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Failure of 3,4-diaminopyridine and edrophonium to produce significant clinical benefit in neurotoxicity following the bite of Papuan taipan (*Oxyuranus scutellatus canni*)

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Abstract

Progressive systemic neurotoxicity is a common feature in patients envenomed following the bite of a Papuan taipan (*Oxyuranus scutellatus canni*). Respiratory paralysis, which commonly results, accounts for considerable morbidity and mortality. Established neurotoxicity does not respond to antivenom. In this study, a combination of clinical and electrophysiological variables was used to assess the effect of edrophonium and 3,4-diaminopyridine in patients with significant neurotoxicity. Both drugs produced minor electrophysiological and clinical changes in envenomed patients. This effect was maximal when the 2 drugs were used in combination, but was insufficient to be of significant clinical benefit. Neither drug can be recommended for use in the management of Papuan taipan bite.

Keywords: snake bite, *Oxyuranus scutellatus canni*, treatment

Introduction

Taipan bite is a considerable problem in the Central Province of Papua New Guinea (TREVETT *et al.*, 1995). Clinical signs of toxicity include ptosis, ophthalmoplegia, restriction in opening the mouth, and paralysis of bulbar, respiratory and peripheral muscles. Specific antivenom may be effective if given early in envenoming but frequently fails to prevent progression to respiratory paralysis. There is a need for a safe intervention in envenomed patients to prevent the requirement for intubation and ventilation or to extend the period before it is required. Options include improving the use of existing antivenoms by giving them earlier or perhaps in larger doses, the development of more specific subspecies or sub-unit antivenoms, or the use of neuroactive drugs to improve neuromuscular transmission.

Anticholinesterases have been shown to be of significant benefit in patients envenomed by snakes with predominantly post-synaptic neurotoxins (WARRELL *et al.*, 1983; WATT *et al.*, 1986; CURRIE *et al.*, 1988; HUDSON, 1988), but have not been shown to be of benefit in ameliorating the effects of pre-synaptic neurotoxins. 3,4-Diaminopyridine (3,4-DAP) improves respiratory function in rabbits poisoned with a presynaptic neurotoxin, β-bungarotoxin, and with whole krait venom (WATT *et al.*, 1994), but has not previously been used in the victims of snake bite. 3,4-DAP, which is available in both oral and intravenous preparations, acts by blocking voltage-dependent potassium channels in the region of the nerve terminal, prolonging the opening of calcium channels. The increased concentration of calcium ions in the presynaptic terminal augments acetylcholine release. The drug has been used in the treatment of various disorders of synaptic transmission (HARVEY, 1988). The action of diaminopyridines is augmented by co-administration of an anticholinesterase (TIERNEY *et al.*, 1985).

Patients and Methods

Patients

Inclusion criteria for the trial were the same as those described by TREVETT *et al.* (1995). The degree of neurotoxicity at which active drugs were given varied from a minimum of ptosis and partial ophthalmoplegia to severe envenoming requiring intubation and mechanical ventilation. Drugs were given only to patients whose clinical condition was deteriorating or who were being ventilated. Several patients received more than one dose of active drug or placebo at different stages of their deterioration, a minimum of 6 h apart. No patient received more than 3 doses. The rate of progression of neurotoxicity varied considerably between patients. For comparative purposes a simple clinical grading system was used, as follows. Stage 1: envenomed, no neurotoxicity; stage 2: ptosis with or without partial ophthalmoplegia; stage 3: ophthalmoplegia and bulbar paralysis; stage 4: bulbar paralysis of sufficient severity to require intubation; stage 5: intubated and ventilated.

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Treatment

Patients received one of the following treatment schedules. (i) Atropine 0·6 mg intravenously followed by a trial dose of 0·4 mL of either 10 mg edrophonium (in 2 mL) or saline by slow intravenous injection. The remaining 1·6 mL of edrophonium solution were given after 30 sec. (ii) Atropine 0·6 mg intravenously followed by either 10 mg 3,4-DAP dissolved in 10 mL saline or 10 mL saline by slow intravenous injection. (iii) Atropine 0·6 mg intravenously followed by 10 mg 3,4-DAP followed by 2 mg of edrophonium at 7 min and 8 mg at 7·5 min.

3,4-DAP (Small Scale Pharmaceuticals, Brighton, UK) was prepared for injection by dissolving 10 mg in 10 mL sterile saline under aseptic conditions. This was then filtered using a sterile micropore filter. Following injection of active drug or placebo through an intravenous cannula, 50 mL of normal saline were given by intravenous drip. Schedules (i) and (ii) were both 'double blind'. Equal volumes of active drugs and placebo were drawn into identical syringes and labelled according to a code by N.C.N. This code was not revealed to the principal investigator (A.J.T.) until after each study. With schedule (iii) only active drugs were given.

Assessment of effect

Clinical and electrophysiological variables were used to assess the effect of the drugs given. Electrophysiological recordings were made using the methods described by TREVETT *et al.* (1995).

The following variables were recorded: (i) Degree of ptosis (mm of iris uncovered); (ii) degree of limitation of lateral gaze; (iii) maximum extent of mouth opening (inter-incisor distance), in non-intubated patients only; (iv) the size of an evoked compound muscle action potential (CMAP) recorded from abductor digiti minimi after supramaximal stimulation of the ulnar nerve at the wrist; and (v) peripheral grip strength, calculated as an average of 3 readings using a hand-held dynamometer (Lafayette, Indiana, USA).

With schedule (i), recordings and observations were made at time 0 (before administration) and after 4, 9, 14, 19 and 24 min. A second injection, of either active drug or placebo by cross-over, was given at 30 min and the same procedure followed. With schedule (ii), recordings

were taken at time 0, 5, 10, 15, 30 and 45 min. A cross-over technique was not employed because of the long half-life of 3,4-DAP. With schedule (iii), observations were made at 0, 5, 10, 15, 30 and 45 min. In all patients, evoked CMAP amplitudes were recorded before grip strength readings to exclude post-exercise facilitation. Three CMAP amplitudes were recorded at least 5 sec apart and the maximum evoked CMAP amplitude was recorded. The percentage change in CMAP amplitude from that recorded at time zero was calculated. It had been hoped to record forced expiratory flow rates and volumes in non-ventilated patients. This proved impractical because patients with severe neurotoxicity had weakness of facial muscles and were unable to form a tight seal around the mouthpiece of a flow meter. The recording of sequential maximum inspiratory pressures in ventilated patients was attempted but it was poorly tolerated by patients and consequently abandoned.

Informed consent was obtained before each study. The trial had the approval of the national ethics committee of Papua New Guinea.

Side effects

Each patient was asked about any side effects which they had experienced, either immediately after the study or after extubation if they had been intubated; these were recorded. Blood pressure and pulse rates were measured sequentially in each study.

Statistics

All results were entered into data files prepared using the Epi-Info package (USD, Atlanta, Georgia, USA) and the Lotus R123® spreadsheet and analysed using the Instat® Biostatistics programme (GraphPad® Software). Numerical data were converted into a percentage change from time zero and the significance of observed differences compared using the paired or unpaired *t* test as appropriate.

Results

Detailed results are shown in the Table. Figures refer to the mean percentage change from values recorded at time zero.

Edrophonium

Eleven patients received the active drug and 11 received the placebo. There was a marginal and unsustained increase in the lateral eye movements in 3 patients, evident at 4 and 9 min. All 3 patients had received active drug. No difference was detected in the degree of ptosis, ophthalmoplegia or mouth opening in any other patient. No side effect was noticed during the course of the studies or subsequently reported.

3,4-Diaminopyridine

Twelve patients received the active drug and 6 received the placebo. One patient, who had received 3,4-DAP, showed a brief increase in lateral eye movements at 10 and 15 min. There was no measurable change in the degree of ptosis or mouth opening. There was no change in any of the other 17 patients. Two patients had a noticeable increase in oral secretions and sweating. Widespread muscle fasciculation was seen in one patient, predominantly in deltoids, pectoral muscles and gastocnemii. Five patients subsequently reported discomfort along the line of the vein after infusion of the drug and 3 reported hot flushes and transient perioral and peripheral paraesthesia. All these patients had received active drug.

3,4-Diaminopyridine and edrophonium

Measurements of CMAP amplitude were made on 10 patients, and grip strength on 9 patients, who were given 3,4-DAP at time zero followed by edrophonium 7 min later. As there was no control in this section of the trial, values were compared with those of the 6 patients who received placebo in schedule (ii). The results of the grip strength measurements from one patient, who showed an increase of over 400%, are excluded from the mean.

An improvement in lateral eye movements was recorded in one patient between 10 and 30 min. Ptosis lessened in severity in another patient between 10 and 45 min. One patient showed an improvement in mouth opening from a baseline of 22 mm to a maximum of 31 mm, but no more than 3 mm variation was noted in any other patient. All these 3 patients also showed an increase in grip strength. There was a marked increase in oral secretions requiring additional suction of the endotracheal tube or pharynx in 6 of the 10 patients, all of whom also had a visible increase in sweating. Muscle fasciculation was noticed in 5 patients. Five patients subsequently reported transient discomfort along the line of the vein through which drugs had been given. Three patients reported perioral paraesthesia.

Comparison of 3,4 diaminopyridine plus edrophonium with 3,4-diaminopyridine alone

Mean and median increases in both CMAP amplitude

Table. Compound muscle action potential (CMAP) amplitude and grip strength of envenomed patients after treatment with edrophonium and/or 3,4-diaminopyridine

Time after treatment (min)	Mean CMAP amplitude[a]			Mean grip strength[a]		
	Active drug	Placebo	*P*	Active drug	Placebo	*P*
Edrophonium (10 mg)						
4	9·0 (8)	−0·5 (0)	0·001	5·0 (0)	0·1 (0)	0·8
9	17·7 (15)	−0·5 (0)	0·001	2·8 (0)	−1·6 (0)	0·9
14	12·1 (5)	−0·5 (0)	0·004	6·9 (4)	−5·8 (0)	0·04
19	10·7 (8)	−0·4 (0)	0·001	2·0 (0)	−6·4 (0)	0·26
24	7·7 (4)	−1·0 (0)	0·005	2·2 (0)	−6·4 (0)	0·22
3,4-Diaminopyridine (10 mg)						
5	2·8 (3·5)	−0·8 (0)	0·28	5·5 (3)	3 (0)	0·58
10	5·4 (5)	−3·7 (−4)	0·01	10·6 (0)	4·6 (0)	0·43
15	6·5 (5)	−3·7 (−3·5)	0·005	12·1 (8)	4·6 (8)	0·09
30	6·0 (9)	−4·7 (−5·5	0·07	13·1 (6)	5·4 (6)	0·44
45	2·0 (4)	−4·4 (−4)	0·04	10·6 (2)	−0·8 (0)	0·31
Edrophonium (10 mg) plus 3,4-diaminopyridine (10 mg)						
5	4·6 (4)	−0·8 (0)	0·13	5·5 (1)	−0·8 (0)	0·94
10	10·2 (5)	−3·7 (−4)	0·05	33·8 (22·5)	−3·7 (−4)	0·01
15	19·3 (9)	−3·3 (−3)	0·002	38·8 (27·5)	−3·3 (−3)	0·01
20	16·8 (13)	−3·6 (−3)	0·013	39·5 (22·5)	−3·6 (−3)	0·01
30	17·6 (13)	−4·7 (−5)	0·002	28·5 (21·5)	−5·1 (−7)	0·04
45	15·9 (13)	−3·8 (−4)	0·04	20·0 (10)	−3·7 (−3)	0·03

[a]Values expressed as percentage increase after treatment (median values in parentheses).

and grip strength were larger in patients who received both drugs but this difference reached significance only in grip strength measurements at 15 min ($P<0{\cdot}02$).

Response according to stage of neurotoxicity

There was no evidence of an association between the stage of neurotoxicity or the time elapsed after envenoming and the extent of the CMAP amplitude response. This was also true of the grip strength measurements in patients in stages 2–4 and in those at stage 5 with a measurable grip. Eight patients who had stage 5 neurotoxicity with particularly profound weakness (no measurable grip strength) did not show any improvement, despite 4 receiving active drugs.

Discussion

Edrophonium, 3,4-DAP and both drugs in combination all produced a measurable increase in CMAP amplitude in most patients. Most patients who were given edrophonium or 3,4-DAP also showed a small increase in grip strength but this was significantly different from control groups only in patients given both drugs together. The onset and time course of changes in both variables was consistent with a response to the drug given. There was little evidence of any sustained improvement in ophthalmic signs of neurotoxicity. In the majority of patients who showed a response to an active drug, increases in CMAP amplitude and recorded grip strength correlated. The amplitude response to active drugs appeared to be proportional to the baseline CMAP amplitude at time zero, irrespective of the stage of neurotoxicity. This also appeared to be true of grip strength measurements except that the 8 patients at stage 5 with the most profound weakness (and lowest zero time CMAP amplitudes, typically of the order of 1 mV) had no measurable grip strength either before or after active drugs (4) and placebo (4). These observations suggest that 3,4-DAP may augment surviving transmitter release but is unlikely to overcome the effect of bound neurotoxin.

A number of criticisms can be levelled at the design of this trial. The numbers of patients in each group were small and the variation in responses recorded was wide. The time course and severity of envenoming varied considerably between patients and, as with any study of interventions in snake bite, direct comparisons are fraught with difficulty. The most important effect that an adjunctive therapy could produce would be to improve respiratory function and this is the ideal variable to assess. Unfortunately, it did not prove possible to measure respiratory function objectively for the reasons given above. The measurement of peripheral grip strength was used as the most practical and objective alternative available. Both schedules (i) and (ii) were planned as 'double blind' studies. With schedule (ii), however, several patients who received 3,4-DAP had a visible response to the drug with increased oral secretions and sweating and it was impossible to 'blind' the principal investigator to this effect. No attempt was made to use placebo groups with schedule (iii). Studies looking at the effect of higher doses of 3,4-DAP were precluded by the frequency and severity of side effects.

Despite the limitations of the study, it answers the questions we set out to resolve. Both edrophonium and 3,4-DAP produced a measurable electrophysiological and clinical response in most patients with neurotoxicity secondary to taipan envenoming. Even when both drugs were used in combination, however, the clinical response was not large enough in any patient to be of significant benefit. The stimulation of oral secretions, only partially blocked by prior administration of atropine, would also be potentially dangerous in patients with paralysis of pharyngeal musculature who could neither be given adequate suction nor intubated until they arrived at hospital. For both reasons, we cannot recommend the use of either edrophonium or 3,4-DAP in the management of victims of taipan bite. The need to find a better way of treating or preventing neurotoxicity following Papuan taipan bite remains.

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References

Currie, B., Fitzmaurice, M. & Oakley, J. (1988). Resolution of neurotoxicity with anticholinesterase therapy in death-adder envenomation. *Medical Journal of Australia*, **148**, 522–525.

Harvey, A. L. (1988). 4-Aminopyridine type of antagonism. Peripheral and central effects. In: *Antagonists in Anaesthesia and Intensive Care*, Lawin, P., Van Aken, H. & Mollmann, M. (editors). Stuttgart & New York. Georg Thieme pp. 20–32.

Hudson, B. J. (1988). Positive response to edrophonium in death adder (*Acanthopis antarcticus*) envenomation. *Australia and New Zealand Journal of Medicine*, **18**, 792–794.

Tierney, P. C., Kim, Y. I. & Johns, T. R. (1985). Synergistic interaction of 4-aminopyridine with neostigmine at the neuromuscular junction. *European Journal of Pharmacology*, **115**, 241–247.

Trevett, A. J., Lalloo, D. G., Nwokolo, N. C., Naraqi, S., Kevau, I. H., Theakston, R. D. G. & Warrell, D. A. (1995). Electrophysiological findings in patients envenomed following the bite of a Papuan taipan (*Oxyuranus scutellatus canni*). *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **89**,

Warrell, D. A., Looareesuwan, S., White, N. J., Theakston, R. D. G., Warrell, M. J., Kosakarn, W. & Reid, H. J. (1983). Severe neurotoxic envenoming by the Malayan krait *Bungarus candidus* (Linnaeus): response to antivenom and anticholinesterase. *British Medical Journal*, **286**, 678–680.

Watt, G., Theakston, R. D. G., Hayes, C. G., Yambao, M. L., Sangalang, R., Ranoa, C. P., Alquizalas, E. & Warrell, D. A. (1986). Positive response to edrophonium in patients with neurotoxic envenoming by cobras (*Naja naja philippinensis*). *New England Journal of Medicine*, **315**, 1444–1447.

Watt, G., Smith, C. D., Kaewsupo, A. & Davis, T. M. E. (1994). 3,4-Diaminopyridine reverses respiratory paralysis induced by a presynaptically active snake venom and its major neurotoxin. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **88**, 243–246.

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