JGI Journal of Global Innovation

BIOprospectum Open access publishing

Mini-Review

Bioprospection of antimicrobial peptides extracted from amphibians: applicability and pharmacological innovation

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Received: 07 Feb 2019; Accepted: 30 May 2020; Published: 28 June 2020.

Abstract: Background: The antimicrobial peptides (AMPs) are mostly derived from the skin secretion of amphibians, which stand out for presenting a wide range of activities against bacteria, fungi, viruses and protozoa. The present study aimed to catalogue the applicability of bioprospection of antimicrobial peptides extracted from amphibians for the development of new drugs in terms of pharmacological innovation; 2) Methods: this is an integrative review, with descriptive and cross-sectional content. The study was carried out by searching scientific articles, in the following databases: BVS, SciELO, Lilacs, Pubmed and Medline. The articles included in this study approached bioprospecting and pharmacological innovation aspects, pointing out to specific information about peptides found on the skin secretion of amphibians; 3) Results: The first reported study about AMPs from South America enabled the discovery of four new peptides identified from the skin of the Patagonian frog *Pleurodema thaul*, one of these denominated Thaulin-I, and its main antimicrobial activity was observed against *Escherichia coli*. The antiviral activity of the AMPs was observed by the study that demonstrated the action of Dermaseptin 01 (DS-01) against *Dengue virus* type 2, *Herpes simplex* virus type I and *Vaccinia virus*; 4) Conclusions: Consequently, it is possible to infer the eminent possibility of bioprospection of antimicrobial peptides extracted from the skin secretions of several species of amphibians and their applicability to research and innovation in health.

Key words: Antimicrobial activity; Amphibians; Bioprospecting; Innovation

1. Introduction

Increasing studies for knowing and/or discovering natural occurring antimicrobial peptides, with undoubtedly ancient origins in the biosphere, provide a possible way to search the objective of discovering or developing new anti-infective therapies. More than 500 different antimicrobial peptides (AMPs) have been reported from several natural sources and, most part of the ones found in the online databases come from the skin of amphibians. One of the most important attributes of these AMPs from the skin of amphibians is their wide range of activities against bacteria, fungi, viruses and protozoa. Skin secretions of amphibians tend to be one of the richest natural sources of different antimicrobial peptides. These peptides showed efficiency against several Gram-negative and Gram-positive bacteria, fungi, protozoa and some viruses, including HIV. AMPs are now known to form a component of the innate immunity, which is the molecular defence in most part of life forms. They are meant to be particularly abundant in the skin secretion of amphibians as a defence against surface microbial colonization, a consequence derived from the fact that both species are common in the same biotopes [1].

The peptidome analysis of the skin secretions from different amphibians has showed to be valuable in the identification of components with potential for the development of antimicrobial products to be used against

drug resistant bacteria and fungi, and also with anticancer and antiviral properties. Besides, peptides identified for the first time based on their cytotoxic activity, showed, afterwards, immunomodulator effect and antidiabetic activity [2]. The present study aimed to catalogue, with the aid of an Integrative literature review, the importance of the bioprospection of antimicrobial peptides extracted from amphibians, as well as the relevant aspects from their applicability for research and innovation in health.

2. Materials and Methods

This study is an integrative review. According to Brehmer [3], an integrative review is a study in which published researches are summarized and provide general conclusions about the topic of interest. Integrative review studies favour the access to the main results of researches and represent the construction of a critical knowledge.

According to Mendes et al. [11], the literature integrative review is one of the research methods that permit the incorporation of the evidences in the clinical practice. This method aims to reunite and summarize the results of researches about a determined theme or question, in a systematic and orderly manner, contributing to deepen the knowledge about the investigated topic. The study was performed by the search of scientific articles in databases accessed by the Virtual Libraries in Public Health. Data collection was carried out in October, 2018. The following uncontrolled descriptors, and their combinations in Portuguese and English, were used: "Peptides", "Amphibians", "Bioprospection" and "Antimicrobial".

The inclusion criteria for the selection of the articles were defined as follows: articles published in Portuguese, English; full articles depicting the topic, articles published and indexed in the database of the Virtual Library in Public Health. After the selection of the articles in the abovementioned sources, the analysis, interpretation and organization of the results were conducted in tabular form, which presents information referring to the title of the article, journal where the article was published and year of publication.

3. Results and discussion

Nowadays, many studies about peptides extracted from the skin secretion of amphibians have been published. **Table I** reports some of these researches. The study carried out by Assis [1], about microbiota and cutaneous secretions of amphibians, highlights that these animals have some mechanisms that grant more resistance and permanence in their natural habits.

Table I – Peptides Secreted by Amphibians.

ANTIMICROBIAL PEPTIDES				
Peptide Name	***Peptide Sequence***	***MIC***	***Strain***	***Author Name***
Tigerinin	RTCIPIPLVMC (N-terminal)	MIC > 100 µM	*Escherichia coli*	McLaughlin CM *et al.*
Thaulin-1	NGNLLGGLLRPVLGVVKGLTGGLGKK (C-terminal)	MIC ≥ 24,7 µM	*Escherichia coli*	Mariani MM *et al.*
Cruzioseptins-1	GFLDIVKGVGKVALGAVSKLF (C-terminal)	MIC ≥ 3,77 µM	*Staphylococcus aureus* *Candida albicans*	Proaño-Bolaños C *et al.*
Balteatide*	LRPAILVRIK (C-terminal)	MIC ≥ 109 µM MIC ≥ 27 µM	*Escherichia coli* *Candida albicans*	Lilin Ge *et al.*
Dermaseptin 01	ALWKTMLKKLGTMALHAGKGAIUIDTISQGTQ (N-terminal)	MIC ≥ 21,5 µM**	Dengue (DENV-02)	Cardoso JLMS *et al.*
Kunitzin-RE	AAKIILNPKFRCKAAFC (N-terminal)	MIC > 30 µM	*Escherichia coli*	Chen X *et al.*
Phylloseptin-1	FLSLIPHAINAVSAIAKHN (C-terminal)	MIC of 3,0 - 7,9 µM	*Staphylococcus aureus* *Enterecoccus faecalis* *Escherichia coli* *Pseudomonas aeruginosa*	Leite JRSA *et al.*

*Even peptide (Balteatide) with different activity at different concentrations.
**Concentration (EC50) of 60 µg / mL (21.5 µM) in LLCMK2 cells for anti-viral activity (DENV-02).

pathogenic microorganisms is found on the skin. In addition to the mechanical components, the skin protection includes biochemical and biological mechanisms, derived from the secretion of bioactive molecules by the dermal glands, as well as the resident microbial community, respectively.

It is also worth to notice that several species seem not to have an AMP system or express these AMPs with null or very weak antimicrobial activity against human pathogenic bacteria. However, when submitted to tests, the same peptides showed strong antimicrobial properties against specific microorganisms for the species, showing an adaptation to the living environment. Marani et al. [9] developed a study of identification and characterization of AMPs obtained from the skin of *Pleurodema taul*, an amphibian that belongs to the *Leptodactylidae* family and *Leiuperinae* Subfamily, by isolating the mRNA, cloning the cDNA and sequencing. They reported the discovery of four new peptides identified in the skin secretion of the Patagonian frog *P. thaul*. This represents the first reported study about amphibians AMPs on this great region of South America. A meticulous characterization of thaulin-I was performed. Thaulin-I is a new amphipathic, cationic peptide, rich in Gly and Leu with 26 residues, with alpha-helix secondary structure. Its main antimicrobial activity is against *E. coli*. It is important to mention that thaulin-I not only presented structural similarity with other AMPs, but also with parts of the sequence found in several transmembrane proteins [4].

Another experiment performed by Proaño-Bolaños [13] in which seven full-length and four partial length cDNAs, which codified new peptides named Cruzioseptins (CZS), were cloned from the skin secretion of *Cruziohylac alcarifer*. They found 10 to 15 Cruzioseptins with antimicrobial activity by RP-HPLC fraction screening of a *C. calcarifer* population from Costa Rica. These samples were re-chromatographed for purification and sequenced by Edman degradation. The study identified antimicrobial activity against *S. aureus* and *C. albicans* [5]. In the study performed by Ge et al. [6], the skin secretion of *Phyllomedusa baltea* was fractioned by RP-HPLC. By the MALDI-TOF analysis and other technologies, the presence of a new peptide, named betiatide was detected, reflecting its species of origin and its structural similarity with a myotropic substance, a peptide called sauvatide, from the skin secretion of *Phyllomedusas auvagei*. The author performed a comparison between both peptides, through data derived from parallel experiments for the determination of antimicrobial MIC: balteatide was moderately active against *E. coli* and sauvatide was essentially ineffective in the highest concentration tested. On the other hand, against *S. aureus*, balteatide was essentially ineffective when compared to sauvatide. The greatest difference on the antimicrobial potential was observed against *C. albicans*, where balteatide exhibited a MIC of 27 μM compared to the one obtained for sauvatide (439 μM).

The study performed by Chen et al. [5], in turn, developed experiments about prototypes of a new class of protease inhibitors from skin secretion of European and Asian frogs, denominated *kunitzins*. The isolation and structural characterization of Kunitzin-RE and kunitzin-OS from fractions obtained by RP-HPLC of the skin secretion enabled prove the relatively potent growth inhibition activity against the Gram-negative species *E. coli*, but were inefficient against *S. aureus* and the pathogenic yeast *C. albicans*.

These peptides, which have recently been discovered on the skin of several amphibians may also present antivirus effects. Cardoso et al. [6], studying the antiviral activity of dermaseptin 01 (DS 01) against *Dengue virus* type 2, *Herpes simplex* virus type I and *Vaccinia virus*, observed that the cytotoxic effect of DS 01 was stronger against the insect cell line than against mammalian cell line and was characterized by cell lysis and membrane disruption. However, how DS 01 discriminates against insect and mammalian cells is still unclear, but this distinction may happen due to differences on the membrane's constitution and structure, such as composition and concentration of phospholipids and cholesterol. Thus, the high cytotoxic activity of DS 01 against insect cells may suggest a new role for the amphibian's antimicrobial peptides.

The pathogenicity and antimicrobial resistance caused by some species of Gram-negative bacteria (*Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) are associated with a biofilm composition [12]. The ability to form called biofilm communities embedded in a matrix exopolysaccharide resistance is one of the mechanisms used by bacteria to survive in the presence of an antibiotic [2]. Studies with ocellatin-PT3, identified by secretion from *Leptodactylus pustulatus*, distinct species in the genus due to its ventral pattern of large bright red to yellow spots on a dark background, found in central Brazil in the Cerrado, in Brazilian States of Goiás, Mato Grosso, Tocantins, Pará, Maranhão, Piauí, and Ceará [10]. Ocellatin-PT3 is particularly strong, and stands out in bactericidal activity against *P. aeruginosa* in just 16 μg/mL and in the control of bacterial biofilm formation [2]. In addition to the microbicidal capability peptides shown to be promising in controlling biofilm increasing the possibility of further studies in search for new molecules to amphibians derived peptide for this purpose.

The placements and discoveries of the above-mentioned authors strengthen the possibility of bioprospecting antimicrobial peptides extracted from the secretions of different species of amphibians, as well as the applicability of these peptides for research and health innovation.

Conclusions

We assess that antimicrobial peptides derived amphibian, demonstrate a wide applicability of the health interest in the control of pathogens, but requires further studies to prospect for antimicrobial molecules seen the enormous possibility of activities described in the above articles.

Funding: This research did not receive any external funding.

Acknowledgements: The authors are grateful to José Roberto Leite and Alexandra Plácido for influencing the choice of the theme for the conduction of this Mini-Review, as well as in the correction and orientation of the study, supporting the development of the present research. This work is financed by national funds through the FCT—Fundação para a Ciência e a Tecnologia, I.P., within the project No. PTDC/BII_BIO/31158/2017. A. Plácido is a recipient of a post-doctoral grant from the same project.

Conflicts of Interest: The authors declare that there is no conflict of interest.

Abbreviations:

1. AMPs - Antimicrobial peptides; 2. HIV - Human Immunodeficiency Virus; 3. DS 01 - Dermaseptin 01; HPLC-High Performance Liquid Chromatography

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