**Introduction**

Chagas’ disease (American trypanosomiasis) is a parasitic disease caused by the kinetoplastid protozoan *Trypanosoma (Schizotrypanum) cruzi*, which afflicts 16–18 million people in Latin America. Specific chemotherapy for this disease is still very unsatisfactory, since the only compounds available for human use, nitrofurans and nitroimidazoles, have poor efficacy in the prevalent chronic form of the disease and significant toxic side effects.  

*T. cruzi* is extremely sensitive to ergosterol biosynthesis inhibitors (SBIs) *in vitro* but currently available SBIs, such as ketoconazole or itraconazole, fail to eradicate *T. cruzi* from experimentally infected animals or patients. We have recently shown that D0870 (Zeneca Pharmaceuticals), a bis-triazole derivative which inhibits the parasite’s sterol C14α demethylase, is capable of inducing parasitological cure in seven of the nine strains tested, including one intermediate and two highly drug-resistant strains. D0870 was able to cure 30–45% of animals chronically infected with various strains, including those harbouring the Colombiana strain, among which no cures were obtained with benznidazole. We also found that the anti-*T. cruzi* activity of D0870 is largely retained even if the hosts are immunosuppressed.

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**In vivo activity of the bis-triazole D0870 against drug-susceptible and drug-resistant strains of the protozoan parasite *Trypanosoma cruzi***

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We report the *in vivo* activity of the bis-triazole derivative D0870 against a variety of strains of *Trypanosoma cruzi*, the causative agent of Chagas’ disease, including nitroimidazole/nitrofuran-resistant strains. In both acute and chronic murine models of the disease, treatment with D0870 at ≥ 10 mg/kg on alternate days for a total of 20 doses provided 60–100% protection against death, regardless of the drug sensitivity of the infecting strain. In the acute model we obtained 70–100% parasitological cure in seven of the nine strains tested, including one intermediate and two highly drug-resistant strains. D0870 was able to cure 30–45% of animals chronically infected with various strains, including those harbouring the Colombiana strain, among which no cures were obtained with benznidazole. We also found that the anti-*T. cruzi* activity of D0870 is largely retained even if the hosts are immunosuppressed.

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partially drug-resistant (Y) and highly drug-resistant (SC-28, YuYu, Colombiana and VL-10) strains. The original isolates have been maintained as trypomastigotes in liquid nitrogen, periodically passaged in mice and refrozen, with full retention of their biological and drug-resistance characteristics. D0870, provided by Dr John Ryley of Zeneca Pharmaceuticals (Macclesfield, UK), was suspended in aqueous 2% methylcellulose plus 0.5% Tween 80. Benznidazole (Rochagan; Roche, São Paulo, Brazil), was dissolved in water containing 1% arabic gum. Both drugs were given by gavage; control (untreated) animals received the vehicle as placebo, which had no detectable toxic effects. For the experimental chemotherapy studies the protocol developed by Filardi & Brener8 was followed. In the acute model, groups of 10–15 immunocompetent or immunosuppressed outbred female Swiss albino mice, weighing 18–20 g, were inoculated ip with 10⁴ blood trypomastigotes of the different strains; oral treatment was initiated 4 days post-infection (p.i.) and given every other day for a total of 20 doses. Surviving animals were followed for 60–110 days p.i. In the chronic model, groups of 12 mice were inoculated ip with 30 blood trypomastigotes of the different strains to allow the development of a chronic, latent infection. After 120 days, surviving animals, which had no parasitaemia detectable by conventional methods, were randomly divided into different treatment groups (12 animals per group) and subjected to oral treatment given daily or every other day for a total of 20 doses. Surviving animals were followed for 201 days p.i. Parasitaemia was measured in tail blood with a haemocytometer. Haemocultures and xenodiagnosis were carried out as described previously.6 Antibodies against live T. cruzi were evaluated by cytofluorometry, using the procedure of Martins-Filho et al.9,10 with minor modifications.

The Kaplan–Meier non-parametric method was used to estimate the survival functions of the different experimental groups and rank tests (log-rank and Peto-Peto–Wilcoxon) were used to compare them. The analyses were done with the Survival Tools package for StatView 4.5 (Abacus Concepts, Berkeley, CA, USA) run on a Power Macintosh 6500/250 computer.

Results and discussion

Table I shows the effects of increasing doses of D0870, given orally on alternate days for a total of 20 doses, on the survival and parasitological eradication in mice acutely infected with nine different strains of T. cruzi. D0870 was given on alternate days based on the long (50 h) terminal half-life of this compound in mice5 and the results of previous studies which demonstrated that this dosing schedule was as effective as daily dosing.6 Treatment with D0870 given at 10 mg/kg on alternate days led to 80–100% protection against death 110 days after infection, regardless of the drug sensitivity of the infecting strain. Furthermore, evaluation of parasitological cures by three independent criteria (xenodiagnosis, haemoculture and presence of antibodies specific for living T. cruzi) showed that the bis-triazole was able to induce high levels (70–100%) of cure in seven of the nine strains tested, including one intermediate (Y) and two highly drug-resistant (SC28 and YuYu) strains. Against the remaining two strains (Colombiana and VL-10), the levels of parasitological cure were significantly higher than those reported previously with benznidazole or nifurtimox at 100 mg/kg given daily.8 The results demonstrate that this triazole derivative can overcome the primary drug insensitivity of natural T. cruzi populations which circulate in endemic regions of Latin America. In a second experiment with the same model, some hosts were immunosuppressed with 50 mg/kg cyclophosphamide, given during two consecutive days before infection; animals were then infected, given anti-parasitic treatment and followed for 60 days, as described above. The strains used were CL

Table I. Effects of D0870 on parasitological cure in a murine model of acute Chagas’ disease with various strains of T. cruzi (cured animals/survivors)

<table>
<thead>
<tr>
<th>T. cruzi strain</th>
<th>Drug resistancea</th>
<th>Dose of D0870 (alternate days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>CL</td>
<td>S</td>
<td>0/0</td>
</tr>
<tr>
<td>J</td>
<td>S</td>
<td>0/0</td>
</tr>
<tr>
<td>Buriti</td>
<td>S</td>
<td>0/6</td>
</tr>
<tr>
<td>Gilmar</td>
<td>S</td>
<td>0/4</td>
</tr>
<tr>
<td>Y</td>
<td>PR</td>
<td>0/4</td>
</tr>
<tr>
<td>SC-28</td>
<td>R</td>
<td>0/0</td>
</tr>
<tr>
<td>YuYu</td>
<td>R</td>
<td>0/0</td>
</tr>
<tr>
<td>Colombiana</td>
<td>R</td>
<td>0/4</td>
</tr>
<tr>
<td>VL-10</td>
<td>R</td>
<td>0/5</td>
</tr>
</tbody>
</table>

aS, susceptible; PR, partially drug-resistant; R, drug-resistant.8

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D0870 effects on drug-resistant *Trypanosoma cruzi*

(susceptible), Y (intermediate drug-resistant) and Colombiana (highly drug-resistant). Immunosuppression led to a significant ($P \leq 0.04$, log-rank test in all cases) reduction in the mean survival time of untreated mice for all infecting strains (data not shown). Nevertheless, very significant differences in survival were found between control and drug-treated animals (D0870 at 20 mg/kg on alternate days or benznidazole at 100 mg/kg, daily), for both immunocompetent and immunosuppressed hosts ($P \leq 0.0001$ in all cases with log-rank and Peto-Peto–Wilcoxon tests). Evaluation of parasitological cure showed that immunosuppression did not affect the capacity of D0870 to eradicate the parasite in animals infected with the susceptible CL or highly drug-resistant Colombiana strains; however, a significant reduction in the percentage of cures attained in animals infected with the partially drug-resistant Y strain was observed (from 100 to 60% of surviving animals).

We also studied a murine model of chronic Chagas' disease: in this case mice were infected with a low inoculum of blood trypomastigotes (30 per animal) of the CL, Y or Colombiana strains, which led in c. 40% of cases to a chronic, latent infection with no circulating parasites. Animals that survived 120 days after infection were treated orally as described above. D0870 20 mg/kg, given daily or on alternate days, led to survival curves with highly significant statistical differences when compared with those of untreated controls ($P \leq 0.02$ (log-rank) and $\leq 0.015$ (Peto-Peto–Wilcoxon)); there were no significant differences in survival between the groups that received the bis-triazole and those that received benznidazole at 100 mg/kg given daily (data not shown). However, assessment of parasitological cures 201 days p.i. showed that D0870 at 20 mg/kg was able to cure 30–45% of chronically infected animals, including those harbouring the Colombiana strain, among which no cures were obtained with benznidazole (Table II).

In conclusion, the results of the present study demonstrate that D0870 is able to eradicate a number of *T. cruzi* strains from both acutely and chronically infected animals, including benznidazole- and nifurtimox-resistant parasites, and that this activity is retained to a large extent even in immunosuppressed hosts. Although the development of D0870 has recently been discontinued, we have obtained similar results with the triazole derivative SCH 56592. Taken together, these results support the notion that fourth-generationazole derivatives with specific anti-*T. cruzi* activity and appropriate pharmacokinetic properties should potentially be useful in the treatment of human Chagas' disease.

### Acknowledgements

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