Experimental Parasitology 123 (2009) 11–16

Contents lists available at ScienceDirect

Experimental Parasitology

journal homepage: www.elsevier.com/locate/yexpr

Antiplasmodial and antileishmanial activities of phylloseptin-1, an antimicrobial peptide from the skin secretion of *Phyllomedusa azurea* (Amphibia)

Selma A.S. Kückelhaus [a,*], José Roberto S.A. Leite [b], Maria Imaculada Muniz-Junqueira [a], Raimunda Nonata Sampaio [c], Carlos Bloch Jr. [d], C. Eduardo Tosta [a]

[a] Laboratório de Imunologia Celular, Área de Patologia, Faculdade de Medicina, Universidade de Brasília, 70910-900 Brasília, DF, Brazil
[b] Grupo de Biodiversidade e Biotecnologia, Campus Ministro Reis Velloso, Universidade Federal do Piauí, 64202-020 Parnaíba, PI, Brazil
[c] Laboratório de Dermatologia, Departamento de Clínica Médica, Faculdade de Medicina, Universidade de Brasília, 70910-900 Brasília, DF, Brazil
[d] Laboratório de Espectroscopia de Massa, EMBRAPA Recursos Genéticos e Biotecnologia, 70770-900 Brasília, DF, Brazil

ARTICLE INFO

Article history:
Received 10 March 2009
Received in revised form 5 May 2009
Accepted 6 May 2009
Available online 19 May 2009

Keywords:
Phylloseptin
Antimicrobial peptide
Leishmania amazonensis
Plasmodium falciparum
Antiparasitic therapy
Phyllomedusa azurea
Hormesis

ABSTRACT

The development of drug resistance by infectious agents represents a major hindrance for controlling parasitic diseases and has stimulated the search for new compounds. We have previously shown that phylloseptin-1 (PS-1), a cationic peptide from the skin secretion of *Phyllomedusa azurea*, exhibited potent antimicrobial activity. Now we evaluate the effect of PS-1 on *Leishmania amazonensis* and *Plasmodium falciparum*. Concentrations as low as 0.5 μg/mL of PS-1 exhibited antileishmanial activity comparable to that of antimoniate of *N*-metilglucamine, while the antiplasmodial effect of PS-1 was evident at the concentration of 16 μg/mL, and reached an activity comparable to that of artesunate, at the concentration of 64 μg/mL. The high antiparasitic activity of PS-1, together with the unrelatedness of its chemical structure to any present antimicrobial drug, which prevents the development of cross-resistance, together with its non-toxicity to mammalian cells make this peptide a promising candidate for the treatment of malaria and leishmaniasis.

1. Introduction

Although several drugs are presently available for treating malaria and leishmaniasis, their efficacy has been threatened by the growing occurrence of resistant parasites. *Plasmodium falciparum*, the species responsible for most deaths associated to malaria, has acquired resistance to all available antimalarial drugs, with the exception of artemisinin derivatives (Wongsrichanalai et al., 2002). Pentavalent antimonial compounds have long been the cornerstone of anti-*Leishmania* chemotherapy and still play a leading role in the treatment of leishmaniasis (Croft and Yardley, 2002). However, these drugs cause severe toxic side effects, and induce the acquisition of resistance by parasites (Croft et al., 2006). These factors represent important hindrances for malaria and leishmaniasis control efforts, and have fuelled the search for new antimalarial and antileishmanial drugs.

Several substances are produced by prokaryotes, fungi, plants and animals for their natural defence against environmental microorganisms (Boman, 1991; Gudmundsson et al., 1996). These antimicrobial substances are especially abundant in amphibians, where they are responsible for defending their naked skin against noxious microorganisms and for assisting in wound repair (Nicolas and Mor, 1995). Several investigators have shown that these peptides exhibit a large spectrum of action against bacteria (Peschel, 2002), fungi (Lee et al., 1999), protozoa (Ghosh et al., 1997; Brand et al., 2002), tumour cells and viruses (Chernysh et al., 2002). These findings make these peptides very attractive candidates for the development of new drugs capable to face the challenges of treating infectious disease.

Our research group has recently characterized in the skin secretion of *Phyllomedusa azurea*[1] a family of cationic peptides named phylloseptins, which exhibit broad spectrum of antimicrobial activity and low hemolytic effect. They consist of 19 or 20 highly conserved residues and present an amidated C-terminus (Leite et al., 2005), a feature associated with their biological activity (Resende et al., 2008). Previous studies with phylloseptin-1 (PS-1) showed that it is endowed with antibacterial activity, presents negligible lytic effect towards human erythrocytes (Leite et al., 2005), and very low toxicity to mice (Kückelhaus et al., 2006).

The possible effect of PS-1 on plasmodium and leishmania organisms has not as yet been evaluated. Considering the impor-

* Corresponding author. Fax: +55 61 3435 6569.
E-mail address: selmak@unb.br (S.A.S. Kückelhaus).

[1] Until 2005 this species, found in the Brazilian cerrado region, was known as *Phyllomedusa hypochondrialis* but, with the process of revalidation, it was renamed *Phyllomedusa azurea* (Caramaschi, 2006).

0014-4894/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.exppara.2009.05.002

tance and urgency to develop new drugs to cope with the problem of drug resistance in malaria and leishmaniasis therapy, and the previous description of the broad spectrum of action of amphibian peptides, the present investigation was designed to evaluate the effects of PS-1 on *Leishmania amazonensis* and *P. falciparum,* and to determine its toxicity to mammalian cells.

2. Materials and methods

2.1. Peptide synthesis

Amidated PS-1 (FLSLIPHAINAVSAIAKHN-NH_2) was synthesized in the Pioneer Synthesis System (Applied Biosystems, Foster, USA), according to the method of Merrifield (1963). F-moc amino acids and F-moc-PALpoly(ethyleneglycol)-polystyrene resin were purchased from Applied Biosystems. Peptide purification was performed through Reverse Phase HPLC system (Shimadzu Corporation, Kyoto, Japan) with a Vydac 218 TP 1022 (Vydac, Columbia, USA) preparative column, and purity was assessed by MALDI/TOF-TOF MS Ultraflex II system (Bruker Daltonics, Bremen, Germany).

2.2. Plasmodium falciparum isolate

To assess the effect of PS-1 on *P. falciparum,* the isolate Pf/UnB-169, obtained from the Laboratory of Malaria, Faculty of Medicine, University of Brasilia was used. This isolate, maintained cryopreserved in liquid nitrogen, was rapidly thawed and cultured in O^+ human erythrocytes with 3% haematocrit, suspended in complete RPMI 1640 culture medium (Sigma–Aldrich, St. Louis, USA), buffered with 20 mM Hepes and 7.5% $NaHCO_3$, supplemented with 0.25 mg/dL hypoxanthine and 10% heat-inactivated AB^+ human serum, and cultured in 75 mL plastic culture flasks (Corning®, Corning, USA) at 37 °C with a mixture of synthetic air plus 5% CO_2.

2.3. Evaluation of the effect of PS-1 on P. falciparum

The effect of PS-1 on the growth of *P. falciparum*-parasitised erythrocytes (initial parasitemia: 0.6% and haematocrit: 3%) was assessed in triplicate samples of cultures in complete RPMI 1640 medium (supplemented with 10% pool of AB^+ human serum) with different concentrations of PS-1 (1, 2, 4, 8, 16, 32, 64 and 128 μg/mL) in 96-well microplates (TTP LabTech, Cambridge, USA). After incubation in a wet chamber at 37 °C, with synthetic air plus 5% CO_2, for 48 or 72 h, the percentage of parasitized erythrocytes was assessed by microscopy (1000× magnitude) in Giemsa-stained thin smears, by examining 1000 erythrocytes. The numbers of *P. falciparum* rings, trophozoites and schizonts were assessed in 1000 erythrocytes per preparation. As positive control, infected erythrocytes were incubated with 8 ng/mL of artesunate (Artemix® Ativus, São Paulo, Brazil) and, as negative control, they were incubated exclusively with the diluent (complete RPMI 1640 medium).

2.4. Leishmania amazonensis isolate

The isolate MHOM/BR/pH8 of *L. amazonensis* was obtained from the Laboratory of Dermatology, Faculty of Medicine, University of Brasilia. It was kept cryopreserved in liquid nitrogen until transferred to NNN-LIT medium and cultured at 22 °C for 48 h. Following, a small aliquot was added to RPMI 1640 culture medium, supplemented with 20% heat-inactivated foetal calf serum (Sigma–Aldrich, St. Louis, USA) and gentamicin sulphate (40 mg/mL) (Schering Plough, São Paulo, Brazil), and cultured until the log phase was reached.

2.5. Evaluation of the effect of PS-1 on L. amazonensis

To assess the effect of PS-1 on *L. amazonensis*, 1.0×10^6 promastigotes were cultured in RPMI 1640 medium with different concentrations of PS-1 (0.5, 1, 2, 4, 8, 16, 32 and 64 μg/mL) in triplicate samples. After incubation at 22 °C for 2 or 6 h, the numbers of viable and dead parasites were assessed by phase contrast microscopy. Parasites were considered as dead when their typical morphology was altered and their integrity was lost. As positive control, antimoniate of *N*-metilglucamine (Glucantime® Rhodia Pharma, São Paulo, Brazil) was used in the concentration of 4 μg/mL, following Brand et al. (2006), and as negative control, parasites were incubated exclusively with complete RPMI 1640 medium. To ascertain whether the morphological alterations of promastigotes caused by incubation with 64 μg/mL PS-1 corresponded to dead parasites, they were sub-cultured with RPMI 1640 medium for seven days and daily examined by microscopy.

2.6. Animals

Two-month-old female Swiss mice, obtained from the Centro Universitário de Brasília, were maintained at 12 h dark/light cycles, with balanced food and water *ad libitum.* The experimental protocols were previously approved by the Animal Research Ethical Committee of the University of Brasília (process number 03/2004).

2.7. Toxicity of PS-1 to peritoneal macrophages

The toxicity of PS-1 towards mammalian cells was evaluated by incubating mouse peritoneal macrophages with different concentrations of the peptide. Cells, obtained by washing mouse peritoneal cavity with 10 mL of cold phosphate buffered saline (PBS) pH 7.2, were centrifuged at 400*g* at 4 °C for 10 min, and quantified with 0.05% nigrosin solution in PBS pH 7.2 in a haemocytometer chamber (viability always higher than 95%). Viable cells (2.5×10^4) were added to 5 mm-diameter glass coverslips in 96-well microplates and incubated in a wet chamber for 1 h at 37 °C, with a mixture of synthetic air plus 5% CO_2. After rinsing the coverslips with PBS, adherent cells (>99% macrophages) were incubated with different concentrations of PS-1 (1, 10, 100, 200 and 300 μg/mL), for 1 h, as described. After incubation, a drop of 0.05% nigrosin solution was laid on coverslips and the percentage of dead cells was immediately evaluated by optical microscopy (400× magnification) in an area corresponding to 10% of that of the coverslip, corresponding to seventeen microscope fields.

2.8. Statistical analysis

The normality of the variables was tested by the Kolmogorov–Smirnov test. ANOVA and Kruskal–Wallis tests were used to compare multiple normal or non-normal samples, respectively. Student's *t*-test and Mann–Whitney test were used to compare two normal or non-normal samples, respectively. The Prism® software package (GraphPad, USA, 2005) was used for performing the statistical tests and for graphical representations. Differences with a two–tailed value of $p < 0.05$ were considered as statistically significant.

3. Results

3.1. Effect of PS-1 on L. amazonensis

The incubation of *L. amazonensis* promastigotes for 2 or 6 h with different concentrations of PS-1 resulted in the reduction of at least 48% of the number of parasites in a dose-dependent pattern

(Fig. 1). The peptide was able to completely inhibit parasite growth at the concentration of 64 μg/mL (IC_{100}) after incubation for 6 h (Fig. 1B). In these cultures promastigotes lost their typical morphology and formed clumps of amorphous material (Fig. 2B), differently from what was found in the control cultures, where the parasites retained their normal structure (Fig. 2A).

3.2. Effect of PS-1 on P. falciparum

The incubation of *P. falciparum* for 48 h (Fig. 3A) or 72 h (Fig. 3B) with different concentrations of PS-1 reduced the number of parasites in a dose-dependent pattern. PS-1 caused the highest inhibition of *P. falciparum* growth at the concentration of 128 μg/mL and 72 h of incubation. In these conditions, the peptide caused 97% inhibition of parasite growth (Fig. 3B).

Surprisingly, in its lowest concentration (1 μg/mL), PS-1 caused a 25% increase in the percentage of trophozoite-infected erythrocytes, as compared to the cultures incubated with RPMI 1640 medium alone (Fig. 4).

3.3. Effect of PS-1 on mammalian cells

To assess the possible toxic effect of PS-1 to mammalian cells, mouse peritoneal macrophages were incubated with different concentrations of the peptide, and modifications of their number and viability were evaluated. It was observed that PS-1 caused some apparent toxicity to peritoneal macrophages exclusively at high concentrations: at 200 μg/mL a reduction of the percentage of adherent cells was detected (Fig. 5A), and 300 μg/mL of PS-1 caused a reduction of cell viability (Fig. 5B), when compared to the control cultures incubated with RPMI 1640 medium alone. Interestingly, at its lowest concentration (1 μg/mL), PS-1 increased the percentage of adherent cells (Fig. 5A).

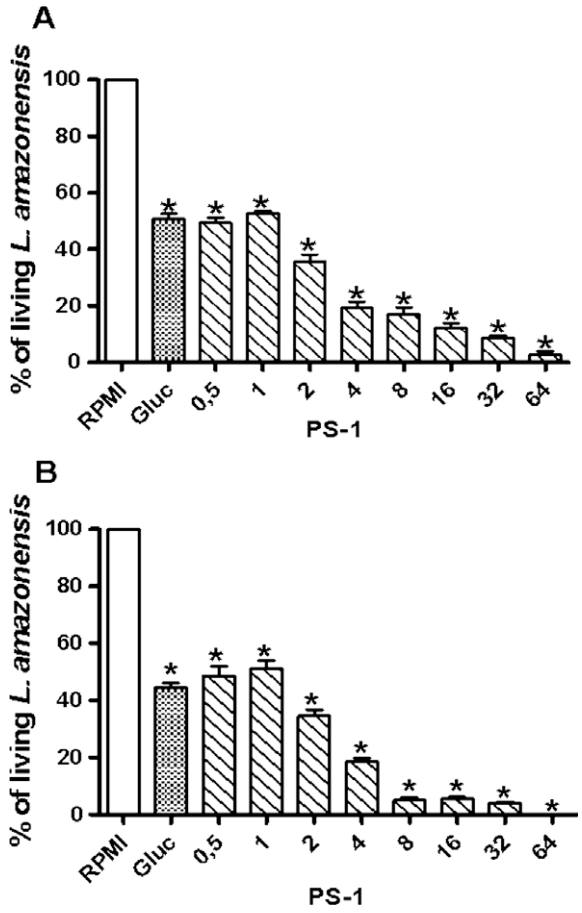

Fig. 1. Percentage of living *Leishmania amazonensis* promastigotes incubated with different concentrations of PS-1 for 2 h (A) or 6 h (B). Antimoniate of *N*-metilglucamine (Gluc) (4 μg/mL) and RPMI 1640 medium were used as positive and negative controls, respectively. Data, based on three experiments, are represented by mean ± SD. The results were analysed by ANOVA, followed by the Student–Newman–Keuls test, and showed differences between negative control and all concentrations of PS-1 (*) $p < 0.001$.

4. Discussion

It has been previously reported that PS-1 presents potent antibacterial activity (Leite et al., 2005). We now show that this peptide exhibits also powerful antiprotozoal activity towards *L. amazonensis* and *P. falciparum*, together with absence of toxicity to mammalian cells, at the range of its antiprotozoal activity.

Our data showed that both *L. amazonensis* and *P. falciparum* present a high affinity for PS-1 since their growths were inhibited by very low concentrations of the peptide. Concentrations as low as 0.5 μg/mL of PS-1 exhibited antileishmanial activity comparable to that of antimoniate of *N*-metilglucamine, the gold-standard drug for the treatment of leishmaniasis. On a concentration basis, PS-1 showed higher antileishmanial activity than other antimicrobial peptides such as tachyplesin-I, clavinin-A (Löfgren et al., 2008) and temporins (Mangoni et al., 2005), but less than some dermaseptins (Brand et al., 2002, 2006; Savoia et al., 2006, 2008).

The antiplasmodial effect of PS-1 was evident at the concentration of 16 μg/mL and reached an activity comparable to that of artesunate, the gold-standard drug for the treatment of *P. falciparum* malaria, at the concentration of 64 μg/mL. On a concentration basis, PS-1 showed antiplasmodial activity higher than that of other peptides, as gomesin (Moreira et al., 2007), although lower than that of lipo-dermaseptin derivatives (Dagan et al., 2002; Marynka et al., 2007) and defensin derivative NK-2 (Gelhaus et al., 2008).

Differences in the antimicrobial activity of peptides have been ascribed to the characteristics of their amino acid structures that allow them to perform adequate interactions with the membranes of microorganisms which are anionic (Dolis et al., 1997; Matsuzaki et al., 1998). These interactions can lead to osmotic instability of the cell, resulting in cell swelling and lysis (El Amri et al., 2006). It is possible that the dramatic antiprotozoal activity of PS-1 we demonstrated, particularly toward *L. amazonensis* promastigotes, was due to the fact that the amidated PS-1 molecule is polycationic, which would favour its interaction with the highly anionic membranes of the parasite. Circular dichroism (CD) spectroscopy and multidimensional nuclear magnetic resonance (NMR) evaluation indicate that PS-1 exhibits random coil conformations in aqueous solution, but adopts continuous helix conformations in membrane environments (Resende et al., 2008). The propensity of small-sized cationic peptides to form α-helical amphiphilic structures in apolar medium has been proposed to be a prerequisite for their membranolytic activity, and responsible for their ability to destabilize protozoan cells, and promote cell lysis and death (Leite et al., 2005; Resende et al., 2008).

In contrast to the relatively well-known effects of antimicrobial peptides against bacteria or protozoa, little is known about their effects on different kinds of mammalian cells. Leite et al. (2005) have shown that PS-1 displays very low lytic effect on erythrocytes, and we reported in the present investigation that this peptide exhibits some degree of toxic effect to mouse peritoneal macrophages exclusively at high concentrations, well above those endowed with antiprotozoal activity. The toxicity of PS-1 towards peritoneal macrophages, also described in association to other antimicrobial peptides (see Arias et al., 2006), af-

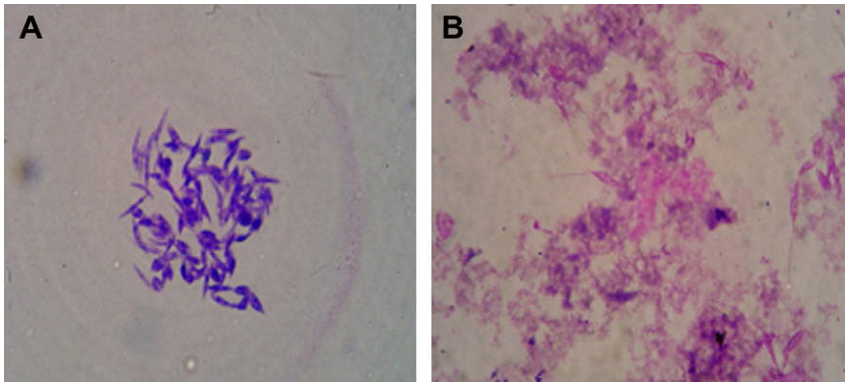

Fig. 2. Photomicrography of *Leishmania amazonensis* promastigotes incubated for 6 h either with RPMI 1640 medium (A) or 64 μg/mL of PS-1 (B). No alteration was detected in the control preparations, while in those incubated with PS-1 an amorphous mass corresponding to dead parasites was observed. Giemsa staining. 1000× magnitude.

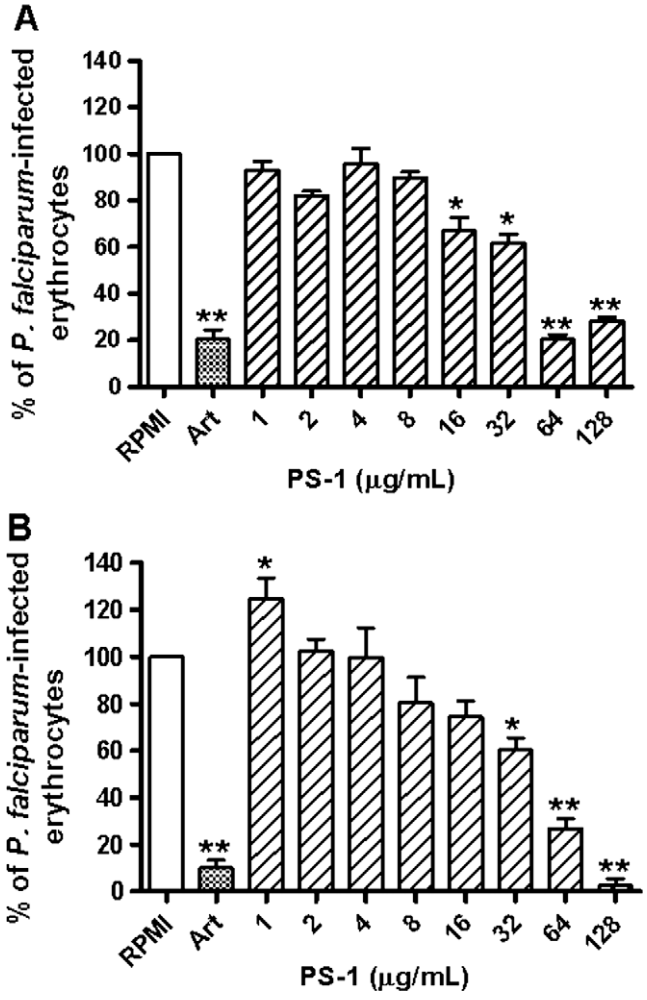

Fig. 3. Percentage of *Plasmodium falciparum*-infected erythrocytes, in vitro incubated with different concentrations of PS-1 for 48 h (A) or 72 h (B). Cultures incubated with RPMI 1640 medium or artesunate (Art) were used as negative and positive controls, respectively. Data correspond to three observations and are represented by mean ± SD. The results, analysed by ANOVA, followed by the Student–Newman–Keuls test, showed differences between control cultures (RPMI) and those incubated with PS-1 or Art (*) $p < 0.01$ and (**) $p < 0.001$.

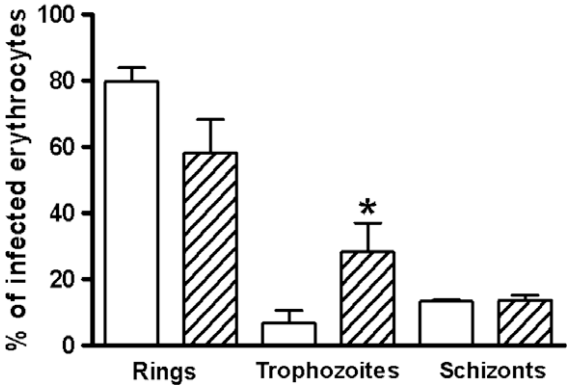

Fig. 4. Percentage of erythrocytes infected with *Plasmodium falciparum* (rings, trophozoites or schizonts) after incubation for 72 h with 1 μg/mL of PS-1 (hatched bars) or RPMI 1640 medium (blank bars). Data, obtained from three observations are represented by mean ± SD. The results showed a 25% increase in the percentage of trophozoite-infected erythrocytes in those cultures treated with PS-1 (*), when compared to those incubated with RPMI 1640 medium alone ($p = 0.014$, Student's *t*-test).

fected both the viability of cells and their ability to adhere to substrates. However, it has been shown that antimicrobial peptides present a degree of selectivity to microorganism cells, when compared to mammal cells. The lower interaction of peptides with mammal cell membranes (Williamson and Schlegel, 1994) could be attributed to their lower external charge (Park et al., 1998; Welling et al., 2000; Lohner and Blondelle, 2005), or to differences in their lipid content, including the presence of cholesterol, absent in the membranes of microorganisms (Sitaram and Nagaraj, 1999).

An unexpected result of our investigation was the increment of *P. falciparum* growth (Fig. 3B), the percentage of adherent macrophages (Fig. 5A), and the acceleration of the transformation of plasmodium ring forms (young trophozoites) into mature trophozoites (Fig. 4), when these cells were incubated with a low concentration of PS-1 (1 μg/mL). A stimulatory effect of antimicrobial peptides has also been described as causing fibroblast proliferation (Reed et al., 1992), and increasing the expression of cell adhesion molecules (Gallo et al., 1994; Gudmundsson and Agerberth, 1999). The reasons for this phenomenon are not as yet clear, but it is possible that it was due to hormesis. Hormesis is defined as an adaptive response of cells and organisms to moderate (usually intermittent) stress (Calabrese, 2002; Rattan, 2004; Mattson, 2008). Cell reaction to either chemical or physical stress involves activation of enzymes such as kinases and deacetylases, and transcription factors such as Nrf-2 and NF-κB, which results in the increased production by cells of cytoprotective and restorative proteins, including growth factors, antioxidant enzymes and protein chaperones (Mattson, 2008; Zhang et al., 2008).

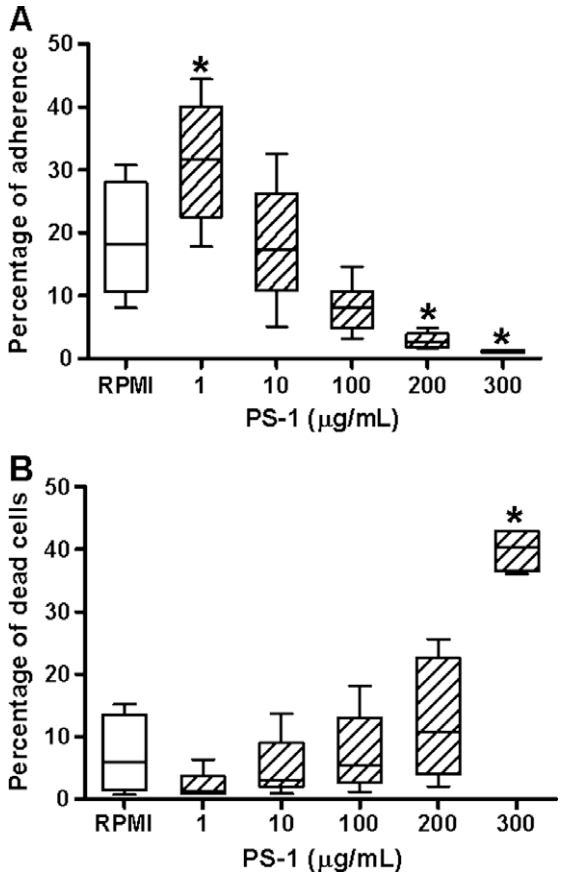

Fig. 5. Toxic effect of different concentrations of PS-1 on mouse peritoneal cells. Toxicity was demonstrated exclusively at very high concentrations of the peptide: at 200 μg/mL or higher a reduction of the percentage of adherent cells was found ($p < 0.001$, Mann–Whitney) (A) and at 300 μg/mL a decrease of viability ($p = 0.004$, Mann–Whitney) (B), when compared to control cultures incubated with RPMI 1640 medium alone. At its lowest concentration (1 μg/mL) PS-1 caused an increase in the percentage of adherent cells ($p = 0.013$, Mann–Whitney). Data, based on eight experiments, are represented by medians, quartiles and extreme values.

In conclusion, we showed that PS-1 was capable to control the growth and to cause in vitro destruction of two important pathogenic protozoa, *L. amazonensis* and *P. falciparum.* Besides, this peptide exhibited some toxic effect to mammalian cells exclusively at very high concentrations, well above those that affect protozoa. These features make PS-1 a potential candidate for further investigation aiming at its future application as a therapeutic agent against malaria and leishmaniasis, either as an alternative or as a complement to conventional treatments.

Acknowledgments

We are grateful to Nelson Nascimento, for excellent technical assistance, to Lucas Souza Kückelhaus, for reviewing the English language of the manuscript, to the Centro Universitário de Brasília, for providing the mice, and to the Brazilian National Research Council, CNPq Universal/2007, PPP/FAPEPI 2006 programs for partially funding the project.

References

Arias, C., Guizy, M., Luque-Ortega, J.R., Guerrero, E., La Torre, B.G., Andreu, D., Rivas, L., Valenzuela, C., 2006. The induction of NOS2 expression by the hybrid cecropin A–melittin antibiotic peptide CA(1–8)M(1–18) in the monocytic line RAW 264.7 is triggered by a temporary and reversible plasma membrane permeation. Biochimica et Biophysica Acta 1763, 110–119.

Boman, H.G., 1991. Antibacterial peptides: key components needed in immunity. Cell 65, 205–207.

Brand, G.D., Leite, J.R.S.A., Silva, L.P., Albuquerque, S., Prates, M.V., Azevedo, R.B., Carregaro, V., Silva, J.S., Sá, V.C.L., Brandão, R.A., Bloch Jr., C., 2002. Dermaseptins from *Phyllomedusa oreades* and *Phyllomedusa distincta*. Anti-*Trypanosoma cruzi* activity without cytotoxicity to mammalian cells. The Journal of Biological Chemistry 277, 49332–49340.

Brand, G.D., Leite, J.R.S.A., de Sa Mandel, S.M., Mesquita, D.A., Silva, L.P., Prates, M.V., Barbosa, E.A., Vinecky, F., Martins, G.R., Galasso, J.H., Kückelhaus, S.A.S., Sampaio, R.N., Furtado Jr., J.R., Andrade, A.C., Bloch Jr., C., 2006. Novel dermaseptins from *Phyllomedusa hypochondrialis* (Amphibia). Biochemical and Biophysical Research Communication 47, 739–746.

Calabrese, E.J., 2002. Hormesis: changing view of the dose–response, a personal account of the history and current status. Mutation Research 511, 181–189.

Caramaschi, U., 2006. Redefinição do grupo de *Phyllomedusa hypochondrialis*, com redescrição de *P. megacephala* (Miranda-Ribeiro 1926), revalidação de *P. azurea* (Cope 1862) e descrição de uma nova espécie (Amphibia, anura, Hylidae). Arquivos do Museu Nacional 64, 159–179.

Chernysh, S., Kim, S.I., Bekker, G., Pleskach, V.A., Filatova, N.A., Anikin, V.B., Platonov, V.G., Bulet, P., 2002. Antiviral and antitumor peptides from insects. Proceedings of the National Academy of Sciences of the United States of America 99, 12628–12632.

Croft, S.L., Yardley, V., 2002. Chemotherapy of leishmaniasis. Current Pharmaceutical Design 8, 319–342.

Croft, S.L., Seifert, K., Yardley, V., 2006. Current scenario of drug development for leishmaniasis. The Indian Journal of Medical Research 123, 399–410.

Dagan, A., Efron, L., Gaidukov, L., Mor, A., Ginsburg, H., 2002. *In vitro* antiplasmodium effects of dermaseptin S4 derivatives. Antimicrobial Agents and Chemotherapy 46, 1059–1066.

Dolis, D., Moreau, C., Zachowski, C., Devaux, P.F., 1997. Aminophospholipid translocase and proteins involved in transmembrane phospholipid traffic. Biophysical Chemistry 68, 221–231.

El Amri, C., Lacombe, C., Zimmerman, K., Ladram, A., Amiche, M., Nicolas, P., Bruston, F., 2006. The plasticins: membrane adsorption, lipid disorders, and biological activity. Biochemistry 45, 14285–14297.

Gallo, R.L., Ono, M., Povsic, T., Page, C., Eriksson, E., Klagsbrun, M., Bernfield, M., 1994. Syndecans, cell surface heparan sulfate proteoglycans, are induced by a proline-rich antimicrobial peptide from wounds. Proceedings of the National Academy of Sciences of the United States of America 91, 11035–11039.

Gelhaus, C., Jacobs, T., Andrä, J., Leippe, M., 2008. The antimicrobial peptide NK-2, the core region of mammalian NK-lysin, kills intraerythrocytic *Plasmodium falciparum*. Antimicrobial Agents and Chemotherapy 52, 1713–1720.

Ghosh, J.K., Shaoo, D., Guillaud, P., Cicéroni, L., Mazieri, D., Kustanovich, I., Shai, Y., Mor, A., 1997. Selective cytotoxicity of dermaseptin S3 toward intraerythrocytic *Plasmodium falciparum* and the underlying molecular basis. The Journal of Biological Chemistry 272, 31609–31616.

Gudmundsson, G.H., Agerberth, B., 1999. Neutrophil antibacterial peptides, multifunctional effector molecules in the mammalian immune system. Journal of Immunological Methods 232, 45–54.

Gudmundsson, G.H., Agerberth, B., Odeberg, J., Bergman, T., Olsson, B., Salcedo, R., 1996. The human gene FALL39 and processing of the cathelin precursor to the antibacterial peptide LL-37 in granulocytes. European Journal of Biochemistry 238, 325–332.

Kückelhaus, S.A.S., Leite, J.R.S.A., Neves, M.P., Frota, K.S., Abdala, L.F., Muniz-Junqueira, M.I., Bloch Jr., C., Tosta, C.E., 2006. Toxicity evaluation to mice of phylloseptin-1, an antimicrobial peptide from the skin secretion of *Phyllomedusa hypochondrialis* (Amphibia). International Journal of Peptide Research and Therapeutics 13, 423–429.

Lee, D.G., Shin, S.Y., Kim, D.H., Seo, M.Y., Kang, J.H.Y.L., Kim, K.L., Hahm, K.S., 1999. Antifungal mechanism of a cysteine-rich antimicrobial peptide, Ib-Amp1, from *Impatiens balsamina* against *Candida albicans*. Biotechnology Letters 21, 1047–1050.

Leite, J.R.S.A., Silva, L.P., Rodrigues, M.I.S., Prates, M.V., Brand, G.D., Lacava, B.M., Azevedo, R.B., Bocca, A.L., Albuquerque, S., Bloch Jr., C., 2005. Phylloseptins: a novel class of anti-bacterial and anti-protozoan peptides from the *Phyllomedusa* genus. Peptides 26, 565–573.

Löfgren, S.E., Miletti, L.C., Steindel, M., Bachère, E., Barracco, M.A., 2008. Trypanocidal and leishmanicidal activities of different antimicrobial peptides (AMPs) isolated from aquatic animals. Experimental Parasitology 118, 197–202.

Lohner, K., Blondelle, S.E., 2005. Molecular mechanisms of membrane perturbation by antimicrobial peptides and the use of biophysical studies in the design of novel peptide antibiotics. Combinatorial Chemistry and High Throughput Screening 8, 241–256.

Mangoni, M.L., Saugar, J.M., Dellisanti, M., Barra, D., Simmaco, M., Rivas, L., 2005. Temporins, small antimicrobial peptides with leishmanicidal activity. The Journal of Biological Chemistry 280, 984–990.

Marynka, K., Rotem, S., Portnaya, I., Cogan, U., Mor, A., 2007. In vitro discriminative antipseudomonal properties resulting from acyl substitution of n-terminal sequence of dermaseptin S4 derivatives. Chemistry and Biology 14, 75–85.

Mattson, M.P., 2008. Hormesis defined. Ageing Research Reviews 7, 1–7.

Matsuzaki, K., Mitani, Y., Akada, K.Y., Murase, O., Yoneyama, S., Zasloff, M., Miyajima, K., 1998. Mechanism of synergism between antimicrobial peptides magainin 2 and PGLa. Biochemistry 37, 15144–15153.

Merrifield, R.B.J., 1963. Solid phase peptide synthesis. Journal of the American Chemical Society 85, 2149–2156.

Moreira, C.K., Rodrigues, F.G., Ghosh, A., Varotii, F.P., Miranda, A., Daffre, S., Jacobs-Lorena, M., Moreira, L.A., 2007. Effect of the antimicrobial peptide gomesin against different life stages of *Plasmodium* spp. Experimental Parasitology 116, 346–353.

Nicolas, P., Mor, A., 1995. Peptides as weapons against microorganisms in the chemical defense system of vertebrates. Annual Review of Microbiology 49, 277–304.

Park, C.B., Kim, H.S., Kim, S.C., 1998. Mechanism of action of the antimicrobial peptide buforin II: buforin II kills microorganisms by penetrating the cell membrane and inhibiting cellular functions. Biochemical and Biophysical Research Communication 244, 253–257.

Peschel, A., 2002. How do bacteria resist human antimicrobial peptides? Trends in Microbiology 10, 179–186.

Rattan, I.S.S., 2004. Aging, anti-aging, and hormesis. Mechanism of Ageing and Development 125, 285–289.

Reed, W.A., White, K.L., Enright, F.M., Holck, J., Jaynes, J.M., Jeffers, G.W., 1992. Enhanced *in vitro* growth of murine fibroblast cells and preimplantation embryos cultured in medium supplemented with an amphipathic peptide. Molecular Reproduction and Development 31, 106–113.

Resende, J.M., Moraes, C.M., Prates, M.V., Cesar, A., Almeida, F.C.L., Mundim, N.C.C.R., Valente, A.P., Bemquerer, M., Piló-Veloso, D., Bechinger, B., 2008. Solution NMR structures of the antimicrobial peptides phylloseptin-1, -2, and -3 and biological activity: the role of charges and hydrogen bonding interactions in stabilizing helix conformations. Peptides 29, 1633–1644.

Savoia, D., Scutera, S., Raimondo, S., Conti, S., Magliani, W., Polonelli, L., 2006. Activity of an engineered synthetic killer peptide on *Leishmania major* and *Leishmania infantum* promastigotes. Experimental Parasitology 113, 186–192.

Savoia, D., Guerrini, R., Marzola, E., Salvadori, S., 2008. Synthesis and antimicrobial activity of dermaseptin S1 analogues. Bioorganic and Medicinal Chemistry 16, 8205–8209.

Sitaram, N., Nagaraj, R., 1999. Interaction of antimicrobial peptides with biological and model membranes: structural and charge requirements for activity. Biochimica et Biophysica Acta 1462, 29–54.

Welling, M.M., Paulusma-Annema, A., Balter, H.S., Pauwels, E.K., Nibbering, P.H., 2000. Technetium-99m labelled antimicrobial peptides discriminate between bacterial infections and sterile inflammations. European Journal of Nuclear Medicine 27, 292–301.

Williamson, P., Schlegel, R.A., 1994. Back and forth: the regulation and function of transbilayer phospholipid movement in eukaryotic cells. Molecular Membrane Biology 11, 199–216.

Wongsrichanalai, C., Pickard, A.L., Wernsdorfer, W.H., Meshnick, S.R., 2002. Epidemiology of drug-resistant malaria. The Lancet Infectious Diseases 2, 209–218.

Zhang, Q., Pi, J., Woods, C.G., Jarabek, A.M., Clewell III, H.J., Andersen, M.E., 2008. Hormesis and adaptive cellular control systems. Dose Response 6, 196–208.