Development of antimicrobial peptide based anti-leishmanial agents: current understandings and future perspective

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The neglected tropical disease, Leishmanisis, is an endemic threat of America, Africa, Asia and Southern Europe. Currently 310 million people over more than 88 countries are at risk. There are no licensed vaccines and chemotherapy is the mainstay to combat the disease which is now threatened due to the emergence of acquired drug resistance, toxicity and resurgence of HIV-VL co-infection. These issues emphasize an urgent need to identify and develop an alternative and safer anti-leishmanial agent. Parasites encounter the immune system of both insects and mammalian hosts and interface with a multitude of different anti-microbial peptides (AMPs). AMPs are multifunctional components of the innate immune system and considered as attractive alternative to conventional pharmaceuticals. This chapter summarizes the research aimed at investigating the potential development of antimicrobial peptide-based anti-leishmanial agents.

Keywords: Leishmaniasis; Chemotherapy; Antimicrobial peptides; Anti-leishmanial agent

1. Introduction

VL caused by *Leishmania donovani* is a major public health problem in the Indian subcontinent and Africa and is classified as a neglected tropical disease. There are no licensed vaccines, and chemotherapy is the mainstay to combat the disease. The armamentarium of drugs currently approved is limited to amphotericin B and its various liposomal formulations, paromomycin, and pentavalent antimonials. Miltefosine, the only well tolerated oral drug approved for VL,[1] cannot be used in children and pregnant women, as gastrointestinal toxicity and teratogenicity were evident from clinical trials carried out in India.[2] The other drugs and combination therapies also suffer from shortcomings, such as unacceptable adverse effects, poor efficacy, limited accessibility due to high cost, and poor compliance, as they require parenteral administration and long treatment regimens.[3] Additionally, the other compounding factors of concern are the emergence of drug resistance, particularly toward pentavalent antimonials in the Indian subcontinent,[4] and resurgence of VL with HIV as a co-infection.[5] These issues emphasize an urgent need to carry out drug discovery programs that would significantly accelerate and facilitate the identification of alternative and safer chemotherapeutic agents against leishmaniasis.

Antimicrobial peptides (AMPs) are structurally diverse highly cationic proteins between 10 and 50 amino acids and are components of the innate immune systems of organisms within all kingdoms. The expression of AMPs in diverse plants, insects, crustaceans, amphibians and mammals as well as in primitive unicellular organisms such as protozoa, fungi and bacteria attest to the conservation of AMP functionality as a primitive immune defense response. Features that make AMPs attractive as alternatives to conventional antibiotics and pharmaceuticals include, rapidity of their action, their broad range of targets, the low likelihood of resistance development and the ability to act in conjunction with existing regimens.[6] AMPs have several functions and are known to interact with and disrupt microbial surface membranes leading to cell death.[7] The amphipathicity of AMPs enable them to interact with negatively-charged microbial membranes. Direct destabilization of surface-membranes by AMPs occurs through a variety of mechanisms and can initiate microbial death by triggering induction of autophagic, necrotic or apoptotic-cell death.[8]

2. Structural Classes of AMPs

Antimicrobial peptides are unique and diverse group of molecules, which are divided into subgroups on the basis of their amino acid composition and structure. The different classes of AMPs are as follows. [9]

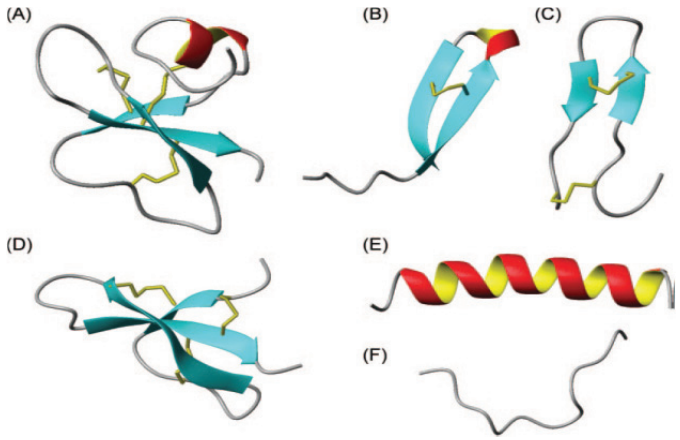

Fig. 1 Structural classes of antimicrobial peptides. (A) Mixed structure of human β-defensin-2 (PDB code 1FQQ) (216); (B) looped thanatin (PDB code 8TFV) (156); (C) β-sheeted polyphemusin (PDB code 1RKK) (202); (D) rabbit kidney defensin-1 (PDB code 1EWS) (165); (E) α-helical magainin-2 (PDB code 2MAG) (76); (F) extended indolicidin (PDB code 1G89) (212). The disulfide bonds are indicated in yellow, and the illustrations have been prepared with use of the graphic program MolMol 2K.1 (132).

3. General Mode of Action of AMPs:

AMPs can interact in different ways and can kill the microorganism, they can directely attach with the cell membrane of the microorganism and form pores or interact with intracellular components like DNA, nucleus, mitochondria and malfunction or inhibit differnt cellular process like transcription, translation, protein synthesis.[10] Different modes of killing of microorganism by diffrent peptides have illustrated in Fig. 1.2.

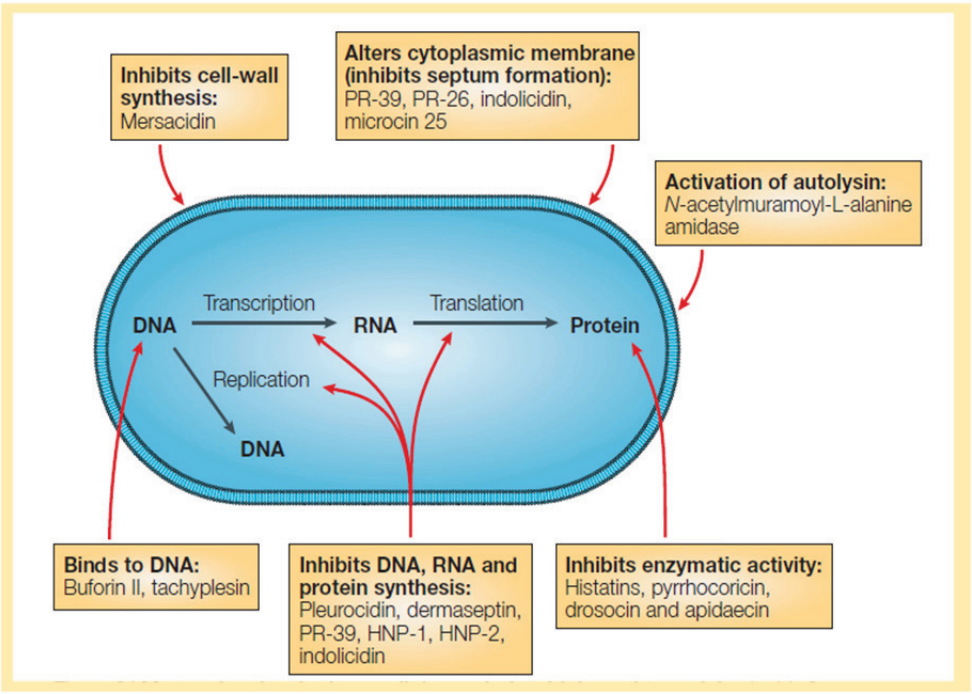

Fig. 2 Mode of action for intracellular antimicrobial peptide activity.[10]

4. Current understanding on antiparasitic Activity of AMPs

Several antimicrobial peptides possess an antiprotozoan mode of action that indicates parallels with their antibacterial, antiviral, or antifungal modes of action. Magainin 2 was one of the first antimicrobial host defense peptides demonstrated to display antiprotozoan activity, leading to swelling and eventual bursting of *Paramecium caudatum.*[11]

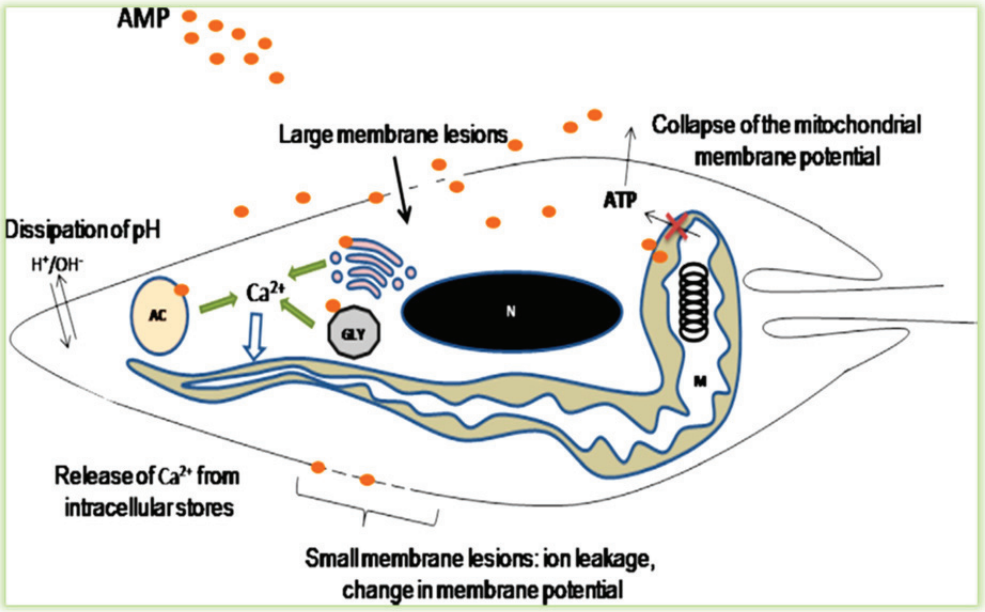

Fig. 3 Targets and effects of antimicrobial peptide attack on parasites.[12]

Peptides isolated from the genera Phyllomedusa (dermaseptins and phylloseptin), Bombina (bombinins), Rana (temporins) and Xenopus (magainins) have anti-leishmanial activity. Dermaseptins are between 24 to 34 amino acids in length and form amphipathic helices within biologic membranes.[13] The prototype peptide for this group, dermaseptin-1, kills multiple *Leishmania* species at concentrations from 2.3 to 12.5 μM.[14] The 25 amino acid AMP, DShypo-01, isolated from Phyllomedusa hypochondrialis, is even more potent, being active as low as 0.2 μM[15] and the 19 residue long phylloseptin-1 (at 0.5 μM) from the related frog *Phyllomedusa azurea* is as active as antimonate against *L. amazonensis* promastigotes.[16] More recently an acylated synthetic antimicrobial peptide, Oct-CA(1-7)-M(2-9), has been shown to be both safe and effective for treating naturally acquired canine leishmaniasis, which is caused by the parasite *Leishmania*, which is also an important cause of morbidity and mortality in humans.[17] The peptide temporin-SHd displayed potent antibacterial activities against Gram-negative and Gram-positive bacteria, including multi-drug resistant *Staphylococcus aureus* strains, as well as antiparasitic activity against promastigote and the intracellular stage (amastigote) of *Leishmania infantum*, at concentration not toxic for the macrophages.[18] Reports from our group explored the antileishmanial activity of spinigerin originally derived from *Pseudacanthotermes spiniger*. *Leishmania donovani* promastigotes present apoptosis-like cell death in a caspase-independent manner upon exposure to spinigerin (IC_{50},150 μM). This antileishmanial property of spinigerin that may be considered for future chemotherapeutic option al one or adjunct with other drug regimens for improved treatment of visceral leishmaniasis.[19] Dragonamides A and E and herbamide B extracted from *Lyngbya majuscula* exhibited antileishmanial activity with IC_{50} values of 6.5, 5.1, and 5.9 microM, respectively.[20.]Studies on different antimicrobial peptides against parasite of Trypanosomatidae family have been listed in Table 1.1.

Table 1 *In vitro* activity of antimicrobial peptides against *Leishmania sp.* Antileishmanial activity based on the ability of the concentration of designated AMP that either kills parasites and/or decrease their ability to replicate: <1 μM = 4+; 1–5 μM = 3+; 6–20 μM = 2+; 21–50 μM = 1+; >50 μM = 0.

Peptide	Parasite	AMP activity	References
Frogs			
Dermaseptins			
Dermaseptin-1	*L. major*; *L. amazonensis*; *L. chagasi*; *L. Mexicana*	3+; 2+; 3+; 2–3+;	15, 21, 22, 23,24
DS-1 analogues	*L. major*	2–3+	21,24
DShypo-01	*L. amazonensis*	4+	15
Phylloseptins			
Phylloseptin-1	*L. amazonensis*	3–4+	16
Temporins			
Temporin A	*L. donovani* and *L. pifanoi* ; *L. major* and *L. amazonensis*	2+; 0[c]	25
Temporin B	*L. donovani* and *L pifanoi* ; *L. major* and *L. amazonensis*	2+	25
Temporin-1Sa	*L. infantum*	2+	26

Peptide	Parasite	AMP activity	References
Temporin-1Sb	*L. infantum*	0	26
Temporin-1Sc Temporin-SHd Temporins A, B and 1Sa	*L. infantum* *L. infantum* *L. Mexicana*	0 0 0	26 18 27
Bombinins			
Bombinin H2	*L. donovani* and *L. pifanoi*	2+	28
Bombinin H4	*L. donovani* and *L. pifanoi*	2+	28
Magainins			
Magainin-2	*L. donovani*	2+	29
Magainin-H1	*L. donovani*	3+	29
Magainin-H2	*L. donovani*	3+	29
Pexiganan	*L. amazonensis* and *L. major*	2+	30
MSI-94	*L. braziliensie*	0	31
Insects			
Cecropins and Melittin			
Cecropin A	*L. donovani*	0	32,33
Cecropin A–melittin hybrids			
CA(1–8)M(1–18)	*L. donovani*	4+	34
Sub-fragments	*L. donovani*	3–4+	34
CA(1–7)M(2–9)	*L. donovani* and *L. pifanoi*	3+ and 2+	35
N-Lipid-CA(1–7)M(2–9)	*L. donovani* and *L. pifanoi*	3–4+ and 2–4	36
Cecropin B			
Melittin (bee venom)	*L. donovani*	4+	37,38
Gomesin (taranula)	*L. amazonensis*	2–3+	39
Sandfly defensin (SD-1)	*L. major* and *L. amazonensis*	0, 0	40
Spinigerin	*L. donovani*	0	19
Aquatic animals (other than frogs)			
Mussel AMPs			
Defensin fragments D and P	*L. major*	1–2+	41
Defensin fragments B, Q and E	*L. major*	0	41
Mylitin A	*L. braziliensis*	1–2+	31
Tachyplesin (horseshoe crab)	*L. braziliensis*	3+	31
Clavanin A (sea squirt)	*L. braziliensis*	0	31
Shrimp AMPs			
Anti-LPS factor	*L. braziliensis*	0	31
Penaeidian-3	*L. braziliensis*	0	31
Plants			
Wheat thionin	*L. donovani*	3–4+	42
Potato defensin (PTH-1)	*L. donovani*	1–2+	42
Potato snakin1	*L. donovani*	0	42
Barley lipid-transfer protein Quinolinic Alkaloids *Nyctanthes arbortristis* *Xylopia discrete*	*L. donovani* *Leishmania sp.* *Leishmania sp.* *Leishmania sp.*	0 - 3+ -	42 43 44

Peptide	Parasite	AMP activity	References
extracts			45
Mammals			
Cathelicidins			
Indolicidin (bovine)	*L. donovani*	4+	46
Myeloid AMP-18 (bovine)	*L. donovani*	1–2+	47
CRAMP (mouse)	*L. major* and *L. amazonensis*	1–2+	30
Protegrin-1 (porcine)	*L. major* and *L. amazonensis*	1–2+; 1+	30,48
SMAP-18, -29 (ovine)	*L. major* and *L. amazonensis*	1–2+; 2+	30,48
Defensins			
Cryptdin-1 and -4 (human)	*L. major* and *L. amazonensis*	1–2+; 1–2+	30,48
β-Defensin-4; -1/2 (human)	*L. major* and *L. amazonensis*	0; 1+	30,48
θ-Defensin-II	*L. major* and *L. amazonensis*	1–2+	30
Other			
Seminalplasmin peptides (bovine)	*L. donovani*	4+	46
Histatin 5 (human)	*L. donovani* and *L. pifanoi*	2–3+	37
NK-2 sub-fragment	*L. chagasi*	3+	38
Agaricus blazei water extract (AbM)	*Leishmania sp.*	-	49
Dragonamide E	*Leishmania sp.*	2+	20

5. Challenges and future directions

Some potential challenges to be keep in mind in the future development of AMP-based antileishmanials. Firstly, the surface metalloprotease GP63, also known as leishmanolysin, may protect *Leishmania* from AMPs. Notably, some AMPs tested against leishmanolysin-knockout mutants of *L. major* promastigotes displayed higher levels of leishmanicidal activity.[50] However, it is unlikely that leishmanolysin activity is a significant factor in the resistance of different *Leishmania spp* toward all AMPs, as certain AMPs display reduced activity toward the clinically relevant amastigote forms of the parasite, despite the fact that these forms express minimal levels of leishmanolysin.[50] Secondly, the *Leishmania* parasites generally reside and proliferate inside the phagolysosome, therefore, any AMP based antileishmanial drug will need to have the ability to cross several physical barriers to reach the parasite. Thirdly, the drug will need to display little or no toxicity toward the host cell. The issue of toxicity is perhaps the least problematic, as several of the AMPs have demonstrated low cytotoxicity toward macrophage.[19,35] Lastly, the identification of AMPs for which promising leishmanicidal activity against axenic extracellular parasites can be transferred to *Leishmania*-infected in vitro macrophage models. Currently, research investigating AMPs in macrophage models has been limited,[25,35] although quantitative, but technically demanding, assays are available.[51] However, if AMPs are to be developed further as antileishmanial agents, more detailed information regarding the modes of action and activity of AMPs against *Leishmania*-infected macrophages will be required.

The number of AMPs screened against *Leishmania* species has been steadily increasing. However, despite this increase, the total number of AMPs that have been tested against this protozoan parasite remains low compared with the number of AMPs that have been screened against bacterial species.[52] Based on the available data, it remains difficult to derive general conclusions that would aid researchers in the rational selection and design of antileishmanial AMPs. In addition to the lack of SAR data, many of the published studies in the field have focused on the insect-stage, promastigote form of *Leishmania* rather than the clinically relevant intra-macrophage amastigote form. Given the evident difference in the surface architecture of these two lifecycle stages, it is important to screen AMPs against amastigotes as well as promastigotes, despite the inherent challenges. The mammalian stage of the *Leishmania* lifecycle that occurs within macrophages presents a hurdle to the development of any antileishmanial drug, including AMPs. In addition to concerns regarding host cytotoxicity, any compound would have to penetrate the phagolysosome within which the amastigotes reside.

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