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# The urothelium enhancive polarization in *CfTX-1* peptide intervened toad urinary bladder

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**Toad urothelium barrier is the model to mimic and investigate urothelium permeability. Thiazide blocked ionic transportation in polarized membrane state. Jellyfish venom causes pores to be adjusted to urothelium permeability which improved polarization. This study aimed at *CfTX-1* peptide in urothelium permeability evoked polarizations. Thiazide pretreated toad urothelium permeability to ions were investigated in modified Ussing chamber. Thiazide urothelium were further intervened by *CfTX-1* peptide and treated with G-protein receptor agonists. 0.1 mol $CaCl_2$ activated transurothelium potential differences were recorded by unipolar lead and computerized by fast Fourier transform technique. Apical chamber was settled as anode. The amplitude of potential differences were evaluated to determine the urothelium polarizations. The results indicated that $CaCl_2$ activation induced a positive monophasic wave in thiazide urothelium, which suggested the urothelium was slightly polarized and significantly enhancive in adrenergic receptor treated urothelium. Furthermore, *CfTX-1* peptide enhanced transurothelium potential difference in thiazide urothelium, therefore, urothelium were supra polarized. NPPB treatment significantly attenuated this supra polarization, which suggested that the $Cl^-$ influx was the main stream ionic compound of this polarization. It is concluded that *CfTX-1* peptide was considered to generate supra polarization in thiazide urothelium. This mechanism is useful to study the improvement of drug delivery crossing urothelium barrier.**

**Key words:** *CfTX-1* partial sequence, toad urothelium, supra polarization, $Cl^-$ permeability.

## INTRODUCTION

*Aurelia aurita* nematocysts were dominated by large quantities of secretory proteolytic enzymes, displayed a wide spectrum of toxic activities that determined clinical relevance of the neurotoxicity, myotoxicity and hemolysis. *Aurelia aurita* crude venom was found to affect irreversible depolarization of the muscle membrane, increase in membrane permeability to sodium ions (Kihara et al., 1988), cell membrane lipid metabolism

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(Helmholz et al., 2007), have a preferentially postsynaptic action at the neuromuscular junction by inhibiting acetylcholine binding to postsynaptic acetylcholine receptors (Ponce et al., 2013). Jellyfish venom, such as *Carybdea rastoni*, *Chiropsalmus quadrigatus*, contained diversified toxic isoforms were classified into at least two distinct subfamilies, type I and II. The box jellyfish *Chironex fleckeri* (*C. fleckeri*) toxin 1 and 2 were the short name of *CfTX-1* and *2*, two highly abundant toxins in the *C. fleckeri* venom, which was confirmed causing rapid cardiovascular collapse accompanied by hemolysis in anaesthetized rats (Yanagihara and Shohet, 2012). *CfTX* toxins shared high structural similarity with pore-forming three-domain Cry toxins produced by the bacterium *Bacillus thuringiensis* that possess the potent insecticidal activities (Podobnik and Anderluh, 2017)). The structural homology of *Chironex fleckeri* toxins and Cry toxins suggest that the toxins may have a similar pore-forming mechanism of action involving helices from the N-terminal domain (Brinkman, 2014), while structural variability within the toxin family may modulate receptor specificity. Therefore, *CfTX*-like toxins may be involved in oligomerization and pore formation not only on erythrocytes, but also other susceptible cells (Brinkman, 2007).

In isolated amphibian urinary bladder wall, mucosal and serosal surfaces have different permeability to solutes and water. Mucosal surface is normally impermeable to water (Peachey and Rasmussen, 1961; Parisi et al., 1981); however urothelium plasma membranes on the serosal surface are normally relatively permeable to circulation released stimulations (Michailova and Usunoff, 2006). Water permeability barrier is at mucous urothelium surface with striking properties of its polarity and the tight junctions (Parsons, 2007), while ionic traverse the barrier are involved in the ionic transporters located in apical membrane (Witten et al., 2018). The active transport in basal membrane induced variation of the permeability is evoked by $Ca^{2+}$ and other solutes across the blood capillaries in serous. It has not been established whether one or the other of these layers is responsible for the unusual permeability characteristics of this cell surface. Cytological evidence indicated that the urothelium were structured by filamentous layer in the outside of the plasma membrane. That was the place to maintain the tight lateral attachment near the mucosal surface to assure an almost leak-proof bladder. Loosely interlocking folds of the lateral membranes probably help to have osmotic equilibrium with the serosal fluid crossing the urothelium barrier. Furthermore, serous as the urothelial sheet were more effect than mucous side in solutes transporting via the circulatory system into the urinary bladder sac. Ions either enter the cell through basal membrane surfaces or act at these surfaces lead to the permeability changes. The amplitude of transurothelium electrical potential difference is proportional to the ionic movement.

Based on the above previously knowledge, ionic permeability induced urothelium potentials in $CaCl_2$ activation in basal membrane on serosal side was investigated. Moreover, the $CaCl_2$ evoked transurothelium electrical potential difference in partial sequence of CfTX-1 pretreated urothelium preparations was analyzed.

## MATERIALS AND METHODS

### *Aurelia aurita* nematocysts crude venom production

The crude venom were extracted from nematocyst of *Aurelia aurita* tentacle that obtained from Qiongzhou strait, Hainan, China. After homogenization nematocyst solution was centrifuged at 20,000×g for 1 h at 4°C. The proteins concentration in resultant supernatant was primarily determined by Bradford method, then immediately frozen at -80°C in condenser chamber and vacuum to extremely dry (VirTis BenchTop freeze dryer, SP industries, Inc., Pennsylvania, U. S. A.). Lyophilized concentrated crude venom was stored in -20°C for further analysis.

### *CfTX-1* peptide identification and synthesize

10% acrylamide gel was used to separate lyophilized concentrated crude venom by SDS-PAGE protocol. The target 43 kDa band cut off. The proteins were digested by trypsinase and the peptide fragments were entered into mass spectrometry analysis. The LC-MS/MS and peptide mapping method were used to identify *CfTX-1* N-terminal fragment. Once the sequence of *CfTX-1* was identified, this partial sequence of *CfTX-1* were synthesized by using commercial resin solid-phase protocol and purified and final quality control by HPLC and electrospray Ionization Tandem Mass Spectrometry (ESI-MS).

### Toad urothelium preparations

The toads were from the Hainan provincial drug safety evaluation center, Hainan province, China. The study protocol was approved in advance by ethics committee, Hainan Medical College. Toads were fed up in a humidity cage in the dark cycle prior to pithing. All toads were maintained under the surveillance of a veterinarian and adherence to policies and procedures from regulations for the administration of affairs concerning experimental animals.

The hemibladder wall were from double pithing toad, then prepared into approximately 9 mm x 9 mm in size, and balanced in isotonic amphibian Ringer's solution for 5 min to maintain the natural existence of urothelium osmotic gradient. The control preparation to the Thiazide pretreated was the urothelium only activated by $CaCl_2$ but had no pretreatment and intervention; the control preparation in Thiazide pretreated was the urothelium only activated by $CaCl_2$ but had no intervention by *CfTX-1* peptide, acetylcholine, norepinephrine or 5-nitro-2-(3-phenylpropylamino) benzoic acid.

Thiazide (HCTZ) pretreated and *CfTX-1* peptide intervened urothelium preparations were detected by using the hemibladder from the same toad urinary bladder. Tissue from at least four hemibladders with appropriate control preparations for comparison was subjected to each experimental condition that was studied. All experimental manipulations were performed within 2 h for avoiding the influences of membrane bioactivities.

### Toad urothelium transurothelium potential difference analysis in mini Ussing chamber

The toad urothelium transurothelium potential differences

measurement referenced Dunning-Davies method (Dunning-Davies et al., 2013). After rinsing with 0.9% saline solution, the intact urothelium layer were apical side down mounted on 80 $mm^2$ circle surface on the modified mini Ussing chamber, where the dual chambers were vertically separated by horizontal mounted urothelium barrier to become apical chamber and basal chamber. The chambers were fulfilled with 0.9% saline solution and stabled in room temperature before investigations. For investigating the ionic crossing urothelium layer induced transurothelium potential difference (TPD), the preparations were initially activated urothelium basal membrane by 0.1 mol $CaCl_2$ in basal chamber to obtain a primary TPD, then inspected the TPD in hydrochlorothiazide (HCTZ) pretreated urothelium preparations (HCTZ urothelium). The ionic crossing urothelium flowing induced TPD were measured by unipolar lead, and recorded with BL-420S biological signal acquisition system (Chengdu Taimeng Technology Ltd., Chengdu, China). The signals were computerized by fast Fourier transform technique with TM_WAVE software (Chengdu Techman Software Co. Ltd., Chengdu, China); apical side was settled as anode. Tissue from at least four hemibladders with appropriate control preparations for comparison was subjected to each experimental condition that was studied. All experimental manipulations were performed within 2 h to avoid the influences of membrane bioactivities (Kerec et al., 2005; Cohen et al., 2007).

#### CfTX-1 peptide evoked transurothelium potential difference in thiazide pretreated urothelium preparations

The HCTZ urothelium were further intervened by *CfTX-1* peptide in basal chamber with dosage of 10 μg/ml. The muscarinic receptor agonist Acetylcholine (*Ach*, 2 mmol) or adrenergic receptor agonist norepinephrine (*NE*, 3 mmol) were treated to observe activating guanine nucleotide-binding proteins signaling induced permeability effect in *CfTX-1* peptide intervened preparations. For understanding the ionic flow components that caused TPD in urothelium, *5-Nitro-2-(3-phenylpropylamino) benzoic acid* (*NPPB*, 2mmol) were used to block the $Cl^-$ channel.

## RESULTS

### CfTX-1 identified in *Aurelia aurita* concentrated crude venom

In Figure 1a, lane 1 was Molecular PageRuler (Thermo Scientific, Inc Lithuania), lane 2, 3 and 4 were the reference bands of bovine serum albumin with the concentration of 10, 5 and 2.5 μg respectively. Lane 5, 6 and 7 were concentrated crude venom of 10, 5 and 2.5 mg, respectively. The compound in 43 kDa band of Figure 1a, lane 5 (the band with the arrow) was the target band for identifying sequence by LC-MS/MS. An 11 amino acid peptide sequence which had a high overlap with the positive strain of amino acid sequences 304 to 314 (*IFNFFDLmKVK*) of *CfTX-1* N-terminal were confirmed (Figure 1b). This specific partial sequence was additionally synthesized by resin solid-phase synthesis. It was final purified to 99.75% (abbreviated as *CfTX-1* peptide in the later). The quality control of peptide was monitored by HPLC and ESI-MS. Figure 1c was the 701.9Da peak value of purified *CfTX-1* 11 amino acid peptide product (Figure 1c).

### *CfTX-1* peptide in *HCTZ* urothelium preparations

In *HCTZ* urothelium, $CaCl_2$ activation induced an increasing ionic current crossing the preparation, further generated a positive amplitude increasing of transurothelium potential difference (Figure 2a, 20.05±1.75 μV, n=5). Because *HCTZ* blocked the $Na^+/Cl^-$ symporter in the apical membrane of urothelium, the remaining main ionic current were the ionic influx from the serosal basal side to the apical side (ionic influx). Furthermore, in *CfTX-1* peptide intervened *HCTZ* urothelium, $CaCl_2$ evoked significant increase in ionic influx current. This further induced secondary enhanced positive amplitude of transurothelium potential difference (Figure 2b, 120.74±23.66μV). This result suggested that *CfTX-1* peptide significantly enhanced the ionic permeability in urothelium.

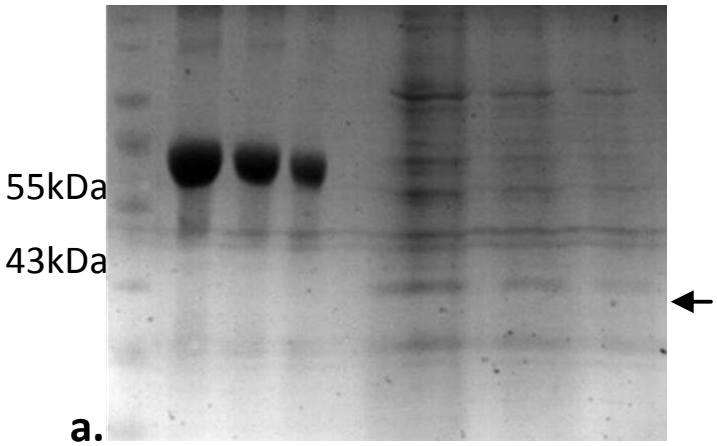
As the anode were settled in the apical of the urothelium in the Ussing chamber, the main stream of the ionic influx have been generated by the anion influx from the basal membrane.

### NPPB attenuated transurothelium potential difference in thiazide pretreated and *CfTX-1* peptide intervened preparation

*NPPB* was an inhibitor of many different $Cl^-$ channels and inhibit ATP release mediated by $Cl^-$ channels. It is an inhibitor of channel-mediated ATP release. In *HCTZ* pretreated and *CfTX-1* peptide intervened preparations, NPPB significantly reduced *CfTX-1* peptide induced ionic current influx and the amplitude of TPD (Figure 2c). The amplitude of TPD were attenuated to 26.67±8.62 μV, reduced 450% when compared with Figure 2b. NPPB attenuated TPD in Figure 2b suggested that *CfTX-1* peptide triggered anion influx and TPD was $Cl^-$ influx.

### *CfTX-1* peptide intervened TPD attenuated in adrenergic receptor activated *HCTZ* urothelium

Figure 2d was the $CaCl_2$ activated ionic permeability increasing induced amplitude of TPD in *HCTZ* urothelium that cholinergic receptor activated by *Ach*. In *HCTZ* urothelium, $CaCl_2$ induced slightly but observable increased positive amplitude of potential difference. However, as shown in Figure 2f, *HCTZ* urothelium activated by adrenergic receptor agonist *NE* induced enhancement amplitude of TPD which suggested adrenergic receptors were the excitatory factor to increasing polarization in *HCTZ* urothelium. In *HCTZ* urothelium, *Ach* treated obtained 18.72±3.11□V of TPD, moreover 82.40±9.55μV of TPD in *NE* treated *HCTZ* urothelium. A significantly results of *CfTX-1* peptide intervene *HCTZ* urothelium was $CaCl_2$ evoked TPD enhancement neither in *Ach* activated *HCTZ* urothelium




a.

1 2 3 4 5 6 7

```
        10         20         30         40         50
MVKMLFFAFL PLLFMTGIAA ESTISSGLNS LKTKIDAKMP SGKQLFDKVV
        60         70         80         90        100
EMQKQIDAKF SNDDERAKVM GAIGSLSTAV GKFQSGDPAK IASGCLDILV
       110        120        130        140        150
GISSVLKDFA KFSPIFSILS LVVGLFSGTK AEESVGSVVK KAVQEQSDQE
       160        170        180        190        200
LQEALYGVKR EYAVSKAFLD GVRNETSDLS PTEVSALAAN VPIYQGVRFI
       210        220        230        240        250
AMVVQRIKYI KPKTESEIKR MLTMLELFTD LCSLRDLILL DLYQLVATPG
       260        270        280        290        300
HSPNIASGIK EVSNLGREEY KKVFEDLLKN DDKETYLFLS YLYPREKNEQ
       310        320        330        340        350
SRKIFNFFDL MKVKYDDRLK QDLTGVKIFS NVHWPNYFMC SSNDYLALIC
       360        370        380        390        400
TKPYGSLKLD KLNDGYYSIK TTQHDPKICH RYGNYILFTH KRNDDLEKFN
       410        420        430        440        450
FVPVKLEKRE IYLLSSKESP NKFAYVPQNA DGALFFVDGI PSKVGYGNQG
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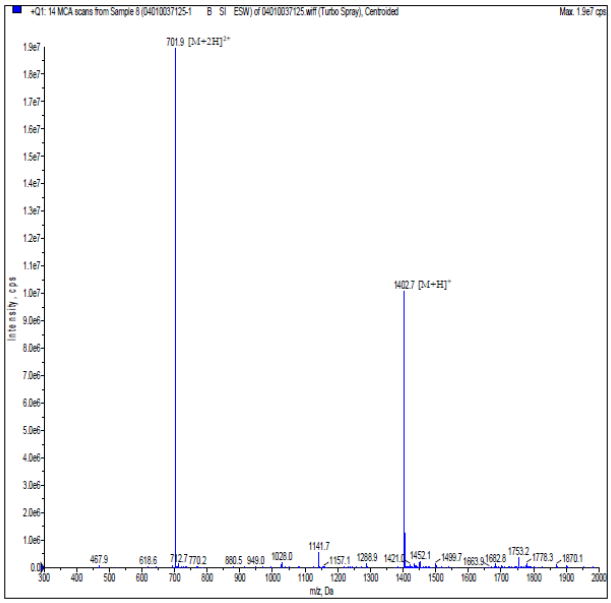
b. YFTLVE




c.

**Figure 1.** *Aurelia aurita* concentrated crude venom and *CfTX-1* partial sequence identification. (a) The candidate proteins in 43kDa band were confirmed from *Aurelia aurita* lyophilized concentrated crude in 10% acrylamide. The arrow marked band location in lane 5, 6, 7 was the 43 kDa band of crude venom. The sampling gradient concentration was 10, 5 and 2.5 µg, respectively. Lane 2, 3, 4 are bovine serum albumin with the sampling gradient concentration 10, 5 and 2.5 µg, respectively. Bovine serum albumin band was the reference band for optical density calculation to confirm the grossly candidate proteins in 43 kDa band (the optical density calculation is not shown). (b) The partial sequence of *CfTX-1* N-terminal fragment were identified from this band. ifnffdlmkvk of Toxin *CfTX-1* was the specific sequence confirmed from Hainan local *Aurelia aurita* crude venom. (c) This partial sequence of *CfTX-1* was laboratory synthesized and final quality control by HPLC and electrospray Ionization Tandem Mass Spectrometry (ESI-MS). The peak value of 1402.7 was the synthesized and purified *CfTX-1* peptide.

nor in *NE* activated *HCTZ* urothelium (Figure 2e and 2g, marked with vertical arrows), which suggested the *CfTX-1* peptide intervene evoked enhancement of urothelium polarization were inhibited by G-protein coupled receptors.

Summary of above results indicated that (1) *CfTX-1* peptide intervene can evoke significantly urothelium polarization in *HCTZ* urothelium preparation. Furthermore, the main compound of ionic current induced supra polarization was $Cl^-$, because *NPPB* can block this specific supra polarization. (2) *CfTX-1* peptide evoked $Cl^-$ current was inhibited by G-protein coupled receptors.

## DISCUSSION

In this study abundant of *Aurelia aurita* tentacles nematocyst proteins were identified from polyacrylamide

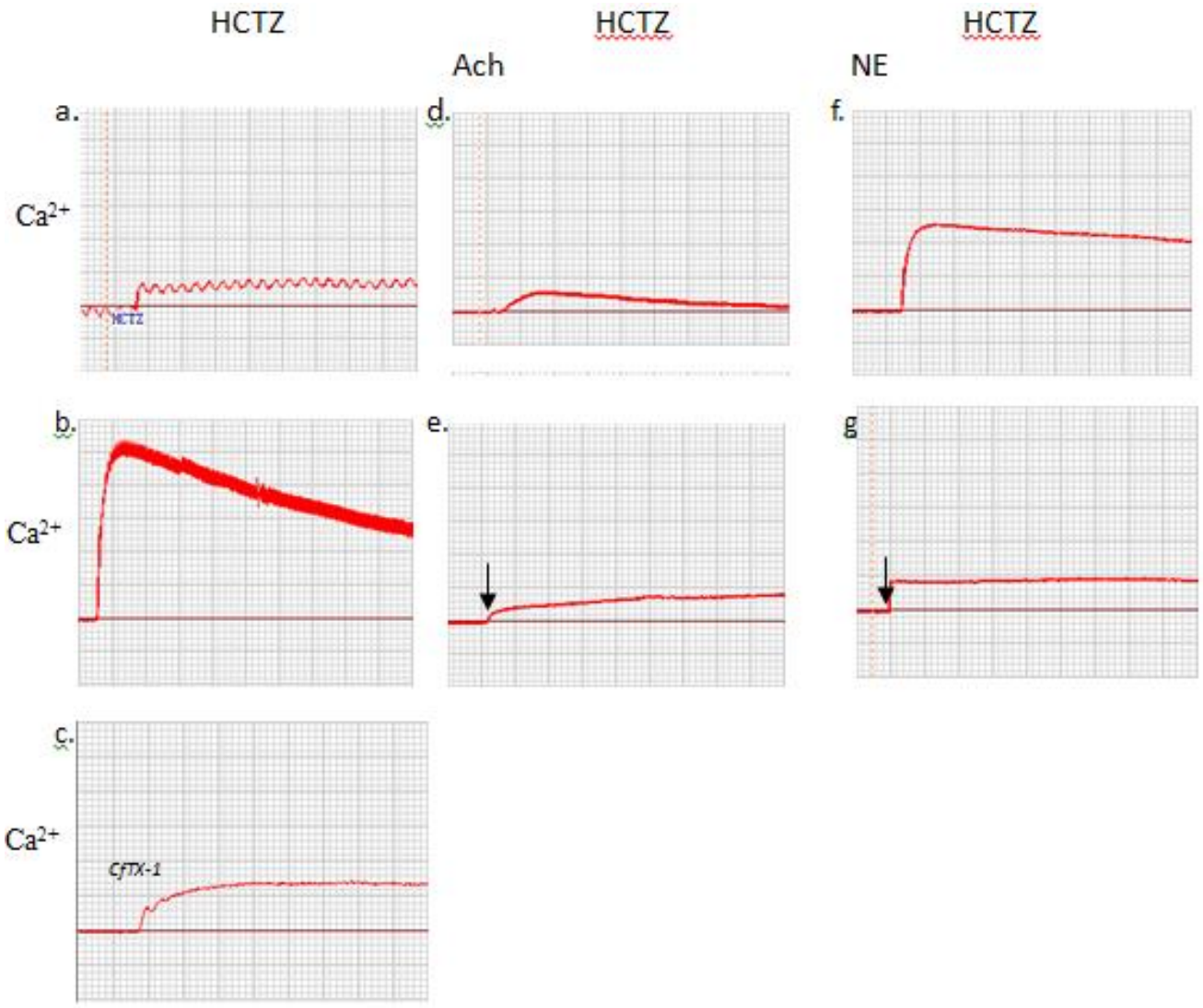



**Figure 2.** *CfTX-1* peptide evoked transurothelium potential difference in *HCTZ* urothelium. (a) $CaCl_2$ activated ionic current and amplitude of TPD in *HCTZ* urothelium, slightly polarized *HCTZ* urothelium. (b) *CfTX-1* peptide intervened evoked an enhancement of TPD in *HCTZ* urothelium. This generated supra polarization on *HCTZ* urothelium. (c) $Cl^-$ channel blockade *NPPB* treatment induced recognized attenuated amplitude of TPD in *CfTX-1* peptide intervened *HCTZ* urothelium. (d and f) $CaCl_2$ activation induced TPD in G-protein receptor agonists treated *HCTZ* urothelium. TPD in *Ach* treated had no significant changes, however *NE* treatment induced a dramatically enhancement of TPD in *HCTZ* urothelium and the urothelium was supra polarized. (**e** and **g)** The *CfTX-1* peptide intervened TPD in *HCTZ* urothelium were significantly attenuated after *Ach* and *NE* treatment.

gel. a specific sequence that belonged to a fragment of *CfTX-1* N-terminal was identified. As the *CfTX-1* N-terminal was function as the cellular outer membrane porin protein (OmpD), this study forward investigated this partial sequence of *CfTX-1* evoking ionic permeability induced TPD in *HCTZ* urothelium. The results revealed that the *CfTX-1* peptide represented enhanced positive peak potentials in anode on apical membrane, generated supra polarization on *HCTZ* urothelium. Moreover, the supra polarization was inhibited in adrenergic receptor activated *HCTZ* urothelium preparation. There is a precious reference report which suggested this result. Thurman and Higgins, 1988) reported that α2-adrenoceptor agonist was most potent for stimulating the amplitude of the short-circuit current in frog urothelium, and related to a simultaneous increase in the transepithelial flux of both chloride and sodium. Jellyfish proteins induced cell membranes pore formation was first reported in box jellyfish proteins (Nagai et al., 2000). Both *CfTX-1* and *CfTX-2* generated ring-shaped pores on cellular membranes which fold in two domains, larger N-terminal domain representing almost 75% of the structure, and a

smaller C-terminal domain. Using tandem mass spectrometry, the proteome of *Aurelia aurita* crude venom profiled in 43 kDa band extracted peptide has significant sequence similarity to *CfTX-1* N-terminal (Figure 1b). *CfTX-1* which belongs to a family of pore-forming cnidarian toxins with N-terminal domain composed exclusively of $\alpha$-helices and connecting loops have significant structural similarity to N-terminal domains of pore-forming 3d-Cry toxins (Brinkman, 2007, 2012). As the domain I of Cry toxin shares a significant structural similarity with the pore-formation domain of $\beta$-PFTs colicin A, *CfTX-1* N-terminal estimated that might play an essential role in membrane penetration and pore formation after binding to the specific receptors.

Amphibian urinary bladder serosal surface was covered by an incomplete mesothelium, urothelium were water infiltrate in hypotonic in serous layer (Peachey, 1961). As is well known, $Ca^{2+}$ applied directly to the mucosal surface has no effect, however, in the living toad the $Ca^{2+}$ supply normally via the capillary network, which is on the serosal side of the epithelial sheet. Furthermore, since the mucosal surface of the bladder is normally impermeable to water, it does not seem that molecule as large as the ion could penetrate this barrier to express their activity. $CaCl_2$ was used to activate serous chamber (basement membrane); this protocol reported increases $[Ca^{2+}]_i$ through acting $Na^+/H^+$ transporter via basolateral membrane of the urothelium (Harvey and Ehrenfeld, 1988). Another cascade is the activation of phospholipase C and inositol 1,4,5-trisphosphate ($IP_3$) pathway (Hanna-Mitchell and Gebruers, 2006). It is final release of $Ca^{2+}$ from cytoplasmic storage. This induced secondary influx of $Ca^{2+}$ via calcium release-activated chloride channels, as well as serous $Ca^{2+}$ induced ATP increased $[Ca^{2+}]_i$ in the absence of extracellular $Ca^{2+}$ (Brodin et al., 1996; Brodin and Nielsen 2000). The thiazide urothelium generated dramatic enhanced positive potential by *CfTX-1* peptide (Figure 2b), however, this enhancement were blocked by $Cl^-$ channel blockade *NPPB* (Figure 2c). It could be summarized that the *CfTX-1* peptide significantly increased urothelium barrier permeability, increased $Cl^-$ influx on urothelium basal membrane. The role of chloride uptake is probably carrier mediated and driven by symporter or counter transporter. In thiazide urothelium chloride may pass through the pore formation of *CfTX-1* peptide. Classic frog serous-urothelium membrane studies revealed that serous ATP activated in tight urothelium increased in $[Ca^{2+}]i$, influenced both apical $Na^+$ channels and basolateral $K^+$ channels, activates chloride channels, plasma membrane proteins $Ca^{2+}$ activated $Cl^-$ channels mediates the secretion of $Cl^-$, bicarbonate and thiocyanate. In smooth muscle and excitable cells of the nervous system, calcium-dependent chloride channel (CaCCs) have an excitatory role coupling intracellular $Ca^{2+}$ elevation to membrane depolarization, causes the appearance of $Ca^{2+}$-activated $Cl^-$ currents in native tissues (Ferrera et al., 2011). However, this classic mechanical is not enough to explain the extremely evoked urothelium hyperpolarization triggered by *CfTX-1* peptide. Cellular membrane pore-forming, resulting in increasing membrane chloride conductance and, thus, depolarization of the urothelium was further dependent on membrane electrochemical gradient.

## Conclusion

*CfTX-1* peptide is a partial sequence of *Chironex fleckeri* toxins, which was identified from *Aurelia aurita* nematocysts from Qiongzhou Strait, Hainan province, China. The primary activation with $CaCl_2$ indicated the increased permeability of anion accross thiazide urothelium, inducing secondary transurothelium potential enhancement. This supra polarization was probably induced by *CfTX-1* peptide improved high permeability to $Cl^-$. The $Cl^-$-induced supra polarization mechanism is meaningful in improving drug delivery crossing the urothelium barrier, and in promoting the drug clearance crossing the barrier.

## ABBREVIATIONS

**CfTX-1,** Chironex fleckeri Toxin 1; **NPPB,** 5-Nitro-2-(3-phenylpropylamino) benzoic acid; **Ach,** acetylcholine; **NE,** norepinephrine; **HCTZ,** hydrochlorothiazide.

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## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

### REFERENCES

Brinkman D, Burnell J (2007). Identification, cloning and sequencing of two major venom proteins from the box jellyfish, Chironex fleckeri. Toxicon 50(6):850-860.

Brinkman DL, Konstantakopoulos N, McInerney BV, Mulvenna J, Seymour JE, Isbister GK, Hodgson WC (2014). Chironex fleckeri (Box Jellyfish) venom proteins: expansion of a cnidarian toxin family that elicits variable cytolytic and cardiovascular effects. Journal of Biological Chemistry 289:4798-4812.

Brinkman DL, Aziz A, Loukas A, Potriquet J, Seymour J, Mulvenna J (2012). Venom proteome of the box jellyfish *Chironex fleckeri*. PLoS One 7(12):e47866.

Brodin B, Rytved KA, Nielsen R (1996). An increase in $[Ca^{2+}]_i$ activates basolateral chloride channels and inhibits apical sodium channels in frog skin epithelium. Pflugers Arch 433(1-2):16-25.

Brodin B, Nielsen R (2000). Evidence for P2Y-type ATP receptors on the serosal membrane of frog skin epithelium. Pflugers Arch 439(3):234-239.

Cohen SM, Ohnishi T, Clark NM, He J, Arnold LL (2007). Investigations of rodent urinary bladder carcinogens: collection, processing, and evaluation of urine and bladders. Toxicologic Pathology 35(3):337-347.

Dunning-Davies BM, Fry CH, Mansour D, Ferguson DR (2013).The regulation of ATP release from the urothelium by adenosine and transepithelial potential. BJU International 111(3):505-513.

Ferrera L, Zegarra-Moran O, Galietta LJ (2011). $Ca^{2+}$-activated $Cl^-$ channels. Comprehensive Physiology 4:2155-2174.

Hanna-Mitchell AT, Gebruers EM (2006). The hydroosmotic response of frog urinary bladder to serosal hypertonicity is dependent on adenylate cyclase for its maintenance and affected by $[Cl^-]_o$ changes. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 291(1):R213-223.

Harvey BJ, Ehrenfeld J (1988). Role of $Na^+/H^+$ exchange in the control of intracellular pH and cell membrane conductances in frog skin epithelium. The Journal of General Physiology 92(6):793-810.

Helmholz H, Ruhnau C, Schütt C, Prange A (2007). Comparative study on the cell toxicity and enzymatic activity of two northern scyphozoan species Cyanea capillata (L.) and Cyanea lamarckii (Péron & Léslieur). Toxicon 50:53-64.

Kerec M, Bogataj M, Veranic P, Mrhar A (2005). Permeability of pig urinary bladder wall: the effect of chitosan and the role of calcium. European Journal of Pharmaceutical Sciences 25(1):113-121.

Kihara H, Anraku M, Ohno M, Hashimura S (1988). Tetrodotoxin-unaffected depolarization of frog muscles induced by the venom of jellyfish (genus Aurelia). Japanese Journal of Physiology 38:839-839.

Michailova KN, Usunoff KG (2006). Serosal membranes (pleura, pericardium, peritoneum). Normal structure, development and experimental pathology. Advances in Anatomy, Embryology and Cell Biology 183:i-vii, 1-144.

Nagai H, Takuwa K, Nakao M, Ito E, Miyake M, Noda M, Nakajima T (2000). Novel proteinaceous toxins from the box jellyfish (sea wasp) *Carybdea rastoni*. Biochemical and Biophysical Research Communications 275(2):582-588.

Parisi M, Montoreano R, Chevalier J, Bourguet J (1981). Cellular pH and water permeability control in frog urinary bladder. A possible action on the water pathway. Biochimica et Biophysica Acta 6;648(2):267-274.

Parsons CL (2007). The Role of the Urinary Epithelium in the Pathogenesis of Interstitial Cystitis/Prostatitis/Urethritis. Urology. 69(Supplement4A):9-16.

Peachey LD, Rasmussen H (1961). Structure of the toad's urinary bladder as related to its physiology. The Journal of Biophysical and Biochemical Cytology 10:529-553.

Ponce D, López-Vera E, Aguilar MB, Sánchez-Rodríguez J (2013). Preliminary results of the in vivo and in vitro characterization of a tentacle venom fraction from the jellyfish *Aurelia aurita*. Toxins. 5(12):2420-2433.

Podobnik M, Anderluh G (2017). Pore-forming toxins in Cnidaria. Semin. Cell and Developmental Biology 72:133-141.

Thurman CL, Higgins JT (1988). Catecholamine stimulation of ion transport in the toad urinary bladder. Biochimica et Biophysica Acta 945(1):81-91.

Witten J, Samad T, Ribbeck K (2018). Selective permeability of mucus barriers. Current Opinion in Biotechnology 52:124-133.

Yanagihara AA, Shohet RV (2012). Cubozoan venom-induced cardiovascular collapse is caused by hyperkalemia and prevented by zinc gluconate in mice. PLoS One 7(12):e51368.