

Major Biological Activities of the Skin Secretion of the Chinese Giant Salamander, *Andrias davidianus*

Wentao Guo[§], Mingzhang Ao[§], Wei Li, Jianwen Wang, and Longjiang Yu*

Institute of Resource Biology and Biotechnology, Department of Biotechnology, College of Life Science and Technology, Huazhong University of Science and Technology, Wuhan, 430074, China; and Key Laboratory of Molecular Biophysics, Ministry of Education, Wuhan, 430074, China. Fax: 86-27-87792264. E-mail: yulongjiang@mail.hust.edu.cn

* Author for correspondence and reprint requests

Z. Naturforsch. **67c**, 86–92 (2012); received March 4/October 31, 2011

Amphibian skin can produce abundant secretion which contains many bioactive compounds. In this work, skin secretion of the Chinese giant salamander (*Andrias davidianus*) was obtained by mild electrical stimulation of the dorsal skin surface, and the main physiopathological properties of the secretion were described. Intraperitoneal administration of the skin secretion caused lethal effects in mice. Low doses of the skin secretion induced significant systemic and local effects like edema and nociception in mice. The activities of phospholipase A₂ and proteolytic enzyme were likely related to the physiopathological activities observed. The work proved the complex toxic effects of the Chinese giant salamander skin secretion and provided clues to study its physiological function further.

Key words: *Andrias davidianus*, Skin Secretion, Toxicity, Biological Activities

Introduction

Amphibians are a transition species from the aquatic ancestors to the reptiles. Their skin is a morphologically, biochemically, and physiologically complex organ which fulfills a wide range of functions necessary for survival, including respiration, water regulation, antipredator and antimicrobial defense, excretion, temperature control, and reproduction (Clarke, 1997). The amphibian skin contains mucous and granular glands; the former ones are usually associated with breathing, reproduction, and water balance (Toledo and Jared, 1993), while the latter ones are responsible for the secretion of toxins and defences against microorganisms and predators (Barra and Simmaco, 1995).

The glands in the skin can produce secretion when the amphibian is provoked. Interestingly, the amphibian skin secretion is one of the richest sources of natural and bioactive compounds. The granular glands in the amphibian skin are responsible for the production of noxious or toxic substances presenting several biological and pharmacological effects. So far, compounds such

as biogenic amines, alkaloids, steroids, proteins, and peptides have been isolated from the skin secretion of anuran amphibians, and their number is still growing. These compounds play different roles either in the regulation of physiological functions of the skin or in the defence against predators and microorganisms (Stebbins and Cohen, 1995). Moreover, they are closely related to a variety of biological effects such as cytotoxic, bactericidal, fungicidal, lytic, neuromimetic, anesthetic, and phenomenal (Apponyi *et al.*, 2004; Barra and Simmaco, 1995; Bevins and Zasloff, 1990; Clarke, 1997; Daly *et al.*, 2005; Giangaspero *et al.*, 2001; Simmaco *et al.*, 1998; Woodley, 2010). However, peptide isolation and characterization seem to be the preferred approach to study amphibian skin secretion, and little attention has been paid to crude skin toxicity assessment, especially to the toxicity of the Chinese giant salamander skin secretion.

The Chinese giant salamander, *Andrias davidianus*, belonging to the family Cryptobranchidae (Amphibia, Urodela), is the largest amphibian species in the world. This family has a combined Asian/North American distribution but contains only three extant species: *Andrias davidianus* in China, *Andrias japonicus* in Japan, *Cryptobran-*

[§] These authors contributed equally to this work.

chus alleganiensis in North America (Zhang *et al.*, 2003). The genetics and ecology of this species have received much attention recently (Katsu *et al.*, 2006; Matsui *et al.*, 2008; Murphy *et al.*, 2000; Yang *et al.*, 2010). Interestingly, the toxicity of the Chinese giant salamander skin secretion is mentioned in the Compendium of Materia Medica, a traditional Chinese codex (Li, 1978). However, few studies have been conducted to demonstrate the toxic activities induced by the skin secretion of the Chinese giant salamander in the murine model. In view of these facts, the aim of the present study was to analyse the biological activities induced by the crude skin secretion of the Chinese giant salamander. This work will provide a better understanding of the role of the proteins and enzymes present in the skin secretion of this species.

Material and Methods

Animals

Kunming mice (Hubei Experimental Animal Center) with a weight of (20 ± 2) g were used. The animals were maintained under standard laboratory conditions of relative humidity (50%), temperature $[(25 \pm 1) ^\circ\text{C}]$, and light (12 h day/12 h night). They were given free access to food and water for at least 5 d before the experiments. All experimental designs and procedures have received approval from the Animal Ethics Committee of Huazhong University of Science and Technology, Wuhan, China (2009-27).

Collection of the salamander skin secretion

The giant salamanders are bred in a simulated natural environment in the Giant Salamander Protection Center in Sangzhi County, Zhangjiajie city, Hunan province of China. Their use in this research has been approved by the Aquatic Wild Animal and Plant Protection Office of the Ministry of Agriculture (2008-4). Adult specimens ($n = 6$; 40–70 cm long and 1.5–2.5 kg in weight) of both male and female (3 males and 3 females) giant salamander were selected randomly. Skin secretion was collected as follows: Animals were first washed with distilled water to remove contaminants from their skin surface. Then the dermal musculature was stimulated electrically as described previously (Tyler *et al.*, 1992). After 1–2 min, the skin surface of salamanders started to

exude secretion which was collected by washing the dorsal area of each animal with distilled water. The collected solutions were quickly centrifuged, and the supernatant was lyophilized to yield the powdered protein sample. The protein concentration of the secretion sample used in all assays was determined by dissolving a specific amount of it in a certain volume of buffer.

Acute toxicity in mice

For the acute lethal toxicity study, the intraperitoneal (i.p.) LD_{50} value for mice was determined following the procedure previously described (Meier and Theakston, 1986). Thirty Kunming mice were divided into three groups and injected i.p. with different doses of the test secretion samples. The test animals were observed for 24 h after injection. Survival time of each animal was recorded and LD_{50} was calculated. A sample incubated at $100 ^\circ\text{C}$ for 10 min was also tested following the same procedure.

Nociceptive activity

Nociceptive activity of the skin secretion was assayed according to the method reported previously (Hunskaar *et al.*, 1985). Thirty μL of the skin secretion (0.8, 2.4, and $7.2 \mu\text{g}$ of protein) were administered by hypodermic injection (i.h.) into the right footpad of mice. Then each mouse was kept in an adapted chamber mounted on a mirror for 10 min. The control group was injected only with saline. Each animal was then returned to the observation chamber, and the time spent on licking or biting each hind paw was recorded.

Vascular permeability

Vascular permeability of the skin secretion was analysed according to the method described previously (Sirois *et al.*, 1988). Evans Blue dye, 20 mg/kg in 200 μL of saline, was administered by intravenous injection (i.v.) 20 min prior to application of the skin secretion (0.8, 2.4, and $7.2 \mu\text{g}$ of protein) or saline i. p. After 2 h, the mice were executed and their peritoneal cavity was washed with 2 mL of ice-cold phosphate-buffered saline (PBS) plus 0.1% bovine serum albumin (BSA). The cells were centrifuged after collection, and the optical density (OD) of the supernatant at 620 nm was measured to evaluate the leakage of Evans Blue into the peritoneal cavity. Five mice were used for each group per experiment, and

the experiment was conducted three times. The results were expressed in μg of Evans Blue per mL and the concentration of Evans Blue was calculated through a standard curve of known concentrations.

Edema

Edema activity of the skin secretion was assayed according to a previously described procedure (Lima *et al.*, 2003). Thirty μL of the skin secretion (0.8, 2.4, and 7.2 μg of protein) were injected i.h. into the right footpad of mice. Local edema was tested by measuring the thickness of the treated paws with a Vernier caliper 0.5, 2, 4, 24, and 48 h after injection. Saline-treated mice were used as the control. The results were expressed as differences of footpad thickness between experimental and control groups.

Cell harvest and counting

Leukocyte migration was assessed 2 or 24 h after the skin secretion (7.2 μg of protein) or saline (30 μL) administration in the footpad. The samples were immediately centrifuged at 3700 $\times g$ for 20 min at 4 °C. The resulting cell pellets were resuspended in 1 mL of PBS for total cell count in a hemocytometer and differential leukocyte count in cytocentrifuge preparations stained with Wright-Giemsa, respectively. Cells were differentially counted by microscopy, evaluating 300 cells per slide.

Hemorrhagic activity

The method of hypodermic injection (Ownby *et al.*, 1978) was used to test the hemorrhagic activity. Skin secretion was injected into mice at a dose of 10 $\mu\text{g}/\text{g}$ body weight. Six h later, all mice were executed and the areas of hemorrhagic spots were measured.

Proteolytic activity

N,N-Dimethylated casein was used as the un-specific protein substrate for the assessment of the proteolytic activity of the skin secretion, as described previously (Menezes *et al.*, 2006). An aliquot of 0.4 mL buffer solution (0.1 M Tris-HCl buffer, pH 7.5, and 0.01 M CaCl_2), 0.1 mL (7.2 μg) sample solution, and 0.5 mL 2% casein solution (dissolved in the same buffer) was maintained at 37 °C for 30 min. The reaction was stopped by adding 1 mL 5% trichloroacetic acid. The mixture

was centrifuged at 15340 $\times g$ for 15 min, and the absorbance at 280 nm was measured. One unit of activity was defined as the amount of sample yielding an increase in OD of 1.0 unit per min at 280 nm. Specific activity was expressed as units/mg protein.

Phospholipase A₂ activity

Phospholipase A₂ activity was determined according to the established method (Holzer and Mackessy, 1996) using 4-nitro-3-(octanoyloxy)-benzoic acid as the substrate. One mL of buffer (10 mM Tris-HCl, 10 mM CaCl_2 , and 100 mM NaCl, pH 8.0) was mixed with 100 μL of the secretion (7.2 μg) and the mixture placed on ice. One hundred μL of substrate (3.0 mM in acetonitrile) were then added. The final concentration of the substrate was 0.25 mM. Each tube was vortexed and kept in a water bath (37 °C) for 20 min. To stop the reaction, tubes were placed on ice, 100 μL of Triton X-100 (2.5%) were quickly added, and tubes were vortexed for 5 s each. Tubes were held at room temperature for 5 – 10 min, and the absorbance at 425 nm was recorded. The activity was expressed as nmol chromophore released per min and mg protein.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was carried out following the previously described method (Laemmli, 1970). Twenty μg skin secretion were analysed by 12% SDS-PAGE gels. Before electrophoresis, the samples were mixed with sample buffer (1:1, v/v). The gel was stained with Coomassie brilliant blue.

Statistical analysis

All results were presented as means \pm SEM of at least four animals in each group. Differences among data were determined by one-way analysis of variance (ANOVA) followed by Dunnett's test. Differences between two means were determined through the unpaired Student's t-test. Data were considered to be different at $p < 0.05$.

Results

Toxicity

Mice injected with the secretion of the Chinese giant salamander showed typical symptoms of poisoning, such as gasping, jerking, and ventral

decubitus. The anatomy of mice in the experimental group revealed hyperemia of the lungs, while the other organs remained unaltered. The LD₅₀ (i.p.) value was determined to be 32.95 mg/kg body weight. After incubation at 100 °C for 10 min, however, the skin secretion lost its ability to cause mortality in mice.

Nociceptive activity

Thirty μ L of the skin secretion (0.8, 2.4, and 7.2 μ g of protein) were injected into the right footpad of mice for nociception, which was characterized by licking, biting, and flinching of the injected paw. Fig. 1 shows that the skin secretion administered i.h. produced a clear dose-dependent nociceptive effect with significant differences among doses of 7.2, 2.4, and 0.8 μ g of protein ($p < 0.05$).

Vascular permeability

Fig. 2 shows that a maximum of Evans Blue extravasation into the peritoneal cavity is observed after injection of 2.4 μ g protein.

Edema

Larger doses of the secretion (2.4 and 7.2 μ g) were significantly edematogenic when compared to the 0.8- μ g dose and the control group (Fig. 3). These higher doses of the skin secretion main-

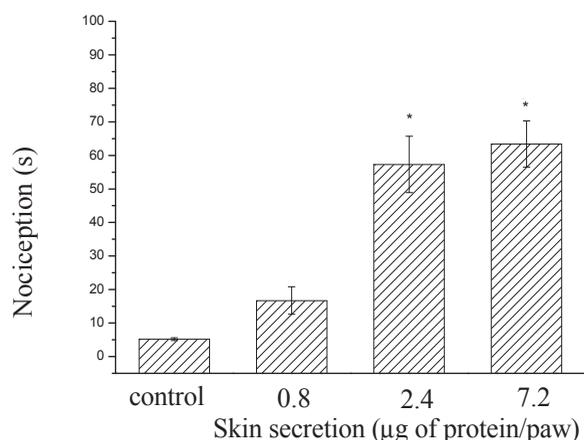


Fig. 1. Estimation of the nociception activity at different doses (0.8, 2.4, and 7.2 μ g of protein) of the skin secretion injected into the right footpad of mice. The time spent on licking or biting each hind paw was recorded during 30 min and taken as the index of nociception. Each point represents mean \pm SEM. * $p < 0.05$ compared with the control group ($n = 5$).

tained the edematogenic response up to 24 h, while only the dose of 7.2 μ g secretion caused a sustained edematogenic response for 48 h after injection compared to the control group.

Leukocyte migration

Leukocyte recruitment to the site of injury after skin secretion injection was evaluated in mice. Fig. 4 shows that the number of total cells became larger after 2 h, characterized by the increment of macrophages. However, cells in the footpad tissue of mice treated with the skin secretion showed no significant change 24 h after injection. Moreover, the number of neutrophils became much larger 24 h after injection. Therefore, the skin secretion induced significant leukocyte influx.

Hemorrhagic activity

The skin secretion did not induce significant hemorrhagic activity.

Enzymatic activities

As seen from Table I, the dose of 1 μ g protein had significant proteolytic activity [2.32 ± 0.26 U/mg] and phospholipase A₂ activity [0.38 ± 0.04 nmol chromophore released/(min mg)].

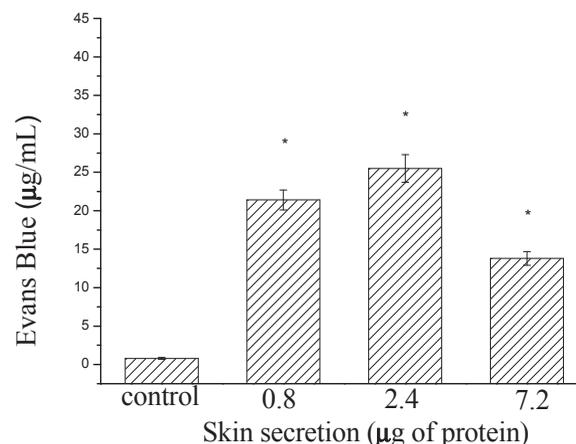


Fig. 2. Evaluation of the vascular permeability. 20 mg/kg Evans Blue was i.v. administered 20 min before the skin secretion (0.8, 2.4, and 7.2 μ g of protein). Vascular permeability was quantified after the peritoneal cavity was washed. Results are expressed in μ g of Evans Blue/mL. Each point represents mean \pm SEM. * $p < 0.05$ compared with the control group ($n = 5$).

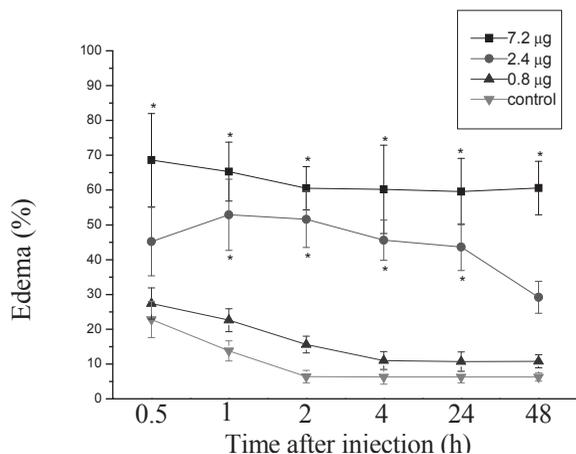


Fig. 3. Estimation of edema-inducing activity. The skin secretion (0.8, 2.4, and 7.2 µg of protein) was injected (i.h.) into the right footpad of mice. Results are expressed as the differences of footpad thickness between experimental and control groups. Each point represents mean \pm SEM. * $p < 0.05$ compared with the control group ($n = 5$).

Electrophoretic protein profile

The electrophoretic profile of the skin secretion revealed numerous proteins with a wide spectrum of molecular masses (Fig. 5).

Discussion

The discovery of toxins in the secretion of animals, especially amphibians, have attracted much attention because of their extremely complex and unique action on the physiology of various mammals (Lewis and Garcia, 2003). This complexity is related to the function of the skin secretion in the defence against various predators. We describe here the toxicity of the crude skin secretion of the Chinese giant salamander for the first time.

The i.p. LD₅₀ value of the skin secretion was 32.95 mg/kg body weight, which was higher than that of *Tylotriton verrucosus* (11.15 mg/kg body weight), *Bombina maxima* (18.18 mg/kg body weight) (Lai *et al.*, 2002), and *Naja naja* venoms (0.565 mg/kg body weight) (Broad *et al.*, 1979), respectively. After incubation at 100 °C for 10 min, the skin secretion had lost its ability to cause mortality in mice, indicating thermolability of the lethal components in the secretion. The anatomical investigation of treated mice revealed that only the lungs had been altered clearly. The hyperemia of the lungs and the convulsion observed in the

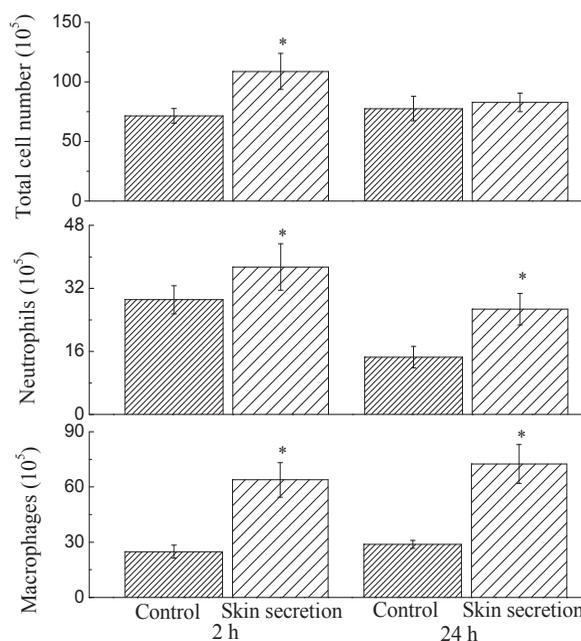


Fig. 4. Effect of the skin secretion on leukocyte recruitment to footpad. Animals were executed 2 and 24 h after the injection of skin secretion (7.2 µg protein in 30 µL PBS). Footpad tissue was processed for cell count. Mice only injected with PBS were the control group. The result represents mean \pm SEM. * $p < 0.05$ compared with the control group ($n = 5$).

mice could contribute to the mortality, and it appears to be proteins that cause the mice's death. The bioactive proteins in the secretion can cause local or systemic physiopathological symptoms in mice. So, different doses (0.8, 2.4, and 7.2 µg of protein) of the skin secretion were injected into the mice to evaluate their effect on nociception, edema, and vascular permeability.

An immediate transient nociceptive response is caused by the release of inflammatory mediators. When the secretion protein Bv8, isolated from *Bombina variegata* skin, was injected into mice, rapid changes occurred in the primary afferent of nociceptive fibers, resulting in an immediate nociceptive response (Negri *et al.*, 2002). In our experiments, the doses of 2.4 and 7.2 µg protein of

Table I. Biological activities of Chinese giant salamander skin secretion.

Biological activity	Value
Proteolytic activity	(2.32 \pm 0.26) U/mg
Phospholipase A ₂ activity	(0.38 \pm 0.04) nmol chromophore released/(min mg)

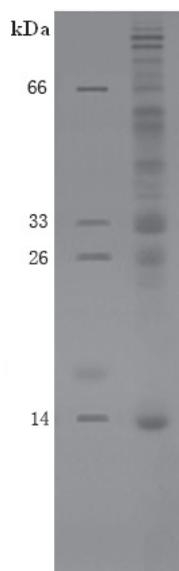


Fig. 5. SDS-PAGE of proteins in Chinese giant salamander skin secretion. Left lane, molecular mass of marker proteins.

the skin secretion induced significant nociception activity in mice.

Edema formation is a common feature in cutaneous inflammatory processes and is dependent on a synergistic reaction between mediators that increase vascular permeability and those that increase blood flow (Brain and Williams, 1985). The local edema induced by the skin secretion was characterized by a rapid onset and a plateau at 30 min. After this, the degree of edema was positively correlated with the dose. The rapid onset of the edematogenic response may also suggest a neurogenic response. An edema is mainly produced by vasodilator peptides released from nociceptors activated by acute noxious stimuli. Neutrophils are recruited rapidly into the sites of acute infection and dominate the initial influx of leukocytes (Issekutz and Movat, 1980). The present work showed that the skin secretion induced the increment of cell recruitment. Later in inflammation, monocytes and macrophages replaced neutrophils and became the predominant leukocytes, suggesting a bimodal recruitment pattern involving a switch from neutrophils to monocytes.

The skin secretion did not possess significant hemorrhagic activity. This result implies that there may be no metalloproteinase hemorrhagin-like compounds in the skin secretion, which is differ-

ent from snake venoms (Gutierrez and Rucavado, 2000).

The detection of phospholipase A_2 activity [(0.38 ± 0.04) nmol chromophore released/(min mg)] in the skin secretion is noteworthy. The secretion of the tree frog *Phyllomedusa hypochondrialis* contains comparable phospholipase A_2 activity [(4.78 ± 0.21) nmol chromophore released/(min mg)] (Conceicao *et al.*, 2007) and also the secretion of *Tylotriton verrucosus* exhibits phospholipase A_2 activity (no quantitative data available) (Lai *et al.*, 2002). Phospholipase A_2 is involved in inflammation, host defence, and several inflammatory diseases, and is specifically distributed in several tissues, suggesting that it may play a role in a number of fundamental physiological processes.

The skin secretion possesses proteolytic activity [(2.32 ± 0.26) U/mg] on casein, a widely used unspecific substrate that can be degraded by both serine proteinases and metalloproteinases. Proteolytic activity may be responsible for the generation of the famous bioactive peptides with antibacterial activity on the amphibian skin (Giangaspero *et al.*, 2001).

The molecular masses of the proteins in the skin secretion of the Chinese giant salamander are distributed over a wide range, while those of the *P. hypochondrialis* skin secretion are above 68 kDa, around 25 kDa, and below 14 kDa (Conceicao *et al.*, 2007).

In conclusion, we propose that the biochemical and pharmacological changes observed in mice were probably due to the injected various proteinaceous and peptidic toxins in the crude skin secretion of the Chinese giant salamander. We have studied the biological activities induced by the skin secretion of the Chinese giant salamander in experimental animals for the first time. The isolation and characterization of the proteins responsible for these effects in the skin secretion are a relevant task and are currently in progress in our laboratory.

Acknowledgements

The authors thank the Jinni Bio-Tech Co., Ltd. for providing Chinese giant salamander crude skin secretion. This work was supported by Special Grant of Chinese giant salamander biological breeding and high-tech industrialization from National Development and Reform Commission in 2007 (2409).

- Apponyi M. A., Pukala T. L., Brinkworth C. S., Maselli V. M., Bowie J. H., Tyler M. J., Booker G. W., Wallace J. C., Carver J. A., Separovic F., Doyle J., and Llewellyn L. E. (2004), Host-defence peptides of Australian anurans: structure, mechanism of action and evolutionary significance. *Peptides* **25**, 1035–1054.
- Barra D. and Simmaco M. (1995), Amphibian skin: a promising resource for antimicrobial peptides. *Trends Biotechnol.* **13**, 205–209.
- Bevins C. L. and Zasloff M. (1990), Peptides from frog skin. *Annu. Rev. Biochem.* **59**, 395–414.
- Brain S. D. and Williams T. J. (1985), Inflammatory mechanism of inflamed-tissue factor. *Agents Actions* **3**, 348–356.
- Broad A. J., Sutherland S. K., and Coulter A. R. (1979), The lethality in mice of dangerous Australian and other snake venoms. *Toxicon* **17**, 661–664.
- Clarke B. T. (1997), The natural history of amphibian skin secretion, their normal functioning and potential medical applications. *Biol. Rev.* **72**, 365–379.
- Conceicao K., Miriane B. F., Antoniazzi M. M., Jared C., Camargo A. C., Lopes-Ferreira M., and Pimenta D. C. (2007), Major biological effects induced by the skin secretion of the tree frog *Phyllomedusa hypochondrialis*. *Toxicon* **49**, 625–633.
- Daly J. W., Spande T. F., and Garraffo H. M. (2005), Alkaloids from amphibian skin: a tabulation of over eight-hundred compounds. *J. Nat. Prod.* **68**, 1556–1575.
- Giangaspero A., Sandri L., and Tossi A. (2001), Amphipathic alpha helical antimicrobial peptides. *Eur. J. Biochem.* **268**, 5589–5600.
- Gutierrez J. M. and Rucavado A. (2000), Snake venom metalloproteinases: their role in the pathogenesis of local tissue damage. *Biochimie* **82**, 841–850.
- Holzer M. and Mackessy S. P. (1996), An aqueous endpoint assay of snake venom phospholipase A2. *Toxicon* **34**, 1149–1155.
- Hunskar S., Fasmer O. B., and Hole K. (1985), Formalin test in mice, a useful technique for evaluating mild analgesics. *J. Neurosci. Methods* **14**, 69–76.
- Issekutz A. C. and Movat H. Z. (1980), The *in vivo* quantitation and kinetics of rabbit neutrophil leukocyte accumulation in the skin in response to chemotactic agents and *Escherichia coli*. *Lab. Invest.* **42**, 310–317.
- Katsu Y., Kohno S., and Oka T. (2006), Molecular cloning of estrogen receptor alpha (ER alpha; ESR1) of the Japanese giant salamander, *Andrias japonicus*. *Mol. Cell. Endocrinol.* **257**, 84–94.
- Laemmli U. K. (1970), Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680–685.
- Lai R., Zhao Y., Yang D. G., Zha H. G., Lee W. H., and Zhang Y. (2002), Comparative study of the biological activities of the skin secretion from six common Chinese amphibians. *Zool. Res.* **23**, 113–119.
- Lewis R. J. and Garcia M. L. (2003), Therapeutic potential of venom peptides. *Nat. Rev. Drug Discov.* **2**, 790–802.
- Li S. Z. (1978), The Ming Dynasty of China. Compendium of Materia Medica. People's Health Publishing House, Beijing, p. 376.
- Lima C., Clissa P. B., Piran-Soares A. A., Tanjoni I., Mourada Silva A. A., and Lopes-Ferreira M. (2003), Characterization of local inflammatory response induced by *Thalassophryne nattereri* fish venom in a mouse model of tissue injury. *Toxicon* **42**, 499–507.
- Matsui M., Tominaga A., and Liu W. Z. (2008), Reduced genetic variation in the Japanese giant salamander. *Mol. Phylogenet. Evol.* **49**, 318–326.
- Meier J. and Theakston R. D. (1986), Approximate LD₅₀ determinations of snake venoms using eight to ten experimental animals. *Toxicon* **24**, 395–401.
- Menezes M. C., Furtado M. F., Travaglia-Cardoso S. R., Camargo A. C., and Serrano S. M. (2006), Sex-based individual variation of snake venom proteome among eighteen *Bothrops jararaca* siblings. *Toxicon* **47**, 304–312.
- Murphy R. W., Fu J. Z., and Upton D. E. (2000), Genetic variability among endangered Chinese giant salamanders, *Andrias davidianus*. *Mol. Ecol.* **9**, 1539–1547.
- Negri L., Lattanzi R., Giannini E., Metere A., Colucci M., Barra D., Kreil G., and Melchiorri P. (2002), Nociceptive sensitization by the secretory protein Bv8. *Brit. J. Pharmacol.* **137**, 1147–1154.
- Owby C. L., Bjarnason J., and Tu A. T. (1978), Hemorrhagic toxins from rattlesnake (*Crotalus atrox*) venom. Pathogenesis of hemorrhage induced by three purified toxins. *Am. J. Pathol.* **93**, 201–218.
- Simmaco M., Mignogna G., and Barra D. (1998), Antimicrobial peptides from amphibian skin: what do they tell us? *Biopolymers* **47**, 435–450.
- Sirois M. G., Jancar S., Braquet P., Plante G. E., and Sirois P. (1988), PAF increases vascular permeability in selected tissues: effect of BN-52021 and L-655,240. *Prostaglandins* **36**, 631–644.
- Stebbins R. C. and Cohen N. W. (1995), A natural history of amphibians. Princeton University Press, New Jersey.
- Toledo R. C. and Jared C. (1993), Cutaneous adaptations to water balance in amphibians. *Comp. Biochem. Physiol. A: Mol. Integr. Physiol.* **105**, 593–608.
- Tyler M. J., Stone D. J., and Bowie J. H. (1992), A novel method for the release and collection of dermal glandular secretions from the skin of frogs. *J. Pharmacol. Toxicol. Methods* **28**, 199–200.
- Woodley S. K. (2010), Pheromonal communication in amphibians. *J. Comp. Physiol. A. Neuroethol. Sens. Neural. Behav. Physiol.* **196**, 713–727.
- Yang L. P., Meng Z. N., and Liu Y. (2010), Growth hormone and prolactin in *Andrias davidianus*: cDNA cloning, tissue distribution and phylogenetic analysis. *Gen. Comp. Endocrinol.* **165**, 177–180.
- Zhang P., Chen Y. Q., Liu Y. F., Zhou H., and Qu L. H. (2003), The complete mitochondrial genome of the Chinese giant salamander, *Andrias davidianus* (Amphibia: Caudata). *Gene* **311**, 93–98.