

Biodistribution of meglumine antimoniate in healthy and *Leishmania (Leishmania) infantum chagasi*-infected BALB/c mice

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Pentavalent antimonials such as meglumine antimoniate (MA) are the primary treatments for leishmaniasis, a complex disease caused by protozoan parasites of the genus Leishmania. Despite over 70 years of clinical use, their mechanisms of action, toxicity and pharmacokinetics have not been fully elucidated. Radiotracer studies performed on animals have the potential to play a major role in pharmaceutical development. The aims of this study were to prepare an antimony radiotracer by neutron irradiation of MA and to determine the biodistribution of MA in healthy and Leishmania (Leishmania) infantum chagasi-infected mice. MA (Glucantime[®]) was neutron irradiated inside the IEA-R1 nuclear reactor, producing two radioisotopes, ¹²²Sb and ¹²⁴Sb, with high radionuclidic purity and good specific activity. This irradiated compound presented anti-leishmanial activity similar to that of non-irradiated MA in both in vitro and in vivo evaluations. In the biodistribution studies, healthy mice showed higher uptake of antimony in the liver than infected mice and elimination occurred primarily through biliary excretion, with a small proportion of the drug excreted by the kidneys. The serum kinetic curve was bi-exponential, with two compartments: the central compartment and another compartment associated with drug excretion. Radiotracers, which can be easily produced by neutron irradiation, were demonstrated to be an interesting tool for answering several questions regarding antimonial pharmacokinetics and chemotherapy.

Key words: leishmaniasis - glucantime - radioisotope - antimony - biodistribution

Leishmaniasis is a complex disease caused by protozoan parasites of the genus *Leishmania* and is transmitted by phlebotomine sandflies. The result of infection can vary from a chronic skin ulcer, known as cutaneous leishmaniasis (CL), to progressive and frequently fatal hepatosplenomegaly, known as visceral leishmaniasis (VL) (Desjeux 2004). Human leishmaniasis is endemic in 98 countries, with a global incidence estimated at approximately 0.2-0.4 million VL cases and 0.7-1.2 million CL cases occurring each year. It is estimated that 20,000-40,000 leishmaniasis deaths occur per year. Brazil is among the 10 countries with the highest estimated case counts (Alvar et al. 2012). Leishmaniasis is the eighth major tropical disease; however, financial support for control and elimination efforts as well as research and new drug development has been insufficient (Hotez et al. 2007).

The mainstays of leishmaniasis treatment are pentavalent antimonials, such as meglumine antimoniate (MA) (Glucantime[®]). Although these antimonials were first introduced in 1945 and remain effective therapies for some forms of leishmaniasis, their mechanisms of

action, toxicity and pharmacokinetics have not been fully elucidated (Frézard & Demicheli 2010). The determination of their pharmacokinetic profiles may suggest a better dosage protocol, administration interval or duration of therapy, which may reduce resistance, relapse and severe side effects.

The analytical methods for the determination of antimony concentrations in biological systems, such as atomic absorption spectrometry or inductively coupled plasma optical emission spectrometry, remain complex and require sample preparation (Rath et al. 2003). Radiotracer studies performed on animals have the potential to play a major role in pharmaceutical development, pharmacology studies and basic biochemistry research (Meikle et al. 2001). Pharmacokinetic studies can be based on bioactive radiolabelled drugs, but antimony radioisotopes are not commercially available. The development of methods to measure antimony concentrations in biological tissue will enable further analysis of pharmacokinetics. Thus, the aims of this study were to prepare an antimony radiotracer by neutron irradiation of MA and to determine the biodistribution of MA in healthy mice and mice experimentally infected with *Leishmania (Leishmania) infantum chagasi*.

MATERIALS AND METHODS

Chemicals - 3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (thiazolyl blue) (MTT), sodium dodecyl sulphate (SDS), M-199 medium and RPMI-PR-1640 medium (without phenol red) were purchased from Sigma (Saint Louis, USA). Foetal bovine serum (FBS)

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was obtained from Gibco (Basel, Switzerland). MA (Glucantime®) was obtained from Sanofi-Aventis [São Paulo (SP), Brazil]. Each ampoule of 5 mL contained 1.5 g of MA, equivalent to 405 mg of pentavalent antimony (Sb⁵⁺) or 81 mg/mL Sb⁵⁺; Sb⁵⁺ represented approximately 27% of the total salt (Sanofi-Aventis). The molecular weight (MW) of MA is 365.98 g/mol (PubChem Compound Database, CID 64953, National Center for Biotechnology Information) (pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=64953).

Experimental animals - Animals were supplied by the animal breeding facility at the Faculty of Medicine of the University of São Paulo and were maintained in sterilised cages in a controlled environment with free access to water and food. All of the animal procedures were performed with the approval of the Research Ethical Committee of the Tropical Medicine Institute of São Paulo (CEP-IMTSP 012/29/042008).

Golden hamsters (*Mesocricetus auratus*) were infected each month with amastigotes obtained from spleens to maintain the *Leishmania* strains. Female BALB/c mice (20-24 g) were used to obtain peritoneal macrophages and all in vivo assays were performed using these mice.

Parasites and macrophages - *L. (L.) infantum chagasi* (MHOM/BR/1972/LD) promastigotes were maintained in M-199 medium supplemented with 10% FBS and 0.25% haemin at 24°C. *L. (L.) infantum chagasi* was maintained in golden hamsters for up to approximately 60-70 days post-infection. The amastigotes were obtained from the spleens of previously infected hamsters by differential centrifugation. The macrophages were collected from the peritoneal cavities of BALB/c mice by washing with RPMI-1640 medium as previously described by Tempone et al. (2005).

RAW 264.7 murine macrophage cells (American Type Culture Collection, Manassas, VA) were cultivated in RPMI-1640 supplemented with 10% FBS and 1% antibiotics (penicillin and streptomycin). The cells were maintained at 37°C in a humidified atmosphere containing 5% CO₂ as previously described by Tempone et al. (2005).

Production and analysis of irradiated MA (IMA) - To produce the antimony radiotracer, 0.5-0.8 mL aliquots of MA were sealed in quartz ampoules and irradiated at the IEA-R1 nuclear reactor facility of Institute of Nuclear and Energy Research-National Commission of Nuclear Energy (SP) using a thermal neutron flux of 1.0×10^{13} n²/cm²/s for 7 min as previously described by Borborema et al. (2005). Antimony radioisotopes were produced by the following nuclear reactions: ¹²¹Sb(n,γ)¹²²Sb and ¹²³Sb(n,γ)¹²⁴Sb. Radionuclidic purity was determined by γ-spectrometry using an HPGe detector coupled to the Genie-PC programme (Canberra Inc, Meriden, CT). The concentrations of radioactive compounds were also measured with the same system after efficiency calibration with standard ⁶⁰Co, ¹³⁷Cs and ¹⁵²Eu sources. Chemical changes in the neutron-IMA and non-neutron-IMA were analysed using an Ultrospec 3000 UV-Visible Spectrometer (Pharmacia Biotech Inc, New Jersey, USA) by scanning the spectrum from 200-700 nm.

L. (L.) infantum chagasi cytotoxicity assays - Promastigotes were seeded in 96-well microplates at a density of 1×10^6 cells/well. MA and cold IMA (after radionuclide decay of at least 10 half-lives) were two-fold serially diluted with growth medium and incubated with the parasites for 24 h at 24°C. Parasite viability was determined using the MTT assay (Tada et al. 1986). Briefly, MTT (5 mg/mL) was dissolved in phosphate-buffered saline (PBS) and sterilised by passage through 0.22-μm membranes. Then, 20 μL of this solution was added to each well and the plates were incubated for 4 h at 24°C. Promastigotes incubated without compounds were used as the viability control. Formazan extraction was performed using 10% SDS for 18 h (80 μL/well) at 24°C and the optical density (OD) was determined using a Multiskan Ascent 354 microplate photometer (Thermo Labsystems, Pennsylvania, USA) at 570 nm. Data analysis was performed using GraphPad Prism 5.03 (GraphPad Software Inc, La Jolla, CA). In this analysis, 100% viability was defined based on the OD of the control promastigotes after normalisation using the mean of three experiments performed in duplicate.

Activity against intracellular *L. (L.) infantum chagasi* amastigotes was determined in infected macrophages. Macrophages were isolated from the peritoneal cavities of BALB/c mice by lavage with RPMI-1640 medium. The cells were then seeded into 24-well plates containing glass cover slips at a density of 4×10^5 cells/well and were incubated for 24 h prior to infection. *L. (L.) infantum chagasi* amastigotes were isolated from the spleens of previously infected hamsters, separated by differential centrifugation and added to the macrophages at a ratio of 10:1 (amastigotes:macrophages). The plates were further incubated for 24 h prior to drug addition. Non-internalised parasites were removed by washing once with medium and the cells were then incubated with the test compounds for five days at 37°C in 5% CO₂. At the end of the assay, the cells were fixed in methanol, stained with Giemsa stain and observed under a light microscope to determine the number of intracellular parasites. The number of amastigotes was determined in 400 macrophages from the drug-treated and control wells. The number of counted amastigotes in the untreated cultures was considered to be 100% for calculating the percentage of parasites suppressed in the drug-treated cultures (Borborema et al. 2011). The 50% inhibitory concentration (IC₅₀) was calculated using a sigmoid dose-response model that was generated with GraphPad Prism 5.03 using the mean of two experiments performed in duplicate.

Macrophage cytotoxicity assay - RAW 264.7 macrophages were seeded in 96-well microplates at a density of 4×10^4 cells/well and were grown under normal culture conditions. After 24 h, the medium was replaced. MA and cold IMA were two-fold serially diluted with growth medium and incubated with cells for 48 h at 37°C in 5% CO₂ (Tempone et al. 2005). The cytotoxic effects of the test compounds were determined using an MTT assay as previously described. Data analysis was performed using GraphPad Prism 5.03 using the mean of

three experiments performed in duplicate. The selectivity index (SI) was calculated using the following equation:

$$SI = IC_{50} \text{ (RAW 264.7 cells)} / IC_{50} \text{ (Leishmania amastigotes)}$$

Treatment of *L. (L.) infantum chagasi*-infected BALB/c mice with IMA - To verify the in vivo efficacy of IMA, female BALB/c mice were infected via the retrobulbar route (i.v.) with *L. (L.) infantum chagasi* amastigotes ($1-2 \times 10^7/150 \mu\text{L}$). Seven days post-infection, three groups of animals ($n = 4$) were treated for four consecutive days with MA, cold IMA and PBS (control). The animals received $50 \text{ mg Sb}^{5+}/\text{kg/day}$ intraperitoneally. The animals were euthanised two weeks post-infection (Yardley & Croft 2000) and their spleens and livers were removed and weighed. The parasite burden was evaluated and quantified using Giemsa-stained imprints by light microscopy. Both organs were weighed and the weights for the drug-treated groups were compared with those for the untreated group (infected group). The number of amastigotes per 400 cell nuclei was determined and multiplied by the organ weight in grams to obtain Leishman-Donovan units (Stauber 1958).

Biodistribution of IMA in healthy and *L. (L.) infantum chagasi*-infected BALB/c mice - Female BALB/c mice were infected via the retrobulbar route (i.v.) with *L. (L.) infantum chagasi* amastigotes ($1-2 \times 10^7/150 \mu\text{L}$). The animals were analysed 19-22 days post-infection. Groups of healthy and infected mice ($n = 6$) received an intraperitoneal injection of IMA containing $0.081 \text{ mg of Sb}^{5+}/100 \mu\text{L}$ (approximately 0.1% of the therapeutic dose) with activity of $2.2 \times 10^4 \text{ Bq}/100 \mu\text{L}$ of ^{122}Sb and $518 \text{ Bq}/100 \mu\text{L}$ of ^{124}Sb . The animals were euthanised after 3, 5, 15, 30, 60, 120, 300, 1,440, 2,880 and 4,320 min by cervical dislocation with blood sampling and their organs were removed, washed and weighed. The injected activity (IA) was measured in an NaI(Tl) scintillation counter (Cobra Auto-Gamma, Canberra Inc, Meriden, CT) and the gamma energy range was established from 500-700 keV and counted for 1 min or until 1,000,000 counts per minutes (cpm) was achieved. Skeletal muscle was calculated as 40% of the body mass and blood volume was calculated as 7% of the body mass. Data were expressed as the percentage of IA per organ and the concentration of radioactivity in the blood was expressed as the percentage of IA per millilitre. The IA was established as 100% of the dose and the percentage of IA per organ was then calculated using the following equation:

$$\% \text{ injected activity/organ} = \frac{\text{cpm/organ}}{\text{injected dose (cpm)}} \times 100$$

Pharmacokinetic analysis - Non-compartmental analysis of the plasma concentrations using PK Solutions Pharmacokinetic software (Summit Research Services, CO, USA) was performed to determine the area under the concentration-time curve (AUC) for these plots. Elimination rate constants (K_{el}) were determined from the linear regression of the last three data points on each of the plots and plasma half-lives ($t_{1/2}$) were calculated as $0.693/K_{el}$. The area under the first moment curve

(AUMC) and the mean residence time (MRT) (equivalent to $AUMC/AUC$) were also calculated. The apparent total clearance (CL) was estimated from the equation $CL/F = \text{dose}/AUC$, where F is the bioavailability of antimony following intramuscular administration. Pharmacokinetic analysis of the liver and spleen was performed using the % IA per gram of organ.

Statistical analysis - The data obtained were reported as the mean and standard deviation of duplicate samples from two independent assays. The IC_{50} values were calculated using sigmoid dose-response curves generated using GraphPad Prism 5.03 and the 95% confidence intervals were included. The Mann-Whitney *U* test (unpaired two-tailed) was used for significance testing and Student's *t* test was applied for statistical analysis of the biodistribution studies. $p < 0.05$ was considered statistically significant.

RESULTS

Production and analysis of IMA - The neutron irradiation of MA produced two radioisotopes of antimony, ^{122}Sb and ^{124}Sb . High radionuclidic purity was verified; only ^{122}Sb ($t_{1/2} = 2.7$ days) and ^{124}Sb ($t_{1/2} = 60.2$ days) gamma peaks were observed. The activity at the end of irradiation was 33.2 MBq of ^{122}Sb and 0.77 MBq of ^{124}Sb , which corresponds to specific activities of $22.12 \text{ MBq } ^{122}\text{Sb}/\text{mL}$ and $0.52 \text{ MBq } ^{124}\text{Sb}/\text{mL}$ for MA. Chemical changes in the neutron-IMA were assessed by UV-visible spectrometry. The samples exhibited a very low absorbance peak (0.02 OD units) at 300 nm accompanied by a slight colour change, which was most likely due to meglumine polymer formation. The same effect was observed when the pharmaceutical was exposed to unsuitable conditions of temperature and light, i.e., harsh storage conditions.

Anti-leishmanial and cytotoxic activity of IMA - The anti-leishmanial activities of cold IMA and MA against *Leishmania* promastigotes were analysed. As summarised in Table I, the evaluation of the IC_{50} demonstrated

TABLE I

Anti-leishmanial activity against *Leishmania (Leishmania) infantum chagasi* and cytotoxicity of RAW 246.7 macrophages of meglumine antimoniate (MA) and irradiated MA (IMA) in infected and non-infected macrophages

Drug	IC_{50} (μM) (95% CI) ^a			SI
	Amastigotes	Promastigotes	Macrophages	
IMA	580.0 (527.5-637.9)	171911 (137412-215195)	11745 (8131-17002)	20
MA	502.2 (432.7-582.3)	342751 (268336-437782)	29240 (19466-43942)	58

^a: values [mean \pm standard deviation and 95% confidence interval (CI)] derived from three independent experiments; IC_{50} : inhibitory concentration 50%; SI: selectivity index (IC_{50} RAW 246.7 cells/ IC_{50} amastigotes).

that the promastigotes were not susceptible to either MA or IMA; however, IMA was slightly more active against *L. (L.) infantum chagasi* promastigotes. No significant difference was observed in the anti-leishmanial activity against *L. (L.) infantum chagasi* amastigotes. The cytotoxic activity of IMA and MA was observed in macrophages and IMA was two-fold more cytotoxic to macrophages. MA and IMA were 680 and 300-fold more active against *L. (L.) infantum chagasi* amastigotes than against promastigotes, respectively; however, both drugs showed similar activity against *L. (L.) infantum chagasi*-infected macrophages.

Treatment of *L. (L.) infantum chagasi*-infected BALB/c mice with IMA - The effectiveness of MA was compared with that of cold IMA in the treatment of *L. (L.) infantum chagasi*-infected BALB/c mice. The parasite burden was evaluated and quantified by light microscopy. The untreated group was used as a control and had an average of 41.69 (8.60) amastigotes per gram in the spleen and 3,331 (1,745) amastigotes per gram in the liver. MA and IMA did not result in therapeutic improvement in the spleen, with an average of 32.17 (19.20) and 43.57 (29.38) amastigotes per gram, respectively. However, MA and IMA reduced the number of amastigotes in the liver by 19% and 23%, with means of 2,696 (1,472) and 2,555 (1,065) amastigotes per gram, respectively. Both drugs showed similar effectiveness, with no statistically significant differences ($p > 0.05$).

Biodistribution of IMA in healthy and *L. (L.) infantum chagasi*-infected BALB/c mice - Antimony did not accumulate in the brain, lungs, heart or uterus, with low uptake of approximately 0.02-1.6% of the IA during the 72 h of the study. The activity in these organs gradually decreased both in healthy and infected mice. In the musculoskeletal system, higher uptake of the drug was observed during the first hour after administration and then gradually decreased, reaching levels of 1-1.5% of the IA by 72 h. Healthy mice showed higher uptake than infected mice at 30 and 60 min.

The biodistributions of IMA in the liver and spleen are presented in Fig. 1A, B, respectively. After intraperitoneal administration, antimony was rapidly absorbed, distributed and slowly eliminated. In the liver, the pharmacokinetic parameters indicated that the drug was rapidly absorbed at 30 min, with mean maximum concentrations (C_{max}) of 61% IA/g and 47.5% IA/g in healthy and infected mice, respectively. Antimony accumulated and was retained in the liver with slow CL, reaching levels of 7% of the IA by 72 h post-administration and healthy animals showed higher uptake of the drug than infected mice. A slight increase in the AUC of the healthy mice compared with that of the infected mice (1.761.4% IA.h/g vs. 1.237.3% IA.h/g) was observed. At 24 h post-injection, no significant activity was observed in any major organ other than the liver in healthy or infected mice.

In the spleen, a rapid drug absorption phase (A phase) occurred, with C_{max} values of 23% IA/g and 21% IA/g at 5 and 3 min in healthy and infected mice, respectively. This absorption was followed by a rapid decrease in the antimony concentration, reaching a level of 0.7% of the

IA 72 h later. Healthy mice had an AUC that was 10-fold higher than that of infected mice (1.742.3% IA.h/g vs. 134.7% IA.h/g). Comparing the %IA/total organ, infected mice had a faster A phase with a higher antimony concentration during the evaluated period compared to healthy mice (Fig. 1B).

IMA was absorbed by the gastrointestinal tract (Fig. 2) and elimination occurred primarily through hepatobiliary excretion after liver processing, reaching the intestinal lumen. There was also rapid renal excretion of a small amount of the drug. High levels of absorption occurred in the stomach by 3 min, with the level then decreasing by 30 min. Next, increasing levels of antimony were observed within 2 h and then the elimination process began. In the small intestine, a significant decrease in the level of antimony was simultaneously observed with an increasing level in the large intestine, representing the passage of drugs metabolised by the liver to the small intestine and then to the large intestine. The elimination phase (E phase) in the large intestine began by 2 h post-injection,

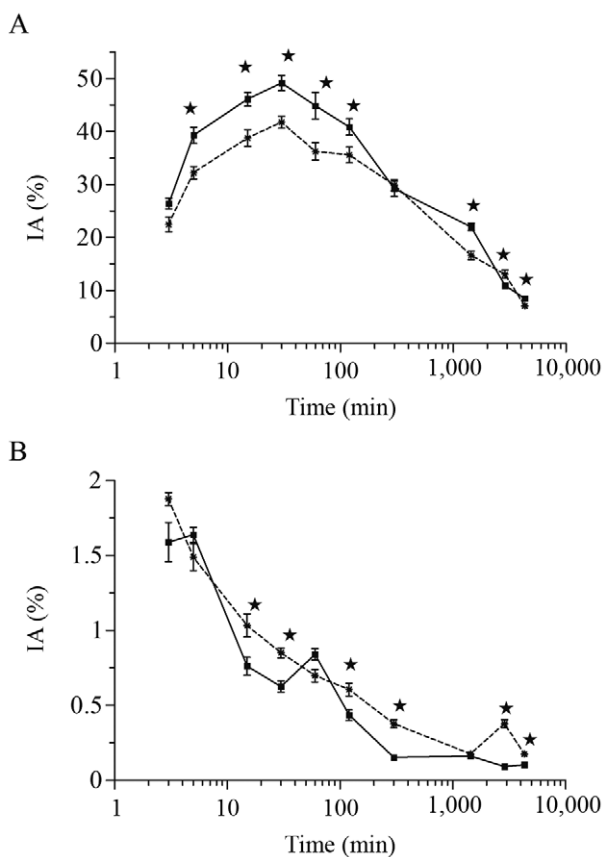


Fig. 1: biodistribution of neutron-irradiated meglumine antimoniate (IMA) in healthy and *Leishmania (Leishmania) infantum chagasi*-infected BALB/c mice ($n = 6$) (mean \pm standard deviation) after intraperitoneal administration containing 0.081 mg Sb⁵⁺/100 μ L with activity of 2.2×10^4 Bq/100 μ L of ¹²²Sb and 518 Bq/100 μ L of ¹²⁴Sb. A: percentage of injected activity (IA) per organ in the liver; B: percentage of IA per organ in the spleen; continuous line: healthy mice; dotted line: *L. (L.) infantum chagasi*-infected mice. Asterisks mean $p < 0.05$ compared to healthy mice group.

with higher levels at 5 h and complete elimination by 24 h. These values corroborate the values observed in the liver, where the decay of the injected dose was equivalent to the concentration eliminated by the intestine within the same time frame. Next, the slow E phase via the kidney and large intestine occurred until 72 h.

The blood concentration-time profile of antimony after the administration of IMA is presented in Fig. 3. Antimony was rapidly absorbed, distributed and slowly eliminated. According to compartmental analysis, antimony follows a two-compartment open model with a

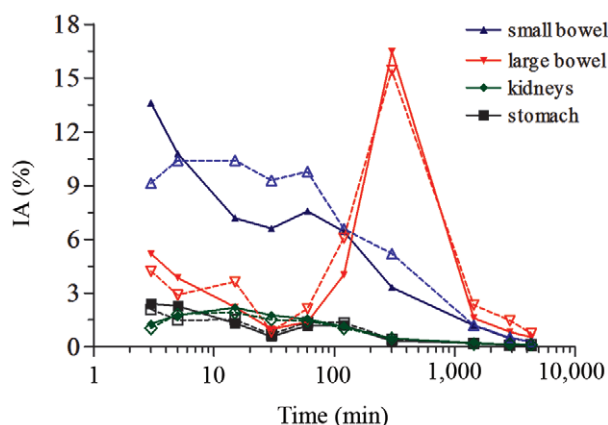


Fig. 2: elimination pathways of neutron-irradiated meglumine antimoniate (IMA) in healthy and *Leishmania (Leishmania) infantum chagasi*-infected BALB/c mice (n = 6) (mean ± standard deviation) after intraperitoneal administration containing 0.081 mg Sb⁵⁺/100 µL with activity of 2.2 x 10⁴ Bq/100 µL of ¹²²Sb and 518 Bq/100 µL of ¹²⁴Sb. Continuous line: healthy mice; dotted line: *L. (L.) infantum chagasi*-infected mice; IA: percentage of injected activity (IA) per organ.

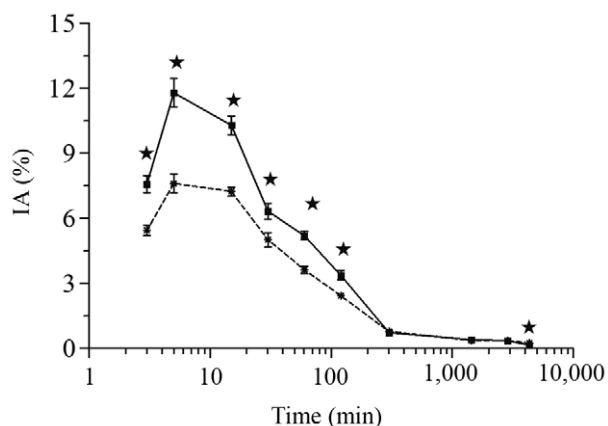


Fig. 3: blood clearance of neutron-irradiated meglumine antimoniate (IMA) in healthy and *Leishmania (Leishmania) infantum chagasi*-infected BALB/c mice (n = 6) (mean ± standard deviation) after intraperitoneal administration containing 0.081 mg Sb⁵⁺/100 µL with activity of 2.2 x 10⁴ Bq/100 µL of ¹²²Sb and 518 Bq/100 µL of ¹²⁴Sb. Continuous line: healthy mice; dotted line: *L. (L.) infantum chagasi*-infected mice; IA: percentage of injected activity per millilitre of blood. Asterisks mean p < 0.05 compared to healthy mice group.

first-order A phase. The means of the pharmacokinetic parameters corresponding to the analysis of the antimony blood concentration curves are listed in Table II. As indicated, the drug was rapidly absorbed, with C_{max} of 11.8% IA/mL and 7.6% IA/mL at 5 min for healthy and infected mice, respectively. The mean times required for the concentration to decrease by one half (t_{1/2}) for the absorption, distribution and E phases were 0.5 vs. 1.0, 17.4 vs. 92.9 and 19.4 vs. 50.2 h for healthy vs. infected mice, respectively. Antimony adsorption was followed by a slow E phase, reaching a level of 0.1-0.2% of the IA by 72 h post-injection. A slight increase in the AUC of infected mice compared with that of healthy mice (58.1% IA.h/mL vs. 48.3% IA.h/mL) was observed and healthy mice had a shorter t_{1/2} with higher antimony concentrations compared to infected mice.

DISCUSSION

In this study, the use of antimony radiotracers produced by neutron irradiation was shown to be a useful tool to answer several questions regarding antimonials. The primary advantage of this procedure is the neutron irradiation of the stable antimony isotopes that are present in the MA formulation. The ¹²²Sb and ¹²⁴Sb radioisotopes were produced at a low thermal neutron flux and during a short period of time and the products had high radionuclidic purity, good specific activity and suitable physiological characteristics for use in biodistribution studies. The use of antimony radiotracers enabled the

TABLE II

Mean pharmacokinetic parameters in the blood of healthy and *Leishmania (Leishmania) infantum chagasi*-infected BALB/c mice (n = 6) following intraperitoneal administration of neutron-irradiated meglumine antimoniate (IMA) containing 0.081 mg Sb⁵⁺/100 µL with activity of 2.2 x 10⁴ Bq/100 µL of ¹²²Sb and 518 Bq/100 µL of ¹²⁴Sb

Parameters	Infected mice	Healthy mice
C _{max} (%IA/mL)	7.6	11.8
T _{max} (h)	0.08	0.08
K _{el} (h ⁻¹)	0.0001	0.0003
t _{1/2} E phase (h)	50.2	19.4
t _{1/2} D/A phase (h)	92.9	17.4
t _{1/2} A phase (h)	1.0	0.5
AUC _{0-∞} (%IA.h/mL)	58.1	48.3
AUMC (%IA.h ² /mL)	3503.4	1246.2
MRT (h)	60.3	25.8
CL (mL/h/kg)	71.7	86.3

A phase: absorption phase; AUC: area under the concentration-time curve; AUMC: area under the first moment curve; CL: total clearance; C_{max}: maximum concentrations; D/A phase: distribution or absorption phase; E phase: elimination phase; K_{el}: elimination rate constant; MRT: mean residence time; t_{1/2}: plasma half-life; T_{max}: time to C_{max}.

determination of small concentrations of MA in biological materials with very good sensitivity. Furthermore, the analysis of antimony radiotracers has the advantage of being independent of the sample matrix and of the efficiency of the digestion or extraction procedures that are required for conventional methods for the evaluation of antimony compounds. Moreover, if a nuclear reactor is available, this type of analysis is easier, faster and less expensive than conventional analytical methods (Osso et al. 2009). Several analytical methods have been used for the determination of antimony concentrations and these methods are characterised by high efficiency, low sample volume, low reagent consumption and improved tolerance of interference; however, these techniques are costly and require trained personnel and sample preparation methods that are not applicable for rapid analyte detection (Rath et al. 2003).

The physicochemical characterisation of IMA showed a slight change in absorbance, most likely due to meglumine polymerisation. MA is produced by the reaction of pentavalent antimony with N-methyl-D-glucamine, a carbohydrate derivative. The extent of polymerisation may influence the pharmacokinetics of drug delivery, uptake by the reticuloendothelial system and the intracellular distribution of pentavalent antimony (Roberts et al. 1998). Meglumine polymerisation can be explained by the Maillard reaction between amines and reducing sugars, which results in brown pigments. In this reaction, an amine reacts with a reducing sugar to form a glycosyl amine and this amine then rearranges via the Amadori rearrangement to form an 1-amino-2-keto sugar that tautomerises and forms coloured compounds (Byrn et al. 2001). Heat exposure intensifies the polymerisation of carbohydrates and consequently changes the colour of the sample. A similar effect was also observed for MA stored under harsh conditions. As reported by Romero et al. (1996), significant differences in osmolarity among samples maintained under different temperature conditions were observed. The UV spectrum of sodium stibogluconate exhibits an absorbance peak in the 270-290-nm region that is associated with conjugated aromatic systems (Roberts & Rainey 1993). Further studies to obtain additional structural information on meglumine antimonite should be performed to support this hypothesis.

Promastigote forms are not susceptible to pentavalent antimonials and this phenomenon is explained by the hypothesis that pentavalent antimonials act as prodrugs that require biological reduction to the trivalent form inside the macrophage or amastigote to exert anti-leishmanial activity (Ouellette et al. 2004). The parasite's life cycle form was evaluated to confirm that IMA contained no trivalent antimony, corroborating the maintenance of the biological properties of MA after the irradiation procedure.

In this study, IMA and MA exhibited similar anti-leishmanial activities against amastigotes and no activity against promastigotes, suggesting that IMA contained no or only a very small amount of trivalent antimony. Similar to the findings of Roberts et al. (1995), our IC_{50} values for pentavalent antimony indicate that it was 100-fold more potent against amastigotes than against promastig-

otes, with a higher therapeutic index; however, trivalent antimony is only 10-fold more potent against amastigotes than against promastigotes and has higher cytotoxicity.

The *in vivo* efficacies of MA and IMA were evaluated in infected mice and both drugs showed similar efficacies, with a reduction of approximately 20% in the parasite burden in the liver. No significant differences were observed between these formulations; therefore, these findings indicate that the biological activity of MA was preserved after the irradiation procedure and exclude the hypothesis that IMA was converted into trivalent antimony. Higher levels of antimony in the liver are related to parasite suppression, which may be associated with selective anti-parasitic effects. However, no significant effect was observed in the spleen (Carter et al. 1988), which may be a site where the parasites are less susceptible to antimonial therapy (Collins et al. 1992).

In the biodistribution studies of the IMA, higher levels of antimony uptake were observed in the livers of healthy mice compared to those of infected mice and this finding may be associated with the hepatic damage caused by the infection (Duarte & Corbett 1987). The antimony concentration peak in tissue was quickly reached after administration, which is an important factor in determining the efficacy of the drug at a particular site of infection. The higher levels of antimony in the liver can be explained by the high rate of blood perfusion (Carter et al. 1988).

Lower levels of antimony in the spleen are associated with relapses (Carter et al. 1988). Higher levels of antimony in the spleen could be needed to achieve a cure because low levels are not sufficient for parasite suppression (Gelhorn & Van Dyke 1946). Further studies should be conducted to correlate the sites of higher drug concentrations with the rate of infection to prevent relapses and treatment failures.

The anti-leishmanial effect of pentavalent antimonials is dependent on the site of action (Carter et al. 1988) and the stage of infection (Baillie et al. 1989). Collins et al. (1992) treated mice with acute infections using sodium stibogluconate and observed significant suppression of the parasite only in the liver, although higher levels of antimony were present in both the spleen and bone marrow. During chronic infection, the absence of parasite suppression in the liver was found to be related to the level of antimony, which was lower than that during the acute infection process (Collins et al. 1992).

Interestingly, IMA was primarily eliminated by biliary excretion after liver processing, reaching the intestinal lumen. There was also rapid renal excretion of a small amount of the drug. Otto and Maren (1950) found the same amount of pentavalent antimony excreted in the faeces and urine of mice that received only one dose of drug. However, they assessed drug elimination in a different mammalian species and higher levels of antimony were observed in the faeces of mice.

IMA was absorbed by the gastrointestinal tract and entered the liver via the portal vein, where it was metabolised before reaching the rest of the body. In biotransformation reactions, original drugs are converted into more polar metabolites by oxidation, reduction or hydrolysis

and the resulting metabolites may be more active than the original molecule (the prodrug) (Roberts et al. 2002). Several studies have suggested that pentavalent antimonials are prodrugs that need to be converted into the corresponding trivalent forms to be active against *Leishmania* (Roberts et al. 1995). After being metabolised, the drug will be eliminated from the body and IMA has been shown to be preferably eliminated via biliary excretion. Biliary excretion is an important route for the elimination of drugs and/or their metabolites. The molecular properties of a compound that affect its biliary excretion include its chemical structure, MW, lipophilicity and polarity. Generally, a high MW, the presence of polar groups and amphipathic characteristics favour biliary excretion (Roberts et al. 2002).

Pentavalent antimonials are generally thought to be excreted through renal elimination. Rees et al. (1980) observed that 80-91% of sodium stibogluconate administered via intramuscular injection was excreted in the urine after 6-8 h and that 96% was excreted in the urine after 6 h when administered intravenously. The differences between the results of the present study and those presented in the literature can be explained by the lack of reports regarding the biodistribution of pentavalent antimonials in organs involved in enterohepatic elimination, particularly the intestines and gallbladder. Furthermore, only analyses of the spleen, liver, bone marrow (Berman et al. 1988, de Yarbuh et al. 1994), skin (Al Jaser et al. 1995), urine (Rees et al. 1980) and blood (Pamplin et al. 1981, Chulay et al. 1988) have been published. These findings underscore the need for more studies to clarify the pharmacokinetic profiles of pentavalent antimonials.

Both healthy and infected mice showed similar biodistribution patterns for MA; however, healthy mice had higher antimony levels in the liver and infected mice had higher antimony levels in the intestines. This finding may be associated with the hepatic damage caused by the infection. Among the different patterns of infection, the typical pattern of morphological alterations is characterised by an increased volume and mass of the liver (Duarte & Corbett 1987). Modifications are also observed in other organs and tissues such as the spleen, bone marrow, lungs, intestines and kidneys. In general, the kidneys exhibit deficiencies in certain proteins that impair the treatment of leishmaniasis. Healthy individuals have been found to have more rapid renal excretion than patients with VL, with complete elimination of MA 24 h after administration. Among ill patients, a delay in elimination occurred and 36 h after drug administration, traces of antimony were still observed in the urine. This delay was caused by the renal dysfunction that is observed in many individuals with VL (Chakravarti & Sem Gupta 1950).

Our findings indicate that antimony is retained longer in the blood of infected mice than the blood of healthy mice (longer $t_{1/2}$ and MRT). The blood antimony concentration as a function of time was best described by a two-compartment pharmacokinetic model (Chulay et al. 1988). The first of these hypothetical kinetic compartments represents a central compartment that includes the blood, the volume into which the drug is absorbed after

intraperitoneal injection and the volume from which the drug is excreted into the urine. The second compartment may represent a peripheral compartment into which the drug is distributed or may be related to the in vivo conversion of pentavalent antimony into trivalent antimony, with fast renal excretion of the pentavalent antimony, following a slow phase that is most likely due to the elimination of trivalent antimony in the liver (Chulay et al. 1988, Valladares et al. 1996).

Determining the pharmacokinetic profile allows the prediction of the residence time of the drug in the organs and the evaluation of its period of action because it is unclear whether the peak antimony concentration is more important for leishmaniasis treatment (Berman et al. 1988) than maintaining the overall level of antimony for a long period of time (Chulay et al. 1988, Tassi et al. 1994).

In conclusion, this study provides important data regarding the biodistribution of antimony during the treatment of leishmaniasis, a neglected disease. Moreover, the use of neutron-irradiated compounds has great potential as a radiopharmaceutical system for evaluating different types of drugs. This study illustrates the benefits of using nuclear reactor facilities for cooperative investigations involving the production of several radiotracers, such as ^{122}Sb and ^{124}Sb , to supply therapeutic radioisotopes for research, radiopharmaceutical development and further clinical applications.

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