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Pharmacological Characterization of the Rat's Paw Oedema Induced by *Echis coloratus* Venom

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Abstract: The present study was conducted to investigate the inflammatory response induced by *Echis coloratus* venom (ECLV) in the rat hind-paw by measuring paw oedema. Non-heated ECLV (50 μg paw^{-1}) caused a marked paw oedema (plateau) accompanied by intense haemorrhage whereas heated venom (97°C, 30 s; 3.125-75 μg paw^{-1}) produced a dose and time-dependent non-haemorrhagic oedema. The response with heated ECLV was maximal within 15 min, disappearing over 24 h. Heated ECLV (50 μg paw^{-1}) was optimized to test the effect of various drugs on oedema induced by the ECLV. The results showed that cyproheptadine (H_1 and H_3) receptor antagonist highly ($P < 0.001$) reduced venom-induced rat paw oedema and was moderately ($P<0.01$) reduced by dexamethasone. A proteinase inhibator (aprotinin), cyclooxygenase inhibitor (indomethacin) and histamine (H_1) receptor antagonist (chlorpheniramine) produced a low but significant inhibition of oedema formation. The commercially available antivenom was found to be ineffective when administered intravenously, whereas its local administration partially reduced rat paw oedema induced by ECLV, but was not statistically significant. The present study concluded that ECLV alone induced oedema and the expected principal mediators of this inflammatory response were serotonin, histamine, cyclo-oxygenase and other prostaglandins (PGs) and cytokines. Finally the polyspecific antivenom given intravenously could not prevent the oedema forming effect in rats.

Key words: Rat paw oedema, *Echis coloratus* venom, Inflammatory mediators, Antagonists Antivenom

Introduction

The eastern (Burton's) carpet viper (*Echis coloratus* Gunther, 1878) is widely distributed from Africa (eastern Egypt) through Palestine, Israel, Jordan, Lebanon and Saudi Arabia (Gasperetti, 1988; Cherlin and Brokin, 1990; Warrell, 1995). In Saudi Arabia it inhabits the desert, rocky terrain and is found in the North, Central (East and West) and South Regions (Gasperetti, 1988; Al-Sadoon, 1989, 1991; Al-Sadoon and Al-Farraj, 1992). This species has got the highest incidence of snake bites, next to *Cerastes gasperettii* (Al-Sadoon and Al-Farraj, 1992) and has also got a similar reputation as the second most common cause of snake envenoming in Israel (Benbassat and

Shalev, 1993). The average total length (TL) of this species (Fig. 1) is 75 - 85 cm with a maximum TL of of 92 cm. The bites of the *Echis* genus could be responsible for the highest mortality level that exceeded all other snake genera, with a death rate of 7 - 15% of untreated victims (Warrell *et al*., 1977; Warrell and Arnett, 1976). Three species (or subspecies) that belong to this genus are found in the Arabian Peninsula, *Echis pyramidum*, *Echis carinatus sochureki* and *Echis coloratus* (Gasperetti, 1988).

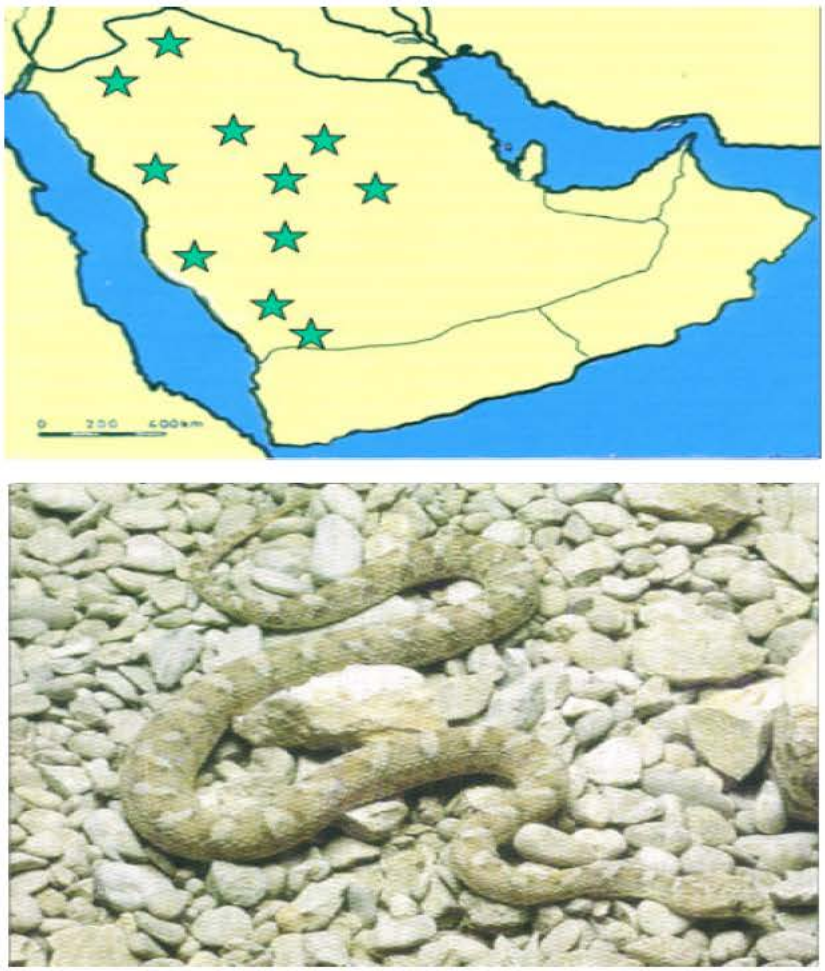

Fig. 1: Panel A shows the distribution of *Echis coloratus* in Saudi Arabia. Panel B shows *E. coloratus*, a specimen from Central Saudi Arabia

Clinical symptoms of *Echis* envenomation are characterized by highly complex pathophysiological features of local as well as systemic nature (Warrell, 1993). The local

manifestations caused by ***Echis*** venoms include oedema, pain, haemorrhage and necrosis, (Rosenfeld, 1971; Kingston, 1981; Kamiguti ***et al.***, 1991; Warrell, 1995; 1993; Milani-Junior ***et al.***, 1997; Al-Jammaz ***et al.***, 1999; Jorge ***et al.***, 1999). The systemic complications are characterized by hypofibrinogenemia, thrombocytopenia and a decline in coagulation factor V and VIII:C (Weiss ***et al.***, 1973; Schaeffer ***et al.***, 1986). Other viper venoms led to the same situations (Lobo ***et al.***, 1994, 1998). Oedema is a common feature of the cutaneous inflammatory response and is dependent on a synergism between the mediators of vascular permeability and blood flow (Williams and Morley, 1973; Williams and Peck, 1977; Williams, 1979; Brain and Williams, 1985). One of the important consequences of altered capillary permeability in local inflammation is the extravasation of leucocytes (Hamblin, 1994). The degree of accumulation of these cells at inflammatory sites in the skin is related to local blood flow (Issekutz and Movat, 1979; Issekutz, 1981; Buckley ***et al.***, 1991).

Several investigators have studied the biochemical and pharmacological effects of the venoms collected from different species that belong to the genus ***Echis*** (Moav ***et al.***, 1963; Theakston ***et al.***, 1982; Theakston, 1983; Theakston and Reid, 1982; Al-Jammaz ***et al.***, 1999). However, to our knowledge, the venom inflammatory effect of ***Echis coloratus***, an inhabitant of the Arabian Peninsula has not been studied so far. This investigation reflects dose-related and time-course inflammatory effects of ECLV using rat's paw oedema model. The effects of various drugs including cyproheptadine, chlorpheniramine, indomethacin, aprotinin, dexamethasone and a commercial antivenom were also examined.

Materials and methods

Reagents

Chlorpheniramine and indomethacin were obtained from Hikma, APM, Jordan; dexamethasone and cyproheptadine from Merck and Co., Inc., Rahway, NJ, USA and aprotinin from Buyer AG, Germany.

Venom and antivenom

ECLV venom was obtained from Zoology department, King Saud University, Riyadh, Saudi Arabia. The venom was dissolved in saline (final concentration 10 mg ml^{-1}) and immediately stored at -20°C until used. The polyvalent antivenom used in this study was a national product, obtained from Al-Hyatt Company Riyadh, Saudi Arabia. The product had been raised in horses using a mixture of ***Echis carinatus***, ***Pyramindum***. ***Echis coloratus***, ***Bitis arietans***, ***Cerastes cerastes***, ***Naja haje*** arabica ***and Walterinnesia aegyptia*** venoms.The antivenom was dialized to remove the preservatives.

Measurement of rat paw oedema

Adult male Wistar rats weighing 120-170 g were used for all the experiments and were provided by the Armed Forces Hospital, Research Center (Animal House Services). All experiments

were carried out according to the methods described by Faria ***et al.*** (2001). The animals were injected into the subplantar region of the right hind paw with 0.1 ml of either heated (30 s, 97°C) or non-heated ECLV (3.125-75 μg paw^{-1}). The left hind paw was used as control and received the same volume of sterile saline. The oedema was measured at 0.25, 0.5, 2, 4, 6 and 24 h using Plethysmometer (Ugo-Basil, Italy). The results were expressed as mean differences between the final and initial volumes (ml) of the injected paws.

Influence of various substances on ECLV-induced oedema

The group of rats (n = 5 each) were pretreated with different classes of drugs, as follows: (1) dexamethasone (1 mg kg^{-1}, s.c., 2 h before); (2) H_1 and H_3 receptor antagonist, cyproheptadine (8 mg kg^{-1}, i.p.,15 min before); (3) H_1 receptor antagonist, chlorpheniramine (16 mg kg^{-1}, i.p., 15 min before); (4) cyclooxygenase inhibitor, indomethacin (30 mg kg^{-1}, i.p., 30 min before) and (5) a proteinase inhibitor, aprotinin (2000 KIU kg^{-1}, i.p., 30 min before). Following the appropriate time intervals, the animals received an intraplantar injection of ECLV (50 μg paw^{-1}) and oedema was measured as described before. The commercial antivenom was either injected intravenously [1.3 mg kg^{-1} of total $F(ab)_2$] or locally together with venom during a 30 min incubation at 37°C.

Statistical analysis

Data were analyzed by analysis of variance (ANOVA) and followed by a Bonferroni test (SPSS Program). A P-value of less than 0.05 was considered to indicate significance.

Results

Effect of ECLV on rat paw oedema

Subplantar injection of non-heated ECLV venom (50 μg paw^{-1}) caused intense haemorrhage and marked paw oedema (0.9 ± 0.03 ml) compared to saline-injected paws (0.09 ± 0.004 ml, n = 5; $P < 0.001$). Since the local haemorrhage interferes with inflammatory oedema development, we decided to heat the venom (97°C, 30 s) in order to destroy the haemorrhagic factors, as previously employed by Perales ***et al.*** (1992).

Subplantar injection of heated ECLV (3.125-75 μg paw^{-1}) produced a dose and time dependent non-haemorrhagic oedema (Fig.2 A,B). The maximal response was observed 15 min after venom injection, decreasing gradually over 24 h. For further experiments, heated venom was routinely used at the dose of 50 μg paw^{-1}.

Pharmacological modulation of heated ECLV rat paw induced oedema

Three of the tested drugs, chlorpheniramine (16 mg kg^{-1}, i.p., 15 min before), indomethacin (30 mg kg^{-1}, i.p., 30 min before) and aprotinin (2000 KIU kg^{-1}, i.p., 30 min before) caused a low, but significant ($P < 0.05$) inhibition of the venom-induced oedema. The treatment of the animals with dexamethasone (1 mg kg^{-1}, s.c., 2 h before) was moderately significant ($P < 0.01$) in reducing

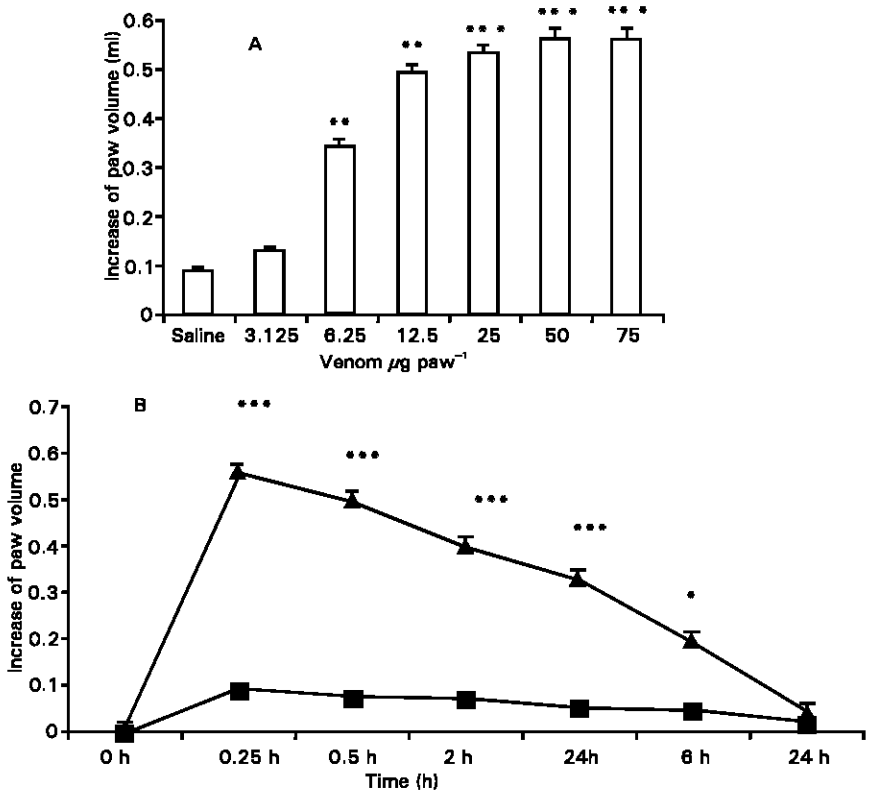

Fig. 2: Rat paw oedema induced by heated ECLV. Panel A shows that the intensity of oedema varies with varying doses (3.125 µg to 75 µg paw^{-1}) of venom. Panel B shows the time course oedema (0.25 to 24 h) using 50 µg paw^{-1} of venom(?), compared to saline (|). The venom was heated at 97 °C for 30 s. The control group received saline 0.1 ml in the same experimental conditions. Each column represents mean ± S.E.M. of five rats. Low significance (* P < 0.05), moderately significant (** P < 0.01) and highly significant (*** P < 0.001)

the venom-induced oedema. Cyproheptadine (8 mg kg^{-1}, i.p., 15 min before) was highly significant (P< 0.001) in the reduction process of the oedema (Fig. 3).

Local injection of the commercial antivenom partially reduced the venom-induced paw oedema (28.6 ±0.084%). In a separate group of experiments, intravenously administered (1.3 mg kg^{-1}) commercial antivenom failed to modify the venom-induced oedema.

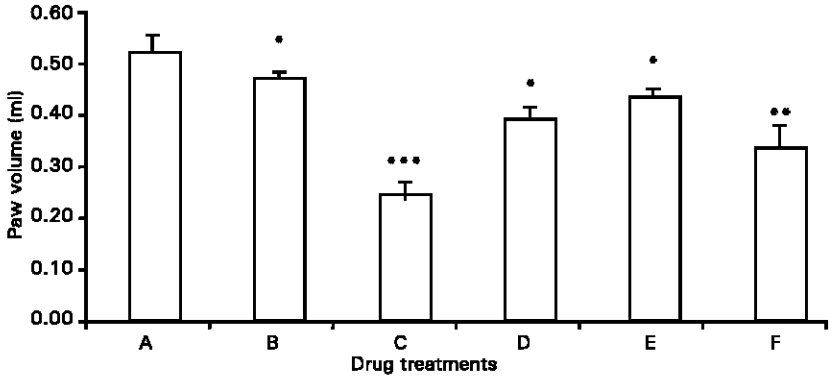

Fig. 3: The effect of (B) aprotinin (2000 KIU kg^{-1}, i.p., 30 min before), (C) cyproheptadine (8 mg kg^{-1}, i.p., 15 min before), (D) chlorpheniramine (16 mg kg^{-1}, i.p., 15 min before), (E) indomethacin (30 mg kg^{-1}, i.p., 30 min before) and (F) dexamethasone (1 mg kg^{-1}, s.c., 2 h before) on the rat paw oedema induced by heated ECLV (50 μg paw^{-1}). (A) the control group received saline instead of drugs in the same experimental conditions. Each column represents mean ± S.E.M. of five rats. Low significance (* $P < 0.05$), moderately significant (** $P < 0.01$) and highly significant (*** $P < 0.001$).

Discussion

Previous studies done on this species (*E. coloratus*) were focussed on serum and tissue (*in vitro*) profile of experimental animals. Snake venom components, especially those of vipers, either activate, inhibit or liberate enzymes by cellular organelles destruction (Moustafa *et al.*,1974; Marsh *et al.*, 1997; Abdel-Nabi *et al.*, 1997). The different toxic effects exhibited by venoms of vipers were due to their contents of proteolytic and lipolytic enzymes (Tan and Ponnudurai, 1990). Common antecedent envenoming signs were hypoglycaemia (Abu-Sinna *et al.*, 1993), general metabolic disturbances (Mahmoud, 1983), muscular dystrophy (Mohamed and Khaled, 1966), nephrotoxicity (Ickowitz *et al.*, 1966) and induction of cytotoxicity (Bertke and Atkins, 1961).

This study shows that subplantar injection of either non-heated or heated ECLV could cause a significant paw oedema in the rat. Viper envenoming produced oedema and altered vascular permeability in the mouse hind paw. Following these responses was an abundant leukocyte infiltration and haemorrhage ensued due to high doses of venom (Lobo *et al.*, 2000). Since haemorrhage appeared soon after venom injection and interfered with the development of inflammatory oedema (Mandelbaun *et al.*, 1975; Assakura *et al.*, 1986; Faria *et al.*, 2001). In this study, heated the venom in order to destroy the heat-labile proteolytic enzymes, the haemorrhagic factors, aiming to observe the inflammatory components of this venom.

Increased vascular permeability and increased blood flow play an important role in oedema formation (Williams and Peck, 1977; Williams, 1979; Brain and Williams, 1985). The capacity of

exogenously applied vasodilators such as calcitonin gene-related peptide (CGRP), prostaglandin E_2 and prostacyclin to potentiate inflammatory oedema in response to different inflammatory mediators is well known (Williams, 1983). These results showed that the ECLV induced rat paw oedema might indicate a direct relationship between local blood flow and the intensity of oedema.

In an attempt to further understand the pharmacological mechanisms involved in ECLV-induced rat paw oedema, different medications were used. These findings showed that treatment of the animals with cyproheptadine (H_1 and H_3 receptor antagonist) and chlorpheniramine (histamine H_1 receptor antagonist) reduced the venom induced paw oedema, indicating the role of ***in vivo*** mast cell degranulation inhibition (Faria ***et al.***, 2001).

Arachidonic acid provides a number of inflammatory mediators, via the action of cyclooxygenase or lipoxygenase (Faria ***et al.***, 2001). The use of cyclooxygenase inhibitor (indomethacin) significantly reduced the paw oedema in response to ECLV. The treatment of the animal with dexamethasone also caused a moderately significant reduction in the venom induced paw oedema. This was expected as corticosteroids were known to indirectly inhibit the phospholipase A_2 action (Flower, 1989). Furthermore, corticosteroids also directly acted on leukocytes and other cell types inhibiting the release of cytokines and other inflammatory mediators (Angeli ***et al.***, 1999). Gutierrez ***et al.*** (1986) and Lomonte ***et al.*** (1993) had a similar report on the kinetics and cell composition of the inflammatory infiltrate observed in the foot pad of the rat. Aprotinin also significantly reduced the induced oedema. It is an inhibitor of many proteases such as kallikrein whose products are bradykinin and kallidin (Erdos, 1963; Goth, 1978). These kinins are potent vasodilators that also increase the capillary permeability and are easilly produced in tissues after injury; thus being cardinal agents in oedema formation (Johnson and Erdos, 1973; Goth, 1978). The primary difference between my findings and others as those of Faria ***et al.*** (2001) was that ECLV iduced oedema without potentiators. Secondly, the cyclooxygenase inhibitors (indomethacin) reduced the swelling induced by ECLV, which indicate that indomethacin metabolite might be involved in mast cell activation by this venom. Furthermore, significantly reduction was obseved from H_1 and H_3 receptor antagonists. Contrast results were obtained by Lobo ***et al.*** (2000), Trebiens (1989) and Perales ***et al.*** (1992).

Finally, we attempted to examine the ability of polyspecific antivenom to neutralize the oedematogenic activity of this venom. Although local effects of antivenoms such as myonecrosis and haemorrhage (of other venoms) were largely studied (Bjarnason and Fox, 1994; Gutierrez and Lomonte, 1995), none of them were done on the oedematogenic effect of ECLV. When a mixture of venom and antivenom was administered in the paws, a partial inhibitory effect was observed, whereas the intravenous administration of antivenom failed to reverse venom-induced oedema. This situation could raise the query about employing ECL antivenom in treating victims. Benbassat and Shalev (1993) had investigated this by reviewing reported data on the effect of ECLV ***in vitro***, laboratory animals and humans and reexamined alternative treatment methods in order to assess the efficacy of using antivenoms. Tilbury ***et al.*** (1987) on ECL, had speculated and reviewed this situation.

In regards to oedema, it could be suggested that antibodies raised against oedematogenic component(s) of ECLV were in very low amounts (highly diluted) in the total antiserum. This finding is in agreement with those of Faria ***et al.*** (2001). Further studies attempting to purify the oedematogenic component(s) present in this venom are necessary to elucidate this aspect.

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