*Rev. Inst. Med. trop. São Paulo*
**11**(6):383-386, novembro-dezembro, 1969

UDC 616-099

# EXPERIMENTAL TREATMENT OF NECROSIS PRODUCED BY PROTEOLYTIC SNAKE VENOMS. I — ACTION OF ISOXSUPRINE

G. ROSENFELD [1], F. G. de LANGLADA and E. M. A. KELEN

## SUMMARY

The subcutaneous injection of proteolytic snake venom (*Bothrops jararaca*) in non treated mice produced 83% necrosis; treatment with antivenin reduced this incidence to 78.5%; treatment with Isoxsuprine alone reduced necrosis to 39.3%; combined treatment with antivenin and Isoxsuprine reduced it even more, to 28.9%. This favorable effect is probably due to the vasodilator effect of Isoxsuprine which increases the venom dissemination thus reducing its concentration at the site of the bite.

## INTRODUCTION

Necrosis is a serious complication in patients bitten by snakes having proteolytic venom. It can only be avoided by specific serumtherapy when applied in sufficient amount within 2 to 3 hours from the accident, or when suction is made at the site of the bite for extraction of part of the venom. No such risk exists when the inoculated amount is small, but it is observed in 15.7% of cases, even if a suitable treatment lowers mortality rate to 0.7% (ROSENFELD [4]). Treatment received far after the indicated time is responsible for necrosis, what makes conclude that death is more easily avoided than necrosis.

The venom kept at the site of the bite allows the action of the proteolytic factors which produce necrosis. Accelerating the circulation of the venom could be a way of avoiding or reducing necrosis production. With this purpose, experiments in mice were made, using a peripheric vasodilator, Isoxsuprine.

## MATERIAL AND METHODS

*Mice* — Four groups of 270 animals weighing from 30 to 35 g were used.

*Venom* — A solution of 1 mg desiccated venom from *Bothrops jararaca* per ml saline was prepared just before inoculation. Injections of 0.5 mg venom per mouse were subcutaneous in the external part of the thigh, corresponding approximately, to a mean dose of 15.0 mg per kg body weight.

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

*Isoxsuprine* — 1-(p-hidroxifenil)-2-(1-metil-2-fenoxietilamino)propanol-1 HCl as a 0.5% solution (*). Subcutaneous injections were made 15 minutes and 8 hours after the venom at the site of the venom injection, in doses ranging from 0.125 to 0.5 ml.

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

## RESULTS

Table I shows numerical results of different experiments and Table II shows their statistical analysis.

Department of Physiopathology, Instituto Butantan, São Paulo, Brasil
(1) Fellowship from the National Council of Research (CNPq) (Head of Research)
(*) Provided by "Companhia Farmacêutica Organon do Brasil S. A." under the name of "Duvadilan"

TABLE I

Necrosis in mice injected subcutaneously with 0.5 mg snake venom (*Bothrops jararaca*) in the right thigh. Isoxsuprine injected at the same site of the venom in all experiments. Specific antivenin injected subcutaneously, an amount neutralizing one or two venom doses, at the same or a different site (dorsal skin) of the venom. Necrosis observed after 48 hours. *SC* = subcutaneous injection; *1 x* and *2 x* = antivenin amount neutralizing one and two venom doses

| Experiment no. | Isoxsuprine SC | | Antivenin SC | | | Group 1 Venom | | Group 2 Venom + Isoxsuprine | | Group 3 Venom + Antivenin | | Group 4 Venom + Antivenin + Isoxsuprine | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Dose mg | Time | Dose | Place of injection | Time | Necrosis | | Necrosis | | Necrosis | | Necrosis | |
| | | | | | | no./total | % | no./total | % | no./total | % | no./total | % |
| 131-132-133 | 0.5 | 15 min | 1 x | same | 15 min | 52/75 | 69.3 | 45/75 | 60.0 | 38/75 | 50.7 | 18/75 | 24.0 |
| 138-139 | 0.5 | 15 min<br>8 hrs | 2 x | Different | 15 min | 31/40 | 77.5 | 13/40 | 32.5 | 27/40 | 67.5 | 5/40 | 12.5 |
| 140-141 | 0.25 | 15 min<br>8 hrs | 2 x | Different | 15 min | 37/40 | 92.5 | 10/40 | 25.0 | 36/40 | 90.0 | 15/40 | 37.5 |
| 142-143-144-145 | 0.25 | 15 min<br>8 hrs | 2 x | Different | 15 min | 87/90 | 96.7 | 24/90 | 26.7 | 86/90 | 96.6 | 18/90 | 20.0 |
| 146 | 0.125 | 30 min<br>8 hrs | 2 x | Different | 15 min | 17/25 | 68.0 | 14/25 | 56.0 | 25/25 | 100.0 | 22/25 | 88.0 |
| Total Results | | | | | | 224/270 | 83.0 | 106/270 | 39.3 | 212/270 | 78.5 | 78/270 | 28.9 |

Group 1 was inoculated with venom, group 2 with venom plus Isoxsuprine, group 3 with venom plus antivenin and group 4 with venom, antivenin and Isoxsuprine. Four groups were used each time an experiment was performed because, a long previous experience acquired in working with venoms showed that different venom solutions vary their biologic activity, even when used from one same dried venom stock. This is a cause of error when only one determination is made in order to find a dose which would produce a certain percentage of necrosis and further using this criterion as a basis for other experiments. Dried venom is not homogeneous since it is a pool of different dried portions; in this way, the scales do not necessarily have same activities. Temptative homogenization by dissolving a great quantity of venom in a small volume of distilled water and subsequent drying resulted in great loss of activity. The variations in different days as to the activity causing necrosis can be observed by the different results within group 1, where 68.0% to 96.7% necrosis were obtained with a mean of 83.0%, although the same venom was always used.

This cause of error was eliminated by the simultaneous performance of all four groups in a same day, and with the same venom solution. It can also be observed that very high doses (15.0 mg/kg) were needed for the production of necrosis in mice. The mouse is a very resistant animal since, in dogs the amount of 1.2 mg/g produced practically 100% necrosis.

The isolated treatment with Isoxsuprine induced a very favorable result by reducing the incidence of necrosis to 39.3%. The statistical analysis showed the drug's highly effective influence.

The percentage of necrosis in animals treated only with antivenin was reduced to 78.5%. Statistically, this difference was not significant.

The combined treatment with antivenin and Isoxsuprine reduced the incidence of necrosis to 28.9%, which indicated an improved effect over the isolated treatment with each therapeutic agent.

TABLE II

Statistical comparison of necrosis between groups treated with Antivenin and Isoxsuprine

| Groups compared | Result | Chi $^2$ n = 1 | Probability P |
|---|---|---|---|
| Group 1 — Venom × Group 2 — Venom and Isoxsuprine | Highly significant Protective effect of Isoxsuprine | 108.49 | P $\ll$ 0.1% |
| Group 1 — Venom × Group 3 — Venom and Antivenin | Not significant No effect of Antivenin | 1.72 | 20% > P > 10% |
| Group 2 — Venom and Isoxsuprine × Group 4 — Venom, Antivenin and Isoxsuprine | Significant Protective effect of Isoxsuprine with Antivenin better than Isoxsuprine alone | 6.46 | 2% > P > 1% |
| Group 3 — Venom and Antivenin × Group 4 — Venom, Antivenin and Isoxsuprine | Highly significant Protective effect of Isoxsuprine against the negative effect of Antivenin | 133.74 | P $\ll$ 0.1% |

## DISCUSSION

It is logical to assume that, if local concentration of proteolytic venom is lowered one may diminish the probability of necrosis formation. For the obtention of this effect BADANO REPETTO [1] (1950) advised the use of a regional sympathic blocking, but he did not try it. Identical suggestion was made by CHIPPAUX et al. [2] in 1961 without having tried this therapeutic method, since all of their patients came to the service late after the accident.

In 1955, REZENDE [3] provoked regional sympathic blocking by lombar infiltration with 20 ml of 1% novocaine in eight patients bitten by *Bothrops* snakes, and obtained a rapid regression of the edema and evolution without necrosis.

These experimental results indicated that, Isoxsuprine alone was more effective than serumtherapy in preventing necrosis produced by proteolytic snake venoms; probably, by the vasodilator effect which increased the diffusion of the venom. Its use must be associated to serumtherapy in order to improve the prevention of necrosis and the neutralization of diffused venom, thus avoiding the aggravation of general envenomation.

## RESUMO

*Tratamento experimental da necrose produzida por veneno ofídico proteolítico. I — Ação da Isoxsuprina*

Camundongos injetados por via subcutânea com veneno ofídico proteolítico (*Bothrops jararaca*), não tratados, apresentaram 83% de necroses; tratados sòmente com soroterapia antiveneno, a incidência foi de 78,5%; tratados ùnicamente com Isoxsuprina, houve 39,3% de necroses, e o tratamento combinado de Isoxsuprina e soroterapia baixou êsse número para 28,9%. Provàvelmente, êsse efeito benéfico é devido à vasodilatação provocada pela Isoxsuprina, o que mobiliza o veneno para a circulação, diminuindo a sua concentração no local onde foi inoculado.

## REFERENCES

1. BADANO REPETTO, J. L. — Ofidismo. Diagnóstico y tratamiento. *Arch. Urug. Med. Cir. Esp.* **37**:324, 1950.

2. CHIPPAUX, C.; O'CONNOR, H. L.; NOSNY, P.; PLESSIS, J.; DUCLOUX, M. & LALUQUE, P. — Nécroses par morsures de serpent. A propos de douze observation. *Presse Méd.* **69**:583, 1961.

3. REZENDE, J. M. de — Infiltração do simpático regional e ofidismo. *Hospital* (Rio), **47**:325, 1955.

4. ROSENFELD, G. — Symptomatology, Pathology and Treatment of Snake Bites in South America. *In*: BÜCHERL, W.; BUCKLEY, E. E. & DEULOFEU, V. — *"Venomous Animals and their Venoms"*. Vol. II, Chapter 4. New York, Academic Press. (In press).

Recebido para publicação em 10/6/1969.