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PHARMACOKINETIC OF ANTIMONY IN MICE WITH CUTANEOUS LEISHMANIASIS

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ABSTRACT

Cutaneous Leishmaniasis (CL) remains a major world health problem, with about 1.5 million new cases each year. Caused by protozoa *Leishmania*, in South America, this infection can vary from a chronic skin ulcer, to an erosive mucosal disease and severe facial disfigurement. Pentavalent antimony (Sb^{+5}) as sodium stibogluconate (Pentostam®) or meglumine antimoniate (Glucantime®) are main drugs for treating most forms of human leishmaniasis. For six decades, despite the recent developments, the effective therapy to cutaneous leishmaniasis has been based on long parenteral courses of such drugs, even though these are fairly costly, toxic and inconvenient to use, without adequate knowledge on their pharmacokinetics or mechanism of action. Pharmacokinetics studies could be based on bioactive traceable drugs, usually with radioactive isotopes, but antimony radioisotopes are unavailable commercially. Neutron irradiation is a powerful tool in the analysis of mineral content of samples, for antimony, there are at least two main isotopes that could be formed after neutron irradiation in nuclear reactor. The aim of the present study was to construct antimony salts with those radioisotopes to obtain tracers to compare the pharmacokinetic and the tissue distribution of neutron irradiated meglumine antimoniate in healthy and cutaneous leishmaniasis experimentally infected mice. Meglumine antimoniate, (Glucantime®, Aventis, S.P, Brazil), was neutron irradiated inside the IEA-R1 nuclear reactor (IPEN/CNEN-SP), producing two radioisotopes ^{122}Sb and ^{124}Sb. Its biodistribution was verified in BALB/c mice experimentally infected with *Leishmania (Leishmania) amazonensis*, which received a single intraperitoneal dose of the drug. At different times after injection, the tissues and blood were excised and activity measured in a NaI (Tl) scintillation counter. Compared with the healthy mice, experimentally infected mice had significantly lower maximum concentration of antimony and high uptake in liver for both healthy or infected mice. Elimination was mostly by biliary route, with healthy mice showing the highest concentrations of antimony, but the elimination of antimony was slower in the infected mice as compared to the healthy mice. Renal excretion was much less evident than biliary excretion and was significantly lower in both groups of mice. Interestingly, the infected footpad has two times more antimony as compared to non-infected contra lateral footpad in the same animal. These data show that antimony is concentrated in cutaneous infected sites, with biliary excretion. Healthy animals are also more efficient in the excretion of the drug, suggesting that the infected animal could be more prolonged and intensely exposed to the drug. The use of the antimony neutron irradiated should be an interesting tool to evaluate pharmacokinetic problems in antimony pharmacology.

1. INTRODUCTION

Leishmaniasis is a parasitic protozoan disease caused by flagellated organisms of the genus *Leishmania.* The parasite is transmitted by the bite of an infected female phlebotomine sand fly. Cutaneous leishmaniasis (CL) is a serious public health problem in many tropical and subtropical regions of the world. The disease is prevalent throughout the world and in at least 88 countries. There are an estimated 12 million cases worldwide, with 1.5 to 2 million new cases each year [1]. The result of infection can vary from a chronic skin ulcer to erosive mucosal disease with progressive destruction of the nasopharynx and severe facial disfigurement. The resulting syndrome depends upon a complex interaction between a specific species of leishmania and the genetic and immunological status of the host [2].

For more than six decades, long parenteral courses of pentavalent antimonial (Sb^{+5}) drugs, as meglumine antimoniate and sodium stibogluconate, have proved to be the best treatment for all forms of leishmaniasis. However, these are fairly costly, toxic and inconvenient to use. The amount of exposure of the infectious parasite to pentavalent antimony is believed to be an important factor in eradicating the cutaneous leishmania disease. Therefore, understanding of its pharmacokinetics and pharmacodynamics is limited [3].

The analytical methods for determination of the amount of antimony in biological systems remain complex and with low sensitivity [4]. Pharmacokinetics studies could be based on bioactive traceable drugs, usually with radioactive isotopes, but antimony radioisotopes are unavailable commercially. Neutron irradiation is a powerful tool in the analysis of mineral content of samples. There are, at least, two main radioisotopes for antimony that could be formed after neutron irradiation in nuclear reactor: ^{122}Sb and ^{124}Sb.

The aim of the present study was to construct antimony salts with those radioisotopes by neutron irradiation to obtain tracers to compare the pharmacokinetic and the tissue distribution of meglumine antimoniate in healthy and cutaneous leishmaniasis experimentally infected mice.

2. MATERIALS AND METHODS

2.1. Animals and Parasites

Female BALB/c mice (20-24g) were supplied by the Animal Breeding Facility at the Faculty of Medicine of Sao Paulo University. They were maintained in sterilized cages under a controlled environment, with free access to food and water. Animal handling complied fully with the institutional policy. *Leishmania (Leishmania) amazonensis* promastigotes were maintained in RPMI 1640^{-} medium supplemented with 20% calf serum and 0.25% hemin at

24°C. Each animal was injected with 0.1 mL of 2 x 10^7 promastigotes/mL into the left footpad. The biodistribution study was done on day 50 post-infection.

2.2. Production of Neutron Irradiated Meglumine Antimoniate

Samples of 0.5-0.8 mL of meglumine antimoniate (Glucantime®; Aventis, S.P., Brazil, 81 mg Sb^{+5}/mL) were sealed in quartz ampoules and irradiated at a thermal neutron flux of 6.8 X 10^{12} n/cm^2.s, for 10.5 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 Geniepc program. Radioactive concentration was also measured with the same system after efficiency calibration with standard ^{60}Co, ^{137}Cs and ^{152}Eu sources. UV-visible spectrometry Ultrospec 3000 (Pharmacia Biotech) was used for the determination of any possible chemical changes in the pharmaceutical, scanning the spectrum from 200 to 700 nm, samples of the neutron-irradiated meglumine antimoniate (IMA) and not neutron-irradiated meglumine antimoniate (NMA) [5].

2.3. Biodistribution of Neutron Irradiated Meglumine Antimoniate

Biodistribution studies of IMA were performed in healthy and *L. (L.) amazonensis* infected female BALB/c mice (20-24g) (n=5). Groups of mice were injected, by intraperitoneal route, with 0.08 mg Sb^{+5}/100µL with approximately activity of 1 x 10^5 Bq/ 100µL of ^{122}Sb (2.7 µCi) and 2 x 10^3 Bq/100µL of ^{124}Sb (0.06 µCi). After 3, 5, 15, 30, 60, 120, 300, 1440 and 2880 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 DISCUSSIONS

3.1. Biodistribution of Neutron Irradiated Meglumine Antimoniate

The mean pentavalent antimony blood concentration-time profiles obtained after single dosing of IMA are depicted in Fig. 1. Compared with the healthy mice, experimentally infected mice had significantly lower maximum concentration of antimony (C_{max}), the mean (S.D.) values of C_{max}, were two-fold higher in the healthy mice [11.8 (1.61) % administered dose/mL] than in the mice with CL [6.17 (0.48) % administered dose/mL] and the time to reach C_{max} (T_{max}) was 5 minutes. Antimony pentavalent was eliminated more slowly in the healthy mice than in the infected mice, which was also reported by others authors [6], that verified that serum concentrations of pentavalent antimonial were higher in healthy treated hamsters than in infected treated hamsters. Analysis of the curve of the concentration in whole blood after administration of IMA, showed two compartments, a distribution in the central compartment and other associated to drug equilibrium and excretion. Pharmacokinetic parameters of pentavalent antimony have been described previously [7] and are similar to

those measured in this study. The first of these hypothetical kinetic compartments represents a central compartment probably the blood, into which the drug is absorbed after intraperitoneal injection and from which the drug is excreted into the urine. The second compartment represent a peripheral compartment into which the drug is distributed and also associated 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 [8]. Pharmacokinetic determination of the pentavalent antimony is useful to evaluate its time of action and residence in the organism. It is not known whether a favourable response to treatment with pentavalent antimonial depends on achieving a high peak drug concentration or maintaining an inhibitory drug concentration for most of a day. For some authors, the latter seems to be more important [8] than the high peak [9].

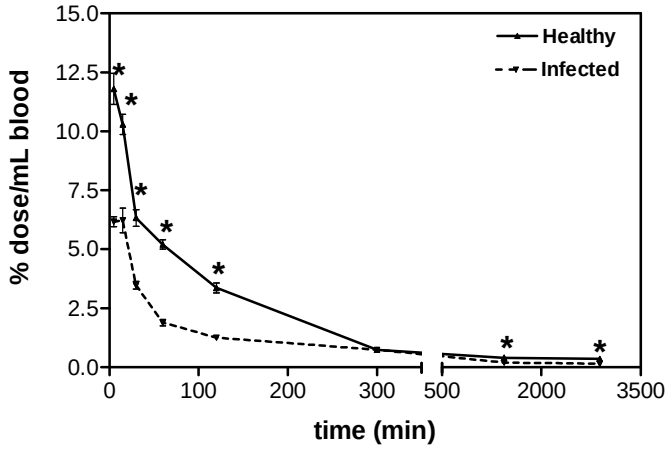

Figure 1. Pharmacokinetic of antimony in the blood of BALB/c mice, after intraperitoneal injection of Irradiated Meglumine Antimoniate (IMA) at 0.081 mg Sb/animal. Data are given as means ± standard deviation (n=5).

Because of infiltration of the skin by the leishmania parasites and the resulting damage to both tissue and blood vessels, it was important to examine the impact of this infection on the pharmacokinetics of antimony in lesion/skin. Several studies have examined the uptake and distribution of antimony in various body tissues (e.g., liver, spleen, bone marrow, etc) following the administration of a pentavalent antimonial drug such as sodium stibogluconate and meglumine antimoniate in animal models [9], but few verified the concentrations of this salt on affected skin in addition to other tissues and serum [10,11]. Thus, in this study, it was found that the infected footpad concentrated two times more antimony as compared to non-infected contra lateral footpad in the same animal. In the lesion on the left hind footpad, the highest Sb concentration (0.3 % administered dose) was observed at 30 minutes after injection, whereas in the healthy right hind footpad it was demonstrated half of this dose. In the lesion, antimony concentrations decreased gradually until 2 hours after the administered dose, however sustained levels of approximately 0.2 % administered dose after 2 days (Fig. 2). Others authors [6] also reported that the lesion contained more antimony than the skin of healthy animals and there was long retention of the pentavalent antimonial by the skin. Accumulation of antimony in the tissue of leishmaniasis animals may therefore reflect the

behaviour of the parasite, particularly how it invades different organs. The decreasing size and final healing of lesion appear to be associated with reduction to pentavalent antimonial to trivalent antimonial in the affected tissue, possibly by activated macrophages [6].

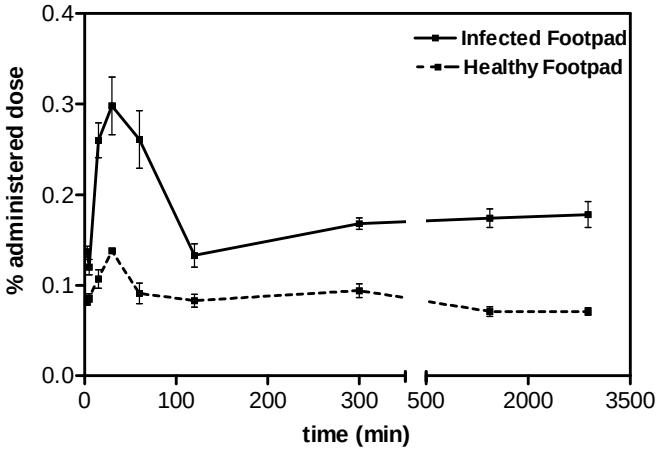

Figure 2. Biodistribution of antimony in the footpad of BALB/c mice, after intraperitoneal injection of Irradiated Meglumine Antimoniate (IMA) at 0.081 mg Sb/animal. Data are given as means ± standard deviation (n=5).

In addition, it was verified higher uptake in liver of healthy or infected mice (40-50 % administered dose, at 30 minutes), but the $T_{½}$ of antimony was more than two-fold higher in the infected mice than healthy mice. Elimination was mostly by biliary route, with healthy mice showing the highest concentrations of antimony, but the elimination of antimony was slower in the infected mice as compared to the healthy mice. Renal excretion was much less evident than biliary excretion and was significantly lower in both groups of mice. It was found by other authors that approximately 37% of the pentavalent antimony dose is excreted in urine 72 hours after administration [12], whereas others [13] found that almost all (80.4%) of the dose given to humans was excreted in the urine. The data from this work are conflicting with those reports, assuming that for pentavalent antimony the main excretion is through the kidney and urine, usually assumed by most authors, but there are no data on intestinal or fecal excretion in those studies, that could be a misinterpretation of biodistribution data. This fact imposes the necessity of more studies to elucidate antimonial pharmacokinetic profile.

4. CONCLUSIONS

The results of this study have consistently shown that antimony is concentrated in cutaneous infected sites, with biliary excretion. The leishmania infection apparently has impact on the pharmacokinetics or penetration of antimony into skin. Healthy animals are also more

efficient in the excretion of the drug, suggesting that the infected animal could be more prolonged and intensely exposed to the drug. The use of the neutron irradiated antimony should be an interesting tool to evaluate pharmacokinetic problems in antimony pharmacology.

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