

A Study on Specific and Nonspecific Effects of Antisnake-Bite Serum

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Summary

In this study monovalent antivenoms of cobra (Naja naja oxiana) Vipera lebetina, Pseudocerastes persicus, Echis carinatus, Vipera latifii and Agkistrodon halys) were produced in the horse. Each specific antivenom and one kind of polyvalent were assayed against the venoms of 6 species of Iranian snakes. The cobra and Iranian pit viper (Agkistrodon halys) antivenoms did not produce any protection (in vivo) or any precipitate band (in vitro) against other venoms. The tests showed that viperid monovalent antivenoms neutralise, to some extent, other viperid venoms.

Introduction

There are 3 families of terrestrial snakes in Iran(1, 2). Injuries and deaths due to snake bites occur in most parts of the country. The polyvalent antivenom are used for treatment of snake-bites poisoning. In the present study 6 specific antivenoms were prepared, in the horse, against the respective venoms of the 6 species(3).

The cross-neutralisation (in vivo) and cross-reactivity (in vitro) tests were carried out to determine the efficacy of each specific antivenom against the venoms of cobra, 4 species of vipers and Iranian pit viper (*A. halys*).

The efficacy of polyvalent antisnake-bite serum was also studied against the venoms of 6 species of Iranian scorpions(4)

Materials and methods

Venoms: The venoms of cobra (*N. n. oxiana*), 4 species of vipers and Iranian pit viper were obtained by pooling the milking of snakes(5). The lethal dose of the venoms, killing 50% of the injected animals (LD50), was determined in mice of 18-20 g body weight by intravenous injection. The results were calculated by Spearman-Kärber method(6).

Monovalent sera: Six monovalent antivenoms were prepared in horses that received subcutaneously 6 afore mentioned snakes venoms.

Potency tests: These tests were carried out by neutralisation test. Briefly, 1 ml of each serum (monovalent and polyvalent) was added to 1 ml serially diluted venoms, incubated at 37°C 1 h and the mixtures were injected into mice (in vivo) (7,8).

Immunodiffusion test: This assay (in vitro) was carried out using Ouchterlony double diffusion method(9,10).

Results and discussion

In vivo neutralisation tests indicated that the specific antivenoms were more effective against the venoms of respective species.

Cobra (*Elapidae*) and Iranian pit viper (*Crotalidea*) antivenoms did not have neutralising effects against the other venoms, but in viperid antivenoms (monovalent) some non-specific effects were found against the venoms of some of the related species as shown in Table 1.

In Ouchterlony double diffusion tests, the cobra and pit viper (*Agkistrodon*) antivenoms produced precipitate bands only against the venoms of their respective species but not against other venoms. However, the *Viperidae* antivenoms (monovalent) showed precipitate bands against the venoms of their respective species and also some of related species as shown in Table 2 and Photos 1-9.

The polyvalent antsnake-bite serum did not have, according to the results of in vivo tests, any neutralising effects against the venoms of Iranian scorpions(Table 3).

More than 12 species of terrestrial venomous snakes from 3 families are found in Iran. They are all dangerous and responsible for the majority of bites. While only a few species such as *Vipera lebetina* or *Echis carinatus* have vast geographical distribution the majority of the species are found

only in limited number of areas. Cobra (*N. n. oxiana*), for example, is limited to north east of Iran, whereas *Vipera albicornuta*, *vipera latifii* and

Table 1. Cross neutralisation tests (in vivo)

antivenom venom	Mon. Cobra No. 69	Mon. Leb. No. 81	Mon. Persica No. 77	Mon. Echis No. 87	Mon. Latifii No. 51	Mon. Agkist. No. 58	Poly. No. 100
Naja naja oxiana	1*	0	0	0	0	0	0.3
Vipera lebetina	0.2	2.4	0.8+	0.2	1+	0	2
Pseudo cerastes	0.2	0.2	3*	1+	1+	0.1	1.8
Persicus							
Echis carinatus	0.1	0.2	0.2	2*	0.2	0	2.6
Vipera latifii	0.2	0.2	0.6	0.1	2.4*	0.1	1.6
Agkistrodon halys	0	0.1	0.1	0.1	0.2	1.4*	1

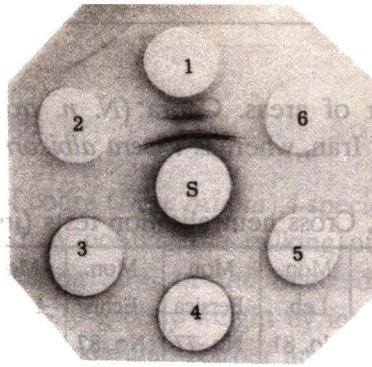
(*): Specific effect
 (+): Nonspecific effect
 Poly: Polyvalent serum
 Mon: Monovalent serum
 (No.): Batch No.

Table 2. Cross reactions of sera (immunogel-diffusion tests)

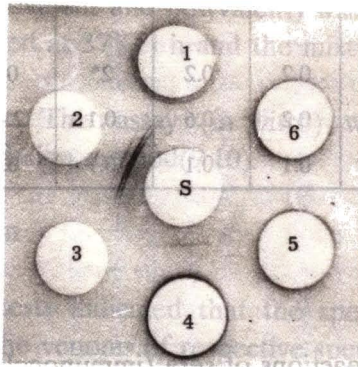
antivenom venom	Mon. Cobra No. 69	Mon. Leb. No. 81	Mon. Persica No. 77	Mon. Echis No. 87	Mon. Latifii No. 51	Mon. Agkist. No. 58	Poly. No. 100
Naja naja oxiana	8*	2	2	2	4	2	8
Vipera lebetina	4	10*	5	7	9+	3	9
Pseudo cerastes	2	7	7*	5	7+	5	9
Persicus							
Echis carinatus	2	5	3	8*	4	3	7
Vipera latifii	4	8+	8+	5	9*	3	11
Agkistrodon halys	2	2	3	2	3	12*	6

(*): Specific effect
 (+): Nonspecific effect
 (No.): Batch No

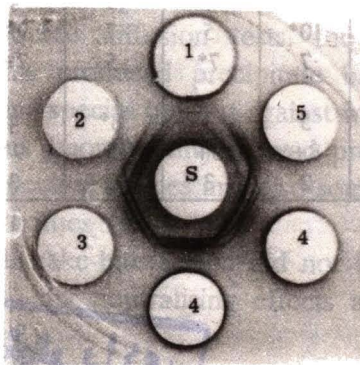




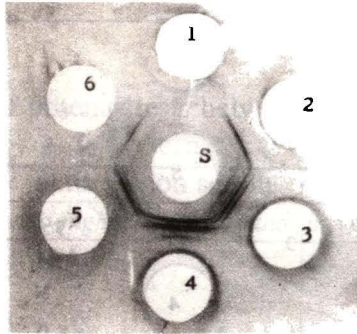
S: Mon. Cobra Lot 69 (0.2 ml.)
Around: Venoms (0.2 ml from 5 mg/ml)
 1-Naja naja 2-Agkistrodon 3-Echis 4-V. latifii
 5-V. lebetina 6-Pseudocerastes.



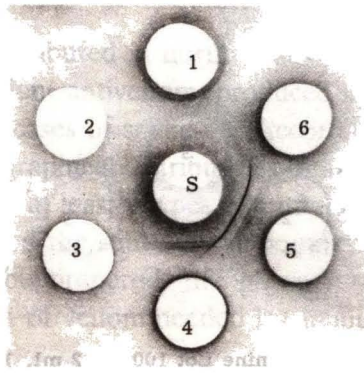
S: Mon. Agkistrodon Lot 58 (0.2 ml.)
Around: Venoms (0.2 ml from 5 mg/ml.)
 1-Naja naja 2-Agkistrodon 3-Echis 4-V. latifii
 5-V. lebetina 6-Pseudocerastes.



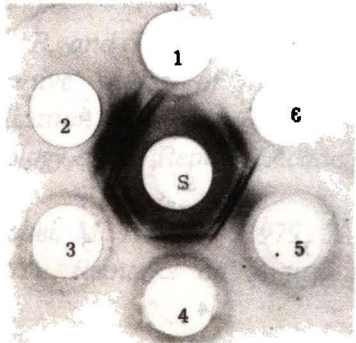
S: Polyvalent Antivenine Lot 100 (0.2 ml)
Around Venoms, (0.2 ml from 5 mg/ml)
 1-N. naja 2-Agkistrodon 3-Echis 4-V. latifii
 5-V. lebetina 6-Pseudocerastes.



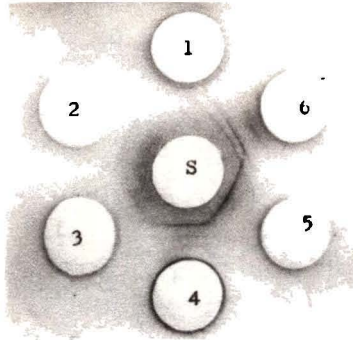
S-Mon.latifii Antivenine(0.2 ml.)
 Venoms:(0.2 ml. from 5 mg/ml) 1-N.naja 2-E.ca-
 rinatus 3-V.lebetina 4-V.latifii 5-V.persica
 6-Agkistrodon halys.



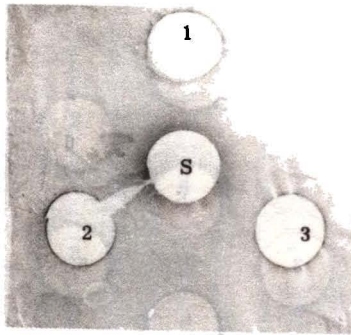
S; Mon. Lebetina Lot 81 (0.2 ml.)
 Around: Venoms (0.2 ml from 5 mg/ml.)
 1-Naja naja 2-Agkistrodon 3-Echis 4-V. latifii
 5-V. lebetina 6-Pesudocerastes.



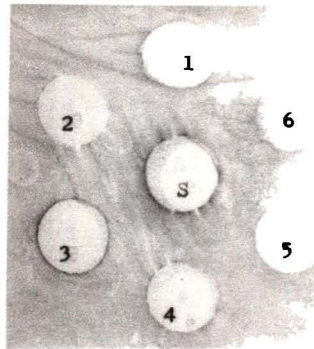
S-Monovalent Echis B. 87
 Venoms: 1-Naja naja oxiana 2-Echis carinatus
 3-V. latifii 4-V. lebetina 5-Pesudocerastes
 6-Agkistrodon halys.



S: Mon. Pseudocerastes Lot 77 (0.2 ml)
 Around: Venoms (0.2 ml from 5 mg/ml)
 1-N.naja 2-Agkist. 3-Echis 4-V.latifii
 5-V.lebetina 6-Pseudocerastes.



S; Poly. Antivenine Lot 100 (0.2 ml.)
 Around Venoms, (0.2 ml from 5 mg/ml.)
 1-Odontobuthus 2-Scorpio 3-Mesobuthus



S: Normal horse, s Serum (0.2 ml)
 Around :Venoms (0.2 ml from 5 mg/ml*
 1-N.naja 2-Agkist. 3-Echis 4-V.latifii
 5-V.lebetina 6-Pseudocerastes.

Table 3. Comparison of potency tests between antsnake and antiscorpion serum

scorpion's venom	antiscorpion serum		anti snake-bite serum	
	<i>In vivo</i> -mg/ml	<i>in vitro</i> band	<i>in vivo</i> mg/ml	<i>in vitro</i> bands
<i>Androctonus</i>	0.04	7	0	0
<i>Buthotus</i>	0.5	3	0.2	0
<i>Odonto buthus</i>	0.3	4	0.2	0
<i>Mesobuthus</i>	0.6	6	0.2	0
<i>Hemiscorpius</i>	1	1	0.4	0
<i>Scorpia maurus</i>	1	6	0.5	0

Agkistrodon halys are distributed in north west of the country. At present, only one type of polyvalent antivenom, produced against the venoms of 6 species, are used for all cases of snake-bit. According to the results of this study and by taking geographical distributions of snakes into consideration, it is necessary to produce at least 3 types of antivenoms for 3 areas, namely, north-east, north-west and the south of the country. Should these regional polyvalant antivenoms be prepared, the potency of the antivenoms will increase and the amount of venom needed for immunisation will decrease.

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