ELSEVIER

Toxicon 49 (2007) 804–809

TOXICON

www.elsevier.com/locate/toxicon

An in vivo examination of the stability of venom from the Australian box jellyfish *Chironex fleckeri*

K.L. Winter[a], G.K. Isbister[a,b], J.E. Seymour[c], W.C. Hodgson[a,*]

[a]*Monash Venom Group, Department of Pharmacology, Monash University, Vic. 3800, Australia*
[b]*Tropical Toxicology Unit, Menzies School of Health Research, NT, Australia*
[c]*Department of Tropical Biology, James Cook University, Qld. 4878, Australia*

Received 29 August 2006; received in revised form 29 November 2006; accepted 29 November 2006
Available online 15 December 2006

Abstract

We have previously characterised the pharmacological activity of a number of jellyfish venoms with a particular emphasis on the profound cardiovascular effects. It has been suggested that jellyfish venoms are difficult to work with and are sensitive to pH, temperature and chemical changes. The current study aimed to examine the working parameters of the venom of the Australian box jellyfish *Chironex fleckeri* to enable fractionation and isolation of the toxins with cardiovascular activity. *C. fleckeri* venom was made up fresh each day and subjected to a number of different environments (i.e. a pH range of 5–9 and a temperature range of 4–30 °C). In addition, the effect of freeze drying and reconstituting the venom was investigated. Venom (50 μg/kg, i.v.) produced a transient hypertensive response followed by cardiovascular collapse in anaesthetised rats. This biphasic response was not significantly effected by preparation of the venom at a pH of 5, 7 or 9. Similarly, venom (50 μg/kg, i.v.) did not display a loss of activity when exposed to temperatures of 4, 20 or 30 °C for 1.5 h. However, the cardiovascular activity was abolished by boiling the venom. Freeze drying, and then reconstituting, the venom did not significantly affect its cardiovascular activity. However, repeated freeze drying and reconstituting of extracted venom resulted in a significantly loss of activity. This study provides a more detailed knowledge of the parameters in which *C. fleckeri* venom can be used and, while supporting some previous studies, contradicts some of the perceived problems of working with the venom.

Keywords: Jellyfish; Venom; *Chironex fleckeri*; Stability; Cardiovascular

1. Introduction

The major Australian box jellyfish *Chironex fleckeri* is arguably the most venomous creature in the world. In the past the pharmacological and biological characterisation of *C. fleckeri* venom has been hindered by many factors (Bloom et al., 1998; Carrette and Seymour, 2004). Nematocyst-derived venom devoid of tentacular debris has been difficult to obtain. It has also been suggested that jellyfish venoms are sensitive to pH, temperature or chemical changes, or a combination of these factors, and have a tendency to aggregate, disaggregate and stick to the surface of equipment (Baxter and Marr, 1969; Othman and Burnett, 1990; Bloom et al., 1998;

*Corresponding author. Tel.: +61 3 99054861; fax: +61 3 99055851.
E-mail address: wayne.hodgson@med.monash.edu.au (W.C. Hodgson).

0041-0101/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.toxicon.2006.11.031

Carrette and Seymour, 2004). These difficulties have hampered the isolation of the active components of *C. fleckeri* venom. Previous methods of venom extraction have yielded samples that may be devoid of the active toxins present in native venom. In addition, the tentacles of *C. fleckeri* have distinct pharmacological activity (Endean and Noble, 1971; Ramasamy et al., 2005a). We previously used a technique developed by Carrette and Seymour (2004), based on the work by Bloom et al. (1998), which provides venom samples that are devoid of tentacular material. In this study we examine the stability of comparatively pure *C. fleckeri* venom in order to develop a better understanding of its lability with a view of identifying and isolating the individual toxins from *C. fleckeri* venom. As cardiovascular collapse appears to be the primary cause of death in envenomed humans (Currie, 2003) and rats (Ramasamy et al., 2004), we utilised the anaesthetised rat as the assay for examining the activity of the venom subjected to a variety of conditions.

2. Methods

2.1. Nematocyst extraction

Mature specimens of *C. fleckeri* were collected from Mission Beach in north Queensland. Tentacles were removed and stored immediately in sea water. The nematocysts were isolated from the tentacles as described by Bloom et al. (1998). The nematocysts were then lyophilised and stored at −20 °C until use. The venom was then extracted from the freeze dried nematocysts (Carrette and Seymour, 2004). Nematocysts were weighed into aliquots of approximately 20 mg in screw top vials. Glass beads (~8000, 0.5 mm in diameter) and 1.5 ml of distilled water (pH 7) were added to the vials. Samples were then shaken four times in a mini bead mill at 5000 rpm for 10 s, and placed on ice for 1 min in between each cycle. The supernatant (venom) was separated from the pellet (nematocyst debris) using a pipette and stored on ice. It was then centrifuged at 4 °C for 3 min at 12000 rpm. Protein content was determined using a Pierce BCA protein assay kit. Samples were read at 562 nm in a Fusion α microplate reader (Packard Bioscience).

2.2. Anaesthetised rat

Male Sprague–Dawley rats were anaesthetised with pentobarbitone sodium (60–100 mg/kg; i.p.) which was supplemented as required. A midline incision was made in the cervical region and cannulae inserted into the trachea, jugular vein and carotid artery, for artificial respiration if needed, administration of drugs/venom and measurement of blood pressure, respectively. Arterial blood pressure was measured using a Gould Statham P23 pressure transducer connected to a MacLab Chart system. Venom was administered via the right jugular vein and flushed through with heparinised (25 U/ml) 0.9% saline (0.2 ml). At the conclusion of the experiment animals were killed by an overdose of pentobarbitone sodium (i.v.). Approval for all animal experiments was obtained from the Monash University Animal Ethics Committee.

2.3. Experimental parameters

Experiments were undertaken to observe the effect of temperature, pH and freeze drying on the cardiovascular activity of *C. fleckeri* venom. Venom (pH 7) was prepared as above (2.1) and then subjected to temperatures of 20 or 30 °C for a period of 1.5 h. The temperatures used in this study were chosen based on the expected temperatures that the venom would typically be exposed to during HPLC analysis. Alternatively, venom was boiled for a period of 5 min before cooling to room temperature. In additional experiments, venom was prepared at a pH of 3, 5 or 9 before use or prepared at pH 7, freeze dried and then reconstituted in distilled water. A further sample was then subsequently freeze dried a second time before being reconstituted in distilled water.

2.4. Statistics

One-way analysis of variance (ANOVA) followed by a Dunnet's post hoc test was used to analyse changes in the cardiovascular effects of venom. In all cases, statistical significance is indicated by $P<0.05$. All values are expressed as mean ± standard error of the mean

3. Results

3.1. The effect of C. fleckeri venom on anaesthetised rats

Venom (50 μg/kg; i.v.) produced a pressor response (38 ± 13 mmHg; $n = 3$; Fig. 1) followed by