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Review

Potential therapeutic applications of multifunctional host-defense peptides from frog skin as anti-cancer, anti-viral, immunomodulatory, and anti-diabetic agents

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ABSTRACT

Frog skin constitutes a rich source of peptides with a wide range of biological properties. These include host-defense peptides with cytotoxic activities against bacteria, fungi, protozoa, viruses, and mammalian cells. Several hundred such peptides from diverse species have been described. Although attention has been focused mainly on antimicrobial activity, the therapeutic potential of frog skin peptides as anti-infective agents remains to be realized and no compound based upon their structures has yet been adopted in clinical practice. Consequently, alternative applications are being explored. Certain naturally occurring frog skin peptides, and analogs with improved therapeutic properties, show selective cytotoxicity against tumor cells and viruses and so have potential for development into anti-cancer and anti-viral agents. Some peptides display complex cytokine-mediated immunomodulatory properties. Effects on the production of both pro-inflammatory and anti-inflammatory cytokines by peritoneal macrophages and peripheral blood mononuclear cells have been observed so that clinical applications as anti-inflammatory, immunosuppressive, and immunostimulatory agents are possible. Several frog skin peptides, first identified on the basis of antimicrobial activity, have been shown to stimulate insulin release both *in vitro* and *in vivo* and so show potential as incretin-based therapies for treatment of patients with Type 2 diabetes mellitus. This review assesses the therapeutic possibilities of peptides from frogs belonging to the Ascaphidae, Alytidae, Pipidae, Dicroglossidae, Leptodactylidae, Hylidae, and Ranidae families that complement their potential role as anti-infectives for use against multidrug-resistant microorganisms.

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1. Introduction

Skin secretions from many species of Anura (frogs and toads) contain a wide range of compounds with biological activity, often in very high concentrations, that have excited interest because of their potential for drug development. Among these substances are host-defense peptides with broad-spectrum antibacterial and antifungal activities, and the ability to permeabilize mammalian cells [16]. These peptides vary in size from as small as 8 up to 48 amino acid residues, and a comparison of their amino acid sequences reveals the lack of any conserved domains that are associated with cytotoxic activity. However, with few exceptions, these peptides are cationic (charge between +1 and +6 at pH 7) and contain between 40 and 70% hydrophobic amino acids [71]. Circular dichroism and nuclear magnetic resonance (NMR) studies have shown that they generally lack stable secondary structure in aqueous solutions but have the propensity to form an amphipathic α-helix in the environment of a phospholipid vesicle or in a membrane-mimetic solvent such as 50% trifluoroethanol–water. There is no single mechanism by which peptides produce cell death but their action generally does not involve binding to a receptor rather a non-specific interaction with the bacterial cell membrane or mammalian plasma membrane that results in loss of integrity and ultimate disintegration [7,13].

It is a common fallacy that all frog species produce peptides with antimicrobial activity in their skin secretions. Although considered to be a component of the animal's system of innate immunity, species distribution of these peptides is sporadic suggesting that their production in the skin may confer some evolutionary advantage to the organism but is not necessary for survival. It has been proposed that cutaneous symbiotic bacteria may provide the major system of defense against pathogenic microorganisms in the environment with antimicrobial peptides assuming a supplementary role in some species [17,74].

The emergence in all regions of the world of strains of pathogenic bacteria and fungi with resistance to commonly used antibiotics constitutes a serious threat to public health. This has necessitated a search for novel types of antimicrobial agent with appropriate pharmacokinetic and toxicological profiles that are active against these multidrug- or pandrug-resistant microorganisms. Over 26 years have passed since the discovery of the magainins in the skin of the African clawed frog, *Xenopus laevis* in the family Pipidae. These peptides, identified independently by Michael Zasloff at the National Institutes of Health, Bethesda, U.S.A. [78] and by the group of Dudley H. Williams at the University of Cambridge, U.K. [35], were the first amphibian peptides with antimicrobial activity to be fully characterized. Since that time several hundred such peptides have been isolated from the skin secretions of many other frog species belonging to different families [71]. However, despite showing potent activity against strains of antibiotic-resistant bacteria and against certain pathogenic fungi and protozoa, the potential of these peptides as therapeutic agents has not been realized. Enthusiasm for frog skin peptides within the pharmaceutical industry declined when the US Food and Drug Administration did not approve marketing of pexiganan, an analog of magainin-2 with potent, broad spectrum antimicrobial activity [34], for treatment of infected foot ulcers in diabetic patients on the grounds that efficacy had not been sufficiently demonstrated. No anti-infective compound based upon the structure of a frog skin peptide has yet been adopted in clinical practice. In consequence, alternative therapeutic applications are being explored.

It is now appreciated that cationic α-helical antimicrobial peptides are multi-functional displaying immunomodulatory, chemoattractant, and insulinotropic properties as well as cytotoxic activities [76]. Consequently, it is more informative to refer to them as host-defense peptides rather than as exclusively antimicrobial peptides. Analogs of naturally occurring amphibian peptides have been developed that show selective cytotoxicity against tumor cells and so have potential for development into anti-cancer agents [47]. As well as producing tumor cell death by disruption of the plasma membrane, certain cationic antimicrobial peptides can instigate apoptosis *via* the mitochondrial pathway and act as anti-angiogenic factors [48,58]. Similarly, certain peptides in frog skin secretions have demonstrated potent antiviral activity, either by directly inactivating the virus particles or by interfering with the initial steps of the viral reproductive cycle such as binding to specific cell surface receptors and subsequent entry into the cytoplasm [58]. These properties, combined with the short contact time required to induce killing, have led to their consideration as candidates for development into novel antiviral agents. The problems posed by the emergence of multidrug resistance in the treatment of bacterial infections are also encountered in cancer and viral chemotherapy [45]. Because of their non-specific and destructive mechanism of action, cell-penetrating peptides show therapeutic potential in cases where the tumor or virus is not responsive to conventional pharmaceutical therapy.

Frog skin host-defense peptides, in common with many other cationic antimicrobial peptides of diverse origins [15,76], display complex cytokine-mediated immunomodulatory properties. Effects on the production of both pro-inflammatory and anti-inflammatory cytokines by peritoneal macrophages and peripheral blood mononuclear cells have been observed so that