ORIGINAL ARTICLE

W-7 (A CALMODULIN ANTAGONIST) INHIBITS CARRAGEENAN-INDUCED PAW EDEMA IN INTACT AND ADRENALECTOMIZED RATS

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ABSTRACT

The aim of present study was to investigate the effect of W-7, a calmodulin antagonist, on carrageenan-induced rat's paw edema. W-7(50 μMol/kg) was given intraperitoneally synchronous with the intraplantar injection of 0.1 ml of 0.5% carrageenan solution. After four hours, paw edema was assessed by calculating the paw volume changes which measured by hydroplethysmometer. In adrenalectomized rats, both adrenal glands were removed. Treatment of animals with W-7 reduced carrageenan-induced paw edema by 50%. In adrenalectomized rats, W-7 was also effective in reduction of paw edema (52%). There was no significant difference between the effect of W-7 in adrenalectomized and control animals. Our findings suggest that W-7 as a calmodulin inhibitor reduce carrageenan-induced paw edema and the inhibitory effect of W-7 on edema formation appears not to be dependent on adrenal function.

Keywords: Paw edema, W-7, Calmodulin, HPA axis.

INTRODUCTION

Edema is a characteristic feature of inflammatory diseases such as asthma and rheumatoid arthritis. Developing a suitable management strategy for reducing inflammatory edema is becoming increasingly recognized as an important route for successful treatment of inflammatory diseases. Despite a great deal of research, the exact mechanisms underlying the development of edema remains unclear.

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Calmodulin is the ubiquitous calcium-sensing regulatory protein which is involved in a variety of cellular function through the activation and regulation of different target enzymes and proteins, including protein kinases, ion channels, and NO synthesis and so on (Biswas *et al*., 2001 and Broeke *et al*., 2004). Increasing evidence indicates that calmodulin is involved in inflammatory reaction. It has been shown that calcium/calmodulin complex activates the membrane-bound phospholipase A_2 which acts on the cell membrane phospholipids producing prostaglandins, leukotrienes and thromboxanes from arachidonic acid (Wong and Cheung, 1979; Craven and DeRubertis, 1983; Moskowitz *et al*., 1983). In mast cells, calcium may bind to calmodulin and it stimulates enzymes important in the synthesis of newly generated mediators including prostaglandins and leukotrienes (Fanta, 1984).

In addition, Impairment of endothelial barrier which is the main physical barrier in the extravasations of blood components to the surrounding tissue leads to an increase in vascular permeability and edema formation (van Nieuw Amerongen *et al*., 1998). Different inflammatory mediators increase vascular permeability through endothelial gap formation which is thought to be due to endothelial cell contraction (Buckley and Ryan, 1969 and Baluk *et al*., 1997). It has been reported that Ca2+/calmodulin interaction activates myosin light chain kinase to phosphorylate myosin light chains, leading to increased actomyosin interaction, force development, and subsequent contraction (Schnittler *et al*., 1990; Lum and Malik, 1994; van Hinsbergh, 1997). Therefore calmodulin, at the cell and molecular level, is involved in edema formation during inflammatory reactions.

The carrageenan induced rat paw edema is a well-established model which is commonly used to study acute inflammation (Bhattacharya *et al*., 1988 and Gualillo *et al*., 2000). Several mechanisms have been reported to be involved in rat paw edema due to carrageenan injection such as prostaglandin's release by injured dermal mast cells (Di Carlo *et al*., 1992 and Gualillo *et al*., 2000), endothelial hyper-permeability (Medeiros *et al*., 1995 and Cirino *et al*., 1996), monocyte activation (Das *et al*., 2006), cytokine expression (Hozumi *et al*., 2006), and so on. However less is known about the involvement of calmodulin in carrageenan-induced edema.

The present study was designed to investigate the effect of W-7, a well known calmodulin antagonist, on acute edema induced by carrageenan in rats.

MATERIALS AND METHODS

Animals

Male Albino rats (200-250g) were supplied by the Animal Care Center, College of Medicine, Rafsanjan University of Medical Sciences and were housed four per cage under a 12 h light/dark cycle in a room with controlled temperature (22±1˚c). Food and water were available ad libitum except in adrenalectomized (ADX) rats. Animals were handled daily (between 9:00 and 10:00 a.m) for 5 days before the experiment day in order to adapt them to manipulation and minimize nonspecific stress responses. Rats were divided randomly into five experimental groups, each comprising 7-9 animals: Groups 1and 2 were control rats received distilled water (as vehicle) or W-7 (50μM/kg). Groups 3 and 4 were ADX rats received vehicle or W-7 (50μM/kg). Group 5 was sham operated. All experiments followed the guidelines on ethical standard for investigation of experimental pain in animals (Zimmermann, 1983).

Drugs

Carrageenan (Sigma, CO, UK) was dissolved in sterile saline yielding to 0.5% carrageenan solution. W-7 was purchased from Alexis and dissolved in distilled water.

Induction and measurement of the inflammation

Animals were anesthetized with thiopental (40 mg/kg) intraperitoneally (i.p). Both paw volumes were measured with the aid of a hydroplethysmometer immediately prior to the injection of carrageenan. Then, 0.1 ml of 5% carrageenan solution was injected into the left hind paw. The right hind paw was received the same volume of saline. 4 h after injection, again both paw volumes were measured and the algebraic difference between the increase in carrageenan-treated and saline-treated paw volume considered as Carrageenan-induced edema (Gualillo *et al*., 2000).

Adrenalectomy

Animals were anesthetized with thiopental (40 mg/kg) i.p. and both adrenal glands were removed through two dorsal incisions. The sham operation consisted of bilateral dorsal incision, plus locating and exposing the adrenals. All adrenalectomized rats were maintained on 0.9% sodium chloride drinking solution; whereas the sham operated rats were kept on tap water. The animals were tested 7 days after adrenalectomy or sham procedure (Khaksari *et al*., 2004).

Statistical analysis

All results are reported as mean ± SEM. The Student's t-test was used for statistical evaluation of the results when two groups were compared. When more than two groups were compared, analysis of variance followed by Tukey's test was used. Two way ANOVA was used

to compare the effects of W-7 on measured parameter, in control and ADX rats. A value of $p<0.05$ was considered significant.

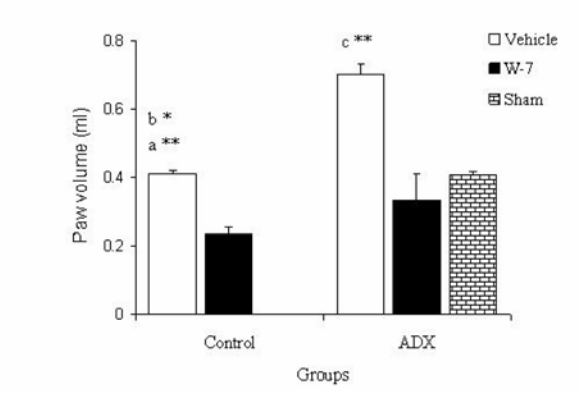

Fig. 1: Effect of W-7 (50 µmol/kg) treatment on carrageenan induced paw volume in control and ADX rats. Edema was induced by intraplantar injection of 0.1 ml of 0.5% carrageenan solution in the left hind paw and the increase in paw volume measured as described in methods. W-7 and vehicle were administered i.p. Results are expressed as the mean ± S.E.M with n = 7-9 rats per group. [a] significant difference between W-7 and vehicle in control group, [b] significant difference between vehicle in control and ADX groups, [c] significant difference between W-7 and vehicle in ADX group. ADX; Adrenalectomized, i.p; intra peritoneal. * P< 0.05, ** P< 0.001.

RESULTS

To investigate the effect of W-7(as a calmodulin antagonist) on edema formation, W-7 (50 µMol/kg) or vehicle (distilled water) was administered i.p simultaneous with intraplantar injection of carrageenan.

As shown in figure 1, paw edema induced by carrageenan administration, was 0.41 ± 0.01 ml in control group. This increased volume was inhibited significantly by w-7 administration (0.23 ± 0.02 vs. 0.41 ± 0.01, unpaired t-test P< 0.001).

In ADX animals, carrageenan induced paw edema was more effective than in control animals (0.7 ± 0.03 vs. 0.41 ± 0.01 ml, ANOVA followed by tukey P< 0.05). W-7 administration in ADX group -similar to control group- inhibited carrageenan induced paw edema (unpaired t-test, P<0.001).

There was no significant difference between paw volume reductions due to W-7 administration in ADX compared to control groups (two ways ANOVA P> 0.05). These data suggest that central mechanisms are not involved in anti-inflammatory effect of W-7.

DISCUSSION

In this study we have evaluated the role of calmodulin in edema formation elicited by carrageenan. Our results demonstrate that inhibition of calmodulin by W-7 effectively attenuated the development of edema in rat's hind paw.

The carrageenan-induced rat paw edema has been used commonly as an acute inflammatory model (Vinegar *et al*., 1987 and Gualillo *et al*., 2000). The dermal inflammatory response by carrageenan consisted of dermal mast cells injury (Klymenko and Pavlova, 1997 and Carvalho *et al*., 2006) and intervention of host of mediators such as oxy-radicals (Broeke *et al*., 2004), nitric oxide (Medeiros *et al*., 1995 and Biswas *et al*., 2001), prostaglandins, cytokines (Das *et al*., 2006) etc. It has been shown that calmodulin is involved in the release of reactive intermediates such as prostaglandins and leukotrienes by injured mast cells (Wong and Cheung, 1979 and Moskowitz *et al*., 1983). In addition, edema formation is partially mediated by disruption of endothelial barrier which is thought to be due to endothelial cell contraction, a process that involves actin-myosin interaction (Lum and Malik, 1994). This interaction requires Ca^{2+}, calmodulin (CaM), and the phosphorylation of the myosin light chain by Ca^{2+}/CaM–dependent protein kinases (Warren, 1993 and Lum and Malik, 1994). According to these reports, it has been proposed that the inhibition of calmodulin activity may abolish edema formation.

Previous studies have been reported that Calmodulin antagonists such as trifluoperazine were effective in reducing inflammatory parameters. However they used different inflammatory models (Beitner *et al*., 1989a and Beitner *et al*., 1989b).

Moreover –similar to control- our data showed that W-7 was also effective in inhibition paw edema in ADX animals (52%). This result suggests that central mechanism (the hypothalamus-pituitary-adrenal axis) does not play a major role in anti-edema action of W-7. However, HPA and their glucocorticoids have the natural anti-inflammatory effect and it has been shown that many factors can modulate the activity of this axis at the level of adrenal, pituitary and hypothalamus (Berczi, 1997 and Harbuz *et al*., 1997). So, further studies need to clarify the effects of W-7 on HPA axis activity at the level of adrenal, pituitary or hypothalamus.

In summary, it seems that calmodulin antagonists might be of pharmacological interest as potential anti-edema agents. Further studies are being carried out to better understand the mechanism of their anti-

inflammatory action and determine the relevance of this finding in human.

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