

Haemodynamic effects of the crude venom from nematocysts of the box-jellyfish *Chiropsalmus quadrigatus* (Habu-kurage) in anaesthetized rabbits

Tomoyuki Koyama, Katsuhiko Noguchi, Toshihiro Matsuzaki, Mayuko Sakanashi, Junko Nakasone, Kanako Miyagi, Makiko Sakanashi and Matao Sakanashi

^a Department of Pharmacology, School of Medicine, Faculty of Medicine, University of the Ryukyus, 207 Uehara, Nishihara-cho, Okinawa 903-0215, Japan

^b Department of Anaesthesiology, School of Medicine, Faculty of Medicine, University of the Ryukyus, 207 Uehara, Nishihara-cho, Okinawa 903-0215, Japan

Received 20 September 2002; accepted 21 January 2003. ; Available online 28 March 2003.

Abstract

Haemodynamic effects of saline-extracted venom from nematocysts isolated from tentacles of the box-jellyfish *Chiropsalmus quadrigatus* (Habu-kurage) were investigated. In anaesthetized rabbits, i.v. injections of the venom produced hypotension following a transient hypertension. Mean femoral arterial blood flow markedly decreased immediately after the injection and femoral vascular resistance increased. Left ventricular dP/dt remarkably decreased after a transient and small increase, and heart rate decreased. Left ventricular end-diastolic pressure markedly elevated. All of the above changes by 0.2–5 $\mu\text{g}/\text{kg}$ of the venom expressed as the amount of protein were seen dose-dependently and occurred without tachyphylaxis. In five of seven animals received an injection of the venom at 10 $\mu\text{g}/\text{kg}$, irreversible cardiac arrest occurred. Changes produced by 1 or 2 $\mu\text{g}/\text{kg}$ of the venom were significantly attenuated either by heating the venom at 40 °C for 10 min or by pretreatment with diltiazem. These results indicate that the venom from Habu-kurage has both vasoconstrictor and cardiodepressive effects, and suggest that these thermolabile actions may be due partly to activation of voltage-dependent calcium channels and probably subsequent calcium-overload.

Author Keywords: Haemodynamics; *Chiropsalmus quadrigatus*; Habu–kurage; Venom; Rabbit

Article Outline

1. Introduction
 2. Materials and methods
 - 2.1. Preparation of crude venom from nematocysts of *C. quadrigatus* ‘Habu–kurage’
 - 2.2. Surgical preparation of rabbits
 - 2.3. Effects of CQV on haemodynamic parameters
 - 2.4. Effects of heated CQV
 - 2.5. Effects of diltiazem treatment
 - 2.6. Drugs
 - 2.7. Data analysis
 3. Results
 - 3.1. Effects of CQV on haemodynamics
 - 3.2. Effects of heated CQV
 - 3.3. Effects of diltiazem treatment
 4. Discussion
- Acknowledgements
- References

1. Introduction

The cubomedusan *Chiropsalmus quadrigatus* (*C. quadrigatus*) named Habu–kurage in Japan has been responsible for many serious accidents including some fatal cases of children in the shallows around Okinawa Island. Despite that the effective treatment for the envenomation is desired to be established, the characteristics and the nature of the venom have not always been well defined to date.

The venom of the fatal cubomedusan *Chironex fleckeri* (*C. fleckeri*) whose size of the bell and the number of the tentacles are known to distinguish *Chironex* from *Chiropsalmus*, which has a name as the sea–wasp or the box–jellyfish in Australia, has extensively been studied by many investigators since the 1960's when the symptoms of envenomation from this stinger were firstly reported (Cleland and

Southcott, 1965; Barnes, 1966 and Baxter et al., 1969). Some studies have shown that the venom of *C. fleckeri* exerts vasoconstriction and cardiotoxicity in the anaesthetized rats and rabbits (Endean et al., 1969 and Freeman and Turner, 1971) and in isolated vascular and cardiac preparations (Turner and Freeman, 1969), and haemolytic activity (Keen and Crone, 1969). However, there were a number of controversies among the previous data concerning the effects of the venom on animals or isolated organs (Barnes, 1967; Freeman and Turner, 1969 and Endean and Henderson, 1969), since the tentacles used in these experiments possessed their own biological effects such as haemolysis and a loss of the activities possibly occurred during preparation of the venom (Endean and Noble, 1971 and Endean et al., 1993).

On the other hand, it is generally known that the cubomedusae including *C. quadrigatus* has highly toxic venom in nematocysts in abundance on their tentacles, which are usually utilized to catch feeds. Therefore, in the present study the crude venom of *C. quadrigatus* 'Habu-kurage' was prepared not from tentacles but solely from nematocysts, which were separately isolated from the tentacles, according primarily to the methods described by Endean et al. (1969). Parenteral administration of such venom is expected to mimic the envenomation from the stinger.

Thus, the present study was conducted to evaluate haemodynamic effects of the intravenously given venom of *C. quadrigatus* 'Habu-kurage', extracted from isolated nematocysts, in anaesthetized rabbits.

2. Materials and methods

The animals used in the study were handled in accordance with Guidelines for the Animal Experimentation of the University of the Ryukyus and the experimental protocol was approved by the Animal Care and Use Committee of the institution.

2.1. Preparation of crude venom from nematocysts of *C. quadrigatus* 'Habu-kurage'

Specimens of *C. quadrigatus* 'Habu-kurage' were collected on Chatan coast in Okinawa Island, from July to September. Tentacles were cut from the pedaliem and were stored at -30°C until use. The contents of the nematocysts were extracted in physiological saline (0.9% NaCl) following the method of a previous report (Endean et al., 1969) with some modifications as described below. After thawing, the

tentacles were gently ground with a pestle on a mortar to separate nematocysts from tentacles in saline. The nematocyst suspension was then placed at 4 °C for 24 h and decanted in a beaker to remove materials from tentacles and discharged nematocysts. This process was repeated daily for several days until disappearance of the effects of supernatants on blood pressure could be confirmed in separate anaesthetized rabbits. The saline suspension of nematocysts was stored at -30 °C. On the day of experiments, an equal volume of cold saline was added to the suspension and homogenized with a glass-glass homogenizer to extract the active components in nematocysts. Then the homogenate was centrifuged at 10,000 rpm for 10 min and filtrated with a 0.45 µm syringe filter (Toyo Roshi, Tokyo, Japan) to remove the fragments and residual intact nematocysts. The supernatant was utilized as the crude venom of *C. quadrigatus* (CQV) in the present experiments. The protein content of the crude venom was 1500–1800 µg/ml, which was measured by the method of Lowry et al. (1951) using bovine serum albumin as the standard. Doses of CQV were expressed as the amount of protein. CQV was diluted to various concentrations of 2–100 µg/ml with cold saline.

2.2. Surgical preparation of rabbits

Male rabbits weighing between 2.4 and 3.5 kg were anaesthetized with pentobarbitone sodium at 30 mg/kg i.v. Additionally, pentobarbitone sodium at 5–6 mg/kg/h was infused via the right ear vein to maintain a constant level of anaesthesia throughout the experimental period. Tracheotomy was performed to make spontaneous ventilation easier. A heparinized catheter was inserted into the jugular vein for intravenous administration of CQV and drugs. Aortic pressure (AoP) was measured through a catheter which was inserted into the abdominal aorta via the right femoral artery. The catheter was connected to a pressure transducer (TP-400T, Nihon Kohden, Tokyo, Japan). A catheter tip manometer (SPR-524, Millar Instruments, Houston, TX, USA) was introduced through the left carotid artery into the left ventricular cavity to measure left ventricular pressure (LVP). The first derivative of LVP ($LVdP/dt$), an index of cardiac contractility, was derived from differentiating the signal of LVP using an electronic differentiator (ED-601G, Nihon Kohden). Heart rate (HR) was continuously counted with a cardiometer (AT-601G, Nihon Kohden) triggered by electrocardiogram. A flow probe (6R668, Transonic Systems Inc., Ithaca, NY, USA), which was connected to an ultrasonic flowmeter (T106, Transonic System Inc.), was positioned around the root of the femoral artery for measurement of mean left femoral arterial blood flow (FBF). Femoral vascular resistance (FVR) was calculated from the following equations: FVR

(mmHg · min/ml)=mean AoP/FBF. Respiratory rate was continuously counted with a thermistor (AA-601H, Nihon Kohden) attached on the opening of the tracheal tube. Data were continuously recorded on a pen recorder (8K-23; NEC San-ei Instrument, Tokyo, Japan).

2.3. Effects of CQV on haemodynamic parameters

After completion of surgical preparations, rabbits were allowed to stabilize for at least 30 min without intervention. The baseline haemodynamics was obtained 3 min before CQV injection. Each dose of the venom at 0.2–10 µg/kg was injected into the jugular vein in a bolus of 0.1 ml/kg with an interval of at least 15 min.

2.4. Effects of heated CQV

Since the venom from *C. fleckeri* has been reported to be thermolabile (Endean and Henderson, 1969), we evaluated the thermostability of CQV by heating. CQV diluted to a concentration of 10 µg/ml was incubated at 40 °C for 10 min before injection. In seven rabbits, the effects of heated CQV at 1 µg/kg on haemodynamic parameters were examined in comparison with those of non-heated CQV.

2.5. Effects of diltiazem treatment

The effects of CQV at 2 µg/kg on haemodynamic parameters were examined in the presence or absence of diltiazem, a selective blocker of the voltage-dependent calcium channel, in six rabbits. Approximately 5 min before CQV injection, diltiazem was given into the jugular vein in a bolus of 100 µg/kg and continuously infused at a rate of 10 µg/kg/min.

2.6. Drugs

The drugs employed in this study were acetylcholine chloride (Dai-ichi, Tokyo, Japan), noradrenaline (Sankyo, Tokyo, Japan), lidocaine (Fujisawa, Osaka, Japan), Phentolamine mesylate (Ciba-Geigy, Tokyo, Japan) and atropine sulfate flecainide and indomethacin (Sigma Chemical Co., St Louis, MO, USA). Diltiazem hydrochloride was kindly donated from Tanabe Pharmaceutical Co., Ltd, Osaka, Japan. Flecainide was dissolved with 5% dimethyl sulfoxide in 5% glucose and then diluted with saline just before use. Indomethacin was dissolved in saline containing 0.2% sodium carbonate just prior to use. Other drugs were dissolved in or diluted with saline.

2.7. Data analysis

The time sequence data were analysed by two-way analysis of variance. The level for statistical significance was $P < 0.05$. All results are expressed as mean \pm SE.

3. Results

3.1. Effects of CQV on haemodynamics

Typical changes in haemodynamic parameters after i.v. injections of CQV to a rabbit are shown in Fig. 1, in which changes in haemodynamics with CQV at 1–10 $\mu\text{g}/\text{kg}$ are represented. AoP showed a decline following a transient elevation at all doses, and a second elevation after the decline was seen with 2–5 $\mu\text{g}/\text{kg}$. At all doses, marked decreases in FBF, HR and LVdP/dt were observed.

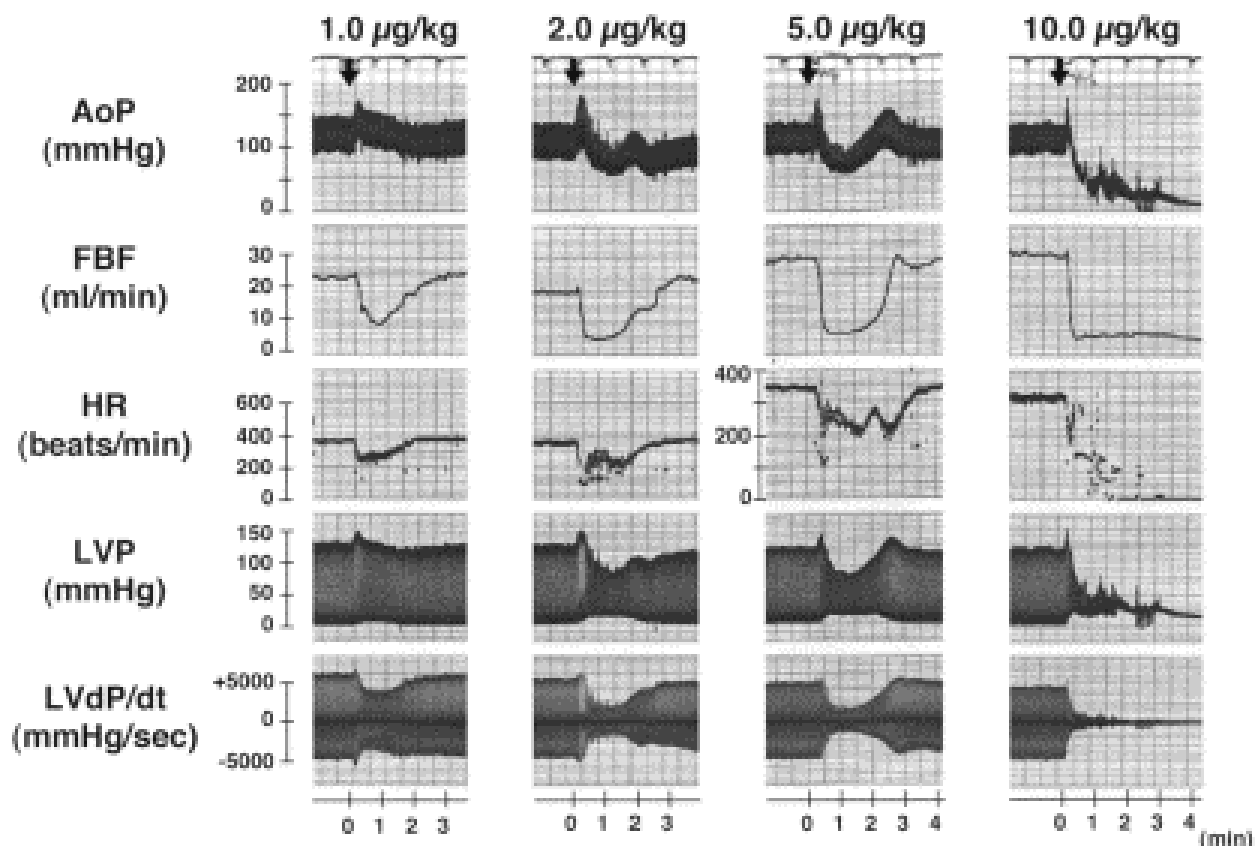


Fig. 1. Representative tracings of haemodynamic changes following an intravenous injection of crude venom form nematocysts of *C. quadrigatus* 'Habu-kurage' (CQV) in a rabbit. AoP=aortic pressure, FBF=mean left femoral arterial blood flow, HR=heart rate, LVP=left ventricular pressure, LVdP/dt=the first derivative of LVP.

Two of nine rabbits, five of seven rabbits and two of two rabbits were dead with 5, 10 and 20 $\mu\text{g}/\text{kg}$ of CQV, respectively. In these fatal cases, irreversible decompensation was seen between 2 and 8 min after administration of CQV as shown in Fig. 1. Thus, the 50% lethal dose for anaesthetized rabbits was estimated to be 7.1 $\mu\text{g}/\text{kg}$ i.v. with 95% confidence limit of 4.8–10.5 $\mu\text{g}/\text{kg}$ using the least-squares method.

Severe arrhythmia such as ventricular fibrillation or ventricular tachycardia was not noted with any doses of CQV in all animals. Apnea occurred only after appearance of the irreversible changes in haemodynamics which was often associated with frothy pink sputum suggesting acute left ventricular failure.

Data from seven rabbits are summarized in Table 1 and Fig. 2. Administration of CQV at 0.2–5 $\mu\text{g}/\text{kg}$ i.v. immediately produced a transient elevation of AoP followed by a decline. FBF decreased to the minimum level of 31.3% of the baseline value with CQV at 5 $\mu\text{g}/\text{kg}$ i.v. FVR calculated from mean AoP and FBF markedly increased. Significant bradycardia to the minimum level of 50.2% of the resting HR occurred immediately after the injection of CQV at 5 $\mu\text{g}/\text{kg}$. (+)LVdP/dt showed a small and transient increase followed by marked depression, which was associated with a marked elevation of LVEDP. These changes elicited by CQV injections at doses ranging between 0.2 and 5 $\mu\text{g}/\text{kg}$ occurred in a dose-dependent manner and disappeared within approximate 5 min after the administration.

Table 1. Baseline values of haemodynamic parameters in anaesthetized rabbits

Haemodynamic parameters	Baseline values
mAoP (mmHg)	110.0±10.5
FBF (ml/min)	18.3 ±4.2
FVR (mmHg · min/ml)	6.9 ±2.6
HR (beats/min)	345.7±18.6
(+)LVdP/dt (mmHg/s)	5471 ±1481
LVEDP (mmHg)	2.9 ±1.5

mAoP=mean aortic pressure, FBF=mean left femoral arterial blood flow, FVR=femoral vascular resistance (mAoP/FBF), HR=heart rate, (+) LVdP/dt=peak positive left ventricular dP/dt and LVEDP=left ventricular end-diastolic pressure. Each value represents mean±SE (n=7).

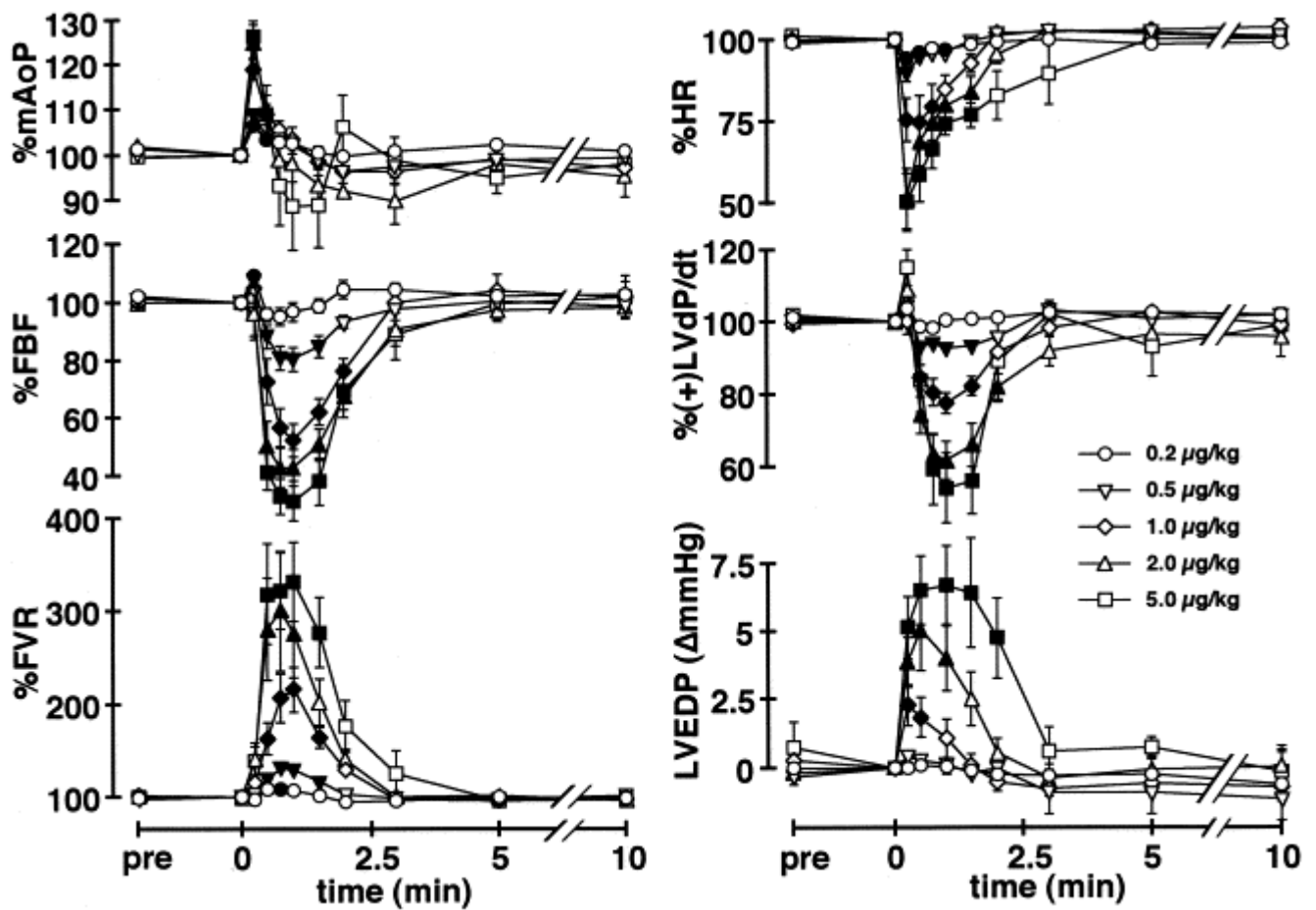


Fig. 2. Effects of CQV on haemodynamic parameters in rabbits. mAoP=mean aortic pressure, FBF=mean left femoral arterial blood flow, FVR=femoral vascular resistance (mAoP/FBF), HR=heart rate, (+)LVdP/dt=peak positive left ventricular dP/dt and LVEDP=left ventricular end-diastolic pressure. Data are shown as percentage of the preinjection value (0 min), but LVEDP is shown as the difference from the preinjection value. Each value represents mean \pm SE ($n=7$). Significant difference ($P<0.05$) from the respective preinjection value was expressed as the solid symbols.

Fig. 3 shows effects of repeated injections of 1 $\mu\text{g}/\text{kg}$ of CQV at an interval of 15 min. Haemodynamic effects of CQV were practically identical between the 1st and 2nd injections, indicating that the effects show no tachyphylaxis. The effects of repeated administration of CQV at 2 $\mu\text{g}/\text{kg}$ were also reproducible (data not shown).

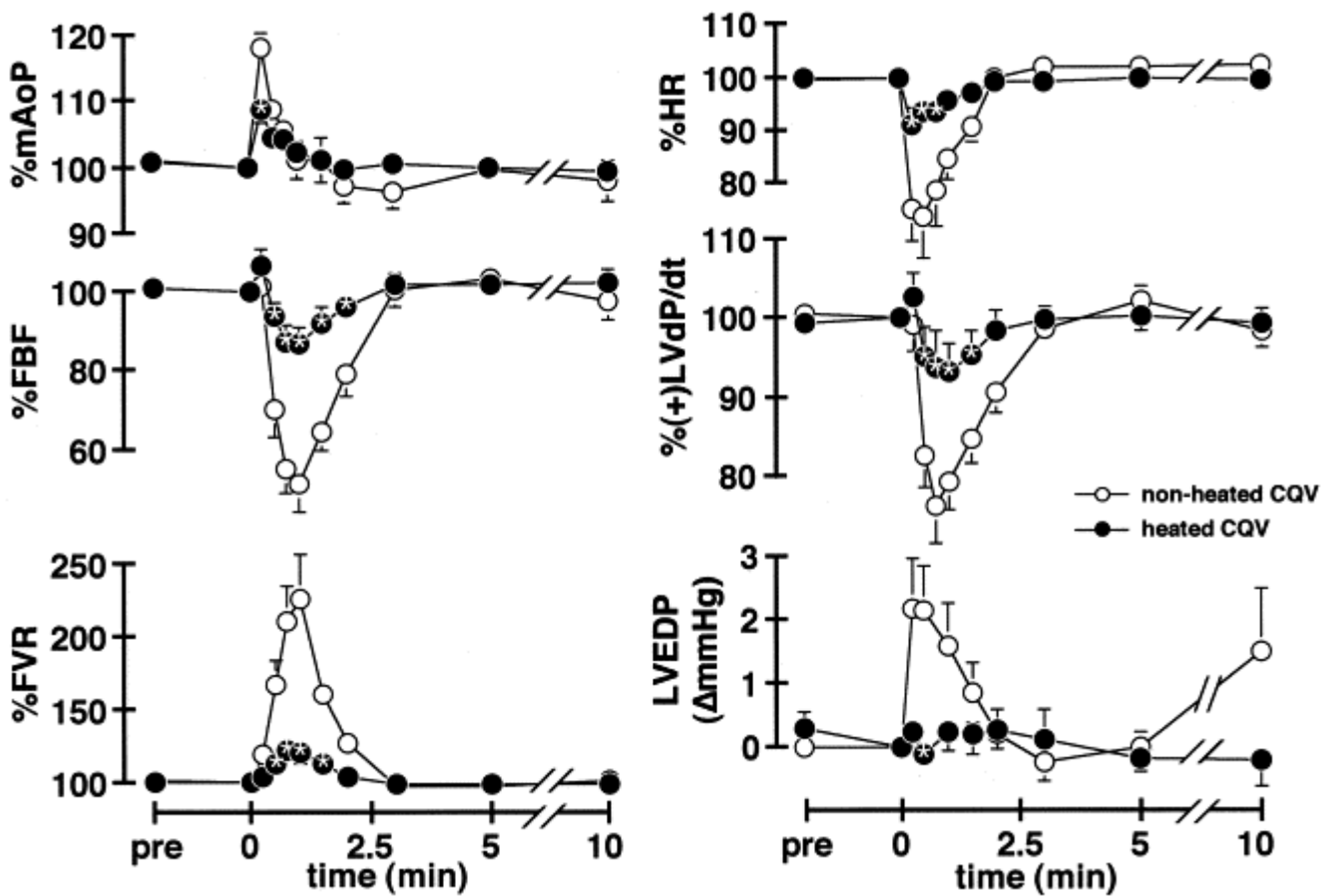


Fig. 3. Reproducibility of haemodynamic effects of CQV. Injection of the venom at 1 $\mu\text{g}/\text{kg}$ was repeated at a 15 min interval. Abbreviations are the same as for Fig. 2. Data are shown as percentage of or the difference from the preinjection value (0 min). Each value represents mean \pm SE ($n=7$). There were no significant differences between the effects induced by the 1st and 2nd injections.

3.2. Effects of heated CQV

As shown in Fig. 4, heat-treated CQV (1 $\mu\text{g}/\text{kg}$ i.v.)-induced increases in AoP, FVR and LVEDP, and decreases in FBF, HR and (+)LVdP/dt were all significantly attenuated in comparison with those produced by non-heated CQV at the same dose ($P < 0.05$, $n=7$).

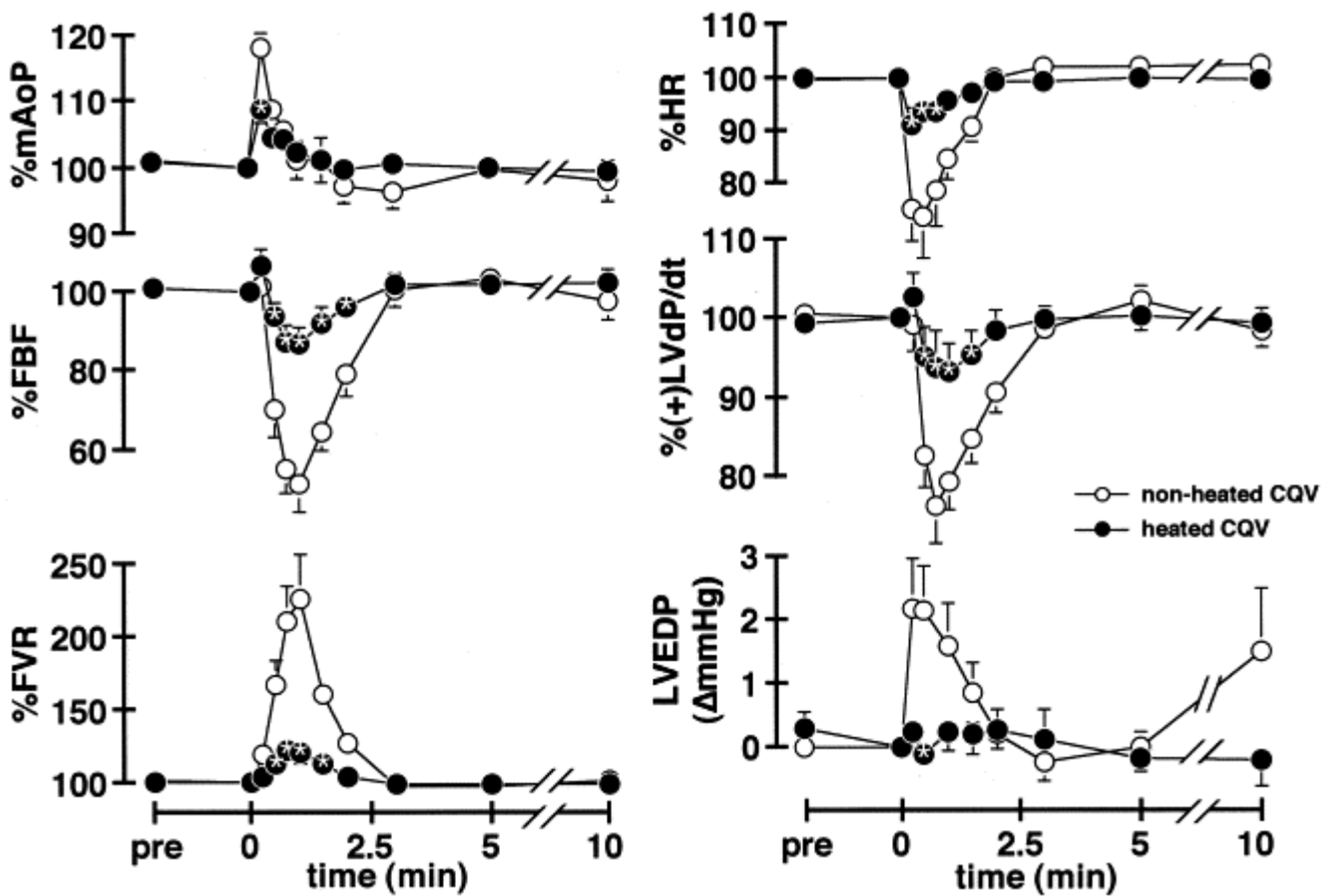


Fig. 4. Effects of heated CQV at 40 °C for 10 min. Abbreviations are the same as for Fig. 2. Data are shown as percentage of or the difference from the preinjection value (0 min). Each value represents mean \pm SE ($n=7$). Effects of heated venom at 1 $\mu\text{g}/\text{kg}$ were compared with those of non-heated venom at 1 $\mu\text{g}/\text{kg}$. *: $P < 0.05$ vs. non-heated venom.

3.3. Effects of diltiazem treatment

Baseline values for haemodynamic parameters were not significantly affected in the presence of diltiazem, although mean AoP and LVEDP were tended to decrease (Table 2). Treatment with diltiazem significantly moderated haemodynamic effects of CQV at 2 $\mu\text{g}/\text{kg}$ i.v. ($P < 0.05$, $n=6$) compared with those in the absence of diltiazem (Fig. 5).

Table 2. Baseline values of haemodynamic parameters before and after diltiazem

Haemodynamic parameters	[Before]	[After]
mAoP (mmHg)	101.1±10.0	89.3 ±9.4
FBF (ml/min)	18.6 ±4.3	18.2 ±4.2
FVR (mmHg · min/ml)	5.9 ±2.4	5.4 ±2.3
HR (beats/min)	344.2±18.5	335.8±18.3
(+)LVdP/dt (mmHg/s)	5239 ±1450	4769 ±1382
LVEDP (mmHg)	4.4 ±2.0	3.4 ±1.7

mAoP=mean aortic pressure, FBF=mean left femoral arterial blood flow, FVR=femoral vascular resistance (mAoP/BBF), HR=heart rate, (+) LVdP/dt=peak positive left ventricular dP/dt and LVEDP=left ventricular end-diastolic pressure. Each value represents mean±SE (n=6). There were no significant differences between values before and after diltiazem.

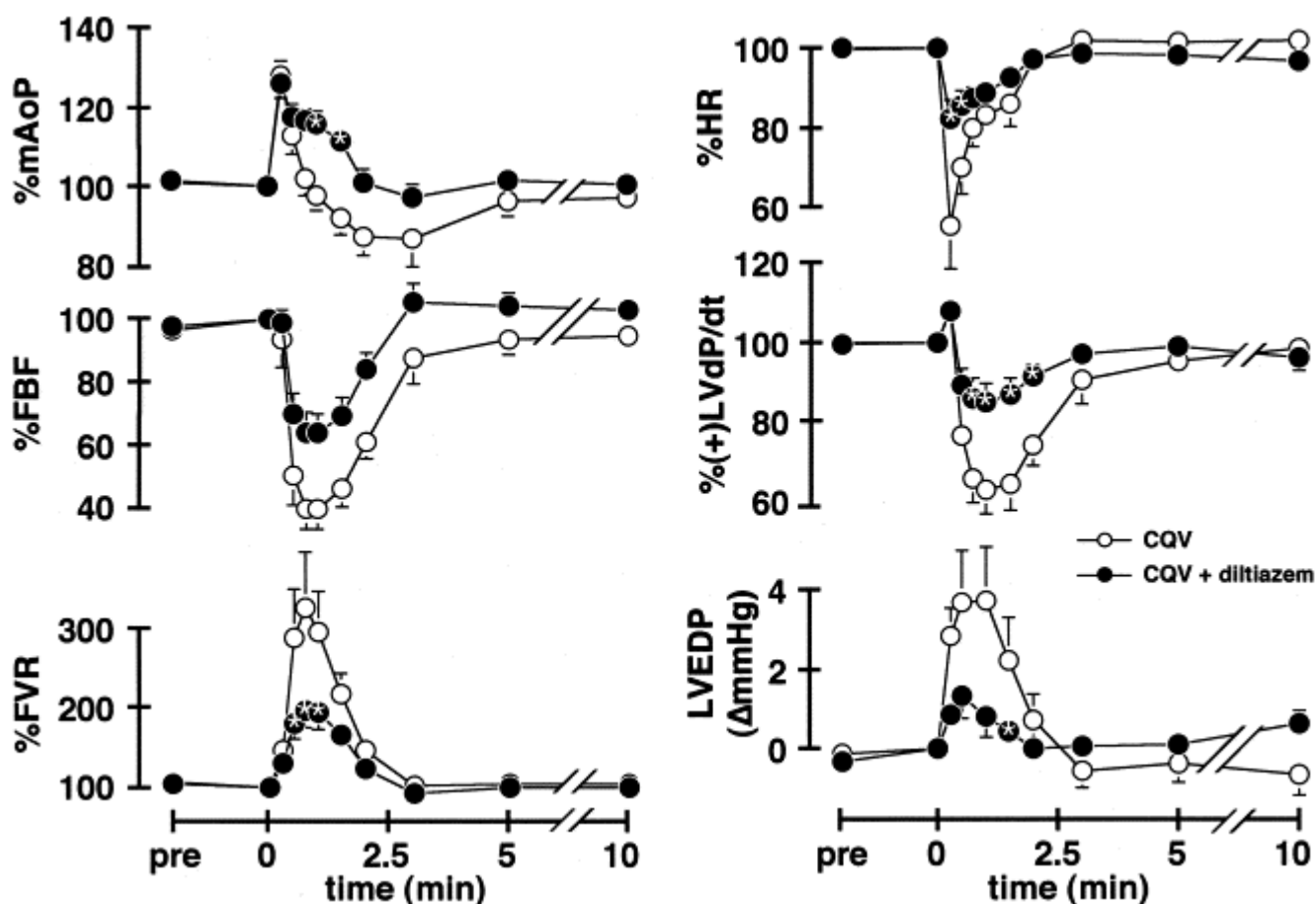


Fig. 5. Effects of diltiazem on haemodynamic changes produced by CQV at 2 µg/kg. Abbreviations are the same as for Fig. 2. Data are shown as percentage of or the difference for the preinjection value (0 min). Each value represents mean±SE (n=6). Effects of the venom in the presence of diltiazem treatment at 100 µg/kg in a bolus+10 µg/kg/min in a continuous infusion were compared with those in the absence of diltiazem treatment. *: P<0.05 vs. in the absence of diltiazem treatment.

On the other hand, pretreatments with the sodium channel inhibitors lidocaine at 4 mg/kg ($n=1$) and flecainide 3 mg/kg ($n=1$), the cyclooxygenase inhibitor indomethacin at 2 mg/kg ($n=2$), the α -adrenergic antagonist phentolamine at 1 mg/kg ($n=2$) and the muscarinic antagonist atropine at 3 or 5 mg/kg ($n=2$) all did not affect the haemodynamic changes with CQV. In these experiments, the effectiveness of blockade with phentolamine and atropine at the above doses was confirmed by disappearance of pressor and depressor responses to noradrenaline (1 μ g/kg, i.v.) and acetylcholine (1 μ g/kg, i.v.), respectively (data not shown).

4. Discussion

With an i.v. injection of CQV at 10 μ g/kg or more, irreversible decompensation in the cardiovascular system occurred within a few minutes in most cases, which was not associated with severe arrhythmia and preceding apnea. Administration of CQV immediately produced small elevations and subsequent dose-dependent declines of AoP, while decreases in FBF appeared to be pronounced. Dose-dependent and monophasic increases in FVR after i.v. injection of CQV clearly indicate that CQV possesses a potent vasoconstrictor activity. This is consistent with the findings of our recent report describing a dose-dependent vasoconstrictor effect of CQV in isolated rat aortae (Sakanashi et al., 2002). On the other hand, (+)LVdP/dt, an index of cardiac contractility, showed a transient increase followed by profound depression, suggesting that CQV may have rapidly and briefly developed small positive inotropic effects followed by the delayed negative inotropic effects that were more evident in the case of higher doses. CQV also exerted a marked negative chronotropic effect, which is possibly attributable to baroreflex-mediated sympathetic depression resulting from the initial hypertension or to a direct inhibitory action on the cardiac conduction system or to both. Thus, hypotension induced by higher doses of CQV seems to be due primarily to a definite decrease in cardiac output derived from the potent negative inotropic and chronotropic effects rather than the effects on blood vessels. Furthermore, it is probable that the marked elevation of LVEDP results from both decreases in HR and profound depression of (+)LVdP/dt. The present data are compatible with the previous findings reporting effects of the venom of *C. fleckeri* on systemic blood pressure and HR in rabbits (Freeman and Turner, 1969).

Tachyphylaxis of the haemodynamic effects produced by CQV administration were not seen in any parameters. This provides the basis for comparison between the effects in the absence and the presence of an inhibitor or treatment within one

animal. In addition, the lack of tachyphylaxis implies that the effects of CQV result mainly from its direct effects on target organs but not from possible indirect effects due to release of endogenous mediators as previously shown in Habu–snake venom (Kinjo et al., 1990). Further investigations may be required in regard to these peculiar actions of CQV.

In our preliminary experiments, we found that the subcutaneous administration of CQV even at an extremely high dose (172 or 500 µg/kg) had no lethal effect in separate two anaesthetized rabbits (data not shown). This indicates that absorption of CQV into blood through vessel wall from the interstitial space may be almost unable or very slow, and/or may show the possibility that the venom is partially inactivated during absorption. Similarly, in the case of crude venom of *C. fleckeri*, it has been reported that intraperitoneal or subcutaneous administration at high doses containing the contents of approximately 200,000 or 2,500,000 nematocysts, respectively, to mice had no lethal toxicity (Endean et al., 1969). These findings suggest that envenomation of a certain amount of the venom directly into the blood stream might be needed to exert the evident lethal effects.

Heating treatment at 40 °C for 10 min significantly attenuated the haemodynamic effects of CQV. This manifests that the active component(s) of CQV causing these haemodynamic effects are thermolabile. Endean and Henderson (1969) reported that the lethal activity of the venom of *C. fleckeri* was perfectly inactivated by heating at 45 °C for 5 min. Freeman and Turner (1972) also demonstrated that haemolytic activity of venoms of *C. fleckeri* and *C. quadrigatus* was decreased to half by heating at 40 °C for 90 and 30 min, respectively. However, the influence of heating on the haemodynamic effects in vivo has not so far been investigated. Taking account of the previous findings that the venom from nematocysts of *C. fleckeri* did not include the bioactive compounds with low molecular weight such as histamine (Freeman and Turner, 1969), it is presumable that the active principle of CQV would be thermolabile protein similar to that of *C. fleckeri*. On the other hand, attenuation of the effects of CQV was revealed through heating at 50 °C for 10 min but not at 40 °C for 10 min in our in vitro experiments (Sakanashi et al., 2002). Discrepancy in the attenuation of CQV activity between in vitro and in vivo data at the same temperature (40 °C) may be explained by the difference in preparing methods for the final CQV solutions: in the in vitro experiment a stocked solution of CQV was diluted just before heating and applied, while in the in vivo experiment a previously diluted CQV solution was heated just before injection.

Since pretreatments with indomethacin, phentolamine or atropine did not influence the haemodynamic effects of CQV, it is unlikely that cyclooxygenase products, α -adrenergic receptors or muscarinic receptors participate in the effects of the venom. On the other hand, diltiazem treatment was significantly effective to inhibit the venom-induced changes in haemodynamic parameters. This finding indicates that voltage-dependent L-type calcium channels will be involved in changes in the haemodynamics after CQV i.v. injection. The beneficial effects of diltiazem against the effects of CQV in vivo coincide with a previous report that verapamil was effective in surviving mice injected with the venom of *C. fleckeri* (Burnett and Calton, 1983). Concerning mechanisms of action of the venom prepared from whole tentacles of *C. fleckeri*, Mustafa et al. (1995) proposed in their in vitro study that cardiovascular effects of the venom of *C. fleckeri* were caused by influx of calcium via voltage-dependent calcium channels following an increase in concentration of intracellular sodium ions through an increase in sodium influx. However, in the present in vivo study, a significant contribution of sodium channels was not found as far as judging from the data that pretreatments with sodium channel inhibitors lidocaine and flecainide did not affect the effects of CQV. Thus, it is possible that vasoconstriction and cardiodepression following transient stimulation induced by CQV may be attributable to an increase in intracellular calcium level and probably subsequent calcium-overload in vascular smooth muscle cells and cardiac cells, as suggested in our recent in vitro study (Sakanashi et al., 2002). Evidently, further experiments, especially using electrophysiological methods, would be needed to clarify the concise mechanisms concerning ion channels.

The biological activity of the venom of *C. quadrigatus* has been compared with that of *C. fleckeri* living in the water of Australia (Freeman and Turner, 1971; Freeman and Turner, 1972 and Keen, 1971). In these studies, the venom was prepared from whole tentacles, while no comparative study concerning the venom from isolated nematocysts has been performed. Since Yamaguchi (1982) has found the difference in shape of gonads between *C. quadrigatus* in Okinawa and Australia, the biological activity of Okinawan *C. quadrigatus* is considered to be different from that of Australian *C. quadrigatus*, and thus investigation on the former will be required. The present study was performed on the biological activity of Okinawan *C. quadrigatus* and we used here the term Habu-kurage, a local name used in Okinawa, toward the cubomedusa *C. quadrigatus* distributing around Okinawa. This is the first report demonstrating the in vivo effects of the venom from isolated nematocysts of *C. quadrigatus* 'Habu-kurage' on haemodynamics in experimental animals.

In conclusion, the present study indicates that the crude venom from isolated nematocysts of *C. quadrigatus* Habu-kurage has both vasoconstrictor and cardiodepressive effects in vivo, and suggests that these actions are thermolabile and may be due partly to activation of voltage-dependent calcium channels and probably subsequent calcium-overload. Further experiments and purification of active components should be conducted to clarify the definite mechanisms of action of the venom on the cardiovascular system.

Acknowledgements

The authors thank Mr Yasutetsu Araki, Okinawa Prefectural Institute of Health and Environment, for collecting of specimens of *C. quadrigatus* (Habu-kurage). We also thank Professor Masashi Yamaguchi, University of the Ryukyus, for valuable advice.

References

- Barnes, J.H., 1966. Studies on three venomous cubomedusae. In: Rees, W.J., Editor, , 1966. *Cnidaria and their Evolution*, Academic Press, London, pp. 307–331.
- Barnes, J.H., 1967. Extraction of cnidarian venom from living tentacle. In: Russell, F.E. and Saunders, P.R., Editors, 1967. *Animal Toxins*, Pergamon Press, Oxford, pp. 115–129.
- Baxter, E.H., Marr, A.G. and Lane, W.R., 1969. Immunity to the venom of the sea wasp *Chironex fleckeri*. *Toxicon* **6**, pp. 45–50.
- Burnett, J.W. and Calton, G.J., 1983. Response of the box-jellyfish (*Chironex fleckeri*) cardiotoxin to intravenous administration of verapamil. *Med. J. Aust.* **2**, pp. 192–194.
- Cleland, J.B., Southcott, R.V. (1965). Injuries to man from marine invertebrates in the Australian region. National Health and Medical Research Council Special Report Series, No. 12, 1–282.

Endean, R. and Henderson, L., 1969. Further studies of toxic material from nematocysts of the cubomedusan *Chironex fleckeri* Southcott. *Toxicon* **7**, pp. 303–314.

Endean, R. and Noble, M., 1971. Toxic material from the tentacles of the cubomedusan *Chironex fleckeri*. *Toxicon* **9**, pp. 255–264.

Endean, R., Duchemin, C., McColm, D. and Fraser, E.H., 1969. A study of the biological activity of toxic material derived from nematocysts of the cubomedusan *Chironex fleckeri*. *Toxicon* **6**, pp. 179–204.

Endean, R., Monks, S.A. and Cameron, A.M., 1993. Toxins from the box-jellyfish *Chironex fleckeri*. *Toxicon* **31**, pp. 397–410.

Freeman, S.E. and Turner, R.J., 1969. A pharmacological study of the toxin of a cnidarian, *Chironex fleckeri* Southcott. *Br. J. Pharmacol.* **35**, pp. 510–520.

Freeman, S.E. and Turner, R.J., 1971. Cardiovascular effects of toxins isolated from the cnidarian *Chironex fleckeri* Southcott. *Br. J. Pharmacol.* **41**, pp. 154–166.

Freeman, S.E. and Turner, R.J., 1972. Cardiovascular effects of cnidarian toxins: a comparison of toxins extracted from *Chiropsalmus quadrigatus* and *Chironex fleckeri*. *Toxicon* **10**, pp. 31–37.

Keen, T.E.B., 1971. Comparison of tentacle extracts from *Chiropsalmus quadrigatus* and *Chironex fleckeri*. *Toxicon* **9**, pp. 249–254.

Keen, T.E.B. and Crone, H.D., 1969. The hemolytic properties of extracts of tentacles from the cnidarian *Chironex fleckeri*. *Toxicon* **7**, pp. 55–63.

Kinjo, N., Noguchi, K., Hirayama, K. and Sakanashi, M., 1990. Characteristics of cardiovascular effects of the venom of Habu (*Trimeresurus flavoviridis*) in rats. *Jpn. J. Pharmacol.* **54**, pp. 151–161.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**, pp. 265–275.

Mustafa, M.R., White, E., Hongo, K., Othman, I. and Orchard, C.H., 1995. The mechanism underlying the cardiotoxic effect of the toxin from the jellyfish *Chironex fleckeri*. *Toxicol. Appl. Pharmacol.* **133**, pp. 196–206.

Sakanashi, M., Matsuzaki, T., Nakasone, J., Koyama, T., Sakanashi, M., Kukita, I. and Sakanashi, M., 2002. Effects of diltiazem on in vitro cardiovascular actions of crude venom obtained from Okinawan box-jellyfish (Habu-kurage), *Chiropsalmus quadrigatus*. *Anaesth. Intens. Care* **30**, pp. 570-577.

Turner, R.J. and Freeman, S.E., 1969. Effects of *Chironex fleckeri* toxin on the isolated perfused guinea pig heart. *Toxicon* **7**, pp. 277-286.

Yamaguchi, M., 1982. Cubozoans and their life histories. *Aquabiology* **21**, pp. 248-254