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ORIGINAL PAPER

Decanethiol functionalized silver nanoparticles are new powerful leishmanicidals in vitro

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Abstract

We evaluated, for the first time, the leishmanicidal potential of decanethiol functionalized silver nanoparticles (AgNps–SCH) on promastigotes and amastigotes of different strains and species of *Leishmania: L. mexicana* and *L. major* isolated from different patients suffering from localized cutaneous leishmaniasis (CL) and *L. mexicana* isolated from a patient suffering from diffuse cutaneous leishmaniasis (DCL). We recorded the kinetics of promastigote growth by daily parasite counting for 5 days, promastigote mobility, parasite reproduction by CFSE staining's protocol and promastigote killing using the propidium iodide assay. We also recorded IC_{50}'s of promastigotes and amastigotes, therapeutic index, and cytotoxicity by co-culturing macrophages with AgNps–SCH or sodium stibogluconate (Sb) used as reference drug. We used Sb as a reference drug since it is used as the first line treatment for all different types of leishmaniasis. At concentrations 10,000 times lower than those used with Sb, AgNps–SCH had a remarkable leishmanicidal effect in all tested strains of parasites and there was no toxicity to J774A.1 macrophages since > 85% were viable at the concentrations used. Therapeutic index was about 20,000 fold greater than the corresponding one for Sb treated cells. AgNps–SCH inhibited > 80% promastigote proliferation in all tested parasites. These results demonstrate there is a high leishmanicidal potential of AgNps–SCH at concentrations of 0.04 μM. Although more studies are needed, including in vivo testing of AgNps–SCH against different types of leishmaniasis, they can be considered a potential new treatment alternative.

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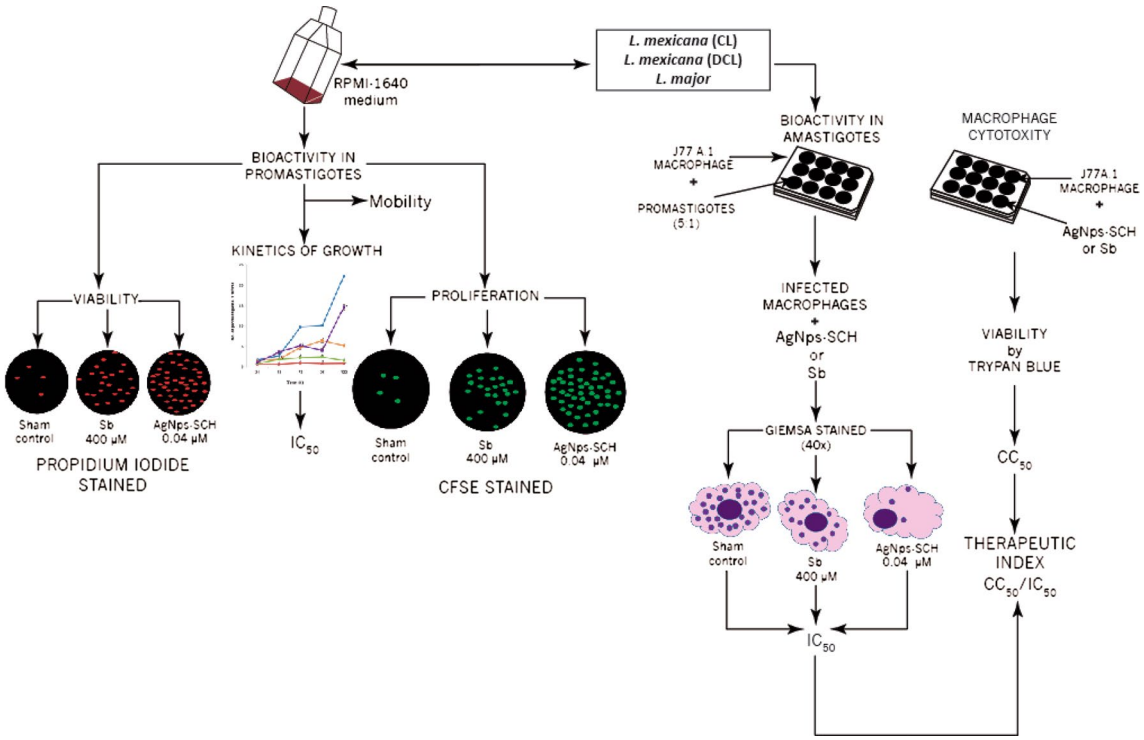
Graphical Abstract



Keywords Decanethiol functionalized silver nanoparticles · Leishmaniasis · Silver nanoparticles · *L. mexicana* · *L. major* · Leishmanicidal activity

Introduction

Leishmaniasis is a parasitic disease endemic in 98 countries and affecting mainly poor rural or peri-urban populations (Abamor and Allahverdiyev 2016). It is transmitted by various species of *Phlebotomus* and *Lutzomyia* sandflies infected with > 20 species of parasites of the genus *Leishmania*. It is classified as re-emerging and neglected disease and after malaria it shows the highest morbidity and mortality rates in the world. About 12 million people suffer from leishmaniasis in different parts of the world and it causes 60,000 deaths and leave 350 million people at risk every year (Gutiérrez et al. 2016).

Leishmaniasis is clinically classified as mucocutaneous leishmaniasis (ML), disseminated cutaneous leishmaniasis (DCL), cutaneous leishmaniasis (CL) and visceral leishmaniasis (VL). CL and VL present an annual incidence of about 1.5 million cases and 500,000 cases respectively. Several conditions such as demographic, sociological, political, and climatological changes contribute to develop a steady increase of their incidence worldwide (Oryan and Akbari 2016). CL is not a fatal medical condition and it is characterized by the presence of nodular or ulcerated skin lesions that can become chronic and/or disfiguring. This condition has negative effects on human communities due to social stigma and psychological consequences that frequently provokes loss of labor productivity. Lack of an effective vaccine and limitations in anti-leishmanial chemotherapy, in some cases, gets the disease out of control in some endemic areas (Kumar and Engwerda 2014).

There is a low number of drugs to treat CL and pentavalent antimonials represent the first line drugs. Second-line drugs include amphotericin B, pentamidine, paromomycin and miltefosine although they are toxic, expensive and cause substantial suffering during parenteral administration. It is well known that pentavalent antimonials are cardiotoxic, hepatotoxic, nephrotoxic while pentamidine and miltefosine can cause diabetes and damage the gastrointestinal tract if administered at high doses (Kalangi et al. 2016; Mlika et al. 2008; Matoussi et al. 2007). Deaths have also been reported due to toxicity of these drugs which is an unacceptable side effect for a non-fatal disease (OPS/OMS 2017).

Many reports underscore the marked increase in parasite resistance to existing anti-leishmanial drugs that result in therapeutic failures and the development of non-responsive clinical forms of the disease. Successful healing rates with currently available drugs have declined during the past 40 years from > 90 to < 50% (Singh et al. 2012; Natera et al.

2007; Isaac-Márquez and Lezama-Dávila 2003). In recent years most therapeutic products developed for CL are reformulated combinations of pre-existing drugs. Therefore, there is an urgent need to develop new anti-leishmanial treatments.

Nanotechnology is a new approach in biomedicine with great potential to treat leishmaniasis since nanoparticles (Nps) present a greater surface area and unique physicochemical properties. Among existing nanometric materials, silver nanoparticles (AgNps) are important because of their high effectiveness against multi-resistant bacteria without in vitro toxicity to mammal cells. They also show an anti-inflammatory effect and an increased wound healing action (Dai et al. 2016; Pourali et al. 2016; Zhang et al. 2016; Gonzalez-Carter et al. 2017). There are also reports of its effective anti-fungal and anti-viral action (Li et al. 2016; Rai et al. 2009). Nevertheless, studies on its anti-leishmanial activity are scarce and are limited to the use of inorganic nanoparticles. There are reports of significant increase of leishmanicidal activity of AgNps by exposing them to ultraviolet light (Mayelifar et al. 2015; Jebali and Kazemi 2013; Allahverdiyev et al. 2011). Nevertheless, Nilforoushzadeh et al. (2012) did not find significant differences in the size of the lesion pre- and post-treatment with AgNps in BALB/c mice infected with *L. major*.

Green synthesis has also been used for the preparation of AgNps, for instance Kalangi et al. (2016) used *Anethum graveolens* leaf extract to obtain AgNps and combined them with different concentrations of miltefosine. This combination resulted in a two fold increase of the anti-*L. donovani* activity of miltefosine while AgNps alone had no effect. Nanoparticles can be conjugated with diverse organic compounds to make them functional (Ravindran et al. 2013). This process is called functionalization and regulates its stability and solubility. This process also gives them unique properties of diffusion through cell membranes and makes them biologically active in the intra-cellular compartment. Functionalized Nps have been used in the treatment of infectious diseases.

Norvancomycin coated with silver nanoparticles has been evaluated for its bactericidal properties (Wei et al. 2006). Ahmad et al. (2016) synthetized green nanoparticles using Amphotericin B bound to AgNps and an extract of the plant *Isatis tinctoria* observing a higher leishmanicidal activity in promastigotes and amastigotes of *L. tropica* compared to amphotericin B alone. Furthermore, it has recently been demonstrated that organosulfur compounds and long chain organic molecules such as sulfones and hexadecanoic acid ethyl ester present strong leishmanicidal activity (Dar et al. 2015; Lezama-Dávila and Isaac-Márquez 2013).

A strategy for the functionalization of NPs consists of coating their surface with thiol groups as reported by Neouze and Schubert (2008). Nevertheless, the effect of functionalized AgNps containing thiol groups bound to long chain alkanes on *Leishmania* parasites have not been evaluated so far and this is the rational basis of the present work. Furthermore, there are no reports of the anti-leishmanial effect of decanethiol functionalized silver nanoparticles (AgNps–SCH). In the present study we evaluated the role of AgNps–SCH in the viability and rate of growth of different species of *Leishmania*.

Materials and methods

Parasites

In this work we used two different parasite's species, one of them with two different parasite's strains. One parasite species was isolated from a patient from Sudan with localized cutaneous leishmaniasis (CL): *L. major* (MRHO/SU/59/P/LV39). We also worked with two strains of *L. mexicana*: One of them is a strain isolated from a patient from Belize with localized cutaneous leishmaniasis (CL): *L. mexicana* (MNYC/BZ/62/M379) and another one originally isolated from a patient from Tabasco, México with chronic disseminated cutaneous leishmaniasis (DCL): *L. mexicana* (MHOM/MX/01/Tab3). These parasites were maintained by serial passage of amastigotes inoculated subcutaneously into shaven rumps of Balb/c mice. All animals were confined to an animal facility according to Universidad Autónoma de Campeche's institutional guidelines. All animal procedures were performed in accordance with the Mexican Official Standard NOM-062-ZOO-1999 (SAGARPA 2001). Amastigotes were recovered from infected lesions to generate promastigotes (Lezama-Dávila et al. 2014, 2016) used to perform all in vitro studies described below. They were suspended in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS), 100 IU penicillin and 100 µg streptomycin (Sigma-Aldrich, México).

Silver nanoparticles and reference drug

Silver nanoparticles solution in hexane (0.1%, W/V) functionalized with decanethiol (AgNps–SCH) were used. Size particle was between 3 and 7 nm and were manufactured by Sigma-Aldrich (México). AgNps–SCH were used at concentrations of 0.04, 0.02, 0.01 and 0.002 µM. AgNps–SCH were originally prepared by slow, drop by drop addition, of a stock solution of Ag_2NO_3 into a stock solution of decanethiol at different temperatures and stirred with a magnetic stirrer. Resulting solution was then dialyzed and finally it was suspended in hexane. Sodium stibogluconate was chosen as a reference drug since antimonials are used as the first line treatment for all different types of leishmaniasis (Lezama-Dávila and Isaac-Márquez 2013; Lezama-Dávila et al. 2014, 2016).

Kinetics of growth and mobility of promastigotes

L. mexicana (CL), *L. mexicana* (DCL) and *L. major* (1×10^6/mL) were seeded into wells of 24 flat-well culture plates. Next, we treated all parasites with 0.04, 0.02 and 0.002 µM of AgNps–SCH or 400, 300 or 200 µM of sodium stibogluconate (Sb). The total volume per well was adjusted to 1 mL using supplemented RPMI-1640 medium. Sham control parasites were treated with different volumes of hexane (40, 20 or 2 µL) used as vehicle. For sham control data shown in Figures and Tables we used the highest volume of hexane of 40 µL. Parasites were daily counted for 5 days using a Neubauer chamber and parasite's mobility was also recorded. These data were used to record the kinetics of parasite growth and to calculate the IC_{50}'s expressed as the mean value of at least three different experiments. IC_{50} was defined as the concentration of AgNps–SCH required to induce 50% reduction in parasite number (Lezama-Dávila et al. 2016; Isaac-Márquez et al. 2010).

Parasite mobility was recorded as we previously reported with a slight modification (Isaac-Márquez et al. 2010). We observed different microscopic fields for each parasite preparation and assigned an arbitrary value of mobility of parasites as follows: 0% = motionless; 25% = only flagellum movements; 50% = slow parasite displacements across the microscopic preparation; 75% = medium parasite displacements across the microscopic preparation; 100% = fast parasite displacements across the microscopic preparation.

Promastigotes' killing assay

L. mexicana (CL), *L. mexicana* (DCL) and *L. major* promastigotes seeded and treated as described before were incubated for 72 h. Untreated and sham controls were also included. After the incubation period, promastigotes were stained with propidium iodide and observed under the fluorescence microscope at the wavelength of 535 nm to show parasite killing. The assay was performed in triplicate for each parasite species. Results were expressed as percent viability (Foglieni et al. 2001).

Promastigotes' proliferation assay

All species and strains of *Leishmania* were stained with CFSE solution (5-Carboxyfluorescein *N*-succinimidyl ester, Sigma-Aldrich, Mexico). Next, stained promastigotes were co-cultured with different concentrations of AgNps–SCH in order to assess parasite's reproduction capacity. Promastigotes (1×10^7/mL) were labeled with 1 mL of 10 µM CFSE in RPMI-1640 for 10 min at 28 °C. Labeling was inactivated with an equal volume of RPMI-1640 medium. Parasites were then washed twice with culture medium and finally suspended in 1 mL of supplemented RPMI-1640. Stained parasites were seeded into wells of a 24-well culture plates treated with AgNps–SCH (0.02 and 0.01 µM) or Sb (300 and 200 µM) at 28 °C for 96 h. Untreated and sham controls were also included. All promastigote cultures were counted daily, in triplicate, using a Neubauer chamber to record kinetics of parasite growth. They were also individually analyzed under a fluorescence microscope at the wavelength of 480 nm by counting the number of fluorescent parasites/field. The results were recorded as percentage of fluorescent parasites/field (Messaritakis et al. 2010).

Amastigote killing inside of macrophages

J774A.1 macrophages [cell line from Balb/c mouse macrophages (CLMM)], suspended in supplemented RPMI-1640 media, were seeded (5×10^5 CLMM/mL) into wells of a 24 well plate, each well contained a glass coverslip on the bottom. Macrophages were infected overnight with *L. mexicana* (CL), *L. mexicana* (DCL) or *L. major* promastigotes (ratio 5:1) and extracellular parasites were washed off with PBS. Infected macrophages were treated with 0.04, 0.02 or 0.002 µM of AgNps–SCH or 400, 300 or 200 µM of Sb at 37 °C in a CO_2 incubator for 72 h. Sham controls were treated with equal volumes of the vehicle. Adherent macrophages on the coverslips were fixed and stained by Giemsa. Leishmanicidal activity was determined by recording the number of parasites per 100 CLMM. These experiments were performed in triplicate. Results were expressed as IC_{50} as we previously described (Lezama-Dávila et al. 2012, 2014).

AgNps–SCH toxicity towards non-infected macrophages

We tested viability of a macrophage line by trypan blue exclusion test to assess possible toxicity of AgNps–SCH. We cultured J774A.1 macrophages (5×10^5 CLMM/mL) in the presence of 0–400 µM of AgNps–SCH, or Sb or sham control (40 µL of hexane) for 72 h. The macrophage cytotoxicity (CC_{50}) was recorded as the concentration of nanoparticle or Sb necessary to destroy 50% of macrophages. The therapeutic index (TI) was calculated as the ratio of CC_{50}/IC_{50} of amastigotes (Lezama-Dávila et al. 2012).

Statistical analysis

Statistical analysis was performed using Student's t test. IC_{50} and CC_{50} values were calculated with LdP Line® (Ehabsoft) and Prism 5® software (GraphPad Software, Inc. La Jolla, CA, USA).

Results

Kinetics of growth and mobility of promastigotes

Chemical structure of a decanethiol functionalized silver nanoparticle is shown in Fig. 1a. Nanoparticles presented a strong inhibition of parasite's growth in all species of *Leishmania*. At 72 h *L. mexicana* (CL) promastigotes co-cultured with AgNps–SCH reached the lowest parasite's number of 0.2×10^6/mL compared with 4.4×10^6/mL of its corresponding sham control (Fig. 1b). *L. mexicana* (DCL) and *L. major* treated with the same nanoparticle concentration showed a significant decrease at 24 h. They showed a reduction of parasite number of 0.6 and 0.4×10^6/mL ($p < 0.001$) compared with 1.7 and 2.4×10^6/mL of their corresponding sham controls (Fig. 1c, d). At 72 h, 75% of promastigotes of all *Leishmania* species treated with 0.04 µM of AgNps–SCH were motionless while treatment with 400 µM of Sb caused variable mobility results and all sham control cultures showed parasites with fast displacements (motionless parasites = 0% and parasites with fast displacements = 100%, Table 1). We also compared IC_{50}'s values after treatment with AgNps–SCH or the reference drug (Sb). Our data show that AgNps–SCH presented a considerable smaller IC_{50} than Sb (Table 2).

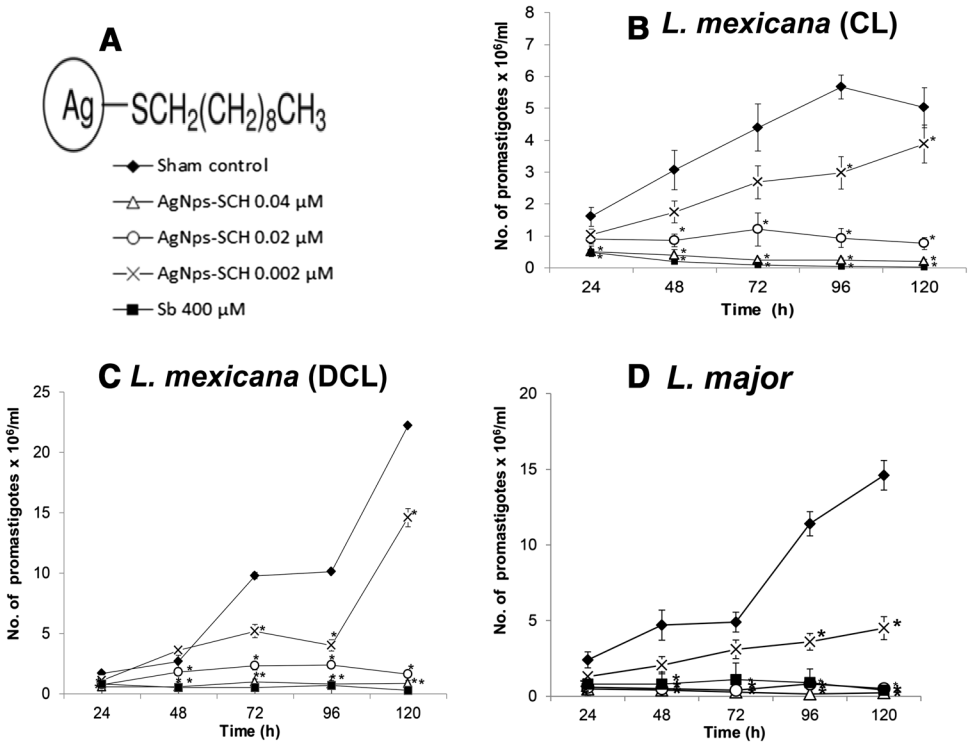


Fig. 1 Chemical structure of a decanethiol functionalized silver nanoparticle (AgNp–SCH) (**a**). Effect of nanoparticles (AgNps–SCH) and sodium stibogluconate (Sb) on the growth of *L. mexicana* (CL) (**b**), *L. mexicana* (DCL) (**c**) and *L. major* (**d**) promastigotes. Data represents a replica of three independent experiments, *$p < 0.05$

Table 1 Mobility of *Leishmania* promastigotes cultured in vitro

Parasites	Compounds	Time (h)				
		24	48	72	96	120
L. mexicana (CL)	Control	100%	100%	100%	100%	100%
	Sb[a]	75%	50%	50%	25%	25%
	AgNps–SCH[b]	50%	50%	25%	25%	0%
L. major (CL)	Control	100%	100%	100%	100%	100%
	Sb[a]	75%	50%	50%	50%	50%
	AgNps–SCH[b]	50%	50%	25%	25%	0%
L. mexicana (DCL)	Control	100%	100%	100%	100%	100%
	Sb[a]	75%	75%	75%	50%	50%
	AgNps–SCH[b]	75%	75%	25%	25%	25%

[a]Tested with Sb 400 µM; % values were set up as described in "Materials and Methods"

[b]Tested with AgNPs–SCH 0.04 µM

Table 2 IC_{50} and cytotoxic activity of AgNps–SCH

Parasites	Compounds	IC_{50} (μM) promastigotes	IC_{50} (μM) amastigotes	Therapeutic index[b]
L. mexicana (CL)	Sb	174.9 ± 11.3	210.9 ± 14.0	2.08 ± 0.148
	AgNps–SCH	0.0057 ± 0.003[a]	0.011 ± 0.002[a]	77419.09 ± 3970.32[a]
L. major (CL)	Sb	215.87 ± 11.35	348.06 ± 9.75	1.26 ± 0.105
	AgNps–SCH	0.0034 ± 0.001[a]	0.0153 ± 0.006[a]	55660.78 ± 2984.63[a]
L. mexicana (DCL)	Sb	200.49 ± 3.21	281.4 ± 4.76	1.56 ± 0.25
	AgNps–SCH	0.0035 ± 0.003[a]	0.0239 ± 0.008[a]	35632.21 ± 3528.3[a]

IC_{50} is the concentration of drug (μM) to achieve 50% killing of 10^6 promastigotes or 2.5×10^6 amastigotes/mL inside of 5×10^5 J774A.1 macrophages/mL after 72 h of culture

J774A.1 macrophages cytotoxicity (CC_{50}) of Sb = 439.80 ± 6.5 μM and AgNPs–SCH = 851.61 ± 18 μM

[a]Significantly different ($p < 0.05$) as compared to Sb

[b]Therapeutic index = CC_{50} of J774A.1 macrophages/IC_{50} of amastigotes

Nanoparticles were approximately 30,000–60,000 times more effective than Sb for promastigotes of *L. mexicana* (CL, DCL) and *L. major*.

Promastigote' killing by AgNps–SCH

We found that using 0.04 μM AgNps–SCH, a dosage 10,000 times lower than 400 μM Sb, AgNps–SCH presented a powerful leishmanicidal activity against *L. mexicana* (CL), *L. mexicana* (DCL) and *L. major*. There was also a marked decrease in viability of promastigotes exposed to different concentrations of AgNps–SCH and measured by following the propidium iodide staining protocol. We found a viability of 15, 27 and 19% of *L. mexicana* (CL), *L. mexicana* (DCL) and *L. major* promastigotes, respectively when treated with 0.04 μM of AgNps–SCH. While the viability of *L. mexicana* (CL), *L. mexicana* (DCL) and *L. major* after Sb treatment at 400 μM was 34, 35 and 41% respectively (Fig. 2). AgNps–SCH induced a loss of parasite viability in a concentration-dependent manner (Fig. 2). An average parasite mortality of 80 and 60% was recorded for the nanoparticle and the Sb respectively (Fig. 2).

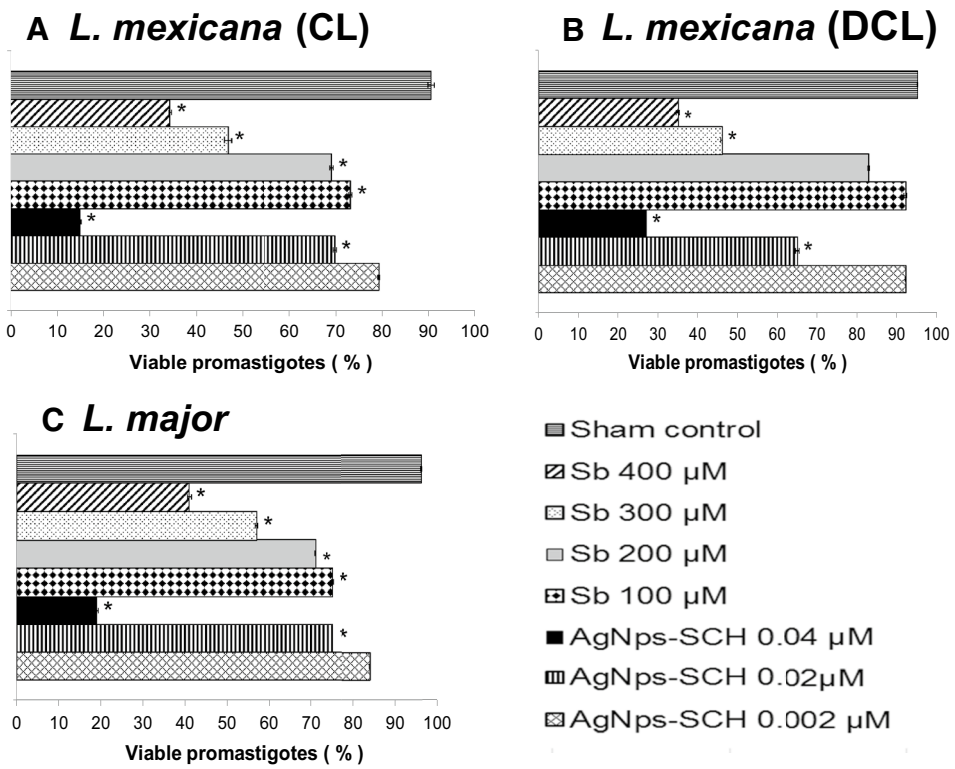


Fig. 2 Viability of *L. mexicana* (CL) (**a**), *L. mexicana* (DCL) (**b**) and *L. major* (**c**) promastigotes co-cultured with nanoparticles (AgNps–SCH) or sodium stibogluconate (Sb) and then stained with propidium iodide. The total number of parasites were counted under the light microscopy while propidium iodide stained parasites (non-viable) were counted by switching the parasite preparation to fluorescent microscopy at a wavelength of 535 nm. Data represents a percentage of viable parasites and it is a replica of three independent experiments, *$p < 0.05$

Promastigote's proliferation

The effect of AgNps–SCH on the proliferative capacity of the different types of *Leishmania* parasites studied was determined by CFSE assay. When a cell is dyed with CFSE and divides, only half of their descendants are stained with CFSE and therefore the fluorescence decreases as parasites divide over time. The sham control population of parasites proliferated at a constant rate and reached the maximum cell density of 3.5, 3.7 and 6.3×10^6/mL at 96 h for *L. mexicana* (CL), *L. mexicana* (DCL) and *L. major* respectively. The percentage of fluorescent promastigotes/field was gradually decreasing every 24 h (Fig. 3). In the case of nanoparticles treated parasites the situation was reversed. At 48 h the percentage of fluorescent parasites/field was above 80% using 0.01 µM of AgNps–SCH (lowest concentration tested). It provoked a marked decrease in cell division rates with parasite counts of 0.2×10^6, 0.4×10^6 and 1×10^6/mL for *L. mexicana* (CL), *L. mexicana* (DCL) and *L. major* respectively at 96 h of incubation ($p < 0.001$, compared to sham control cultures). A similar inhibition effect of promastigote proliferation was observed with Sb treated parasites (Fig. 3).

AgNps–SCH killing of amastigotes inside of macrophages

We evaluated the effectiveness of AgNps–SCH against amastigotes internalized in murine macrophages J774A.1 (CLMM). At low concentration of nanoparticles, they showed higher killing activity on amastigotes of *L. mexicana* (CL, DCL) and *L. major* compared to Sb (Table 2). Nanoparticles were approximately 10,000–20,000 more effective than Sb (Table 2).

AgNps–SCH toxicity towards non-infected macrophages

The toxicity assay was performed in non-infected CLMM treated with different concentrations of AgNps–SCH. The viability of treated cells at all tested concentrations was similar to sham controls (> 80%). Sb treated CLMM showed 50–70% of viability. AgNPs–SCH showed a $CC_{50} = 851.61 \pm 18$ µM and the Sb displayed a $CC_{50} = 439.80 \pm 6.5$ µM. These results indicate that nanoparticles require a two fold concentration of Sb to kill 50% of CLMM. The therapeutic index of nanoparticles was approximately 20,000–40,000 times higher than Sb (Table 2).

Discussion

Our results represent the first report of antileishmanial activity of silver nanoparticles functionalized with decanethiol (AgNps-SH) on promastigotes and amastigotes of *L. mexicana* (CL), *L. mexicana* (DCL) and *L. major*. We found that parasites co-cultured with AgNps-SH provoked a significant

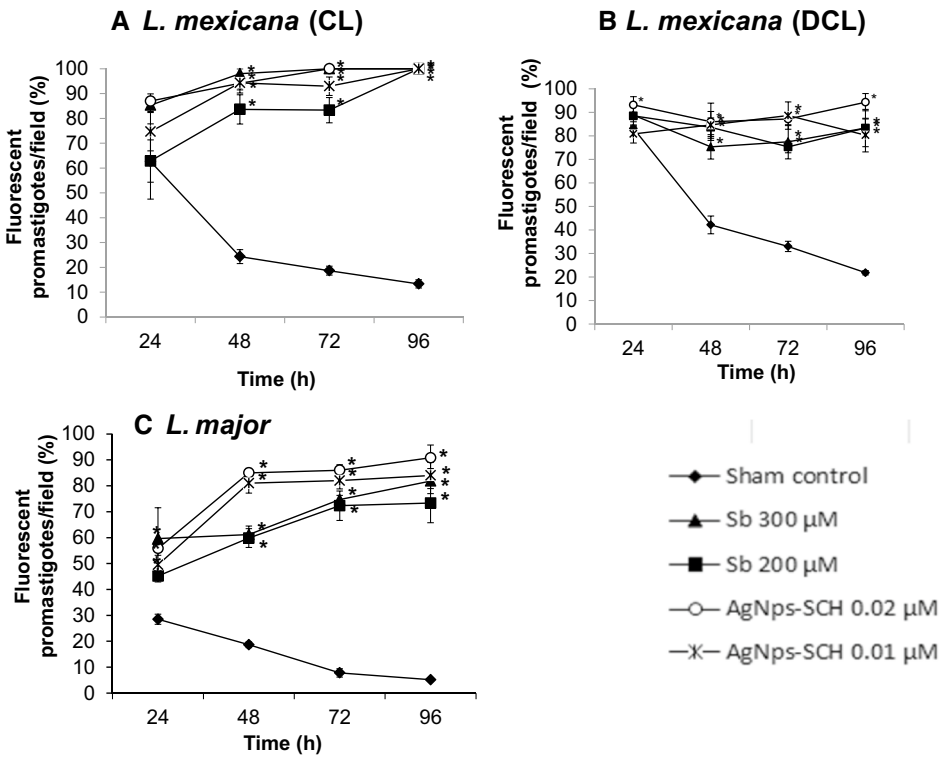


Fig. 3 Proliferation of *L. mexicana* (CL) (**a**), *L. mexicana* (DCL) (**b**) and *L. major* (**c**). Cell division of promastigotes was measured after CFSE staining. The total number of parasites was recorded under the light microscope while fluorescent parasites were observed under the fluorescent microscope at a wavelength of 480 nm. Data represents a percentage of CFSE stained parasites and it is a replica of three independent experiments, *$p < 0.05$

decrease ≥ 90% in the number of promastigotes and showed a time-dependent leishmanicidal effect. Moreover, AgNps-SH presented a more efficient leishmanicidal activity with significantly lower IC_{50} compared to values reported in the literature for inorganic nanoparticles (Jebali and Kazemi 2013). Nanoparticles tested in our work were non-toxic to non-infected macrophages. Our work also showed that AgNps-SH presented a CC_{50} considerable lower than the reference drug (Sb). AgNps–SCH presented a therapeutic index 20,000–40,000 fold greater that the corresponding one for Sb. Our data suggest AgNps-SH would be safe anti-leishmanial drugs for human use.

We also showed that AgNps-SH can induce high level of parasite killing towards all parasite species tested using the propidium iodide staining protocol. The reproductive capacity of these parasites was also tested using the CFSE technique. AgNps-SH stopped parasite division at 48 h in a proportion ≥ than 80%. These results indicate that one mechanism used by nanoparticles is inhibition of parasite division and killing of the remaining parasites.

We also measured nitric oxide (NO) in supernatants from CLMM infected with different *Leishmania* species studied, using Griess reagent (Lezama-Dávila et al. 2016). We found that AgNps–SCH did not induce NO production (data not shown). This result suggests that parasite death occurs by a NO independent pathway.

A possibility to explain the strong anti-leishmanial effect of AgNps-SH could be, in part, due to its small size of 3–7 nm. These nanoparticles exhibited a more potent leishmanicidal activity at much lower concentrations than nanoparticles tested in other studies. Thus, AgNps with average size of 10–40 nm (Allahverdiyev et al. 2011; Abamor and Allahverdiyev 2016) and amphotericin B-bound silver nanoparticles with a size range of 15–20 nm (Ahmad et al. 2016) displayed a less efficient activity than AgNps-SH. It is also possible that the lipid nature of the sided long chain of AgNps-SH could favor interaction with parasite membrane components and this way speed up its death by membrane instability and membrane damage. This would be possible by binding to sulfur containing proteins. This binding would favor its intracellular diffusion where they can bind to sulfur containing enzymes and/or the phosphorus present in DNA affecting biochemical pathways and cell transcription. The glycoprotein (GP 63) related to the infectivity of *Leishmania* may also be affected (Jebali and Kazemi 2013; Arvizo et al. 2010; Rai et al. 2009). Another plausible explanation would be the activation of the oxidative stress which produces reactive oxygen species (ROS). This would increase efficacy of nanoparticles to interfere with the functions of cell membranes and to induce apoptosis (Ahmad et al. 2016; Allahverdiyev et al. 2013; Carlson et al. 2008). Nevertheless, additional studies are required to fully evaluate the mechanistic aspects of AgNps-SH on *Leishmania p*arasites.

According to data presented in this work, the leishmanicidal activity in vitro of AgNps–SCH was 99% more powerful than the reference drug (Sb) and also inhibited parasite reproduction. Therefore AgNps-SH could be considered in the future as the basis for a new treatment for CL.

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