Antileishmanial Effect of 3-Aminooxy-1-Aminopropane Is Due to Polyamine Depletion

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The polyamines putrescine, spermidine, and spermine are organic cations that are required for cell growth and differentiation. Ornithine decarboxylase (ODC), the first and rate-limiting enzyme in the polyamine biosynthetic pathway, catalyzes the conversion of ornithine to putrescine. As the polyamine biosynthetic pathway is essential for the growth and survival of Leishmania donovani, the causative agent of visceral leishmaniasis, inhibition of the pathway is an important leishmaniacidal strategy. In the present study, we examined for the first time the effects of 3-aminooxy-1-aminopropane (APA), an ODC inhibitor, on the growth of L. donovani. APA inhibited the growth of both promastigotes in vitro and amastigotes in the macrophage model, with the 50% inhibitory concentrations being 42 and 5 μM, respectively. However, concentrations of APA up to 200 μM did not affect the viability of macrophages. The effects of APA were completely abolished by the addition of putrescine or spermidine. APA induced a significant decrease in ODC activity and putrescine, spermidine, and trypanothione levels in L. donovani promastigotes. Parasites were transfected with an episomal ODC construct, and these ODC overexpressers exhibited significant resistance to APA and were concomitantly resistant to sodium antimony gluconate (Pentostam), indicating a role for ODC overexpression in antimonial drug resistance. Clinical isolates with sodium antimony gluconate resistance were also found to overexpress ODC and to have significant increases in putrescine and spermidine levels. However, no increase in trypanothione levels was observed. The ODC overexpression in these clinical isolates alleviated the antiproliferative effects of APA. Collectively, our results demonstrate that APA is a potent inhibitor of L. donovani growth and that its leishmaniacidal effect is due to inhibition of ODC.

Leishmaniasis, caused by a protozoan parasite, constitutes a wide spectrum of diseases ranging from the simple self-limiting cutaneous form to the debilitating visceral form, which is often fatal if it is left untreated. Although pentavalent antimonials (sodium antimony gluconate [SAG]) are the standard first-line treatments for visceral leishmaniasis (VL) (30), in more recent times, increasing resistance to SAG has emerged as a major barrier in the treatment of VL. There has been an epidemic of visceral form to the debilitating visceral form, which is often fatal if it is left untreated. Although pentavalent antimonials (sodium antimony gluconate [SAG]) are the standard first-line treatments for visceral leishmaniasis (VL) (30), in more recent times, increasing resistance to SAG has emerged as a major barrier in the treatment of VL. There has been an epidemic of visceral leishmaniasis. Antileishmanial therapy (18). Polyamines are organic cations required for cell growth and differentiation (31). Ornithine decarboxylase (ODC), the first and rate-limiting enzyme in the polyamine biosynthetic pathway, catalyzes the conversion of ornithine to putrescine (21), and therefore, its inhibition offers a promising approach to antiparasitic therapy (18). α-Difluoromethylornithine (DFMO), the enzyme-activated, irreversibly ODC inhibitor, is an effective trypanocidal drug for the treatment of patients with West African sleeping sickness (18), even those refractory to currently available antitypransosomal drugs and those with central nervous system involvement (22). DFMO is also effective against other parasitic protozoa, e.g., Plasmodium falciparum and Plasmodium berghei (2). It has been demonstrated earlier that DFMO treatment results in lowering of the intracellular polyamine content and has been shown to be effective against promastigotes of the 1S strain of Leishmania donovani (12). In addition to DFMO, several inhibitors of ODC, like 3-aminooxy-1-aminopropane (APA), have been reported to be effective in blocking the proliferation of parasites and tumor cells (9, 13, 14, 16, 25, 29).

In the present study we show for the first time the inhibitory effect of APA, a competitive inhibitor of mammalian ODC, on parasite growth, ODC activity, and cellular polyamine and trypanothione [T(SH)2] concentrations in L. donovani. Furthermore, the L. donovani ODC gene was used to generate ODC overexpressers, and these ODC overexpressers proved to be resistant to sodium antimony gluconate, pointing toward a role for ODC overexpression in antimonial drug resistance. To understand the role of ODC in antimonial drug resistance, we analyzed the polyamine synthesis pathway in field isolates collected from patients with visceral leishmaniasis who did not respond to SAG treatment. All the SAG-resistant isolates showed an increase in ODC activity concomitant with high levels of putrescine and spermidine. Furthermore, the ODC overproducers raised in the laboratory and clinical isolates...
resistant to sodium antimylocuneate were also resistant to APA, confirming that ODC is the primary intracellular target.

MATERIALS AND METHODS

Chemicals. Growth media and antibiotics were purchased from Sigma (St. Louis, MO), and fetal bovine serum (FBS) was purchased from Gibco/BRL (Life Technologies, Scotland, United Kingdom). All other chemicals were of analytical grade. Sodium antimylocuneate powder was obtained from Glaxo-Wellcome.

Parasite and culture conditions. Promastigotes of Indian Leishmania donovani clone GE1 (MHOM/IN/80/GE1F8R) (1), Leishmania donovani strain (MHOM/IN/80/AG83) (wild type), and three untyped clonal strains (strains S-1, R-1, and R-2) were isolated from patients with VL and were routinely cultured at 22°C in medium M-199 with Hanks’ salts, including 25 mM HEPES buffer (Sigma) supplemented with 10% FBS and 100 μg/ml gentamicin (Sigma).

Clinical isolates sensitive to SAG included S-1 and AG83, whereas the three SAG-resistant isolates were GE1, R-1 and R-2. The SAG-resistant isolates were maintained in the absence of drug pressure in vitro. The isolates have been passaged through hamsters or BALB/c mice to retain their virulence; and importantly, their chemosensitivity profiles have remained unchanged, as measured periodically by the amastigote-macrophage infectivity assay described below.

Effect of drugs on cell growth. To estimate the 50% inhibitory concentrations (IC50) of the drugs, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) micromethod was used, as described previously (15). Briefly, late-log-phase promastigotes were seeded in a 96-well flat-bottom plate (200 μl per well, Nunc) in the presence or absence of drugs. To examine the effects of exogenous polyamines, the cultures were additionally supplemented with 1 mM putrescine or spermidine. After 72 h of incubation, MTT (10 mg/ml, 10 μl per well) was added to each well and the plates were incubated for an additional 4 h. The enzyme reaction was then stopped by addition of acidic isopropanol (0.4 ml 10 N HCl in 100 ml isopropanol, 100 μl per well), and the absorbances were measured at 570 nm. Two or more independent experiments were performed in triplicate for each drug.

Constructs and transfection. Construct pGEM7Zf-ODC clone GE1 (MHOM/IN/80/GE1F8R) (1), Leishmania donovani promastigotes expressing the luciferase gene (pGL-NEO), which was transfected with pGEM7Zf-ODC, which contains the ODC gene of L. donovani cloned into a Leishmania expression vector (pGEMTz-ODC-HYG-a) (6), and an episomal Leishmania expression vector (pGGL-neoNeOLUC) containing luciferase-encoding DNA and neomycin phosphotransferase-selectable marker (26) were used in the present study. Clinical isolates were transfected with pGGL-neoNeOLUC. Twenty micrograms of the construct was transfected into L. donovani promastigotes by electroporation in 2-mm-gap cuvettes at 450 V and 500 μF (ELECTRO Cell Manipulator 600). Transfectants were selected for resistance to either hygromycin B (100 μg/ml) or G418 (50 μg/ml), as described previously (20). L. donovani strain AG83 was used for overexpression of the L. donovani ODC gene by transfection. Transfectants overexpressing ODC were routinely maintained in α-minimum essential medium supplemented with 50 μg/ml hygromycin B. FBS was excluded to avoid polyamine interference (12).

Effects of drugs on amastigote-macrophage model. Stationary-phase Leishmania promastigotes expressing the luciferase gene (pGGL-neoNeOLUC) were used to infect J774A.1 macrophages. Macrophage cell line J774A.1 (American Type Culture Collection) was maintained at 37°C in RPMI 1640 medium (Sigma) containing 10% FBS, as described previously (11). Briefly, J774A.1 murine macrophages (1 × 105 cells/250 μl/well) were infected with 1 × 105 promastigotes in medium M-199 with 10% FBS (26). After 3 h, the noninternalized parasites were washed off and drug was added at different concentrations. After 5 days of drug exposure, plates containing adherent macrophages were washed and luciferase activity was determined (26). The IC50 was determined from a graph representing different concentrations of drug plotted against the number of relative light units produced by luciferase-expressing parasites.

Enzyme assay. L. donovani promastigotes (1 × 107) in late log phase were treated with APA, and the cells were harvested 48 h later by centrifugation at 2,000 × g for 15 min at 4°C. The cell pellet was washed with phosphate-buffered saline (pH 7.4) and resuspended in 50 mM Tris-HCl (pH 7.5), 10 μM EDTA, and 2.5 mM diethyliothreitol (DTT). The cells were lysed by freezing-thawing in liquid nitrogen. The lysate was centrifuged at 15,000 × g (20 min, 4°C), and the supernatant was assayed for ODC activity and polyamine and protein concentrations. Protein concentrations were determined by the method of Bradford (3), with bovine serum albumin used as the standard. ODC activity was assayed by monitoring the release of 14CO2 from [1-14C]ornithine (24, 27). The standard assay mixture, which contained the supernatant, 200 μM pyridoxal phosphate, 12.5 mM DTT, 250 mM Tris (pH 7.5), 2 mM ornithine, and 3 μCi of the radiolabeled ornithine, was incubated at 37°C for 1 h. The reaction was terminated by injecting 5 N H2SO4 and the activity was expressed in enzyme units, in which 1 unit is the number of nmol of CO2 produced per minute.

TABLE 1. Effects of APA and DFMO on promastigotes and amastigotes of Leishmania donovani and macrophage cell line J774A.1

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Promastigote IC50 (μM)</th>
<th>Amastigote IC50 (μM)</th>
<th>Macrophage cell line IC50 (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>APA</td>
<td>42 ± 8</td>
<td>5 ± 2.1</td>
<td>&gt;200</td>
</tr>
<tr>
<td>DFMO</td>
<td>&gt;10,000</td>
<td>50 ± 3.2</td>
<td>ND</td>
</tr>
</tbody>
</table>

The results are the means ± SDs of three independent experiments for all data sets. IC50 for promastigotes were determined after 72 h of drug addition. IC50s for intracellular amastigotes were determined after 5 days of drug addition. IC50s for macrophage cell line J774A.1 were determined after 24 h of drug addition. ND, not determined.

RESULTS

Effect of APA on growth of promastigotes and amastigotes. L. donovani promastigotes were cultured in the presence of increasing concentrations of APA. APA inhibited the growth of promastigotes in a dose-dependent manner; the effective concentration that caused 50% inhibition of growth (IC50) after 72 h of drug addition was 42 μM (Table 1). The sensitivities of the amastigotes were tested in an intracellular amas-
Effects of APA on ODC activity and polyamine and trypanothione levels in promastigotes. APA inhibited the ODC activities of the *L. donovani* promastigotes (Fig. 2A). Treatment with 50 μM APA for 48 h inhibited ODC activity by ~76%. Treatment with APA (50 μM) for 48 h also resulted in the concomitant reduction of putrescine and spermidine levels, with 50 μM APA resulting in an ~70% reduction of putrescine levels (Fig. 2B) and a 44% reduction of spermidine levels compared to those for the untreated cells (Fig. 2B). APA (50 μM) inhibited trypanothione levels by ~79% (Fig. 2C).

Characterization of *L. donovani* transfectants overexpressing ODC. Promastigotes from the *L. donovani* AG83 strain overexpressing ODC were isolated after transfection of an episomal ODC gene construct, as described in Materials and Methods. The growth patterns of the wild-type and ODC-overexpressing cells were very similar, with population doubling times of ~32 h and ~29 h, respectively (results not shown). Northern blot analysis of total *L. donovani* RNA prepared from promastigotes of the wild type and ODC overexpressers revealed the presence of the 4.8-kb and 6.5-kb ODC transcripts in both cell lines (Fig. 3A). The presence of two ODC transcripts in *L. donovani* species has been reported earlier (8).

However, the 4.8-kb transcript was more abundant in the ODC overexpressors than in the wild-type cells (~6.2-fold). Interestingly, no major difference in the expression of the 6.5-kb transcript between the ODC-overexpressing and wild-type cells was observed (Fig. 3A). Western blot analysis showed that in the ODC-overexpressing cells there was an increase in the level of ODC protein (~4.4-fold) compared to that in wild-type promastigotes, which corresponded to a similar difference in ODC activity (~3.8-fold) (Fig. 3B and C). Although overexpression of ODC in the transfected cells was observed both by Northern analysis and by Western blot analysis, the increase in the level of ODC mRNA expression was higher than the increase in the level of the ODC protein. This discrepancy may be due to posttranscriptional regulation, which seems to be the mechanism of choice for gene expression in *Leishmania* and other lower organisms (4).

The increased activity of ODC in the promastigotes of the overexpressers resulted in a twofold increase in the putrescine content compared to that in wild-type cells (Table 2), but no corresponding difference in the spermidine content was observed between the two cell lines. The absence of spermine in both the wild type and the ODC overexpressers is consistent with the previous observation that *L. donovani* lacks spermine synthase (12). We also quantified the T(SH)2 levels in the wild type and the ODC overexpressers (Table 2). Surprisingly, there were no significant differences in the T(SH)2 levels between the wild type and the ODC overexpressers.

A link between polyamine metabolism and antimony drug resistance in leishmaniasis has been suggested (17, 32). To evaluate whether the level of expression of ODC in *L. donovani* affected sensitivity to the antimonial drug sodium antimony gluconate, the effect of this drug was determined in the wild-type and the ODC-overexpressing cell lines. As shown in Table 3, the ODC overexpressers exhibited significant resistance to sodium antimony gluconate compared to the resistance of wild-type cells for both promastigotes and amastigotes. The concentrations of sodium antimony gluconate that
inhibited growth of the wild-type promastigotes and amastigotes by 50% were 57 μM and 15 μM, respectively, whereas the IC_{50} values for promastigotes and amastigotes of the ODC overexpressers were 190 μM and >76 μM, respectively, indicating that overexpression of ODC can confer resistance to antimonials in both forms of the parasite. Similarly, the competitive ODC inhibitor APA effectively inhibited growth, with the IC_{50} values being 42 μM and 5 μM for wild-type promastigotes and amastigotes, respectively, and 155 μM and >100 μM for ODC-overexpressing promastigotes and amastigotes, respectively (Table 3). In comparison, no difference in growth inhibition was observed between the two cell lines when the antitrypanosomal diamidine compound berenil was added to the growth medium (IC_{50}, 40 μM for both cell lines [results not shown]).

Characterization of ODC and polyamine and trypanothione levels in promastigotes of clinical isolates. The sensitivities of various clinical isolates to sodium antimony gluconate was tested both in promastigotes and in intracellular amastigotes. The IC_{50} values for the wild type strain and strain

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**TABLE 2. Polyamine and trypanothione levels in wild-type and ODC-overexpressing L. donovani promastigotes**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Putrescine level (nmol/mg protein)</th>
<th>Spermidine level (nmol/mg protein)</th>
<th>Trypanothione level (nmol/10^8 cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>14.3 ± 2.3</td>
<td>39.6 ± 17.0</td>
<td>6.1 ± 1.2</td>
</tr>
<tr>
<td>ODC overexpresser</td>
<td>27.5 ± 4.3</td>
<td>42.0 ± 6.5</td>
<td>5.9 ± 0.5</td>
</tr>
</tbody>
</table>

* Polyamines were measured in acid extracts of promastigotes (strain AG83) harvested at 48 h of growth. Data are means ± SDs (n = 3).

* Intracellular levels of trypanothione were measured by derivatization with monobromobimane and separation by high-performance liquid chromatography. Data are means ± SDs (n = 3).

* Not significantly different from the results for the wild type.

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The polyamine biosynthetic pathway is a potential target for the development of new drugs with activities against parasitic protozoa (18). The polyamines are essential for cell proliferation and differentiation, and interference with their biosynthesis is an established strategy for the treatment of West African trypanosomiasis caused by Trypanosoma brucei gambiense (18, 22). Recent results obtained with null mutants of L. donovani in which genes in the polyamine biosynthetic pathway have been silenced have clearly demonstrated that this pathway is also a promising target for drugs with activities against the protozoa that cause leishmaniasis (10, 23).

In the present study we examined the effect of APA, an ODC inhibitor, on L. donovani growth. APA is a structural analog of putrescine, and the aminooxy group in this compound forms an oxime with the pyridoxal phosphate cofactor in the active site of ODC. APA inhibited the growth of both promastigotes in vitro and amastigotes in the macrophage model, with the 50% inhibitory concentrations being 42 and 5 μM, respectively. All drugs with activities against parasitic organisms that cause disease should exhibit selective activity against the pathogen and not the host; and in this regard, APA at concentrations up to 200 μM had no effect on macrophages, thus establishing the usefulness of L. donovani ODC as a target for the treatment of leishmaniasis. Earlier data on the effect of APA on human T24 bladder carcinoma cells (IC50, ~24.4 μM) (29) further confirm that Leishmania amastigotes exhibit higher sensitivities to APA than the mammalian cells. The antileishmanial effect of APA is mediated by the decrease in ODC activity and putrescine and spermidine levels, as corroborated by the abolition of antileishmanial activity following the addition of putrescine or spermidine. It is likely that the effect of putrescine is due to its conversion to spermidine, as has been reported previously (25). In those studies, APA (50 μM) was found to significantly inhibit trypanothione levels in the wild-type cells.

Surprisingly, in this study, addition of an irreversible inhibitor of ODC, DFMO, to promastigotes even up to a concentration of 10 mM showed no effect on growth. In an earlier study by Kaur et al. (12), DFMO was reported to cause polyamine depletion and growth inhibition of L. donovani promastigotes. Interestingly, in the present study, DFMO inhibited

### TABLE 3. IC50s for wild-type parasites and ODC-overproducing parasites with sodium antimony gluconate and APA

<table>
<thead>
<tr>
<th>Parasite form</th>
<th>Wild type</th>
<th>ODC overexpressers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Promastigotes</td>
<td>Pentostam</td>
<td>APA</td>
</tr>
<tr>
<td>S-1</td>
<td>57 ± 0</td>
<td>47 ± 6 (1)</td>
</tr>
<tr>
<td>R-1</td>
<td>128 ± 3.3 (2.7)*</td>
<td>&gt;400</td>
</tr>
<tr>
<td>R-2</td>
<td>68 ± 4.2 (1.4)*</td>
<td>&gt;400</td>
</tr>
<tr>
<td>GE1</td>
<td>164 ± 5.1 (3.4)*</td>
<td>&gt;950 (7.6)*</td>
</tr>
</tbody>
</table>

* The effects of sodium antimony gluconate and APA on the growth of promastigotes and amastigotes of wild-type and ODC-overexpressing L. donovani strains are indicated as IC50s, which were determined as described in Materials and Methods. The results are means ± SDs (n = 3).

### DISCUSSION

The polyamine biosynthetic pathway is a potential target for the development of new drugs with activities against parasitic protozoa (18). The polyamines are essential for cell proliferation and differentiation, and interference with their biosynthesis is an established strategy for the treatment of West African trypanosomiasis caused by Trypanosoma brucei gambiense (18, 22). Recent results obtained with null mutants of L. donovani in which genes in the polyamine biosynthetic pathway have been silenced have clearly demonstrated that this pathway is also a promising target for drugs with activities against the protozoa that cause leishmaniasis (10, 23).

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Surprisingly, in this study, addition of an irreversible inhibitor of ODC, DFMO, to promastigotes even up to a concentration of 10 mM showed no effect on growth. In an earlier study by Kaur et al. (12), DFMO was reported to cause polyamine depletion and growth inhibition of L. donovani promastigotes. Interestingly, in the present study, DFMO inhibited

### TABLE 4. Comparative analysis of the susceptibilities of the sodium antimony gluconate-sensitive and -resistant clinical isolates to sodium antimony gluconate and APA

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Sodium antimony gluconate</th>
<th>APA</th>
<th>Sodium antimony gluconate</th>
<th>APA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>57 ± 0</td>
<td>42 ± 8</td>
<td>15.0 ± 0.6</td>
<td>5 ± 2</td>
</tr>
<tr>
<td>S-1</td>
<td>47 ± 5.6 (1)</td>
<td>19 ± 0.7</td>
<td>13.0 ± 1.5 (1)</td>
<td>10 ± 2.5</td>
</tr>
<tr>
<td>R-1</td>
<td>128 ± 3.3 (2.7)*</td>
<td>&gt;400</td>
<td>62.0 ± 3.4 (5)*</td>
<td>&gt;200</td>
</tr>
<tr>
<td>R-2</td>
<td>68 ± 4.2 (1.4)*</td>
<td>46 ± 2.8</td>
<td>23.0 ± 1.4 (1.8)*</td>
<td>&gt;200</td>
</tr>
<tr>
<td>GE1</td>
<td>164 ± 5.1 (3.4)*</td>
<td>&gt;950 (13.0)*</td>
<td>&gt;950 (7.6)*</td>
<td>&gt;200</td>
</tr>
</tbody>
</table>

* Stationary-phase L. donovani isolates transfected with pGL-neOOnLUC were used to infect J774A.1 macrophages, as described in Materials and Methods. Luciferase activity was determined after 5 days of drug exposure. IC50s were determined from the graph representing the concentration of drug plotted against the number of relative light units produced by luciferase-expressing parasites. IC50s are given as the means ± SDs of at least three independent determinations. The fold increase in the IC50 compared to that of strain S-1 is given in parentheses. * The resistance levels observed in the SAG-resistant isolates are statistically different from that in SAG-sensitive strain S-1 (P < 0.002).
amastigote growth in the macrophage model, with an IC₅₀ of 50 μM. Differences in sensitivities to DFMO and also to APA between promastigotes and amastigotes is interesting and may be due to differences in the uptake of the inhibitors. Amastigotes of the *L. donovani* strain used in this study were shown to be highly sensitive to the ODC inhibitors DFMO and APA, which inhibited growth in the micromolar range. However, APA was 10-fold more effective than DFMO against the amastigote stage.

Previous results have shown that resistance to antimonial compounds in *Leishmania* is multifactorial, with contributions made by several independent mechanisms (6). A link between polyamines and antimonial resistance has been suggested (19).

In trypanosomatids, like *Leishmania*, glutathione is replaced by trypanothione, which is a conjugate between the polyamine spermidine and glutathione. A correlation between resistance to antimonial drugs and increased trypanothione levels appears to exist in *Leishmania* (17, 32). Thus, increased resistance to antimonial drugs and increased trypanothione levels appears to exist in *Leishmania* (17, 32). This implies that APA is a potent inhibitor of ODC activity and that the target was indeed ODC. APA was 10-fold more effective than DFMO against the amastigote stage. However, APA was 10-fold more effective than DFMO against the amastigote stage. APA was 10-fold more effective than DFMO against the amastigote stage. APA was 10-fold more effective than DFMO against the amastigote stage. APA was 10-fold more effective than DFMO against the amastigote stage. APA was 10-fold more effective than DFMO against the amastigote stage.

### TABLE 5. Quantification of intracellular polyamine and trypanothione levels in promastigotes of sodium antimony gluconate-sensitive and -resistant isolates

<table>
<thead>
<tr>
<th>Strain</th>
<th>Putrescine level (nmol/h/mg protein)</th>
<th>Spermidine level (nmol/h/mg protein)</th>
<th>Trypanothione level (nmol/10⁸ cells)</th>
<th>ODC activity (nmol/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-1</td>
<td>17.7 ± 1.8 (1)</td>
<td>12.39 ± 4.2 (1)</td>
<td>7.8 ± 1.1</td>
<td>28.2 ± 3.4 (1)</td>
</tr>
<tr>
<td>R-1</td>
<td>33.1 ± 5.8* (1.8)</td>
<td>19.97 ± 3.5* (1.6)</td>
<td>6.6 ± 0.4*</td>
<td>58.7 ± 4.5** (2.0)</td>
</tr>
<tr>
<td>R-2</td>
<td>59.7 ± 3.0** (3.4)</td>
<td>23.85 ± 6.5* (1.9)</td>
<td>4.7 ± 0.5**</td>
<td>144.3 ± 2.8*** (5.1)</td>
</tr>
<tr>
<td>GE1</td>
<td>52.67 ± 4.78*** (2.9)</td>
<td>22.75 ± 2.1* (1.8)</td>
<td>5.7 ± 0.6**</td>
<td>62.6 ± 5.5*** (2.2)</td>
</tr>
</tbody>
</table>

* Each value is the mean ± SD of at least four determinations for putrescine and spermidine levels and three determinations for ODC activity from two independent experiments. For trypanothione levels, each value is the mean ± SD of triplicate experiments. Concentrations were determined by using the values from known amounts of standards. The fold increase compared to the level in strain S-1 is given in parentheses. *, P < 0.05 compared to the corresponding values obtained for strain S-1; **, P < 0.01 compared to the corresponding values obtained for strain S-1; ***, P < 0.001 compared to the corresponding values obtained for strain S-1.

b Not significantly different from the value for strain S-1.

Our results demonstrate a link between ODC overexpression and antimonial resistance. We have also demonstrated that APA is a potent inhibitor of *L. donovani* and that its leishmaniacidal effect is due to inhibition of ODC. The inhibitory effect of APA on ODC activity correlates well with its effect on parasite growth and depletion of putrescine and spermidine levels. Furthermore, the sodium antimony gluconate-resistant clinical isolates exhibited significant resistance to APA. However, APA exhibited selective activity against the pathogen and not against the host, establishing the usefulness of *L. donovani* ODC as a target for the treatment of leishmaniasis.

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