

NEOSTIGMINE IN THE TREATMENT OF SNAKE ACCIDENTS CAUSED BY *MICRURUS FRONTALIS*: REPORT OF TWO CASES (1)

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SUMMARY

Antivenom in order to be effective in the treatment of coral snake accidents must be injected very soon after the bite owing to the rapid rate of absorption of the venom neurotoxins. As this is not always possible, other forms of treatment besides serotherapy must be employed to avoid asphyxia and death. Neostigmine and artificial respiration are used for this purpose. Neostigmine restores neuromuscular transmission if the venom-induced blockade results from a reversible interaction of its neurotoxins with the end-plate receptors. This is the mechanism of the neuromuscular blockade produced by the venom of *M. frontalis* snakes from centereastern and southern Brazil, and Argentine. Neostigmine is able, therefore, to antagonize the blockade, and has been shown to be very effective in the treatment of the experimental envenomation of dogs and monkeys. In the present communication, two cases of *M. frontalis* accidents treated with antivenom and neostigmine are reported. In both, neostigmine was successful in producing regression of the paralysis, confirming the effectiveness shown in the treatment of the poisoning induced in animals by *M. frontalis* venom.

KEYWORDS: Coral snake; Venom; Neostigmine; Anticholinesterase drugs; Envenomation.

INTRODUCTION

Coral snakes in contrast to the main representatives of the Asian, African and Australian Elapidae are burrowing, timid and low aggressive snakes. This explains why accidents caused by them are so infrequent. In the state of São Paulo, for instance only about 1% of all snake bite accidents are caused by the *Micruri*¹⁴. However, the accidents are usually severe due to the high toxicity^{2, 20, 23, 26} of their venoms.

Micrurus venoms are neurotoxic, producing motor paralysis and death by paralysis of the respiratory muscles. They give rise in man and monkey to a facies identical to that produced by other neurotoxic venoms^{3, 15}

and occurring in myasthenia gravis patients: bilateral ptosis of the eyelids, ophthalmoplegia, mandibular ptosis and paralysis of other facial muscles. Blurred vision, diplopia, dysphagia and dysphonia also occur. Salivation and in some patients nausea and vomits before paralysis are other symptoms observed in coral snake accidents. Pain irradiating from the site of the bite and paresthesias are local effects produced by the venoms. Locally there is no oedema but myonecrosis of the muscles at the venom inoculation region has been revealed histologically⁶. However, myoglobinuria and renal complications (oliguria, anuria) do not occur in patients bitten by coral snakes or in animals injected

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with their venoms. The paralysis and respiratory arrest caused by *Micrurus* venoms as those produced by all neurotoxic snake venoms are exclusively due to blockade of neuromuscular transmission^{7, 9, 13, 18, 22, 23}.

Due to the rapid rate of absorption of the coral snake venom neurotoxins and evolution of the envenomation, the specific antivenom to be effective in preventing paralysis and respiratory insufficiency must be injected very soon after the bite and preferentially intravenously. As this is not always possible, other forms of treatment must be employed to avoid asphyxia and death. Neostigmine and artificial respiration are used for that purpose. Two conditions are needed for the effectiveness of the anticholinesterase drug to revert the signs and symptoms of the envenomation: first, the neuromuscular blockade must result from interaction of the venom neurotoxin(s) with the end-plate nicotinic receptor, and second, this interaction must be reversible. Only *M. frontalis* venom among those of the coral snakes studied to the present date satisfy these two conditions¹⁷. In the present communication, we report two cases of patients bitten by *M. frontalis* treated with antivenom and neostigmine (one of the accidents was reported by VIEIRA et al. in the Brazilian Congress of Toxicology held in São Paulo in 1989).

CASE HISTORIES

Patient No. 1 - A 20 years old male attended the University Hospital (Hospital das Clínicas da UNICAMP) about two hour after being bitten by a coral snake in the left leg with muscle weakness and difficulty in speaking and walking. He complained of pain and paresthesias in the bitten member and blurred vision. When one of us (R. J. V.) examined him, he presented bilateral ptosis (Fig. 1, I), ophthalmoplegia, impossibility of standing upright (Fig. 1, II), and respiratory distress. After administration i. v. of betasone (8.0 mg) and pheniramine maleate (45.0 mg), atropine sulfate (0.5 mg) and neostigmine methylsulfate (1.0 mg) were injected i. v.. Neostigmine administration induced partial recover of the patient muscle strength. Disappearance of the myasthenic facies (Fig. 1, III), complete recovery of the muscle strength and possibility of standing upright (Fig. 1, IV) and walking followed a second injection of neostigmine (1.0 mg) 10 min after the first one. Antivenom (100 ml) against *M. frontalis* and *M. corallinus* venoms ("soro antielapídico" from Butantan Institute) was then injected i. v..

There was no relapse of the signs and symptoms of envenomation, and after two days the patient was discharged.

Patient No. 2 - A 38 years old male attended the University Hospital (Hospital das Clínicas da UNICAMP), eleven hours after being bitten by a coral snake in the second finger of the left hand.

Soon after bite, the patient felt local pain, itching along the member and a sensation that he would faint. In the nearest Hospital from the site of the accident, 50 ml of the specific antivenom were injected, and as he presented muscle weakness besides blurred vision and diplopia, he was sent to the University Hospital.

When one of us (R. J. V.) examined him, he was drowsy, could not stay sitting, and walked with great difficulty. He presented bilateral eyelid ptosis, ophthalmoplegia, muscle fasciculations and muscle weakness. After being injected with 50 ml of the specific antivenom, atropine sulfate (0.5 mg) and neostigmine methylsulfate (1.0 mg) were administered i. v.. There was some improvement. However as the eyelid ptosis persisted, a new injection of atropine (0.5 mg) and neostigmine (1.0 mg) was given. The ptosis disappeared, he could sit without help, and get up to urinate. There was relapse of the signs and symptoms of envenomation 10 min thereafter. A new injection of neostigmine was then given and 250 ml of glucosidic physiological solution with 4.0 mg of neostigmine were infused at rate of 50 microdrops per min. An infusion apparatus was used for that purpose. An infusion of 250 ml of physiological solution with 4.0 mg of atropine was also made at a rate of 30 microdrops per min. The infusion of neostigmine and atropine was stopped after 6 hours without the reappearance of the signs and symptoms of envenomation. The patient was discharged after three days.

COMMENTS

Nearly all accidents produced by coral snakes in Centereast and South of Brazil are caused by *M. frontalis* or *M. corallinus* snakes.

The main cause of the neuromuscular blockade produced by *M. corallinus* venom is inhibition of evoked Ach release²⁰. Hence, neostigmine does not antagonize the blockade (Fig. 2) and seems therefore to

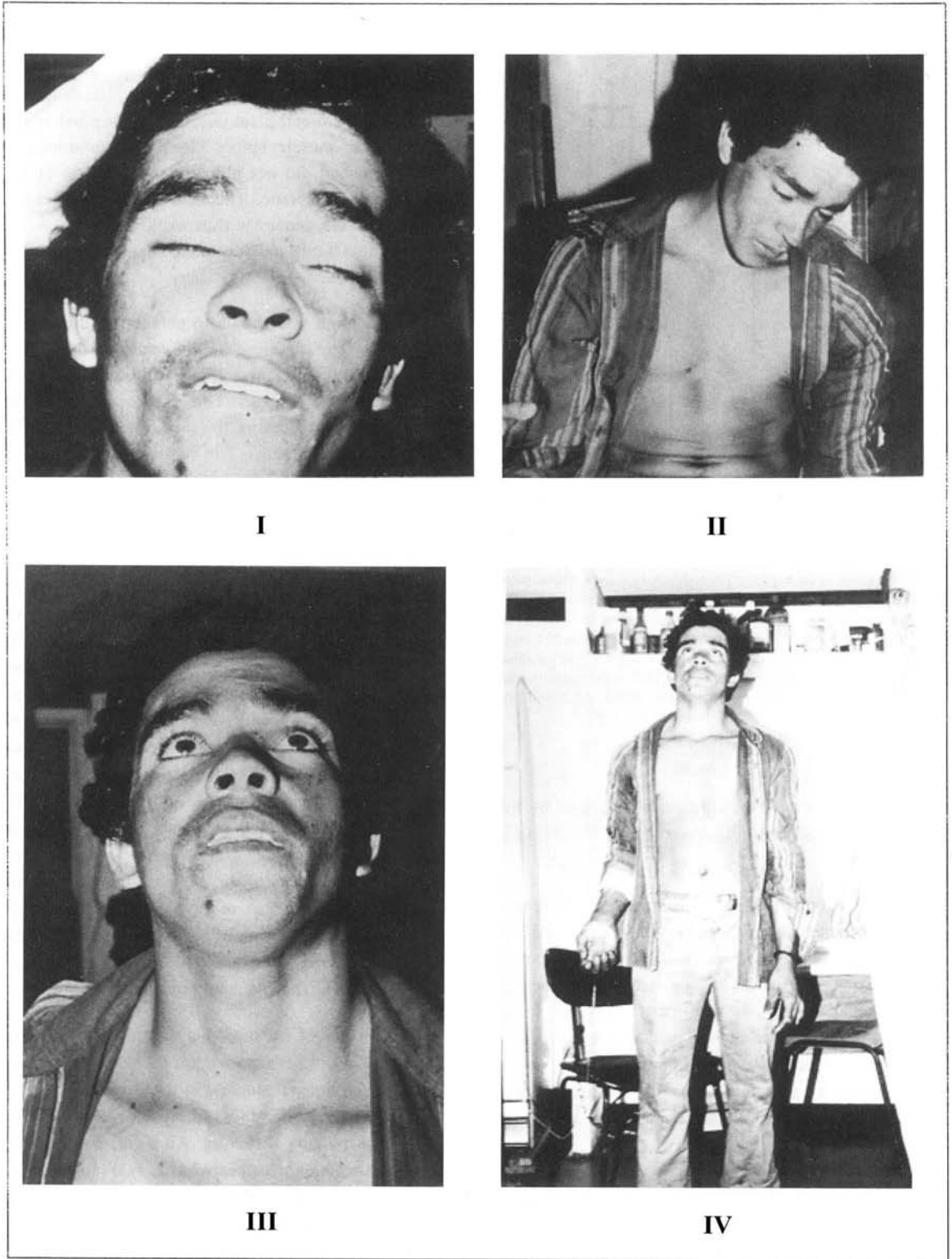


Fig. 1 - Patient bitten by a coral snake (*Micrurus frontalis*) about 2 h. before; I and II before neostigmine methylsulfate injection; III and IV after a second injection of 1.0 mg of neostigmine methylsulfate and 0.5 mg of atropine sulfate. (See Case histories, Patient No. 1).

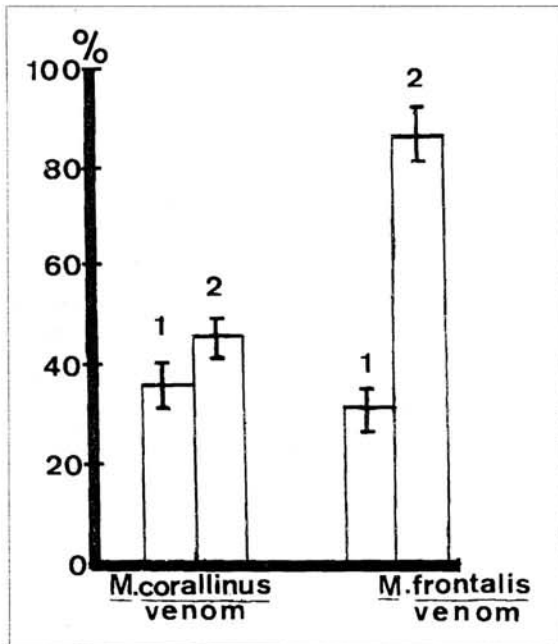


Fig. 2 - Effect of neostigmine on the blockade produced by *Micrurus corallinus* and *M. frontalis* venoms in the sciatic nerve-tibialis anterior muscle preparation *in situ* of the dog. Nerve stimulation with supramaximal pulses of 0.5 Hz, 0.2 ms. Twitch tensions (% of the twitch tension before venom): 1. after the injection i.m. of venom and immediately before neostigmine administration; 2. about 10 min after neostigmine methylsulfate (0.1 mg/kg i.v.) injection. Mean \pm S.E.M. of seven (7) experiments with *M. corallinus* venom and five (5) with *M. frontalis* venom. The difference between 1 and 2 in *M. corallinus* venom experiments is not statistically significant; it is highly significant in the experiments of *M. frontalis* venom ($P < 0.01$).

be useless in the treatment of accidents caused by this snake. In contrast, artificial respiration is lifesaving in the accidents.

The antagonistic effect of neostigmine on the neuromuscular blockade produced by *M. frontalis* and *Naja naja* venoms was demonstrated in 1950 in experiments carried out on the sciatic nerve-gastrocnemius muscle preparation *in situ* of the rat¹⁹. In 1976²³, the mechanism of the neuromuscular blockade induced by *M. frontalis* venom was investigated as well as the effect caused by neostigmine on the venom-produced blockade in dogs and on the experimental envenomation of dogs and monkeys.

The venom of *M. frontalis* of snakes from center-eastern and southern Brazil induced a reversible neuromuscular blockade in the rat phrenic nerve-diaphragm preparation and did not decrease evoked Ach release nor increased the spontaneous one in experiments car-

ried out in that preparation. This shows that the venom does not act presynaptically. In the chronically denervated hemidiaphragm of the rat, the venom blocked irreversibly the contracture produced by Ach. It inhibited the miniature end-plate potentials (m.e.p.s) in the toad sartorius muscle before blocking neuromuscular transmission and did not induce depolarization of the muscle fiber membrane. The neuromuscular blockade produced by the venom is thus exclusively due to its postsynaptic action, and reversible in innervated muscle preparations.

Neostigmine was shown to antagonize the neuromuscular blockade induced by the venom in the sciatic nerve-anterior tibialis muscle preparation *in situ* of anesthetized dogs (Fig. 2). This antagonism was also demonstrated by recording the electromyogram of the diaphragm *in situ* of dogs. The curative action of neostigmine on *M. frontalis* envenomation was investigated in 10 nonanesthetized dogs injected intramuscularly with a lethal dose (0.95 mg/kg) of the venom. Five were treated with neostigmine methylsulfate (0.05 mg/kg) administered intravenously after atropinization when the dogs presented respiratory difficulties; five were not injected with the anticholinesterase drug (controls). All of the treated dogs survived; all of the controls died in less than 3 hours (table 1). A monkey was injected intramuscularly with *M. frontalis* venom. About 3 hours thereafter, the animal was paralysed, voiceless and presented a myasthenic-like facies; its respiration was diaphragmatic. It was then injected intravenously with atropine and neostigmine. The aphonia and dysphagia as well as the myasthenic facies disappeared and there was complete recovery of the muscle strength in about 10 min. There was relapse of the paralysis 40 min later but after a second injection of neostigmine, the monkey recovered again his strength and all signs of envenomation disappeared. The monkey survived²¹.

The results of neostigmine administration in the two cases of accidents reported are entirely confirmatory of the experimental studies. In the first one, besides the myasthenic facies and loss of muscle strength, there was some respiratory difficulty. All signs and symptoms of envenomation disappeared after two injections of 1.0 mg of neostigmine and did not return. In the second case, two injections of neostigmine were also necessary for complete recovery of the muscle strength and disappearance of the myasthenic facies. However, there was relapse of the eyelid ptosis after 10 min. A new injec-

TABLE 1

Treatment with neostigmine (neostigmine methylsulfate) of experimental poisoning induces by *Micrurus frontalis* venom in dogs.

Dog (weight)	Exp. No.	Venom (0.95 mg/kg i.m.) (hour)	Respiratory depression (hour)	Neostigmine (0.05 mg/kg i.v.) (hour)	Result
4.9 Kg	1	9h 55 min a.m.	11h a.m.	11h 55 min a.m.*	Survived
4.0 Kg		9h 50 min a.m.	11h a.m.	-----	Dead at 11h 50 min a.m.
6.3 Kg	2	11h 50 min a.m.	1h 20 min p.m.	1h 30 min p.m.	Survived
5.8 Kg		1h 30 min a.m.	0h 50 min p.m.	-----	Dead at 1h 20 min p.m.
7.7 Kg	3	9h 40 min a.m.	10h 30min a.m.	10h 40min a.m.	Survived
6.4 Kg		9h 25 min a.m.	11h a.m.	-----	Dead at 11h 20 min a.m.
5.8 Kg	4	0h 5min p.m.	2h p.m.	2h 5min p.m.**	Survived
5.3 Kg		0h 10min p.m.	2h p.m.	-----	Dead at 2h 30 min p.m.
5.4 Kg	5	10h a.m.	11h 55min a.m.	0h 15min p.m.	Survived
4.8 Kg		10h 5min a.m.	0h 10min p.m.	-----	Dead at 1h p.m.

* A second injection of neostigmine was made after 45 min.

** A second injection of neostigmine was made after 20 min.

tion of neostigmine caused the disappearance of this sign, and an infusion of a solution with neostigmine for 6 hours prevented the relapse of the signs and symptoms of envenomation. A third very severe accident caused by *M. frontalis* occurring in Santa Catarina, South of Brazil was also successfully treated with neostigmine ⁴.

Anticholinesterase drugs have also been used in Asia and Oceania for the treatment of accidents of the neurotoxic type caused by elapid snakes. Disappearance of the myasthenic facies, recovery of muscle strength and, when there was paralysis of the respiratory muscles, return of spontaneous respiration were reported following neostigmine or edrophonium administration in accidents caused by the Indian cobra (*N. n. naja*) ^{8, 10, 16}, Malayan krait (*Bungarus candidus*) ²⁴ or death-adder (*Acanthophis antarcticus*) ⁵. A much higher survival of patients treated with antivenom and neostigmine than with antivenom alone in accidents caused by the India cobra was reported by BANERJEE et al. ¹ and PANDEY et al. ¹¹. In an interesting study, a placebo

controlled double blind crossover trial of edrophonium in 10 patients bitten by the Philippine cobra (*N. n. philippensis*) was used by WATT et al. ²⁵ to assess the effectiveness of anticholinesterase drugs to revert the signs of envenomation. They reported that edrophonium induced a significant attenuation or abolished the signs of the poisoning and concluded that the anticholinesterase drugs are beneficial in the management of neurotoxic envenomation caused by the Asian cobras. The edrophonium test was recommended in any patient with signs of neurotoxic envenomation after snake bite. In contrast to the results of the preceding reports, PAWAR & SINGH ¹² did not observe regression of the motor or respiratory paralysis following neostigmine administration in four patients bitten by cobra (*Naja naja*) or by krait (*Bungarus* sp.). Artificial respiration was lifesaving in all the four patients. The ineffectiveness of neostigmine could be due to the irreversibility of the postsynaptic action of the cobra venom neurotoxin(s) or to the presynaptic action of the neurotoxin of the krait venom.

In conclusion, neostigmine effectiveness in the treatment of the poisoning produced by *M. frontalis* venom was demonstrated experimentally and is confirmed in the two cases here reported. It is also effective in the treatment of accidents of the neurotoxic type caused by other elapidic snakes whose venom neurotoxins block neuromuscular transmission by interacting reversibly with the end-plate nicotinic receptor.

RESUMO

Neostigmina no tratamento dos acidentes ofídicos causados por *Micrurus frontalis*: relato de dois casos

O soro antipeçonhento específico para ser eficaz no tratamento dos acidentes causados pelas cobras corais deve ser administrado logo após a mordedura, uma vez que as neurotoxinas da peçonha são absorvidas muito rapidamente. Como isto não é sempre possível, outras formas de tratamento, além do soroterápico, devem ser empregadas para evitar a asfixia e morte do paciente. A administração da neostigmina e a respiração artificial são utilizadas com esse objetivo. A neostigmina restabelece a transmissão neuromuscular se o efeito da peçonha resultar de interação reversível de suas neurotoxinas com os receptores colinérgicos da placa terminal. É esse o mecanismo de ação das neurotoxinas da peçonha de *M. frontalis* de serpentes do centroeste e sul do Brasil, e da Argentina. Em consequência a neostigmina antagoniza o bloqueio neuromuscular produzido pela peçonha dessas serpentes e é muito eficaz no tratamento do envenenamento experimental de cães e símios. Na presente comunicação relatamos dois acidentes causados por *M. frontalis*, tratados com a administração do soro específico e da neostigmina. Nos dois pacientes a administração da neostigmina produziu regressão completa dos fenômenos de paralisia, confirmando sua eficiência demonstrada no tratamento do envenenamento experimental de animais.

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