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Comparative Efficacies of TAK-187, a Long-Lasting Ergosterol Biosynthesis Inhibitor, and Benznidazole in Preventing Cardiac Damage in a Murine Model of Chagas' Disease

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We carried out a comparative study of benznidazole and TAK-187, a long-lasting ergosterol biosynthesis inhibitor, with a murine model of Chagas' disease. The results indicated that TAK-187 was more effective than benznidazole in preventing *Trypanosoma cruzi*-induced cardiac damage in experimental animals.

Chagas' disease is still the parasitic disease with the heaviest burden on the American continents, despite recent advances in the control of the vectorial and transfusional transmission of its etiological agent, *Trypanosoma cruzi* (7, 11, 15). Available chemotherapy, based on the nitrofuran nifurtimox (Bayer) and the nitroimidazole benznidazole (BZL; Roche), is unsatisfactory as these compounds are effective only against recent infections and have frequent toxic side effects and drug resistance is commonly encountered (3, 11). *T. cruzi* has an essential requirement for endogenous sterols, such as ergosterol and its analogs, and we have shown that novel ergosterol biosynthesis inhibitors can induce radical parasitological cures in animal models of both acute and chronic experimental Chagas' disease, with very low or no toxic side effects (9–11, 13). One of these compounds is TAK-187 (Takeda Chemical Company, Osaka, Japan) (Fig. 1A), a long-lasting triazole derivative which is a potent inhibitor of *T. cruzi*'s sterol C14α demethylase and has in vitro and in vivo trypanocidal activity and no detectable toxicity for its mammalian hosts (14).

For experimental chemotherapy studies, 60 Swiss male mice (2 months old) were used. The mice were infected with 10^3 blood trypomastigotes of the Tulahuen strain. Thirteen days postinfection (p.i.), when parasitemia could be detected in all infected mice, the animals were randomly assigned to three groups of 20 mice each and treated with 30 doses of TAK-187 at 20 mg/kg of body weight given every other day for a total of 60 days or the same number of doses of BZL at 200 mg/kg given daily (30 days of treatment); control (nontreated) animals received 30 daily doses of the vehicle used to prepare the drugs' suspensions (100 μl of 1% carboxymethylcellulose). Parasitemia determinations and hemocultures were carried out as described previously (12). Quantitative evaluation of circulating anti-*T. cruzi* antibodies was carried out by the use of an enzyme-linked immunospot assay method in a microwell plate format, with as antigen a soluble homogenate of *T. cruzi* epimastigotes, which was reacted with sera diluted 1:100. Blood samples for PCR (1, 2) were taken on two different dates (days 111 and 118 p.i.) and pooled to obtain 700 μl of blood from each mouse. On day 198 p.i., all surviving animals were sacrificed and autopsied, and samples of skeletal muscle, heart, urinary bladder, gut, and liver were taken and fixed for histological studies. The Mann-Whitney U test was used to compare the antibody levels (Fig. 2A) and the quantitative evaluations of histopathological findings (see Fig. 4), while the Fisher exact test was used for the semiquantitative evaluations (Fig. 3).

Specific treatment with both drugs led to a complete and

TABLE 1. Effects of BZL and TAK-187 in a murine model of acute Chagas' disease as determined by hemoculture and blood PCR assays[a]

Treatment group	No. of positive hemocultures/total (91 days p.i.)	Blood PCR result (111 days p.i.)		
		No. positive/total[b]		No. negative/total
		Strong	Weak	
Control (untreated)	14/20	12/14	1/14	1/14
TAK-187	0/18	0/18	13/18	5/18
BZL	0/19	0/19	6/19	13/19

[a] Animals were infected with 10^3 trypomastigotes/mouse, and treatment started 13 days p.i. Hemocultures were carried on day 91 p.i. (19 days after the end of TAK-187 treatment), while blood samples for PCR were obtained on days 111 and 118 p.i. (39 and 46 days posttreatment) and pooled to carry out the assays.

[b] "Strong" and "weak" refer to the intensities of bands in the limit of detection in agarose gels stained with ethidium bromide.

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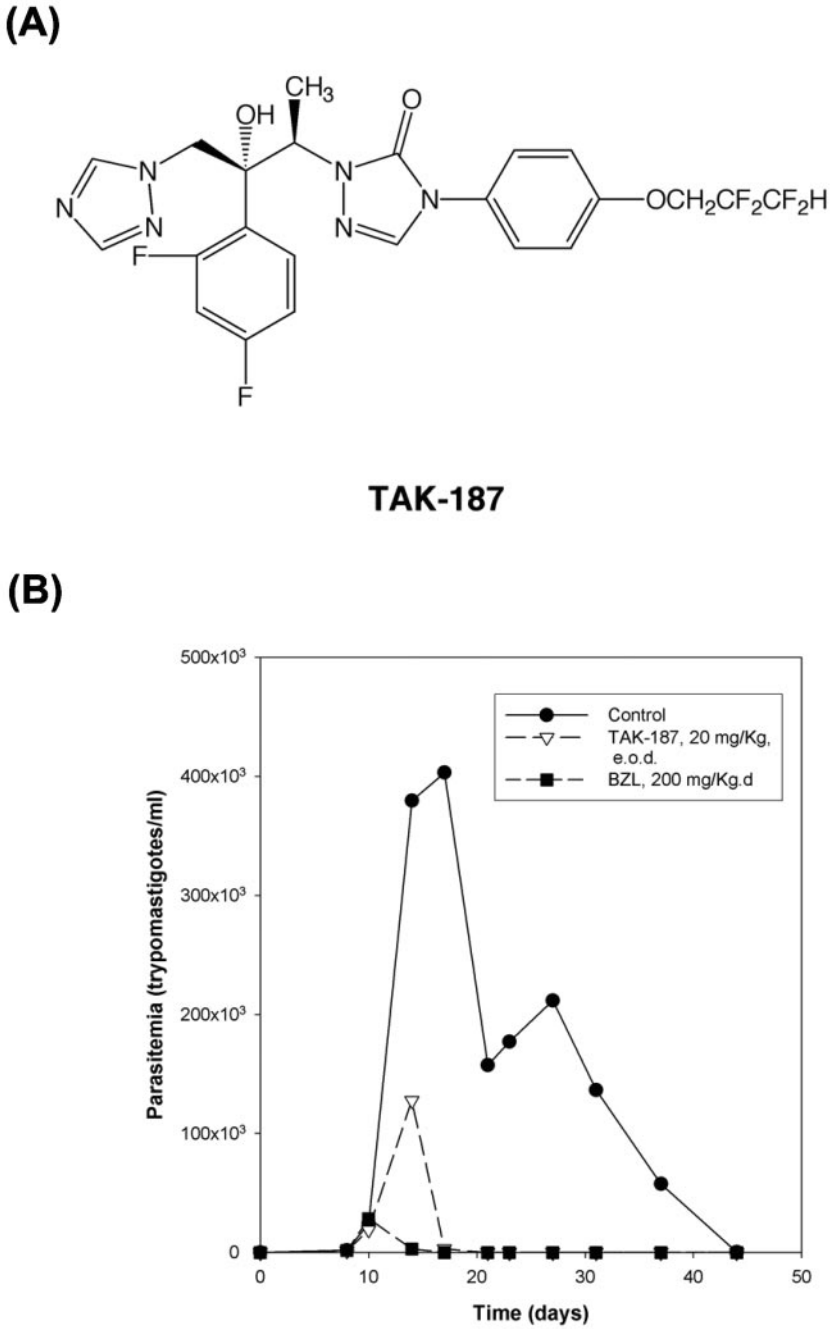

FIG. 1. (A) Chemical structure of TAK-187; (B) effects of TAK-187 and BZL on parasitemia in a murine model of acute Chagas disease. Swiss mice were inoculated with 10^3 blood trypomastigotes of the Tulahuen strain, and oral treatment was started 13 days later, at the stated doses and frequencies, for a total of 30 doses. The cutoff for each reaction was the mean of the values determined for the negative controls plus three times the standard deviation. Abbreviation: e.o.d., every other day.

permanent suppression of parasitemia on day 17 p.i., just 4 days after the start of treatment (Fig. 1B). In neither treatment were group deaths attributable to the parasitic infection observed, while 30% of control (nontreated) animals were dead at the end of the observation period. The rapid suppression of parasitemia and absence of relapses indicated a profound suppression of the parasite infection by both drugs, an interpretation supported by hemoculture, PCR, and serological assays (Table 1 and Fig. 2). Hemocultures were carried on day 91 p.i. (18 days after the end of TAK-187 treatment). Table 1 shows that while 14 out of 20 of the infected but nontreated animals tested positive for parasitemia, no positive results were obtained among treated animals. A sensitive PCR test for the parasite's mini-circle DNA (1, 2) was carried out with blood samples collected on days 111 and 118 p.i. While 13 out of 14 surviving controls tested positive for parasitemia, the majority

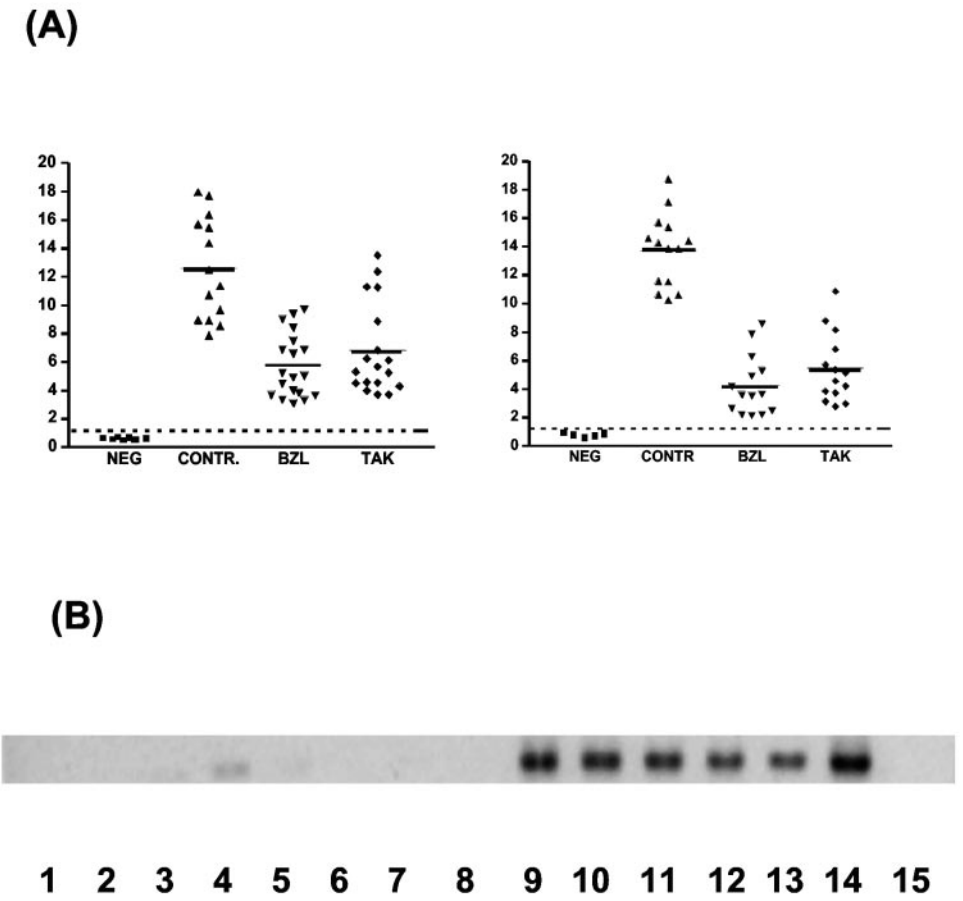

FIG. 2. Effects of TAK-187 and BZL on anti-*T. cruzi* antibody levels (A) and circulating *T. cruzi* DNA (B). (A) Dispersion diagrams of antibody levels in control (untreated) animals and those receiving TAK-187 or BZL treatments at 111 days p.i. (39 days posttreatment [left]) and 198 days p.i. (126 days posttreatment [right]). The results are expressed as the ratio of the absorbance of each serum sample at 490 nm to the cutoff value. The cutoff for each reaction was the mean of the values determined for the negative controls plus three times the standard deviation; values greater than 1 were considered reactive. Both BZL ($P < 0.0001$ at both time points) and TK-187 ($P = 0.0002$ at 39 days posttreatment and $P = 0.0001$ at 126 days posttreatment) reduced antibody levels compared with those of untreated controls. (B) PCR assays of control (untreated) and TAK-187-treated animals. Lane 1, blank; lane 2, negative-control sample; lanes 3 to 8, duplicated extractions from blood specimens from three infected, TAK-187-treated mice (day 39 posttreatment); lanes 9, 10, 11, and 12, duplicated extractions from blood samples of two infected but not treated animals; lanes 13 and 14, infected-blood control; lane 15, blank. Positive bands have a 330-bp band. NEG, negative; CONTR., control.

of treated animals tested negative for parasitemia or gave PCR bands in the limit of detection (Table 1 and Fig. 2B). Samples for serology were obtained on days 111 and 198 p.i. (days 39 and 126 posttreatment). Infected, nontreated mice presented high anti-*T. cruzi* antibody levels at both time points (Fig. 2A). Both treatment schemes (TAK-187 and BZL) significantly reduced the circulating-antibody levels ($P < 0.001$) (Fig. 2A), but these did not reach the basal levels of noninfected mice, even at the latter time point.

Heart tissue sections of infected, nontreated mice presented moderate interstitial or subpericardial inflammatory infiltrates in the ventricular wall associated with *T. cruzi* amastigote nests (Fig. 3A). A significant reduction of these alterations was associated with TAK-187 treatment ($P = 0.0159$) but not with BZL treatment ($P > 0.1$) (Fig. 3A). Histopathological analyses of skeletal muscle showed that animals of the control, nontreated group displayed different degrees of lymphomonocytic inflammatory infiltrates surrounding or replacing muscle fibers associated with amastigote nests and necrotic areas with calcium deposits (Fig. 3B). Nuclei of muscle cells were often enlarged and displaced toward the center of the cell (nuclear displasia). These histopathological findings were significantly prevented in mice treated with either TAK-187 or BZL ($P < 0.01$), but the protective effect was more evident in animals treated with TAK-187 than in those receiving BZL (Fig. 3B). In the livers of the majority of nontreated animals, discrete foci containing 10 to 40 inflammatory cells were found. These were most often placed in the interlobular spaces but were also found in the trabecular spaces or under the Glisson capsule. The density of these foci was quantified as the number of foci per 10 mm^2 of liver section. Treatment with both TAK-187 and BZL significantly reduced inflammatory foci in the liver ($P < 0.0001$) (Fig. 4). Inflammatory infiltrates were also found in urinary bladder sections of most infected, nontreated mice. These were often placed interstitially at the muscular wall or at the submucous space. A significant reduction of inflammation ($P < 0.05$), becoming slight or absent, was caused by both the TAK-187 and BZL treatments (data not shown).

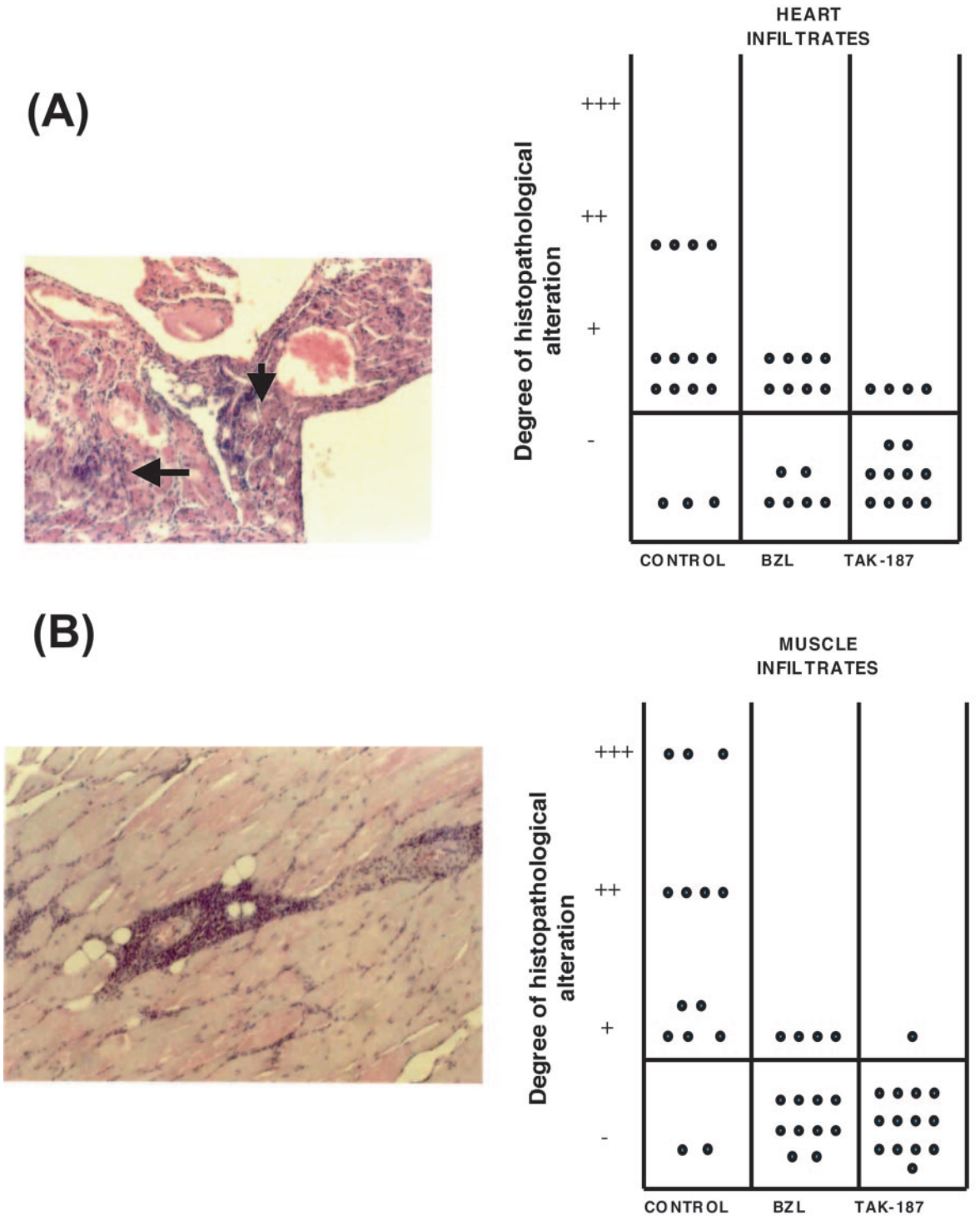

FIG. 3. Inflammatory infiltrates in the heart (A) and skeletal muscle (B) of mice chronically infected with *T. cruzi*. (Left) Microphotograph of cardiac or muscle tissue from mice that were infected but not treated; note inflammatory infiltrates in the atria and bases of ventricles (arrows). Magnification, ×100. (Right) Dispersion diagrams indicating the intensities of inflammatory infiltrates in the three experimental groups for both tissues. TAK-187 significantly prevented these lesions in the heart (A) ($P = 0.0159$), but BZL did not ($P > 0.1$). For muscle (B), both compounds were able to induce a significant ($P < 0.01$) reduction, but the effect was more complete with TAK-187.

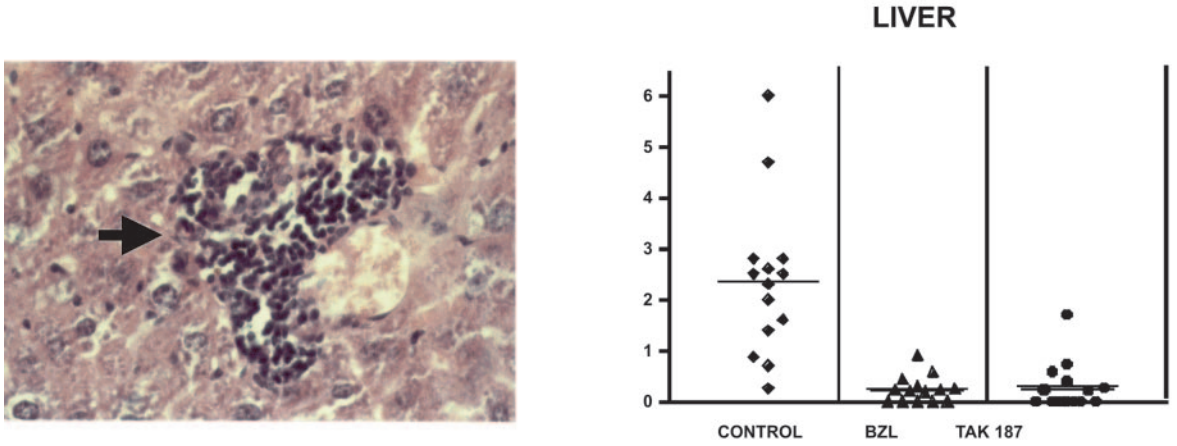

FIG. 4. Inflammatory foci in the liver of a mouse chronically infected with *T. cruzi*. (Left) Microphotograph of liver tissue from a mouse infected but not treated; note discrete inflammatory foci (arrow). Magnification, ×400. (Right) Dispersion diagram showing the number of foci per 10 mm^2; a very significant reduction was observed in both treatment groups compared with levels in untreated controls ($P < 0.0001$).

In the present study, we confirmed the remarkable in vivo anti-*T. cruzi* activity of the triazole derivative TAK-187 reported previously (14). The compound given at 20 mg/kg every other day was able to sharply reduce or eliminate the parasite loads of animals with fully established *T. cruzi* infections (Fig. 1 and 2), a result comparable to that obtained with BZL but with a 10-fold-higher dose (200 mg/kg) and daily dosing. Moreover, the results of our histological studies indicated that TAK-187 was significantly superior to BZL in preventing inflammatory infiltrates and tissue damage, particularly in the hearts and skeletal muscles of infected animals (Fig. 3). The higher efficacy of TAK-187 in this experimental model may be explained by its superior intrinsic anti-*T. cruzi* activity (14) and its long terminal half-life (35.6 h in mice; Takeda Chemical Industries, data on file), which allowed dosing on every other day and thus a two-fold-longer drug exposure time for the same number of doses.

In conclusion, our findings support the notion that ergosterol biosynthesis inhibitors could be a superior alternative to currently available therapy in the management of chronic Chagas' disease patients (4–6, 8, 11).

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