Activities of the Triazole Derivative SCH 56592 (Posaconazole) against Drug-Resistant Strains of the Protozoan Parasite Trypanosoma (Schizotrypanum) cruzi in Immunocompetent and Immunosuppressed Murine Hosts

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We have studied the in vivo activity of the new experimental triazole derivative SCH 56592 (posaconazole) against a variety of strains of the protozoan parasite Trypanosoma (Schizotrypanum) cruzi, the causative agent of Chagas’ disease, in both immunocompetent and immunosuppressed murine hosts. The T. cruzi strains used in the study were previously characterized as susceptible (CL), partially resistant (Y), or highly resistant (Colombiana, SC-28, and VL-10) to the drugs currently in clinical use, nifurtimox and benznidazole. Furthermore, all strains are completely resistant to conventional antifungal azoles, such as ketoconazole. In the first study, acute infections with the CL, Y, and Colombiana strains in both normal and cyclophosphamide-immunosuppressed mice were treated orally, starting 4 days postinfection (p.i.), for 20 consecutive daily doses. The results indicated that in immunocompetent animals SCH 56592 at 20 mg/kg of body weight/day provided protection (80 to 90%) against death caused by all strains, a level comparable or superior to that provided by the optimal dose of benznidazole (100 mg/kg/day). Evaluation of parasitological cure revealed that SCH 56592 was able to cure 90 to 100% of the surviving animals infected with the CL and Y strains and 50% of those which received the benznidazole- and nifurtimox-resistant Colombiana strain. Immunosuppression markedly reduced the mean survival time of untreated mice infected with any of the strains, but this was not observed for the groups which received SCH 56592 at 20 mg/kg/day or benznidazole at 100 mg/kg/day. However, the overall cure rates were higher for animals treated with SCH 56592 than among those treated with benznidazole. The results were confirmed in a second study, using the same model but a longer (43-dose) treatment period. Finally, a model for the chronic disease in which oral treatment was started 120 days p.i. and consisted of 20 daily consecutive doses was investigated. The results showed that SCH 56592 at 20 mg/kg/day was able to induce a statistically significant increase in survival of animals infected with all strains, while benznidazole at 100 mg/kg/day was able to increase survival only in animals infected with the Colombiana strain. Moreover, the triazole was able to induce parasitological cures in 50 to 60% of surviving animals, irrespective of the infecting strain, while no cures were obtained with benznidazole. Taken together, the results demonstrate that SCH 56592 has in vivo trypanocidal activity, even against T. cruzi strains naturally resistant to nitrofurans, nitroimidazoles, and conventional antifungal azoles, and that this activity is retained to a large extent in immunosuppressed hosts.

Chemotherapy of Chagas’ disease (American trypanosomiasis), a parasitic disease caused by the kinetoplastid protozoan Trypanosoma (Schizotrypanum) cruzi which afflicts 16 to 18 million people in Latin America, remains an enormous scientific and social challenge, as the drugs currently available, nitrofurans (nifurtimox; Bayer) and nitroimidazoles (benznidazole; Roche, São Paulo, Brazil), have little or no activity in the prevalent chronic form of the disease and can also produce serious toxic effects in the host (7, 8, 24, 26). Like many fungi and yeasts, T. cruzi has a strict requirement of specific endogenous sterols for cell viability and growth and is extremely sensitive to sterol biosynthesis inhibitors in vitro (13, 16, 29, 31–33, 35, 36). However, currently available sterol biosynthesis inhibitors, which are highly successful in the treatment of fungal diseases, are not powerful enough to eradicate T. cruzi from experimentally infected animals or human patients (3, 18, 20). Recent work from our laboratories has shown that new azole derivatives (inhibitors of fungal cytochrome P-450-dependent C14 sterol demethylase), such as D0870 (Zeneca Pharmaceuticals) and SCH 56592 (Schering-Plough Research Institute, Kenilworth, N.J.), are capable of inducing parasitological cures in murine models of both acute and chronic Chagas’ disease (13, 29, 30, 33, 34) and are the first compounds ever to display such activity. It has been shown that this special anti-parasitic activity results from the potent and selective anti-T. cruzi activity and special pharmacokinetic properties of these compounds, particularly their long terminal half-lives and large volumes of distribution (13, 29, 30, 33, 34). Although the development of D0870 has recently been discontinued, numerous studies have consistently shown that SCH 56592 has a

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It has been known for a number of years that T. cruzi strains differ widely in terms of their biological properties as well as their susceptibility to nitrofurans and nitroimidazoles (2). Filardi and Brener (10), in a study of a large number of clinical and natural isolates from different geographical areas, found three main groups characterized as susceptible, partially drug resistant, and highly drug resistant. Both nitrofuran/nitroimidazole-susceptible and -resistant strains have recently been shown to be resistant in vivo to conventional antifungal azoles, such as ketoconazole, also an inhibitor of the parasite’s C14 sterol demethylase (1). In this work, as in a previous study (10), a strain is defined as resistant to a given drug if the drug is unable to induce a parasitological cure with an experimental protocol (inoculum, dose and duration of treatment) which produces sterilization of animals infected with reference (susceptible) strains.

Many studies have also been devoted to the characterization of the role of the immune system in the resistance of vertebrate hosts, including humans, to T. cruzi and its involvement in the pathogenesis of Chagas’ disease, particularly in its chronic form (4, 25, 30). The crucial role of the immune system in the maintenance of the host-parasite balance in the indeterminate phase of Chagas’ disease has been highlighted recently by reports of dramatic reactivation phenomena observed in chronic chagasic patients immunosuppressed due to AIDS or pharmacological intervention (9, 27). Finally, recent studies have demonstrated the participation of stimulatory cytokines such as gamma interferon or interleukin-12 (IL-12) in the antiparasitic activity of benznidazole in murine models of acute Chagas’ disease (19).

In the present study we investigated the in vivo activity of SCH 56592 against a series of T. cruzi strains selected from among those previously characterized by Filardi and Brener (10) in a variety of murine models, including immunosuppressed hosts.

MATERIALS AND METHODS

Parasites. The CL, Y, Colombiana, SC-28, and VL-10 strains were previously characterized (10); the original isolates have been maintained as trypomastigotes in liquid nitrogen, periodically transferred to mice, and refrozen, with full retention of their biological and drug resistance characteristics. Handling of live T. cruzi was done according to established guidelines (11).

Models of acute infection. The protocol developed by Filardi and Brener (10) was followed for studies of acute infections. Briefly, groups of 10 immunocompetent or immunosuppressed (see below) outbred female Swiss albino mice, weighing 18 to 20 g, were inoculated intraperitoneally (i.p.) with 10⁷ blood trypomastigotes of the different strains; oral treatment was initiated 4 days postinfection (p.i.) and given daily for a total of 20 doses. Surviving animals were monitored for 60 days. In a second study, normal (immunocompetent) animals were infected with the same inoculum (10⁷) and treatment was started at 4 days p.i. but given daily for 28 days, followed by a 7-day rest and another 15 days of treatment (16, 29, 32–35). Surviving animals were monitored for up to 113 days p.i. SCH 56592 was suspended in aqueous 2% methylcellulose plus 0.5% Tween 80, while benznidazole was dissolved in water containing 1% arabic gum; both drugs were given by gavage. Control (untreated) animals received the vehicle as a placebo, which had no detectable toxic effects.

Model of chronic infection. Outbred female Swiss albino mice weighing 18 to 20 g were inoculated i.p. with 30 blood trypomastigotes of the different strains to allow the development of a chronic, latent infection. After 120 days, surviving animals with no circulating parasites were randomly divided into different treatment groups (12 animals per group) and subjected to oral treatment for 20 consecutive days, as described above. Surviving animals were monitored for up to 191 days p.i.

Parasitological and serological tests. Parasitemia was measured in a hemacytometer with tail blood. Hemocultures were carried out by inoculating 5 ml of liver infusion medium with 0.2 to 0.4 ml of blood obtained from the orbital sinuses of experimental animals. Cultures were incubated without agitation at 28°C and examined for the presence of proliferative epimastigote forms at 30 and 60 days. Xenodiagnosis was done with 10 second-stage Rhodius prolitus and Rodnius prolixus.
Triatoma infectans nympha per mouse; 30 to 40 days after feeding, the insect feces were analyzed for T. cruzi metacyclic forms. Antibodies against live T. cruzi were evaluated by the procedure of Martins-Filho et al. (17), with minor modifications. Briefly, 5 × 10⁶ live trypomastigotes were incubated at 37°C for 30 min in the presence of different dilutions (1:1,500 to 1:3,000) of serum from experimental animals. The parasites were then washed once with phosphate-buffered saline (PBS) containing 10% fetal bovine serum (FBS) and incubated at 37°C for 30 min in the dark in the presence of fluorescein isothiocyanate (FITC)-conjugated anti-mouse immunoglobulin G (IgG) antibody solution (Sigma Immunochemical Reagents, St. Louis, Mo.), diluted 200-fold with PBS containing 10% FBS. Each assay included a control in which parasites were not exposed to mouse serum but were incubated with FITC-conjugated anti-mouse IgG. FITC-labeled parasites were washed once with PBS containing 10% FBS and fixed at 4°C with FACS FIX solution (1% [wt/vol] paraformaldehyde, 0.01% sodium azide, 1% sodium cacodylate [pH 7.2]). Labeled parasites were analyzed by cytofluorometry in a Becton Dickinson FACScan interfaced to a digital Micro HP 9153C as described before (17).

Statistical analysis. The Kaplan-Meier nonparametric 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 run on a Power Macintosh 6500/250 computer.

RESULTS AND DISCUSSION

Effects of SCH 56592 on acute experimental infections caused by drug-resistant T. cruzi strains in immunocompetent and immunosuppressed mice. In our initial studies, a murine model of acute Chagas disease previously designed to characterize drug resistance among different T. cruzi strains (10) was used. In this protocol mice were infected with 10⁴ bloodstream trypomastigotes of different T. cruzi strains; oral treatment was started 4 days p.i. and given daily for 20 consecutive days. As can be seen in Fig. 2, with this protocol SCH 56592 at 20 mg/kg/day provided a high level of protection (80 to 90%) against death, which was comparable (CL and Y strains) or superior (Colombiana strain) to that observed with benznidazole at 100 mg/kg/day, which is the optimal dose of this drug (10). Highly significant statistical differences in survival were obtained between control (untreated) and both drug-treated groups (P values in log-rank and Peto-Peto-Wilcoxon tests were ≤0.0001 and ≤0.0002 for SCH 56592 and ≤0.01 and ≤0.03 for benznidazole, respectively). Parasitological cures in surviving animals were verified by three independent criteria: hemoculture, xenodiagnosis, and flow cytometry analysis for anti-live T. cruzi antibodies. Animals were considered cured when all three tests were negative (for details see Materials and Methods).

### TABLE 2. Effects of SCH 56592 and benznidazole in a murine model of acute Chagas disease with different strains of T. cruzi

<table>
<thead>
<tr>
<th>Strain</th>
<th>No drug (control)</th>
<th>Benznidazole, 100 mg/kg/day</th>
<th>SCH 56592, 5 mg/kg/day</th>
<th>SCH 56592, 10 mg/kg/day</th>
<th>SCH 56592, 20 mg/kg/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL</td>
<td>0/10</td>
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<td>9/10</td>
</tr>
<tr>
<td>Y</td>
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<td>9/10</td>
<td>10/10</td>
<td>9/10</td>
</tr>
<tr>
<td>Colombiana</td>
<td>0/10</td>
<td>6/10</td>
<td>9/10</td>
<td>9/10</td>
<td>8/10</td>
</tr>
<tr>
<td>SC-28</td>
<td>0/10</td>
<td>7/10</td>
<td>9/10</td>
<td>10/10</td>
<td>9/10</td>
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<td>VL-10</td>
<td>0/10</td>
<td>7/10</td>
<td>4/10</td>
<td>10/10</td>
<td>9/10</td>
</tr>
</tbody>
</table>

* Female albino Swiss mice (18 to 20 g) were inoculated with 10⁴ bloodstream trypomastigotes of the indicated strain, and treatment was started 4 days p.i. The drugs were given orally for 28 days, followed by a 7-day rest and another 15 days of treatment. Animals were monitored for 113 days p.i.
The results described above suggest that the anti-
*T. cruzi* effects of SCH 56592 may be further enhanced when it is used in
combination with IL-12 and related cytokines.

Figure 2 shows survival plots for the different experimental
groups. It was found that immunosuppression led to a signifi-
cant (*P* ≤ 0.04 [log-rank test] in all cases) reduction in the
mean survival time for all strains (mean survival times for
control and immunosuppressed animals were 26.9 and 18.9
days, 25.2 and 14.8 days, and 29.1 and 22.3 days for the CL, Y,
and Colombiana strains, respectively). Nevertheless, as in im-
munocompetent animals, there were very significant differ-
cences in survival between control and drug-treated groups
(*P* values in log-rank and Peto-Peto-Wilcoxon tests of ≤0.0001
and 0.0002 for SCH 56592 and 0.001 and 0.003 for benznidazole,
respectively), while there were no significant differ-
cences in survival between immunocompetent and immunosup-
pressed animals which received either drug treatment. For all
strains, survival levels for immunosuppressed animals which
received SCH 56592 at 20 mg/kg/day were higher than those
for mice receiving benznidazole at 100 mg/kg/day, but there
was no statistically significant difference between data for the
two drugs. These results clearly show that both drugs can pro-
tect from a lethal parasitic infection, even in the presence of a
severe immunosuppression.

Evaluation of the parasitological cure (Table 1) revealed
that, while immunosuppression did not affect significantly the
level of cures induced by SCH 56592 in animals infected with
the susceptible CL or drug-resistant Colombiana strain, it had
a significant effect in animals infected with the partially drug-
resistant Y strain (Table 1). However, even in immunosup-
pressed animals, the overall cure rates for animals infected with
the Y or Colombiana strain and treated with the triazole
remained higher than values for those treated with benznidazole.
These results indicate that the potent anti-*T. cruzi* effects of SCH 56592 against the Y strain in normal hosts are partially
dependent on cooperation from the immune system but also
that its trypanocidal activity is higher than that of benznidazole
even in immunosuppressed animals. Recent studies have dem-
onstrated that early activation of the immune system by cyto-
kines such as IL-12 may be involved in the trypanocidal activity
of benznidazole in acute murine infections with *T. cruzi* (19).
The results described above suggest that the anti-*T. cruzi*
effects of SCH 56592 may be further enhanced when it is used in
combination with IL-12 and related cytokines.

In a second set of experiments the same acute model was
used but with a longer treatment course, starting 4 days p.i. and
given daily for 28 consecutive days followed by a 7-day rest and
another 15 days of treatment, for a total of 43 doses. This course
of treatment was used in our previous studies that first
demonstrated the *in vivo* trypanocidal effects of both D0870
and SCH 56592 (13, 16, 32–35). In addition, other *T. cruzi*
strains (SC-28 and VL-10, both nifurtimox and benznidazole
resistant [10]) were included in the study. As shown in Table 2,
with this protocol SCH 56592 given at ≥10 mg/kg/day was able
to induce very high (90 to 100%) survival levels for all strains,
comparable or superior to those obtained with benznidazole at
100 mg/kg/day. It can be seen in Table 3 that SCH 56592 at just

<table>
<thead>
<tr>
<th>Strain</th>
<th>No. of negative mice/no. tested</th>
<th>Benznidazole, 100 mg/kg/day</th>
<th>SCH 56592, 5 mg/kg/day</th>
<th>SCH 56592, 10 mg/kg/day</th>
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<tr>
<td>CL</td>
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<td>9/9</td>
<td>9/9</td>
</tr>
<tr>
<td>Y</td>
<td>0/10</td>
<td>4/8</td>
<td>5/9</td>
<td>6/10</td>
<td>7/9</td>
</tr>
<tr>
<td>Colombiana</td>
<td>0/10</td>
<td>3/6</td>
<td>5/9</td>
<td>4/9</td>
<td>6/8</td>
</tr>
<tr>
<td>SC-28</td>
<td>0/10</td>
<td>2/7</td>
<td>5/9</td>
<td>6/9</td>
<td>8/8</td>
</tr>
<tr>
<td>VL-10</td>
<td>0/10</td>
<td>1/7</td>
<td>2/4</td>
<td>3/10</td>
<td>5/9</td>
</tr>
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</table>

* Female albino Swiss mice (18 to 20 g) were inoculated with 10³ bloodstream trypomastigotes of the indicated strain, and treatment was started 4 days p.i. The drugs
were given orally for 28 days, followed by a 7-day rest and another 15 days of treatment. Parasitological cure of animals which survived until the end of the observation
period (113 days p.i.) was assessed by hemoculture, xenodiagnosis, and flow cytometry analysis for anti-live *T. cruzi* antibodies. Animals were considered cured when
all three tests were negative (for details see Materials and Methods).
azole resistance in a chronic model were lower than those obtained in a previous study with the Bertoldo strain (33), a fact which could be attributed with the higher affinity of this triazole derivative to its biological target, cytochrome P-450-dependent C14 sterol demethylase (Sanglard et al., 37th ICAAC).

In conclusion, our results indicate that SCH 56592 has trypanocidal activity against a variety of T. cruzi strains, including benznidazole-, nifurtimox-, and ketoconazole-resistant organisms, in murine models of both acute and chronic Chagas’ disease and that this activity is retained to a large extent even if the host is immunosuppressed. Such results have been obtained before only with the bis-triazole D0870 (J. Molina, M. S. S. Araujo, M. E. S. Pereira, Z. Brener, and J. A. Urbina, Abstr. 37th Intersci. Conf. Antimicrob. Agents Chemother., abstr. B-41b, 1997; Molina et al., unpublished data) but are consistent with recent reports on the efficacy of SCH 56592 in the treatment and prevention of invasive pulmonary aspergillosis in persistently neutropenic rabbits (Petrattiene et al., 39th ICAAC) as well as in the treatment of azole-refractory candidiasis in human immunodeficiency virus-infected patients with advanced AIDS (Skiest et al., 39th ICAAC). Taken together, these results support the proposal that SCH 56592 be considered for clinical trials in human Chagas’ disease.

ACKNOWLEDGMENTS

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REFERENCES


<table>
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<th>Strain</th>
<th>No. of negative mice/no. tested</th>
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</thead>
<tbody>
<tr>
<td>Control (untreated)</td>
<td>0/12</td>
</tr>
<tr>
<td>Benznidazole, 100 mg/kg/day</td>
<td>0/12</td>
</tr>
<tr>
<td>SCH 56592, 5 mg/kg/day</td>
<td>0/12</td>
</tr>
<tr>
<td>SCH 56592, 10 mg/kg/day</td>
<td></td>
</tr>
<tr>
<td>SCH 56592, 20 mg/kg/day</td>
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</tbody>
</table>

Female albino Swiss mice (18 to 20 g) were inoculated with 30 bloodstream trypomastigotes of the indicated strain, and treatment was started 120 days p.i. The drugs were given orally for 20 days. Parasitological cure of animals which survived until the end of the observation period (191 days p.i.) was assessed by hemoculture, xenodiagnosis, and flow cytometry analysis for anti-live T. cruzi antibodies. Animals were considered cured when all three tests were negative (for details see Materials and Methods).


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