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Short communication

Effects of fluconazole on the pharmacokinetics and pharmacodynamics of antimony in cutaneous leishmaniasis-infected hamsters

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Abstract

Pentavalent antimony (Sb^V) compounds are the drugs of choice for the treatment of all forms of leishmaniasis. For 20 years there has been an interest in antifungal azoles for treating leishmaniasis, with variable success. In the current study, we examined the effects of co-administration of fluconazole (FLZ) on the pharmacokinetics and pharmacodynamics of Sb^V in cutaneous leishmaniasis-infected hamsters. Hamsters were divided into four groups. All hamsters were injected with 0.1 mL of 1×10^8 promastigotes/mL into the right foot on Day 1. Treatment was started 5 days after the infection. The antimony group received 80 mg/kg/day of Pentostam® intramuscularly whilst the FLZ group received FLZ 20 mg/kg/day orally for 14 days. The combination group received both Pentostam® and FLZ at the above mentioned doses for 14 days. Animals in the control group received no treatment. The infected footpads were measured on Days 1 and 14. A pharmacokinetic study was conducted on Days 1 and 14 of treatment, representing single- and multiple-dose pharmacokinetics, respectively. Blood samples were collected at different time intervals up to 24 h. Sb^V was determined using flameless atomic absorption spectrophotometry. Pharmacokinetic parameters were calculated using a non-compartmental analysis. In the single-dose study, there was no statistically significant difference in any of the pharmacokinetic parameters of Sb^V when given alone or with FLZ. However, on Day 14 a significant increase in peak plasma concentration (C_{max}) (three-fold) and area under the concentration–time curve (AUC) (four-fold) of antimony was observed when Sb^V was co-administered with FLZ. A statistically significant prolongation of the terminal half-life from 1.63 to 8.67 h ($P < 0.05$) was also observed. A significant reduction in clearance was detected. However, FLZ had no effect on the pharmacodynamics of Sb^V as measured by footpad sizes. In conclusion, FLZ did not improve the therapeutic effect of Sb^V when given concomitantly despite the significant increase in blood concentration and prolongation of the elimination half-life of Sb^V.

Keywords: Antimony; Sodium stibogluconate; Leishmaniasis; Pharmacokinetics; Fluconazole

1. Introduction

Leishmaniasis is a devastating protozoan disease caused by flagellated organisms of the genus *Leishmania*, which infect man by transmission via the bite of a sandfly. Cutaneous leishmaniasis (CL) is caused by the species *Leishmania major*. Most CL lesions manifest as indolent ulcers on exposed skin. Over 90% of cases heal spontaneously within 3–18 months depending upon the infecting species and the host's immune response.

For six decades, long parenteral courses of pentavalent antimonial drugs have proved to be effective antileishmanial agents and have been used for all forms of leishmaniasis, but their utility is limited by their cost, toxicity and inconvenience [1]. Sodium stibogluconate (Pentostam®) is a complexed pentavalent antimony (Sb^V) mainly used in patients with disfiguring or relapsing cutaneous or mucocutaneous leishmaniasis [2]. Second-line drugs include amphotericin B and pentamidine, both of which are nephrotoxic. The difficulties of treatment are exacerbated by the spread of resistance to

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antimony in India and the intractability of the disease to all drugs in patients co-infected with human immunodeficiency virus (HIV) [3].

Over the last few years there have been a number of reports of new therapies, but none has proved to be effective, safe and easily administered [4]. Imiquimod, an immunomodulator for genital warts, produced a 90% cure rate when the ointment was used in conjunction with antimonials in 12 patients who had not responded to antimony alone [5].

For 20 years there has been an interest in antifungal azoles for treating leishmaniasis. Owing to the similarity between the *Leishmania* and fungal cell membranes, these agents have potential in the treatment of leishmaniasis. Ketoconazole and itraconazole have been used to treat CL with variable success [6,7].

The mechanism of action of the azoles is inhibition of cytochrome P450-dependent ergosterol synthesis, which results in the inhibition of conversion of lanosterol to ergosterol and subsequently to inhibition of cell membrane formation.

Most trials have been limited and results are equivocal. Recently, the use of 200 mg/kg fluconazole (FLZ) for 6 weeks led to healing of CL (*L. major*) in 79% of patients compared with 34% receiving placebo [8].

It is theorised that combining FLZ with Sb^V would produce a synergistic therapeutic effect that would heal CL more completely and in a shorter period, thus reducing exposure of patients to the toxicities and difficulties of using Sb^V.

There are no clinical data available on the possible interaction of Sb^V and FLZ. It is well known that different diseases affect the pharmacokinetics of drugs in general. We have some evidence indicating that *Leishmania* affects the clearance of antimony in hamsters (unpublished data). Therefore, this study was undertaken to investigate the effect of administration of single and multiple doses of FLZ on the pharmacokinetics of Sb^V in CL-infected hamsters. In addition, the therapeutic effect of FLZ on healing of a CL ulcer in the footpad of hamsters when administered alone and in combination with Pentostam® was studied.

2. Materials and methods

2.1. Parasite

L. major parasites were preserved in liquid nitrogen with subsequent culturing until they were used for infecting hamsters. At that time, promastigotes were grown in 3N biphasic medium until they reached their stationary phase, i.e. ≥4 days.

2.2. Animals

Age-matched Syrian hamsters with an average weight of 140 ± 5 g (institution animal house) were used. There were 18 hamsters in each treatment group and 6 in the control group. Animals were housed six per cage with free access to food and water. Animal handling complied fully with our institutional policy.

Each hamster was injected with 0.1 mL of 1×10^8 promastigotes/mL into the right foot. This infective dose was selected to ensure development of a significant lesion. The day of infection was considered Day 1. Treatment was started on Day 5 post-infection and continued for 14 days.

2.3. Drugs

Pentostam® (100 mg/mL antimony; Burroughs Wellcome, Research Triangle Park, NC) was administered intramuscularly. FLZ (Pfizer, Memphis, TN) was purchased as Diflucan® and was administered orally as a suspension. All other chemicals and solvents used in the analysis of antimony were of analytical grade.

2.4. Single- and multiple-dose pharmacokinetic protocol

Three groups of 18 hamsters were selected randomly to receive either Pentostam® 80 mg/kg intramuscularly daily for 14 days (antimony group), FLZ orally at a daily dose of 20 mg/kg for 14 days (FLZ group) or both Pentostam® and FLZ for 14 days (combination group) at the above mentioned doses. The last group of six animals was selected randomly to receive no treatment (control group). The pharmacokinetics of antimony was determined for a single dose (following the first day of treatment) and for multiple doses (following the last dose on Day 14 of treatment). Following drug administration, 0.5 mL of blood was collected from the orbital venous plexus from each group at 0.08, 0.25, 0.5, 1, 2, 3, 4, 6, 8 and 24 h. Animals were anaesthetised with CO_2 during blood sampling. Six animals from each group were bled at each sampling time and each hamster was bled only four times to avoid damage to the eye. Blood samples were maintained at 4 °C until the concentration of Sb^V could be determined. In the single-dose study, blood collection was performed after the first dose from the antimony and combination groups to give two sets of blood samples named Group 1 and Group 2, respectively. In the multiple-dose study, blood collection was performed on Day 14 of daily dosing from the antimony and combination groups to give two sets of blood samples named Group 3 and Group 4, respectively.

2.5. Measurement of infected footpads

Animals were checked daily for survival and overall status. The infected footpads were measured at 2-week intervals from the date of infection using a micrometer.

2.6. Blood analysis of antimony using atomic absorption spectrometry

The antimony concentration in plasma was measured by flameless atomic absorption spectrophotometry, using a