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Stonefish antivenom neutralises the inflammatory and cardiovascular effects induced by scorpionfish *Scorpaena plumieri* venom

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ABSTRACT

Venomous fish are often involved in human accidents and symptoms of envenomation include local (intense pain and swelling) and systemic effects (cardiovascular and neurological disorders). However the only commercially available antivenom is against the Indo-Pacific stonefish *Synanceja trachynis* Stonefish Antivenom (SFAV). The aim of the present study was to evaluate the potential of SFAV in neutralising the *in vivo* effects of some toxic activities of scorpionfish *Scorpaena plumieri* venom (SpV), and the *in vitro* immuno cross-reactivity. The SpV (7.5–100 μg/animal) caused nociceptive and dose-dependent edematogenic responses in the mice footpad. In rats SpV (300 μg/kg, i.v.) produced immediate and transient increase in arterial blood pressure and decrease in heart rate. Prior incubation of SpV with SFAV (1 μg SpV/1 U SFAV) abolished the inflammatory response, and significantly attenuated the cardiovascular effects induced by SPV. Western blotting analysis on two-dimensional SDS-PAGE of *S plumieri* venom proteins using SFAV proved that the epitopes recognized by SFAV are shared with the ~98 kDa proteins. This is the first report of venom similarities between Indo-Pacific and Atlantic venomous fish, suggesting that the SpV compound responsible for inflammatory and cardiovascular effects possesses similar biochemical and antigenic properties to those found in stonefish venom.

1. Introduction

A large number of venomous fish are encountered in freshwater and marine environments worldwide. As described for the terrestrial venomous animals, the development of an arsenal of noxious substances by some aquatic animals was an important adaptation that aids these species in their fight for survival in a highly competitive ecosystem (Russell, 1971; Magalhães et al., 2006).

Among the aquatic animals often involved in human accidents, a special attention is devoted to fish belonging to the Scorpaenidae (lionfish and scorpionfish) and Synanceiidae (stonefish) families due to the severe injuries caused, which include local and systemic manifestations. The venom apparatus of these fish comprises 11–17 dorsal, 3 anal and 2 pelvic fin spines with venomous glandular tissue of different morphology located in grooves along opposing sides of each spine (Gwee et al., 1994; Haddad, 2000; Smith and Wheeler, 2006).

All venomous fish use their venom primarily for defensive purposes. This can be deadly for any human unlucky enough to step on them. Therefore, human envenomation occurs when swimmers or fishermen mishandle or step on the spines of the dorsal fin (Halstead, 1951; Roche and Halstead, 1972). The intensity of the clinical features triggered by fish envenomation is related to the amount of

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venom injected in the puncture wounds; and patients can be stung by one or several spines present in the dorsal region of the fish (Gwee et al., 1994).

Only a few studies have been dedicated to the venom of specimens of *Scorpaena* genus scorpionfish. Some works were performed using the venom of *Scorpaena guttata*, the sculpin or California scorpionfish (Carlson et al., 1971, 1973; Schaeffer et al., 1971; Coats et al., 1980). However there is little information about the venom of *Scorpaena plumieri*, one of the most abundant scorpionfish found along the Brazilian coast (Figueiredo and Menezes, 1980; Carvalho-Filho, 1999).

In previous works, we have found that *S. plumieri* venom is lethal (LD_{50} in mouse 0.28 mg/kg, i.v.) displaying hemorrhagic, hemolytic and proteolytic activities (Carrijo et al., 2005). Injections of the venom in the footpad or peritoneal cavity of mice lead to deposition of venom in the lung, endothelial barrier dysfunction and microvascular hyperpermeability (Boletini-Santos et al., 2008).

In addition, the fresh venom was able to induce cardiovascular effects (changes in mean arterial pressure and heart rate) in anaesthetized rats (Carrijo et al., 2005). Recently, we have demonstrated that *S. plumieri* venom induces coronary vasoconstriction, positive chronotropic, lusitropic and inotropic effects on isolated rat hearts (Gomes et al., 2010). A new vasoactive cytolytic toxin, referred to as Sp-CTx, has been purified from this venom. This protein has proved to possess a potent hemolytic activity on washed rabbit erythrocytes and induces vaso-relaxation followed by constriction on rat aortic rings (Andrich et al., 2010).

In spite of the low risk of death, the envenomation caused by scorpionfish is serious and the symptoms are similar to those observed in accidents with stonefish and lionfish. The clinical manifestations of accidents with *S. plumieri* and *S. brasiliensis* include intense pain, irradiation of the pain, edema, erythema, occasional skin necrosis, adenopathy, nausea, vomiting, agitation, malaise, sweating, diarrhea, tachycardia and arrhythmias (Haddad et al., 2003). The treatment protocol of the victims is symptomatic and antivenom therapy for fish envenoming is only available against stonefish (*Synanceia trachynis*) envenomation.

Commercial Stonefish Antivenom (SFAV) is a horse Fab'2 preparation made by CSL in Melbourne, Australia (White, 1995) which is effective in neutralising all known clinical effects of serious *S. trachynis* envenomation, annulling the lethal, vascular permeability-increasing and hemolytic properties of the venom (Church and Hodgson, 2003).

It is also known that SFAV neutralises the hemolytic and toxic effects of other stonefish (*S. verrucosa*) and lionfish (*Pterois volitans, P. lunulata, P. antennata* and *Dendrochirus zebra*) (Shiomi et al., 1989). It has been reported that the endothelium-dependent relaxation activity in porcine coronary arteries, the inotropic and chronotropic responses in rat atria, and the biphasic cardiovascular responses in anaesthetized rat produced by *Gymnapistes marmoratus* and *P. volitans* venoms are abolished by SFAV (Church and Hodgson, 2001, 2002a). Recently, we demonstrated that the potent hemolytic activity of Sp-CTx is strongly reduced after treatment with SFAV (Andrich et al., 2010). The effectiveness of SFAV in neutralizing the activity of some other piscine venoms is explained by the notion that venomous fish belonging to different genus may share similar venom compounds (Church and Hodgson, 2002b). Consequently, it has been proposed that the venoms of most venomous fish are chemically and pharmacologically similar and that their effects only differ quantitatively (Church and Hodgson, 2002b).

Therefore, the aim of the current study was to investigate the cross-reactivity between the venom of the Atlantic scorpionfish *S. plumieri* and the commercial antivenom raised against the venom of Australian stonefish *Synanceja trachynis* (SFAV) through an array of binding and neutralisation studies *in vivo* and *in vitro*. This work also attempts to characterize and document the edema-inducing and nociceptive activities of *S. plumieri* venom.

2. Material and methods

2.1. Venom extraction

Venom was obtained from wild specimens of *S. plumieri*, collected on shallow water beaches on the coast of Espírito Santo State — Brazil, and maintained alive in oxygenated seawater. The venom extraction was carried out according to the batch method, previously described by Schaeffer et al. (1971). The whole venom apparatus or 15 spines from a medium size fish (about 20 cm in length and ~400 g weight) yielded an average of 10–16 mg of protein. The fresh venom extract (SpV) was immediately used for cardiovascular, edema-inducing and nociceptive assays. The protein concentration of the *S. plumieri* venom was determined by the method of Lowry et al. (1951), using bovine serum albumin as standard.

2.2. In vivo assays

All procedures were conducted in accordance with the Biomedical Research Guidelines for the Care and Use of Laboratory Animals (1996), as stated by the Brazilian College of Animal Experimentation (COBEA).

2.2.1. Edema-inducing activity

The ability of *S. plumieri* venom to induce edema was studied in male Swiss mice (20–25 g) according to Lima et al. (2003). Samples of 30 μl of sterile phosphate buffer saline (PBS) containing different doses of SpV (7.5, 15, 60 μg of protein/animal) were injected via intraplantar (i.pl.) route in the right hind paw of mice. Local edema was quantified periodically in 0.5, 2, 4, 6, 12, 24, 48, 72 and 96 h after injection ($N = 4$) by measuring the thickness of injected paws with a digital caliper (Zaas Precision). Mice injected with sterile PBS were considered as control group. Results were expressed as percentage increase of paw thickness after venom administration.

2.2.2. Nociceptive activity

Nociceptive activity of the SpV was assayed according to Hunskaar et al. (1985). Each mouse (20–25 g) was kept in a chamber mounted on a mirror. After an adaptation period (10 min), 30 μl of sterile PBS containing different doses of *S. plumieri* venom (7.5, 15, 30, 60 and 100 μg of protein/animal) were injected (i.pl.) in the right hind paw of mice

($N = 4$). Afterwards, each animal was returned to the observation chamber and the period of time spent licking or biting the right hind paw was recorded during 30 min and taken as index of nociception. Mice injected with sterile PBS were considered as control group ($N = 4$).

2.2.3. Cardiovascular assays

Effects of SpV on blood pressure and heart rate were evaluated in male Wistar rats (250–300 g, $N = 7$) anesthetized with urethane (1.2 g/kg, i.p.). A midline incision was made in the cervical region and polyethylene catheters (PE-50) were implanted into the carotid artery and jugular vein of rats for cardiovascular recordings and venom injections, respectively. During all procedures, depth of anesthesia was checked through the pinch of the rear paw. When necessary, additional doses of anesthetic were injected. During all experiments, animals breathed spontaneously. The venom extract was administrated *in bolus* in a dose of 300 μg protein/kg in 100 μL of saline. The dose used was selected according to our previous work (Gomes et al., 2010). The pulsatile arterial pressure (PAP) was recorded through a blood pressure transducer (Grass Instrument Div., Warnick, USA) and signals were processed using the BIOPAC-System (MP100, Model PT300, Santa Barbara, USA). The mean arterial pressure (MAP) and the heart rate (HR) recordings were obtained simultaneously from the PAP signal. After the injection of the *S. plumieri* venom, the peak values of MAP and HR were measured.

2.2.4. Estimation of neutralisation of S. plumieri venom pharmacological activities

Cross-neutralisation experiments were performed in order to determine if stonefish antivenom (SFAV, obtained from CSL, Melbourne, Australia) was able to neutralise the nociceptive, edematogenic and cardiovascular effects induced by SpV. For neutralisation of nociceptive and edematogenic activities, samples of SpV were incubated at 25 °C for 30 min with SFAV at different ratios (1:0.25, 1:0.5, 1:1.0 and 1:1.5 μg of SpV/U of SFAV). After that, 30 μl of each mixture containing 15 μg of SpV were injected in the right hind paw of mice. Nociceptive and edematogenic activities were evaluated after 0.5 h according to items 2.2.1 and 2.2.2 ($N = 4$).

For the cardiovascular assays, *S. plumieri* venom was pre incubated with SFAV (1:1 SpV/U of SFAV for 5 min at 25 °C), and subsequently, the mixture was administrated *in bolus* (300 μg protein of SpV/kg) according to item 2.2.3 ($N = 7$).

Samples of *S. plumieri* venom (15 μg and 300 μg), in appropriate vehicle, were submitted to the same incubation conditions (25 °C for 30 or 5 min) and used as positive control for inflammatory and cardiovascular assays, respectively.

2.3. Two-dimensional (2D) electrophoresis

SpV (100 μg of protein) was applied to each of 7 cm immobilized linear pH gradients (pH 3–10 and 4–7) strips (IPG, Bio-Rad), with Deastreak rehidration solution (Amersham, Uppsala, Sweden) for 12 h, 50 V at 20 °C. Isoelectric focusing (IEF) was performed in an IEFCell system (Bio-Rad, Hercules, CA). Electrical conditions were set as described by the supplier. After the first-dimension run, the IPG gel strip was incubated at room temperature for 15 min in equilibration buffer (50 mM Tris–HCl pH 8.8, 6M urea, 2% SDS, 30% glycerol and traces of bromophenol blue) containing 125 mM DTT, followed by a second incubation step (15 min at room temperature) in equilibration buffer containing 125 mM iodacetamide instead of DTT. The second dimension electrophoresis was performed in a vertical system with uniform 10% separating gel (mini PROTEAN 3 cell; Bio-Rad) at 25 °C, according to the method described by Laemmli (1970). Protein spots in the gel were stained with colloidal coomassie blue brilliant CBB G-250 following procedures described elsewhere (Neuhoff et al., 1988).

2.4. Western mmunoblotting

S. plumieri proteins separated by 2D electrophoresis (according to item 2.3) were transferred to a nitrocellulose membrane for 1 h at 350 mA/100 V. Membrane was blocked in 5% low fat milk, 0.3% tween 20 in phosphate buffered saline (PBS). Following blockade, membrane was washed with PBS and probed (1 h at 25° C) with a 1:500 dilution of stonefish antivenom. Another washing step was performed and the bound antibodies were probed (1 h, 25 °C) with a diluted peroxidase-conjugated antibody (1:5000 in PBS containing 0.05% tween 20). Immunodetection was carried out using hydrogen peroxide as substrate in the presence of diaminobenzidine and 4-chloro-1-naphthol.

2.5. Enzyme linked immunosorbant assay (ELISA)

ELISA was used with the aim of evaluating the antigenic cross-reactivity of *S. plumieri* whole venom with Stonefish antivenom. The assays were performed as described previously by Chávez-Olórtegui et al., 1991.

Falcon flexible microtitration plates purchased from Becton Dickinson Labware Europe (Becton Dickinson France S.A.) were coated with 100 μl of a 5 μg/ml solution of the *S. plumieri* venom in 0.02 M sodium bicarbonate buffer, pH 9.6 and incubated overnight at 5 °C. After blocking non-specific sites with 2% (w/v) casein solution for 1h at 37 °C, the immobilized venom proteins were titrated with decreasing concentrations of stonefish antivenom (from 1:200 to 1:204800 dilution) and incubated at 37 °C for 1h. Non-specific binding was measured in the presence of pre-immune horse serum at the same conditions. Bound IgG was detected via peroxidase conjugated antibody raised against horse IgG diluted 1:1000. Wells coated with 2% casein were taken as blank and subtracted from all values. Absorbance values were determined at 492 nm with a Titertek Multiscan spectrophotometer. All measurements were made in triplicate and the results expressed as the mean of two assays.

2.6. Statistical analyses

Results were expressed as mean ± SEM (Standard Error of the Mean) and were evaluated using one- or two-way

analysis of variance (ANOVA) followed by the Tukey *post hoc* test. Results were also evaluated by Student's *t*-test. In all cases, differences were considered significant at $p < 0.05$.

3. Results

3.1. S. plumieri venom edematogenic and nociceptive activities in mice

For determination of the edematogenic response induced by *S. plumieri* venom, doses of 7.5, 15 and 60 μg of venom/animal were used. Fig. 1A shows the time-course evaluation of edematogenic effect. It is possible to observe that the venom induced an intense and sustained dose-dependent edematogenic response with a maximal activity observed 30 min after injection of 58 ± 6% with 7.5 μg, 61 ± 6% with 15 μg, and 82 ± 2% with 60 μg of protein/animal. The edema remained significantly elevated compared to control group over 6 h at the dose of 7.5 μg, 24 h at the dose of 15 μg and 72 h at the dose of 60 μg. Higher doses were unable to increase the edematogenic response compared to the response induced by 60 μg of SpV (data not shown).

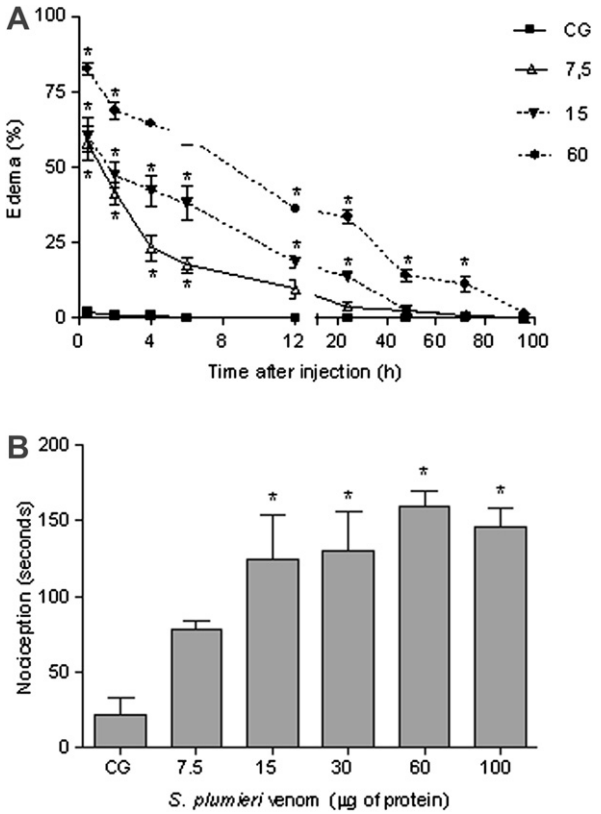

Fig. 1. Edematogenic and nociception-inducing activities of *S. plumieri* venom in mice. Different doses of *S. plumieri* venom (SpV) were injected i.pl. route and local edema (A) and nociception (B) were quantified. The results were expressed as percent increase in paw thickness or the amount of time spent licking or biting the injected hind paw. Control-group (CG). *$p < 0.05$ compared with control group.

Likewise, a significant nociceptive response was observed. Fig. 1B shows that the SpV induced an increase of paw licking duration that reached its maximum with 15 μg of protein/animal (124.5 ± 29.3 s). Doses >15 μg of *S. plumieri* venom were unable to increase the paw licking duration in a dose-related way, nevertheless each dose presented significant values (Fig. 1B).

The vehicle control (PBS) had no significant effect on the experiment.

3.2. Neutralisation of S. plumieri venom edematogenic and nociceptive effects using stonefish antivenom

The ability of SFAV in neutralizing the inflammatory activity induced by *S. plumieri* venom was evaluated by pre-incubation of SpV with SFAV. Fig. 2 shows that SFVA succeeded in neutralizing the *in vivo* edematogenic and nociceptive effects of SpV. The intensity of the protective effect of SFAV was achieved in a dose-dependent ratio. At ratios of 1/1 and 1/1.5 μg protein of venom/U of antivenom almost a complete neutralisation of edema (90.3 ± 1.6% and 90.8 ± 1.2%, respectively) and nociception responses (97.1 ± 1.7% and 94.8 ± 2.2%, respectively) were observed. At lower ratios (1/0.5 and 1/0.25 μg of SpV/U of antivenom) only a partial neutralisation of edema (68.4 ± 4.8% and 46.1 ± 9.8%, respectively) and nociception responses (50.5 ± 7.3% and 5.6 ± 3.9%, respectively) was observed.

3.3. Neutralisation of S. plumieri venom cardiovascular effects using stonefish antivenom

In the cardiovascular assays, systolic pressure, diastolic pressure and HR values of anesthetized rats prior to the start of the experiments were 120 ± 4.5 mmHg, 80 ± 7.0 mmHg and 320 ± 20 bpm respectively. SpV (300 μg/kg, i.v.) caused a pressor response of 36.9 ± 4.0 mmgH increase in mean arterial pressure and bradycardia of 65.6 ± 9.2 bpm decrease in heart rate in anaesthetized rats (Fig. 3). These effects were immediate and transient, and the values of MAP and HR returned to the basal levels after 2–6 min. When the same dose of SpV was pre-mixed with SFAV (1 μg of SpV/1 U of

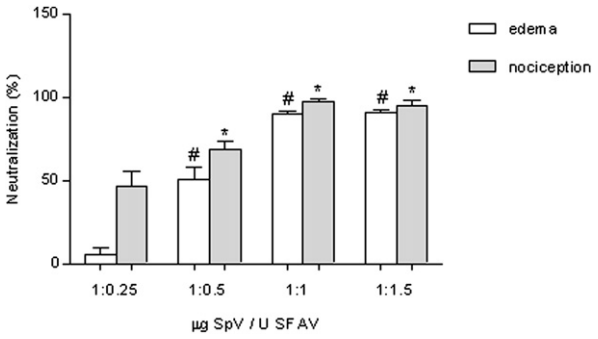

Fig. 2. The effect of stonefish antivenom on edematogenic and nociceptive responses induced by scorpion fish venom in mice hind paw. Sample of *S. plumieri* venom (SpV) were prior incubated with different amounts of stonefish antivenom (SFAV) and than injected (i.pl.) in mice. Local edema and nociception were then quantified. * and #$p < 0.05$ compared with respective 1:0.25 group.

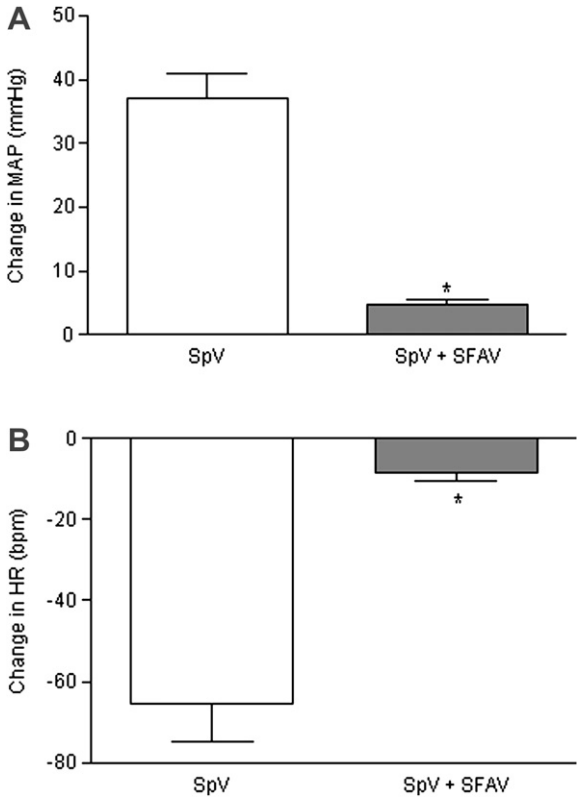

Fig. 3. Alterations induced by scorpionfish venom (SpV) observed upon the mean arterial blood pressure - MAP (A) and heart rate HR (B) and the reduced effect of the *in bolus* injection of the venom pre-mixed with stonefish antivenom (SFAV, 1 U/μg SpV). $^{*}p < 0.05$ compared to SpV injection alone. Bpm: beats per minute.

SFAV during 5 min at 25 °C), the pressoric and bradycardic responses were reduced in 88% (4.6 ± 0.8 mmHg increase in MAP) and 87% (8.3 ± 2.2 bpm decreased in HR), respectively (Fig. 3).

3.4. Cross-reactivity of S. plumieri venom and stonefish anti-venom

Titration of SpV with SFAV demonstrated that SFAV sera displayed consistent immunoreactivity with the *S. plumieri* venom antigens coated to the microtiter plate (Fig. 4). Under our experimental conditions, SpV showed cross-reactivity with anti-stonefish anti-serum at 1:1000 dilution, whereas pre-immune sera did not react significantly.

3.5. Protein profile of S. plumieri venom and immunoreactivity to stonefish anti-venom

When the crude venom of *S. plumieri* was subjected to 2D-PAGE, distinct protein spots possessing masses between 6 and 120 kDa were identified using Coomassie Blue staining, and the majority of these spots reached the isoelectric point between pH 4 and 7 (Fig. 5A). In order to achieve a better separation profile, an attempt of focalization using a narrow pH gradient range (4–7) strip was

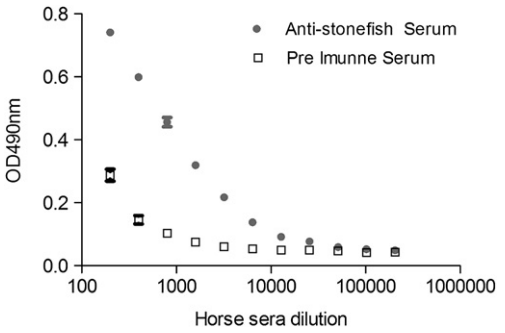

Fig. 4. Characterization by ELISA of the reactivity of *S. plumieri* venom with *Synanceja* antivenom. Microtiter plates were coated with *S. plumieri* venom. Horse anti-serum against stonefish venom (●) was tested at a 1:1000 dilution. The pre-immune serum (□) was used as negative control. The reaction was visualized by incubation with peroxidase-coupled anti-horse IgG.

performed. As it is possible to see in Fig. 5B, this new IEF improved the resolution of the acidic protein spots. This gel was used for a further Western blot cross-reactivity analysis with SFAV where only few protein spots, with apparent molecular mass around 98 kDa and pI ranging from 6 to 7 were recognized by the anti-*Synanceja* serum (Fig. 5C).

4. Discussion

Both clinical and experimental envenomation with the Atlantic black scorpionfish (*S. plumieri*) venom caused pronounced cardiovascular effects, intense pain and edema (Haddad et al., 2003; Carrijo et al., 2005; Gomes et al., 2010). These symptoms are qualitatively similar to those observed after envenomation by stonefish (Sutherland, 1983). The treatment protocol of scorpionfish victims is symptomatic, and some of the local symptoms are alleviated by immersing the affected member in warm water and administrating local anesthetics or analgesics, resulting in slight decrease of the symptoms of the envenomation (Haddad et al., 2003; Haddad, 2000).

In life-threatening accidents involving poisonous animals, the antivenom administration is the major therapeutic option. However, commercial anti-serum against venomous fish is only available for the stonefish *Synanceja trachynis* (StoneFish AntiVenom, SFAV), which together with *Synanceja verrucosa* and *Synanceja horrida*, are the deadliest fish in the world (Khoo et al., 1992; Church and Hodgson, 2001).

The similarities between the envenomation symptoms and the pharmacological activities induced by stone- and scorpionfish venoms (Kreger, 1991; Khoo et al., 1992; Garnier et al., 1995; Carrijo et al., 2005; Gomes et al., 2010), prompted us to investigate whether *in vivo* cardiovascular and inflammatory activities of *S. plumieri* venom could be neutralized by SFAV.

After injection of *S. plumieri* venom in hind paw of mice, a local inflammatory lesion, characterized by intense edema and pain, was observed. The intensity and persistence of the edema were dose-dependent. For all doses tested, the maximal edematogenic response occurred 30 min after venom injection and it remained significantly

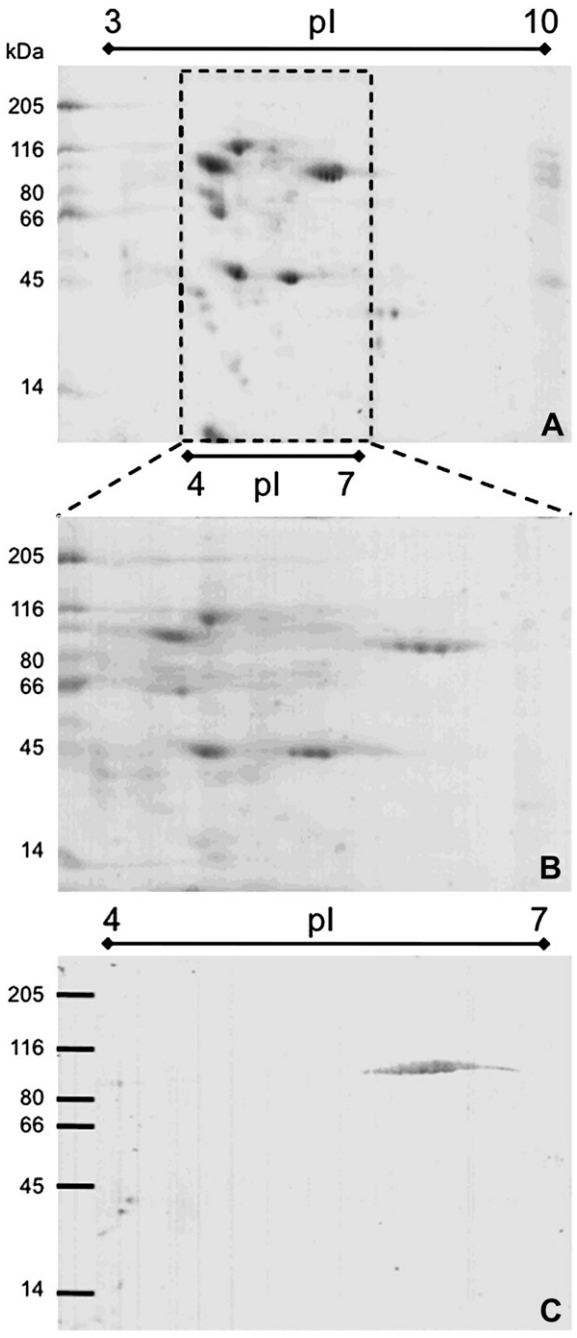

Fig. 5. Two-dimensional gel electrophoresis patterns obtained for venom of *Scorpaena plumieri* (SpV). (A) Gel covering pH 3.0–10; (B) gel covering pH 4.0–7.0; (C) Western immunoblot of SpV with stonefish antivenom (SFAV).

elevated over 6, 24 or 72 h according to the dose administered. In addition, we observed a pronounced nociceptive response which reached its maximum at doses ≥15 μg/paw. This local reaction is similar to that observed on human victims of accidents with scorpionfish *S. plumieri* (Haddad et al., 2003).

Similar inflammatory responses have also been observed in previous studies with other fish venoms. Magalhães et al. (2006) described that both stingrays *Potamotrygon cf. scobina* and *P. gr. orbignyi* venoms induce significant edematogenic activity, which was sustained up to 10 h after injection. Experimental studies carried out with *Thalassophryne nattereri* and *T. maculosa* venoms showed that doses ≤30 μg of venom/paw induce intense edema and nociception (Lopes-Ferreira et al., 1998; Sosa-Rosales et al., 2005b). The *Scatophagus argus* fish venom also produces dose-dependent edema until 48 h after venom injection (Sivan et al., 2007).

Besides the inflammatory response, *S. plumieri* venom caused profound alterations on the cardiovascular system *in vivo* as reported previously (Carrijo et al., 2005; Gomes et al., 2010). The cardiovascular response was characterized by a hypertensive response and bradycardia.

Both inflammatory and cardiovascular responses induced by SpV were neutralized by SFAV. The same assays were carried out with antibothropic antivenom, which was not able to neutralize the SpV cardiovascular effects, suggesting the SFAV specificity (data not shown). Pre-mixing the *S. plumieri* venom with the stonefish antivenom resulted in a protective effect, which was achieved at ratios of 1/1 and 1/1.5 μg protein of venom/U of antivenom. This neutralisation activity demonstrates that the pro-inflammatory and cardioactive venom compounds are mainly proteins.

These results are in accordance with those of Carlson et al. (1971), which suggested that the cardiovascular effects induced by the *Scorpaena guttata* venom are probably mediated by the release of endogenous stores of neurotransmitters by protein compounds, rather than the presence of either transmitter in the venom. However, we cannot exclude the possible presence of neurotransmitters or low molecular mass mediators in the *S. plumieri* venom, since they have been found in *S. verrucosa* and *S. horrida* stonefish venoms (Garnier et al., 1996).

The two-dimensional SDS-PAGE analyses showed that the majority of the *S. plumieri* venom components are in the mass range of 6–120 kDa and are predominantly anionic proteins (pI 4–7). A similar MW range has been described for the protein components of other fish venoms: 20–295 kDa in *Synanceja trachynis* (Hopkins and Hodgson, 1998), 11–109 kDa in *Gymnapistes marmoratus* (Hopkins and Hodgson, 1998), 14–100 kDa in *Thalassophryne maculosa* (Sosa-Rosales et al., 2005a), 15–130 kDa in *Potamotrygon falkneri* (Haddad et al., 2004).

Despite the fact that various proteins are found in the SpV, only the major spot observed in the two-dimensional electrophoretic profile of *S. plumieri* venom was recognized by the SFAV after immunoblotting analysis. These *in vitro* observations correlate well with the results obtained in the *in vivo* assays and also corroborate that *S. plumieri* venom compounds responsible for inflammatory and cardiovascular effects are similar to those found in stonefish venom. In addition, ELISA analysis of *S. plumieri* venom proteins suggested that the epitope(s) detected by the neutralizing polyclonal SFAV antibody is (are) shared by proteins present in both fish venoms.

Interestingly, Andrich et al. (2010) demonstrated that SFAV was able to cross-react and neutralise the hemolytic activity of Sp-CTx, a dimeric (73 kDa/subunit) cytolytic and vasoactive glicoprotein isolated from *S. plumieri* venom

(Andrich et al., 2010). Thus, due to its MW it is possible that the SFAV-recognized spot in the present work is the previously identified scorpionfish venom cytolysin. The isoeletric point variation of the SFAV-recognized protein spot could be due to the different glycosilation levels exhibited by Sp-CTx (Andrich et al., 2010), being an additional evidence that the SFAV-recognized spot is the scorpionfish cytolysin.

Both the molecular mass (98 kDa) and isoeletric point (6.0–7.0) values of SFAV-recognized protein spot are similar to the stonustoxin (SNTX; α subunit = 71 kDa, β subunit = 79 kDa, pI 6.9) and trachynilysin values (TLY; α subunit = 76 kDa, β subunit = 83 kDa, pI 5.7), the dimeric cytolytic toxins isolated from *Synanceja horrida* and *S. trachynis* venoms, respectively (Poh et al., 1991; Kreger, 1991; Colasante et al., 1996). The cytolysins from fish venoms are reported as multifunctional toxins, triggering an array of biological actions, including *in vitro* hemolysis, increase in vascular permeability, cardiovascular disorders and death (Perriere et al., 1988; Poh et al., 1991; Kreger, 1991; Chhatwal and Dreyer, 1992; Garnier et al., 1995; Colasante et al., 1996; Ueda et al., 2006), probably as the result of a non-specific cell membrane disturbing action (Kreger, 1991; Chen et al., 1997). Albeit few pharmacological activities were described for Sp-CTx (hemolysis and vascular tonus modulation) (Andrich et al., 2010), the neutralisation of the local inflammatory and cardiovascular effects of crude *S. plumieri* venom by SFAV-treatment offers evidence that this scorpionfish cytolysin may possess other pharmacological actions. However, further studies are necessary to assess such potential features of this cytolysin.

The results obtained demonstrated that the stonefish antivenom evoked an immune cross-reactive response with scorpionfish venom. SFAV is efficient in neutralising the most prominent toxic effects of scorpionfish venom. This is in accordance with the hypothesis that venomous fish belonging to different genera or inhabiting different regions may share venom compounds with similar antigenic properties (Church and Hodgson, 2002b). This resemblance may rely on the fact that piscine venoms have evolved for a same defensive purpose and possess similar multifunctional cytolytic toxins (Saunders, 1960; Russell, 1965; Kreger, 1991; Church and Hodgson, 2002b; Andrich et al., 2010).

Finally, the study of such venoms and/or toxins may be useful for developing new and more specific antivenoms (or even antibodies) targeting specifically the fish venoms membrane-disturbing toxins and helping in alleviating the major symptoms of scorpionfish envenomation.

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Conflict of interest

None declared.

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