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27. *IN VIVO* NEUTRALIZATION OF CORAL SNAKE VENOM

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INTRODUCTION

Treatment of persons bitten by venomous snakes with antivenin is generally considered one of the most effective forms of therapy. The potency of the particular antivenin usually is based on an *in vitro* neutralization test where venom and antiserum are mixed in a tube, incubated and injected into mice. The results of this type of test largely reflect the degree of neutralization that occurs in the tube. However, in the case of snakebite, and in the subsequent antivenin therapy, the true measure of potency and efficacy of antiserum is more logically based on its capacity to neutralize venom *in vivo*.

The present studies were designed to evaluate the effectiveness, *in vivo*, of coral snake antiserum in neutralizing the lethal effects of coral snake venom. Similar tests were also conducted with other venoms and antisera.

IN VIVO NEUTRALIZATION OF *MICRURUS FULVIUS* VENOM IN MICE BY HOMOLOGOUS ANTISERUM PRODUCED IN GOATS

Neutralization experiments were carried out with a randomly bred strain of albino, male, mice, weighing 16-18 grams. The *Micrurus fulvius* venom used was a freeze-dried pool of numerous milkings of a large number of snakes. The antiserum was a pool of two hyperimmune goat sera (1) specific for *M. fulvius* venom.

The *in vivo* neutralization tests were carried out by injecting groups of mice with venom. Each group of envenomed mice was then injected with goat antiserum at a different time interval. Control mice received normal goat serum following envenoming. Different combinations of routes of injections were used for the venom and antiserum.

The initial group of experiments was conducted with *M. fulvius* venom and anti-*M. fulvius* goat serum. The results of these experiments are shown in Table I. The intraperitoneal LD_{50} for the venom was 13 μg. All mice received 2 LD_{50} of venom. The antiserum dose was contained in 0.25 ml, and had an *in vitro* neutralization potency of 13.6 mouse LD_{50} per dose. When venom was given intraperitoneally and antiserum was given intravenously, mice were completely protected by antiserum given immediately after the venom and some of the mice were protected when it was given after 30 minutes. Mice were not protected when antiserum was given after 1 hour. However, the time of death for these mice was prolonged 3 to 5 hours beyond the controls. Control mice which received normal serum died between 4 to 6 hours.

When 2 LD_{50} of venom were given subcutaneously and antiserum intravenously, mice were not protected nor were death times prolonged as compared to controls. The subcutaneous LD_{50} of the venom was 32.5 μg which indicated greater tolerance by mice to venom administered by this route than by the intraperitoneal route. This suggests that even though the mouse is more resistant to venom deposited subcutaneously, neutralization of this venom by antiserum given intravenously is not as effective as when the venom was deposited intraperitoneally. To further test this phenomenon, venom was again given subcutaneously but the same antiserum dose was given intraperitoneally. The antiserum failed to protect the mice further confirming the observation that the route of venom injection — in this case subcutaneously — is a factor in the ability of a given dose of antiserum to protect mice *in vivo*. In addition to the route of venom injection, the ability to bring about *in vivo* neutralization by antiserum should depend on the potency and volume of antiserum used. This is illustrated by the last experiment reported in Table I which included antiserum over three times more potent. Protection was obtained when the stronger antiserum was given immediately and some of the mice were protected when it was given after 30 minutes. These results suggest that the routes of envenomation determine the dose of antiserum required for *in vivo* neutralization of the venom.

TABLE I — *IN VIVO* NEUTRALIZATION OF *M. FULVIUS* VENOM IN MICE BY HOMOLOGOUS GOAT ANTISERUM

Route venom injected	Route antiserum injected	Survivors as a function of time antiserum given				Prolongation of death time by antiserum (*Hours*)
		Time		*Alive*	*Dead*	
Intraperitoneal	Intravenous	0 min	Control	0	6	
			Antiserum *	6	0	
		30 min	Control	0	6	
			Antiserum	2	4	3 to 5
		1 hr	Control	0	6	
			Antiserum	0	6	3 to 5
Subcutaneous	Intravenous	0 min	Control	0	6	
			Antiserum *	0	6	0
Subcutaneous	Intraperitoneal	0 min	Control	0	6	
			Antiserum *	0	6	3 to 5
			Antiserum **	5	1	
		30 min	Control	0	6	
			Antiserum *	0	6	
			Antiserum **	2	4	0

* Antiserum dose neutralized 13.6 mouse LD_{50} *in vitro*.
** Antiserum dose neutralized 48.0 mouse LD_{50} *in vitro*.

IN VIVO NEUTRALIZATION OF *M. FULVIUS* VENOM IN MICE BY SÔRO ANTIELAPÍDICO

A similar experiment using Sôro Antielapídico, a purified antivenin produced in horses at the Instituto Butantan, was conducted. Although this serum is produced with venoms from South American coral snakes, it is more than

90% effective in neutralizing *M. fulvius* venom. The results of this experiment are summarized in Table II. The same *M. fulvius* venom dosage and routes of injection used previously were employed. Protection of most mice was obtained if antiserum was given within 30 minutes of envenomation and the time to death was prolonged for several hours when the antiserum was given as late as 1 hour after envenomation. These results are in general agreement with the data from experiments with goat antiserum.

TABLE II — *IN VIVO* NEUTRALIZATION OF *M. FULVIUS* VENOM IN MICE BY SÔRO ANTIELAPÍDICO

Route venom injected	Route antiserum injected	Survivors as a function of time antiserum given				Prolongation of death time by antiserum
		Time		*Alive*	*Dead*	*(Hours)*
Intraperitoneal	Intravenous	0 min	Control	1	5	
			Antiserum *	6	0	
		30 min	Control	1	5	
			Antiserum	4	2	6 to 8
		1 hr	Control	0	6	
			Antiserum	1	5	1 to 3
		2 hr	Control	0	6	
			Antiserum	0	6	0

* Antiserum dose neutralized 16.2 mouse LD_{50} *in vitro*.

IN VIVO NEUTRALIZATION OF *M. FULVIUS* VENOM IN GUINEA PIGS BY HOMOLOGOUS GOAT ANTISERUM

An *in vivo* neutralization experiment was conducted in guinea pigs. The LD_{50} for Hartley, female, 120-130 gram guinea pigs was found to be 23.3 μg by the subcutaneous route, as compared to 32.5 μg for this route in 16-18 gram mice, 30% less venom was required to achieve an LD_{50}. This indicated that these guinea pigs were considerably more sensitive to the venom than the mice and for these two species a toxicity relationship based on a unit of body weight is not valid. The guinea pigs received 2 LD_{50} of venom subcutaneously and the goat antiserum intraperitoneally. The antiserum had a neutralizing potency *in vitro*, of 40.8 mouse LD_{50} per dose. The results of this experiment are shown in Table III. Complete protection was obtained when antiserum was given 30 minutes after envenomation. Antiserum given after 1 hour did not prevent death; however, the time of death was prolonged by as much as 24 hours. Antiserum given as late as 2 hours after envenomation still prolonged the time of death several hours. Control guinea pigs which received normal serum died after 4 hours.

In experiments with both guinea pigs and mice, antiserum gave complete protection only when it was injected within 30 minutes of envenomation. However, the time of death for guinea pigs was prolonged nearly 24 hours longer than in mice even though they both received the same dose of antiserum.

TABLE III — *IN VIVO* NEUTRALIZATION OF *M. FULVIUS* VENOM IN GUINEA PIGS BY HOMOLOGOUS GOAT ANTISERUM

Route venom injected	Route antiserum injected	Survivors as a function of time antiserum given				Prolongation of death time by antiserum
		Time		*Alive*	*Dead*	*(Hours)*
Subcutaneous	Intraperitoneal	0 min	Control	0	3	
			Antiserum *	3	0	
		30 min	Control	0	3	
			Antiserum	3	0	
		1 hr	Control	0	3	
			Antiserum	0	3	24
		2 hr	Control	0	3	
			Antiserum	0	3	2 to 4

* Antiserum dose neutralized 40.8 mouse LD_{50} *in vitro.*

IN VIVO NEUTRALIZATION OF *NAJA ATRA* VENOM IN MICE BY HOMOLOGOUS ANTISERUM PRODUCED IN HORSES

Experiments were designed to determine whether the need to administer antiserum within 30 minutes was a characteristic unique to the coral snake venom system. For this purpose *in vivo* neutralizations were conducted with the venom of *Naja atra,* the Formosan cobra. Crude, horse, anti-*Naja atra* serum was obtained from Cmdr. G. S. Huber, MSC, USN, NAMRU-2, Taipei, Taiwan. The venom and antiserum dosages were the same as those used in previous tests. Results are presented in Table IV. The data are similar to the results obtained with coral snake venom neutralizations. Antiserum must be given within 30 minutes for protection of mice and it failed to prolong death times.

IN VIVO NEUTRALIZATION OF *CROTALUS D. TERRIFICUS* VENOM IN MICE BY HOMOLOGOUS ANTISERUM PRODUCED IN HORSES

To study the *in vivo* neutralization of a crotalid venom mice were given *Crotalus d. terrificus* venom followed by a commercially prepared antivenin * produced in horses with a mixture of four crotalid venoms. This product has wide neutralizing activity for many species of the CROTALIDAE of the Americas. In establishing the dose the antivenin potency was based on its ability to neutralize *Crotalus d. terrificus* venom. The antivenin dosage was adjusted to the same range as those used in the previous studies. The results of this experiments are shown in the lower half of Table IV. Mice were protected only if antivenin was given immediately after envenomation. There was only slight prolongation of death times when antivenin was given after 30 minutes. These neutralizations were slightly less effective than those observed with cobra and coral snake venom.

* Wyeth Laboratories, Philadelphia, Pennsylvania, U.S.A.

TABLE IV — *IN VIVO* NEUTRALIZATION OF *NAJA ATRA* VENOM IN MICE BY HOMOLOGOUS HORSE ANTISERUM

Route venom injected	Route antiserum injected	Survivors as a function of time antiserum given				Prolongation of death time by antiserum (*Hours*)
		Time		*Alive*	*Dead*	
Intraperitoneal	Intravenous	0 min	Control	0	6	
			Antiserum *	6	0	
		30 min	Control	0	6	
			Antiserum	3	3	0

IN VIVO NEUTRALIZATION OF *CROTALUS D. TERRIFICUS* VENOM IN MICE BY CROTALID ANTIVENIN

Route venom injected	Route antiserum injected	Survivors as a function of time antiserum given				Prolongation of death time by antiserum (*Hours*)
		Time		*Alive*	*Dead*	
Intraperitoneal	Intravenous	0 min	Control	0	6	
			Antiserum **	5	1	
		30 min	Control	1	5	
			Antiserum	1	5	2
		1 hr	Control	0	6	
			Antiserum	0	6	1

* Antiserum dose neutralized 13.4 mouse LD_{50} *in vitro*.
** Antiserum dose neutralized 16.2 mouse LD_{50} *in vitro*.

References

1. COHEN, P., and SELIGMANN, E. B. JR., *Mem. Inst. Butantan, Simp. Internac.*, 33 (1), 339-347, 1966.