

## EXPERIMENTAL TREATMENT OF NECROSIS PRODUCED BY PROTEOLYTIC SNAKE VENOMS. III — ACTION OF A COMBINATION OF ANTIHISTAMINIC, ADRENALIN, AND HYDROCORTISONE (AAC)

G. ROSENFELD<sup>(1)</sup>, F. G. de LANGLADA and E. M. A. KELEN

### S U M M A R Y

Subcutaneous injection of proteolytic snake venom (*Bothrops jararaca*) in not treated mice produced 95.5% necrosis; treatment with antivenin reduced incidence only to 92.0%; treatment with a combination of antihistaminic, adrenalin, and hydrocortisone (AAC) reduced the number of necrosis to 64.5%; when this combination was given associated with antivenin the number of necrosis was reduced to 16.5%. Probably, the favorable effect of the drug is due to the neutralization of histamine added to the mobilization of the venom by corticosteroid with the consequent diminishing of venom concentration at the site of the injection.

### I N T R O D U C T I O N

Antihistaminics are of current clinical use in the treatment of snake bitten patients<sup>9</sup>. Adrenalin is also employed when shock may occur as a consequence of antivenin treatment. Different opinions exist about the utilization of corticosteroids, some being favorable<sup>2, 4</sup> others unfavorable<sup>1, 10</sup> besides the opinion on the inactivity of this hormone in such cases<sup>7</sup>. However, all observations were made in relation to the protective effect against lethality produced by the venom. ROSENFELD & LANGLADA<sup>7</sup> observed that ACTH and Dexamethasone do not increase protection against lethality in mice injected with proteolytic snake venom (*Bothrops jararaca*), but Dexamethasone was useful as protecting against necrosis.

The introduction in therapy of the combination of the three therapeutic agents lead to the experimental investigation as to its possible advantage in the protection against necrosis produced by proteolytic snake venom.

### M A T E R I A L A N D M E T H O D S

*Mice* — Four groups of 200 animals weighing from 30 to 35 g were used.

*Venom* — A solution of 1 mg desiccated *Bothrops jararaca* venom was prepared in 1 ml saline just before injections. These were subcutaneous in the external part of the thigh in a dose of 0.016 mg per g body weight, corresponding to a proportion of 16.0 mg/kg.

*Antivenin* — Polyvalent anti-*Bothrops* serum from the "Instituto Butantan" with a potency of 2 units per ml. It was injected subcutaneously in the back in a dose neutralizing the venom amount, 15 minutes after the venom.

*Drugs combination (AAC)* — Solution containing in 1 ml: pirilamine maleate 25 mg, hydrocortisone sodium succinate 25 mg, and adrenalin 0.25 mg (\*). The drug was injected subcutaneously at the site of the venom. Another group received the drug in

Department of Physiopathology, Instituto Butantan, São Paulo, Brasil

(1) Fellowship from the National Council of Research (CNPq) (Head of Research)

(\*) Provided by "Indústria Brasileira de Produtos Químicos S.A." under the name of "Lisochoque"

the opposite thigh. The dose was 0.05 ml, injected 15 minutes after the venom.

*Observation of necrosis* — Necrosis appearing as a sharp black scar was observed 48 hours after injections.

### RESULTS

Table I shows numerical results of different experiments, and Table II the statistical analysis of them.

Group 1 was only injected with venom, group 2 with venom and the drugs combination AAC, group 3 with venom and antivenin, and group 4 with venom, antivenin and the drugs combination. Four groups were simultaneously used each time an experiment was performed. The reason for such a procedure was explained previously<sup>7</sup>.

Exclusive treatment with the drugs combination AAC reduced the incidence of necrosis from 95.5% to 64.5% which was statistically significant.

TABLE I

Necrosis in mice inoculated subcutaneously in the right thigh with 0.016 mg/g snake venom (*Bothrops jararaca*). Specific antivenin injected subcutaneously in the back, in a neutralizing dose corresponding to the amount of the venom. Association of Antihistaminic, Adrenaline and Hydrocortisone (AAC) in a dose of 0.05 ml injected subcutaneously at the same or at a different site of the venom. All treatments made 15 minutes after the venom inoculation. Necrosis observed 48 hours after

Experiment no.	AAC 0.05 ml	1	2	3	4
		Venom	Venom + AAC	Venom + Antivenom	Venom + Antivenom + AAC
		Necrosis Total	Necrosis Total	Necrosis Total	Necrosis Total
160	injected	20/25	14/25	23/25	1/25
169	in the	21/25	18/25	23/25	5/25
171	opposite	25/25	13/25	22/25	6/25
167	thigh	25/25	15/25	25/25	3/25
		91/100	60/100	95/100	15/100
170	injected	25/25	16/25	24/25	5/25
168	in the	25/25	15/25	25/25	2/25
155	same	25/25	25/25	18/25	3/25
172	thigh	25/25	13/25	22/25	8/25
		100/100	69/100	89/100	18/100
Total		191/200	129/200	184/200	33/200
Percentage		95.5%	64.5%	92.0%	16.5%

ROSENFELD, G.; LANGLADA, F. G. de & KELEN, E. M. A. — Experimental treatment of necrosis produced by proteolytic snake venoms. III — Action of a combination of antihistaminic, adrenalin, and hydrocortisone (AAC). *Rev. Inst. Med. trop. São Paulo* 11:390-393, 1969.

TABLE II

Statistical comparison of number of necrosis between groups treated with Antivenin and an association of Antihistaminic Adrenaline and Hydrocortisone (AAC)

Groups compared	Result	Chi <sup>2</sup> n = 1	Probability P
Group 1 — Venom × Group 2 — Venom and AAC	Highly significant Protective action of AAC	60.062	P << 0.1%
Group 1 — Venom × Group 3 — Venom and Antivenin	Possibly significant There might be some protective effect of antivenin	3.484	10% > P > 5%
Group 2 — Venom and AAC × Group 4 — Venom, Antivenin and AAC	Highly significant Associated treatment with antivenin and AAC protects much better than isolated AAC	95.611	P << 0.1%
Group 3 — Venom and Antivenin × Group 4 — Venom, Antivenin and AAC	Highly significant Associated treatment with antivenin and AAC protects much better than isolated antivenin	229.669	P << 0.1%

Treatment with antivenin alone reduced necrosis to 92.0% which was not significant. However, its association with the drugs combination AAC caused a drastic diminishing of the number of necrosis to 16.5%.

#### DISCUSSION

The drugs combination AAC was described by CARDOSO et al.<sup>3</sup> as an efficacious agent in preventing the experimental anaphylactic shock in guinea-pigs. No other application was studied. Experiments here described showed that the combination of antihistaminic, adrenalin, and hydrocortisone reduced better than antivenin the incidence of necrosis produced by the venom of *Bothrops jararaca*. The utilization of this drug associated with antivenin was highly protective against necrosis.

Same results were obtained with the medicament injected either at the same or at

a different site of the venom. As to the mechanism of protective action, one may try to give an explanation. Antihistaminics currently utilized in envenomations<sup>9</sup> have a triple indication<sup>5</sup>: to neutralize a possible and probable histamine liberation by the venom; to prevent a possible reaction to serumtherapy; and sedation of the patient by its collateral hypnotic effect. As already described by ROSENFELD et al.<sup>8</sup>, corticosteroids have a protective action in relation to necrosis production by proteolytic snake venom. We could not find a satisfactory explanation for the effect of adrenalin in relation to necrosis.

It is out of doubt however, that the combination of the three therapeutic agents showed its indication as a choice for the treatment of envenomation caused by the bite of poisonous animals having proteolytic venom, in order to diminish the probability of necrosis formation, which is a quite frequent complication in this kind of accidents.

RESUMO

*Tratamento experimental da necrose produzida por veneno ofídico proteolítico. III — Ação de combinação de anti-histamínico, adrenalina e hidrocortisona (AAC)*

Camundongos injetados por via subcutânea com veneno ofídico proteolítico (*Bothrops jararaca*), não tratados, apresentaram 95,5% de necroses; tratados somente com soroterapia antiveneno, a incidência foi de 92,0%; tratados unicamente com uma associação de anti-histamínico, adrenalina e hidrocortisona houve uma redução para 64,5% e essa combinação medicamentosa, juntamente com a soroterapia, diminuiu o número de necroses para 16,5%. Provavelmente, o efeito benéfico foi devido à neutralização de histamina somada a uma maior mobilização do veneno pelo corticóide que diminuiu sua concentração no local da inoculação.

REFERENCES

1. ALLAM, M. W.; WEINER, D. & LUKENS, F. D. W. — Comparison of cortisone and antivenin in the treatment of crotaline envenomation. In: "Venoms". BUCKLEY, E. E. & PORGES, N. — Eds., *Amer. Assoc. Adv. Sci.* (Washington), p. 393, 1956.
2. ARORA, R. B.; WIG, K. L. & SOMANI, P. — Effectiveness of hydrocortisone and hydrocortisone-antivenene combination against *Echis carinatus* snake venom. *Arch. Int. Pharmacodyn.* 137:299, 1962.
3. CARDOSO, D. M.; ALMEIDA, W. F. & FRANÇA, H. N. — Tratamento profilático do choque anafilático experimental. *Rev. Quim. Farm.* 29:13, 1964.

4. MORALES, F.; ROOT, D. H. & PERRY Jr., J. F. — Protection against *Naja naja* venom in dogs by hydrocortisone. *Proc. Soc. Exp. Biol. Med.* 108:522, 1961.
5. ROSENFELD, G. — Symptomatology, Pathology and Treatment of Snake Bites in South America. In: "Venomous Animals and their Venoms". BUCHERL, W.; BUCKLEY, E. E. & DEULOFEU, V., Vol. II, Chapter 4. New York, Academic Press. (In press).
6. ROSENFELD, G. & LANGLADA, F. G. de — Corticosteroid and ACTH in experimental poisoning with animal venoms. *Mem. Inst. Butantan* 31:171, 1964.
7. ROSENFELD, G.; LANGLADA, F. G. de & KELEN, E. M. A. — Experimental treatment of necrosis produced by proteolytic snake venoms. I — Action of Isoxsuprine. *Rev. Inst. Med. trop. São Paulo* 11:383-386, 1969.
8. ROSENFELD, G.; LANGLADA, F. G. de & KELEN, E. M. A. — Experimental treatment of necrosis produced by proteolytic snake venoms. II — Action of Dexamethasone. *Rev. Inst. Med. trop. São Paulo* 11:387-389, 1969.
9. ROSENFELD, G.; NAHAS, L.; CILLO, D. M. de & FLEURY, C. de T. — Envenenamentos por Serpentes, Aranhas e Escorpiões. In: "Atualização Terapêutica", CINTRA DO PRADO, F.; RAMOS, J. de A. & RIBEIRO DO VALLE, J., Rio, Livraria Luso-Espanhola e Brasileira, 1957, pp. 931.
10. RUSSELL, F. E. & EMERY, J. A. — Effects of corticosteroids on lethality of *Ancistrodon contortrix* venom. *Amer. J. Med. Sci.*, 241:507, 1961.

Recebido para publicação em 10/6/1969.