Cardiac ventricular diastolic phase and papillary muscle lusitropic response to Aurelia venom in abdominal arteriovenous fistulas induced murine circulation volume overloading heart.

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Abstract

Purpose: *Aurelia* venom was reported to inhibit muscle cholinergic receptor elicited cross cellular membrane currents and introduced muscle physiologic tetanus. However, the recording and analyzing of inotrope effectiveness in cardiac muscles was not well established. In this study, we investigated *Aurelia* venom and its analogue intervene induced cardiovascular hemodynamics variations in cardiac cycle *in vivo* and cardiac papillary muscle passive tension responsiveness *in vitro.*

Methods: *Aurelia* venom was separated by 10% acrylamide gel. *CfTX-1* homology 11 amino acid sequence was identified. The sequence was resin solid-phase synthesized subsequently, and defined as *CfTX-1* analogue. *In vivo* fistulas mice left ventricle haemodynamics were measured through carotid artery cannula and venom intervene by local irrigation. Arterial pressure waveforms, diastole and systole dP/dt_{max} were evaluated. Cardiac papillary muscle lusitrope was investigated *in vitro*. Myogenic activities during passive tension deceleration and deceleration velocity were determined in several step length stretching.

Results: 100 µl volume challenge with *Aurelia* venom or *CfTX-1* analogue reduced 10% diastolic pressures, however systolic pressure did not significantly increased with significant enhancing of pulse pressure. Furthermore, $-dP/dt_{max}$ in diastolic phase was improved rather than systolic dP/dt_{max} in *CfTX-1* analogue intervened fistulas heart. The cardiac papillary muscle velocity attenuation after active stretching was significantly accelerated in severe stretched higher preload muscle.

Conclusion: *Aurelia* crude venom especially *CfTX-1* analogue significantly improved ventricle lusitropic responses in diastolic phase, induced a rapidly relaxation velocity reducing. This further promoted ventricular filling to fit volume and pressure strained having great worth to provide accommodation to protect ventricle from mechanical stressing.

Keywords: *Aurelia venom,* lusitrope, ventricle diastole.

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Introduction

Aurelia was considered as inshore genus and harmless jellyfish, however, the extracted tentacle venom bring out a certain extent neurotoxic characteristics in excitation of skeletal muscle membranes [1]. *Aurelia* crude venom contains a cocktail of toxins. The basic chemical stimulation induced responses consists of several mechanisms, such as sustained steadily increasing of smooth muscle active tension in aortic preparations which were significantly interacted in extracellular Ca^{2+} [2], declined mammalian heart left ventricle pressures and cardiac muscle contractile induced pressure variations within the cardiac cycle (dP/dt) [3], reduced the cardiac ventricular inotropic response [4], and facilitated with internal environment of calcium and its ion channels [5]. The crude venom included multiple content of proteins, carbohydrates, and non-proteinaceous compound, were considered functions through calcium sensitive manner. The effectiveness in toxic way or physiological therapeutics were implicated in target cell signaling mechanisms. *Chironex fleckeri* venom-implicated genes expressed and encoded bioactive proteins, which had profound nociceptive effects on the cardiovascular system [6]. The venom bi-phasic responses may link to the cardiac muscle positive inotrope during blood ejection or a markable relaxation during blood filling during cardiac ventricular cycles.

The optimal performance of the left ventricle depends on the cycle of diastole that allows the left ventricle to fill from low lung arterial pressure, and ejects the stroke volume at arterial pressures in systole phase. Ventricle filling volume and pressures during filling phase were determined mainly by passive properties of cardiac muscles. Systemic circulation

volume overload induced cardiac diastole dysfunctions partlyattributed to the incomplete myocardial relaxation. Relaxation characteristics and intracellular Ca^{2+}-homeostasis were important to preserve ejection fraction in hypertension and heart failure. The prolonged myocardial maximal relaxation time was prolonged in hypertension and heart failure. Elevated diastolic Ca^{2+} were associated with incomplete relaxation and an increase in diastolic tension [7]. In addition the volume overload adaptations in ventricle diastolic phase are important in extreme environment acclimatization, such as rapidly body postural changes, deep water immersion and gravity variations. The consequence of diastolic dysfunction in extreme conditions may caused by elevated filling volume and pressures induced overload. However, quite a few studies have devised in positive lusitropic regulation for improving systolic functions; very few study focused on diastolic functions. The jellyfish crude venom was reported either toxic or physiologic affects that depended on dosages and internal environment thermal conditions. Conditioned crude venom settles on cellular membrane appropriate substrate, improved biphasic changes in cardiac ventricle muscle excitation-contraction coupling.

There were significant differences in the poisonousness of *Aurelia* because of the varieties developed under multiple combinations of temperature, salinity and food regimen [8-11]. *Aurelia* defined as a "non-stinging scyphozoan species" was because of low total lipids content in fresh tissue [12]. Further recently years reports elaborated the transformation research of venom with its complex mixture of bioactive substances-including thermolabile enzymes such as phospholipases, metalloproteinases and pore-forming proteins [13,14]. The development of new standpoint explained cellular membrane permeability to sodium ions and how depolarization of muscle membrane eliciting muscle twitches discovery by Kihara et al. [1]. The direction of development in cardiovascular pharmacology study strengthened further [15,16].

Aurelia venom triggers multiple reactions with cardiotoxic mechanism was reported relative to porins formation. In cnidarian, pore forming toxins self-assemble to form a robust membrane of pores. These pores provided potentials to mend lusitropic functions of cardiac muscle, therefore, ameliorated myocardium compliance, promote cardiac stroke and output. Together with the reacquaint knowledge of aquatic venom and previous works, in this study we investigated the effects of concentrated *Aurelia* venom and its specific sequence *CfTX-1* analogue in creatures the essential of lusitropic response in fistulas mice cardiac ventricles.

Material and Methods

Volume challenge with venom infusion and hemodynamic variations

Specific pathogen free (SPF) grade BALB/c mice (Male 4wks) were fed on a 12h/12h light/ dark cycle. The research protocol was approved in advance by Laboratory Animal Center, Hainan Medical College. The research was conducted in accordance with the guidelines put forth by the Institutional Animal Care and Use Committee (8th Edition, International Standard Book Number-13: 978-0-309-15401-7). All mice were kept under the surveillance of veterinarian. The living conditions were in accordance to the policies and procedures described in the Regulations for the Administration of Affairs Concerning Experimental Animals; these regulations were approved by the State Council and promulgated as per decree No. 2 of State Science and Technology Commission. Crude venom and chemical compounds induced mice postural systolic and diastolic pressures were monitored by carotid artery cannulation method.

Mouse left ventricle postural hemodynamic monitoring

Calligaris and colleague's method was referred and refined [17] for mice carotid arterial cannula and blood pressure monitoring. After 50 µl, 3% Pentobarbital Sodium intraperitoneal injection, 1.4F polypropylene cannula was catheterized into left common carotid artery, then connected to a pressure transducer (YP100 model, Shanghai Yi Lian medical instrument development Co. ltd. Shanghai, China) with the following technical specification: Natural measurement range: 0-1000 mmHg; Accuracy: non-linear ≤ 0.5% full scale (FS); Hysteresis ≤ 0.5% FS and repeatability ≤ 0.5% FS. The carotid pressures were recorded and analyzed with BL-420S biological data acquisition and analysis system (BL420S, Chengdu Techman Software Co. Ltd. Chengdu, China). Thereafter, postural systolic and diastolic waveforms, pulse pressures, arterial dP/dt_{max} on 6 steady-state cycles were processed with TM_WAVE software Ver. 1.1, (Chengdu Techman Software Co. Ltd. Chengdu, China). The carotid maximal slope of systolic pressure increment ($+dP/dt_{max}$) and diastolic pressure decrement ($-dP/dt_{max}$) were algorithms using calculus of TM_WAVE software options.

Fistulas mice ventricle papillary muscle inotropy test

In vitro papillary muscle of cardiac ventricle isometric passive tension velocity was investigated by strip stretching method. Papillary muscle strip was removed from the inner wall of left ventricle, rinse with 4°C cooling *Ringer's* solution. Strip was tightly attached to hook stainless hook, another side were fixed on the reed of Wheatstone bridge type piezoelectric strain sensor (Model JH-2 10 g, Beijing aerospace medical engineering institute, Beijing China). Papillary muscle specimen length were adjusted by the attached tuning micrometer (SV-4 series, Chengdu TME Technology Co. Ltd. Chengdu, China). Specimen was stretched and primarily preload with 1gram, then tuning the micrometer to obtain a 1mm length stretching. Total 5 steps of stretching generated. Mechanical stretching induced myogenic isometric passive tensions regression and its velocity changes were recorded by BL-420S system (Chengdu TME Technology Co. Ltd. Chengdu, China) and evaluated by TM_WAVE software.

Venom preparation

The *Aurelia Aurita* was captured in PuQian coast of QiongZhou strait, Hainan Island. *Aurelia* tentacles were frozen and autolysis in +4°C in a laboratory shaker, and filtered with 40 mesh filter. The filtered solution was centrifuged at 900×g for 5 min. The sediment was obtained by sampling syringe, and the nematocysts were confirmed and counted under the microscope with a magnification of 400x. The sediment was diluted with pure water at a temperature of 4°C. Then, it was again filtered with 200 mesh filter. The purified nematocysts had a fine shape, and they were mechanically homogenized for 20 sec. The

homogenized nematocysts were incubated with ice. Finally, they were centrifuged at 20,000×g for 1h at 4°C. The resultant supernatant venom was immediately frozen at -80°C in condenser chamber and vacuum to extremely dry (VirTis BenchTop freeze dryer, SP industries, Inc. PA, USA). Lyophilized venom was further dissolved in pure water for further examination use. The protein concentration was determined using Bradford method; the albumin of fetal bovine serum was used as the standard.

Molecular masses determination by 10% acrylamide gel SDS-PAGE

The purified venom powder was separated using 10% SDS polyacrylamide gel. The venom proteins (10 µL; 2.5 mg per milliliter of 2xSDS sampling buffer, pH 8.3, containing 0.01% SDS) were then eluted. Polyacrylamide gel electrophoresis was performed at 100V and 38 mA for 2 h. Page Ruler Unstained Protein Ladder (Thermo Scientific, Vilnius, Lithuania) was used to determine the approximate size and molecular mass of eluted proteins. The target molecular bands were stained with Coomassie Brilliant Blue Solution (Solarbio Life Sciences, Beijing, China), the candidate proteins between 40-50kDa were excised for further analysis.

LC-MS/MS and Peptide Mapping

LC-MS/MS based peptide sequencing method was used in this study. For exploring the candidate proteins within 40 kDa-50 kDa, molecular in bands were extracted by in-gel sequence of grade-modified trypsin digestion in 50 mM ammonium bicarbonate at 37°C overnight, and then extracted twice with 1% trifluoroacetic acid in 50% acetonitrile aqueous solution for 30 min. The peptide mixture from the proteolytic digestion was separated HPLC with 75 µM ID column with flow rate less than 1 µl/min. Tandem mass spectrometer was on-line coupled with HPLC to elute fragmented of peptides from HPLC column. MS/MS spectra were acquired for each fragmented peptide. Each MS/MS spectrum that was correspond specific peptide sequence was used to search protein database (uniprot of jellyfish) for matched peptides. The target amino acids sequence then synthesized by using commercial resin solid-phase technology and purified using HPLC. The synthesized signaling peptide was finally lyophilized. The final quality control were analyzes by HPLC and Electrospray Ionization Tandem Mass Spectrometry (ESI-MS).

Statistical analysis

Hemodynamic parameters *in vivo* and specimens elastic recoil biomechanical response were presented as mean ± SEM. Differences between groups were analyzed using unpaired test by Statistica (ver 7, StatSoft).

Results

Hemodynamic patterns of venom intervened relative changes in fistulas mice

Fistulas mice carotid artery postural pressures were monitored in each volume challenge. In anesthesia control mice without volume challenge (50 µl, 3% Pentobarbital Sodium, Intraperitoneal injection), carotid arterial blood pressure and average heart rate (HR) were slightly different between supine and standing postures, but no statistical significant difference between postural changes (Table 1, annotation a, e and b, f. HR were 360 bpm and 382 bpm in horizontal supine and vertical standing posture respectively). The carotid arterial pressure waveform had no significantly difference between horizontal and vertical position. Cardiac ventricle volume challenge with 100 µL of 0.9% saline solution did not induced HR significantly changes. From the collected data it is suggested that slowly postural changes do not disturbing the hemodynamic results in anesthesia mice. No significantly difference between distinct postural mice which were in volume challenged or control. The incisura in the arterial pressure waveform dicrotic limb could be distinguished during mice inspiratory phase. This is referred to as the reflected pressure wave by aortic valve closure (Figure 1a and 1b, arrow mark). However, 100 µl norepinephrine (0.1 mg) challenge induce a continuously enhancement of systolic and diastolic pressure, moreover pulse pressure were dramatically expanded in both posture (Table 1, annotation a**, e** and b**, f** in horizontal and vertical posture respectively, ** P<0.001). In the pressure waveform systolic and diastolic pressure phases were more sharply. The dicrotic limb in the carotid arterial pressure waveform lost the dicrotic notch. Norepinephrine induced positive inotrope reduced the valve closure reflected wave induced incisura (Figure 1c).

When immediate 100µL challenge with *cVN*, pulse pressure were significantly enhanced (Table 1, annotation e*, f* that compared to control e, f; * P<0.05), however, diastole but not systole pressures were significantly reduced 10% for both

Table 1. *Distinct solution volume challenge and postural haemodynamic parameter changes in fistulas mice carotid artery (n=21).*

Grouping	BW (g)	Posture	Systolic	Diastolic	PP	Carotid dP/dt_{max} (mmHg/sec)		Heart Rate (bpm)
			(mmHg)			dP/dt_{max}	$-dP/dt_{max}$	
Concentrated Crude Venom (*cVN*) (n=5)	35.27 ± 0.97	supine	113.07 ± 11.87	49.36 ± 8.22^{c*}	64.71 ± 6.21^{e*}	692.57 ± 36.61	-594.39 ± 3.70^{i*}	692.00 ± 100.00
		stand	111.89 ± 12.72	50.11 ± 2.36^{d*}	61.82 ± 7.97^{f*}	680.81 ± 27.75	-542.44 ± 4.90^{j*}	712.00 ± 121.00
CfTX-1 analogue (n=5)	36.13 ± 1.47	supine	121.25 ± 23.74	61.22 ± 9.32$^{c\#**}$	79.75 ± 10.19$^{e\#*}$	692.57 ± 36.61	-623.33 ± 7.17^{i*}	700.00 ± 90.00
		stand	128.44 ± 30.01	61.91 ± 10.51$^{d\#**}$	76.06 ± 11.14$^{f\#*}$	680.81 ± 27.75	-660.51 ± 9.13^{j*}	720.00 ± 87.00
Norepinephrine (n=5)	37.14 ± 1.14	supine	346.21 ± 31.65^{a**}	176.44 ± 11.28^{c**}	170.98 ± 15.75^{e**}	990. 80 ± 124.98^{g**}	-878.17 ± 13.30^{i**}	730.00 ± 122.00
		stand	352.37 ± 37.27^{b**}	182.02 ± 13.09^{d**}	171.02 ± 16.10^{f**}	930.50 ± 113.71^{h**}	-815.32 ± 11.77^{i**}	741.00 ± 131.00
0.9% Saline (n=3)	36.12 ± 0.90	supine	114.32 ± 10.11	55.36 ± 7.25	60.23 ± 3.47	710.63 ± 19.05	-430.87 ± 3.54	673.00 ± 111.00
		stand	110.58 ± 11.04	57.11 ± 3.31	53.97 ± 9.19	650.33 ± 37.10	-430.11 ± 2.98	700.00 ± 97.00
Control (n=3)	36.71 ± 1.11	supine	111.82 ± 17.53^{a}	54.00 ± 3.11^{c}	57.23 ± 2.17^{e}	690.12 ± 27.78^{g}	-428.95 ± 2.87^{i}	720.00 ± 126.00
		stand	102.73 ± 19.68^{b}	56.82 ± 1.90^{d}	46.97 ± 7.29^{f}	660.10 ± 15.70^{h}	-427.65 ± 2.63^{j}	660.00 ± 134.00

Control means no volume challenge; BW body weight in gram; PP pulse pressure; dP/dt_{max}=systolic velocity max; $-dP/dt_{max}$=diastolic velocity max; values are in Mean ± SEM;

*means P<0.05, ** means P<0.001.

postures. Furthermore, 100 μL challenges with synthesized CfTX-1 analogue (sVN) generated a dramatically transient enhancement of arterial pressure pulsatility and increasing of systemic pulse pressure (Table 1, annotation c#**, e#** and d#**, f#** when compared with control mice; ** P<0.001). Venom compound intervene, unlike the norepinephrine, were partly improved aortic valve closure reflected wave. The incisura image can be captured in some respiratory phase (Figure 1d and 1e). The carotid maximal slope of systolic increment (+dP/dt_{max}) and diastolic decrement (−dP/dt_{max}) were both increased in volume challenges with norepinephrine (Table 1, annotation g**, i** and h**, j** when compared with control mice; ** P<0.001), however *cVN* and *sVN* mainly improved diastolic phase decrement −dP/dt_{max} (Table 1, annotation i#**, j#** when compared with control mice; ** P<0.001).

Figure 1 Volume challenge induced carotid artery pressure variations in concentrated crude venom and *CfTX-1* analogue intervene with 100μL volume challenge (0.1mg/ml) through carotid artery cannula, caused 10% reduction in diastolic pressure; however, systolic pressure had no dramatically changes. Diastolic carotid -dP/dt_{max} increased 38.8%, whereas systolic carotid dP/dt_{max} have no statistic significantly changes. Adrenergic responsiveness with norepinephrine challenge induced 45.4% in systole dP/dt_{max} and 105% in diastole -dP/dt_{max}. In the control groups, there was no significantly dP/dt_{max} difference between 0.9% saline infusion and non-infusion fistulas mice.

In vitro istulas mice papillary muscle lusitropic measurement

As show in Table 2, fistulas mice papillary muscle strips were active stretched continuously to elicit lusitropic responses. In each active stretching step, instantaneously sustained PT velocity and rapidly attenuated PT velocity differed significantly with respect to time. In control specimens, strips bath in *Ringer's* solution during active stretching. In each stretching step, PT velocity was instantaneously sustained and attenuated PT velocity was rapidly reduced (in 5th stretching, Figure 2a

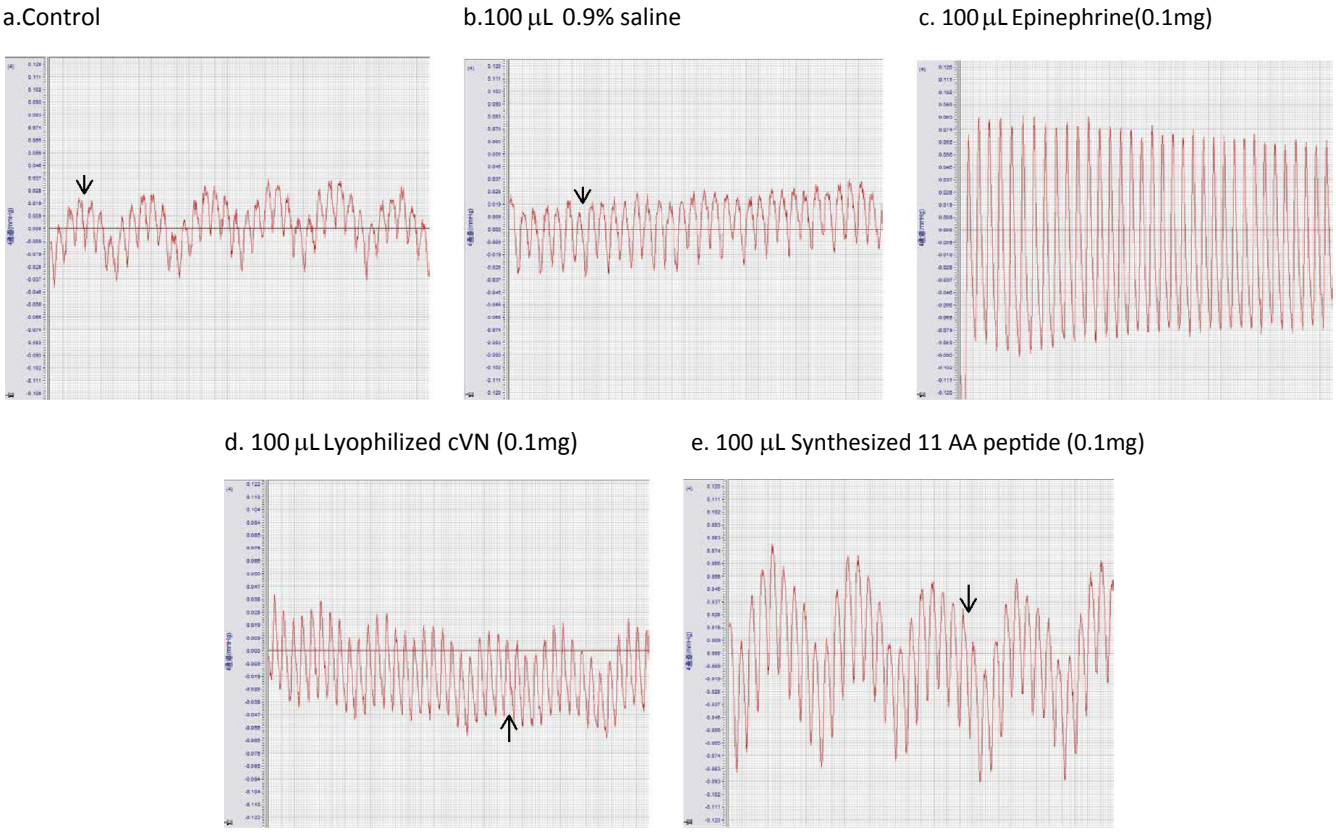

Figure 1. *Volume challenge induced carotid artery pressure variations in concentrated venom and CfTX-1 intervene.*

Table 2. *Fistulas mice isolated ventricular papillary muscle lusitropic response to static-passive stretching (n=18).*

Grouping	Strech induced max velocity and isometric tension velocity attenuation in each stretching step					
		1st	2nd	3rd	4th	5$^{th\$}$
Concentrated Crude Venom (*cVN*) (n=5)	Velocity max (V_{max}, g/s)	121.80 ± 11.72	131.13 ± 13.76	155.29 ± 19.41	197.65 ± 21.72	210.71 ± 23.22
	Velocity Attenuation (ΔV, g/s^2)	20.05 ± 3.08	22.91 ± 4.71	24.58 ± 9.18^{c*}	25.94 ± 7.72	27.87 ± 9.91^{b*}
CfTX-1 analogue (n=5)	Velocity max (V_{max}, g/s)	119.33 ± 14.22	122.54 ± 17.45	131.97 ± 21.36	178.33 ± 31.55	194.84 ± 33.18^{f**}
	Velocity Attenuation (ΔV, g/s^2)	23.15 ± 4.14^{a*}	25.78 ± 7.99^{b**}	27.82 ± 11.03^{c**}	28.78 ± 11.84^{d**}	29.12 ± 10.01^{e**}
Norepinephrine (n=5)	Velocity max (V_{max}, g/s)	120.73 ± 12.68	120.82 ± 19.60	182.73 ± 21.68	200.73 ± 19.68	218.82 ± 22.60
	Velocity Attenuation (ΔV, g/s^2)	20.79 ± 2.07	21.59 ± 3.09	24.99 ± 8.81	25.19 ± 7.78	27.42 ± 9.76^{e*}
Ringer's solution (n=3)	Velocity max (V_{max}, g/s)	123.11 ± 9.84	139.27 ± 12.33	157.89 ± 17.07	199.89 ± 1.72	221.11 ± 22.56^{f}
	Velocity Attenuation (ΔV, g/s^2)	21.49 ± 2.37^{a}	22.70 ± 5.11^{b}	23.74 ± 5.07^{c}	24.60 ± 3.45^{d}	25.36 ± 5.61^{e}

Values were in Mean ± SEM; * means significantly increasing (P<0.05)** means significantly increasing (P<0.001), $ means severe stretching induced overload

with arrow mark). *Aurelia* crude venom were incubated 5min to decrease instantaneously sustained PT velocity and to reduce the attenuation of PT velocity rate in each stretching step (Figure 2b with arrow mark). In norepinephrine intervened strips, PT velocity attenuation rate was significantly attenuated only in high preload grade (Table 2, with e*); however, instantaneously sustained PT velocity did not change significantly in control specimens (Figure 2c with arrow mark).

Specimens obtained from *in vitro* ventricular papillary muscle were stabilized in Ringer's solution with an initial preload 1gram. a) The additional stretching-with preload 1mm in length-was represented as an instantaneously sustained PT velocity in isometric status. Furthermore, it rapidly reduced the attenuated PT velocity back to baseline. Prolonged sustained PT velocities were observed in server stretching (5th stretching); however, attenuation in PT velocity rate was reduced. b) Fraction proteins of crude venom softened the instantaneously sustained PT velocity and improved the attenuation of PT velocity rate through server stretching. c) Norepinephrine promoted the attenuated PT velocity rate such that it receded back to baseline; there were no significant changes in the instantaneously sustained PT velocity.

SDS-PAGE analysis of lyophilized crude venom

The nature *Aurelia* tentacle crude venom extraction (nature venom, *nVN*) and the lyophilized crude venom (concentrated venom, *cVN*) were compared in 10% gel in SDS-PAGE protocol. In Figure 3, Molecular PageRuler (Thermo Scientific, Inc Lithuania) and bovine serum albumin solution (0.2 mg/ml, Solarbio Beijing Solarbio Science & Technology Co. Ltd. Beijing, China) were in lane 1 and lane 5. Lane 3 and lane 7 were the lyophilized concentrated venom (*cVN*) and nature venom (*nVN*) respectively (sampling were concentration balanced to 0.2 mg/ml in each well). The lyophilization process increased proteins extraction efficiency from nature crude venom. Specially the venom molecular between 40-50kDa were well concentrated (Figure 3, band with arrow).

Figure 3 Lyophilized concentrated crude venom in 10% acrylamide gel. The Aurelia tentacles autolysis and centrifuged crude venom extraction were further concentrated by deep frozen and vacuum dryer. The crude venom molecular compounds between 40-50 kDa were significantly well concentrated in *cVN* (arrow band in lane 3). The band in lane 5 and 7 was the

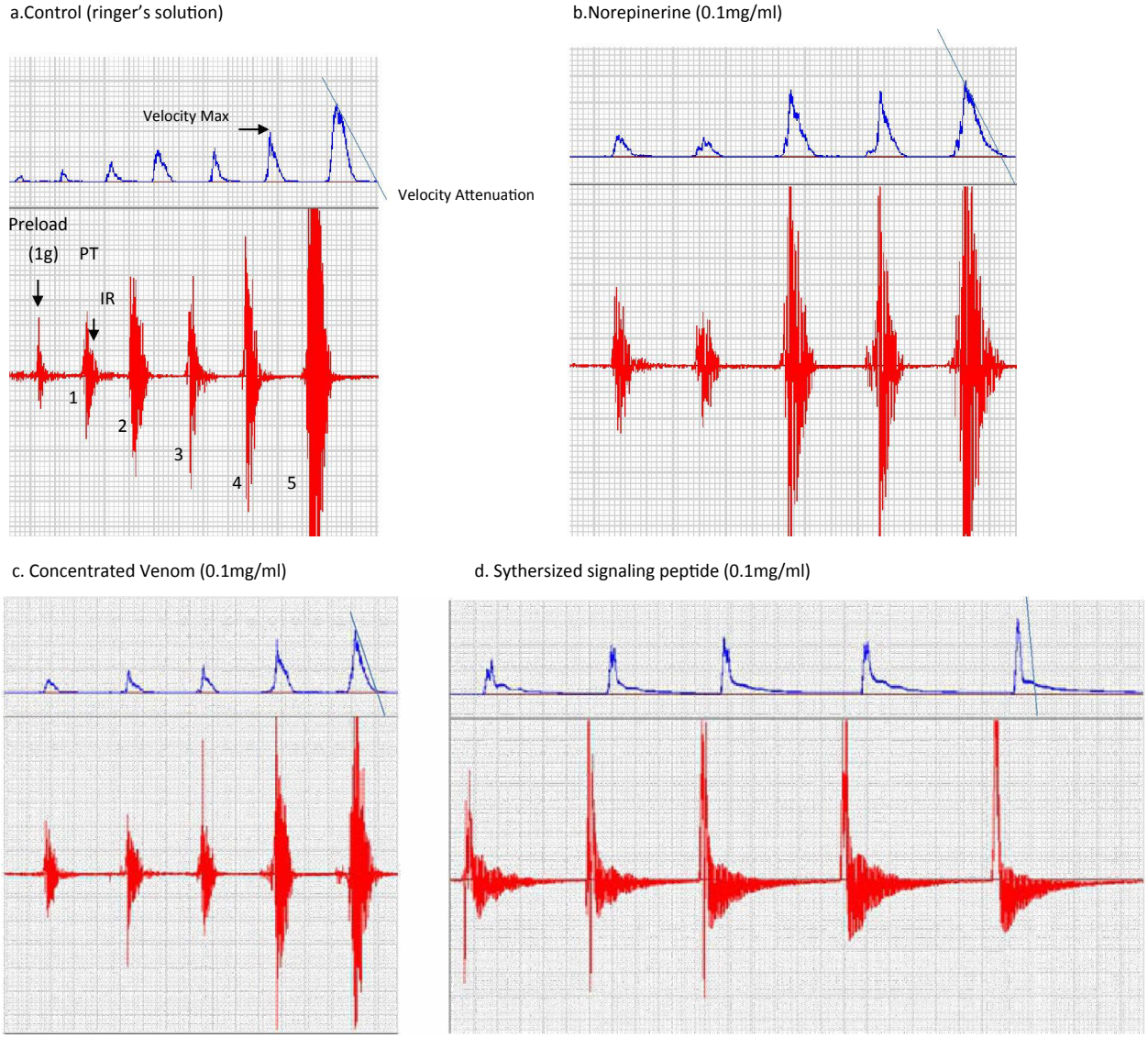

Figure 2. *Mechanical stretching in fistulas mice isolated ventricular papillary muscle and isometric tension velocity attenuations.*

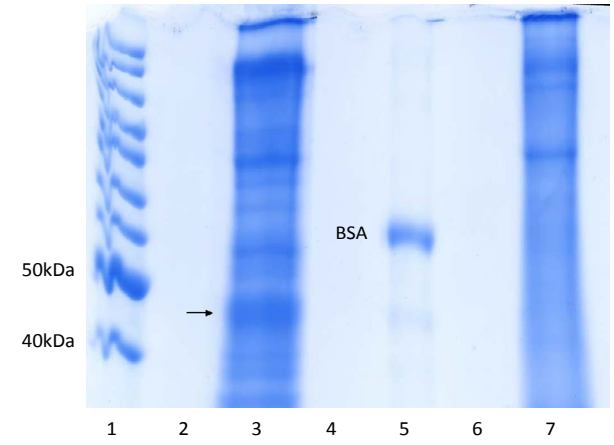

Figure 3. *Lyophilized concentrated crude venom fractions 10% acrylamide gel.*

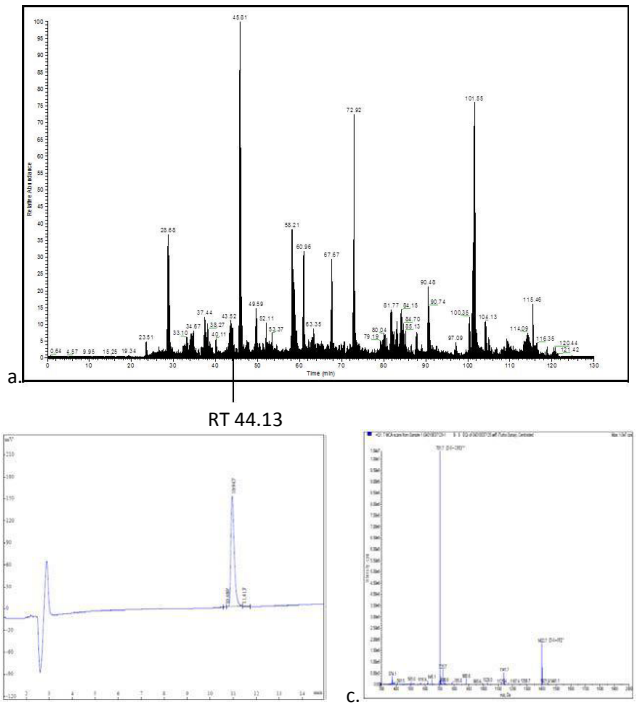

Figure 4. *LC/MS/MS quantitive analyzed cVN 40-50 kDa band in 10% acrylamide gel.*

BSA in 0.2 mg/ml and *nVN* solution respectively. *cVN* and *nVN* sampling in the well concentration were balanced, reference BSA concentration with Bradford method.

Molecular data analysis in 40-50 kDa band

In the *LC-MS/MS* analyzing of 40-50 kDa band of lyophilized venom, 7 types of peptides were confirmed that relative to the jellyfish protein in uniprot database. Most peptides were functionally associated with development and genesis of nematocytes, however, one kind of toxin type signaling peptide was identified at RT 44.13 (Figure 4). The calculated molecular weight was 51.4 (isoelectrical point 8.32) which representing a unique sequence of 11 amino acids which had a high overlap with the positive strain of amino acid sequences 304–314 (IFNFFDLmKVK) of toxin-1 *Chironex fleckeri* (*CfTX-1*).

Figure 4, LC/MS/MS quantitive analyzed 40-50 kDa band in 10% acrylamide gel. a) An *CfTX-1* type N-terminal peptide fraction was identified at the peak of RT 44.13. This 11 amino acid peptide (*CfTX-1* analogue) with the unique sequence IFNFFDLmKVK were further prepared by resin solid-phase synthesis. *CfTX-1* analogue was final purified to 99.75%. The quality control of synthesized signaling peptide was analyzed by HPLC and ESI-MS, b) RP-HPLC chromatogram, c) MS spectrum.

Discussion

Aurelia are innocuous aquatic animal, and were the most common jellyfish species on Hainan Island shores. However, some reported noticed that the large bell (more than 20cm) can be pathogenic [10]. The sting induced toxicity that severely damaged the cardiovascular system [18-23] by proteolytic enzymes. The presented in the venom extract increased permeability of membranes toward sodium ions were reported, generally causing irreversible depolarization of the myocyte membrane and eliciting skeletal muscle twitches [1]. *Aurelia* extracts also exhibit phospholipase A2 (PLA2) activity which governs lipid metabolism in cell membranes [24]. However, toxic compounds of *Aurelia* have not been identified clearly till date. In this study, we used the -20°C frozen *Aurelia* tentacle to separated and extracted effective compounds. We focus our analysis on the 40-50kDa molecular masses because most muscle twitch toxicity compounds were reported based on this molecular range. Our laboratory protocol reveal the frozen drying lyophilized venom were well concentrated the proteinaceous molecular masses in 40-50 kDa. LC-MS/MS peptide mapping explored 7 types of peptides which were expressed in majority of the jellyfish tentacles. The most striking peptide sequence of concentrated *Aurelia* tentacles venom in 40-50 kDa range was the 11 amino acid peptide that was a fragment of *CfTX-1* N-terminal. *CfTX-1* were reported most rich in tentacles of Cubozoan (*Chironex fleckeri*) and Japanese scallop (*Mizuhopecten yessoensis*), however rarely reported in *Aurelia* venom. Cubozoan *CfTX* protein group revealed active on cellular membrane permeability through pore-forming mechanism. The structural homology has similar pore-forming mechanism of action involving α-helices of the N-terminal domain. The identified *Aurelia CfTX-1* analogue were one specific fragment of 456 amino acid *CfTX-1*, located in 304 to 314 of positive strand. This specific fragment functions is not clear, however as a part of the N-terminal, *CfTX-1* analogue may have the responsibility in pore-forming, regulating membrane permeability and membrane excitation. As the expected result, both concentrated crude venom (*cVN*) and *CfTX-1* analogue, induced a significantly improved in fistulas mice diastole functions that was decreased diastolic pressure (Table 1, diastolic c* and d*) and enhanced pulse pressure (Table 1, PP e* and f*). Carotid dP/dt_{max} and $-dP/dt_{max}$ were indirectly reflected the inotropic variations of ventricular muscle during systolic and diastolic phase. The results indicated that relaxation relative indirect parameters were ameliorated after *cVN* and *CfTX-1* analogue intervene (Table 1, $-dP/dt_{max}$ i* and J*). However, the adrenergic effects was influencing both systolic phase and diastolic phase (Table 1, g*, h*, i* and j* in norepinephrine column). *In vitro* fistulas mice papillary muscle active stretching tests indicated that *CfTX-1* analogue

[25]. As well as under normal gravity, whole body immersion in water or postural changes causes cardiac rhythmicity and ventricle filling phase adaptation [26-28]. Aurelia venom and *CfTX-1* analogue hopefully become the cardiac muscle inotropy regulator to prolong myogenic myocardium dilation and rectify incomplete relaxation in high heart rate period. As a conclusion, Aurelia venom especially the synthesized *CfTX-1* analogue induced improve of lusitropic response in tension and velocity that further presented the potential affect to improve cardiac diastole functions in volume and pressure stressed heart.

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Disclosure Statement:

The authors do not have any conflict of interest.

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