Original Article

Inflammatory mediator gene expression of the kidneys induced by *Daboia siamensis* venom and its components

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Abstract

Phospholipase A_2 (PLA_2) and metalloprotease (MP) from *Daboia siamensis* (*Ds*) venom are believed to contribute to renal tissue toxicity. Imbalance of the cytokine system induced by snake envenomation causes tissue injury and cell damage which leads to acute toxic kidney injury. A better understanding of the pathological mechanism involved in renal injury after venom administration is needed. In the present study, we aimed to investigate inflammatory mediator genes (IL-2, IL-10, TNFα, IFNγ, and COX-2) in mouse kidney tissue after venom injection. Quantitative real time PCR was performed to detect the cytokine gene profiles. Beta actin was used as a house-keeping gene in order to normalize calculations throughout the experiment. Our results indicated that crude *Ds* venom, PLA_2 and MP enzymes from the snake venom induced both pro- and anti-inflammatory cytokines. These cytokines significantly increased the highest level detected at 60 mins except IL-2. IL-2 gene expression prolonged increased high levels until the end of the experiment. In conclusion, PLA_2 induced an up-regulation of COX-2 expression meanwhile MP caused down-regulation in mouse renal tissue. MP had no significant effect on COX-2 expression in this experiment. The results pointed out that IL-2 gene expression plays an important role to induce inflammatory response in kidneys by PLA_2 and MP from *Daboia siamensis* (*Ds*) venom.

Keywords: *Daboia siamensis* venom, Phospholipase A_2, Metalloproteinase, Mouse kidney, Inflammatory cytokine

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Received June 23, 2020
Accepted October 27, 2021
https://doi.org/10.14456/tjvm.2022.8

Introduction

Daboia siamensis (Siamese Russell's viper) is a venomous viper snake that causes common medical problems throughout the Asia-Pacific region. This venom causes acute toxic kidney injury (ATKI), renal hypoperfusion, intravascular hemolysis, rhabdomyolysis and myoglobinuria and disseminated intravascular coagulation (Mohamed *et al*., 2015). The mechanisms of nephrotoxicity by the venom are complicated and not well understood. A few previous reports have stated that *Daboia siamensis* venom and its fractions can cause changes to systemic effects and enhance renal hemodynamic in dogs. The venom induces a decrease of heart rate, pulse and blood pressure in the initial post-injection period. In addition, increased total peripheral vascular resistance and renal vascular resistance are believed to decrease renal blood flow and glomerular filtration rate due to the effect of PLA_2 and proteolytic enzyme activities (Suwansrinon *et al*., 2006). Both PLA_2 and MP of *Daboia siamensis* venom have been assessed to elevate inflammatory effects and renal hemodynamic changes (Mitrmoonpitak *et al*., 2013). Hemodynamic changes initially occur due to catecholamine release and renin-angiotensin activation by prostaglandins (Tungthanathanich *et al*., 1986). Acute renal failure is one medical problem in victims of Russell's viper bites. *Daboia siamensis* venom and its components cause acute kidney injury (AKI) and animal death, which have been reported in 5% of snakebite patients in Thailand (Athappan *et al*., 2008; Wijewickrama *et al*., 2018).

Hemodynamic changes caused by vasoactive inflammatory mediators, are endogenously generated in the host and affect renal blood flow. Decreased renal blood flow and the inflammation process act synergistically in causing renal injury. However, a number of toxins, hemodynamic changes, cytokines and inflammatory responses are involved in renal injury and renal failure (Sitprija and Sitprija, 2012; Sathe *et al*., 2018). The severity of renal injury and renal failure in AKI causes morbidity and mortality in the host in terms of the association between AKI and the immune system dysregulation (Singbartl *et al*., 2019; Song *et al*., 2019).

Several extracted enzymes such as phospholipase A_2 (PLA_2) and metalloprotease (MP) from snake venom are believed to contribute to acute renal toxicity and change pro-inflammatory cytokine effects (Menaldo *et al*., 2017). These enzymes are involved in many levels of venom actions inducing inflammatory mediators leading to local tissue injury, early onset of pain, edema, bleeding and bullous lesions, and are complicated by abscess with tissue necrosis (Albuquerque *et al*., 2013).

Phospholipase A_2 evolves into hydrolyzes phospholipids of membranes, generates lysophospholipids and serves free fatty acids as a substrate for the synthesis of many pro-inflammatory mediators. Metalloprotease enzyme digests the extracellular matrix protein network of cell surfaces and induces the secretion of chemokines and cytokines induced leukocyte migration and inflammation (Teixeira *et al*., 2009; Sitprija and Sitprija, 2012). It has been associated with the release of many inflammation mediators, such as Interleukin-1β (IL-1β), Interleukin-6 (IL-6), Interleukin-10 (IL-10), prostaglandin E2 (PGE_2) and Tumor necrosis factor-alpha (TNF-α) (Boda *et al*., 2018). Thus, envenomation by this snake venom induced inflammatory mediators which is associated with both local and systemic effects.

The immune system is normally released after foreign matter, including microorganism, envenomation and abnormal cell infringement. Whereas the immune overreaction or imbalance due to matter such as snake envenoming causes damage to body tissue and cells lead to nephrotoxic acute kidney injury (Wang *et al*., 2018). Snakebite is not only a condition mediated directly by venom proteins but also by the reaction of the body to the venom. Understanding the co-opting prey to the venom cause of toxicity amplification is challenge when designing effective therapies in snakebite treatment (Bickler *et al*., 2020).

Daboia siamensis venom and its fractions have been clearly described to cause hemadynamics and physiological changes without molecular information in kidney injury. For a better understanding of the mechanisms of renal tissue injury after venom was administrated, we aimed to investigate inflammatory mediator gene profiles in mouse kidney tissue where the venom and its fraction was directly administered intraperitoneum; phospholipase A_2 (PLA_2) and metalloprotease (MP) at different times of toxic exposure. The results might describe the physiological response to acute toxic kidney injury induced by *Daboia siamensis* envenomation.

Materials and Methods

Animal: A total of 80 IRC mice weighing 20 to 25 g were enrolled in this study. The study protocol and experimental method were approved by the Ethics and Animal Welfare Committee of Queen Saovabha Memorial Institute (No. 03/2017). All mice were housed under specific pathogen-free conditions with commercial food and tapped water *ad lib*.

Study design: The mice were divided into four groups including a control group, a crude venom, a PLA_2 and an MP exposure group. They were intraperitoneally injected with 1 ml of normal saline (Control group; Gr 1) and 0.5 µg/mouse in the *Ds* venom exposure group (Gr 2), 0.15 µg/mouse in the PLA_2 exposure group (Gr 3) and 0.35 µg/mouse in the MP exposure group (Gr 4). After 30, 60, 120 and 240 mins of post exposure injection, five mice from each time point were euthanised. Necropsy was performed and the left and right kidneys were dissected and directly kept at –70°C until use.

Sample preparation: *Daboia siamensis* venom was provided from the snake farm at the Queen Saovabha Memorial Institute, Thai Red Cross Society. PLA_2 was purified according to Khunsap *et al*. (2016). Briefly, *Daboia siamensis* venom in lyophilized form was dissolved in 0.02 M phosphate buffer (PB) pH 6.0 and applied to ion exchange chromatography on an HiTrap™ CM FF column. The proteins were eluted with 0-1 M NaCl linear gradient

in 0.02 M PB pH 6.0. The highest PLA_2 activities peaks were further purified by size exclusion chromatography by being applied to a pre-equilibrated Superdex™ 75 10/300 GL column. Whereas, MP was isolated using a three steps combination of Superdex™ 75 10/300 GL column pre-equilibrated with 0.1M ammonium acetate buffer pH 6.7. The proteins were purified followed by a Mono Q 5/50 GL and Resource™ S column (Pharmacia Biotech, USA). The proteins were collected and measured at absorbance 280 nm under an AKTA pure Fast Protein Liquid Chromatography system (FPLC, GE Healthcare; Sweden). The isolated fractions were characterized followed biochemical techniques.

Total RNA extraction: Mouse kidney tissue was blended by being sterile. Total ribonucleic acid (RNA) was extracted by Tri reagent kit (MRC, USA). Briefly, ground kidney was put in 1.5 ml eppendorf, 200 µl of Tri reagent added and vortexed for 30 mins at maximum speed. Fifty microliters of absolute chloroform was added to the mixture and continually vortexed for 5 mins. The supernatant was collected and added with absolute isopropanol being added for total RNA precipitation. RNA concentration was measured by Qubit™ RNA HS assay kit (Thermo, USA).

Quantitative Real-Time PCR: Quantitative Real-Time PCR was performed by a Bio-Rad manufactured kit. Eight hundred nanograms of total RNA were used as a template for gene expression. The cycling condition was performed under default by the CFX96 Touch™ Real-Time PCR detection system (BioRad, USA) by using iTaq™ universal SYBR® Green One-Step kit (BioRad, USA). The reverse transcription reaction was started at 50°C for 10 mins, then polymerase activation and DNA denaturation at 95°C for 60 secs. After an initial denaturation at 95°C for 10 secs and 40 cycles at 60°C for 10 secs, melting analysis was collected at 95°C for 10 secs, 65°C for 5 secs and 95°C for 50 secs. The sequence primers for the RT-PCR of mice beta actin, IL-2, IL-10, TNFα, IFNγ and COX-2 were purchased from Bio-Rad (PrimePCR Unique assay ID. qMmuCED0027505, qMmuCED0060978, qMmuCE D0044967, qMmuCED004141 and qMmuCED006268, respectively), while COX-2 sequence was followed by McCarthy *et al*., 2013. The specific primer for each cytokine quantitation using real-time RT-PCR follows in Table1:

Table 1 Oligonucleotide primers of inflammatory cytokines used in this study

Genes	Sequences
Beta actin forward: Beta actin reverse:	5'CGGGACCTGACAGACTACCT'3 5'TGCTCGAAGTCTAGAGCAACA'3
IL-2 forward:	5'AGCCTTATGTGTTGTAAGCAGGA'3
IL-2 reverse:	5'CTGTGGTGGACTTTCTGAGGA'3
IL-10 forward:	5'CGTTTGTACCTCTCTCCGAAA'3
IL-10 reverse:	5'TTGGGCTTCTTTCTAAATAGTTCA'3
TNFα forward:	5'CCACAAGCAGGAATGAGAAGA'3
TNFα reverse:	5'TCCCTCCAGAAAAGACACCA'3
IFNγ forward:	5'TTTGAGGTCAACAACCCACA'3
IFNγ reverse:	5'AAATTCAAATAGTGCTGGCAGA'3
COX-2 forward:	5'TGACCCCCAAGGCTCAAAT'3
COX-2 reverse:	5'GAACCCAGGTCCTCGCTTATG'3

IL-2 = Interleukin-2; IL-10 = Interleukin-10; TNF-α = Tumor necrosis factor alpha; IFN-γ = Interferon gamma; COX-2 = Cyclooxygenase-2 enzyme

All data analyses were performed by the CFX manager software detection system under Bio-Rad license. Relative quantification analysis determined the levels of expression of Genes Of Interest (GOI) and expressed it on the levels of a Reference Gene (RefG). Reference genes are genes whose expression level does not change which are commonly used for normalization of target genes expression. Beta actin was the reference gene and control group as calibrator in this work.

Statistical analysis: The mRNA expressions of interest genes were normalized to the beta actin gene and quantified with the control group under the same conditions. The results of the definitive statistic were reported as mean ± SEM of fold changes. Statistical differences were evaluated using t-test analysis (Primer program version 3.02). A P value < 0.05 was considered as statistically significant.

Results

Inflammatory mediator gene expression: Evaluation of inflammatory mediator gene expression in mouse kidney tissues after intraperitoneum induced by Gr 2 (*Ds* venom 0.5 µg/mouse), Gr 3 (PLA_2 0.15 µg/mouse) and Gr 4 (MP 0.35 µg/mouse) for 30, 60, 120 and 240 min exposure time. The mRNA expression in each group was compared with the negative control (normal saline, Gr 1). The sublethal doses of venom were used in this experiment (data not shown).

The quantification of inflammatory mediator gene expression was revealed after 60 mins stimulus of Gr 2 (*Ds* venom exposure). Most genes showed high-levels of expression at 60 mins post-exposure. The expression of the IL-2 and IL-10 levels achieved the highest at 60 mins post-exposure and was maintained at this level until 120 mins post-exposure, then it slightly decreased to normal at 240 mins post-exposure. Whereas the level of IFNγ and TNFα was highly increased within 60 mins post exposure and decreased to basal level at 120 and 240 mins post-exposure, respectively. COX-2

expression was significantly down-regulated at 120 mins post exposure induced by *Ds* venom (Gr 2) (Fig. 1).

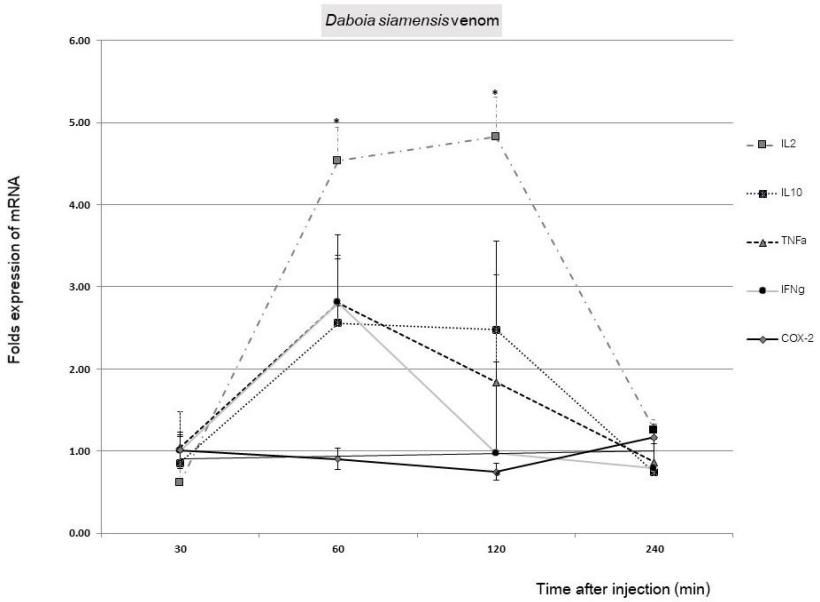

Figure 1 The expression of inflammatory genes in kidney tissue induced by *Ds*V (Gr 2).
(*) indicate significant difference between experimental and control groups ($P < 0.05$).

Gr 2 (PLA_2 exposure) revealed IFNγ and IL-10 significantly expressed at early 30 mins post exposure. At a dosage of 0.15 µg/mouse of PLA_2 promoted a high level of all inflammatory mediator gene expression at 60 mins and was slightly decreased within 240 mins post exposure. The expression level of IFNγ was the highest, followed by TNFα and IL-10 which were as same as IL-2 gene expression, respectively. However, IL-2 was consistently prolonged at the same level until 120 mins and suddenly dropped at 240 mins. COX-2 expression was significantly up-regulated at 120 mins post exposure induced by PLA_2 (Gr 3) (Fig. 2).

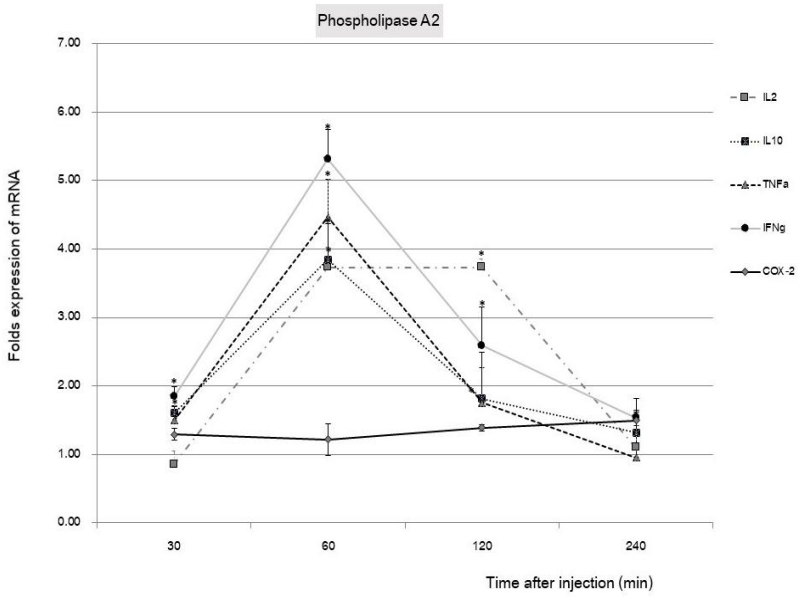

Figure 2 The expression of inflammatory genes in kidney tissue induced by PLA_2 (Gr 3).
(*) indicate significant difference between experimental and control groups ($P < 0.05$).

The level of TNFα mRNA expression was the highest at 60 mins post exposue induced by MP (Gr 4). The expression levels of IL-10 and IFNγ were 2.25 and 2.62 fold, respectively. A high-level of IL-2; pro-inflammatory gene appeared at 120 mins post exposue (Gr 4). However, the expression level of all genes decreased to normal at 240 mins post exposure. COX-2 gene expression was within normal level in Gr 4 (Fig. 3).

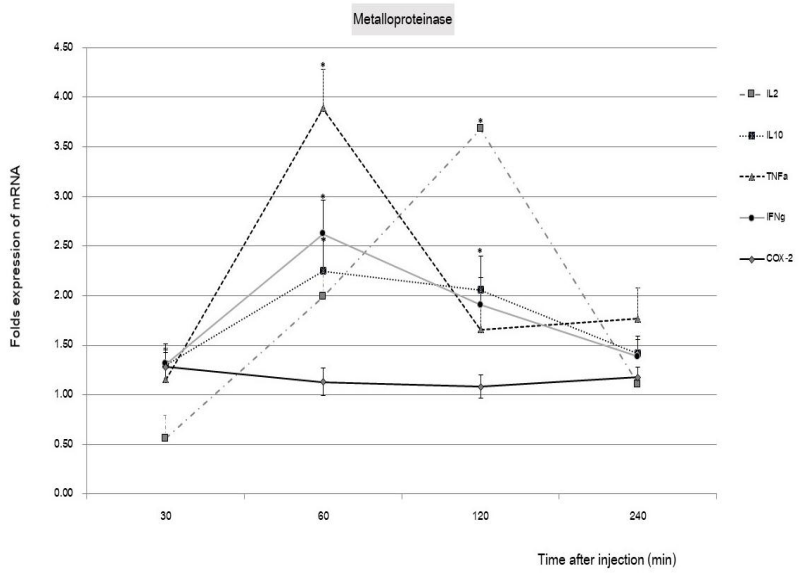

Figure 3 The expression of inflammatory genes in kidney tissue induced by MP (Gr 4).
(*) indicate significant difference between experimental and control groups ($P < 0.05$).

Discussion

The inflammatory process is a normal physiological response to remove pathogens and restore homeostasis. During envenomation, the complex network of cytokine is responsive. *Ds* venom induces high levels and prolongs IL-2 pro-inflammatory mediators, caused by tissue damage and regulator inflammation (Gao *et al*., 2017; Stremska *et al*., 2017). Similar to our results, acute inflammatory events that lead to tissue damage after snake envenomation, are mainly caused by increased IL-2. However, IL-2/anti-IL-2 complex can attenuate acute renal damage and improve renal recovery via Treg expansion which may be a therapeutic strategy for renal ischemia reperfusion injury (Kim *et al*., 2013). On the other hand, IL-2 may play an important role in T-cell proliferation and the differentiation of Th1 and Th2 effector cells (Luna *et al*., 2019). Incidentally, the expression of IL-2 in kidney tissue was not related to COX-2 function. IL-2 was significantly increased, whereas COX-2 was inhibited by *Ds* venom (Gr 2) at the same periods (Fig. 1). Increasing levels of IFNγ and TNFα seem to support the inflammatory process of IL-2. The expression level of IL-2 was significantly higher than IL-10 in a similar pattern. On the other hand, IL-10 might have inhibited IFNγ and TNFα at 120 mins causing an increase in IL-2 level (Kessler *et al*., 2017) or a balanced reaction of IL-2 for cell prevention. The increasing of IL-10 was reported to protect the patients after envenomation with various snake venom such as *Daboia siamensis*, *Crotalus durissus terrifitus* and *Bothrops spp* (Boda *et al*., 2018).

PLA_2 affects renal function through its ability to mediate inflammatory response causing cell lysis (Sitprija and Sitprija, 2012). It induces both pro- and anti-inflammatory cytokine linked to enzymatic and non-enzymatic activity (Boda *et al*., 2018). PLA_2 induced a pro-inflammatory response particularly IFNγ, TNFα and IL-2 levels in Gr 3 in this study. This PLA_2 enzyme might up-regulate a high level of IFNγ and TNFα in the kidneys and spleens of mice (Yacoub *et al*., 2018). Ribeiro et al. (2014), reported that *Crotalus durissus collilineatus* venom induced high expression of both TNFα and IFNγ in PBMC cells (Ribeiro *et al*., 2014) which was similar to PLA_2 effect in this study. TNFα and IFNγ might play an important role in host response to the venom and the immune response to infection after snake envenomation (Cruz *et al*., 2008). On the other hand, IL-10 was considered to be an anti-inflammatory cytokine which inhibited pro-inflammatory cytokines in various tissues (Cruz *et al*., 2008; Ribeiro *et al*., 2014; Yacoub *et al*., 2018). In our study, the IL-10 profile correlated with the TNFα and IFNγ pattern on the same exposure timescale as their pro-inflammatory counterparts. It might play a balancing factor to TNFα and IFNγ after snake envenomation. This result supports the potential role of IL-10 in modulating an inflammatory response to prevent cell injury (Fernández and Kaski, 2002). Unfortunately, IL-10 did not affect levels of IL-2 in this study. Although decreasing the level of TNFα and IFNγ, the level of IL-2 stayed relatively high until 120 mins, indicating that the inflammatory process has a continual progression (Fig. 2). Therefore, PLA_2 enzyme may be dominant factor promoting inflammatory pathogenesis of *Daboia siamensis* venom.

MP is a proteolytic enzyme which belongs to a zinc-dependence enzyme. It plays a major role in the hemorrhagic event and is associated with the inflammatory response. It also activates the complementary system and release of various cytokines (Luchini *et al*., 2019). Imbalance of inflammatory mediators in the complement system leads to inflammatory diseases. Many studies have reported numerous inflammatory mediators such as IL-6, TNFα, IL-10, IL-1β and IL-1 induced by MP of *Botrops* sp. after 1 to 4 hours of injection (Mitrmoonpitak *et al*., 2013; Bernardes *et al*., 2015; Burin *et al*., 2018). Similar to our study, TNFα significantly increased the expression following the first hour of IFNγ, IL-10 and IL-2. The expression of TNFα and IFNγ declined slowly to baseline after 60 mins post-exposure. However, the expression of the IL-10 was

still at a high level at 120 mins which, at the same period, was the highest expression of IL-2. The result indicated that IL-10 is able to modulate both TNFα and IL-2 effects for balancing inflammatory events (Fig. 3).

Cyclooxygenase-derived eicosanoids such as prostaglandins are involved in the inflammatory responses and play a major role in local tissue damage. There have been reports that PGE_2 represented in snake venom-related inflammatory response causes COX-2 immuno suppression (Wanderley *et al.,* 2014; Burin *et al.,* 2018). PGE_2 is also produced via a COX-2 pathway after intraperitoneal injection of PLA_2 from the *Botrops asper* venom in mice (Moreira *et al.,* 2014). According to our results, the action of PLA_2 from *Ds* venom is involved in the COX-2 pathway. COX-2 was significantly increased by PLA_2 meanwhile it was down-regulated by *Ds* venom. In contrast, MP had no effect on COX-2 in kidney tissue (Fig. 3). The results indicated that the expression of IL-2 and IFNγ genes was related to COX-2 function induced by PLA_2 activities (Fig. 2). They may be mainly inducers for pro-inflammatory cytokines cause local tissue damage. In contrast, *Ds* venom and MP effects on kidney injury were not involved in the COX-2 expression. Therefore, *Ds* venom, PLA_2 and MP are enzymes that seem to involve the local myonecrosis, skin damage and inflammatory reaction (de Fátima Pereira Teixeira *et al.,* 2005). These venoms can induce both anti- and pro-inflammatory mediator in kidney tissue. Our results indicated that pro-inflammatory cytokines were considered to be the prominent products induced by *Ds* venom. The anti-inflammatory; IL-10 had been increased to modulate the immunosuppressive effect which might be a reversible mechanism (Greenberg *et al.,* 2015). The balancing system in kidney tissue between pro- and anti-inflammatory cytokines is a reversible mechanism after snakebite envenomation.

In conclusion, the impact of *Ds* venom in terms of inflammatory activity was a profiled analysis of IL-10, IL-2, TNFα and IFNγ in mouse kidney stimulation. The optimized time of cytokine mRNA expression in mouse kidney was 60 mins post-exposure. *Ds* venom and its components induce all cytokines, especially pro-inflammatory mediators which related to cell injury and immune response. PLA_2 had a predominant enzyme effect on kidney tissue which was better than *Ds* and MP. Therefore, PLA_2 inhibitors might relay toxicity and should be further investigated. Information on these toxins might help to understand the potential mechanisms that modulate kidney damage after snake envenoming as supportive information for inflammatory mediator gene expression. Anti-pro inflammatory cytokines, known as pro-inflammatory inhibitors, should be studied further for modulating inflammatory response to snake venom. Pro-inflammatory cytokine detected from serum of venom exposure host needs to be further investigated in order to fine some correlation to renal gene expression.

Acknowledgements

This work was supported by the Queen Saovabha Memorial Institute, Thai Red Cross Society. The authors would like to thank Dr. Anudep Rungsripipat for the revised manuscript and Dr. Lawan Chanhome for the venom provided.

References

Albuquerque MMLP, Jacinto NC, Silva Junior BG, Lima BJ, Veras BM and Daiier FE 2013. Acute kidney injury caused by *Crotalus* and *Botrops* snake venom: a review of epidemiology, clinical manifestations and treatment. Rev Int Med Trop Sao Paulo. 55(5):295-301.

Athappan G, Balaji MV, Navaneethan U and Thirumalikolundusubramanian P 2008. Acute renal failure in snake envenomation: A large prospective study. Saudi J Kidney Dis. Transplant. 19(3): 404-410.

Bernardes PC, Menaldo LD, Mamede CNC, Zoccal FK, Cintra COA, Faccioli HL, Stanziola L, de Oliveira F and Sampaio VS 2015. Evaluation of the local inflammatory events induced by BpirMP, a metalloproteinase from *Botrops pirajai* venom. Mol. Immunol. 68:456-464.

Bickler PE 2020. Amplification of snake venom toxicity by endogenous signaling pathways. Toxins. 68: doi:10.3390/toxins12020068.

Boda F, Banfai K, Garai K, Curticapean A, Berta L, Sipos E and Kvell K 2018. "Effect of *Vipera ammodytes ammodytes* Snake venom on the human cytokine network." Toxins. 10(7):259. doi:10.3390/toxins10070259.

Burin MS, Menaldo LD, Sampaio VS, Frantz GF and Castro AF 2018. An overview of the immune modulating effects of enzymatic toxins from snake venoms. Int J Biol Macromol. 109:664-671.

Cruz HA, Garcia-Jimenez S, Mendonca ZR and Retricevich LV 2008. "Pro- and anti-inflammatory cytokine release in mice injected with *Crotalus durissus terrificus v*enom." Med Inflamm. doi10.1155/2008/874962.

de Fátima Pereira Teixeira C, Fernandes MC, Zuliani PJ and Zamuner FS 2005. Inflammatory effects of snake venom metalloproteinase. Mem Inst Oswaldo Cruz, Rio de Janeiro. 100:181-184.

Fernández PR and Kaski CJ 2002. Interleukin-10 and coronary disease. Rev Esp Cardiol. 55(7): 738-750.

Gao W, Li F, Zhou Z, Xu X, Wu Y, Zhou S, Yin D, Sun D, Xiong J, Jiang R and Zhang J 2017. "IL-2/anti-IL-2 complex attenuates inflammation and BBB disruption in mice subjected to traumatic brain injury. Front Neurol." doi: 10.3389/fneur.2017.00281.

Greenberg HJ, Whitlock R, Zhang RW, Thiessen-Philbrook RH, Zappitelli M, Devaraian P, Eikelboom J, Kavsak AP, Devereaux PJ, Shortt C, Garg XA and Parikh RC 2015. Interleukin-6 and interleukin-10 as acute kidney injury biomarkers after pediatric cardiac surgery. Pediatr Nephol. 30(9):1519-1527.

Harshavardhan L, Lokesh AJ, Tejeshwari HL, Halesha BR and Siddharama SM 2013. A study on the acute kidney injury in snake bite victims in tertiary care centre. J Clin Diagn Res. 7(5):853-856.

Kessler B, Rinchai D, Kewcharoenwong C, Nithichanon A, Biggart R, Hawrylowicz MC,

Bancroft JG and Lertmemongkolchai G 2017. "Interleukin 10 inhibits proinflammatory cytokine responses killing of Burkholderia pseudomallei." Sci Rep. doi:10.1038/srep42791.

Khunsap S, Khow O, Buranapraditkun S, Suntrarachun S, Puthong S and Boonchang S 2016. "Anticancer properties of phospholipase A_2 from *Daboia siamensis* venom on human skin melanoma cells." J Venom Anim Toxins Incl Trop Dis. doi:10.1186/s40409-016-0061-z.

Kim MG, Koo YT, Yan JJ, Lee E, Han HK, Jeong CJ, Ro H, Kim SB, Jo KS, Oh HK, Surh DC, Ahn C and Yang J 2013. IL-2/anti-IL-2 complex attenuates renal ischemia-reperfusion injury through expansion of regulatory T cells. J Am Soc Nephrol. 24:1529-1536.

Luchini GSL, Pidde G, Squaiella-Baptistão CC and Tambourgi VD 2019. "Complement system inhibition modulates the pro-inflammatory effects of a snake venom Metalloproteinase." Front Immunol. [Online]. Available: doi:10.3389/fimmu.2019.01137.

Luna PK, Melo MC, Pascoal PV, Martins-Filho AO and Pereira RV 2019. "Bothrops erythromelas snake venom induces a proinflammatory response in mice splenocytes." Int J Interferon Cytokine Mediat Res. [Online]. Available: http://www.dovepress.com/by 103.44.217.57

McCarthy MK, Levine RV, Procario MC, McDonnell PJ, Zhu L, Mancuso P, Crofford LJ, Aronoff DM and Weinberg JB 2013. Prostaglandin E_2 induction during mouse adenovirus type 1 respiratory infection regulates inflammatory mediator generation but does not affect viral pathogenesis. PLOS one. 8(10): e77628. doi:10.1371/journal.pone.0077628.

Menaldo DL, Bernardes CP, Zoccal KF, Jacob-Ferreira AL, Costa TR, Lama MPFMD, Naal RMZG, Frantz FG, Faccioli LH and Sampaio SV 2017. Immune cells and mediators involved in the inflammatory responses induced by a P-I metalloprotease and a phospholipase A_2 from *Bothrops atrox* venom. Mol Immunol. 85:238-247.

Mitrmoonpitak C, Chulasugandha P, Khow O, Noiprom J, Chaiyabutr N and Sitprija V 2013. Effects of phospholipase A_2 and metalloprotease fractions of Russell's viper venom on cytokines and renal hemodynamics in dogs. Toxicon. 61:47-53.

Mohamed F, Endre ZH and Buckley NA 2015. Role of biomarkers of nephrotoxic acute kidney injury in deliberate poisoning and envenomation in less developed countries. Br J Clin Pharmacol. 80(1): 3-19.

Moreira V, Lomonte B, Vinolo MAR, Curi R, Gutiérrez JM and Teixeira C 2014. An Asp49 phospholipase A_2 from snake venom induces Cyclooxygenase-2 expression and prostaglandin E_2 production via activation of NF-κB, p38MAPK, and PKC in macrophages. Med Inflamm. [Online]. Available: http://dx.doi.org/10.1155/2014/105879

Ribeiro BC, dos Santos CJ, Silva MJ, de Godoi SHP, Magalhães RM, Spadafora-Ferreira M, Fonseca GS and Pfrimer HAI 2014. "*Crotalus durissus collilineatus* venom induces TNF-α and IL-10 production in human peripheral blood mononuclear cells." ISRN Inflamm. http://dxdoi.org/10.1155/2014/563628

Sathe P, Sakhavalkar P, Borse R, Parathody A, Huparikar A and Rayte N 2018. "Snake bite induced sepsis with multi organ failure successfully treated with extracorporeal cytokine adsorption device (ECAD) therapy along with standard of care-a case series" [Online]. Available: https://www.ijmdat.com/wp-content/uploads/sites/3/2018/10/e158-Snake - bite-induced-sepsis.pdf

Singbartl K, Formeck LC and Kellum AJ 2019. Kidney-immune system crosstalk in AKI. Sem Nephro. 39(1):96-106.

Sitprija V and Sitprija S 2012. Renal effects and injury induced by animal toxins. Toxicon. 60:943-953.

Song J, Yu J, Prayogo WG, Cao W, Wu Y, Jia Z and Zhang A 2019. Understanding kidney injury molecule 1: a novel immune factor in kidney pathophysiology. Am J Transl Res. 11(3):1219-1229.

Stremska EM, Jose S, Sabapathy V, Huang L, Baiwa A, Kinsey RG, Sharma RP, Mohammad S, Rosin LD, Okusa DM and Sharma R 2017. IL233, a novel IL-2 and IL-33 hybrid cytokine, ameliorates renal injury. J Am Soc Nephrol. 28:2681-2693.

Suwansrinon K, Khow O, Mitmoonpitak C, Daviratanasilpa S, Chaiyabutr N and Sitprija V 2006. Effects of Russell's viper venom fractions on systemic and renal hemodynamics. Toxicon. 49(1):82-88.

Teixeira C, Cury Y, Moreira, Picolo G and Chaves F 2009. Inflammation induced by *Botrops asper* venom. Toxicon. 54:67-76.

Tungthanathanic P, Chaiyaburt N and Sitprija V 1986. Effect of Russell's viper (*Vipera russelli siamensis*) venom on renal hemodynamics in dogs. Toxicon. 24:365-371.

Wang S-Z and Qin Z-H 2018. "Anti-inflammatory and immune regulatory actions of *Naja naja naja* venom." Toxins. 10:100. doi:10.3390/toxins10030100.

Wijiewickrama ES, Kurukulasooriya I, Gunatilake M, Priyani AAH, Gnanathasan A, Gawarammana I and Isbister GK 2018. Determination of the sub-lethal nephrotoxic dose of Russell's viper (*Daboia russelii*) venom in Wistar rats. Toxicon. 152:43-45.

Wanderley CWS, Silva CMS, Wong DVT, Ximenes RM, Morelo DFC, Cosker F, Aragao KS, Fernandes C, Palheta-Júnior RC, HavtA, Brito GA, Cunha FQ, Ribeiro RA and Lima-Júnior RC 2014. *Bothrops jararacussu sn*ake venom-induces a local inflammatory response in a prostanoid- and neutrophil-dependent manner. Toxicon. 90:134-147.

Yacoub T, Rima M, Sadek R, Hleihel W, Fajloun Z and Karam M 2018. *Montivipera bornmuelleri* venom has immunomodulatory effects mainly up-regulating pro-inflammatory cytokines in the spleens of mice. Toxicol Reports. 5:318-323.