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PRODUCTION OF NEUTRON-IRRADIATED MEGLUMINE ANTIMONIATE AND ITS BIODISTRIBUTION IN HEALTHY AND *LEISHMANIA (L.) CHAGASI* INFECTED MICE

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ABSTRACT

Pentavalent antimony, as meglumine antimoniate (Glucantime®) or sodium stibogluconate (Pentostam®), is the main treatment for leishmaniasis, a complex of diseases caused by protozoan parasite *Leishmania,* and an endemic and neglected threat in Brazil. Despite over half a century of clinical use of these antileishmanial agents, their mechanism of action, toxicity and pharmacokinetics data remain mostly unknown. The analytical methods for determination of the amount of antimony in biological systems remain complex and with low sensitivity. Radiotracer studies performed on animals have the potential to play a major role in pharmaceutical development. The aim of this study was to obtain a radiotracer, by neutron irradiation of antimony, with suitable physics and biological properties, allowing easy determination of its biodistribution. Meglumine antimoniate (Glucantime®, Aventis, S.Paulo, Brazil) was neutron irradiated inside the IEA-R1 nuclear reactor, producing two radioisotopes ^{122}Sb and ^{124}Sb, with high radionuclidic purity and good specific activity. This compound presented slightly color change, probably due to the meglumine polymer formation. In its biodistribution studies, it was found higher uptake in the liver of healthy or infected mice and elimination is mostly by biliary excretion with a small and fast proportion of the drug excreted by kidney. The serum kinetic curve is bi-exponential, showing two compartments, a distribution in the central compartment and other associated to drug excretion. The use of the radiotracers, easily created by neutron irradiation, showed to be an interesting instrument to elucidate some questions about antimonials.

1. INTRODUCTION

The leishmaniasis is a complex of diseases caused by protozoan parasite of the genus *Leishmania*, transmitted by phlebotomine sandfly vector. Human leishmaniasis is distributed worldwide, but mainly in the tropics and subtropics, with a prevalence of 12 million cases per year, causing diseases ranging from skin lesions in cutaneous leishmaniasis (CL) to a progressive and frequently fatal hepatosplenomegaly in visceral leishmaniasis (VL) [1]. Co-infection with HIV makes VL a priority for the World Health Organization [2].

Pentavalent antimonials, such as meglumine antimoniate (Glucantime®) or sodium stibogluconate (Pentostam®), are the main drugs recommended in the treatment of all forms of leishmaniasis [3]. Others alternative drugs used in the treatment are pentamidine and amphotericin B, but their use have been limited by high toxicity and cost [4]. Despite several gaps on the knowledge of action, toxicity and pharmacokinetics, pentavalent antimonials have been used over 60 years [5], and the definition of its pharmacokinetic profile may suggest a better therapeutic protocol for doses, administration interval and duration of the antimonial therapy, reducing resistance, relapse and severe side effects.

The analytical methods for determination of the amount of antimony in biological systems remain complex and with low sensitivity [6]. Radiotracer studies performed on animals have the potential to play a major role in pharmaceutical development, pharmacology studies and basic biochemistry research [7]. Therefore, the aim of this study was to obtain a radiotracer, by neutron irradiation of Glucantime®, resulting in radioactive antimony salts, allowing easy determination of its biodistribution in healthy and experimentally infected mice.

2. MATERIALS AND METHODS

2.1. Production and Analysis of Antimony Radiotracer

For standardization, samples of 0.5-0.8 mL of meglumine antimoniate (Glucantime®; Aventis, S.P., Brazil, 81 mg Sb^V/mL) were sealed in quartz ampoules and irradiated at a thermal neutron flux of $1x10^{13}n/cm^2.s$, for 20, 15 and 7 minutes, inside the IEA-R1 nuclear reactor (IPEN-CNEN/SP). Radionuclidic purity was determined by γ-spectrometry, using an HPGe detector (Canberra Company) coupled to the Geniepec program. Radioactive concentration was also measured with the same system after efficiency calibration with standard ^{60}Co, ^{137}Cs and ^{152}Eu source. UV-visible spectrometry Ultrospec 3000 (Pharmacia Biotech) was used for the chemical determination, scanning the spectrum from 200 to 700 nm, samples of the neutron-irradiated meglumine antimoniate (IMA) and not neutron-irradiated meglumine antimoniate (NMA).

2.2. Biodistribution of Radioactive Meglumine Antimoniate

Biodistribution studies of IMA were performed in healthy and *L. (L.) chagasi* infected female BALB/c mice (20-24g) (n=6). Groups of mice were injected, by intraperitoneal route, with 0.081mg Sb^V/100μL with activity of 2.2×10^4 Bq/ 100μL of ^{122}Sb (0.6 μCi) and 518 Bq/100μL of ^{124}Sb (0.014 μCi). After 3, 5, 15, 30, 60, 120, 300, 1440, 2880 and 4320 minutes, mice were sacrificed by cervical dislocation with blood sampling. The organs were excised and activity was measured in a NaI(Tl) scintillation counter (Cobra Auto-Gamma - Canberra Company). The student´s t- test was applied for statistical analysis of the biodistribution studies. The level of $P<0.05$ was considered to be statistically significant.

3. RESULTS AND DISCUSSION

3.1. Production and Analysis of Antimony Radiotracer

After analysis of the IMA, it was verified that the neutron irradiated samples with longer time (20 and 15 minutes) showed intense color change, with spectrophotometric modifications (data not shown) and with physical-chemical alterations as compared to NMA. Neutron irradiated samples to 7 minutes presented very little absorbance peak at 300 nm, with slight color change, probably due to the meglumine polymer formation. The same effect is verified when the product is exposed to unsuitable conditions of temperature and lighting, bad storage conditions [8]. This sample showed maintenance of the biological activity (data not shown), giving evidence of its use in the next studies. High radionuclidic purity was verified, where ^{122}Sb ($t_{1/2}$ = 2.7 days) gamma peaks were observed in 563.99 keV (69.3%) and 692.94 keV (3.78%) and ^{124}Sb ($t_{1/2}$ = 60.2 days) in 602.66 keV (97.8%), 645.77 keV (7.38%), 668.87 keV, 709.26 keV, 713.73 keV, 722.75 keV (10.76%), 790.46 keV, 968.31 keV, 1045.15 keV, 1368.43 keV (2.62%) e 1691.51 keV (47.34%). Activity in the end of irradiation was 33.2 MBq (0.897mCi) of ^{122}Sb and 0.77 MBq (0.021mCi) of ^{124}Sb corresponding to a specific activity of 22.12 MBq ^{122}Sb/mL of meglumine antimoniate (0.598 mCi/mL) and 0.52 MBq ^{124}Sb/mL of meglumine antimoniate (0.014 mCi/mL).

3.2. Biodistribution of the Radioactive Meglumine Antimoniate

In the biodistribution study of the IMA administrated by intraperitoneal injection, shown in Fig. 1, it was observed that healthy or infected mice did not accumulate IMA in brain, lungs, heart and uterus, with an low uptake, 0.02-2% of the injected activity during 72h of the study. Based on these findings, it can be supposed that IMA has not toxic side effects to these organs. In the spleen, the uptake was low, 0.1-2% of the injected activity during the study. The liver uptake was highest, ~55% of the injected activity at 30 minutes, the activity is accumulated and retained in liver. At 24h post injection, no significant activity was seen in any major organ other than liver, both in healthy or infected mice, which was also reported by other authors, which could also be associated with selective anti-parasitic effect [9]. The high levels of antimony in the liver can be explained by high rate blood perfusion [10]. It was found higher uptake in the liver of healthy mice than in the liver of infected mice, this finding can be the result of hepatic damage caused by infection [11]. The IMA showed elimination mostly by biliary excretion, after liver processing, reaching the intestinal lumen, with a small and fast renal excretion. These data are conflicting with reports assuming that for pentavalent antimony the main excretion is through the kidney and urine, usually assumed by most authors [12], but there are no data on intestinal or fecal excretion in those studies, that could be a misinterpretation of biodistribution data [13, 14]. This fact imposes the necessity of more studies to elucidate antimonials pharmacokinetic profile.

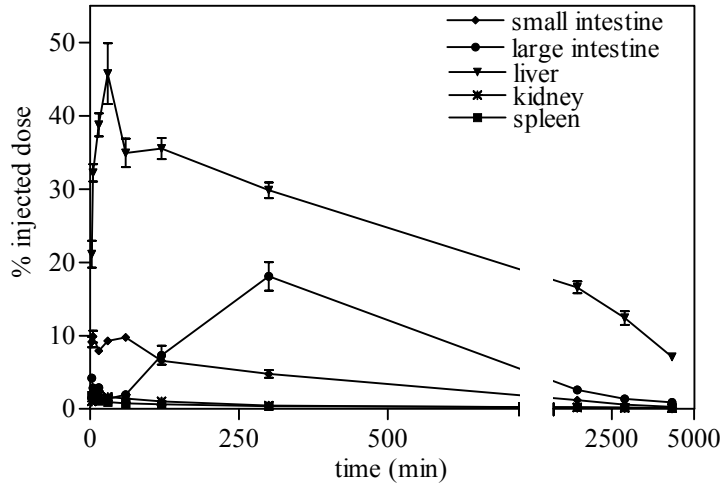

Figure 1. Biodistribution of neutron-irradiated meglumine antimoniate in *L. (L.) chagasi* infected Balb/c mice. The results are expressed as means± standard deviations (n=6).

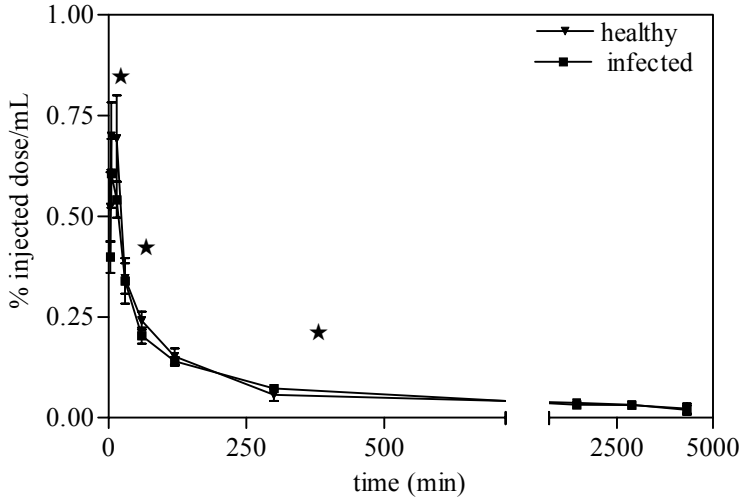

Figure 2. Blood clearance of neutron-irradiated meglumine antimoniate. The results are expressed as means± standard deviations (n=6). *= significant statistic difference between healthy and infected mice (P<0.05).

The antimony serum profile has been described in the other studies [15], which corroborates with data obtained in this study, showed in Fig. 2. Blood antimony concentration as a function of time was best described by two compartments pharmacokinetic model. The first of these hypothetical kinetic compartments represents a central compartment that includes the blood, volume into which the drug is absorved after intraperitoneal injection and 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 *in vivo* conversion of

pentavalent antimony to trivalent antimony, with fast renal excretion of pentavalent antimony, following a slow phase probably elimination of trivalent antimony in the liver [15]. It is suggested that antimony pentavalent acts as a prodrug that is converted to trivalent antimony at or near the site of action, after its administration [16]. This hypothesis was further supported by the observations that trivalent antimony is more toxic than pentavalent antimony against both parasite stages of different *Leishmania species* [17].

4. CONCLUSIONS

1. The neutron irradiation of meglumine antimoniate in nuclear reactor produced two radioisotopes of antimony: ^{122}Sb and ^{124}Sb. This compound showed high radionuclidic purity, good specific activity and suitable physiological characteristics for use in biodistribution studies;
2. In biodistribution studies of the IMA, it was found higher uptake in the liver of healthy or infected mice;
3. The IMA was mostly eliminated by biliary excretion, after liver processing, reaching the intestinal lumen, with a small and fast proportion of the drug excreted by the kidney;
4. The use of the radiotracers, easily created by neutron irradiation, showed to be an interesting instrument to elucidate some questions about antimonials. This study presents, along with direct results for antileishmanial therapy, meaningful indirect advances to pharmacokinetic study of the compound containing atoms that can be irradiated by neutrons producing radiotracers.

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