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Research paper

Layered double hydroxides (LDHs) as carrier of antimony aimed for improving leishmaniasis chemotherapy

Janaína Menezes [a], Tamires da Silva [a], Jailma dos Santos [b], Edgar Catari [b], Mário Meneghetti [b], Carolina da Matta [c,d], Magna Alexandre-Moreira [c], Nereide Santos-Magalhães [e], Luciano Grillo [a], Camila Dornelas [a,*]

[a] Departamento de Farmácia, Universidade Federal de Alagoas, UFAL, 57072-900 Maceió, AL, Brazil
[b] Instituto de Química e Biotecnologia, IQB, Universidade Federal de Alagoas, UFAL, 57072-900 Maceió, AL, Brazil
[c] Instituto de Ciências Biológicas e da Saúde, ICBS, Universidade Federal de Alagoas, UFAL, 57072-900 Maceió, AL, Brazil
[d] Rede Nordeste de Biotecnologia, RENORBIO, Universidade Federal de Alagoas, UFAL, Brazil
[e] Laboratório de Imunopatologia Keizo Asami, LIKA, Universidade Federal de Pernambuco, UFPE, 50670-901 Recife, PE, Brazil

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ABSTRACT

A leishmanicidal drug, antimony potassium tartrate, was used to produce a binary system of SbIII with an inorganic nanocarrier Mg, Al layered double hydroxide via ion exchange. X-ray powder diffraction and inductively coupled plasma-atomic emission spectrometry showed that the intercalated/exfoliated anion was the antimony trioxide. Binary systems were also successfully characterized by Raman and Fourier-transform infrared spectroscopy, thermogravimetric analysis, energy-dispersive X-ray spectroscopy, and scanning transmission electron microscopy. These materials can be an important alternative for leishmaniasis chemotherapy. In addition to the low cost and easily laboratorial synthesis, clay has no cytotoxicity and its uptake by macrophages, amastigotes reservoir leads to a vectorization of antimony and a reduction in its side effects.

1. Introduction

Leishmaniasis, classified by the World Health Organization (WHO) as Category I of the Programme for Tropical Diseases (Special Programme for Research and Training in Tropical Diseases, TDR), concerning emerging or uncontrolled diseases (WHO, 2004), is an infectious zoonotic disease caused by about 20 species of a heteroxenic flagellate (Protozoa: Kinetoplastida: Trypanosomatidae). Leishmaniasis is endemic in 88 countries, of which 72 are in the development, but its impact on global public health is underestimated, since the declaration of the disease is compulsory in only 32 of them. This disease can be manifested in two main forms: cutaneous and visceral leishmaniasis, which, together with Chagas disease and sleeping sickness, is classified as extremely neglected. A recent study on the global funding of innovation for neglected diseases revealed that less than 5% of the total has been invested in this group of parasitic diseases, although more than 500 million people are vulnerable by them (Moran et al., 2009). This justifies the use of Sb-containing drugs, or antimonial drugs, in chemotherapy of leishmaniasis for 100 years, so far little is known about their toxicity and pharmacological mechanisms, and yet they continue to be the first choice drugs for this disease. Antimony is a semimetal element and the most common oxidation states are trivalent (Sb^{+3} or SbIII) and pentavalent (Sb^{+5} or SbV). The first antimonial agent used was tartar emetic [potassium antimony(III) tartrate]. In the search for agents with higher therapeutic indices, a number of other antimonial agents were prepared and evaluated until the less toxic pentavalent compounds in the 1920s. But is still credited to SbIII the leishmanicidal action of antimonial drugs. Goodwin and Page (1943) were the first to propose that SbV could act as a pro-drug that has to be converted into active SbIII, and this is the most accepted mechanism today. Both presently available products, sodium stibogluconate (Pentostam®, Glaxo-Wellcome, UK) and meglumine antimonate (Glucantime®, Specia, France) were developed in the 1940s (Goodwin, 1995) and are pentavalent antimony–carbohydrate complexes. According to Roberts et al. (1995), carbohydrates form water-soluble complexes with antimony may serve to deliver antimonial drugs to host macrophages and for Goodwin and Page (1943) they are simply solutions of antimony pentoxide in a highly hydroxylated organic medium. Even so, the fact is that their use comes with major limitations, such as: high incidence of toxicity, expensive treatment, lengthy treatment and necessity of parenteral administration. So, alternatives are being investigated that aimed for improving chemotherapy with antimonials. In the context of nanotechnology, liposomes and cyclodextrins have been highlighted.

* Corresponding author at: Laboratório de Tecnologia de Nanosistemas Carreadores de Substâncias Bioativas (TecNano), Departamento de Farmácia, Universidade Federal de Alagoas, Campus A. C. Simões, Av. Lourival Melo Mota, s/n, Cidade Universitária, 57072-900 Maceió, AL, Brazil. Tel.: +55 82 3214 1154.
E-mail address: dornelascb@yahoo.com.br (C. Dornelas).

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Frézard et al. (2008) are developing a meglumine antimoniate–cyclodextrin system in order to an oral therapy. Results suggest increased oral absorption of antimony, although has not yet justified this action — since the habitual use of cyclodextrins is through the inclusion of lipophilic substances. The product is in the process of patenting.

Lipid formulations of amphotericin B, an alternative drug, were developed and the highlight in the chemotherapy of leishmaniasis, approved for use by the FDA in 1997, is the AmBisome® (Fujisawa Healthcare Inc., Deerfield, IL), a lyophilized preparation of small unilamellar vesicles consisting of hydrogenated soy phosphatidylcholine (HSPC), cholesterol, dipalmitoyl phosphatidyl glycerol (DMPG) and drug molar ratio 2:1:0.8:0.4. The system has proven advantages, because it is well absorbed by the mononuclear phagocytic system, where *Leishmanias* reside, associated with lower renal toxicity (Soares-Bezerra et al., 2004).

However, we question the choice of nanocarriers, liposomes and cyclodextrins, notoriously high-cost carriers, besides the technologies involved, i.e. lyophilization. Thus, characteristics such as ease of laboratorial synthesis using water as solvent, inexpensive, dispersability in water and degradation at acid pH, generating ions which already exist in the human body prompted the investigation of the viability of layered double hydroxides (Mg, Al LDHs) as carrier of antimony. Mg, Al LDH is biocompatible and has already found pharmaceutical applications as an excipient, as a drug stabilizer, and as a component in adhesive for transdermal delivery, for the symptomatic treatment of peptic ulcers, and for the therapy of digestive disorders (Ambrogi et al., 2003). LDHs are represented by the general formula $[M^{+2}_{1-x}M^{+3}_{x}(OH)_2]^{+x}\,A^{-n}_{x/n}\cdot mH_2O$, where M^{+2} is a divalent metal, M^{+3} a trivalent metal and A^{-n}, an anion n valent; usually where the ratio between M^{+2}/M^{+3} is $0.1 \leq \times \leq 0.5$ (Olfs et al., 2009). LDHs, or the so-called anionic clays, consist of cationic brucite-like layers, compensated by anions in the interlamellar space (Conceição and Pergher, 2007). It is the ion exchange property that justifies its use as host, which the interlayer region may be considered a container of nanometric dimensions where intercalated guest is protected (Ambrogi et al., 2003). In a drug delivery topic, its use is mature in the scientific community and much work has been done by using LDH as host for vitamins, such as ascorbic acid and retinoic acid and non-steroidal anti-inflammatory drugs (NSAID), such as salicylate, naproxen and diclofenac (Costantino et al., 2008).

The unique report of the association of LDH with antimonials refers to Kim et al. (2008), however they did not yield intercalation compounds; the idea was to use antimony as one of the cations present in the lamellae of the inorganic clay precursor Mg, Al LDH. The authors succeeded in about of 10% of replacement of Al^{+3} by Sb^{+3}.

Based on these findings, we investigated in this work the preparation of a binary system by the association of SbIII (the source was the tartar emetic or antimony potassium tartrate, Fig. 1), to a LDH precursor, resulting in a site-specific system of release of antimony, as a powder, with a simple and inexpensive scaling-up, in order to reach the potential patients of leishmaniasis.

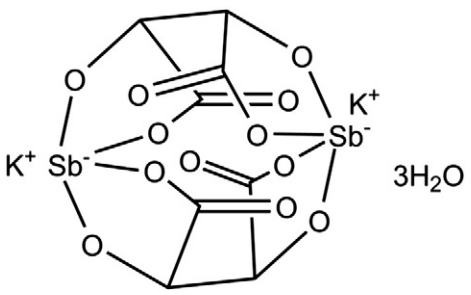

Fig. 1. Antimony potassium tartrate.

2. Experimental

2.1. Materials

Aluminum chloride hexahydrate and magnesium chloride were purchased from Sigma-Aldrich Co. (USA). The drug, antimony potassium tartrate, was provided by Vetec (Brazil).

2.2. Synthesis of LDH precursor

The LDH precursor was synthesized by the coprecipitation method from a mixed metal chloride salt solutions with Mg/Al ratio of 2:1 at pH greater than 10. The resulting white slurry was aged for 24 h at room temperature. The product was centrifuged, washed with deionized water 3–4 times and then dried and reserved for characterization.

2.3. Characterization of LDH precursor

Dynamic light scattering (DLS, Microtrac, Betatek Inc.) was used to analyze the particle size distribution of LDH suspensions. X-ray powder diffraction (XRPD) data were obtained in a Shimadzu XRD 3000 diffractometer using Cu Kα radiation at 30 mA, 30 kV. XRPD patterns were recorded over the 2θ ranging from 2 to 75° in steps of 0.02° with a count time of 2 s. The d-spacing can be calculated by using Bragg's equation. Thermogravimetric (TG) studies were carried out in Shimadzu equipment, model DTG-60, with a heating rate of 20 °C min^{-1}. The IR spectra were obtained on a Shimadzu spectrophotometer, SSU-8000, mode diffuse reflection infrared Fourier transform spectroscopy (DRIFT). The FT-Raman spectra were obtained on a Renishaw spectrophotometer, employing a HeNe laser and a wavelength of 775 nm. Scanning electron microscopy (SEM) measurements were performed using Shimadzu microscope, model SSX-550. The sample was previously coated with colloidal gold in a Quick Coater SC-701 for 5 min, with a current of 10 mA. Transmission electron microscopy (TEM) measurements were performed using a Jeol microscopy, 100 CX II. The sample was dispersed in ethanol before being spread and fixed on the surface of carbon grids.

2.4. Preliminary biological assays — LDH precursor

2.4.1. Isolation of the peritoneal macrophages and cell culture

Experiments were performed using Swiss mice 6–8 weeks old, weighing 20–25 g. Animals were maintained in accordance with the International Commission on Ethics for handling animal (Zimmermann, 1983) and approved by the ethical committee for animal handling of Federal University of Alagoas, Brazil (no. 003570-2011-63). All efforts were made to minimize animal suffering and to reduce the number of animals used. To obtain macrophages, thioglycolate (TG)-elicited macrophages were harvested 3 days after ip injection of 1 mL TG medium. Swiss mice were killed by cervical dislocation and macrophages were isolated by peritoneal lavage after washing with ice cold phosphate buffer saline (PBS). Care was taken not to cause internal bleeding while collecting macrophages in the exudates. Cells were grown on Dulbecco's modified eagle medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS), L-glutamine (2 mM), non-essential amino acids (0.1 mM) and gentamicin (50 μg/mL) for 24 h at 37 °C with 5% CO_2. Non-adherent cells were removed by vigorously washing three times with ice-cold PBS, so that only the macrophages remained adherent culture plates. The macrophages obtained were used to perform all the experimental protocols described below (Davies and Gordon, 2004).

2.4.2. Citotoxicity assay

The cytotoxicity of the synthesized Mg, Al LDH towards cells was determined using the biochemical MTT assay method on cultured murine peritoneal macrophages, based on the protocol described for