

Vipera palaestinae antivenin

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ABSTRACT

A review is presented of the methods developed to raise the neutralizing capacity of anti-*Vipera palaestinae* (Vp) venom. These include formaldehyde detoxification of the venom, immunization with carboxymethyl-cellulose-bound neurotoxin, and enzymatic fragmentation of the neutralizing antibody.

INTRODUCTION

Vipera xantina paelestinae is the most widely distributed poisonous snake in Israel. Its venom is composed mainly of hemorrhagins, proteases, phospholipase A, hyaluronidase, esterases, phosphodiesterase, and L-amino oxidase (1,2)

The need to develop an antiserum against *V. palaestinae* (Vp) venom became apparent in the early sixties in view of the low neutralizing potency of the commercial antiserum in use at the time. The available horse antivenin was found capable of protecting envenomated mice against the hemorrhagic action of the Vp venom, but its antilethal activity was insufficient and the animals were dying with neurotoxic signs. Electrophoretic and chromatographic fractionation of the venom revealed its composition of two main toxins, hemorrhagin and neurotoxin (1). The hemorrhagin, a protein of high molecular weight (2), proved to be an effective antigen, whereas the isolated neurotoxin had poor antigenicity (3).

Our subsequent efforts to obtain a more effective antiserum were made in three stages, which will be reviewed chronologically.

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1. DETOXIFICATION OF Vp VENOM WITH FORMALDEHYDE

Formaldehyde treatment of Vp venom detoxified the hemorrhagin, both at acidic and alkaline pH. The immunogenicity of the hemorrhagin, however, was preserved only when detoxification was carried out at acidic pH (4).

The neurotoxin was detoxified by formaldehyde at alkaline pH; its poor immunogenicity was unchanged (4). Treatment of whole venom with formaldehyde at pH above neutral, which detoxified both the hemorrhagin and the neurotoxin, was not only ineffective in the production of antineurotoxin, but also abolished the immunogenic activity of the hemorrhagic component (4). It was evident that immunization with formalinized Vp venom alone would not provide a potent antiserum. On the other hand, with formaldehyde treatment at pH 7.4, which detoxified both the hemorrhagin and the neurotoxin, the immunogenicity of the venom hemorrhagin decreased, but was still adequate for primary immunization and enabled continued immunization with native venom.

2. ENHANCEMENT OF IMMUNIZATION WITH ISOLATED Vp NEUROTOXIN

Isolation of the neurotoxin from Vp venom by ion exchange chromatography and salt precipitation yielded a homogeneous basic protein with an estimated molecular weight of 12,000 (5). Attempts were made to increase the weak immunogenicity of the isolated neurotoxin by binding to albumin through azo-bonds and binding to carboxymethyl cellulose (CMC) (3,6); only the latter procedure proved to be effective. CMC-bound Vp neurotoxin was found to be nontoxic but had markedly enhanced immunogenicity. Immunization of rabbits with CMC-bound neurotoxin produced an effective anti-neurotoxic serum (6).

This principle was then applied to the large-scale preparation of anti-Vp serum in horses, as follows (7): primary immunization was carried out with one injection of whole Vp venom formalinized at pH 7.4, followed by repeated injections of increasing doses of native whole venom and finally by repeated booster injections with CMC-bound neurotoxin.

Effective horse antiserum was obtained by this procedure, reaching an antitoxic titer of up to 400 LD₅₀/ml.

3. NEUTRALIZATION OF Vp VENOM HEMORRHAGIN BY ANTIBODY FRAGMENTS

Further studies were carried out to investigate neutralizing activity of antibody fragments in order to increase the specific activity of the antibody while eliminating its immunogenicity, thus attempting to avoid anaphylactic reactions.

The neutralizing activity against Vp hemorrhagin of antibody fragments obtained by enzymic digestion of specific anti-Vp hemorrhagin rabbit IgG was studied.

IgG was isolated from antisera produced in rabbits against the hemorrhagin preparation. The antitoxic activities of the monovalent 3.5S (Fab) antibody fragment obtained by papain digestion of IgG, and of the bivalent 5S F(ab)₂ fragment obtained by pepsin digestion, were compared to the antitoxic activity of the nontreated IgG.

As seen in Table 1, the antitoxic activity of the monovalent 3.5S antibody, when expressed on a weight basis, exhibited about half the antitoxic activity of the bivalent complete antibody.

The bivalent 5S antibody fragment obtained after pepsin digestion exhibited increased antitoxic activity.

The results described above revealed a marked increase in specific anti-hemorrhagin neutralizing activity of the F(ab)₂ fragments and a pronounced decrease in that of the F(ab) fragments. The increased specific activity of the F(ab)₂ antibody fragments indicates that the Fc fragment removed by pepsin digestion does not contribute to the neutralization of toxicity of the hemorrhagin.

Table 1

Neutralization of toxic activity of Vipera palaestinae venom hemorrhagin by specific antibody and antibody fragments

Bleeding	Immunoglobulin preparation	Antitoxic activity	
		LD ₅₀ neutralized	/mg globulin %
1	IgG	14.5	100
	3.5S F(ab) fragment	8	55
2	IgG	17	100
	5S F(ab) ₂ fragment	23	135

The clinical experience with the above-described anti-Vp horse antivenin for the treatment of Vp snake bites in Israel was recently reported (8). It was found that this antivenin was highly effective, even when administered more than 24 h after the snakebite, in patients with envenomation complicated by marked and progressive local signs, delayed systemic signs, and laboratory abnormalities (8).

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