

Oral therapy with sertraline, a selective serotonin reuptake inhibitor, shows activity against *Leishmania donovani*

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Objectives: This study was executed to investigate the efficacy of oral therapy and preliminary leishmanicidal mechanism of sertraline, a selective serotonin reuptake inhibitor widely used for the management of depression, against *Leishmania donovani*, a causative agent of visceral leishmaniasis (VL).

Methods: The effect of the drug was determined for: (i) direct promastigote killing by inhibition of MTT reduction and (ii) killing activity against intracellular amastigotes in mouse peritoneal macrophages by microscopic evaluation of surviving amastigotes in macrophages in Giemsa-stained slides. Furthermore, the oral therapy of sertraline against established VL in BALB/c mice was evaluated through estimation of splenic and liver parasite burdens by Leishman Donovan units. Moreover, the preliminary mechanism of action of the drug against promastigotes was assessed by measuring the intracellular ATP levels and oxygen consumption of treated cells.

Results: Sertraline killed *L. donovani* promastigotes and intracellular amastigotes with 50% inhibitory concentrations (IC₅₀s) of 2.2 and 2.3 mg/L, respectively. The drug was also effective in eliminating splenic (72%) and liver (70%) parasite loads in infected BALB/c mice through oral therapy. A sertraline-induced fall in cytoplasmic ATP levels and oxygen consumption rate in promastigotes suggests the involvement of an apoptosis mode of cell death in the treated parasites.

Conclusions: Sertraline could be a promising pharmacological tool for the oral treatment of VL.

Keywords: antileishmanial chemotherapy, BALB/c mice, ATP, oxygen consumption

Introduction

Leishmania donovani, a protozoan parasite, inflicts a fatal disease, visceral leishmaniasis (VL). Pentavalent antimonials, first introduced 60 years ago, remain the first-line treatment. However, treatment with these drugs suffers from several limitations such as cost, specific toxicities, parenteral administration, emergence and spread of drug resistance, and relapses in HIV–*Leishmania* co-infected patients.¹ Amphotericin B, originally identified as a systemic polyene antifungal, is currently used as an efficient second-line antileishmanial.¹ However, it is more toxic than pentavalent antimonials causing infusion-based reactions such as rigour and chills, thrombophlebitis with occasional myocarditis, severe hypokalaemia, renal dysfunction, and even death. The launching of miltefosine into the therapeutic armamentarium of leishmaniasis is a landmark event for the therapy of VL. However, teratogenicity, gastrointestinal upset, potential

of resistance development, and a low therapeutic window pose limitations on its use.² These issues emphasize the urgent need for the development of effective, safer and cheaper chemotherapeutic agents without the prevalent limitations of the current therapeutics for treating leishmaniasis, preferably by oral route. An extensive literature survey suggests that sertraline, a selective serotonin reuptake inhibitor used as an oral antidepressant agent, also exhibits antifungal,³ antimicrobial,⁴ spermicidal and antitrichomonas activities,⁵ as well as anticancer activities.⁶ The diverse biological activities of sertraline, with striking findings of a broad-spectrum antifungal activity and pronounced anticancer effect, have inspired us to assess its antileishmanial activity against *L. donovani* parasites *in vitro* and in extending our observations through oral administration *in vivo*. In addition, we have also investigated whether this drug has any effect on intracellular ATP levels and oxygen consumption of the treated parasites.

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Materials and methods

Parasites

L. donovani (MHOM/IN/1983/AG83) parasites, originally isolated from an Indian kala-azar patient, were maintained in hamsters and cultured in medium 199 (Sigma Immunochemicals, St Louis, MO, USA) (pH 7.4) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Sigma Immunochemicals), 100 U of penicillin G-sodium/mL, and 100 µg of streptomycin sulphate (Sigma Immunochemicals)/mL at 22°C, for *in vitro* and *in vivo* experiments.

Drug and chemicals

The antidepressant drug, sertraline (C₁₇H₁₇NCl₂), mol. wt 342.7, a gift from Sun Pharmaceuticals (Baroda, India), was used for *in vitro* studies in the pure form, and the marketed oral tablets (Sun Pharmaceuticals) were used for *in vivo* studies by suspending them in PBS at the desired dose. Other chemicals and reagents, used for this investigation, were of analytical grade.

Animals

BALB/c mice (25 g) of 4–6 weeks were used for the *in vivo* studies in accordance with the procedure standards approved by the Ethics Committee of the institute.

Effect of sertraline on *L. donovani* promastigotes and intracellular amastigotes *in vitro*

To investigate the effect of sertraline on promastigotes, freshly transformed promastigotes of *L. donovani* AG83 (2×10^6 /mL) in medium 199 containing 10% FBS were incubated with graded concentrations (3–30 mg/L) of drug at 22°C for 2 h. After treatment, the parasites were centrifuged at 1734 g for 10 min and subsequently washed with PBS (0.02 M). The pellets were resuspended in 100 µL (2 mg/mL) of PBS solution of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). After incubation for 4 h at 22°C, their viability⁷ was determined in a spectrophotometer (Hitachi High Technologies, USA) at 570 nm by measuring the optical density of reduced formazan (soluble in dimethyl sulphoxide; DMSO).

To investigate the effect of sertraline on intracellular amastigotes, resident peritoneal macrophages (10^6 cells) from BALB/c mice were infected with *L. donovani* promastigotes at a ratio of 1:10 at 37°C. Following infection for 6 h, the macrophages were treated for 48 h with graded doses (3–15 mg/L) of drug. After treatment, the antileishmanial efficacy of sertraline towards the intracellular amastigotes was evaluated through microscopic counting of the number of amastigotes per 200 macrophages by the Giemsa staining method, in comparison with untreated controls.

Determination of antileishmanial activity of sertraline *in vivo*

To examine the therapeutic efficacy of sertraline, BALB/c mice were infected intravenously (iv) with 2×10^7 amastigotes isolated from spleens of infected hamsters. After 8 weeks of infection, the mice were treated orally, twice weekly for 1 month with 10 mg/kg body weight⁸ of sertraline (marketed tablet formulation, Sun Pharmaceuticals) triturated and dispensed in PBS. Mice were sacrificed 4 weeks post-treatment, and the parasite burdens in spleen and liver were estimated and expressed as Leishman Donovan units (LDUs).⁹

ATP assay after sertraline treatment

The cytoplasmic ATP content, after 2 h of treatment with sertraline at graded doses (3–30 mg/L), was estimated by the luciferin–luciferase method.¹⁰ The assay is based on the requirement of luciferase for ATP in producing light (emission maximum 560 nm at pH 7.8). Briefly, *L. donovani* promastigotes (2×10^6) were harvested (after treatment) and resuspended in reaction buffer prepared as per the kit manual. Whole cell suspensions were assayed for ATP using the Sigma chemical luciferase ATP assay kit. The amount of ATP in test samples was calculated against the standard curve prepared with different concentrations of ATP, and cellular ATP levels were expressed as nmol/ 2×10^6 cells.

Measurement of oxygen consumption

L. donovani AG83 promastigotes (2.5×10^6) were treated with different concentrations (3–30 mg/L) of sertraline for 2 h at 22°C. Cells were harvested and washed in respiration buffer (50 mM sucrose, 145 mM KCl, 5 mM NaCl, 1 mM EDTA, 1 mM MgCl₂ and 10 mM sodium-phosphate buffer, pH 7.4) and ultimately resuspended in the same buffer. Oxygen uptake¹¹ was determined with a Clarke type oxi-electrode 54 having a cell capacity of 2 mL and was considered a direct measure of mitochondrial respiration.

Statistical analysis

Data are expressed as means \pm SE unless otherwise stated. Comparisons were assessed between different treatments through an unpaired Student's *t*-test using Graphpad Prism 4 software. $P < 0.05$ was considered significant.

Results

Sertraline killed 97.5% ($P < 0.0001$) of the promastigotes effectively at a dose of 30 mg/L after 2 h of treatment, compared with untreated controls (Figure 1a). At the lowest concentration (3 mg/L) of sertraline, promastigotes exhibited significant loss of viability (61%, $P < 0.001$). The 50% effective concentration of sertraline was 2.2 mg/L (calculated by the sigmoidal regression analysis using Microsoft Excel, 2007). Treatment of intracellular *L. donovani* amastigotes within peritoneal macrophages for 48 h with graded doses of sertraline showed significant killing of the parasites with substantial elimination of amastigotes at 15 mg/L (94.7%, $P < 0.0001$). The lowest concentration, 3 mg/L, killed 62.5% ($P < 0.001$) of the intracellular parasites. The data plotted in Figure 1(b) revealed that the IC₅₀ value of sertraline against intracellular amastigotes was 2.3 mg/L. Hence, the antileishmanial effect of sertraline on promastigotes and intracellular amastigotes was dose-dependent.

The effect of 4 weeks of oral therapy with sertraline on BALB/c mice infected for 8 weeks with *L. donovani* is shown in Figure 1(c and d). The dose given to each animal was 10 mg/kg body weight, two times per week for 4 weeks. This oral treatment regimen of sertraline reduced the parasite load in spleen by 72% ($P < 0.0001$) and in liver by 70% ($P < 0.0001$), compared with controls.

In promastigotes, exposure to graded doses (3–30 mg/L) of sertraline for 2 h led to a dose-dependent decrease in luminescence and, thus, down-regulation of cytoplasmic levels of ATP

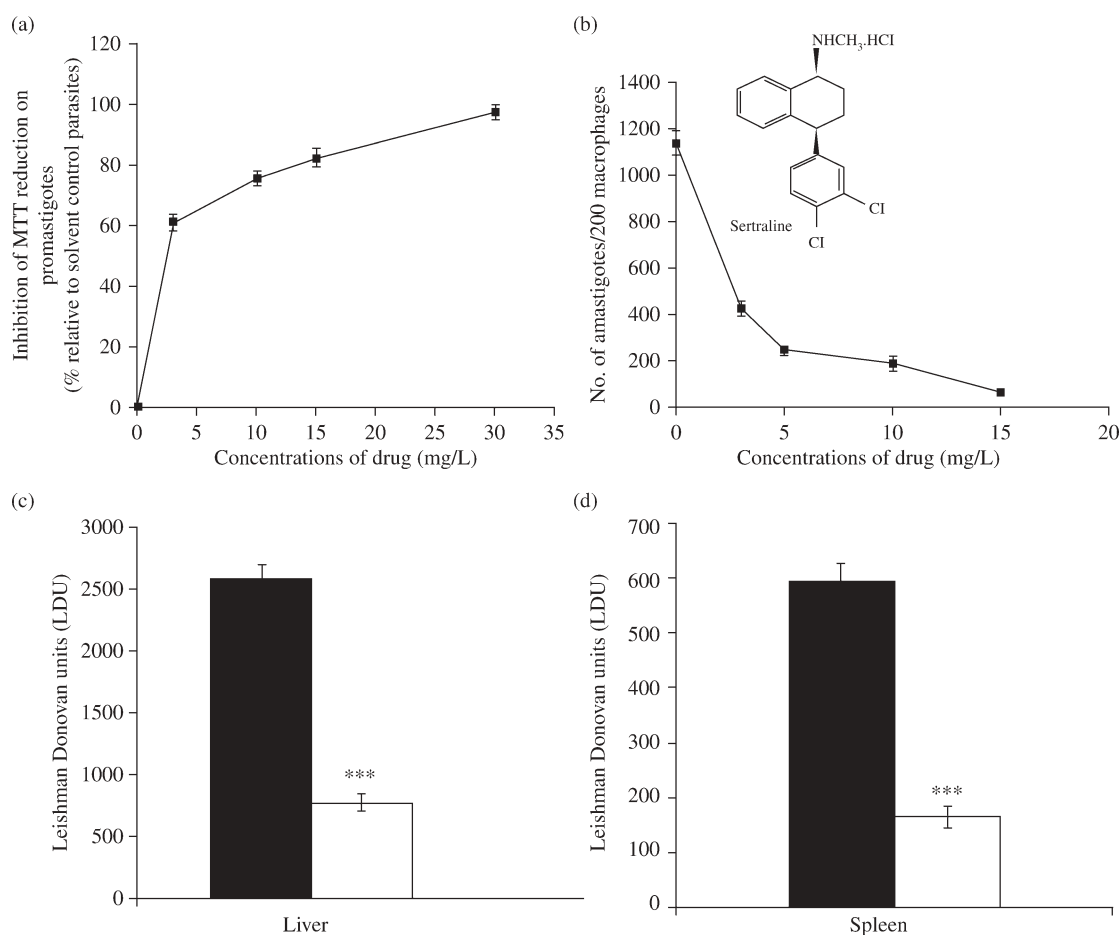


Figure 1. Antileishmanial activity of sertraline against *L. donovani* AG83 promastigotes and intracellular amastigotes *in vitro*, and *in vivo* against established VL infection in BALB/c mice. Promastigotes of AG83 strain (a) were cultured in M199 medium with graded doses (3, 10, 15 and 30 mg/L) of sertraline for 2 h at 22°C. Parasite viability was estimated by the MTT assay and expressed as the cell number relative to those for solvent control (0.2% DMSO) cultures. Effect of the drug at various doses (3, 5, 10 and 15 mg/L) on the survival of *L. donovani* amastigotes (b) internalized in murine peritoneal macrophages 48 h post-treatment. Amastigotes were counted by Giemsa staining and are represented in the figure as the number of amastigotes per 200 macrophages with respect to solvent controls (0.2% DMSO). The results are expressed as the means \pm SE of triplicate values from one experiment representative of two performed. (a) and (b) Sertraline-treated parasites are represented by filled squares. The molecular structure of sertraline has been incorporated as an inset in (b). Eight-week infected mice were treated orally with a dose of 10 mg/kg of body weight of sertraline in PBS (0.02 M), twice weekly for 1 month. Control untreated group received only PBS (0.02 M). Mice were sacrificed 4 weeks post-treatment. Levels of parasite burden in liver (c) and spleen (d) were counted and expressed as LDUs. Values represent the means \pm SE. Untreated control group, black bars; drug treated, white bars. *** $P < 0.0001$ when compared with untreated controls.

(Figure 2a). The ATP levels in parasites exposed to 15 and 30 mg/L of sertraline were brought down to 0.80 and 0.65 nmol/ 2×10^6 cells, respectively ($P < 0.001$), in comparison with untreated controls (3.34 nmol/ 2×10^6 cells).

The inhibition of the respiratory chain as evidenced by the decrease in the cytoplasmic ATP levels was substantiated by the fall in the oxygen consumption rate of sertraline-treated promastigotes when compared with untreated controls. Results demonstrate that the suppression of the rate of oxygen consumption was dose-dependent with maximum decreases at 15 mg/L (57%, $P < 0.001$) and 30 mg/L (83%, $P < 0.0001$) (Figure 2b).

Discussion

Herein, we report an antileishmanial activity of sertraline against *L. donovani* parasites. To the best of our knowledge, this is the

first report of sertraline, as a promising leishmanicidal agent, which is effectual orally for significant reduction in the parasite burden of *L. donovani*-infected BALB/c mice. The therapeutic dose (10 mg/kg) is 55 times lower than the LD₅₀ value (acute toxicity, 548 mg/kg in mice) of this drug.¹² Toxicity studies with the treated dose (10 mg/kg) on normal mice showed that kidney, liver and heart functions were within safe and normal limits at post-treatment (data not shown and Tuynder *et al.*⁶). Sertraline is widely used in the treatment of depression disorder. Our finding of an antileishmanial potentiality for sertraline adds to the versatility of this drug as a chemotherapeutic agent. An extensive literature survey reveals that well-known neuroleptic drugs such as chlorpromazine and its analogues have lethal effects on *Leishmania* and other protozoa. Their protozoacidal effects occur either through enzymatic (trypanothione reductase) inhibition or through some other mechanism, which is completely different from their parent antipsychotic and anxiolytic

Antileishmanial activity of sertraline

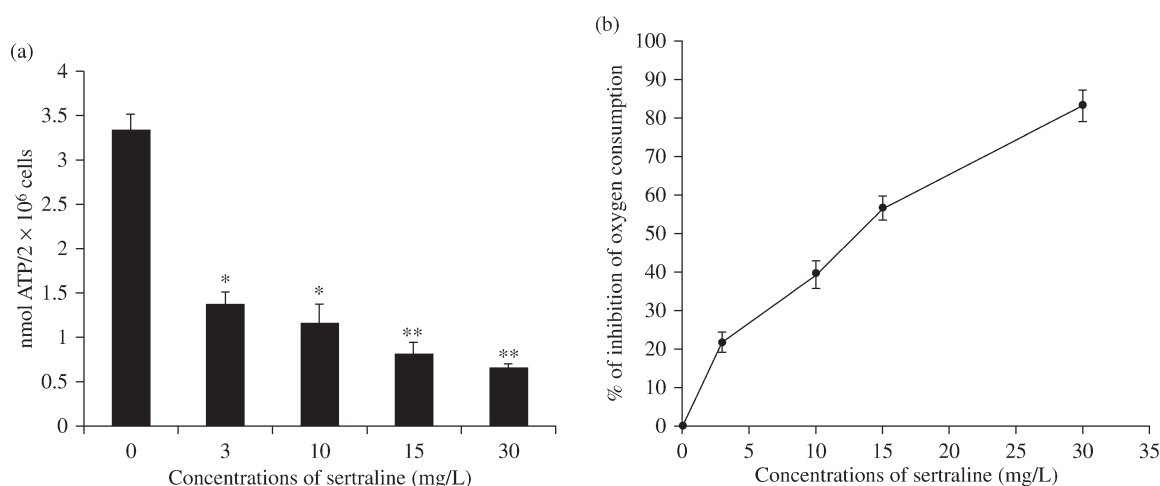


Figure 2. Sertraline exposure leads to a decrease in the cytoplasmic ATP levels and oxygen consumption rate in treated promastigotes. (a) Relative ATP levels were determined as per the manufacturer's protocol (details in the Materials and methods section) after 2 h treatment of *L. donovani* promastigotes with sertraline at different concentrations (3, 10, 15 and 30 mg/L) with respect to solvent controls (0.2% DMSO). * $P < 0.002$ and ** $P < 0.001$ when compared with untreated controls. Mean ATP levels of parasites are represented by black bars. Percentage inhibition of oxygen consumption (b) of *L. donovani* promastigotes was determined in the presence of sertraline for 2 h at different doses (3, 10, 15 and 30 mg/L). The percentage inhibition of oxygen consumption of treated parasites is presented with respect to untreated controls (0.2% DMSO) and depicted as filled circles. The values are expressed as means \pm SD.

properties.^{13,14} Prior to our present report on its antileishmanial activity, sertraline has been reported to have antifungal, antibacterial, spermicidal, antitrichomonas and anticancer activities.^{3–6} However, the mechanism by which sertraline acts as an antimicrobial agent is at present not clearly understood. The antifungal activity probably results from an interaction of sertraline with the fungal membrane transporter system.³ In addition, diminishing of extracellular phospholipase activity by sertraline may be responsible for the loss in the fungal virulence.¹⁵ It has recently been reported that the presence of the phospholipase enzyme system (phospholipase D and related phospholipaseA2) in *Leishmania* is necessary for its cell survival and propagation,¹⁶ and inhibition of phospholipaseA2 activity, in ovarian cancer cells, leads to a modest increase in apoptosis.¹⁷ Previous reports suggest that a fall in oxygen consumption and reduced cytoplasmic ATP levels, resulting from inhibition of the respiratory chain, are essential events in the initiation of apoptosis in leishmanial cells.^{18–21} Herein, our preliminary studies on the leishmanicidal mechanism of action of sertraline reveal reduction in the oxygen consumption rate endowed with a significant decline in cytoplasmic ATP levels of treated parasites. From these observations, we hypothesize that drug-induced cell death and inhibition of the respiratory chain in *L. donovani* parasites could possibly be due to the effects of sertraline on the phospholipase enzyme system of *Leishmania*,¹⁶ which could initiate an apoptosis-like process for parasite cell death as has been shown in ovarian carcinoma cells.¹⁷ Detailed investigations are required to understand the sertraline-induced mode of cell death in *L. donovani* parasites, which will be carried out in our future studies.

In conclusion, leishmanicidal therapy with sertraline associated with an effective drug carrier such as oral liposomes²² to target the drug directly to the spleen and the liver could make it a valuable pharmacological tool against the neglected disease of VL.

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Transparency declarations

None to declare.

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