Carbohydrate Polymers 92 (2013) 414–420

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Proinflammatory activity of an alginate isolated from *Sargassum vulgare*

Kézia O.A.L. Lins, Mariana L. Vale, Ronaldo A. Ribeiro, Letícia V. Costa-Lotufo*

Departamento de Fisiologia e Farmacologia, Universidade Federal do Ceará, P.O. Box 3157, 60430-270 Fortaleza, Ceará, Brazil

ARTICLE INFO

Article history:
Received 21 February 2012
Received in revised form 22 August 2012
Accepted 26 August 2012
Available online 1 September 2012

Keywords:
Sargassum vulgare
Alginate
Inflammation
Paw edema
Neutrophil migration

ABSTRACT

Alginates are unbranched polymers of polysaccharide presented as the structural components of marine brown algae. The proinflammatory activity of SVHV, an alginate isolated from *Sargassum vulgare*, was investigated using models of paw edema, mast cells degranulation and neutrophil migration *in vivo*. SVHV induced a dose dependent paw edema, with a peak at 2 h, associated with an increased myeloperoxidase activity and production of TNF-α and IL-1β. Pharmacological modulators, remarkably dexamethasone and indomethacin, inhibited the edema. SVHV (1.0 mg) also led to a significant induction of neutrophil migration in the peritoneal cavity of rats. This neutrophil migration was significantly reduced by peritoneal resident macrophages depletion, but was not affected by the depletion of mast cells. Our data suggest that SVHV has proinflammatory activity dependent of the activation of resident cells, being the macrophages the main cells involved.

1. Introduction

Alginates, unbranched polymers of polysaccharide with gel-forming properties made up by blocks of β-(1-4)-D-mannuronic acid (M) and α-(1-4)-L-guluronic acid (G), have emerged as valuable biomaterials with interesting biomedical and biotechnological applications (Gomes d'Ayala, Malinconico, & Laurienzo, 2008; Vériter et al., 2010; Zimmermann, Shirley, & Zimmermann, 2007). According to several authors, alginates present low toxicity, favorable mechanical properties and the capacity for bioresorption of the constituent materials, characteristics that make them suitable biopolymers for the development of controlled-release systems (Lai, Abu'Khalil, & Craig, 2003; Wang & He, 2010). In such context, they have been used for immobilization of Langerhans islets in the treatment of experimental diabetes mellitus in rats and for microencapsulation of hormone-producing cells for the treatment of diabetes mellitus and parathyroid disease (Darquy & Sun, 1987; Fan et al., 1990; Soon-Shiong et al., 1994; Tze & Tai, 1982; Vériter et al., 2010; Zimmermann et al., 2007).

It has been shown that alginates induce monocytes and macrophages stimulation through a NF-κB involved pathway, resulting in an increase of cytokine production (Otterlei et al., 1991; Pasquali et al., 2005; Son, Moon, Rhee, & Pyo, 2001; Thomas, Harding, & Moore, 2000; Yang & Jones, 2009). The macrophage stimulation seems to be related to the alginates ability to enhance the healing process (Thomas et al., 2000). Experimental data suggested that low-molecular weight alginates could be useful in the prevention of obesity, hypercholesterolemia, and diabetes (Kimura, Watanabe, & Okuda, 1996; Wang & He, 2010).

Torres et al. (2007) reported the extraction and physicochemical characterization of two *Sargassum vulgare* alginates, denoted as SVLV (*S. vulgare* low viscosity) and SVHV (*S. vulgare* high viscosity). Based on ^{1}H NMR and fluorescence data, as well as rheological measurements, the authors concluded that SVLV and SVHV presented a higher M/G ratio, while being much richer in mannuronic block structures than other alginates isolated from *Sargassum* spp. Further, the antitumor activity of SVLV and SVHV has been demonstrated against sarcoma 180 cells transplanted in mice, where SVHV was more active and less toxic (Sousa et al., 2007, 2008). It was additionally shown that the referred alginates presented no cytotoxicity *in vitro*, suggesting that this anticancer activity may be mediated by a modulation of the immune system (Sousa et al., 2007). This being said, the aim of the present study was to investigate proinflammatory activities of the alginate with high viscosity (SVHV) isolated from the brown seaweed *S. vulgare* and the inflammatory mediators involved in this process.

2. Experimental

2.1. Drugs and reagents

Carrageenin, compound 48/80, dextran, indomethacin (Sigma–Aldrich, St Louis, MO, USA) pentoxifylline (Trental®, Hoechst, São Paulo, Brazil, 100 mg ampoule), diphenhydramine

* Corresponding author at: Department of Physiology and Pharmacology, UFC, Rua Cel. Nunes de Melo, 1127, P.O. Box 3157, 60430-270 Fortaleza, Ceará, Brazil. Tel.: +55 85 3366 8255; fax: +55 85 3366 8333.
E-mail addresses: costalotufo@gmail.com, lvcosta@secrel.com.br (L.V. Costa-Lotufo).

0144-8617/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carbpol.2012.08.101

(Difenidrin; Cristália – São Paulo, Brazil, 4 mg ampoule), thalidomide (Talidomida®, CEME, Minas Gerais, Brazil, 100 mg tablet) were used in the experiments. ELISA kits for TNF-α, IL-1β and IL-10 quantitation were purchased from R&D Systems (Minneapolis, USA). All other reagents were of analytical grade. SVHV was obtained essentially as described by Torres et al. (2007).

2.2. Animals

The animals were obtained from the Central Animal House of the Universidade Federal do Ceará, Brazil, where they were produced. Rats Wistar (150–180 g) were housed in cages with free access to food and water. All animals were kept under a 12 h:12 h light–dark cycle (lights on at 6:00 a.m.). Animals were treated according to the ethic principles of animal experimentation of COBEA (Colégio Brasileiro de Experimentação Animal), Brazil. The Animal Studies Committee of Universidade Federal do Ceará approved the experimental protocol.

2.3. Inflammatory paw edema induced by SVHV

SVHV (0.1, 0.3 and 1 mg/0.1 mL) was injected subcutaneously into the right hind paw of the rats. Carrageenan (0.3 mg/0.1 mL) and Dextran (0.3 mg/0.1 mL) were used as positive controls. Paw volume was measured immediately before SVHV injection and at selected time intervals (1, 2, 3 and 4 h) with a hydroplethysmometer apparatus (Ugo Basile, Italy). The results were expressed as the increase in paw volume (mL) calculated by subtracting the basal volume. After the experiment, the paw skin was used to measure the enzyme MPO and cytokines TNF-α, IL-1β and IL-10.

2.4. Measurement of paw tissue myeloperoxidase (MPO) activity

The neutrophil infiltration into the paw with edema induced by SVHV was investigated indirectly by measuring myeloperoxidase (MPO) activity, as previously described by Bradley, Priebat, Christenses, and Rothstein (1982). The right hind paw skin was placed in 50 mM potassium phosphate buffer, pH 6.0, containing 0.5% hexadecil-trimethyl-ammonium bromide (1 ml/50 mg of tissue) in a Potter homogenizer. The homogenate was vortex-mixed and centrifuged for 5 min at 2500 rpm. A volume of 10 μl of the supernatant was obtained and added to a 96-well microplate, in triplicate. The followings were added: 200 μl of a buffer solution containing O-dianisidine dihydrochloride (16.7 mg), distilled water (90 μl), potassium phosphate buffer (10 μl) and 1% H_2O_2 (50 μl). The enzyme activity was determined by measuring the absorbance (460 nm) in a plate reader, and recorded at 15 s intervals for 2 min.

2.5. Citokines determination

The amount of TNF-α, IL-1β, IL-10 of tissue supernatants were determined by ELISA kit (purchased from R&D Systems, Minneapolis, USA) following the instruction of manufacturers.

2.6. Effect of pharmacological modulators on the paw edema induced by SVHV

In a second set of experiments, some animals were pretreated with saline (control group), indomethacin (2 mg/kg, s.c.); dexamethasone (2 mg/kg, s.c.), thalidomide (45 mg/kg, s.c.), diphenhydramine (10 mg/kg, i.p.), pentoxifylline (45 mg/kg, i.p.) and L-NAME (10 mg/kg, i.p.) 1 h before the subcutaneously administration of SVHV (1 mg/0.1 mL) into the right hind paw of the rats. Paw edema were evaluated at selected time intervals (1, 2, 3 and 4 h), and then compared to the corresponding controls.

2.7. Effect of SVHV on peritoneal mast cell degranulation

The animals were i.p. treated either with saline, carrageenan (500 μg), compound 48/80 (0.6 mg/kg), or SVHV (1 mg). After 30 min, the mesentery was harvested, placed and fixed with albumine on microscope slides and dyed with a solution of 0.1% toluidine blue (in 0.9% sodium chloride) for 60 s followed by extensive rinsing in deionized water as described previously (Zhong, Chunn, Volmer, Fozard, & Blackburn, 2001). The percentage of degranulated mast cells was determined by counting one hundred stained cells per tissue section.

2.8. In vivo neutrophil migration to peritoneal cavities induced by SVHV

The neutrophil migration to peritoneal cavity was induced by the intraperitoneal injection of saline, carrageenan (0.3 mg) or SVHV (1 mg). After 4 h the animals were sacrificed under anesthesia and cells were recovered by washing the peritoneal cavity with 10 ml of sterile saline containing 5 UI heparin/ml. The fluid was recovered for total and differential cell counts as described previously (Souza & Ferreira, 1985). The results were reported as mean ± SEM of the number of cells per ml.

2.9. Evaluation of the role of peritoneal resident cell population (mast cells and macrophages) in SVHV-induced neutrophil migration

For the investigation of the influence of peritoneal resident mast cells in the SVHV-induced neutrophil migration, this cell population was depleted by chronic treatment with compound 48/80, according to the method described by Di Rosa, Giroud, and Willoughby (1971). The animals were i.p. treated with compound 48/80 for 4 days (0.6 mg/kg twice a day for 3 days and 1.2 mg/kg twice on the day 4). On the fifth day the mast cell depletion was assessed in six rats submitted to 48/80 treatment by counting the number of mast cells stained by toluidine blue in mesenteric tissue. The other rats submitted to depletion procedure received i.p. injections of saline, carrageenan (0.3 mg) or SVHV (1 mg) with the purpose of inducing neutrophil migration. Four hours later the neutrophil migration was evaluated as described above.

For the investigation of the influence of peritoneal resident macrophages in the SVHV-induced neutrophil migration, the rats were anesthetized with a mixture of ketamine (100 mg/kg, vetanarcol®, Konig do Brasil, São Paulo, Brasil) and xilazine (10 mg/kg, Kensol®, Konig do Brasil, São Paulo, Brasil) and three hypodermic needles were inserted into the abdominal cavity. Sterile saline was injected through the needle placed next to the sternum. The abdominal cavity was then gently massaged for 1 min and the peritoneal fluid collected *via* the two needles inserted into the inguinal region. This operation was repeated 3 times. Over 80% of the peritoneal resident macrophages were removed in the lavage fluid and more than 90% of the saline injected was recovered. Sham animals were submitted to the same procedure, but no fluid was injected or recovered. If blood was visually detected in the recovered saline, the animal was discarded. This method was initially described by Faccioli, Souza, Cunha, Poole, and Ferreira (1990) and modified by Ribeiro et al. (1997). Thirty minutes later the peritoneal macrophage population was estimated in 6 rats that were submitted to peritoneal wash and 6 rats that were submitted to sham procedures injecting 10 ml of sterile saline containing 5 UI heparin/ml. The fluid was recovered for total and differential cell counts. The other sham and washed rats were submitted to neutrophil migration induction by i.p. injections of saline, carrageenan (0.3 mg) or SVHV (1 mg). Four hours later the neutrophil migration was evaluated as described above.

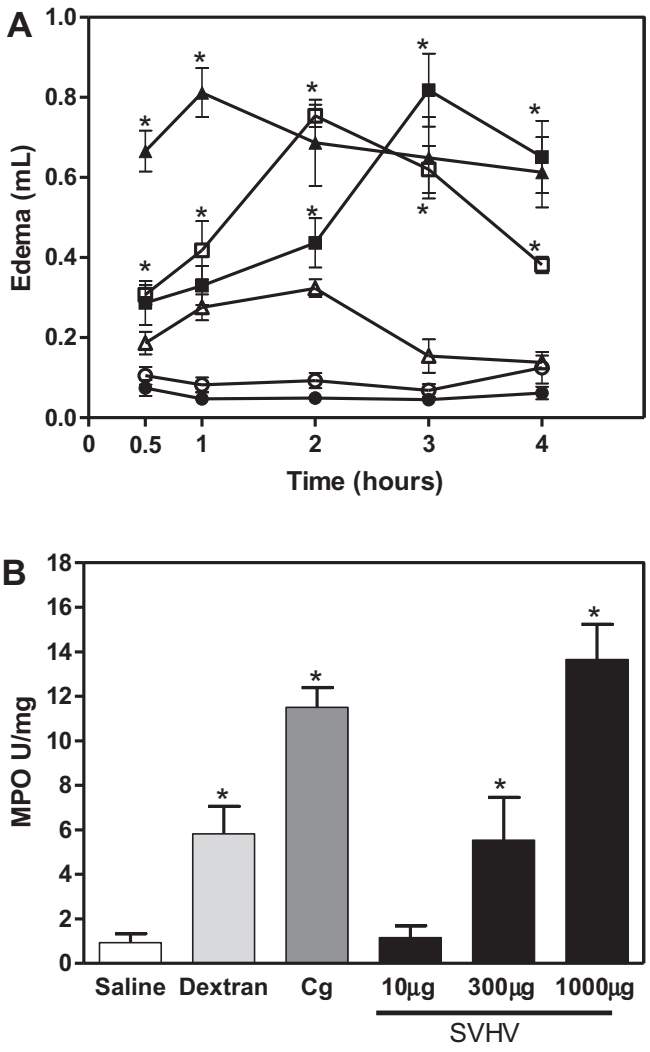

Fig. 1. (A) Effect of the alginate isolated from *Sargassum vulgare* (SVHV) on paw edema formation. Rats were injected subcutaneously into the right hind paw with saline (●), carrageenan (0.3 mg/0.1 mL, ■), dextran (0.3 mg/0.1 mL, ▲), SVHV (0.1 mg/0.1 mL, o), (0.3 mg/0.1 mL, △) or (1 mg/0.1 mL, □). (B) Effect of SVHV on myeloperoxidase (MPO) activity. Paw skins were collected after paw edema test and used to measure MPO activity. Data are presented as mean ± S.E.M. from 6 animals. $^{*}p < 0.05$, compared with saline by ANOVA followed by Student Newman–Keuls.

2.10. Statistical analysis

All results were expressed as mean ± SEM for n experiments. Statistical evaluation was undertaken by analysis of variance (ANOVA) followed by Bonferroni's test for multiple comparisons and *t*-test for comparisons between two groups. A *p* value below 0.05 was considered statistically significant.

3. Results

3.1. Paw edema induced by SVHV: neutrophill recruitment and cytokine production

The subplantar injection of SVHV (0.1, 0.3 and 1.0 mg/paw) caused an intense and dose-dependent (maximal volume of 0.75 ± 0.03 mL at a dose of 1.0 mg/paw) paw edema (Fig. 1A). The increase in paw volume was significant 0.5 h after SVHV (1 mg/paw) administration, peaked at 2 h and remained significantly elevated up to 4 h after the challenge. The maximum response was comparable to that observed with carrageenan after 3 h (0.82 ± 0.09 mL) and dextran after 1 h (0.82 ± 0.06 mL).

SVHV proinflammatory effect is associated with a dose dependent migration of neutrophils, as indicated by the significant increase in the MPO activity (0.3 and 1.0 mg/paw) in the s.c. tissue of the rat paw at 4 h (Fig. 1B). As expected, both, carrageenan and dextran, also led to a massive neutrophill migration to the damaged tissue. Additionally, SVHV (0.3 and 1.0 mg/paw) also led to TNF-α and IL-1β, but not IL-10, production on the site of inflammation (Fig. 2).

3.2. Effect of pharmacological modulators on the paw edema induced by SVHV

In order to examine which inflammatory mediators may be involved in SVHV-induced paw edema, it was studied the effects of several different inhibitors: a TNF-α production inhibitor (thalidomide), a cyclooxigenase inhibitor (indomethacin), an antihistamine (diphenidramine), a nitric oxide synthase inhibitor (L-NAME), a PDE4 inhibitor (penthoxyphiline) and a glucocorticoid (dexamethasone). As shown in Fig. 3, all substances were able to inhibit, at some extension, the SVHV-induced paw edema. However, dexamethasone (2 mg/kg s.c.) and indomethacin (2 mg/kg s.c.) were more efficient, inhibiting SVHV activity by 86 and 78% at 2 h, respectively.

3.3. The involvement of mast cells in SVHV proinflammatory activity

The onset of SVHV proinflammatory activity (0.5 h) and the inhibition of SVHV activity observed after the pre-treatment of rats with diphenidramine suggested the involvement of mast cells on the response elicited by SVHV. Thus, the activity of SVHV in peritoneal rat mast cells was assessed by counting the number of degranulated mast cells present in the peritoneal cavity of treated rats (Fig. 4). In fact, SVHV (1.0 mg) led to a significant degranulation of the mast cell population (52.3 ± 2.8%) compared to control animals (9.2 ± 0.8%) and carragenan-treated animals (17.7 ± 1.7%), but in a weaker fashion than that observed to compound 48/80 itself (96.0 ± 1.5%).

3.4. The involvement of neutrophils in SVHV activity and the role of resident cells

The edema kinetics associated to the MPO activity observed after the subcutaneous plantar injection of SVHV in the rat paw suggests that neutrophils are involved in this proinflammatory activity, which is corroborated by induction of neutrophil migration to peritoneal cavity by SVHV single injection (Figs. 5B and 6B).

In a second set of experiments, it was verified whether resident mast cells or macrophage depletions interfered with neutrophill migration elicited by SVHV. Fig. 5A shows that the peritoneal wash significantly reduced the resident macrophage in 81.50% when compared to sham group and that this depletion reduced the SVHV-induced neutrophil migration to peritoneal cavity in about 75.38% when compared to the sham group (Fig. 5B). Fig. 6A shows that subchronic treatment of animals with compound 48/80 completely depleted the mast cell population in comparison with the control group. Intraperitoneal injection of SVHV (1.0 mg) induced a significant neutrophil migration into rat peritoneal cavities despite pre-treatment with compound 48/80 (Fig. 6B). Compared with non-treated rats (44.1×10^4 neutrophils/mL), the neutrophil migration induced by SVHV (38.8×10^4 neutrophils/mL) was only slightly reduced in the mast cell-depleted animals, but with no statistical significance ($p > 0.05$).

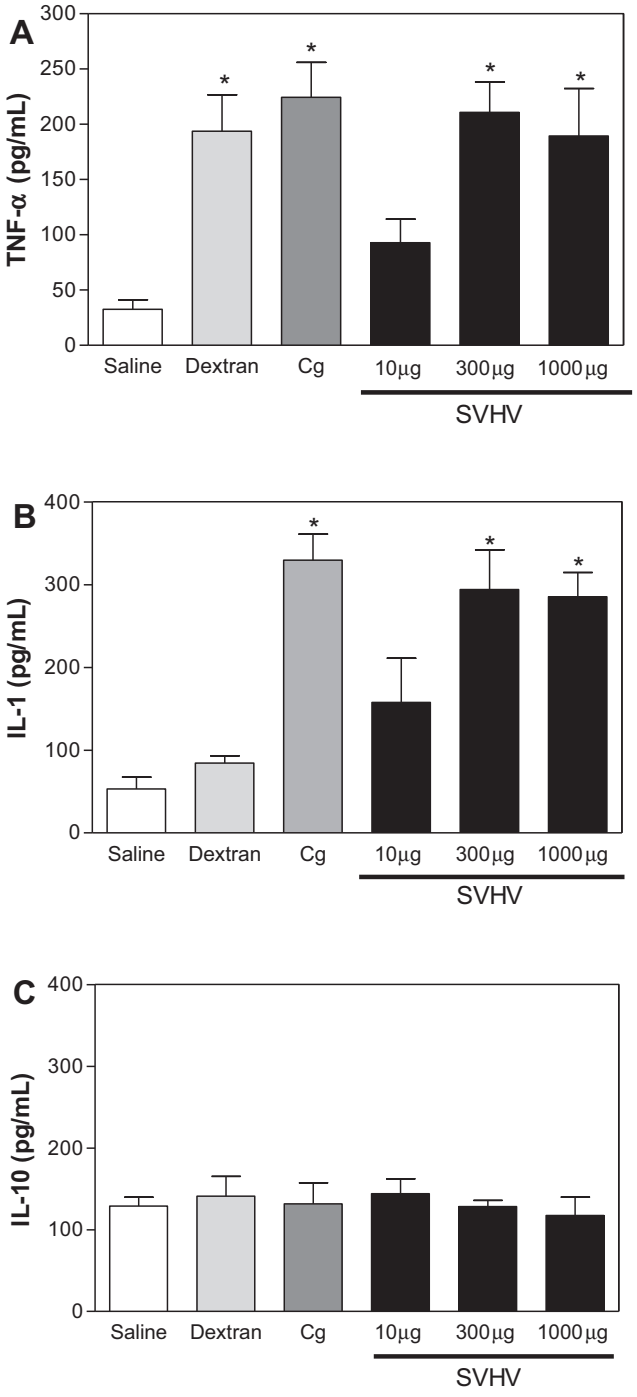

Fig. 2. Effect of the alginate isolated from *Sargassum vulgare* (SVHV) on cytokines production in tissue harvested from rat paw. Rats were injected subcutaneously into the right hind paw with saline, carrageenan (0.3 mg/0.1 mL), dextran (0.3 mg/0.1 mL), or SVHV (0.1, 0.3, 1 mg/0.1 mL). Four hours later the paw tissue was harvested and TNF-α (A), IL-1β (B), and IL-10 (C) levels were measured by ELISA. Data are presented as mean ± S.E.M. from 6 animals. $^{*}p < 0.05$, compared with saline by ANOVA followed by Student Newman–Keuls.

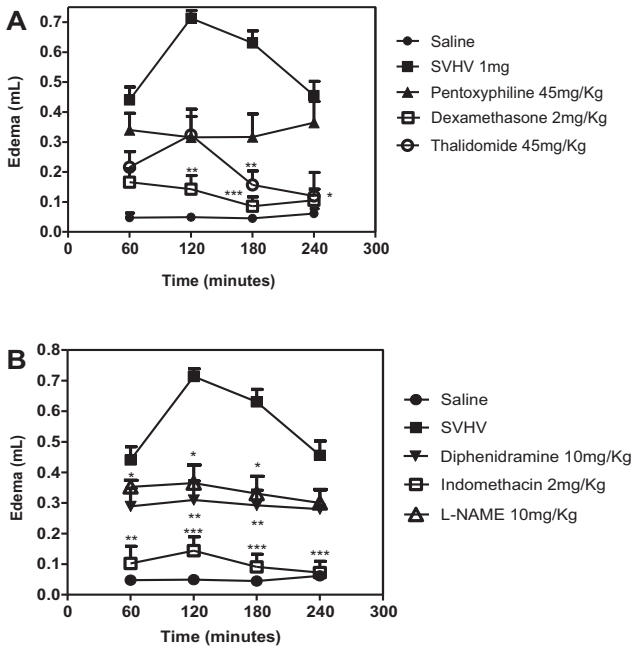

Fig. 3. Effect of pharmacological modulators on the paw edema induced by the alginate isolated from *Sargassum vulgare* (SVHV). Rats were pretreated with saline (control group), indomethacin (2 mg/kg, s.c.); dexamethasone (2 mg/kg, s.c.), thalidomide (45 mg/kg, s.c.), diphenidramine (10 mg/kg, i.p.), pentoxyphiline (45 mg/kg, i.p.) and L-NAME (10 mg/kg, i.p.) before the subcutaneously administration of SVHV (1 mg/0.1 mL) into the right hind paw of the rats. Data are presented as mean ± S.E.M. from 6 animals. $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ compared with saline by ANOVA followed by Student Newman–Keuls.

4. Discussion

Many polysaccharides can induce inflammation when locally administered, as these molecules target different cells related to the immune system, such as macrophages, lymphocytes and natural killer cells, stimulating cytokine release and immune responses (Leung, Liu, Koon, & Fung, 2006; Yang & Jones, 2009). In this context, carrageenan, a complex group of polysaccharides made up of repeating galactose-related monomers also isolated from algae, is greatly characterized as an inflammatory stimulus, and the carrageenan-induced rat paw edema is amongst the most used model for the study of the inflammatory response and inhibitors (Morris, 2003).

In the present work, it was demonstrated that SVHV induces a potent rat paw edema with the same magnitude, but a different timing, than that observed with carrageenan or dextran. The SVHV-induced edema reached a maximum volume after 2 h, while

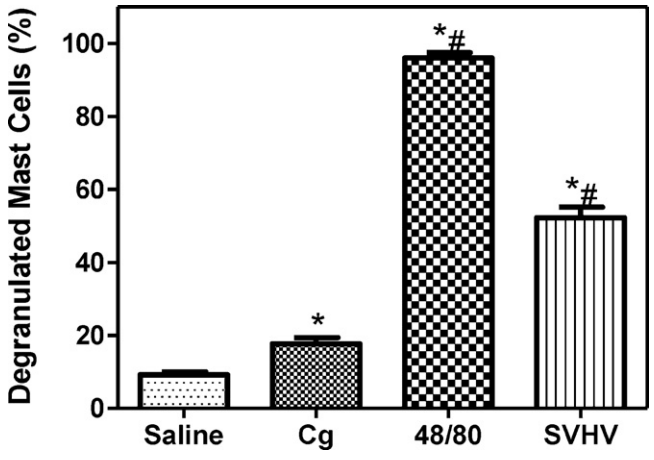

Fig. 4. Effect of the alginate isolated from *Sargassum vulgare* (SVHV) upon mast cell degranulation. Rats were i.p. treated either with saline, carrageenan (500 μg), compound 48/80 (0.6 mg/kg), or SVHV (1 mg). After 30 min, depletion of the mast cell mesenteric population was estimated by counting of the granulated and degranulated cells stained with toluidine blue. Data are presented as mean of the percentage of degranulated cells ± S.E.M. from 6 animals. $^{*}p < 0.05$, compared with saline and $^{\#}p < 0.05$, compared with carrageenan by ANOVA followed by Student Newman–Keuls.

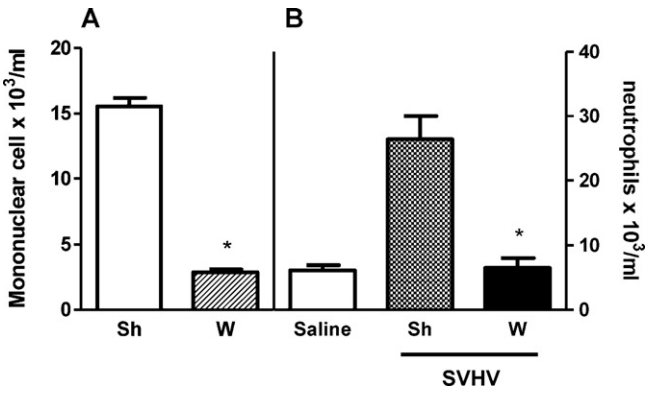

Fig. 5. Role of resident macrophages in the neutrophil migration to peritoneal cavity induced by the alginate isolated from *Sargassum vulgare* (SVHV). Rats were submitted to the depletion of the total peritoneal cell population by peritoneal lavage. To confirm the peritoneal depletion 30 min later the number of peritoneal macrophage was estimated by counting the number of mononuclear cell harvested from peritoneal cavity of washed animals (W) and Sham animals (Sh) (Panel A). Neutrophil migration was induced by SVHV (1 mg) in washed and sham animals (Panel B). Data are presented as mean ± S.E.M. from 6 animals. $^{*}p < 0.05$, compared with saline and $^{\#}p < 0.05$, compared with sham group by ANOVA followed by Student Newman–Keuls.

carrageenan-induced edema reached a maximum volume after 3–5 h and dextran effects could be observed as soon as 15 min after injection with a peak 0.5–1 h later (Di Rosa et al., 1971; Jori, Bentivoglio, & Garattini, 1961; Metcalfe, 2008; Morris, 2003; Winter, Risley, & Nuss, 1962).

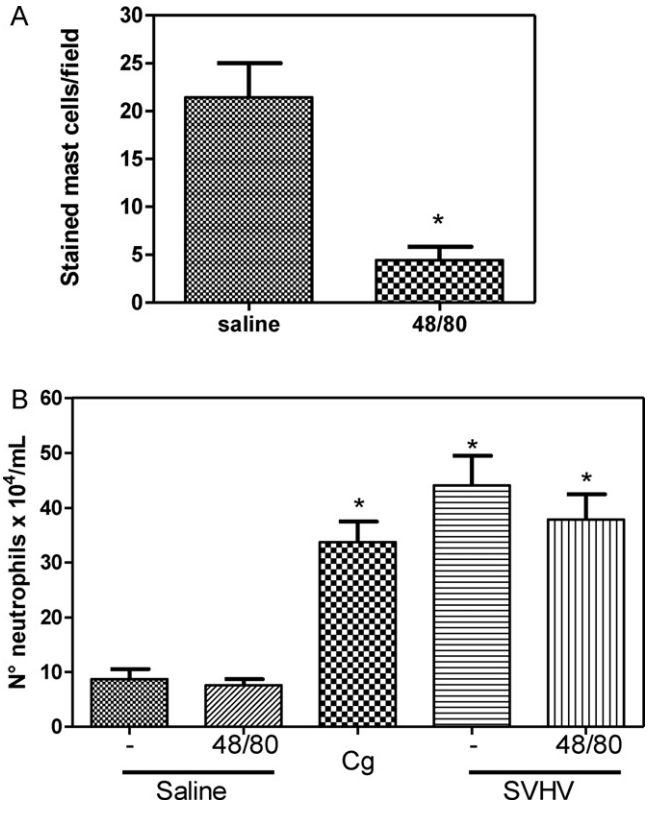

Fig. 6. Role of mast cells in the neutrophil migration to peritoneal cavity induced by the alginate isolated from *Sargassum vulgare* (SVHV). Rats peritoneal mast cells were depleted by sub chronic pretreatment with compound 48/80 for 4 days (0.6 mg/kg twice a day for 3 days and 1.2 mg/kg twice on the day 4). Panel (A): peritoneal mast cell population in saline and compound 48/80 pre-treated animals stained by toluidine blue. Panel (B): neutrophil migration induced by saline, carrageenan (0.3 mg) or SVHV (1 mg). Data are presented as mean ± S.E.M. from 6 animals. $^{*}p < 0.05$, compared with Saline by ANOVA followed by Student Newman–Keuls.

The timing of the inflammatory response is highly related to the released mediators and consequently to the elicited biological phenomena. Evidence found in the literature demonstrates that carrageenan-induced paw edema has two phases (Vinegar, Schreiber, & Hugo, 1969). The early phase is the result of increased histamine and serotonin concentrations in the extracellular space (Vinegar, Truax, Selph, & Voelker, 1982), while the late edema phase is known to be dependent on cytokine production by resident cells and neutrophil infiltration (Di Rosa et al., 1971; Kulkarni, Mehta, & Kunchandy, 1986; Vinegar et al., 1969). On the other hand, the edema induced by dextran is only mediated by an increase in vascular permeability, through fluid accumulation and mast cell degranulation (Metcalfe, 2008).

The alginate edematous activity seems to have the involvement of neutrophils, since the damage tissue presented an increase in MPO activity. Moreover, it also seems to be dependent on the production of proinflammatory cytokines, as SVHV was able to induce the production of TNF-alpha and IL-1 beta, two cytokines with known proinflammatory activities (Feldmann & Saklatvala, 2001; Haddad, 2002; Hopkins, 2003). The production of IL-10, on the other hand, was not affected by inflammatory stimuli including alginates. IL-10 is a cytokine recognized as antiinflammatory due to its ability to inhibit the production of proinflammatory cytokines and inflammatory mediators, such as prostaglandins and nitric oxide (Haddad, 2002).

In fact, the inflammation response elicited by exogenous stimuli, such as carrageenan, zymosan and lipopolissacharide (LPS), or by chemotactic mediators such as IL-1 and TNF, which induces neutrophil migration by an independent mechanism, involves resident macrophages and mast cell as the key cells controlling neutrophil migration. These resident cells respond to those exogenous stimuli with the release of proinflammatory cytokines with chemotactic activity on neutrophils (Faccioli et al., 1990; Ribeiro et al., 1997; Souza & Ferreira, 1985).

The effects of pharmacological modulators such as thalidomide and pentoxifylline, two inhibitors of cytokine production (Aarestrup, Goncalves-Da-Costa, & Sarno, 1995; Doherty, Jensen, Alexander, Buresh, & Norton, 1991; Moreira et al., 1993; Sampaio, Sarno, Galilly, Cohn, & Kaplan, 1991; Sampaio et al., 1998), a cyclooxygenase inhibitor indomethacin, the glucocorticoid dexamethasone, an H1 histamine receptor antagonist diphenhydramine, and an inhibitor of nitric oxide synthesis L-NAME were further investigated seeking to evaluate, *in vivo*, the mechanisms underlying the edematous properties of SVHV. In fact, all these inhibitors are able to prevent the paw edema induced by SVHV at some extension. However, dexamethasone, indomethacin and thalidomide were more efficient, inhibiting SVHV activity while L-NAME and diphenidramine had a reduced activity. Thus, it can be suggested that SVHV edema is dependent on the release of various types of inflammatory mediators, such as cytokines, chemokines, histamine, nitric oxide and prostaglandins. In such a complex process, histamine, released by mast cell degranulation, may exhort its main contribution to SVHV edematogenic activity during the first and second hours.

The importance of mast cells in the genesis of SVHV-induced edema and the possible role of these cells in the neutrophil migration was further explored. To investigate this hypothesis, the population of mast cells in the peritoneal cavity of rats was depleted by treatment with compound 48/80, however neutrophil migration induced by SVHV was not significantly affected, indicating that mast cells are not the key cells in the genesis of this inflammatory parameter. Previous studies indicate that mast cells are important for the inflammatory activity of carrageenan and dextran, although being more important for the latter stimulus. In addition, previous data have shown that the treatment with 48/80 did not change the peritoneal macrophage population (Ribeiro et al., 1997). However,

when we investigated the activity of SVHV upon mast cell degranulation, we found that it is capable to induce degranulation in a lower degree than compound 48/80, but higher than carrageenan. Taking these results together, we suppose that SVHV induces mast cell degranulation, which may be important for the early phase of edema, but not for the late phase, which seems to be dependent on neutrophil migration, since mast cell depletion did not modify neutrophil migration. In addition we can observe that edema elicited by SVHV is different from that elicited by carrageenan, mainly in the first 2 h. During the first and second hours, SVHV seems to be more potent than carrageenan, resembling dextran-induced edema, which is fast and mast cell dependent. Afterwards, it was investigated the importance of resident macrophages in the genesis of proinflammatory effect of SVHV, since it was demonstrated that some endogenous substances such as TNF-α and IL-1β and exogenous stimuli, such as carrageenan and LPS, induces neutrophil migration through the activation of resident cells (Ribeiro et al., 1997). Indeed, animals, whose resident macrophage depletion was induced by peritoneal lavage presented a decrease in neutrophil migration induced by SVHV injection, suggesting the alginate induced neutrophil migration by an indirect mechanism, dependent on the presence of macrophages but not of mast cells. When confronted these results with those described in the literature for different stimuli, it can be suggested that the SVHV has a similar profile to carrageenan and LPS, and the release of cytokines IL-1β and TNF-α should be related to macrophage activation. Thus, SVHV has a proinflammatory activity that depends on the stimulation of resident cells, specifically, macrophages. Probably these resident cells are initially activated by the SVHV and start to produce proinflammatory cytokines such as TNF-α and IL-1β that, in turn, could be inducing the generation of chemotactic mediators and vasoactive substances like prostaglandins. In accordance, Yang and Jones (2009) have demonstrated that low viscosity sodium alginates isolated from brown algae induces activation of macrophages through NF-κB pathway leading to the release of proinflammatory cytokines.

5. Conclusion

Gathering all this information, it can be concluded that the SVHV has proinflammatory activity, as demonstrated in the model of paw edema and neutrophil migration into the peritoneal cavity of rats. The referred edematous activity seems to depend much more on the migration of neutrophils than on the activation of mast cells and release of vasoactive substances, such as histamine and nitric oxide. These, in turn, may have a role in the initial phase, but not in the migration phase, of the edema. It may also be suggested that SVHV-proinflammatory activity depends on the activation of resident cells, which seems to have macrophages as the main cell involved.

References

Aarestrup, F. M., Goncalves-Da-Costa, S. C., & Sarno, E. N. (1995). The effect of thalidomide on BCG-induced granulomas in mice. *Brazilian Journal of Medicinal and Biological Research*, *28*, 1069–1076.

Bradley, P. P., Priebat, D. A., Christenses, R. D., & Rothstein, G. (1982). Measurement of cutaneous inflammation: Estimation of neutrophil content with an enzyme marker. *The Journal of Investigative Dermatology*, *78*, 206–209.

Darquy, S., & Sun, A. M. (1987). Microencapsulation of parathyroid cells as a bioartificial parathyroid *in vitro* studies. *ASAIO Journal*, *33*, 356–358.

Di Rosa, M., Giroud, J. P., & Willoughby, D. A. (1971). Studies of mediators of the acute inflammatory response induced in rats indifferent site by carrageenan and turpentine. *Journal of Pathology*, *104*, 15–21.

Doherty, G. M., Jensen, J. C., Alexander, H. R., Buresh, C. M., & Norton, J. A. (1991). Pentoxifylline suppression of tumor necrosis factor gene transcription. *Surgery*, *110*, 192–198.

Faccioli, L. H., Souza, G. E., Cunha, F. Q., Poole, S., & Ferreira, S. H. (1990). Recombinant interleukin-1 and tumor necrosis factor induce neutrophil migration 'in vivo' by indirect mechanisms. *Agents Actions*, *30*, 344–349.

Fan, M. Y., Lum, Z. P., Fu, X. W., Levesque, L., Tai, I. T., & Sun, A. M. (1990). Reversal of diabetes in BB rats in transplantation of encapsulation pancreatic islets. *Diabetes*, *39*, 519–522.

Feldmann, M., & Saklatvala, J. (2001). Proinflammatory cytokines. In S. K. Durum, J. J. Oppenheim, & M. Feldmann (Eds.), *Cytokine reference: A compendium of cytokines and other mediators of host defense* (pp. 291–306). Boston: Academic Press.

Gomes d'Ayala, G. G., Malinconico, M., & Laurienzo, P. (2008). Marine derived polysaccharides for biomedical applications: Chemical modification approaches. *Molecules*, *13*, 2069–2106.

Haddad, J. J. (2002). Cytokines and related-receptor mediated signalling pathways. *Biochemical and Biophysical Research Communication*, *297*, 700–713.

Hopkins, S. J. (2003). The pathological role of cytokines. *Legal Medicine*, *5*, S45–S47.

Jori, A., Bentivoglio, A. P., & Garattini, S. (1961). The mechanism of the local inflammatory reaction induced by compound 48/80 and dextran in rats. *Journal of Pharmaceutical Pharmacology*, *13*, 617–619.

Kimura, Y., Watanabe, K., & Okuda, H. (1996). Effects of soluble sodium alginate on cholesterol excretion and glucose tolerance in rats. *Journal of Ethnopharmacology*, *54*, 47–54.

Kulkarni, S. K., Mehta, A. K., & Kunchandy, J. (1986). Anti-inflammatory actions of clonidine, guanfacine and B-HT 920 against various inflammagen-induced acute paw oedema in rats. *Archives Internacionales de Pharmacodynamie et de Therapie*, *279*, 324–334.

Lai, H. L., Abu'Khalil, A., & Craig, D. Q. (2003). The preparation and characterization of drug-loaded alginate and chitosan sponges. *International Journal of Pharmaceutics*, *251*, 175–181.

Leung, M. Y. K., Liu, C., Koon, J. C. M., & Fung, K. P. (2006). Polysaccharide biological response modifiers. *Immunology Letters*, *1005*, 101–114.

Metcalfe, D. D. (2008). Mast cells and mastocytosis. *Blood*, *112*(4), 946–956.

Moreira, A. L., Sampaio, E. P., Zmuidzinas, A., Frindt, P., Smith, K. A., & Kaplan, G. (1993). Thalidomide exerts its inhibitory action on tumor necrosis factor alpha by enhancing mRNA degradation. *Journal of Experimental Medicine*, *177*, 1675–1680.

Morris, C. J. (2003). Carrageenan-induced paw edema in the rat and mouse. *Methods in Molecular Biology*, *225*, 115–121.

Otterlei, M., Ostgaard, K., Skjak-Braek, G., Smidsrod, O., Soon-Shiong, P., & Espevik, T. (1991). Induction of cytokine production from human monocytes stimulated by arginate. *Journal of Immunotherapy*, *10*, 286–291.

Pasquali, P., Zalcman, A., Murtas, S., Adone, R., Brambilla, G., Marianelli, C., et al. (2005). In vitro stimulation of murine peritoneal monocytes induced by alginates. *Archives of Pharmacal Research*, *28*, 936–941.

Ribeiro, R. A., Souza-Filho, M. V., Souza, M. H., Oliveira, S. H., Costa, C. H., Cunha, F. Q., et al. (1997). Role of resident mast cells and macrophages in the neutrophil migration induced by LTB4, fMLP and C5a des arg. *International Archives of Allergy and Immunology*, *112*, 27–35.

Sampaio, E. P., Moraes, M. O., Nery, J. A., Santos, A. R., Matos, H. C., & Sarno, E. N. (1998). Pentoxifylline decreases *in vivo* and *in vitro* tumour necrosis factor-alpha (TNF-alpha) production in lepromatous leprosy patients with erythema nodosum leprosum (ENL). *Clinical and Experimental Immunology*, *111*, 300–308.

Sampaio, E. P., Sarno, E. N., Galilly, R., Cohn, Z. A., & Kaplan, G. (1991). Thalidomide selectively inhibits tumor necrosis factor alpha production by stimulated human monocytes. *Journal of Experimental Medicine*, *173*, 699–703.

Son, E. H., Moon, E. Y., Rhee, D. K., & Pyo, S. (2001). Stimulation of various functions in murine peritoneal macrophages by high mannuronic acid-containing alginate (HMA) exposure in vivo. *International Immunopharmacology*, *1*, 147–154.

Soon-Shiong, P., Henitz, R. E., Merideth, N., Yao, Q. X., Zheng, T., Murphy, M., et al. (1994). Insulin independence in a type I diabetic patient after encapsulated islet transplantation. *Lancet*, *343*, 950–951.

Sousa, A. P. A., Barbosa, P. S. F., Torres, M. R., Martins, A. M. C., Martins, R. D., Alves, R. S., et al. (2008). The renal effects of alginates isolated from brown seaweed *Sargassum vulgare*. *Journal of Applied Toxicology*, *28*, 364–369.

Sousa, A. P. A., Torres, M. R., Pessoa, C., Moraes, M. O., Rocha Filho, F. D., Alves, A. P. N. N., et al. (2007). In vivo growth-inhibition of sarcoma 180 tumor by alginates from brown seaweed *Sargassum vulgare*. *Carbohydrate Polymers*, *69*, 7–13.

Souza, G. E. P., & Ferreira, S. H. (1985). Blockade by antimacrophage serum of the migration of PMN neutrophils into the inflamed peritoneal cavity. *Agents Actions*, *17*, 97–103.

Thomas, A., Harding, K. G., & Moore, K. (2000). Alginates from wound dressings activate human macrophages to secrete tumour necrosis factor-α. *Biomaterials*, *21*, 1797–1802.

Torres, M. R., Sousa, A. P. A., Filho, E. A. T. S., Melo, D. F., Feitosa, J. P. A., Paula, R. C. M., et al. (2007). Extraction and physicochemical characterization of *Sargassum vulgare* alginate from Brazil. *Carbohydrate Research*, *342*, 2067–2074.

Tze, W. J., & Tai, J. (1982). Biocompatibility and immunological studies of microencapsulation with cross-linked alginate capsules. *Transplantation*, *33*, 563–564.

Vériter, S., Mergen, J., Goebbels, R., Aouassar, N., Grégoire, C., Jordan, B., et al. (2010). In vivo selection of biocompatible alginates for islet encapsulation and subcutaneous transplantation. *Tissue Engineering Part A*, *16*, 1503–1513.

Vinegar, R., Schreiber, W., & Hugo, R. (1969). Biphasic development of carrageenan edema in rat. *Journal of Pharmacology and Experimental Therapeutics*, *166*, 96–103.

Vinegar, R., Truax, J. F., Selph, J. L., & Voelker, F. A. (1982). Pathway of onset, development, and decay of carrageenan pleurisy in the rat. *Federation Proceedings*, *41*, 2588–2595.

Wang, T., & He, N. (2010). Preparation, characterization and applications of low-molecular-weight alginate-oligochitosan nanocapsules. *Nanoscale*, *2*, 230–239.

Winter, C. A., Risley, E. A., & Nuss, G. W. (1962). Carrageenan induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. *Proceedings of the Society of Experimental Biology*, *111*, 544–547.
Yang, D., & Jones, K. S. (2009). Effect of alginate on innate immune activation of macrophages. *Journal of Biomedical Material Research*, *90*, 411–418.
Zimmermann, H., Shirley, S. G., & Zimmermann, U. (2007). Alginate-based encapsulation of cells: Past, present, and future. *Currents Diabetes Reports*, *7*, 314–320.
Zhong, H., Chunn, J. L., Volmer, J. B., Fozard, J. R., & Blackburn, M. R. (2001). Adenosine-mediated mast cell degranulation in adenosine deaminase-deficient mice. *Journal of Pharmacology and Experimental Therapeutics*, *298*, 433–440.