Oral Therapy with Amlodipine and Lacidipine, 1,4-Dihydropyridine Derivatives Showing Activity against Experimental Visceral Leishmaniasis

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Amlodipine and lacidipine, conventional antihypertensive drugs, inhibited *Leishmania donovani* infection in vitro and in BALB/c mice when administered orally. These 1,4-dihydropyridine derivatives functioned through dose-dependent inhibition of oxygen consumption, triggering caspase 3-like activation-mediated programmed cell death of the parasites.

The recommended drugs used for the treatment of visceral leishmaniasis (VL), i.e., pentavalent antimonials, were first introduced 60 years ago. Over the last decade, alternative new drugs and newer formulations have become available or are under clinical trial to combat this deadly disease. However, they all suffer from limitations of cost, specific toxicities, parenteral administration, emergence and spread of drug resistance, or extended treatment regimens (2, 6). The most remarkable advance has been the introduction of the first effective oral treatment of VL with miltefosine, an alkyl lysophospholipid analogue. However, teratogenicity, gastrointestinal upset, potential of resistance development, and a low therapeutic window pose limitations on its use (5, 20). Hence, the ambition to develop an orally effective drug or drug formulation which requires a short course of treatment without the prevalent limitations of toxicity and drug resistance remains unfulfilled. Amlodipine and lacidipine, dihydropyridine Ca²⁺ channel blockers, are used orally for the treatment of hypertension. Previous reports suggested that amlodipine can also inhibit the proliferation of different cancer cells (9, 21). In addition, amlodipine has been reported as a potential antimicrobial agent (8). It has also been reported that lacidipine (15) and some 3-chloro-phenyl (11) and nitro aryl 1,4-dihydropyridine (16) derivatives are cytotoxic towards *Trypanosoma cruzi* through respiratory chain inhibition. Moreover, nifedipine, another dihydropyridine Ca²⁺ channel blocker, can inhibit *Leishmania*-macrophage attachment during initiation of the disease (13). Amlodipine and lacidipine both have a phenyl-1,4-dihydropyridine moiety (Fig. 1A) and are structurally unrelated to other Ca²⁺ channel blockers. In view of the diverse biological activities observed for amlodipine and lacidipine, we were interested in assessing their activity against *Leishmania donovani* (MHOH/IN/1983/AG83) parasites in vitro and in extending our observations through oral administration in vivo.

To evaluate the effects of the drugs on promastigotes, freshly transformed promastigotes of *L. donovani* AG83 (2 × 10⁶/ml) in medium 199 containing 10% fetal bovine serum were incubated with graded concentrations of drugs at 22°C for 2 h, and their viability was determined by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) assay (14). The 50% effective concentrations of amlodipine and lacidipine were 2 and 2.5 µg/ml (calculated by sigmoidal regression analysis using Microsoft Excel, 2007), respectively (Fig. 1B). Both drugs killed (98.76% for amlodipine [P < 0.0001] and 90.5% for lacidipine [P < 0.001]) promastigotes effectively at a dose of 30 µg/ml after 2 h of treatment, in contrast to verapamil- and diltiazem-treated and untreated controls (assessed through unpaired Student’s t test). The 50% inhibitory concentrations for amlodipine and lacidipine were significantly reduced, to 0.875 and 1.45 µg/ml, respectively, for long-term growth inhibition study when viable *L. donovani* promastigotes were exposed to these drugs for three continuous days at doses ranging from 0.3 to 3 µg/ml (Fig. 1C). In order to investigate the effects of these drugs on intracellular amastigotes, peritoneal macrophages (10⁶ cells) isolated 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 of drugs. A dose of 15 µg/ml led to significant killing of intracellular amastigotes by amlodipine and lacidipine (96.39% [P < 0.0001] and 85.66% [P < 0.001], respectively). At 3 µg/ml, >50% of intracellular parasites were killed, in contrast to untreated controls. The data plotted in Fig. 1D revealed that the 50% inhibitory concentrations of amlodipine and lacidipine against intracellular amastigotes were 2.1 and 2.8 µg/ml, respectively. Similar to the case for promastigotes, the killing effect of the drugs on intracellular amastigotes was dose dependent. The doses of amlodipine and lacidipine that were toxic for macrophages were >100 and 150 µg/ml, respectively, indicating that the experimental doses were safe for the host cells.

To examine the therapeutic efficacy of these two drugs, BALB/c mice (4 to 6 weeks old) were each infected intravenously with 2 × 10⁷ amastigotes isolated from spleens of infected hamsters. After 8 weeks of infection, the mice were treated orally with 10 mg/kg of body weight (4, 17) of marketed formulations (oral tablets; Sun Pharmaceuticals Ltd.) of amlodipine and lacidipine (4.5 and 325 times lower than the 50% lethal doses of amlodipine [45 mg/kg] and lacidipine [3,250...
mg/kg] for mice) in phosphate-buffered saline (PBS), in single doses administered weekly for four consecutive weeks, for a total of four doses. The control untreated group received only PBS. Mice were sacrificed at 30 days posttreatment, and the parasite burdens in the spleen and liver were estimated and expressed as Leishman Donovan units (1). Treatment with amlodipine and lacidipine showed significant decreases in the spleen and liver weights compared to those of untreated controls (data not shown). Moreover, these therapies led to significant reductions in splenic (85.27% [\( P < 0.0001 \)] and 75.03% [\( P < 0.0001 \)]) and liver (86.01% [\( P < 0.0001 \)] and 72.01% [\( P < 0.0001 \)]) parasite burdens at 30 days posttreatment with amlodipine and lacidipine, respectively, compared to controls (Fig. 2A and B).

In order to elucidate the mode of cell death through possible inhibition of oxygen consumption, we measured the oxygen uptake of drug-treated (graded concentrations) promastigotes with a Clarke type oxi-electrode (18). The results showed that although verapamil and diltiazem had negligible effects, the rate of oxygen consumption decreased 86% and 78% after treatment with amlodipine and lacidipine, respectively, at 30 \( \mu \)g/ml for 2 h (Fig. 3A). This suppression of oxygen consumption was dose dependent. It was reported earlier that an increase in the inhibition of oxygen uptake by parasites after drug treatment causes up-regulation in the number of apoptotic cells (19). To investigate the role of caspase-like proteases in the apoptotic cascade of these drug-treated parasites, we carried out a fluorometric assay of caspase 3, a member of the CED-3/CPP32 group of proteases, in the cytosol of parasites following treatment, per the manufacturer’s protocol (Calbiochem). The results demonstrated that caspase 3-like activity in treated cells increased significantly (\( P < 0.0001 \)) with increasing concentrations of amlodipine and lacidipine, from 3 to 30 \( \mu \)g/ml, in comparison to untreated controls (Fig. 3B).

Herein we report a remarkable inhibitory activity of amlodipine and lacidipine on the in vitro and in vivo growth of \( L. \)
**donovani** parasites. The antileishmanial effect of these drugs correlated with reduced oxygen consumption of the treated parasites and the activation of caspase 3-like protease. We also measured the intracellular Ca\(^{2+}\) concentrations of parasites after treatment with amlodipine, lacidipine, and two other Ca\(^{2+}\) channel blockers having antihypertensive activity, i.e., verapamil and diltiazem. All four drugs inhibited Ca\(^{2+}\) uptake by the parasites to similar levels (data not shown). However, although amlodipine and lacidipine demonstrated significant antileishmanial activity, verapamil and diltiazem were completely ineffective in killing the parasites and showed no inhibitory effect on oxygen consumption. This negates a possible correlation between the leishmanicidal activity and the Ca\(^{2+}\) channel blocking action of the drugs. The antileishmanial effect is therefore not related to a disruption in Ca\(^{2+}\) homeostasis of the parasites. Amlodipine and lacidipine both contain a phenyl-1,4-dihydropyridine ring which is absent in verapamil and diltiazem. This suggests that the antimicrobial activity of dihydropyridine derivatives observed by us and others (8, 11, 15, 16) could be due to the presence of the phenyl-1,4-dihydropyridine ring. In *Leishmania*, inhibition of the mitochondrial respiratory chain, comprising complexes I to IV (3, 18), causes down-regulation of oxygen consumption. Many antileishmanials that inhibit the respiratory chain complexes also induce apoptosis (12). Inhibition of oxygen consumption causes an increase in the intracellular reactive oxygen species, leading to a loss of mitochondrial membrane potential (19) and a release of cytochrome c into the cytoplasm. This then activates caspase-like proteases (7), which play major roles in the apoptotic cascade of these parasites (19, 22). We postulate that interference in the mitochondrial respiratory chain complexes of the parasite might be responsible for the lethal effects of amlodipine and lacidipine, which cause a reduction of oxygen consumption and death, apparently by apoptosis, via activation of caspase 3-like protease, with mitochondria as the key intracellular target (10).

To our knowledge, this is the first report of amlodipine and lacidipine as potential antileishmanial drugs which were effec-

FIG. 2. Evaluation of leishmanicidal activity of amlodipine and lacidipine in established infection model of VL in BALB/c mice. Mice infected for 8 weeks were treated orally with a dose of 10 mg/kg of body weight of amlodipine and lacidipine in PBS weekly for 1 month. Control infected animals received only PBS. Mice were sacrificed at 30 days posttreatment. Levels of parasite burden in spleens (A) and livers (B) are expressed as Leishman Donovan units. Values represent the means ± standard errors for four animals per group.

![Graph A](image1.png)

**A**

**B**

![Graph B](image2.png)

FIG. 3. Effects of amlodipine and lacidipine on oxygen uptake and caspase 3 activity of parasites after treatment. (A) Percentages of inhibition of oxygen consumption of the parasites were determined in the presence of drugs (amlodipine, lacidipine, verapamil, and diltiazem) at graded doses (3, 10, 15, and 30 \(\mu\)g/ml) after 2 h of treatment. Oxygen uptake measurements were carried out polarographically with a Clark electrode. (B) Spectrofluorometric detection of caspase 3 activity in the cytosol of *L. donovani* promastigotes after 2 h of treatment with graded doses (3, 10, 15, and 30 \(\mu\)g/ml) of amlodipine and lacidipine with respect to controls (0.2% DMSO). The results are expressed as means ± standard deviations for triplicate values. Bars 1, DMSO control; bars 2, 3 \(\mu\)g/ml; bars 3, 10 \(\mu\)g/ml; bars 4, 15 \(\mu\)g/ml; bars 5, 30 \(\mu\)g/ml.
tual orally for satisfactory reduction in the parasite burdens of *L. donovani*-infected BALB/c mice. Our results demonstrate that amlodipine is more effective than lacidipine at identical doses. Both of these drugs are widely used as Ca\(^{2+}\) channel antagonists for the treatment of hypertension (4, 17). Chronic toxicity tests with healthy mice showed that the therapeutic dose of amlodipine and lacidipine (10 mg/kg) was within a safe and acceptable margin, and liver, kidney, and heart functions were normal posttreatment. Since these drugs also demonstrate strong antileishmanial activity and are apparently devoid of the severe side effects associated with the currently available antileishmanial drugs, amlodipine and lacidipine could be good therapeutic tools for oral treatment of VL. This observation is redolent of the analgesic aspirin, which is also useful in treating heart disease. Based on the phenyl-1,4-dihydropyridine ring as the lead structure, future drugs may be synthesized to optimize the antileishmanial efficacy of these compounds for cost-effective oral combination therapy of the neglected disease VL.

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REFERENCES


