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EFFECTS OF DRUGS AGAINST CYTOTOXIC COMPOUNDS FROM MARINE ANIMALS IV-HEPATOTOXIC ACTIVITY OF *SEA-SNAKE HYDROPHIS CYANOCINCTUS* VENOM

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ABSTRACT

Hepatotoxic activity and pathogenesis of sea snake *Hydrophis cyanocinctus* venom have been studied in guinea pigs and albino rats by histopathological examination and determining total Ca^{2+}. Concentration in liver tissue. Efficacy of two Ca^{2+}-antagonist drugs, verapamil and diltiazem was also studied against the cytotoxic activity of this venom. Administration of both lethal and sublethal doses *of Hydrophis cyanocinctus* crude venom induced in-creased cellularity within the portal area in liver accompanied by increased tissue Ca^{t+} concentration. This elicit a close correlation between tissue necrosis and increased Ca^{2+} content in tissues. Both verapamil and diltiazem administration after an injection of *H. cyanocfncros* venom showed significant decrease in Ca^{2+} concentration and tissue necrosis. Increase in survival time was also noted in almost all animals receiving Ca^{2+} - antagonist drugs. It is thus suggested that cytotoxic compounds present in this marine venom cause tissue and cellular necrosis by increasing Ca^{2+} influx within the cells which in turn stimulates Ca^{2+} -dependent processes resulting in both organ dysfunction and sometimes fatality in few cases. But both these effects could be inhibited by using antagonizing drugs which could reduce the pathological conditions induced by such compounds by blocking the cation influx and its dependent processes.

Introduction

A number of clinical studies have been carried out during last decades on sea snake venom and its components (Cheah et *al.,* 1987; Marsden and Reid, 1961; Reid, 1961; Yang and Lee; 1978) having myotoxic, neurotoxic and cytotoxic potencies but the mechanism of their cytotoxic activities, or in other words, pathogenesis of their

cytotoxins, is still not clear. Similarly no detailed report is available on drug therapy against specific clinical effects of sea-snake venom and their components.

It has been reported earlier that cytotoxins act by increasing the permeability of cell membrane to cations such as Ca^{2+} and Na^{+} (Muhvich *et al,* 1991; Terao *et al,* 1989; 1991) which in turn destroy the cells. Jellyfish venom and shellfish poison are two main agents reported to cause an increase influx of Ca^{t+} into vascular smooth muscle cells resulting in muscle paralysis and cardio-respiratory arrest (Alam *et al*, 1993; Muhvich *et a1*., 1991; Terao *et al,* 1989; 1990).

Recently in our laboratories, studies have been initiated to investigate the pathogenesis of cytotoxic compounds in marine animals in general and sea snakes in particular and the protective efficacy of several drugs against their general lethal and toxic effects (Alam, 1992 a, b, c; Alam *et al.,* 1993).

The present communication deals with the hepatotoxic activity of sea snake *Hydrophis* cyanocincus venom, its pathogenesis and protective efficacy of two Ca^{2+}-antagonist drugs, verapamil and diltiazem against venom's cytotoxic effects.

Material and Methods *Venom Extraction:*

Sea-snake species *of Hydroplls cyanocinctus were* collected near and around coastal areas of Karachi. Venom from live snakes was collected manually and lyophilized. Lyophilied venom was treated for bioassay according to the method described by Alam *et al.* (1993). For histopathological studies LDSO was serially diluted to increase the survival time upto 12 h.

Animals:

Guinea pigs of either sex (500-600 gm) were used for histopathological examination and drug efficacy according to the protocol described by Alam (1992a) and Alam *et al.* (1993). Albino rats of either sex (150-200 gm) were used only for drug efficacy experiment because of the easy handling and the usage of low quantities of both venom and drugs. Both guinea pigs and albino rats were divided into two groups each, one with venom only and other with venom + both drugs.

Controls were 6 each of guinea pigs and albino rats which were given equi-pH and equi-protein solution of bovine serum albumin intraperitoneally.

Histopathological Analysis:

In guinea pigs the target organs were assessed for cellular degeneration and tissue ecrosis according to the procedure described by Alam *et al.* (1993).

Assay for Calcium Content:

Total calcium content in tissues of treated guinea pigs and rats were estimated as described earlier (Terao *et a1.,* 1989).

Efficacy of Ca^{2+}-Antagonist Drugs:

This was performed according to the methods of Alam *et al.* (1993).

Statistical Analysis:

Ca^{Z+} content and drugs efficacy data were analyzed by student's t-test. Results were considered significant when $P < 0.05$.

Results

Histopathology:

Fatty changes (Fig. 1) and increased cellularity (Fig. 2) were observed at both lethal and sublethal doses in liver of evenomated guinea pigs. Those guinea pigs who received verapamil and diltiazem after venom injection showed non-significant levels of necrosis.

Total Ca^{2+} Content:

Table I shows the total calcium content in liver of evenomated guinea pigs and rats with venom only and with venom + drugs. In groups injected with venom only, total Ca^{Z+} content of tissues increased significantly as compared to controls ($P < 0.05$).

Verapamil and Diltiazem Efficacy:

In groups injected with venom followed by verapamil or diltiazem, no significant increase in total Ca^{2+} content in liver was noted as compared to those given venom only (Table I). The total Ca^{2+} content of venom + drug groups, comparable to control group. An increase in survival time upto 24 hours (double than original LD_{50} level of 12 h) and a significant decrease in clinical signs was also observed in groups treated with both drugs.

Discussion

In the present *study, Hydrophis cyatocinems* venom induced fatty change, necrosis and hemorrhage in liver of envenomated guinea pigs accompanied by a significant increase in total Ca^{2+} -content of necrotic liver as compared to controls in both guinea pigs and albino rats. This suggest that the cellular and tissue necrosis were possibly due to an increase in Ca^{2+}-dependent processes resulted due to an increase in Ca^{2+} concentration within the cells. It has been well documented that the venom and poisons of jellyfish and shellfish are known to be "cytotoxic" because it increases cell membrane permeability to

cations like Na^+ and Ca^{2+} (Muhvih *et al.,* 1991; Terao *et al.,* 1988). We have also recently reported the pathogenesis of cytotoxic compounds from jellyfish *Physalia* ulnculus and shellfish Perna *vindis* in which cellular and tissue necrosis in all organs were found to be due to an increase in Ca^{2+} concentration within the cells (Alam *et al.,* 1993). However, i.p. doses of two Ca^{2+} -antagonist drugs, verapamil and diltiazem in present study cause significant decrease in Ca^{2+} content. An increase in survival time of animals was also noted in almost all cases.

Previously several scientists have demonstrated that antihistamines and Ca^{2+}-antagonist drugs not only protect against the irregular Ca^{2+} -dependent processes but also control Ca^{2+} influx (Terao *et al.,* 1988; 1990). It was also reported that vcrapamil inhibited Ca^{2+} -dependent processes, specially the toxin activity on pre- and postsynaptic L-voltage dependent Ca^{2+} channels (Crosland, 1991). Diltiazem induced its protective effectiveness not only by inhibiting the influx of Na^+ and Ca^{2+} within the cells but also possibly by inhibiting pre- and post - synaptic action of phospholipases, a major component of sea-snake venom which require Ca^{2+} for its activity (Crosland, 1991). Previously in our laboratories, Ca^{2+}-antagonist drugs, verapamil, diltiazem and nifedipine, were tested against animals treated with sea-snake venoms from *Hydrophis spirally H. lapemoides* and *Lapemis* corms and jellyfish venom from *Physalia physalis* (Alam, 1992a). All three groups exhibit wide range of protective efficacy, in some cases upto 100% level.

Table 1
Total calcium content (μg/mg protein) in liver of Guinea pigs (A) and Albino rats (B) treated with *Hydrophis cyanocinctus* venom and drugs

	*Groups			
	Venom	Venom + Verapamil	Venom + Diltiazem	Control
A)	3.70 ± 0.09	1.90 ± 0.03	2.03 ± 0.04	1.13 ± 0.04
B)	4.72 ± 0.04	2.08 ± 0.03	2.05 ± 0.05	1.64 ± 0.04

*Number of animals in each group = 6.
Number of control animals = 6
Results are expressed as mean ± S.E.

It is thus concluded from present study that, sea-snake *Hydrophis cyanocinctus* venom is cytotoxic and induce hepatoxicity in animals by an abnormal stimulation of Ca^{2+}-dependent process through an increase Ca^{2+} influx within the cells. This is also confirmed by administration of verapamil and diltiazem, two potent Ca^{2+}-antagonist drugs, which cause significant decrease in total Ca^{2+} content in liver tissue as well as necrosis.

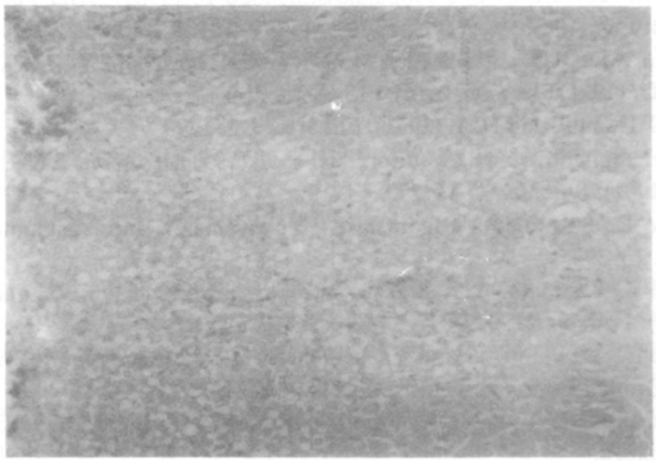

Fig. 1: Photomicrograph of liver showing fatty changes H x E. x 175.

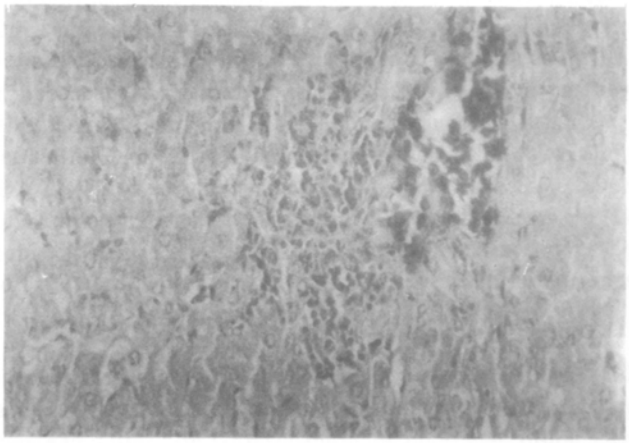

Fig. 2: Photomicrograph of liver showing increase cellularity H x E. x 350.

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