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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 *Leishmania* (*Leishmania*) *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, but combined therapy reduced lesion progression even in the glibenclamide-resistant strain.

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, for the treatment of visceral leishmaniasis (16). Glibenclamide (Gb), an inhibitor of K^+_{ATP} channels (2) and P-glycoprotein (7), has been reported to inhibit the uptake and multiplication of *Leishmania* within macrophages in vitro (13). This effect has been associated with increased responsiveness to gamma interferon and with stimulation of Th1 mechanisms in general (13, 14). In the present study, we evaluate the effect of glibenclamide against *Leishmania* (*Leishmania*) *mexicana* infection of BALB/c mice and the efficacy of a combined treatment with glibenclamide and glucantime. The 50% effective concentration against promastigotes of *L.* (*L.*) *mexicana* (MHOM/VE/90/9012) growing in Schneider's *Drosophila* medium was 50 μM, and a glibenclamide-resistant line was selected at 50 μM Gb (Gb^{50r} strain). Both the glibenclamide-sensitive (Gb^s) and Gb^{50r} strains showed a moderate susceptibility to glucantime; however, a fixed concentration of 50 μM glibenclamide in combination with various concentrations of glucantime caused an inhibition of 80 to 90% in cell growth that was independent of the 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^s amastigotes were administered glibenclamide on the 20 days after infection; subsequent lesion enlargement was inhibited by 1.25 mg Gb/kg of body weight/day. Similar to in vitro results, the effect in vivo was dose dependent. The effect of glibenclamide at 80 mg/kg/day, which is forty times lower than the 50% lethal dose (3,250 mg/kg) for mice, was compared to the effect of 100 mg/kg/day glucantime. Although both drugs inhibited lesion enlargement in mice infected with the Gb^s strain (Fig. 2A), glibenclamide proved more effective than glucantime in reducing lesion size. In contrast, mice infected with amastigotes of the Gb^{50r} strain failed to respond to treatment with either glibenclamide or glucantime at the same concentrations used for the Gb^s strain (Fig. 2B); such unresponsiveness to glibenclamide confirmed the genetic stability of the resistant phenotype of the Gb^{50r} strain, and the lack of an effect of glucantime on the course of the infection with the Gb^{50r} strain suggests the occurrence of cross-resistance to both drugs. After 49 days of treatment with either drug alone, mice were treated with

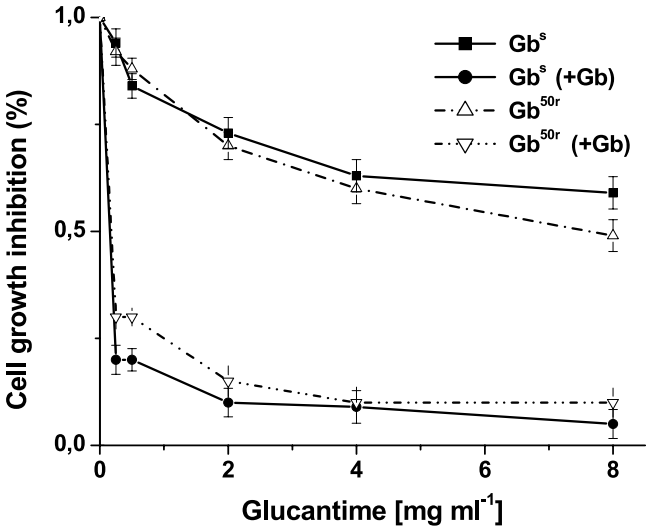

FIG. 1. Effect of glucantime on the growth of *L.* (*L.*) *mexicana* wild-type and glibenclamide-resistant strains. Promastigotes of *L.* (*L.*) *mexicana* Gb^s and Gb^{50r} strains were cultured in Schneider's *Drosophila* medium supplemented with 10% fetal calf serum. Aliquots of stationary-phase Gb^s strain culture were incubated with increasing concentrations of glucantime (1.5 g/5 ml; Aventis Pharm Ltda, Brazil) in the absence or presence of 50 μM glibenclamide (Research Biochemical International, Natick Mass.) (13); promastigotes of Gb^{50r} were subjected to the same glibenclamide treatment, i.e., absence or presence. Parasite growth was estimated by direct counting in a Newbauer chamber and expressed as the cell number relative to those for control cultures. The results are expressed as means ± standard errors of the means ($n = 3$). The 50% effective concentration value, which is given without correction due to the extensive binding to serum proteins of glibenclamide, was 54.3 ± 1.0 μM according to the linear interpolation method of Huber and Koella (9).

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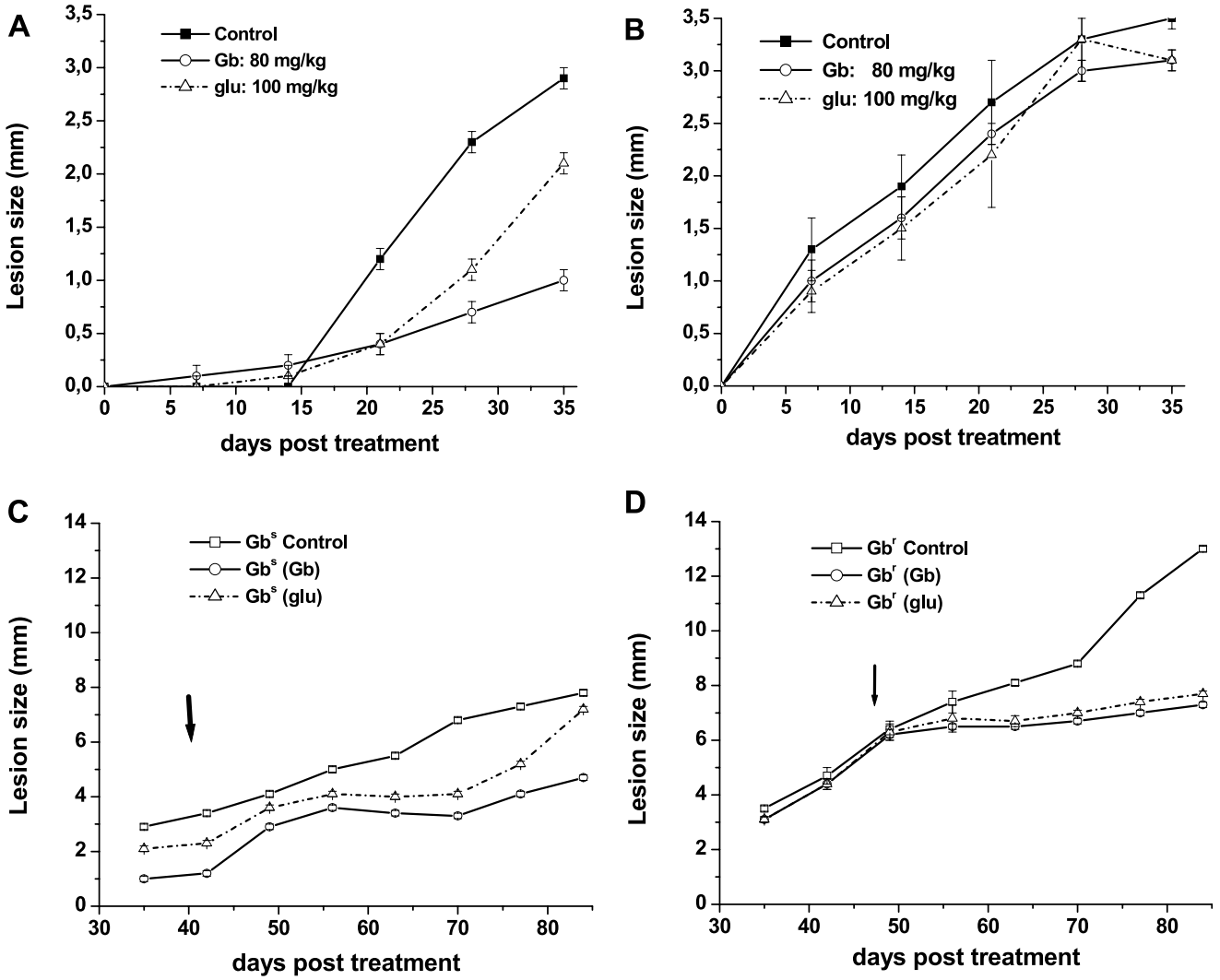

FIG. 2. Comparison of antileishmanial treatment with glucantime and glibenclamide on the course of infection with *L.* (*L.*) *mexicana* in BALB/c mice. Mice (5 to 6 weeks old, 20 to 25 g) were infected in the footpad on day 0 with 1.25×10^6 amastigotes of Gb^s (A and C) or Gb^{50r} (B and D) *L.* (*L.*) *mexicana*. Twenty days after infection, mice were intraperitoneally treated daily for 20 days with 5% dimethyl sulfoxide (control), 100 mg/kg of glucantime (glu), or 80 mg/kg of glibenclamide. The 50% lethal dose of glibenclamide estimated for mice is 3,250 mg/kg. Lesion appearance was monitored, and lesion size was measured daily starting with the initiation of treatment. The same groups of mice received daily for 20 more days the combined regimen 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 and Duncan's multiple comparison tests at a P value of <0.05. Each point represents the mean ± standard error of the mean ($n = 7$). For mice receiving glucantime treatment versus untreated mice, mice receiving glibenclamide treatment versus untreated mice, and mice receiving glucantime treatment versus glibenclamide-treated mice, the P values were <0.0001.

both drugs in combination for 20 more days. Lesion development in mice infected with the Gb^s strain diminished but did not stop when mice were 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. In contrast, lesion development in mice infected with the Gb^{50r} strain 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 *Leishmania* has not yet been established; the drug has been described as a classical inhibitor of the K^+_{ATP} channels in pancreatic β cells whose target is the SUR receptor, a protein belonging to the ABC transporter family, which has not been identified in *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 for *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 into *Leishmania* organisms 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, 8, 15). Probably, the uptake of glibenclamide, a sulfonylurea, occurs through a mechanism different 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 to evaluate the eventual coexpression 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 gamma interferon on the clearance of *L.* (*L.*) *major* by macrophages has been found (14); also, a 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* than glucantime does. 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 line and the resistant line.

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