
Alligators and Frogs: A More Pharmacovigilant Source of Drugs for Multiple Resistant Microorganisms and Other Bodily Diseases

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ABSTRACT

Frogs and alligators promise to provide an alternative source of newer drugs in the community of drug discoverers and developers. It is of a great importance to note that some of the alkaloids found in herbal plants can also be found in animals, or at least similar groups of compounds. There is therefore need to concert efforts in research on animal venoms, secretions and other peptides that have the potential to develop more pharmacovigilant drugs against multi drug resistant microorganisms and other bodily disorders. The effort are highly required in both drug structure activity relationships as well as structure property relationships in order to determine bioavailability predictions at earlier stages of drug discovery. Besides pharmacognosy, this paper urges researchers to also invest in animal secretions for potential drug development.

KEYWORDS

Animal secretions; Disease management; Drug development; Drug discovery; Frog venom; Lizard venom.

1. Introduction

Humans use animals and animal parts for medicinal purposes. Anurans (frogs and toads) are one of the animals used in the ancient traditional medicine. The Chinese have traditionally used frog skin and secretions from toad parotid glands to regulate internal corporal functions and fertility or as a treatment for dog bites. Amphibian skins from the genus *Rana* was used during the Vietnam War in 1960s to treat burns [1]. Amphibians living environments are full of various kinds of microorganisms, have developed a unique survival strategy for their own protection from potential pathogens [2]. The dermatous exocrine glands of many species of Anura (frogs and toads) are capable of synthesizing substantial quantities of a rich variety of hormones and neuropeptides. This includes thyrotropin-releasing hormone, bradykinins, tachykinins, angiotensins, bombesin/gastrin-releasing peptides, xenopsin, crinia-angiotensin, and some D-amino acid containing opioids, dermorphin and deltorphins. Anura glands release their contents, including antimicrobial

peptides (AMPs) onto the surface of the skin through a holocrine mechanism. glands [3]. The antimicrobial peptides are specifically secreted by nonlymphoid cells on the mucosal surfaces of the gastrointestinal and respiratory tracts, and by the granular glands of the skin [1].

Studies have shown that antimicrobial peptides synthesized in the skins of certain frogs represent a promising source of potential therapeutic agents. For instance, a compound effective against *Staphylococcus aureus* and against viruses that are resistant to antibiotics was discovered from a frog species of the genus *Rana* [1].

Peptides From Frogs and Their Medicinal Use

2. Frog Skin Antimicrobial Peptides

Antimicrobial peptides from frog skin vary in size from as small as 8 to as much as 48 amino acid residues. These peptides are generally cationic ($Z=+2$ up to $+6$; at pH 7) due to the presence of multiple lysine residues, and they contain at least 50% hydrophobic amino acids of which isoleucine and leucine are usually the most abundant [4]. This cationic nature allows some peptides to actually penetrate the surface of pathogens and mortify them [5].

According to Kumar et al., the genus *Amolops* (family: Ranidae), is endemic to China. Eight species of frogs from this genus have been studied and their peptides isolated. The most common peptides isolated are of the Esculentin 2, Brevinin 1 and 2, and Palustrin 2. All of them have been shown to be active against fungi, gram-negative and gram-positive bacteria, and cancer cells. The *Clinotarsus* genus is endemic to the Western Ghats of India where new peptides are being discovered. The peptidomic approach revealed the presence of five novel peptide amides homologous to the Brevinin 1 family from *C. curtipes*. All of the five showed promising effective pharmacological activities against gram-negative and gram-positive bacteria [5]. Studies conducted in Turkey indicated that crude skin-parotid gland secretions of *B. variabilis*, *B. bufo* and *B. verrucosissimus* demonstrated significant cytotoxic activities against both cancer cell lines and non-cancerous cell lines. This requires optimization to avoid killing or injuring non-cancerous cells. Skin secretions from these species also showed the most potent activity against *S. epidermidis*, *E. faecium* and *E. faecalis* where as skin secretions of *B. verrucosissimus* and *B. bufo* alone showed moderate antibacterial activity against *S. aureus* [6].

Temporin-DRa

Temporin-DRa is a dodecapeptide which was first isolated from skin secretions of *Rana draytonii*, a Californian redlegged frog. It showed potential for the treatment of infections produced by methicillin resistant strains of *Staphylococcus aureus* (MRSA) [7]. Despite their low cationicity and small sizes, some temporins (Tb, Ta, Tl, Tf, SHd and SHa) were also able to kill *Leishmania*, a human parasitic protozoan, which is the causative agent of leishmaniasis [3, 7].

Brevinin-2-Related Peptide

Brevinin-2-related peptide (B2RP) was first isolated from the mink frog of North America, *Lithobates septentrionalis*. They represent peptides with therapeutic potential for the treatment of infections produced by multidrug-resistant strains of *Acinetobacter baumannii* (MDRAB) [8].

Ascaphin-8

Ascaphin-8 is a cationic α -helical peptide isolated from skin secretions of *Ascaphus truei*, a tailed frog with broad-spectrum antibacterial activities. The peptide showed potential for the treatment of infections produced by β -lactamase-producing microorganisms [9].

Pseudin-2

A 24 amino-acid-residue antimicrobial peptide, Pseudin-2, was first isolated from the skin of *Pseudis paradoxa*, a South American paradoxical frog. It showed potential for the treatment of infections caused by extended spectrum beta lactam-producing Gram-negative bacteria, predominantly *E. coli* [10].

Brevinin-1BYa

Brevinin-1BYa is a naturally occurring cationic α -helical peptide with an intramolecular disulphide bridge first isolated from skin secretions of *Rana boylei*, the foot hill yellowlegged frog. The peptide showed potential for the treatment of infections caused by azole-resistant *Candida* spp [11].

Brevinin-2PRa

Brevinin-2PRa was isolated from an extract of the skin of the Hokkaido frog, *Rana pirica* and is a candidate for development into an anti-infective agent for use against antibiotic-resistant *Pseudomonas aeruginosa*. The peptide was also active against reference strains of other Gram-negative (*E. coli*, *Enterobacter cloacae*, and *K. pneumoniae*) and Gram positive (*S. aureus*, *S. epidermidis*) bacteria [12, 13].

The mode of antimicrobial action of most of the frog skin AMPs is believed to be the disruption or permeation of lipid plasma membranes of target cells through binding of peptides that are cationic in nature to the outer leaflet of bacterial bilayers. The high content of anionic lipids in prokaryotic membranes and their absence from the neutral matrix of erythrocytes account for the preferential binding of cationic peptides to bacterial membranes through long-range electrostatic interactions [3].

Bufadienolides

Toad venoms are found in skin secretions and have been isolated mainly from parotoid glands of species from the Bufonidae family. Bufadienolides are composed of one of the most interesting groups of bioactive substances from secretions of amphibians. They are also found in reptiles such as the Asian snake *Rhabdophis tigrinus* as well as in several plant families. Bufadienolides are usually characterized by the presence of a 14-hydroxyl group. Some bufadienolides have been pharmacologically assayed in various in-vitro and in-vivo models. They have shown anticancer and antiproliferative activity. The most relevant pharmacological effect for these steroids is their specific inhibition of Na^+/K^+ -ATPase activity [14].

Guanidine Alkaloids

Guanidine alkaloids have been found in the skin of frogs of the genus *Atelopus*. Although their physiological role is unknown in *Atelopus* frogs, they are thought to be defensive molecules against predators. Guanidine alkaloids in *Atelopus* are water soluble toxins that present some guanidine and hydroxyl groups in their structures. The alkaloids are better extracted with an acidic solution rather than water, suggesting that these toxins could be present as bound precursors in oocytes and skin of *Atelopus*. They possess neurotropic and myotropic activities. Guanidine alkaloids from *Atelopus* act as blockers of voltage-active sodium channels (Nav) [13].

Indole Alkaloids

The family Bufonidae represents a rich source of indole alkaloids. These alkaloids are found in the genera; Bufo, Ansonia stoliczka, Duttaphrynus, Bufotes rafinesque, Epidalea Cope and Incilius cope. Indoles get easily extracted by polar solvents, such as the smallest primary alcohols, ethanol or methanol. In fact, acetone is the best organic solvent for preparing a toad extract to obtain indole alkaloids [13].

The alkaloids have general anti cancer and antiviral activities whose mechanisms of action for the later is apparently by inhibiting the viral infections through competition for appropriate nicotinic acetylcholine receptors[13]

Huachansu (Cinobufacini)

It is one of the most widely-used commercial preparations which are sterilized hot water extracts of dried toad skin secretions. Cinobufacini, officially approved in 1991 by the Chinese Food and Drug Administration as a regimen for treating patients with Hepatitis B virus and several types of cancers including lung, liver, pancreatic and colon cancers. An in-vivo assay showed that Cinobufacini injections inhibit the growing of mouse Lewis lung cancer cells with the response rate of between 45 and 50% and prolong their life [6].

3. Methods of Collection of Secretions from Frogs

Three methods are used for the collection of the secretions: electrical stimulation, chemical stimulation and skin harvesting. In electrical stimulation skin secretions are obtained by inducing mild electrical stimulation. Secretions are thoroughly washed from the skin surface with distilled water, collected in a beaker and then lyophilized. Chemical stimulation is widely applied either by exposing the frog to irritant chemicals or by the physiological stimulation of the parasympathetic nervous system. In physiological stimulation, norepinephrine is injected to induce secretion. Chemical irritant technique has been successfully applied and appears to be the least complex and least invasive method. Several frogs are put into a cylinder containing a piece of absorbent cotton highly saturated with ether (anhydrous). Following exposure to the ether for 1 to 2 mins, the frogs' skins produces copious secretions which are then collected by washing the back region of each animal with an appropriate buffer solution. The secretions are obtained through homogenization and cleaned up using solid phase extractions. Skin harvesting involves sacrificing the frogs and then excising their skins [2, 3, 14, 15].

4. Drug /Peptides Derived from Lizards

Byetta®

Byetta®, a synthetic exenatide, was approved in 2005 as the first class of a new type of agent for treating type2 diabetes mellitus. They were obtained from venom of the American poisonous lizard, the Gila monster, Heloderma suspectum. Their venom is produced by a number of perimandibular glands that secrete onto grooved teeth in the lower jaws [16, 17]. In this species, venoms contain exendin-3 and exendin-4, which are responsible for the activation of adenylyl cyclase. Exendin-4 reduced fasting and postprandial blood glucose in healthy volunteers where the peptide was shown to reduce blood glucose and improve b-cell sensitivity to glucose when administered subcutaneously twice a day, in one month to type 2 diabetes mellitus patients [17-19].

Exendin-4 inhibits glucagon secretion, stimulates insulin synthesis, protects against b-cell apoptosis in response to different insults and promotes b-cell proliferation. GlucagonLike Peptide-1 Receptor Agonist. In normal animals and in experimental models of obesity it mimics GLP-1 in promoting satiety, reducing food intake, reducing fat deposition and reducing body weight [17, 20]. Exendin-4 also inhibits gastric emptying [17, 21]. For a very long time, exendin-4 has been synthesized chemically at a high price and is therefore

unsuitably too costly for mass production. It therefore needs to be produced in quantities by genetic recombinant technology or modified cheaper-to-synthesize analogues. Exendin-4 has been successfully expressed in *E. coli* systems and it proved having glucose-lowering action in-vivo.

Alligators

Alligator *mississippiensis* (American alligator), a member of order Crocodylia, dwells in bacteria-laden environments but is not known to yield to bacterial infections. Their serum has been demonstrated to have anti-bacterial activities much better than that of human serum, and it is considered that this activity is partially due to cationic antimicrobial peptides (CAMPs). CAMPs are small, hydrophobic proteins produced by most eukaryotic organisms as part of the immune system. Alligator cathelicidins and related peptides have shown stronger activities against Gram-negative bacteria than Gram-positive ones. AM-CATH36 was found to be the most effective peptide against most Gram-negative bacteria such as *K. pneumoniae*, *A. baumannii*, and *P. aeruginosa*. AM-CATH28 and AM-CATH21 was less effective against *A. baumannii* but was more effective against *P. aeruginosa* and *K. pneumoniae* [22]. Apo5 and Apo6 were novel peptides discovered from plasma of alligator *mississippiensis*. They were reported to have in vitro antimicrobial activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus* [23].

5. Conclusion

Frogs and alligators promise to provide an alternative source of newer drugs in the community of drug discoverers and developers. It is of a great importance to note that some of the alkaloids found in herbal plants can also be found in animals, or at least similar groups of compounds. There is therefore need to concert efforts in research on animal venoms, secretions and other peptides that have the potential to develop more pharmacovigilant drugs against multi drug resistant microorganisms and other bodily disorders. The effort are highly required in both drug structure activity relationships as well as structure property relationships in order to determine bioavailability predictions at earlier stages of drug discovery. Besides pharmacognosy, this paper urges researchers to also invest in animal secretions for potential drug development.

References

- Park P, Wang TT, Kim E, Zhong-Ji Q. "Nutraceuticals," Recent Advances of Bioactive Food Components. Journal of Chemistry, 2016:1-2.
- Chen X, Wang H, Wang L, Zhou M, Chen T, et al. "Identification of Miscellaneous Peptides from the Skin Secretion of the European Edible Frog, *Pelophylax kl. Esculentus*," Protein J, 2016, 35:291-299.
- Ladram A, Nicolas P. "Antimicrobial peptides from frog skin: biodiversity and therapeutic promises," Front Biosci (Landmark Ed), 2016, 21:1341-1371.
- Rajasekaran K, Cary JW, Jaynes JM, Montesinos E, "In Small Wonders: Peptides for Disease Control; ACS Symposium Series; American Chemical Society: Washington, 2012.
- Kumar VT, Holthausen D, Jacob J, George S, "Host Defense Peptides from Asian Frogs as Potential Clinical Therapies," Antibiotics (Basel), 2015, 4:136-159.
- Nalbantsoy A, Karis M, Yalcin HT, Gocmen B, "Biological activities of skin and parotoid gland secretions of bufonid toads (*Bufo bufo*, *Bufo verrucosissimus* and *Bufoes variabilis*) from Turkey," Biomed Pharmacother, 2016, 80:298-303.
- Popovic S, Urbán E, Lukic M, Conlon JM, "Peptides with antimicrobial and anti-inflammatory activities that have therapeutic potential for treatment of acne vulgaris.," Peptides, 2012, 34:275-282.
- Conlon JM, Ahmed E, Condamine E, Condamine, "Antimicrobial properties of brevinin-2-related peptide and its analogs: Efficacy against multidrug-resistant *Acinetobacter baumannii*," Chem Biol Drug Des, 2009, 74:488-493.

- Eley A, Ibrahim M, Kurdi SE, Conlon JM, "Activities of the frog skin peptide, ascaphin-8 and its lysine-substituted analogs against clinical isolates of extended-spectrum beta-lactamase (ESBL) producing bacteria," *Peptides*, 2008, 29:25-30.
- Jeon D, Jeong MC, Jacob B, Bang JK, Kim EH, et al. "Investigation of cationicity and structure of pseudin-2 analogues for enhanced bacterial selectivity and anti-inflammatory activity," *Scientific Reports*, 2017, 7:1455.
- Pál T, Abraham B, Sonnevend A, Jumaa P, Conlon JM. "Brevinin-1BYa: a naturally occurring peptide from frog skin with broad-spectrum antibacterial and antifungal properties.," *Int J Antimicrob Agents*, 2006, 27:525-529.
- Sarin R, Manisha S, Singh R, Kumar S. "Nutraceuticals: A review," *International Research Journal of pharmacy*, 2012, 3:95-99.
- Rodríguez C, Rollins-Smith L, Ibáñez R, DurantArchibold AA, Gutiérrez M, Durant-Archibold, and M. Gutierrez, "Toxins and pharmacologically active compounds from species of the family Bufonidae (Amphibia, Anura)," *J Ethnopharmacol*, 2017, 198:235-254.
- Perera Córdova WH, Leitão SG, Cunha-Filho G, Bosch RA, Alonso IP, "Bufadienolides from parotoid gland secretions of Cuban toad *Peltophryne fustiger* (Bufonidae): Inhibition of human kidney Na(+)/K(+)ATPase activity," *Toxicon*, 2016, 110: 27-34.
- Govender T, Dawood A, Esterhuysen A, Katerere D, "Antimicrobial properties of the skin secretions of frogs." *S Afr J Sc*, 2012, 108:1-6.
- Raufman JP, "Bioactive peptides from lizard venoms," *Regul Pept*, 1996, 61:1-18.
- Furman B, "The development of Byetta (exenatide) from the venom of the Gila monster as an anti-diabetic agent," *Toxicol*, 2012, 59:464-471.
- Edwards CM, Stanley SA, Davis R, Brynes AE, Frost GS, et al. "Exendin-4 reduces fasting and postprandial glucose and decreases energy intake in healthy volunteers," *Am J Physiol Endocrinol Metab*, 2001, 281:E155-E161.
- Kolterman OG, Buse JB, Fineman MS, Gaines E, Heintz S, et al. "Synthetic exendin-4 (exenatide) significantly reduces postprandial and fasting plasma glucose in subjects with type 2 diabetes," *J Clin Endocrinol Metab*, 2003, 88:3082-3089.
- Silvestre RA, Rodríguez-Gallardo J, Egidio EM, Marco J, "Interrelationship among insulin, glucagon and somatostatin secretory responses to exendin-4 in the perfused rat pancreas," *Eur J Pharmacol*, 2003, 469:195-200.
- Szayna M, Doyle ME, Betkey JA, Holloway HW, Spencer RG, et al. "Exendin-4 decelerates food intake, weight gain, and fat deposition in Zucker rats," *Endocrinology*, 2000, 141:1936-1941.
- Barksdale SM, Hrifko EJ, van Hoek ML, "Cathelicidin antimicrobial peptide from *Alligator mississippiensis* has antibacterial activity against multidrug resistant *Acinetobacter baumannii* and *Klebsiella pneumoniae*," *Dev Comp Immunol*, 2017, 70:135-144.

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