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Antimicrobial Peptide Action on Parasites

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Abstract: Diseases caused by protozoan parasites can pose a severe thread to human health and are behind some serious neglected tropical diseases like malaria and leishmaniasis. Though several different drugs have been developed in order to eradicate these diseases, a successful candidate has not yet been discovered. Among the most active compounds tested, antimicrobial peptides (AMPs) are particularly appealing because of their wide spectrum of action. AMPs have been described to perturb protozoan homeostasis by disrupting the cellular membranes but also by interfering with key processes in the parasite metabolism.

In this review we describe the diverse mechanisms of action of AMPs on protozoan targets and how they can be exploited to treat diseases. Moreover, we describe with detail the antimicrobial action of AMPs on two major parasitical infections: leishmaniasis and malaria.

All the features reviewed here show that AMPs are promising drugs to target protozoan parasites and that further understanding of the mechanism of action of these compounds will lead to improved drugs that could be worth to test in a clinical phase.

Keywords: Antimicrobial peptides, leishmaniasis, malaria, protozoa.

1. INTRODUCTION

Protozoa are unicellular eukaryotic organisms that usually live in aqueous environments and can display either sexual or asexual reproduction. Some of them are obligated parasites that can cause important human diseases such as malaria, dysentery, cryptosporidiosis or leishmaniasis, among others. Despite their severe threat to human health, protozoan diseases have been somehow neglected in basic and applied clinical investigation, one possible reason being the fact that they affect mainly poor tropical and subtropical areas of the world. Another important reason for the comparative disregard of protozoan-caused diseases is the rather peculiar life cycle of protozoans, involving multiple stages with dramatic differences in metabolism, protein expression and membrane composition [1]. Further, genetic tools are not as developed for protozoan as for mammalian cells, e.g. in *Leishmania* (excepting *L. braziliensis*), RNAi is unfeasible due to lack of a functional RNAi pathway [2]. Though many diverse drugs have been used to treat parasitic infections, in this review we will focus on antimicrobial peptides (AMPs), which constitute a first line of defense against pathogen invasion and dissemination, including *Protozoa,* and are currently undergoing intense investigation [3] with a view to improve their druggability [4].

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2. ANTIMICROBIAL PEPTIDES AS ANTIPARASITIC COMPOUNDS

AMPs are broad-spectrum antimicrobial agents targeting microorganisms by multiple mechanisms: i) directly perturbing the plasma membrane; ii) interacting with internal targets and iii) modulating the immune response [5]. As most AMPs are cationic, the expected differences in behavior towards microbial (negatively charged) and eukaryotic (neutral) membranes [6] underlie the preferential action of AMPs against microbial pathogens [7]. Some AMPs, however, are inhibited by salt at physiological concentrations, or neutralized by binding to various serum components, or too large for efficient chemical synthesis or difficult to obtain by recombinant expression [8, 9]. Various experimental and computational platforms have been proposed to develop more efficient AMPs [10-15].

In *Protozoa*, like in other lower eukaryotes, anionic phospholipids at the outer leaflet of the membrane account for the relative specificity of AMPs towards parasite over host cells [16]. Other components of protozoan membranes such as sterols also modulate AMP activity, usually by hindering it when in high amounts [17]. Also, and despite the lack of a permanent cell wall (in contrast to fungi or yeast), other structural traits confer *Protozoa* high resistance to AMPs and other antimicrobials:

Encystment

Some parasites develop encapsulated ookinetes, or oocysts, consisting of a thick-walled capsule made of glycoprotein [18], of extensively cross-linked protein mesh [19], of

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quinone-tanned protein, or even of chitin [20], all of them highly impermeable to water-soluble substances hence hampering access of antiparasitic compounds to the plasma membrane.

Glycocalix

The term describes a glycoconjugate interface between the parasite and the external environment. In *Leishmania* promastigotes, it is made up of lipophosphoglycan (LPG), a highly anionic component anchored to the membrane through glycosylphosphatidylinositol (GPI) and other glycolipids and glycoproteins (see below); it covers ca. 40% of the parasite surface and acts as flypaper by capturing polycationic peptides and preventing their action on the membrane [21].

Membrane-Anchored Proteases

Metalloproteases such as Gp63 of *Leishmania* promastigotes degrade external peptide-based compounds with broad specificity, hence exert a protective effect against AMPs [22].

2.1. Mechanism of AMP Action in Parasites

As stated above, AMPs commonly target the cytoplasmic membrane, disrupting the electrochemical gradient and consequently inducing an osmotic shock in pathogen cells. In addition, other mechanisms not involving severe membrane damage and dependent on internal parasite targets have been described (Fig. (**1**)).

2.1.1. Membrane Damage

The best-characterized mechanism of AMP action is the disruption of membrane integrity resulting in osmotic imbalance, cell swelling and lysis. This mechanism involves (i) rapid collapse of membrane potential and drop of intracellular ATP levels; (ii) consistent increase in membrane permeability, and (iii) morphological alteration such as blebs and breakages [1] or formation of anionic phospholipid-rich domains that cause defective phospholipid packing and ensuing membrane permeability [23]. The various types of membrane damage by AMPs (reviewed in [1, 24, 25]) can be fitted into three major basic mechanisms: i) barrel-stave; ii) carpet-like (or detergent-like) and iii) toroidal-pore.

2.1.1.1. Barrel-Stave

This model applies to AMPs that promote loss of the electrochemical potential at low, well-defined peptide:phospholipid ratios. Peptides that form barrel-stave pores have high partition coefficients and a tendency to self-assemble in membrane environments.

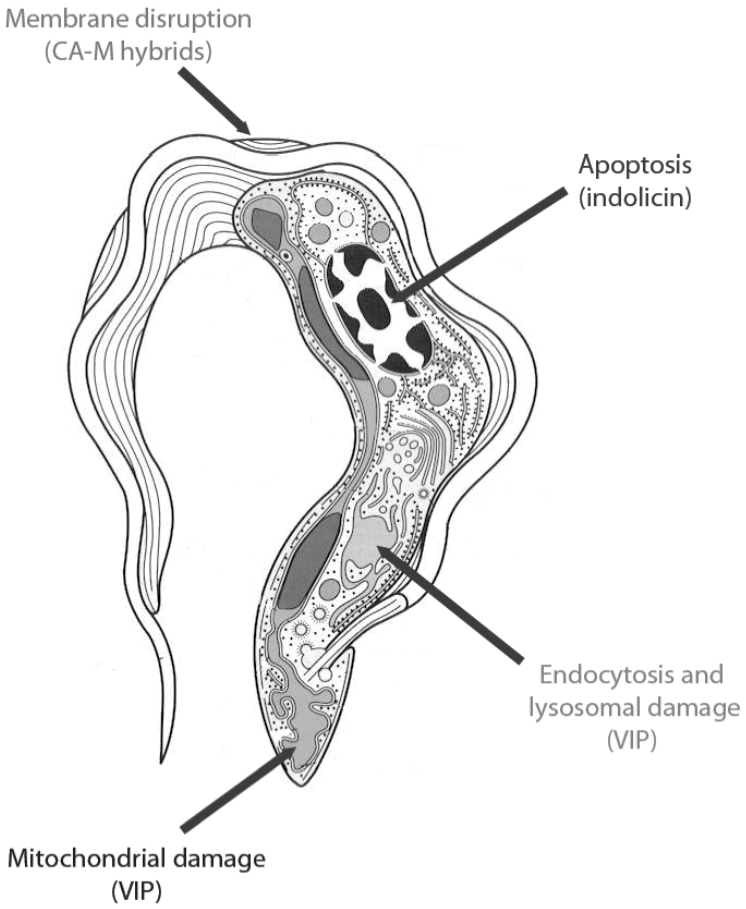

Fig. (1). Modes of action and targets of AMPs in trypanosomatids.

2.1.1.2. Carpet-Like Model

In this model, AMPs accumulate on the membrane surface by electrostatic interaction with anionic phospholipids; once a threshold concentration is reached, partial membrane solubilization in a detergent-like fashion takes place, favored by the amphipathic nature of the peptide.

2.1.1.3. Toroidal Pore or Two-State

In this intermediate model, AMPs accumulate parallel to the membrane surface, with partial insertion causing expansion of the outer but not the inner leaflet, with ensuing mechanical stress. Once a critical threshold is reached, tension is relieved by reorientation of the peptides perpendicular to the bilayer, which undergoes a positive curvature and modifies its thickness, creating transient pores made of both peptide and lipid molecules that in turn catalyze phospholipid flip-flop hence promote further membrane asymmetry.

As examples of membrane-damage mechanisms exerted by AMPs on *Protozoa*, temporins A and B and cecropin A (CA)-melittin (M) analogs such as CA(1-8)M(1-18) are paradigmatic. The former are short (~13 residues) amphibian AMPs with only one cationic amino acid (lysine or arginine) [26] whereas CA-M analogs are longer and richer in cationic residues [27]. Both AMP types adopt amphipathic helical structures in hydrophobic media and permeabilize bacterial membranes and liposomes. However, their different size and cationicity bear upon their interaction with LPG. Thus, for temporin (low charge) antiparasitic activity is independent of the length of the LPG phosphoglycan moiety [26]: the peptide has similar activity against wild type *L. donovani* and its LPG-defective R2D2 mutant [28]. In contrast, for CA(1-8)M(1-18), with larger size and higher charge, the mutant strain is more susceptible to peptide action [29]. It would thus appear that the small size and low charge of the temporins favor diffusion across the glycocalix into the plasma membrane, while the stronger electrostatic interaction of CA(1-8)M(1-18) with the LPG matrix impairs membrane access and worsens activity.

Along with charge, positional hydrophobicity is an important factor for the antiparasitic effects of magainins [30]. Accumulation of hydrophobic residues at a particular stretch of the sequence can promote aggregation; once the aggregate hits the membrane, the resulting high peptide concentration may cause local permeabilization. On the other hand, excess aggregation may result in micelle formation and lead to a complete loss of AMP efficacy.

While all above examples apply to AMPs targeting *Leishmania*, similar observations have been made for other trypanosomatids. Thus, cathelicidins such as SMAP-29, ovispirin and protegrin disrupt membrane integrity in African trypanosomes as well as in bacteria [31].

Different AMP antiparasitic activities may be displayed depending on the stage of the parasite targeted. Again in *Leishmania,* the amastigote is consistently more resistant to AMPs even when in axenic (macrophage-free) form; different parasite surface compositions most likely account for such differences. Similarly, the surface of bloodstream form of *T. brucei* contains high amounts of the variable surface glycoprotein, while the most abundant component of the procyclic form is procyclin. While both proteins are GPI-anchored, differences in their structure [32] may translate into different AMP susceptibility [33].

Other membrane-based mechanisms, though not actually involving mechanical damage, have been observed. For example, the small hydrophobic peptides (SHP) derived from the signal sequences of human apolipoproteins [34] intercalate in the acyl chain region of the membrane of *T. brucei* and modify membrane fluidity. It is known that many membrane proteins are highly dependent on lipid fluidity for their activity; hence a severe perturbation of this parameter will lead to impaired protein functionality. Finally, other possible ways in which AMPs might interact with membranes include redistribution of membrane components such as sterols, formation of specific phospholipid microdomains, or even direct interaction with membrane proteins and alteration of their function.

2.1.2. Action on Internal Targets

Aside from membrane injury, some AMPs gain access into the cytoplasm without significant, persistent damage to the bilayer, causing instead parasite death by interaction with internal targets. This requires their translocation across the lipid bilayer, in a manner similar to cell-penetrating peptides (CPPs). Among the pathways proposed to explain how CPPs can cross pathogen membranes [35], spontaneous lipid-assisted translocation, closely similar to the two-state model (see 2.1.1) of AMP action, is prevalent. As discussed above, hydrophobic residues of peptides laying parallel to the bilayer would perturb phospholipid packing, thereby inducing bilayer thinning and curvature, and partial peptide internalization. Next, peptides would adopt an orientation perpendicular to the membrane plane and form transient toroidal pores, thus favoring low-level peptide translocation to the inner leaflet of the membrane. Given the transient nature of these structures, some peptide molecules might dissociate from the membrane and eventually reach the cytoplasm.

Given that the boundaries between AMPs and CPPs are somewhat fuzzy [36], and that some CPPs have indeed been described to possess antimicrobial activities, it seems worthwhile to discuss some examples and their purported mechanism of action on intracellular targets (see Fig. (**1**)).

2.1.2.1. Bioenergetic Exhaustion

Histatin 5 (Hst5), a human salivary AMP [37] that promotes reversible depolarization of *Leishmania* plasma membranes, actually causes minimal membrane damage, suggesting that membranolysis plays a minor role, if any, in leishmanicidal action. The mitochondrion has been shown to be a major target for Hst5 in *Leishmania* [38], based on various evidences: i) Hst-5-treated parasites show extensive mitochondrial morphological alterations including a swollen matrix and poor cristae definition; ii) fluorescein-labeled Hst5 distributes largely in overlap with mitochondrial markers, and Hst5 uptake is precluded in metabolically inhibited parasites; iii) respiration rate and mitochondrial electrochemical potential decrease upon Hst5 incubation. The final outcome of the Hst5 attack is bioenergetic collapse of the parasite. Interestingly, the all-D Hst5 enantiomer has stronger activity than the natural peptide, possibly due to

higher buildup inside the parasite resulting from increased proteolytic resistance.

2.1.2.2. Endocytosis-Mediated Killing

Some cationic α-helical neuropeptides bind anionic glycoproteins such as the variant surface glycoprotein (VSG) of African trypanosomes [39], upon which they are quickly endocytosed through the flagellar pocket, entering the main trafficking pathway of the parasite [40], eventually accessing the lysosome and disrupting its membrane, which releases hydrolases that cause parasite death.

2.1.2.3. Apoptosis-Mediated Killing

While AMPs killing by membrane disruption interact mainly with the parasite surface, setting off osmotic lysis as described before [31], those killing by apoptosis interact with intracellular organelles or proteins, usually by caspase-3/7-like activation. The precise apoptosis mechanism is unclear but may involve leakage of cytochrome c from mitochondria and activation of a caspase-9-like enzyme, which in turn activates caspase-3/7. Alternatively, activation of a caspase-8-like enzyme via a yet unknown death signal similar to the Fas/FADD system of mammalian cells, could also lead to caspase-3/7 activation [41].

3. ANTIMICROBIAL PEPTIDES AGAINST NEGLECTED TROPICAL DISEASES

Tables **1** and **2** show a representative list of AMPs targeting *Leishmania* and *Plasmodium*, the two protozoan parasites causing diseases with higher mortality and morbidity.

3.1. AMPs Against *Leishmania*

Leishmaniasis, a neglected tropical disease endemic in over 80 countries, affects 12 million people a year with more than 350 million at risk of infection. It is one of the most prevalent causes of death and animal morbidity in underdeveloped countries. Infection is caused by several species of the genus *Leishmania,* intracellular obligate parasites with a dimorphic life cycle (Fig. (**2A**)). The extracellular promastigote stage infects a sandfly vector and, after massive multiplication in the gut of the insect, migrates into the salivary glands and is transmitted by the bite. In the mammalian host promastigotes are taken up by mononuclear phagocytes of the parasitophorus vacuole and transformed into amastigotes [42]. Current treatments against leishmaniasis are based on rather outdated, highly toxic drugs (e.g., antimonials) whose efficacy –with the possible exception of amphotericin B– is threatened by increasing resistance ([43]; WHO. WHO Neglected Tropical Diseases: htpp://www.who.int).

The discovery of AMPs opened new prospects for antimicrobial agents (Table **1**, Fig. (**2A**)). For instance, chimeric CA(1-8)M(1-18) [44] showed improved activity over the parental peptides, causing collapse of membrane potential and subsequent ATP loss [29]. Substantial efforts have been invested into designing more effective CA-M hybrids, with shortened sequences [45], lipidated N-termini [46], or N-methylated Lys residues [47]. The therapeutic potential of such CA-M analogues against canine leishmaniasis was evidenced by the 80% reduction in parasitemia and transitory clearance of disease symptoms achieved by octanoyl-CA(1-7)M(2-9) given intravenously to infected dogs [48].

Defensin-type antileishmanial peptides such as 18-residue gomesin from the spider *Acanthoscurria gomesiana* reduced to 50% the population of *L. amazonensis* promastigotes at micromolar concentration [49]. SD-1, a defensin expressed in the sandfly vector *Phlebotomus duboscqi,* showed only moderate *in vitro* activity against *L. major* promastigotes but was intriguingly active on bacteria, so it may enhance *Leishmania* parasite proliferation in infected sand flies by clearing other microorganisms [50].

Amphibian cutaneous secretions are one of the most abundant AMP reservoirs in nature, often with potent antileishmanial activities. For instance, 20-residue bombinins H2 and H4 from *Bombina* frogs inhibited *L. donovani* and *L. pifanoi* proliferation in a submicromolar range in both promastigote and amastigote stages [51, 52]. Similarly, *Phyllomedusa* frog-derived peptides dermaseptin and phylloseptin displayed a characteristic biphasic killing of *Leishmania* amastigotes and promastigotes [53-55]. The activity of temporins, short peptides from the genus *Rana*, against *Leishmania* promastigotes and axenic amastigotes, has been mentioned above [26]. Particularly interesting is temporin-1Sa which, lacking cationic residues, is nonetheless able to inhibit *Leishmania* proliferation at micromolar range [51, 56, 57].

Leishmanicidal AMPs from mamamalian sources have been more recently reported. Thus cathelicidins such as SMAP-29, with a conserved N-terminal cathelin-like domain and a C-terminal highly variable antimicrobial domain, inhibit *Leishmania* at low or submicromolar concentration. Similar behavior has been described for other cathelicidins such as indolicin, myeloid AMP-18 and AMP-28, protegrin-1 and LL-37 [31, 50, 58-60].

Mammalian defensins are structurally classified into three families, named α-, ß- and θ-. Both α- and ß-defensins adopt a triple-stranded antiparallel ß-sheet structure stabilized by three disulfide bridges whereas θ-defensins display a cyclized peptide backbone, made by ligation of two identical or similar nonapeptides and stabilized by three disulfide bridges [61]. Cryptdin-1 and -4, ß-defensin-1, -2 and -4 and θ-defensin-II [22] have been reported to have leishmanicidal activities. Other human mammalian AMPs with leishmanicidal activity include histatin-5 [38] and seminalplasmin [58].

Plants also represent an important source of antileishmanial agents. Some examples are wheat thionin, barley lipid-transfer protein, or defensins and snakins from potato, all of them cysteine-rich peptides able to disrupt *L. donovani* promastigotes and amastigotes without altering mitochondrial respiration [62, 63].

Leishmanicidal AMPs from marine organisms include (i) tachyplesin from the limulid *Tachypleus tridentatus*, active against *L. braziliensis* promastigotes and amastigotes [64] (ii) mytilin A from the mussel *Mytilus edulis*, with antileishmanial activity against *L. braziliensis* [64], (iii) dragomide E, a linear lipopeptide isolated from the cyanobacteria *Lyngbya majuscula* with an antileishmanial activity against *L. donova*ni promastigotes [65]; and (iv) the cy-

Table 1. Activity of Antimicrobial Peptides on *Leishmania*

AMP	*Leishmania* spp.	Parasite Stage	Antiparasitic Activity	References
Invertebrates				
CA(1-8)M(1-18)	*L. donovani*	Promastigotes	IC_{50} 1-5 μM	[29]
CA(1-7)M(2-9)	*L. donovani* /*L. pifanoi*	Axenic amastigotes	IC_{50}<1 μM	[46]
Oct-CA(1-7)M(2-9)	*L. infantum*	Blood circulating amastigotes	IC_{50} <1 μM	[48]
Gomesin	*L. amazonensis*	Promastigotes	IC_{50} 2.5 μM	[49]
SD-1	*L. amazonensis* / *L. major*	Promastigotes	IC_{50} >50 μM	[89]
Amphibians				
Bombinin H2 and H4	*L. donovani* /*L. pifanoi*	Promastigotes/axenic amastigotes	IC_{50} 7/11 μM	[26, 51, 52]
Dermaseptin-S1 and H-3	*L. amazonensis* / *L. major*	Promastigotes/Amastigotes	IC_{50} 4.5/13.5 μM	[53]
SPYY	*L. major*	Promastigotes/amastigotes	MIC 5.9/6.2 μM	[90]
Phylloseptin-1	*L. amazonensis*	Promastigotes	IC_{50} 0.5 μM	[91]
Temporins A and B	*L. infantum* /*L. mexicana*	Promastigotes/amastigotes	IC_{50} 12/50 μM	[56]
Mammalians				
Cathelicidins				
Indolicin	*L.donovani*			
Myeloid AMP-18	*L. major*	Promastigotes/amastigotes	IC_{50} 0.5/12.5 μM	
Myeloid AMP-28				[31, 50, 58-60]
Protegrin-1				
CRAMP				
Defensins				
Cryptdin-1 and -4				
ß-defensin-1, -2 and 4	*L. amazonensis/L, major*	Promastigotes/amastigotes	IC_{50} 20/50 μM	[22]
θ-defensin-II				
Histatin 5	*L. donovani*	Promastigotes/amastigotes	IC_{50} 7.3/14.2μM	[38]
Seminal plasmin	*L. donovani*	Promastigotes	IC_{50} <1 μM	[58]
Plants				
Wheat thionin	*L. donovani*	Promastigotes/amastigotes	IC_{50} 1/42 μM	[62, 63]
Barley lipid transfer				
Protein	*L.donovani*		IC_{50} > 50 μM	
PTH-1				
Potato snakin 1				
Other				
Tachyplesin	*L. braziliens*	Promastigotes/amastigotes	IC_{50} 6 -20 μM	[64]
Mytilin A	*L. braziliens*	Promastigotes/amastigotes	IC_5 50 μM	[64]
Dragomide E	*L. donovani*	Promastigotes	IC_{50} 5 μM	[65]

Table 2. Activity of Antimicrobial Peptides on *Plasmodium*

AMP	*Plasmodium* spp.	Parasite Stage	Antiparasitic Effect	References
Invertebrates				
CA(1-8)M(1-18)	*P. falciparum*	Erythrocytic stages	IC_{50} 10 μM	[71]
Cecropin A and B	*P. falciparum, P. berghei, P. knowlesi* and *P. cymonolgi*	Oocyst and ookinete	IC_{50} 1.5 μM	[72, 77]
Shiva 3	*P. berghei*	Oocyst and ookinete	MIC 100 μM	[78]
Shiva 1 + anti-Pbs21	*P. berghei*	Ookinete	90% inhibition at 10 μM	[85]
Drosomycin	*P. berghei*	Ookinete	IC_{50} 10 μM	[81]
Scorpine	*P. berghei*	Gametocyte	98% inhibition at 15 μM	[79]
Meucine-24 and -25	*P. berghei*	Ookinetes	IC_{50} ~10-20 μM	[80]
Gomesin	*P. falciparum* and *berghei*	Gametocyte and oocyst	56% and 53% reduction at 50 μM	[82]
Gambicin	*P. berghei*	Ookinetes	54-64% lethality at 10-100 μM	[92]
Defensins	*P. gallinaceum*	All mosquito stages	IC_{50} 200 μM	[83]
TP10	*P. falciparum*	Gametocyte, zygote and ookinete	35-45% reduction at 30 μM	[84]
Amphibian				
Magainin II	*P. falciparum, knowlesi* and *cymonolgi*	Oocyst, trophozoite and schizont	90% inhibition at 100 μM	[71, 72]
PGla	*P. falciparum*	Trophozoite and schizont	IC_{50} 40 μM	[71]
Dermaseptin S3 and S4	*P. falciparum*	Trophozoite	IC_{50} 1.4 μM	[73]
K_4K_{20}-S4 and K4S4(1-13)	*P. falciparum*	Trophozoite	IC_{50} 0.2 -3.3 μM	[75]
Mammalian				
Angiotensin II and VC5	*P. gallinaceum*	Sporozoites	88% and 76% reduction at 5-60 μM	[69]
NK-2	*P. falciparum*	Erythrocytic stages	IC_{50} 6.2 μM	[70]
Other				
CEL-III	*P. falciparum* and *berghei*	Oocyst and ookinete	IC_{50} 15 nM	[85]
Tyrothricin	*P. falciparum*	Erythrocytic stages	IC_{50} 0.6 nM	[86]
AdDLP	*P. berghei*	Ookinetes	IC_{50} 20 μM	[80]

clodepsipeptide IB-012012 from the marine fungus *Clonostachys* [66].

4. AMPS AGAINST *PLASMODIUM*

Malaria, with 225 million cases reported worldwide in 2009 (World Malaria Report summary. WHO, 5 November 2011) and 1,238,000 estimated deaths in 2010 [67], is caused by several species of *Plasmodium* parasites and transmitted by an invertebrate vector of the genus *Anopheles*. The parasite life cycle is complex, involving multiple developing stages and locations both in mosquitos and humans. After a mosquito female ingests intracellular gametocytes from a human, these differentiate into gametes that, upon fertilization and *via* a diploid zygote, give rise to a motile ookinete that infects the midgut epithelia of the mosquito and differentiates into an oocyst. After replication, oocysts release large numbers of sporozoites that invade the human host upon the mosquito bite and reach the bloodstream by transmigration through the dermis. Once in circulation, sporozoites reach liver hepatocytes in whose parasitophorous vacuole they multiply and eventually invade erythrocytes, subse-

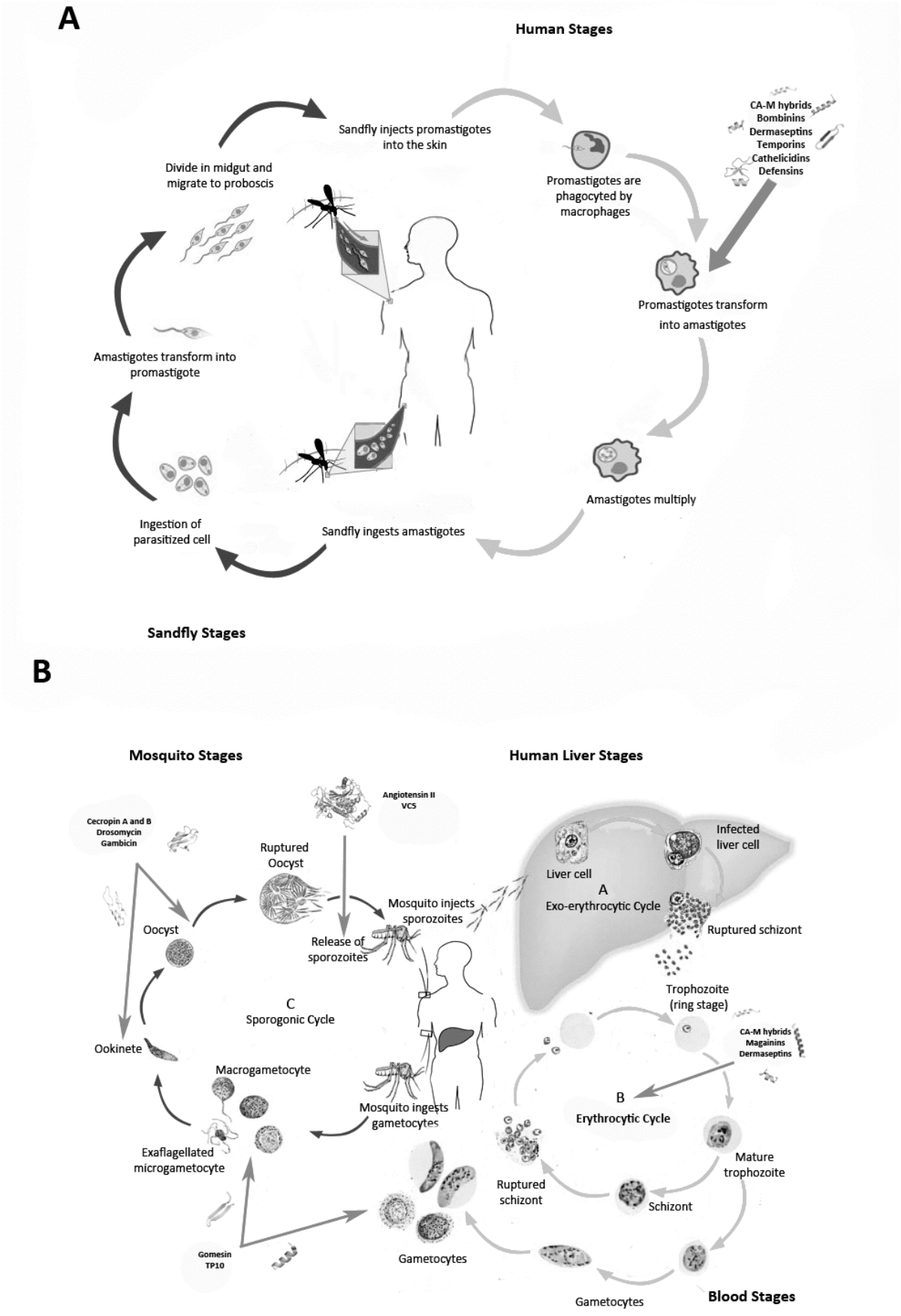

Fig. (2). The activity of some AMPs on specific stages of the life cycles of (**A**) *Leishmania* and (**B**) *Plasmodium*. Adapted (A and B, respectively) from http://www.cdc.gov/malaria/about/biology/index.htmland http://www.cdc.gov/parasites/leishmaniasis/biology.html.

quently differentiating into trophozoites. Through an asexual process of schizogony these yield thousands of trophozoites that can further differentiate into gametocytes and close the cycle [68] (Fig. (**2B**)).

Finding a universal drug against malaria has proven a challenging task. Nonetheless, its complex infectious cycle offers multiple sites where specific drugs, including AMPs, can act (Table **2**, Fig. (**2B**)). AMPs such as angiotensin II and its related vanicere-5 peptide can target disease transmission by selectively disrupting the cell membrane of *P. gallinaceum* sporozoites. Parasite load on salivary glands of infected mosquitoes is reduced by 75% when treated with these AMPs, with a dramatic decrease in infectivity in the vertebrate host [69]. Erythrocyte stages, composed of infective trophozoites and replicative schizonts responsible for human pathology, are also possible AMP targets. Thus, NK-lysin–derived NK-2 peptide permeabilizes infected cells –selectively exposing phosphatidylserine residues– and drastically reduces *P. falciparum* viability while almost totally sparing non-infected –phosphatidylserine non-exposing– cells [70]. CA-M hybrids are also active on *P. falciparum*-infected erythrocytes at low culture [71].

Amphibian AMPs have also been reported as good antimalarial agents. For example, magainin II and its amidated homolog promote oocyst degeneration in *P. falciparum*, *P. cymonolgi* and *P. knowlesi* infections. In addition, magainin II inhibits reinfection of erythrocytes by *P. falciparum* trophozoites and schizonts at 100 μM concentration [71, 72] and PGLa, another amphibian peptide, also displays antiparasitic activity against *P. falciparum* [71]. Dermaseptins S3 and S4 kill *P. falciparum* trophozoites although their selectivity between parasites and erythrocytes is not as high as for other peptides [73, 74]. Optimization of S4 has led to the 28-mer K4K20-S4 or the short K4S4(1-13) analog with enhanced activity towards infected red blood cells [75, 76].

AMPs can also target mosquito stages. Thus, cecropins A and B are active on ookinetes and oocysts of *P. falciparum*, *P. berghei*, *P. knowlesi* and *P. cymonolgi* [72, 77], and Shiva 3, a CA-M derivative, is harmful to *P. berghei* ookinetes and oocytes in the mosquito midgut after a few minutes of exposure [78].

Venom peptides are also an important source for antimalarial AMPs. Scorpine, similar to cecropin and isolated from the tarantula *Pandinus imperator,* inhibits proliferation of *P. berghei* gametocytes [79]; meucine-24, an α-helical peptide with an N-terminal homologous to melittin, and meucine-25, a singular peptide devoid of similarity with any other, have been isolated from the venom of of the scorpion *Mesobuthus eupeus* and shown to inhibit ookinete stages in *P. berghei* [80].

Several insect defensins also display antimalarial activity. Drosomycin, a 44 mer from *Drosophila,* inhibits the development of *P. berghei* ookinetes at micromolar concentrations [81], and gomesin, an insect defensin, causes a dramatic reduction of gametocyte exflagelation and oocyst population in both *P. falciparum* and *P. berghei* [82]. Other insect defensins from dragon fly and flesh fly reduce gametocyte viability in mosquitos infected by *P. gallinaceum* [83]. Finally, TP10, a CPP isolated from wasp venom, is very effective against zygote/ookinete stage of *P. falciparum* [84].

Other miscellaneous examples of antimalarial AMPs are CEL-III from sea cucumber, active at 15 nM against *P. berghei* ookinetes and oocysts, but with only moderate inhibition of *P. falciparum* [85]. Tyrocidins, a heterologous family of cyclic decapeptides from *Bacillus brevis*, showed a high percentage of lysis for the bloodstages of *P. falciparum* [86].

Finally, *de novo* peptides mimicking the structure and/or function of native counterparts were tested against *Plasmodium*. Thus, oligoacyl-lysyls (OAKs), with an alternance of cationic residues an acylamino chains recreating an amphipatic pattern, showed both *in vivo* and *in vitro* antimalarial activity with low hemolytic activity [87]. Synthetic analogues of the LAH4 peptide showed high (>100) selectivity index high potency against *P. falciparum* [88]. Other synthetic peptides such as Vida 1-3 have been designed to target ookinete development of *P. berghei* and *P. yoelii nigeriensis,* thus preventing oocyst infection in *Anopheles gambiae* [84].

5. CONCLUSIONS AND FUTURE DIRECTIONS

As extensively described in this review, there are multiple protozoan-targeting AMPs that can be pharmaceutically attractive to fight parasitic diseases. It is, however, difficult to predict if AMPs will stimulate future therapeutic strategies against diseases such as malaria or leishmaniasis. At present, an obvious shortcoming of AMP therapy is the higher production cost of synthetic peptides over conventional organic molecule drugs. However, computational and experimental strategies aimed at size reduction, proteolytic stabilization and other druggability issues are likely to cut prices down in the near future. Even so, mechanisms of action typically requiring concentrations in the μM range will continue to burden AMP-based therapies vs. often nanomolar-active classical drugs. The fact that parasitic diseases, despite their high prevalence, usually affect low-income communities also bears on the issue, as returns on pharma investment are unlikely to be plentiful. This lack of financial incentives might hopefully be corrected by governmental or not-for-profit interventions targeting neglected diseases at a global level, in a context where AMPs as antiparasitic agents receive adequate attention.

6. CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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