Acta Tropica 122 (2012) 224–229

Contents lists available at SciVerse ScienceDirect

Acta Tropica

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

Short communication

Anti-*Trypanosoma cruzi* activity of nicotinamide

Milena B.P. Soares[a,b,*], Cinara V. Silva[a], Tanira M. Bastos[a], Elisalva T. Guimarães[a], Claudio P. Figueira[a], Despina Smirlis[c], Walter F. Azevedo Jr.[d,e]

[a] Centro de Pesquisas Gonçalo Moniz, Fundação Oswaldo Cruz, Rua Waldemar Falcão, 121, Candeal 40296-710, Salvador, BA, Brazil
[b] Hospital São Rafael, Av. São Rafael, 2152, São Marcos 41253-190, Salvador, BA, Brazil
[c] Laboratory of Molecular Parasitology, Microbiology Dpt, Hellenic Pasteur Institute, 127 Vasilissis Sofias avenue, 11521 Athens, Greece
[d] Faculdade de Biociências, Instituto Nacional de Ciência e Tecnologia em Tuberculose-CNPq, Laboratório de Bioquímica Estrutural, Pontifícia Universidade Católica do Rio Grande do Sul – PUCRS, Porto Alegre, Brazil
[e] Programa de Pós-Graduação em Medicina e Ciências da Saúde, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, RS, Brazil

ARTICLE INFO

Article history:
Received 13 May 2011
Received in revised form
13 December 2011
Accepted 1 January 2012
Available online 18 January 2012

Keywords:
Sirtuin
Trypanosoma cruzi
Nicotinamide
Virtual screening

ABSTRACT

Inhibition of *Trypanosoma brucei* and *Leishmania* spp. sirtuins has shown promising antiparasitic activity, indicating that these enzymes may be used as targets for drug discovery against trypanosomatid infections. In the present work we carried out a virtual screening focused on the C pocket of Sir2 from *Trypanosoma cruzi*. Using this approach, the best ligand found was nicotinamide. In vitro tests confirmed the anti-*T. cruzi* activity of nicotinamide on epimastigote and trypomastigote forms. Moreover, treatment of *T. cruzi*-infected macrophages with nicotinamide caused a significant reduction in the number of amastigotes. In addition, alterations in the mitochondria and an increase in the vacuolization in the cytoplasm were observed in epimastigotes treated with nicotinamide. Analysis of the complex of Sir2 and nicotinamide revealed the details of the possible ligand–target interaction. Our data reveal a potential use of TcSir2 as a target for anti-*T. cruzi* drug discovery.

1. Introduction

Trypanosoma cruzi is a hemoflagellate protozoan parasite causative of Chagas' disease, or American Trypanosomiasis, a disease affecting 16–18 millions of persons mainly in Latin American countries (WHO, 2002). It is transmitted by reduviid bugs to mammalian hosts, and in humans the infection courses with two phases. The acute phase is characterized by intense blood parasitemia and tissue parasitism, whereas in the chronic phase the parasitism is scarce, but persistent. The chronic symptomatic form of the disease appears in about 30% of the individuals, and manifests as cardiomyopathy, megasyndromes (megacolon and megaesophagus), or both forms (Soares et al., 2001). Currently there are two drugs available for the treatment of Chagas' disease, Benznidazole and Nifurtimox, which are endowed with high toxicity and low efficacy in the chronic phase of infection. Thus, there is a great need for new drugs more effective against the parasite and less toxic to humans (Moreira et al., 2009).

Sirtuins are NAD-dependent deacetylases conserved from bacteria to mammals, and genes coding for seven sirtuins (SIRT 1–7) have been found in the human genome. Sirtuin catalyzes the deacetylation of acetylated lysine residues of histone and non-histone substrates. The structural basis for inhibition of sirtuins has been established through previous structural and functional studies (Denu, 2003, 2005; García-Salcedo et al., 2003; Hoff et al., 2006). Involvement of sirtuins in the cell cycle strongly suggests a role for these enzymes in cancer and the potential use of their inhibitors as anticancer drugs (Irwin and Shoichet, 2005). In addition, inhibition of sirtuins from *Trypanosoma brucei* and *Leishmania* sp. showed promising results, indicating that these enzymes may be considered as targets for drug discovery in parasite infection (Jackson et al., 2003; Kadam et al., 2006, 2008; Kowieski et al., 2008).

Several structures of complexes involving sirtuins and inhibitors have been reported (Hoff et al., 2006; Lipinski et al., 1997; Moreira et al., 2009). Nicotinamide, a well known sirtuin inhibitor, is a water-soluble vitamin of the B complex, which together with nicotinic acid belongs to vitamin B3 or vitamin PP and it acts as constituent of the enzyme cofactors NAD (nicotinamide adenine dinucleotide) and NADP (nicotinamide adenine dinucleotide phosphate) (pyridine nucleotides). These molecules function as electron carriers in cell metabolism of carbohydrates, fatty acids and amino acids. Nicotinamide has been used to treat pellagra, osteoarthritis and is currently in trials as a therapy to prevent cancer recurrence

* Corresponding author at: Centro de Pesquisas Gonçalo Moniz, FIOCRUZ, Rua Waldemar Falcão, 121, Candeal 40296-710, Salvador, BA, Brazil. Tel.: +55 71 3176 2260; fax: +55 71 3176 2272.
E-mail address: milena@bahia.fiocruz.br (M.B.P. Soares).

0001-706X/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.actatropica.2012.01.001

and insulin-dependent (type I) diabetes. This vitamin is safe even when administered at high dosage (6 g/day) in human (Flodin, 1988; Sereno et al., 2005; Gazanion et al., 2011).

In this context, this study reports homology modeling of Sir2 from *T. cruzi* (TcSir2) and structure-based virtual screening (SBVS) focused on the C pocket of TcSir2. The best hit identified in the SBVS, nicotinamide, was submitted to biological activity test, which confirmed its anti-*T. cruzi* activity.

2. Materials and methods

2.1. TcSir2 modeling, docking analysis and virtual screening

The web server PARMODEL was used to model the structure of Sir2 (EC 3.5.1.) from *T. cruzi* (gene name: Tc00.1047053447255.20) (Nguewa et al., 2004; Oprea et al., 2001). The complete amino acid sequence of TcSir2 was obtained from the NCBI protein database (Q4CNV0_TRYCR; SWISSPROT Accession Number Q4CNV0-1). We used the atomic coordinates of Sir2 from *Thermotoga maritima* (TmSir2) (PDB access code: 1yc5), which presents 33% identity with Sir2 from *T. cruzi* (TcSir2). We applied the flexible docking protocol available in the program MolDock (Sali and Blundell, 1993). In order to validate the present docking protocol we performed the docking simulation against the nicotinamide-binding pocket (also known as C pocket) of TcSir2 and compared with the modeled structure, obtained directly from the crystallographic structure of complex Sir2-nicotinamide (1yc5). We used the default protocol of MolDock with center at coordinates $x = 5.73$, $y = 24.93$ and $z = 8.31$ Å and docking sphere with radius of 7 Å. It has been proposed that nicotinamide is an important template for inhibition of Sir2 from *Leishmania* (Kowieski et al., 2008). Based on this observation, we employed the nicotinamide core to carry out a search in the ZINC database to build a small-molecule database, using the fingerprint of nicotinamide with a Tanimoto coefficient cutoff of 90% (Sauve et al., 2001). A total of 159 molecules were retrieved and used to build this database (Sauve and Schramm, 2004).

2.2. Anti-T. cruzi assay on epimastigote and trypomastigote forms

Epimastigotes of *T. cruzi* (Y and Colombian strains) were cultured at 26 °C in liver infusion tryptose medium (LIT) supplemented with 10% fetal bovine serum (FBS) (Cultilab, Campinas, SP, Brazil), 1% hemin (Sigma, St. Louis, MO, USA), 1% R9 medium (Sigma), and 50 μg/mL gentamycin (Sigma). Parasites (10^7 cells/mL) were cultured in fresh medium in the absence or presence of nicotinamide (Sigma, St. Louis, MO, USA) at various concentrations (2.46 mM, 0.82 mM, 0.27 mM, 0.09 mM, 0.03 mM, and 0.009 mM), in triplicates. Cell growth was determined after culture for 3–6 days by counting viable forms in a hemocytometer. Trypomastigote forms of Y and Colombian *T. cruzi* strains were obtained from supernatants of infected LCC-MK2 cell cultures and cultured in 96-well plates (4×10^5/well) in DMEM supplemented with 10% FBS and 0.10 mM gentamycin in the absence or presence of different concentrations of nicotinamide, in triplicates. Viable parasites were counted in a hemocytometer 24 h later. The percentage of inhibition was calculated in relation to untreated cultures.

2.3. In vitro T. cruzi infection assay

Peritoneal exudate cells were obtained by washing with saline solution the peritoneal cavity of BALB/c mice five days after injection of 3% thioglycollate (1.5 ml per mouse). All animals were maintained at the animal facilities at the Gonçalo Moniz Research Center – FIOCRUZ, Salvador, Bahia, Brazil and the protocol was approved by the Animal Ethics Committee of the Centro de Pesquisas Gonçalo Moniz – Fiocruz (protocol number L-029-09). Peritoneal exudate cells were washed twice with saline and resuspended in RPMI medium (GIBCO-BRL, Gaithersburg, MD) supplemented with 10% FBS, L-glutamine (2 mM), sodium pyruvate (1 mM), HEPES (10 mM), and gentamycin (0.10 mM). To evaluate the trypanocidal activity of nicotinamide on amastigote forms, peritoneal exudate cells were plated at 2×10^5 cells/well in 24-well plates with glass coverslips in the bottom and cultured during 24 h prior to infection. Macrophages were infected with trypomastigotes of Y strain at a ratio of 10 parasites per macrophage. After 2 h of infection, the free trypomastigotes were removed by successive washes using saline solution. Cultures were incubated in complete medium alone or with nicotinamide (0.09 mM and 0.9 mM) or 0.08 mM benznidazole (used as a reference trypanocidal drug). Six hours later, wells were washed and cultures were incubated in complete medium for 4 days. Cells were then fixed in methanol and the percentage of infected macrophages and the mean number of amastigotes/infected macrophages were determined by counting the slides after Giemsa staining using an optical microscope (Olympus, Tokyo, Japan), by counting 100 cells per slide.

2.4. Electron microscopy analysis

Epimastigotes of Y strain *T. cruzi* were incubated for 6 days at 26 °C in the absence or presence of nicotinamide. After incubation, the parasites were fixed for 24 h at 4 °C with 2.0% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) and were post-fixed for 1 h at 4 °C with 1% OsO_4 with the same buffer. The samples were then routinely processed for transmission electron microscopy and were examined in an EM109 electron microscope (Zeiss, Germany).

2.5. Statistical analyses

To determine the IC_{50} value, we used nonlinear regression on Prism 5.02 GraphPad software. Student's *t* test was applied to ascertain the statistical significance of the observed differences in IC_{50} values. The one-way ANOVA and Bonferroni tests were used to determine the statistical significance of the group comparisons in the in vitro infection study. Results were considered statistically significant when $P < 0.05$.

3. Results

3.1. TcSir2 modeling

First, we performed a multiple alignment of TcSir2 with the canonical fold of the sirtuins from *T. brucei* (Tb927.7.1690), *L. infantum* (LinJ.26.0200) and the *Homo sapiens* SIRT1 (uniprot id: Q96EB6) (Fig. 1). We then performed homology modeling of TcSir2. The structure obtained from homology modeling presents the canonical fold of sirtuins, as shown in Fig. 2. The TcSir2 structure presents a bilobal structure with the larger domain consisting predominantly of a modified Rossmann fold, found in many diverse NAD(H)/NADP(H) binding enzymes, and the minor domain that contains a structural zinc atom. At the interface of the two domains, there is a large groove formed by the four crossovers and three loops of the large domain. The larger lobe is formed by six β-strands that shape a parallel β-sheet. This central β sheet is tightly surrounded by six α helices. Between the two lobes there is a cleft where NAD^+ binds. There is a zinc-binding pocket, where the zinc atom is coordinated by Cys 121, Cys 144, and Thr124.

3.2. Docking studies on TcSir2

The use of the MolDock to the structure of TcSir2 in complex with nicotinamide was capable of correctly predicting the

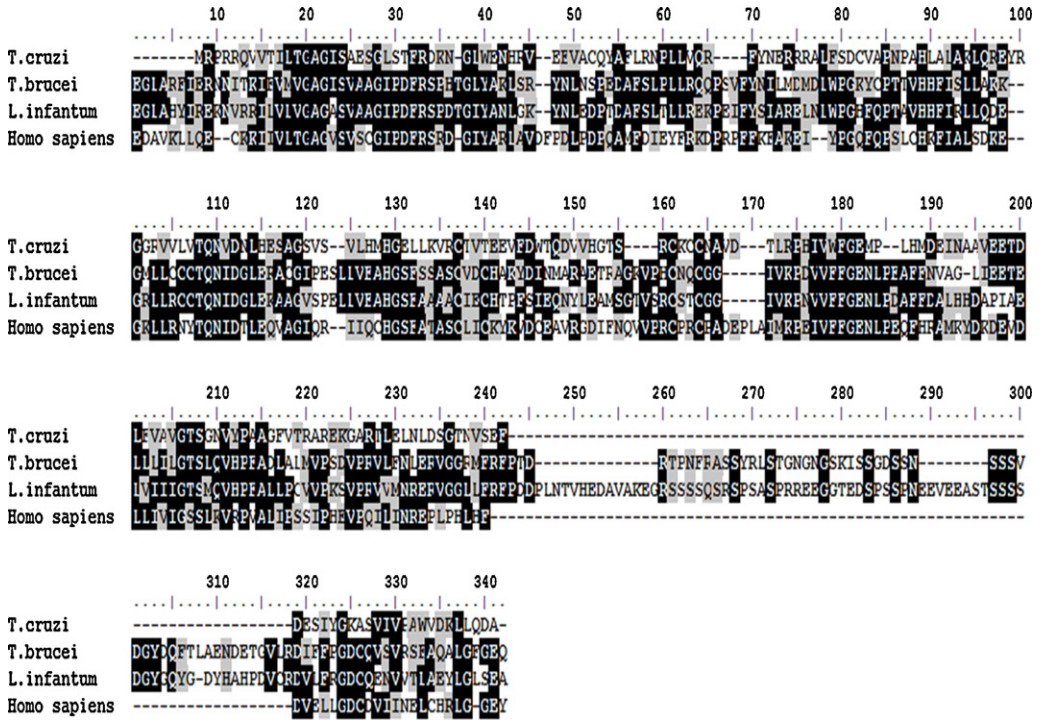

Fig. 1. Multiple-sequence alignment of the sirtuin catalytic domain. Multiple-sequence alignment of the sirtuin catalytic domain in *Tc*Sir2 (template Tc00.1047053447255.20, whole protein), in *T. brucei* (template Tb927.7.1690, aminoacid residues 18–327), in *L. infantum* (template LinJ.26.0200, aminoacid residues 20–351) and in *Homo sapiens* SIRT1 (template uniprot id: Q96EB6). Identical residues are indicated in black shading and similar residues in gray. The multiple sequence alignment was based on the Clustal W algorithm.

nicotinamide positioning in the binding pocket of TcSir2, the RMSD of superposition is 0.59 Å. Similar docking protocols have been employed in SBVS focused on Sir2 from *Leishmania* (Kowieski et al., 2008). Since this docking protocol seems capable of reproducing the crystallographic structure, we applied it to a dataset of molecules which presents nicotinamide core. Nicotinamide presents several structural properties that make it a promising template for SBVS, such as cLog $P = -0.4$, molecular weight of 122.13 g/mol, number of rotatable bonds = 1, polar surface area = 56 Å^2, H-bond donor = 1 and H-bond acceptor = 2. All these values satisfy the Lipinski and Veber rules (Schmidt et al., 2004; Schuetz et al., 2007). In addition, combination of the ideas of lead-likeness, template conservation and fragment-based screening has generated guidelines for the structural properties of scaffolds employed in screening libraries. This knowledge is incorporated in the 'rule of 3', which is also satisfied by nicotinamide (Soares et al., 2001). It has been observed that nicotinamide inhibits the deacetylation activity of SIRT1 by interacting with a reaction intermediate. The catalyzed deacetylation conducts to production of 2′- and 3′-O-acetyl-ADP-ribose and deacetylated lysine. If nicotinamide binds to the enzyme when it contains the O-akyl-amidate intermediate, nicotinamide can react with the

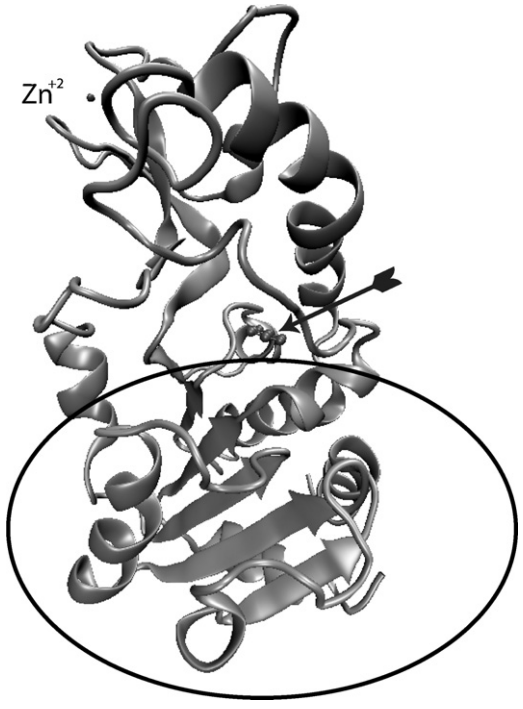

Fig. 2. Structure of TcSir2. The ellipse indicates the larger domain, and the arrow indicates the C pocket.

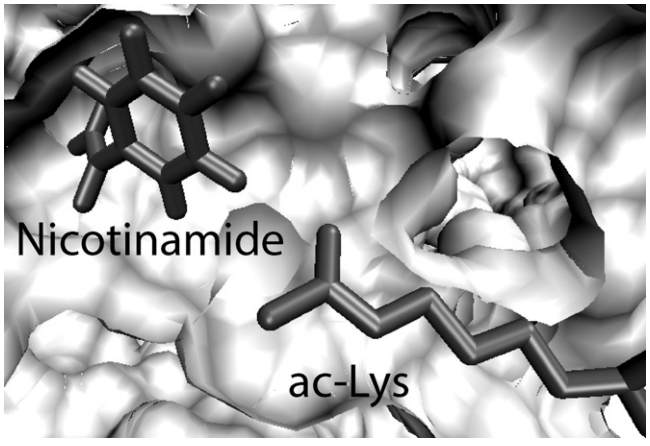

Fig. 3. C pocket of TcSir2 showing nicotinamide and acetylate lysine.

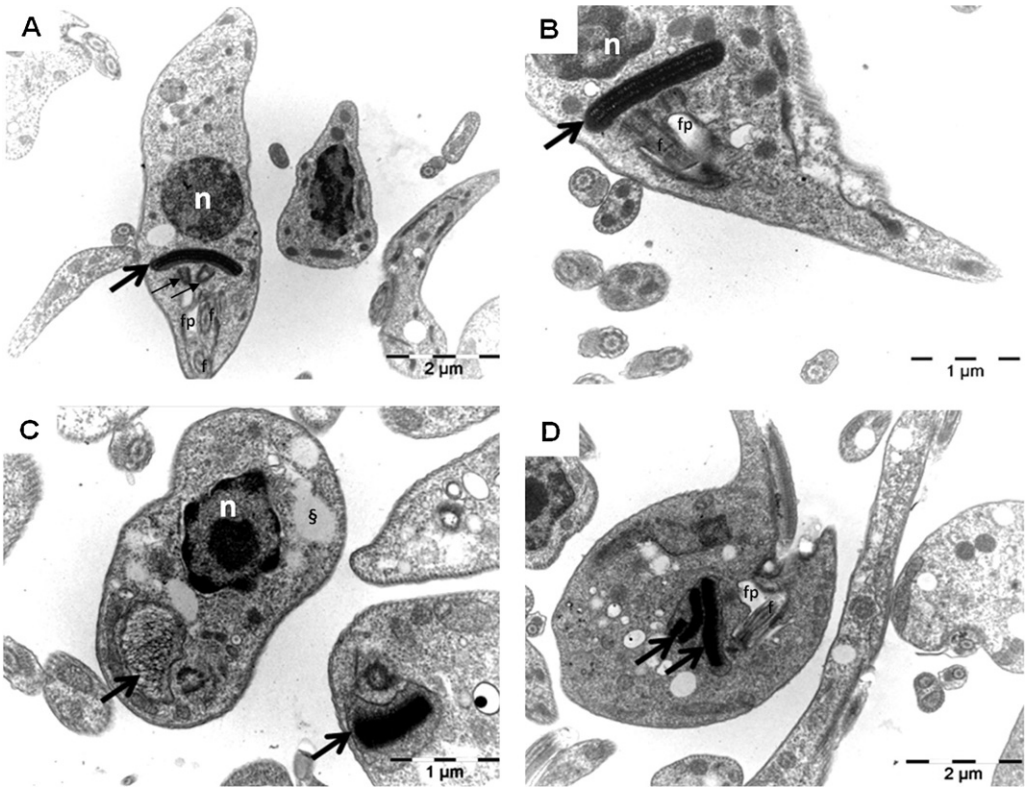

Fig. 4. Alterations in *T. cruzi* epimastigotes caused by nicotinamide. Y strain *T. cruzi* epimastigotes were cultured for 6 days in the absence (A and B) or presence (C and D) of nicotinamide (2.46 mM). Transmission electron micrographs showing alterations in the mitochondria and kinetoplast in C and D, compared to untreated controls (A and B). Arrows indicate the kinetoplasts; (n) nucleus; (fp) flagellar pocket; (f) flagellum; (↑) basal corpuscle; (§) lipid inclusions.

intermediate in a procedure known as nicotinamide exchange, in which NAD^+ and N6-acetyl-lysine are reformed (Szczepankiewicz and Ng, 2008; Taunton et al., 1996; Thomsen and Christensen, 2006; Timmers et al., 2008). Furthermore, the rate of the nicotinamide exchange reaction can be increased by raising the nicotinamide concentration, which happens at expense of the deacetylation activity. These studies suggest that this moiety deserves further investigation as a potential sirtuin inhibitor.

Docking simulations of these molecules against the active site of TcSir2 returned nicotinamide as the best ligand (lowest score: −48.67). Fig. 3 shows nicotinamide docked to TcSir2. Analysis of the positioning of nicotinamide in the C pocket indicates that it binds in the substrate binding cleft contiguous to the acetyl lysine side chain (Fig. 3). Nicotinamide presents intermolecular interactions with main-chain oxygen from Trp32, side-chain oxygen of Asp98, and main chain nitrogen from Asp98. These residues outline a hydrophobic pocket, known as the C pocket, and forms hydrogen bond interactions that rotate the carboxamide approximately 150° from its favored coplanar conformation with the nicotinamide ring. The residues Asp 98 presents strong intermolecular hydrogen bonds with the carboximide moiety, which functions as an anchor to fix nicotinamide in the C pocket. The positioning of nicotinamide in the C pocket and the rotation of the carboxamide group are comparable to what has been observed in the structure of Sir2Af2 bound NAD^+ in the "productive" conformation (Szczepankiewicz and Ng, 2008).

3.3. Nicotinamide inhibits the growth and viability of T. cruzi

The growth of epimastigotes from Y and Colombian *T. cruzi* strains in the presence of nicotinamide was inhibited in a concentration-dependent manner in axenic cultures. The IC_{50} values are shown in Table 1. The observation by light microscopy of epimastigotes cultured in the presence of nicotinamide showed the presence of rounded parasites, some of them in division, whereas in control cultures epimastigotes were highly mobile and elongated (data not shown). Transmission electron microscopy analysis was performed in order to evaluate morphological and ultra-structural alterations in nicotinamide-treated parasites. As shown in Fig. 4A and B, control parasites presented normal mitochondria with bar-shaped kinetoplasts. In contrast, epimastigotes treated with 2.46 mM, but not 0.82 mM nicotinamide, had alterations in the mitochondria, such as swelling and disorganization of the kinetoplast and an increase in the vacuolization in the cytoplasm (Fig. 4C and D). Approximately 60% of the parasites presented these ultra-structural alterations. Treatment of trypomastigotes of Y and Colombian *T. cruzi* strains with nicotinamide had a cytotoxic effect in a concentration-dependent manner (Fig. 5A and B).

The effects of nicotinamide on the intracellular form of the parasite were also evaluated in cultures of macrophages infected with *T. cruzi*. Treatment of macrophage cultures with nicotinamide caused a significant reduction in the percentage of macrophages infected by *T. cruzi* and in the number of intracellular parasites, in concentrations non-toxic to macrophages (Fig. 6A and B).

Table 1
IC_{50} values of nicotinamide on *T. cruzi* epimastigotes.

T. cruzi strain	3 days	6 days	P^a
Y	0.037 ± 0.016	0.126 ± 0.025	0.0145
Colombian	0.025 ± 0.001	0.100 ± 0.007	0.0004

Data are expressed as means ± SEM in mM of 3–6 independent experiments performed.

[a] Compared between the third and sixth day/each strain.

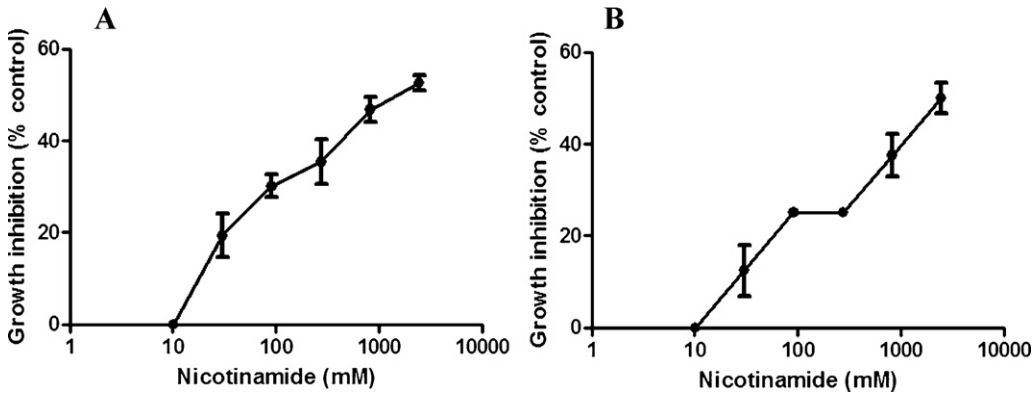

Fig. 5. Effects of nicotinamide on trypomastigote forms of *T. cruzi*. Trypomastigotes of Y (A) and Colombian (B) *T. cruzi* strains were incubated with nicotinamide at different concentrations. The number of viable trypomastigotes was determined 24 h later by counting the parasite preparations in a hemacytometer. Results are expressed as means ± SEM of three independent experiments performed.

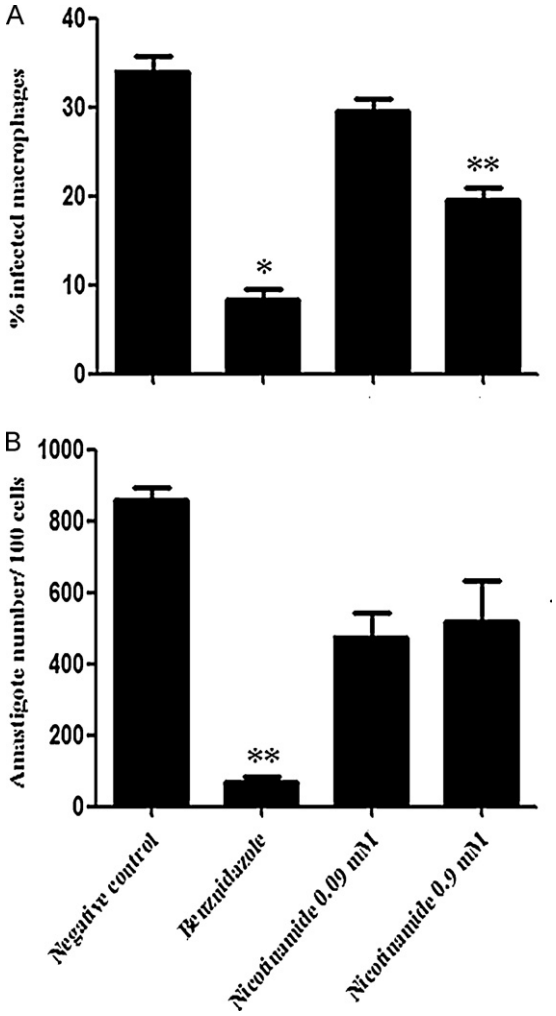

Fig. 6. Effects of nicotinamide on macrophages infected with *T. cruzi*. Peritoneal macrophages were infected with Y stran trypomastigotes at a ratio 10:1 (parasites/macrophages), and treated with benznidazole (0.08 mM) and nicotinamide (0.09 and 0.9 mM) or not (control), as described in Section 2. The percentage of infected macrophages (A) and the number of amastigotes (B) were determined by counting Giemsa-stained slides using a light microscope. Results are expressed as means ± SD of triplicates, and represent one of two independent experiments performed. $^{*}P < 0.05$ vs. control; $^{**}P < 0.01$ vs. control.

4. Discussion

In this study we show the antitrypanosomal properties of nicotinamide. Although not much is known on the mechanisms of entry of nicotinic acid into trypanosoma parasites, we believe that since these parasites rely on NADP levels for their survival (Leroux et al., 2011), they should possess functional nicotinic acid/nicotinamide transporters. Glucose transporters and proteins belonging to the major facilitator superfamily have been implicated in the transport of nicotinate/nicotinamide (Jeanguenin et al., 2011; Sofue et al., 1992). Homologs of this major facilitator superfamily are also present in *T. cruzi* (i.e. glucose transporter). We have shown here that nicotinamide is a potential inhibitor of *T. cruzi* Sir2. We have also performed a molecular modeling of TcSir2. Little is known about sirtuins from *T. cruzi*, compared to other trypanosomatids. The expression of Sir2 homolog in amastigotes of Y strain *T. cruzi* was suggested by using antibodies raised against the *Leishmania major* Sir2 recombinant protein *Lm*Sir2 (Uchoa et al., 2004). In *T. brucei*, a Sir2-related protein (TbSir2RP1) was cloned and characterized from the insect form of the parasite (Veber et al., 2002). This protein is found located in the nucleus and kinetoplast, associated to chromosome, and is a NAD-dependent ADP-ribosyltransferase which also catalyzes the deacetylation of histones, mainly H2A and H2B. TbSir2RP1 confers resistance to DNA damage caused by the DNA alkylating agent MMS (Veber et al., 2002). Moreover, Alsford et al. (2007) identified, in the mammalian-infective bloodstream-stage of *T. brucei*, three SirT2 homologs, Sir2rp1-3 (Zemzoumi et al., 1998). Sir2rp1 had a nuclear localization, while Sir2rp2 and Sir2rp3 were found in the mitochondria. The role of the *T. cruzi* Sir2 homolog is unknown, but the alterations in the kinetoplast and growth arrest observed in our study suggest that this protein also play an important role in DNA repair in *T. cruzi*.

It has been proposed that Sir2 is a promising target for development of antitrypanosomal, antiplasmodial and antileishmanial drugs (Kadam et al., 2008; Prusty et al., 2008; Kowieski et al., 2008). The sequence alignment between the *T. cruzi* TcSir2 and the *Homo sapiens* SIRT1 protein suggests that despite the homology there are many differences in the aminoacid sequence of the sirtuin catalytic domain that could be useful for the evaluation of this protein as a putative drug target. In addition, an in silico structural and surface analysis of trypanosomal and human sirtuins suggested the presence of potentially important structural differences in the inhibitor binding domain of these proteins, indicating a possible selectivity of an inhibitor to a specific protein (Kaur et al., 2010). Sereno et al. (2005) showed that overexpression of a *Leishmania* cytoplasmic SIR2-related protein promoted the survival of amastigote forms by preventing programmed cell death. The authors suggested that,

since nicotinamide is an inhibitor of certain deacetylase SIR2 proteins, it may have an impact on the parasite growth by interfering with metabolic processes involving deacetylase activities dependent on SIR2-like proteins (Sereno et al., 2005). In our tests, SBVS focused on TcSir2 identified nicotinamide as the best ligand from a small-molecule database of 159 molecules. In vitro tests confirmed the anti-*T. cruzi* activity of this molecule, both against epimastigote and trypomastigote forms of the parasite, in two different strains. In addition, nicotinamide was found active against amastigote forms in an in vitro infection model. The inhibition of TcSir2 arrested the parasite growth, implicating that it deregulated the parasite cell-cycle. This is in accordance with Sereno et al. (2005), who suggested that even at concentration as high as 80 mM, nicotinamide does not kill instantly the *T. brucei* and *Leishmania*, but inhibits the parasite growth.

Since nicotinamide presents low molecular weight and low number of hydrogen donors and acceptors, its scaffold is adequate for further optimization in order to enhance target affinity. Nicotinamide is very cheap, has low toxicity and can be administered at a high dose (10 g/day) (Prusty et al., 2008). Further studies are needed in order to understand the role of Sir2 on *T. cruzi*.

Acknowledgments

This work was supported by grants from CNPq, PRONEX, FAPESB and RENORBIO. MBPS and WFA are senior researchers for CNPq (Brazil).

References

Alsford, S., Kawahara, T., Isamah, C., Horn, D., 2007. A sirtuin in the African trypanosome is involved in both DNA repair and telomeric gene silencing but is not required for antigenic variation. Mol. Microbiol. 63, 724–736.

Denu, J.M., 2003. Linking chromatin function with metabolic networks: Sir2 family of NAD(+)-dependent deacetylases. Trends Biochem. Sci. 28, 41–48.

Denu, J.M., 2005. Vitamin B3 and sirtuin function. Trends Biochem. Sci. 30, 479–483.

Flodin, N.W., 1988. Niacin and niacinamide. Curr. Top. Nutr. Dis. 20, 129–138.

García-Salcedo, J.A., Gijón, P., Nolan, D.P., Tebabi, P., Pays, E., 2003. A chromosomal SIR2 homologue with both histone NAD-dependent ADP-ribosyltransferase and deacetylase activities is involved in DNA repair in *Trypanosoma brucei*. EMBO J. 22, 5851–5862.

Gazanion, E., Vergnes, B., Seveno, M., Garcia, D., Oury, B., Ait-Oudhia, K., Ouaissi, A., Sereno, D., 2011. In vitro activity of nicotinamide/antileishmanial drug combinations. Parasitol. Int. 60, 19–24.

Hoff, K.G., Avalos, J.L., Sens, K., Wolberger, C., 2006. Insights into the sirtuin mechanism from ternary complexes containing NAD+ and acetylated peptide. Structure 14, 1231–1240.

Irwin, J.J., Shoichet, B.K., 2005. ZINC—a free database of commercially available compounds for virtual screening. J. Chem. Inf. Model. 45, 177–182.

Jackson, M.D., Schmidt, M.T., Oppenheimer, N.J., Denu, J.M., 2003. Mechanism of nicotinamide inhibition and transglycosidation by Sir2 histone/protein deacetylases. J. Biol. Chem. 278, 50985–50998.

Jeanguenin, L., Lara-Núñez, A., Rodionov, D.A., Osterman, A.L., Komarova, N.Y., Rentsch, D., Gregory 3rd, J.F., Hanson, A.D., 2011. Comparative genomics and functional analysis of the NiaP family uncover nicotinate transporters from bacteria, plants, and mammals. Funct. Integr. Genomics, Sep 28, 2011 (Epub ahead of print).

Kadam, R.U., Kiran, V.M., Roy, N., 2006. Comparative protein modeling and surface analysis of *Leishmania* sirtuin: a potential target for antileishmanial drug discovery. Bioorg. Med. Chem. Lett. 16, 6013–6018.

Kadam, R.U., Tavares, J., Kiran, V.M., Cordeiro, A., Ouaissi, A., Roy, N., 2008. Structure function analysis of *Leishmania* sirtuin: an ensemble of in silico and biochemical studies. Chem. Biol. Drug Des. 71, 501–506.

Kaur, S., Shivange, A.V., Roy, N., 2010. Structural analysis of trypanosomal sirtuin: an insight for selective drug design. Mol. Divers. 14, 169–178.

Kowieski, T.M., Lee, S., Denu, J.M., 2008. Acetylation-dependent ADP-ribosylation by *Trypanosoma brucei* Sir2. J. Biol. Chem. 283, 5317–5326.

Leroux, A.E., Maugeri, D.A., Cazzulo, J.J., Nowicki, C., 2011. Functional characterization of NADP-dependent isocitrate dehydrogenase isozymes from *Trypanosoma cruzi*. Mol. Biochem. Parasitol. 177, 61–64.

Lipinski, C.A., Lombardo, F., Dominy, B.W., Feeney, P.J., 1997. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv. Drug Deliv. Rev. 23, 3–26.

Moreira, D.R., Leite, A.C., dos Santos, R.R., Soares, M.B., 2009. Approaches for the development of new anti-*Trypanosoma cruzi* agents. Curr. Drug Targets 10, 212–231.

Nguewa, P.A., Fuertes, M.A., Valladares, B., Alonso, C., Pérez, J.M., 2004. Programmed cell death in trypanosomatids: a way to maximize their biological fitness. Trends Parasitol. 20, 375–380.

Oprea, T.I., Davis, A.M., Teague, S.J., Leeson, P.D., 2001. Is there a difference between leads and drugs a historical perspective. J. Chem. Inf. Comput. Sci. 41, 1308–1315.

Sali, A., Blundell, T.L., 1993. Comparative protein modelling by satisfaction of spatial restraints. J. Mol. Biol. 234, 779–815.

Prusty, D., Mehra, P., Srivastava, S., Shivange, A.V., Gupta, A., Roy, N., Dhar, S.K., 2008. Nicotinamide inhibits *Plasmodium falciparum* Sir2 activity in vitro and parasite growth. Microbiol. Lett. 282, 266–272.

Sauve, A.A., Celic, I., Avalos, J., Deng, H., Boeke, J.D., Schramm, V.L., 2001. Chemistry of gene silencing: the mechanism of NAD+-dependent deacetylation reactions. Biochemistry 40, 15456–15463.

Sauve, A.A., Schramm, V.L., 2004. SIR2: the biochemical mechanism of NAD(+)-dependent protein deacetylation and ADP-ribosyl enzyme intermediates. Curr. Med. Chem. 11, 807–826.

Schmidt, M.T., Smith, B.C., Jackson, M.D., Denu, J.M., 2004. Co-enzyme specificity of Sir2 protein deacetylases: implications for physiological regulation. J. Biol. Chem. 279, 40122–40129.

Schuetz, A., Min, J., Antoshenko, T., Wang, C.L., Allali-Hassani, A., Dong, A., Loppnau, P., Vedadi, M., Bochkarev, A., Sternglanz, R., Plotnikov, A.N., 2007. Structural basis of inhibition of the human NAD+-dependent deacetylase SIRT5 by suramin. Structure 15, 377–389.

Sereno, D., Monte Alegre, A., Silvestre, R., Vergnes, B., Ouaissi, A., 2005. In vitro antileishmanial activity of nicotinamide. Antimicrob. Agents Chemother. 49, 808–812.

Soares, M.B., Pontes-de-Carvalho, L., Ribeiro-dos-Santos, R., 2001. The pathogenesis of Chagas' disease: when autoimmune and parasite-specific immune responses meet. An. Acad. Bras. Cienc. 73, 547–559.

Sofue, M., Yoshimura, Y., Nishida, M., Kawada, J., 1992. Possible multifunction of glucose transporter. Transport of nicotinamide by reconstituted liposomes. Biochem. J. 288, 669–674.

Szczepankiewicz, B.G., Ng, P.Y., 2008. Sirtuin modulators: targets for metabolic diseases and beyond. Curr. Top. Med. Chem. 8, 1533–1544.

Taunton, J., Hassig, C.A., Schreiber, S.L., 1996. A mammalian histone deacetylase related to the yeast transcriptional regulator Rpd3p. Science 272, 408–411.

Thomsen, R., Christensen, M.H., 2006. MolDock: a new technique for high-accuracy molecular docking. J. Med. Chem. 49, 3315–3321.

Timmers, L.F.S., Pauli, I., Caceres, R.A., De Azevedo Jr., W.F., 2008. Drug-binding databases. Curr. Drug Targets 9, 1092–1099.

Uchoa, H.B., Jorge, G.E., Da Silveira, N.J.F., Camera, J.C., Canduri, F., De Azevedo, W.F., 2004. Parmodel: a web server for automated comparative modeling of proteins. Biochem. Biophys. Res. Commun. 325, 1481–1486.

Veber, D.F., Johnson, S.R., Cheng, H.Y., Smith, B.R., Ward, K.W., Kopple, K.D., 2002. Molecular properties that influence the oral bioavailability of drug candidates. J. Med. Chem. 45, 2615–2623.

WHO, 2002. Control of Chagas Disease. World Health Organ. Tech. Rep. Ser. 905, pp. 1–109.

Zemzoumi, K., Sereno, D., François, C., Guilvard, E., Lemesre, J.L., Ouaissi, A., 1998. *Leishmania major*: cell type dependent distribution of a 43 kDa antigen related to silent information regulatory-2 protein family. Biol. Cell 90, 239–245.