Glibenclamide, a blocker of $K^{+}_{ATP}$ channels, shows antileishmanial activity in experimental murine cutaneous leishmaniasis

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Glibenclamide reduced the rate of lesion growth in BALB/c mice infected with 
*L. (L.) mexicana*, the effect was dose-dependent and the highest dose proved more 
effective than glucantime. Cross-resistance to glucantime was found in animals 
infected with a glibenclamide resistant line; drastic effect was observed with a 
combined drug treatment.
The chemotherapy currently available for leishmaniasis relies on the administration of antimonial compounds; their toxicity and the emergence and spread of drug resistance emphasize the urgent need for affordable alternative drugs (3, 5). The most significant advance has been the introduction of the first effective oral treatment with miltefosine, an alkyl-lysophospholipid (ALP), for the treatment of visceral leishmaniasis (16). Glibenclamide, an inhibitor of \( K^+_{\text{ATP}} \) channels (2) and Pgp-glycoprotein (7), has been reported to inhibit uptake and multiplication of \textit{Leishmania} within macrophages \textit{in vitro} (13). The effect was associated with increased responsiveness to \( \gamma \)-interferon and to stimulation of Th1 mechanisms in general (13, 14). In the present study we evaluate the effect of glibenclamide against \textit{L. (L.) mexicana} infection on BALB/c mice and the efficacy of a combined treatment with glibenclamide and glucantime. The EC\textsubscript{50} against promastigotes of \textit{L. (L.) mexicana} (MHOM/VE/90/9012) growing in Schneider’s \textit{Drosophila} medium was 50 \( \mu \)M and a glibenclamide resistant line was selected at 50 \( \mu \)M Gb (Gb\textsuperscript{50R}). Both the glibenclamide-sensitive (Gb\textsuperscript{S}) and Gb\textsuperscript{50R} strains showed a moderate susceptibility to glucantime; however, a fixed concentration of 50 \( \mu \)M glibenclamide in combination with a variable concentration of glucantime caused an inhibition of 80-90% in cell growth independently of sensitivity of the strain to glibenclamide (Fig. 1).

Significant reduction in lesion size (\( P < 0.0001 \)) was evident when BALB/c mice infected with Gb\textsuperscript{S} amastigotes were administered glibenclamide on the 20 days after infection; subsequent lesion enlargement was inhibited by 1.25 mg Gb/kg/day. Similarly to \textit{in vitro} results, the effect \textit{in vivo} was dose-dependent. Because a much larger amount of glibenclamide can be given to mice, the effect of 80 mg/kg/day
glibenclamide was compared to the effect of 100 mg/kg/day glucantime. Although both drugs inhibited lesion enlargement in mice infected with the Gb\textsuperscript{S} strain (Fig 2A), the highest dose (80 mg/kg/day) proved more effective than glucantime (100 mg/kg/day) in reducing lesion size. In contrast, mice infected with amastigotes of the Gb\textsuperscript{50R} strain failed to respond to treatment with either glibenclamide or glucantime at the same concentrations used for Gb\textsuperscript{S} (Fig. 2B); such unresponsiveness to glibenclamide confirmed the genetic stability of the resistant phenotype of Gb\textsuperscript{50R}, and the lack of an effect of glucantime on the course of the infection with Gb\textsuperscript{50R} suggests the occurrence of cross resistance to both drugs. After 49 days of treatment with either drug alone, mice were treated with both drugs in combination for 20 more days. Lesion development in mice infected with Gb\textsuperscript{S} diminished but did not stop when treated with either drug for 49 days, ceased completely when drug combination was used for 20 days, and recovered when the treatment was stopped (Fig. 2C); this suggests that each drug adds to the antileishmanial activity of the other drug. In contrast, lesion development in mice infected with Gb\textsuperscript{50R} was significantly (P< 0.0001) reduced by the combined drugs (Fig. 2D). This result is surprising; although there is cross-resistance between the drugs, they are effective when administered together.

The mode of action of glibenclamide against \textit{Leishmania} is not yet established; the drug has been described as a classical inhibitor of the K\textsuperscript{+}\textsubscript{ATP} channels in pancreatic β cells whose target is the SUR receptor, a protein belonging to the ABC transporters family which has not been identified in \textit{Leishmania}. Recently, the inhibitory effect of glibenclamide on different ABC transporters with dissimilar functions, including P-glycoprotein, was demonstrated (7); such transporters have been described
in *Leishmania* spp. (4, 10, 11, 12). Also, a role for Ca\(^{2+}\) homeostasis seems to be related to the antileishmanial activity of glibenclamide (14).

Glibenclamide and glucantime do not seem to share the same route of entry to *Leishmania* and probably have different mechanisms of action. Systems for arsenic detoxification have been identified in all living organisms and an aquaglyceroporin system was identified in *L. tarentolae* and *L. (L.) major* (6, 15). Probably, the uptake of glibenclamide, a sulphonylurea, occurs through a different mechanism from that for glucantime. It would be interesting to know whether glucantime and glibenclamide share an efflux system and whether there are differences between the drugs in the affinity of this system for them; also, it would be worthwhile evaluating the eventual co-expression of different drug transporters.

The combination of glibenclamide and glucantime enhanced the antileishmanial effect *in vitro* as well as *in vivo*. The mechanism of this effect is not known; an additive effect could explain the lesion reduction found in mice infected with Gb\(^{S}\) but not with Gb\(^{50R}\). Experiments are in progress in order to evaluate the possible occurrence of a synergistic effect of glibenclamide and glucantime. A synergistic effect of glibenclamide and IF-\(\gamma\) on the clearance of *L. (L.) major* by macrophages has been found (14); also, synergistic effect has been found in the treatment of leishmaniasis with glucantime combined with other drugs (1, 6).

In summary, glibenclamide affects the viability of *L. (L.) mexicana* *in vivo* and *in vitro*; in both cases drug effects were dose-dependent. Glibenclamide has a higher efficacy and tolerance at the concentration used for the treatment of *L. (L.) mexicana* compared with glucantime. A cross-resistance to both glibenclamide and glucantime
was evidenced. Treatment of experimental mice with the combination of these drugs was highly effective against infections with both the glibenclamide sensitive and the resistant line.
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FIG. 1. Effect of glucantime on the growth of *L. (L.) mexicana* wild type and glibenclamide resistant strains. Promastigotes of *L. (L.) mexicana* Gb<sup>S</sup> and Gb<sup>50R</sup> were cultured in Schneider’s *Drosophila* medium supplemented with 10 % FCS. Aliquots of stationary phase of Gb<sup>S</sup> strain culture were incubated with increasing concentrations of glucantime (1.5g/5ml; Aventis Pharm Ltda, Brasil), in the absence (■) or in the presence (□) of 50 µM glibenclamide (Research Biochemical International, Natick Mass., USA. 33); promastigotes of Gb<sup>50R</sup> were subject to the same glibenclamide treatment, absent (▲) or present (△). Parasite growth was estimated by direct counting on Newbauer chamber and expressed as the cell number relative to control cultures. The results are expressed as means ± standard error of the mean (n = 3). The EC<sub>50</sub> value, without correction due to extensively binding to serum proteins of glibenclamide, was 54.3 ± 1.0 µM according to the linear interpolation method of Huber and Koella (9).
FIG. 2. Comparison of antileishmanial treatment with glucantime and glibenclamide on the course of infection with *L. (L.) mexicana* in BALB/c mice. Mice (5-6 week old, 20-25 g) were infected in the footpad on day 0 with $1.25 \times 10^6$ amastigotes of Gb$^5$ (A and C) or Gb$^{50R}$ (B and D) *L. (L.) mexicana*. Mice were treated daily i.p., twenty days after infection, for 20 days with 5 % DMSO (■; control), 100 mg/kg of glucantime (△) and 80 mg/kg of glibenclamide (○). The LD$_{50}$ of glibenclamide estimated for mice is 3250 mg/kg. Lesion appearance was monitored and size of lesion was measured daily, starting with the initiation of treatment. The same groups of mice received daily for 20 more days the combined regime with 75 mg/kg glucantime and 60 mg/kg glibenclamide (arrow). Statistical assessment of differences between treatments was done through one-way analysis of variance (ANOVA) and Duncan’s multiple comparison tests at P< 0.05. Each point represents the mean ± standard error of the mean (n = 7). P < 0.0001 for glucantime treatment versus untreated mice, glibenclamide treatment versus untreated mice and glucantime treatment versus glibenclamide treated mice.
Cell growth inhibition (%) vs Glucantime [mg ml$^{-1}$] for different strains of T. cruzi:

- $G_b^S$
- $G_b^S (+Gb)$
- $G_b^{50R}$
- $G_b^{50R} (+Gb)$

The graph shows the percentage of cell growth inhibition as a function of Glucantime concentration for each strain.
Lesion size (mm)
days post treatment

Control
Gb: 80 mg/kg
glu: 100 mg/kg

Lesion size (mm)
days post treatment

Control
Gb: 80 mg/kg
glu: 100 mg/kg