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Structural features and assessment of zymosan-induced arthritis in rat temporomandibular joint model using sulfated polysaccharide

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ABSTRACT. The green seaweed *Caulerpa cupressoides* var. *lycopodium* contains three SPs fractions (Cc-SP1, Cc-SP2 and Cc-SP3). Cc-SP1 and Cc-SP2 had anticoagulant (*in vitro*), pro- and antithrombotic, antinociceptive and/or anti-inflammatory (*in vivo*) effects. This study analyzed structural features and the antinociceptive and anti-inflammatory effects of Cc-SP1 on zymosan-induced acute arthritis of the rat temporomandibular joint (TMJ). Cc-SP1 was investigated by infrared technique. Male Wistar rats (200-240 g) received subcutaneously (s.c.) Cc-SP1 1h prior to intra-articular (i.art.) injection of zymosan (2 mg joint^{-1}) or saline (0.9%) into the left TMJ. Mechanical hypernociception was measured by the electronic Von Frey method in the basal and 4h after zymosan injection. Animals were euthanized 6h after zymosan injection and the TMJ cavity was removed for total leukocyte counts from the synovial fluid and myeloperoxidase (MPO) activity assessment. Cc-SP1 (1, 3 or 9 mg kg^{-1}) containing sulfate ester, galactose-6-sulfate, uronic acid and glycosidic linkages reduced zymosan-induced hypernociception (78.12, 81.13 and 87.43%, respectively, $p < 0.01$), and inhibited the total leukocyte influx (85, 88.14 and 89.95%, respectively, $p < 0.01$), being confirmed by MPO activity ($p < 0.05$). Therefore, Cc-SP1 reveals a pharmacological tool for treating inflammatory arthropathies.

Keywords: Chlorophyta, sulfated polymers, bioactivity, inflammatory arthropathies.

Características estruturais e ensaio de artrite induzida por zimosam em modelo de articulação temporomandibular de ratos usando polissacarídeo sulfatado

RESUMO. A alga marinha verde *Caulerpa cupressoides* var. *lycopodium* contém três frações de PSs (Cc-PS1; Cc-PS2 e Cc-PS3). Cc-PS1 e Cc-PS2 apresentaram efeitos anticoagulante (*in vitro*), pró- e antitrombótico, antinociceptivo e/ou anti-inflammatório (*in vivo*). Analisaram-se as características estruturais e os efeitos antinociceptivo e anti-inflamatório de Cc-PS1 sobre artrite aguda induzida por zimosam na articulação temporomandibular (ATM) de ratos. Foi investigada a Cc-PS1 por técnica de infravermelho. Ratos machos Wistar (200-240 g) receberam subcutaneamente (s.c.) Cc-PS1 1h antes de injeção intra-articular (i.art.) de zimosam (2 mg articulação^{-1}) ou salina (0,9%) na ATM esquerda. A hipernocicepção mecânica foi mensurada por método Von Frey elétrico em zero e 4h após injeção de zimosam. Os animais foram entanasiados após 6h de injeção de zimosam e a cavidade da ATM foi removida para contagem de leucócitos totais do fluído sinovial e ensaio da atividade de mieloperoxidase (MPO). A Cc-PS1 (1; 3 ou 9 mg kg^{-1}) contendo éster sulfato, galactose-6-sulfato, ácido urônico e ligações glicosídicas reduziu a hipernocicepção induzida por zimosam (78,12; 81,13 e 87,4%, respectivamente; $p < 0,01$), além de inibir o influxo de leucócitos totais (85; 88,14 e 89,95%, respectivamente; $p < 0,01$), sendo, ainda, confirmado pela atividade de MPO ($p < 0,05$). Portanto, a Cc-PS1 revela como uma ferramenta farmacológica para tratar de artropatias inflamatórias.

Palavras-chave: Chlorophyta, polímeros sulfatados, bioatividade, artropatias inflamatórias.

Introduction

Inflammation is a physiological event of the body caused by several factors ranging from microorganism infection and chemical injury to environmental pollution, leading to cell damage. It is characterized by pain, heat, redness, swelling and loss of function that results in the movement of leukocytes into the inflamed zones (KULINSKY, 2007). Epidemiological data have revealed an increase of certain chronic and acute inflammatory diseases (e.g., atherosclerosis, arthritis, diabetes, asthma, acquired immunodeficiency syndrome, Crohn, Alzheimer and depression) in recent years (IWALEWA et al., 2007).

Temporomandibular joint (TMJ) disorders have been considered a group of pathophysiological conditions that cause high levels of TMJ pain-

related disability, negatively impacting the quality of life of human (CAIRNS, 2010). In a previous study, Chaves et al. (2011) established an experimental model of zymosan-induced TMJ acute arthritis in rat to assess potential tools for therapies. In addition, the use of non-steroidal anti-inflammatory drugs and glucocorticoids to modulate the exaggerated and uncontrolled inflammatory responses presents several adverse effects (e.g., gastrointestinal symptoms, peptic ulceration, hemorrhagic effects and decrease in immunity) (IWALEWA et al. 2007; KULINSKY, 2007). Thus, there is a continuous need for the development of new analgesic and/or anti-inflammatory agents with novel modulatory effects from natural products derived from different origins (DE ARAÚJO et al., 2012; DORE et al., 2013; IWALEWA et al., 2007; VANDERLEI et al., 2010; YOUNG, 2008).

Seaweeds comprise a heterogeneous group of autotrophic organisms widely reported as a rich source of bioactive compounds with pharmacological importance (JIAO et al., 2011; QUINDERÉ et al., 2014; VANDERLEI et al., 2010). These bioactive products include cell-walls sulfated polysaccharides (SPs) naturally occurring as structural components of the extracellular space in marine algae (POMIN; MOURÃO, 2008). In red seaweeds, sulfated galactans occur as the most common source of SPs (CAMPO et al., 2009; FONSECA et al., 2008); the fucans or fucoidans are present in brown seaweeds (LI et al., 2005; POMIN; MOURÃO, 2008); and the heteropolysaccharides are the most frequently found in green seaweeds (GHOSH et al. 2004; JIAO et al., 2011). The chemical structures of these compounds vary among different algal species (JIAO et al., 2011; POMIN; MOURÃO, 2008). SPs were also identified in marine angiosperms, mangrove (AQUINO et al., 2005), microalgae (MAJDOUB et al., 2009), animals (vertebrates and invertebrates) (POMIN; MOURÃO, 2008) and, more recently, in freshwater plants, especially in *Eicchornia crassipes*, a species usually found in nutrient-enriched environments (DANTAS-SANTOS et al., 2012).

Over the years many studies have been focused on the modulatory potential of SPs from different organisms as anticoagulant and/or pro- and antithrombotic agents (AMORIM et al., 2011; ASSREUY et al., 2008; DANTAS-SANTOS et al., 2012; FONSECA et al., 2008; MAJDOUB et al., 2009; QUINDERÉ et al., 2014). However, SPs play roles on other biological processes (e.g., antioxidant, antiviral, antitumor and antimicrobial effects) (AMORIM et al., 2012; CAMPO et al., 2009; DORE et al., 2013).

With the recent advent of glycomics (POMIN, 2012), some SPs have also been tested in animal models of pain and/or inflammation. For example, De Araújo et al. (2011) and Coura et al. (2012) reported SPs from the red seaweeds *Solieria filiformis* and *Gracilaria cornea* with antinociceptive effects in mice, respectively. Cardoso et al. (2010) evaluated the pharmacological effect of fucoidans from *Fucus vesiculosos* (Phaeophyta) on zymosan-induced arthritis and found anti-inflammatory actions by reduction of cellular influx and nitric oxide concentration into the knee joint of rats. It was demonstrated by Siqueira et al. (2011) that the anti-inflammatory effect of a SP from the brown seaweed *Lobophora variegata* occurred by inhibition of nitric oxide and cyclooxygenase activities. In another study, an anti-inflammatory response of SPs from *L. variegata* on zymosan-induced arthritis into the knee joint of rats was reported by Paiva et al. (2011). Recently, Dore et al. (2013) discovered that a SP isolated from *Sargassum vulgare* (Phaeophyta) displayed a strong anti-inflammatory effect by reduction of edema and cellular infiltration. To the best of our knowledge (CHAVES et al., 2011; GONDIM et al., 2012), there are no studies about the use of SPs on experimental arthritis of the rat TMJ.

The *Caulerpa* species of green seaweeds (Caulerpaceae, Bryopsidales) are recognized to have high invasive capacity in the marine environment (PIAZZI et al., 2006), contributing to the algal biomass of coral reefs and lagoons in tropical and subtropical zones (TRI, 2009). Polysaccharides from this genus consisting of sulfate, galactose, glucose, arabinose and xylose, and small amounts of mannose and rhamnose and traces of fucose residues have been documented with pharmacological efficacies (e.g., anticoagulant, antiviral, antitumor and immunostimulatory effects) (GHOSH et al., 2004; JI et al., 2008; MAEDA et al., 2012). *Caulerpa cupressoides* var *lycopodium* contains three different SPs fractions (Cc-SP1, Cc-SP2, and Cc-SP3). Cc-SP2 had anticoagulant (*in vitro*) (RODRIGUES et al., 2011a, 2013a), antithrombotic, pro-thrombotic (*in vivo*) (RODRIGUES et al., 2011b), antinociceptive and anti-inflammatory (*in vivo*) (RODRIGUES et al., 2012) effects. Cc-SP1 and Cc-SP3 had no anticoagulant actions (RODRIGUES et al., 2011a and b), but an *in vivo* antinociceptive effects of Cc-SP1 has been recently reported in mice, without modifying the locomotor activity of the animals (RODRIGUES et al., 2013b). In order to explore new therapeutic options of SPs from this algal species, it was assessed the antinociceptive and anti-inflammatory properties of Cc-SP1,

using the model of experimental arthritis of the rat TMJ. A structural analysis of this fraction was also conducted.

Material and methods

Experimental design

A non-anticoagulant SPs fraction (Cc-SP1) was obtained from crude SP from *C. cupressoides* var. *lycopodium* and its structural features were analyzed by infrared spectroscopy. The effects on nociception (mechanical hypernociception) and inflammation (cell counting and myeloperoxidase activity assessment) were also examined *in vivo* using the experimental model of zymosan-induced arthritis in the rat TMJ.

Marine algae and SPs

The selected vegetative thalli of *C. cupressoides* var. *lycopodium* C. Agardh (Chlorophyta) were collected from the intertidal zone of Flecheiras beach, Ceará State, Brazil. A voucher (#4977) specimen was deposited in the Prisco Bezerra Herbarium (Department of Biology, Federal University of Ceará, Brazil). The animal experimentation was performed at Physiology and Pharmacology laboratory, Campus Sobral. The crude SP was extracted from dehydrated algal tissue (room temperature) by papain digestion (60°C, 6h), and then fractionated by anion-exchange chromatography on a DEAE-cellulose column using a NaCl gradient (0→1.5 M, with 0.25 M of intervals). Fractions (Cc-SP1, Cc-SP2 and Cc-SP3 eluted with 0.5, 0.75, and 1 M of NaCl, respectively) were obtained, as previously described (RODRIGUES et al., 2011a).

Animals

Wistar rats (200-240 g) were randomly obtained from the Animal House of the Federal University of Ceará, maintained on a 12h light/dark cycle, in temperature-controlled rooms and received water and food ad libitum. For the experiment, a total of 36 animals were used. All procedures and animal treatments were conducted in accordance with the guidelines for Institutional Animal Care and Use of the FUC, Brazil, previously approved by 80/10 protocol. In addition, the tested doses and administration route of Cc-SP_1 used in the experimental model were based on Rodrigues et al. (2012, 2013b).

Infrared (IR) spectroscopy

To analyze the structural features, Fourier Transform IR (FT-IR) spectra of the Cc-SP1 fraction was determined using a SHIMADZU IR spectrophotometer (model 8300) between 4000 and 500 cm^{-1}. A sample containing 5 mg of Cc-SP1 was pressed as KBr pellets.

Mechanical hypernociception

This test was based on Chaves et al. (2011). Groups of six rats received subcutaneously (s.c.) Cc-SP1 (1, 3 or 9 mg kg^{-1} body weight) or indomethacin (5 mg kg^{-1} body weight) (Indocid, Merk Sharp & Dohme; Campinas, São Paulo State, Brazil). After 1h of pretreatment, rats were anesthetized with 1% intraperitoneal (i.p.) tribromoethanol and received a single intra-articular (i.art.) injection of zymosan (40 µL of 2 mg zymosan $joint^{-1}$, dissolved in 0.9% sterile saline) into the left TMJ. Control group received saline (i.art.). For arthritis induction, the TMJ skin region of the animals was carefully shaved. After that, the specific volume of zymosan or only saline was injected and the facial hyperalgesia was measured using electronic Von Frey method in the basal and 4h after zymosan injection (arthritis) by threshold of force intensity that needed to be applied to the TMJ region until that a reflex response of the animal was noted (e.g., head withdrawal). On the fifth day, the basal force threshold value was recorded (in triplicate) before the i.art. or s.c. injections of zymosan, saline, indomethacin or Cc-SP1 and after these treatments (4h).

Cell counting

To examine the effect of Cc-SP1 on cellular influx, rats were euthanized under anesthesia and exsanguinated to analyze the responses after 6 h zymosan injection. For this, the superficial tissues of the animals were dissected, and the TMJ cavity was washed with 0.1 mL sterile saline (0.9%) to collect the synovial fluid by a pumping and aspiration technique using 0.5 mL of EDTA in neutral buffered PBS (twice). Total number of white cells present in the synovial lavage fluid was counted using a Neubauer chamber (CHAVES et al., 2011).

Myeloperoxidase (MPO) activity assessment

In order to confirm the cellular response in the collected synovial fluid after zymosan injection (6h), it was also determined the MPO activity, an enzyme found primarily in the azurophilic granules of the neutrophils and has been widely used as a biochemical marker of granulocyte infiltration into various tissues. This test was performed as previously described by Bradley et al. (1982).

Statistical analysis

Data are presented as mean ± S.E.M. or medians. Differences between means were compared using a one-way ANOVA followed by the Bonferroni test. Values of $p < 0.05$ were considered statistically significant.

Results and discussion

It was previously noticed that the fractionation of the crude SP from the green seaweed *C. cupressoides* var. *lycopodium* by anion exchange chromatography (DEAE-cellulose column) yielded three SPs fractions (Cc-SP1, Cc-SP2 and Cc-SP3) (RODRIGUES et al., 2011a, 2011b, 2012). Increasing molarities of NaCl (Cc-SP1 (0.5 M), Cc-SP2 (0.75 M) and Cc-SP3 (1 M), respectively) it was observed differences in relative proportions of total sugars (13-39%) and sulfate (16-29%), but no contaminant proteins were found among the fractions (RODRIGUES et al., 2013b), and in comparison with other investigated algae SPs (CHATTOPADHYAY et al., 2007; DE ARAÚJO et al., 2011).

Differences on normal coagulation time (*in vitro*) were also previously observed, where only Cc-SP2 displayed *in vitro* anticoagulant effect (RODRIGUES et al., 2011a, b, 2013a). Li et al. (2005) reported that the use of anticoagulant SPs could be considered an adverse consequence for treating patients subjected to chronic renal diseases. SPs from *L. variegata* (Phaeophyta) (SIQUEIRA et al., 2011) and *S. filiformis* (Rhodophyta) (DE ARAÚJO et al., 2012) had no *in vitro* anti-clotting effect, but exhibited *in vivo* anti-inflammatory effects in Wistar rats. Similar results were found for Cc-SP1, when an antinociceptive response in mice was observed (RODRIGUES et al., 2013b).

Based on these reports, it was evaluated the structural features of Cc-SP1, as well as its effects on experimental acute arthritis of the rat TMJ.

Infrared (IR)

The IR spectrum of Cc-SP1 showed a typical absorption band related to the presence of ester sulfate group (at 1264 cm^{-1}, S = O stretching) (CHATTOPADHYAY et al., 2007; GHOSH et al., 2004; PAIVA et al., 2011) in sample of the native polysaccharide, as seen in Figure 1. However, the intensity of this signal in the IR spectrum of Cc-SP1 was weak in comparison with Cc-SP2 in another study (RODRIGUES et al., 2013a). Along with literature data, it is justified due to the differences in the sulfate content found (AMORIM et al., 2012; DE ARAÚJO et al., 2011). Characteristic bands in the IR spectrum of Cc-SP1 proved information on the presence of 3445 (O-H stretching), 2371 (CH stretching), 1650 (uronic acid, COO^- or O-H stretching), 1403 (carboxylic group of the pyruvic acid stretching), 1075 (arabinogalactan sulfate backbone stretching) (RODRIGUES et al., 2013a) and 820 cm^{-1} (galactose-6-sulfate stretching) (CHATTOPADHYAY et al., 2007), respectively.

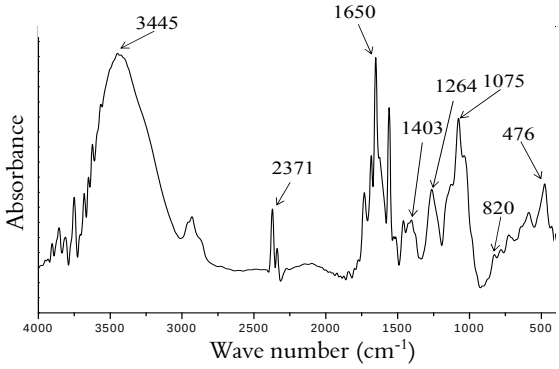

Figure 1. The IR spectrum of the Cc-SP1 fraction from the green seaweed *Caulerpa cupressoides* var. *lycopodium* at 500-4000 cm^{-1} in kBr pellets.

Effect of Cc-SP1 on mechanical hypernociception

The Figure 2 shows that the s.c. treatment of rats with Cc-SP1 elicited a dose-dependent antinociceptive response, at all doses tested, on the mechanical threshold for head withdrawal. The s.c. Cc-SP1-injected animals produced, at the highest dose (9 mg kg^{-1}), reduction of mechanical hypernociception by i.art injection of zymosan into the left TMJ after 4h by 87.43% (87.84 ± 1.5); at the intermediate dose of Cc-SP1 (3 mg kg^{-1}), the inhibitory effect was displayed by 81.13% (81.37 ± 1.8); and at the lowest dose (1 mg kg^{-1}), the reduction of process was manifested by 78.12% (77.17 ± 2.1), respectively, compared with the zymosan group (40.54 ± 0.7, $p < 0.01$). As expected, indomethacin (5 mg kg^{-1}, s.c.) (positive control) also produced antinociception (91.20%, 94.15 ± 8.9, $p < 0.01$).

Inflammatory and hypernociceptive dose-response effect of zymosan-induced TMJ arthritis was previously standardized and validated by Chaves et al. (2011). The i.art. 2 mg injection of zymosan significantly decreased the mechanical threshold for head withdrawal during the 4 and 6thh. Similar mechanical nociceptive responses were observed.

Inflammatory disorders are frequently associated with spontaneous and subsequent events (e.g., secondary hyperalgesia, allodynia, and referred pain) that results in various pathological processes, being the administration of non-steroidal anti-inflammatory drugs, which inhibit pain, hyperthermia and several inflammatory mediators, such as leukotriene B_4,

prostaglandins E_2 and I_2, usually required as the preferential inhibitors of cyclooxygenase-2 (KULINSKY, 2007; IWALEWA et al., 2007).

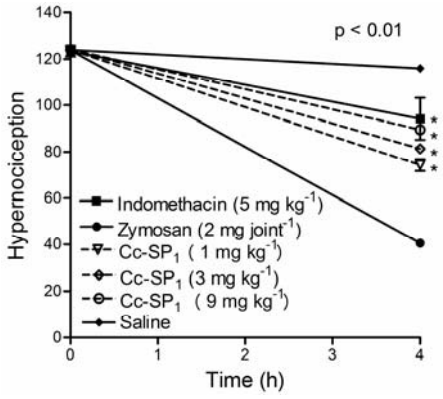

Figure 2. Effect of Cc-SP1 on mechanical hypernociception of zymosan-induced TMJ arthritis in rats. Data are expressed as mean ± S.E.M. (n = 6). *p < 0.01 compared with zymosan group (ANOVA, Bonferroni test).

The results obtained in the present study are in accordance with *in vivo* antinociceptive effects found for *C. cupressoides* var. *lycopodium* SPs fractions (Cc-SP1 and Cc-SP2) using classical tests of nociception in mice. For Cc-SP1 (3, 9 or 27 mg kg^{-1}), it was observed an *in vivo* analgesic effect predominantly through a peripheral mechanism (RODRIGUES et al., 2013b). For Cc-SP2, it was manifested an *in vivo* antinociceptive property in terms of distinct inhibitory forms of the formalin response, with effect at low dose (3 mg kg^{-1}) similar to non-steroidal drugs and corticosteroids; but, at higher dose (9 and 27 mg kg^{-1}), it was observed a pharmacological profile related to drugs that interact with the opioid systems, like morphine, being it subsequently confirmed by the hot-plate test (RODRIGUES et al., 2012). Data corroborated to an important role of these molecules in painful conditions (DE ARAÚJO et al., 2011; ASSREUY et al., 2008; COURA et al., 2012; QUINDERÉ et al., 2013).

Chaves et al. (2011) established the experimental model of zymosan-induced acute arthritis in the rat TMJ to assess both the mechanisms underlying TMJ inflammation and to the design of new therapeutic alternatives for the treatment of arthropathies. According to the authors, zymosan also caused changes on some inflammatory parameters (e.g., leukocyte migration, plasma extravasation and nitric oxide expression).

Recently, Gondim et al. (2012) investigated the antinociceptive and anti-inflammatory effects of electroacupuncture on experimental arthritis of the rat TMJ. It was observed that this technique was capable of inhibiting neutrophil migration, vascular permeability, tumoral necrosis factor, cyclooxygenase-2 and inducible nitric oxide synthase compared with the saline group. The authors postulated the electroacupuncture as an important therapeutic strategy for treating TMJ disorders.

Literature describes some algae SPs to be capable of inhibiting the *in vivo* acute inflammatory response induced by zymosan (CARDOSO et al., 2010; PAIVA et al., 2011; SIQUEIRA et al., 2011). Based on the hypothesis that there is an association between the antinociceptive effect and inflammatory pain (COURA et al., 2012; QUINDERÉ et al., 2013; RODRIGUES et al., 2012; VANDERLEI et al., 2010), the effects of Cc-SP1 on leukocyte recruitment and MPO activity were also evaluated in the present study.

Effect of Cc-SP1 on leukocyte influx

The s.c. treatment of rats with Cc-SP1 resulted in significant decreases (dose-dependent) in the number of polymorphonuclear cells in comparison to the zymosan group, as seen in Figure 3.

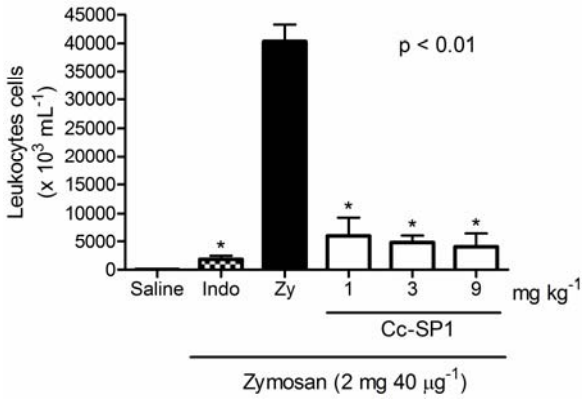

Figure 3. Effect of Cc-SP1 on leukocyte influx of zymosan-induced TMJ arthritis in rats. Data are expressed as mean ± S.E.M. (n=6). *p < 0.01 compared with zymosan group (ANOVA, Bonferroni test).

The anti-inflammatory response of CcSP1 on the total leukocyte count from the synovial lavage fluid was similar at all doses tested and in comparison with indomethacin (positive control) ($p > 0.05$). The rats pretreated with Cc-SP1 (1, 3 or 9 mg kg^{-1}), injected 1 h prior to inflammatory stimuli (2 mg zymosan, i.art.) (40.270 ± 2.9 cells mm^{-3}), inhibited ($p < 0.01$) the leukocyte migration by 85% (6.038 ± 3.2 cells mm^{-3}, 1 mg kg^{-1}), 88.14% (4.775 ± 1.3 cells mm^{-3}, 3 mg kg^{-1}) and 89.95% (4.044 ± 2.4 cells mm^{-3}, 9 mg kg^{-1}), respectively. As expected, animals pretreated with indomethacin (5 mg kg^{-1}, s.c.) also showed anti-inflammatory effects (95.50%, 1.810 ± 6.3 cells mm^{-3}, $p < 0.01$).

In the inflammatory process, the selectin-adhesion molecules mediate leukocyte-rolling along the vascular endothelium at zones of inflammation, leading to mechanical inflammatory hypernociception. When there is an excessive leukocyte activation occurs intravascular aggregation and the release of toxic oxygen radicals and proteolytic enzymes that lead to vascular and tissue damage. Glucocorticoids are thought to be more efficient in the inhibition of phospholipase A2 and multiple inflammatory genes; suppression of synthesis of cytokines, nitric oxide, tumoral necrosis factor and cellular adhesion molecules (KULINSKY, 2007).

Some SPs with anti-inflammatory activity and minimal adverse effects have been examined (SIQUEIRA et al., 2011; COURA et al., 2012; QUINDERÉ et al., 2013; RODRIGUES et al., 2012). Cc-SP1 inhibited cell infiltration in the rat TMJ fluid during the inflammatory response stimulated by zymosan (Figure 3), and it could perhaps interact with cell-receptors, such P and L-selectins, thus causing a reduction on the leukocyte recruitment (YOUNG, 2008; CARDOSO et al., 2010; SIQUEIRA et al., 2011; RODRIGUES et al., 2012).

It is believed that the key to trigger an anti-inflammatory response is the modulating of leukocytes around the inflamed sites (IWALEWA et al., 2007; YOUNG, 2008; POMIN, 2012; RODRIGUES et al., 2012). However, the involved mechanisms for the anti-inflammatory effects of SPs are complex and incompletely understood (YOUNG, 2008). Pomin (2012) reported that the anti-inflammatory activity of SPs could also involves sequestration of chemokines responsible to drive and to activate the leukocytes because of the presence of conserved heparin-binding sites in some chemokines.

The nature of the biological interactions of SPs has proven to be not only a mere consequence from charge originated from sulfation content, but also by stereospecific features (e.g., monosaccharide composition, sulfation and glycosylation sites, anomericity, and conformational structure) (CAMPO et al., 2009; JIAO et al., 2011; POMIN; MOURÃO, 2008). In addition, owing to the structural variations among the different algae SPs, structure-function relationship analysis of the effects of these polymers on the inflammatory response has not yet been elucidated (POMIN, 2012).

The current study, together with the literature data, pointed galactose-6-sulfate (Figure 1) as a conserved structural sugar in Caulerpaceae (CHATTOPADHYAY et al., 2007; GHOSH et al., 2004). This sulfated residue shows to be important for the anticoagulant action of some SPs (DANTAS-SANTOS et al., 2012; RODRIGUES et al., 2013a). On the other hand, our group demonstrated that the lack of *in vitro* anti-clotting effect of Cc-SP1 did not influence its *in vivo* analgesic effect (RODRIGUES et al., 2013b). It also demonstrates the lack of data concerning the action of these molecules in both nociceptive and inflammatory processes.

In order to confirm the pharmacological action of Cc-SP1 on zymosan-induced arthritis in the rat TMJ, the MPO activity was also measured from the synovial lavage fluid. MPO is an enzyme widely used as a biochemical marker of granulocyte infiltration (neutrophils) into various tissues (BRADLEY et al., 1982). According to Chaves et al. (2011), the predominant cell type which peaked at 6th h was neutrophils (characterizing acute inflammation) in this animal model.

Based on our findings (Figure 4), the MPO activity was reduced by 51% (20.71 ± 2.90 U joint fluid^{-1}, 1 mg kg^{-1}), 56.58% (18.35 ± 2.71 U joint fluid^{-1}, 3 mg kg^{-1}) and 77.92% (9.33 ± 1.10 U joint fluid^{-1}, 9 mg kg^{-1}), respectively, in comparison with zymosan group (42.27 ± 1.73 U joint fluid^{-1}) ($p < 0.05$). These results were corroborated by the decrease in total leukocyte count (Figure 3), suggesting the predominance of neutrophils in this process (COURA et al., 2012). As expected, indomethacin (5 mg kg^{-1}, s.c.) also reduced MPO activity in TMJ fluid (75.82%, 10.22 ± 0.67 U joint fluid^{-1}) ($p < 0.05$).

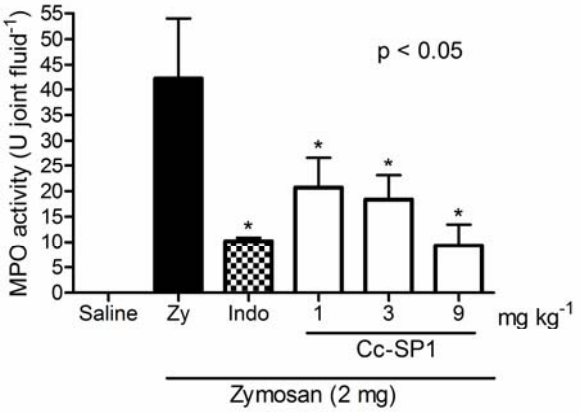

Figure 4. Effect of Cc-SP1 on MPO activity of zymosan-induced TMJ arthritis in rats. Data are expressed as mean ± S.E.M. (n=6). *p < 0.05 compared with zymosan group (ANOVA, Bonferroni test).

The anti-inflammatory activity of Cc-SP1 with both reductions in total leukocyte migration and MPO activity (Figures 3 and 4) could also be associated with a significant inhibition of nitric oxide

released by macrophages in animals TMJ exudates. Cardoso et al. (2010) discovered fucoidans from the brown seaweed *F. vesiculosus* exhibiting anti-inflammatory activity in a zymosan-induced arthritis model of acute inflammation by reduction of nitric oxide concentration into the knee joint of rats. Siqueira et al. (2011) found inhibitory effect of a SP from the brown seaweed *L. variegata* on the nitric oxide concentration from the peritoneal fluid of rats induced with zymosan in the SP anti-inflammatory effect. In addition, heterofucans isolated from *L. variegata* presented antioxidant effect with an important modulatory role in the cellular redox state in a study performed by Paiva et al. (2011). Further investigations should be conducted to infer the effect of Cc-SP1 on nitric oxide concentration and oxidative stress (that contribute to articular destruction, edema and pain) in TMJ disorders (CHAVES et al., 2011).

Conclusion

A non-anticoagulant sulfated polysaccharide from the green seaweed *Caulerpa cupressoides* var. *lycopodium* has promising antinociceptive and anti-inflammatory effects on zymosan-induced acute arthritis in rat temporomandibular joint.

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