonaja affinis (Dugite)	0·660 (0·550–0·800)	0·560 (0·505–0·620)
Pseudechis papuanus (Papuan black snake)	1·09 (0·865–1·35)	1·36 (1·23–1·51)
Hoplocephalus stephensii (Yellow banded snake)	1·36 (1·12–1·66)	1·44 (1·27–1·63)
Tropidechis carinatus (Rough scaled snake)	1·36 (1·19–1·56)	1·09 (0·980–1·21)
Ophiophagus hannah (King Cobra)	1·80 (1·50–2·18)	1·91 (1·55–2·37)
Pseudechis guttatus (Blue-Bellied black snake)	2·13 (1·79–2·51)	1·53 (1·24–1·89)
Pseudechis colletti (Collett's snake)	2·38 (2·08–2·74)	Not done
Pseudechis australis (King brown snake)	2·38 (1·93–2·92)	1·91 (1·57–2·33)
Pseudechis porphyriacus (Red-Bellied black snake)	2·52 (2·09–3·04)	2.53 (2·05–3·14)
Cryptophis nigrescens (Small-Eyed snake)	2·67 (2·40–2·96)	Not done
Crotalus adamanteus (Eastern Diamond-Back Rattlesnake)	11·4 (9·10–14·25)	7·70 (6·30–9·40)
Demansia olivacea (Spotted snake)	>14·2	Not done
Bothrops atrox (Barba amarilla)	>27·8	Not done

Venoms are arranged in order of decreasing lethal potency in Table 1. The data in Table 2 were obtained from a literature survey. The remarkable potency of the Australian elapid venoms is clearly confirmed by this study. Eleven of these venoms are more toxic than the Indian cobra (*Naja naja*) and 20 are more toxic than the eastern diamond-back rattlesnake (*Crotalus adamanteus*). Considering that the average yield of venom upon milking for *O. scutellatus* is 120 mg and is 35 mg for *N. scutatus*, it is not surprising that

TABLE 2. TOTAL MOUSE LD_{50} DOSES PRESENT IN THE AVERAGE AND MAXIMUM YIELDS OBTAINED BY MILKING SNAKES*

Species of snake	Average venom yield (mg)	Average venom yield Total LD_{50} doses	Maximum venom yield (mg)	Maximum venom yield Total LD_{50} doses
P. microlepidotus	44 (BROAD *et al.*, in press)	217,821	110 (BROAD *et al.*, in press)	544,554
O. scutellatus	120 (CAMPBELL, 1967)	94,488	400 (CAMPBELL, 1967)	314,961
P. textilis	2 (WORRELL, 1970)	2469	67 (KELLAWAY, 1931)	82,716
N. scutatus	35 (WORRELL, 1970)	14,893	189 (FREEMAN and KELLAWAY, 1934)	80,426
N. ater serventyi	75 (WORRELL, 1970)	13,838	388 (KELLAWAY and THOMSON, 1932)	71,587
N. naja	169 (DEORAS, 1971)	16,900	610 (MINTON and MINTON, 1969)	61,000
A. antarcticus	78 (FAIRLEY and SPLATT, 1929)	11,538	236 (FAIRLEY and SPLATT, 1929)	34,911
O. hannah	421 (GANTHAVORN, 1969)	11,050	Not known (MINTON, 1974 suggests 500)	13,123
C. adamanteus	410 (BROWN, 1973)	2662	848 (BROWN, 1973)	5,505

*Figures based on LD_{50} results obtained with 0·1% bovine serum albumin in saline dilutions. Dose was administered by s.c. route into 18–21 g mice. Values from Table 1 are used for calculation of total LD_{50} doses.

in the days before antivenom, the mortality rate in humans following a taipan bite was likely to be 100% and after a tiger snake bite was 45%. There has been only one proven case of envenomation by *P. microlepidotus* and this patient was critically ill for weeks (SUTHERLAND *et al.*, 1978). This snake inhabits an area with little human settlement and case reports of bites are rare.

The decision to use 0·1% bovine serum albumin in saline as diluent was made after experience with toxicity studies on a potent neurotoxin isolated from *Pseudonaja textilis*. When saline was used as diluent with this toxin inconsistent results were obtained. The dilute solutions required in the assay of this toxin (0·5 μg/ml) meant that substantial losses could occur by adsorption to glass tubes, plastic syringes, etc. When 0·1% bovine serum albumin was used in place of saline, more consistent results were obtained as well as up to a six-fold increase in the measured lethality. The LD_{50} s.c. in 18–21 g mice was 7·0 μg/kg when saline was used as a diluent. When 0·1% bovine serum albumin in saline was employed the LD_{50} s.c. was 2·5 μg/kg. If the i.v. route was used the values obtained were 3·0 μg/kg in saline vs 0·5 μg/kg in 0·1% bovine serum albumin in saline. Since adsorption takes place with this toxin, it might also occur with very dilute crude venom solutions. This was found to be the case (Table 1).

Acknowledgements—The authors are indebted to Mr. ALWYN MATHEWS for helpful discussions and advice. The excellent technical assistance of Mrs. MIRELLA BARBARO and Mrs. ANN BOSSIO is greatly appreciated.

REFERENCES

BAXTER, E. H. and GALLICHIO, H. A. (1976) Protection against sea snake envenomation: comparative potency of four antivenenes. *Toxicon* **14**, 347.

BROAD, A. J., SUTHERLAND, S. K., TANNER, C. and COVACEVICH, J. (In press.) Electrophoretic, enzyme and preliminary toxicity studies of the venom of *Parademansia microlepidotus* (the small-scaled snake) with additional data on its distribution (Serpentes: Elapidae). *Mem. Qd Mus.*

BROWN, J. H. (1973) Toxicity and variation in yields of venoms from poisonous snakes. In: *Toxicology and Pharmacology of Venoms from Poisonous Snakes*. Springfield: Charles C. Thomas.

CAMPBELL, C. H. (1967) The Taipan (*Oxyuranus scutellatus*) and the effects of its bite. *Med. J. Aust.* **1**, 735.
COVACEVICH, J. and WOMBEY, J. (1976) Recognition of *Parademansia microlepidotus* McCoy (Elapidae), a dangerous Australian snake. *Proc. R. Soc. Qd* **87**, 29.
DEORAS, P. J. (1971) The story of some Indian poisonous snakes. In: *Venomous Animals and Their Venoms*, (BUCHERL, W. and BUCKLEY, E. E., Eds.). New York: Academic Press.
FAIRLEY, N. H. and SPLATT, B. (1929) Venom yields in Australian poisonous snakes. *Med. J. Aust.* **1**, 336.
FINNEY, D. J. (1964) *Statistical Method in Biological Assay*, 2nd Edn., p. 524. London: Griffin.
FREEMAN, M. and KELLAWAY, C. H. (1934) The venom yields of common Australian poisonous snakes in captivity. *Med. J. Aust.* **2**, 190.
GANTHAVORN, S. (1969) Toxicities of Thailand snake venoms and neutralization capacity of antivenene. *Toxicon* **7**, 239.
KELLAWAY, C. H. (1931) Observations on the certainly lethal dose of venom of the common brown snake (*Demansia textilis*) for the common laboratory animals. *Med. J. Aust.* **2**, 747.
KELLAWAY, C. H. and THOMSON, D. F. (1932) Observations on the venom of a melanotic insular variety of the tiger snake (*Notechis scutatus*). *Aust. J. exp. Biol. med. Sci.* **19**, 35.
MINTON, S. A. and MINTON, M. J. (1969) Properties and composition of venoms. In: *Venomous Reptiles*, p. 34. London: George Allen & Unwin Ltd.
MINTON, S. A. (1974) Snakes and snake venoms. In: *Venom Diseases*. Springfield: Charles C. Thomas.
RUSSELL, F. E. (1966) To be, or not to be . . . the LD_{50}. *Toxicon* **4**, 81.
SUTHERLAND, S. K., BROAD, A. J., TANNER, C. and COVACEVICH, J. (1978) Australia's potentially most venomous snake: *Parademansia microlepidotus*. *Med. J. Aust.* **1**, 288.
WORRELL, E. (1970) *Reptiles of Australia*. Sydney: Angus & Robertson.

Toxicon, Vol. 17, pp. 664–667.

0041-0101/79/1101-0664$02.00/0

DISRUPTION OF ELECTROCARDIOGRAPHIC ACTIVITY BY STREPTOLYSIN O IN RATS

SUSHMA GUPTA and R. K. GUPTA

Departments of Physiology and Pathology, Maharani Laxmi Bai Medical College, Jhansi-284001, India

(*Accepted for publication* 29 *May* 1979)

RHEUMATIC fever is a post-streptococcal sequel whose pathogenesis is not well known. Streptolysin O, a potent streptococcal exotoxin produces cardiac damage (GUPTA *et al.*, 1973; GUPTA *et al.*, 1976*a*; HALBERT *et al.*, 1961) and derangement of cardiac activity in experimental animals (GUPTA *et al.*, 1976*b*, 1976*c*, 1976*d*; GUPTA and GUPTA, 1977; SPIRA *et al.*, 1968). Streptolysin O has also been shown to be toxic to mammalian heart cells in tissue culture (THOMPSON *et al.*, 1970). In the present study the effect of streptolysin O on the electrocardiogram (ECG) of rats has been investigated.

The experiments were performed on 52 Swiss albino rats of either sex weighing 150 ± 20g and anaesthetized with i.p. pentobarbitone sodium (35 mg/kg). Half an hour after induction of anaesthesia electrocardiograms were recorded using needle electrodes. The effect