Experimental Parasitology 120 (2008) 314–319

Contents lists available at ScienceDirect

Experimental Parasitology

journal homepage: www.elsevier.com/locate/yexpr

Trypanosoma cruzi: Effect of benznidazole therapy combined with the iron chelator desferrioxamine in infected mice

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ARTICLE INFO

Article history:
Received 16 January 2008
Received in revised form 15 April 2008
Accepted 18 August 2008
Available online 24 August 2008

Keywords:
Trypanosoma cruzi
Chagas' disease (CD)
Reactive oxygen species (ROS)
Analysis of variance (ANOVA)
Deoxyribonucleic acid (DNA)
desferrioxamine (DFO)
benznidazole (BZ)
Days post-infection (dpi)
Ethylenediamine tetraacetic acid (EDTA)
Intraperitoneal (ip)
Liver infusion tryptose (LIT)
Polymerase chain reaction (PCR)

ABSTRACT

Iron chelators have been employed in various studies aimed at evaluating the relationship between the iron status of the host and the development of infection. In the present study, the effects of benznidazole (BZ) therapy in combination with the iron chelator desferrioxamine (DFO) on the development of infection in mice inoculated with *Trypanosoma cruzi* Y strain have been investigated. Infected mice treated with DFO presented lower levels of parasitemia compared with infected untreated animals. Therapy with BZ for 21 days, with or without DFO, led to decreased parasitemia and reduced mortality, but BZ in combination with DFO treatment for 35 days (BZ/DFO-35) gave 0% mortality. All infected groups presented lower levels of iron in the liver, but serum iron concentrations were greater in DFO-35 and BZ/DFO-35, whereas hemoglobin levels were higher in BZ/DFO-35 and lower in DFO-35 compared with other treated groups. The percentage cure, determined from negative hemoculture and PCR results in animals that had survived for 60 days post-infection, was 18% for BZ and BZ/DFO-35, 42% for BZ combined with DFO for 21 days, and 67% for DFO-35. The results demonstrate that modification in iron stores increases BZ efficacy.

1. Introduction

Chagas' disease (CD), the causative agent of which is the parasitic protozoan *Trypanosoma cruzi*, affects 13 million inhabitants in Central and South America, although it is estimated that *ca.* 25% of the population of Latin America may be at risk of infection (WHO, 2005). The only drug currently available in Brazil for the treatment of CD is benznidazole (BZ; Roche). Whereas this compound can eliminate the symptoms associated with the acute phase and provides a cure rate of *ca.* 60% (Coura and de Castro, 2002), it is much less effective in the chronic phase of the disease (Galvão et al., 1993; Viotti et al., 1994). Moreover, BZ is reported to give rise to severe side effects in patients and can generate resistance in the parasite, probably by virtue of the heterogeneity of *T. cruzi* populations (Coura and de Castro, 2002). In the murine model, the efficacy of specific chemotherapy varies according to the *T. cruzi* strain and, as for humans, treatment is more efficient during the acute phase of infection (Filardi and Brener, 1987). Although the mechanism of action of nitroheterocyclic derivatives against *T. cruzi* is poorly understood, it is believed that the involvement of the immune system is crucial to the efficacy of the therapy (Murta et al., 1999; Revelli et al., 1999).

Iron, which is by far the most abundant transition metal in body fluids and tissues (Crichton and Ward, 1992), is an essential element for the continued growth of almost all living cells, including parasitic protozoa. Moreover, iron homeostasis is fundamental to the regulation of the human immune system (Weinberg, 1984; Kent et al., 1990), affecting both humoral and cellular immunity (Blakley and Hamilton, 1988; Galan et al., 1988). On this basis, modulation of the availability of iron could represent a potential strategy for augmenting host defense levels against *T. cruzi* and in restricting the development of human CD.

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doi:10.1016/j.exppara.2008.08.002

In this context, iron chelators have been employed in various studies aimed at evaluating the relationship between the iron status of the host and the development of infection (Dhur et al., 1989). In early studies concerning the correlation between iron levels and infection by *T. cruzi* (Lalonde and Holbein, 1984; Loo and Lalonde, 1984), it was found that the depletion of iron stores in mice that had been maintained on an iron-deficient diet, or treated with desferrioxamine (DFO) on the 5th and 6th day post-infection (dpi), generated a reduction in both parasitemia and mortality of the infection. Subsequently, Lima and Villalta (1989) showed that amastigote forms of *T. cruzi* possess receptors for human transferrin, the major iron transport protein in mammalian plasma. It would thus appear that the iron that is essential for amastigote growth is delivered by receptor-mediated transferrin endocytosis.

In mice, the effect of iron deficiency on the evolution of experimental *T. cruzi* infection is dependent on the strain of the parasite. Thus Pedrosa et al. (1990) demonstrated that, in comparison with a control group, mice infected with the YuYu strain developed a less severe form of the disease when treated on the 5th dpi with DFO at a dose of 10 mg/mouse, whereas no differences were observed in animals infected with Y and CL strains. More recently, Arantes et al. (2007) showed that mice that had been treated with DFO 14 days prior to infection with *T. cruzi* Y strain and for 21 days after infection, presented lower levels of parasitemia and reduced rates of mortality compared with infected but untreated animals. Furthermore, the infected groups exhibited lower levels of iron in the liver compared with non-infected animals of both treated and untreated groups. The serum iron levels of the infected but untreated group were, however, higher on the 21st dpi in comparison with the infected and treated group and the control group.

In order to extend the investigation outlined above, the effect of BZ therapy on the course of infection by *T. cruzi* Y strain has been evaluated in mice that had been submitted to a reduction in iron supplies through treatment with DFO according to the procedure employed by Arantes et al. (2007). The aim of the study was to determine whether treatment with BZ in an experimental model infected by *T. cruzi* and with reduced iron supplies would be more effective than that achieved when the iron supplies were normal.

2. Materials and methods

2.1. Animals and experimental design

All procedures were approved by the Committee on Ethics in Research of the Universidade Federal de Ouro Preto, MG, Brazil, and followed international guidelines pertaining to the treatment of experimental animals. Two hundred Swiss male mice, aged between 6 and 8 weeks and with weights in the range 16–20 g were fed throughout the 74 day experimental period on a non-purified commercial diet consisting of Purina Rodent Chow (Purina, São Paulo, SP, Brazil) provided in pellet form. Animals were randomly divided into 6 experimental groups ($n = 30$ each) together with a control group (the NINT group; $n = 20$) that would remain both non-infected with *T. cruzi* and untreated with DFO or BZ. The mice constituting groups DFO-35 and BZ/DFO-35 received a daily dose (5 mg; 0.05 mL) of DFO (Desferal®, Novartis, Basel, Switzerland) by intraperitoneal (ip) injection for 14 days prior to infection and for a further 21 days post-infection. On day 14 of the experimental period, animals of the BZ, DFO-21, BZ/DFO-21, DFO-35, BZ/DFO-35 and INT groups were infected with *T. cruzi* Y strain (Silva and Nussenzweig, 1953) by ip injection of 500 blood stream forms each. Mice belonging to the DFO-21 and BZ/DFO-21 groups received a daily dose (5 mg; 0.05 mL) of DFO by ip injection for 21 consecutive days beginning on the first day of the *T. cruzi* patent period, whilst animals of the INT and NINT groups received a daily ip injection of 0.05 mL of sterile water over the same period. Animals forming groups BZ, BZ/DFO-21 and BZ/DFO-35 were administered a daily dose of 100 mg/kg body weight of BZ by gavage for 21 consecutive days beginning on the first day of the *T. cruzi* patent period. BZ (Rochagan®, Roche, Rio de Janeiro, Brazil) was prepared by suspending one pulverized tablet (containing 100 mg of active principle) in 10 mL of distilled water and adjusting the final concentration of the drug according to the dose required.

2.2. Parameters evaluated

The levels of hemoglobin in blood and of iron in the serum and liver were evaluated in mice from all 7 groups on the 10th and 16th dpi, as appropriate. Parasitemia was measured in all infected animals starting on the 4th dpi and daily thereafter according to the method of Brener (1962). The prepatent period, the patent period, the maximum parasitemia, and the day of maximum parasitemia were thus determined. Mortalities were recorded on a daily basis and expressed as a cumulative percentage up to the 40th dpi. In the case of animals that survived the acute phase of the infection, blood samples were collected in the acute phase (on the 60th dpi) and submitted to parasitological tests (hemoculture and PCR).

2.3. Iron levels in the liver

Liver samples, which had been obtained by necropsy and stored at −70 °C, were digested in nitric acid at 100 °C, evaporated to dryness, and the iron quantified colorimetrically using the orthophenanthroline method of the Association of Official Analytical Chemists (1980). An external iron standard solution of concentration 89.5 μmol/L was employed.

2.4. Iron levels in serum samples

Serum iron concentrations were determined spectrophotometrically in non-hemolyzed serum samples using the Ferrozine® dye-binding method (Labtest Kit catalogue no. 38; Labtest Diagnostica and Bioclin Química Básica, Belo Horizonte, MG, Brazil). An external iron standard solution of concentration 89.5 μmol/L was employed.

2.5. Levels of hemoglobin

Hemoglobin concentrations were determined in blood samples collected from the tails of mice (Henry et al., 1974) immediately prior to infection and in the 10th and 16th dpi, as appropriate, using a commercial assay procedure (Labtest Kit catalogue no. 43). A solution containing 10 g/L of cyanmethemoglobin (Labtest Standard catalogue no. 47) was used as standard.

2.6. Hemoculture

Hemocultures were carried out on the 60th dpi according to the method of Filardi and Brener (1987). Blood collected from the orbital sinus vein was inoculated into tubes containing 3 mL of LIT medium (Camargo, 1964) and incubated at 28 °C for 30, 60 and 90 days prior to examination for the presence of parasites.

2.7. PCR amplification

Blood samples were mixed in a proportion of 1:2 with 6 M guanidine in 0.2 M EDTA (pH 8.0) and stored at room temperature until required for assay (Ávila et al., 1991). DNA was extracted according to the method of Wincker et al. (1994) as modified by

Gomes et al. (1998). PCR amplifications were performed according to Gomes et al. (1998) in 9 μL of reaction mixture containing 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 75 mM KCl, 3.5 mM $MgCl_2$, 0.2 mM of each deoxynucleotide (Sigma, St. Louis, MO, USA), 0.5 U of Platinum Taq DNA polymerase (Invitrogen, São Paulo, SP, Brazil), and 10 pmole of each oligonucleotide primer (S35 and S36 described by Ávila et al., 1991; Invitrogen). The reaction mixture was added to 2 μL of the sample and overlaid with 30 μL of mineral oil in order to avoid evaporation. Amplifications were performed in a MJ Research (Ramsey, MN, USA) model PTC-150 thermal cycler employing an initial denaturation step of 5 min at 94 °C followed by 35 cycles consisting of 1 min denaturation at 95 °C, 1 min annealing at 65 °C and 1 min extension at 72 °C, and a final extension step of 10 min at 72 °C. Amplicons were separated by electrophoresis on 6.0% polyacrylamide gels and revealed by silver staining (Santos et al., 1993). Positive, negative and reagent controls were processed in parallel with each assay.

2.8. Statistical analysis

Statistical analyses of the data were carried out using GraphPad Prism software (GraphPad Software, San Diego, CA, USA). Data were initially assessed by one-way analysis of variance (ANOVA): when interactions were significant, the Tukey test was used to determine the specific differences between mean values. The Kolmogorov–Smirnov test was employed to compare parasitemia between infected groups that had been treated or remained untreated. One-way variance analysis or Mann–Whitney U tests were used to compare values of the prepatent period, the patent period, the maximum parasitemia, and the day of maximum parasitemia between the different groups. Values are expressed as means ± standard deviation: differences in mean values were considered significant at the $p < 0.05$ level.

3. Results and discussion

3.1. Effect of treatment with BZ and DFO on parasitemia and mortality

The parasitemia curves of mice that had been infected with *T. cruzi* Y strain and either treated with BZ and/or DFO (groups BZ, DFO-21, BZ/DFO-21, DFO/35 and BZ/DFO-35), or had received no such treatment (the INT group), are shown in Fig. 1A. The patent periods of animals in the BZ-treated groups were shorter than those of groups that had received either DFO alone or no treatment (Table 1), and all differences were statistically significant except for BZ/DFO-35 vs. DFO-21. In contrast, no significant differences were observed in the prepatent periods, which were estimated as 6 days for DFO-35 and BZ/DFO-35 and 7 days for the other groups, or in the day of the peak of parasitemia (Table 1). The average parasitemia value in BZ/DFO-21 mice (16,000 trypomastigotes/0.1 mL of blood) was determined to be some 58-times lower than in INT animals (932,000 trypomastigotes/0.1 mL of blood).

The cumulative mortality rates for groups of animals that had been infected with *T. cruzi* and either left untreated or treated with BZ and/or DFO are presented in Fig. 1B. In the INT and DFO-21 groups, 100% of the animals died within 40 dpi with mortality commencing on the 12th dpi. In contrast, the mortality rates in the BZ/DFO-21 and BZ groups were 25%, with mortality commencing on the 12th dpi in the former and on the 18th dpi in the latter group. With respect to the DFO-35 group, the mortality rate was 70% with mortality commencing on the 15th dpi, but in the BZ/DFO-35 group no animals died before the 40th dpi. These results demonstrate in a quantitative manner the effectiveness of combination therapy with both drugs in comparison with DFO treatment alone.

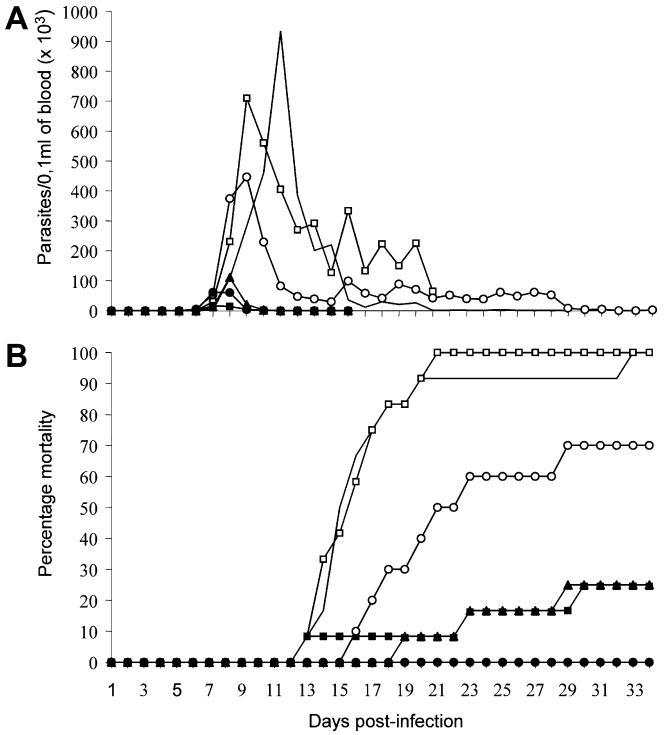

Fig. 1. Parasitemia (A) and percentage of mortality (B) in a population of Swiss mice (n = 30 per group; N = 180) inoculated with 500 blood trypomastigotes of *Trypanosoma cruzi* Y strain and submitted to different treatments: —— (INT, infected and untreated); —▲— (BZ, infected and treated with benznidazole); —□— (DFO-21, infected and treated with desferrioxamine for 21 days); —■— (BZ/DFO-21, infected and treated with benznidazole and desferrioxamine for 21 days); —○— (DFO-35, infected and treated with desferrioxamine for 35 days); —●— (BZ/DFO-35, infected and treated with benznidazole for 21 days and desferrioxamine for 35 days).

In agreement with previous reports (De Souza et al., 2000; Olivieri et al., 2002, 2006), the present results confirm that BZ promotes a decrease in parasitemia levels and in mortality rates independent of any association with DFO. However, when the two therapies were applied in combination over a longer period (i.e. in group BZ/DFO-35) a 100% survival level was obtained suggesting that iron levels could influence the course of infection.

In animals of the DFO-35 group, a reduction in the parasitemia level and an increase in the patent period was observed, indicating

Table 1
Biological parameters related to the parasitemia curve of Swiss mice inoculated with 500 blood trypomastigotes of *Trypanosoma cruzi* Y strain

Group	Prepatent period	Patent period	Day of maximum parasitemia	Maximum parasitemia (trypomastigotes/0.1 mL)
INT[a]	7	21	11	932,000
BZ[b]	7	4[g,h]	8	111,000
DFO-21[c]	7	13	9	720,000
BZ/DFO-21[d]	7	7[g,h]	7	16,000
DFO-35[e]	6	25	9	447,000
BZ/DFO-35[f]	6	9[g]	7	61,000

[a] Infected and untreated group.
[b] Infected and treated with benznidazole.
[c] Infected and treated with desferrioxamine for 21 days.
[d] Infected and treated with benznidazole and desferrioxamine for 21 days.
[e] Infected and treated with desferrioxamine for 35 days.
[f] Infected and treated with benznidazole for 21 days and desferrioxamine for 35 days.
[g] Value significantly different from those of the INT and DFO-35 groups.
[h] Value significantly different from that of the DFO-21 group.

that the severity of infection by the Y strain could be attenuated by treatment with iron chelator as has been previously reported (Arantes et al., 2007). This effect was not evident, however, in animals treated with DFO for 21 days post-infection (group DFO-21), suggesting that treatment with DFO prior to infection is crucial to the efficacy of the therapy. This finding is in agreement with that of Lalonde and Holbein (1984), who demonstrated that depletion of iron supplies, either by treatment with DFO or by administration of a diet deficient in iron, promoted the reduction of mortality in mice infected with *T. cruzi* Brazil strain.

The demonstration that parasite reproduction is negatively correlated with plasma iron level would appear to support the original hypothesis. In contrast to the results outlined above, however, Pedrosa et al. (1990) reported that *T. cruzi* Y strain was not sensitive to DFO treatment, and that an effect on parasite replication could only be observed when mice were fed on an iron-free diet. The authors explained this result in terms of the higher multiplication rate of the Y strain compared with the YuYu strain, which permitted earlier attainment of the parasitemia peak by the Y strain. In the study by Pedrosa et al. (1990), each mouse received 1,400 trypomastigote forms of the parasite and only one dose of DFO (administered on the 5th dpi). These experimental conditions were very different from those employed in the present investigation and most likely account for the divergence of the results obtained. However, the findings of Arantes et al. (2007) and Pedrosa et al. (1990), together with the data presented here, suggest that the effect of DFO is dependent on whether the therapy is initiated pre- or post-infection and on the length of time over which administration is continued.

3.2. Hemoculture and PCR assays

Table 2 shows the numbers of animals in the various groups that survived to the 60th dpi, together with the numbers displaying negative hemoculture and PCR results. Taken together, the two parasitological assays reveal that the cure rate (based on surviving animals) of the BZ and BZ/DFO-35 treatment was 18% that of the BZ/DFO-21 treatment was 42%, whilst that of the DFO-35 treatment was 67%. It should be pointed out, however, that the later treatment also showed the highest mortality rate (70%) at 40 dpi (Fig. 1B). Considering the higher sensitivity of the PCR assay (Miyamoto et al., 2006), the results suggest that treatment with DFO over a period of 35 days could have eliminated the infection in animals of the DFO-35 group. The higher frequencies of negative hemoculture and PCR results, tests that are typically positive in infected mice, in conjunction with the significantly reduced parasitemia curve and decreased mortality rates (in comparison with infected but untreated mice) recorded in these animals, indicates that DFO treatment certainly plays an important protective role during the course of the infection.

Table 2
Results of hemoculture and PCR assays during acute phase (60th dpi) of infection and percentage of cure in mice ($n = 52$) that had survived inoculation with *Trypanosoma cruzi* Y strain

Group (surviving animals)	Animals with negative hemoculture	Animals with negative PCR	Percentage of cure
BZ ($n = 17$)[a]	7 (41%)	4 (24%)	3 (18%)
BZ/DFO-21 ($n = 12$)[b]	7 (58%)	7 (58%)	5 (42%)
DFO-35 ($n = 6$)[c]	5 (83%)	4 (67%)	4 (67%)
BZ/DFO-35 ($n = 17$)[d]	8 (47%)	6 (35%)	3 (18%)

[a] Infected and treated with benznidazole.
[b] Infected and treated with benznidazole and desferrioxamine for 21 days.
[c] Infected and treated with desferrioxamine for 35 days.
[d] Infected and treated with benznidazole for 21 days and desferrioxamine for 35 days.

Table 2 further reveals that, when employed in combination with BZ, the chelator DFO is more effective if administered for a shorter period of time. Thus, the combined treatment received by group BZ/DFO-35, although producing a 100% survival rate at 40 dpi, exhibited a cure rate identical to that of the BZ group, namely 18%, whereas the cure rate in the BZ/DFO-21 group was more than 2-fold higher. As an intracellular parasite, *T. cruzi* is exposed to different reactive oxygen species (ROS) generated by its own aerobic metabolism, by the host immune response (Docampo, 1990) and, occasionally, released by drugs used in the treatment of CD (Turrens, 2004). The results from the present study suggest that DFO might act against oxidative stress (Clark and Hunt, 1983; Srivastava et al., 1999) resulting in some protection for the parasite and the host against ROS intermediates. In this way, DFO may to some extent increase the survival of the parasite by inhibiting one mechanism of its elimination.

Clark and Hunt (1983) showed that DFO would be useful in arresting malarial pathology arising from damage to the vascular endothelium or erythrocytes. More recently, Wen et al. (2006) showed that increased oxidative stress is correlated with mitochondrial dysfunction in chagasic patients leading to a compromised antioxidant defense.

3.3. Effect of treatment with BZ and DFO on iron levels in the liver

Significantly lower concentrations of iron were detected on the 10th (data not shown) and 16th dpi in liver samples of all infected animals in comparison with those of the NINT group (Fig. 2A). Pedrosa et al. (1990) reported that treatment of mice with DFO led to a reduction in iron levels in the liver of animals infected with *T. cruzi* Y and CL strains, together with a reduction in iron concentration in the spleen of those infected with the Y strain. These authors further established that the levels of iron in the liver and spleen of germ-free mice remained unaltered when DFO was administered 15 days after infection with *T. cruzi* (Pedrosa et al., 1993). Moreover, in a study concerning the effect of iron on *T. cruzi* infection in mice, Lalonde and Holbein (1984) demonstrated a reduction in iron supplies (48% in the liver and 15% in the spleen) following either treatment with DFO or the administration of an iron deficient diet.

The present study demonstrates that infection itself provokes a reduction in iron supplies in all experimental groups when compared with non-infected and untreated animals. The data also imply that DFO does not remove the iron linkage to transferrin and, although iron becomes unavailable for the parasite, there is no modification of the status of the element within the organism.

3.4. Effect of treatment with BZ and DFO on serum iron levels

On the 16th dpi, iron levels in the sera of infected animals belonging to the BZ/DFO-35 group had increased in comparison with those of all other groups, whilst the DFO-35 group showed increases in serum iron concentrations compared with the NINT, INT, BZ, DFO-21 and BZ/DFO-21 groups (Fig. 2B). It appears that administration of DFO over a 35 day period provokes a large release of iron from tissues that rely on mononuclear phagocyte cells for the supply of iron, and this gives rise to an increase in the level of the transition metal in the serum of the treated animals prior to elimination.

Previously, Lalonde and Holbein (1984) reported that no significant changes occurred in the serum iron levels of non-infected mice that had been treated with DFO, whereas *T. cruzi* infected animals that had received the same treatment presented iron supplies that were sufficient to maintain a normal immune response. It is important to note, however, that the protocols regarding inoculation with *T. cruzi* and administration of DFO used by these authors were very different from those employed in the present study.

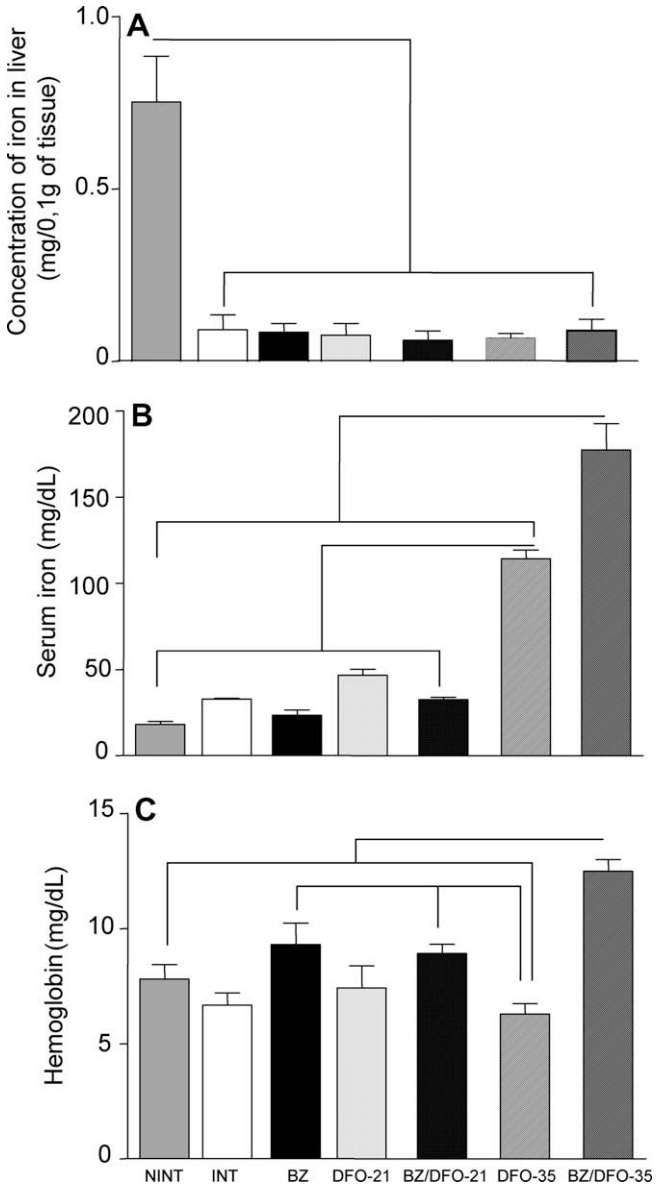

Fig. 2. Evaluation of iron levels in liver (A), serum (B) and hemoglobinemia (C) at the 16th dpi in Swiss mice inoculated with 500 blood trypomastigotes of *Trypanosoma cruzi* Y strain and submitted to different treatments: NINT, non-infected untreated control; INT, infected and untreated; BZ, infected and treated with benznidazole; DFO-21, infected and treated with desferrioxamine for 21 days; BZ/DFO-21, infected and treated with benznidazole and desferrioxamine for 21 days; DFO-35, infected and treated with desferrioxamine for 35 days; BZ/DFO-35, infected and treated with benznidazole for 21 days and desferrioxamine for 35 days. Values shown are means ± SD ($n = 30$ per group of infected animals; $n = 20$ for non-infected group; $n = 200$). The lines indicate statistically significant differences between groups as determined by one-way ANOVA.

Moreover, Letendre (1985) demonstrated that during infection there was a decrease in the level of circulating iron, occasioned by its diminished release from the mononuclear phagocyte system, and that this restricted the amount of iron available to extracellular parasites. However, the beneficial aspects of this process are questionable in the case of intracellular parasites such as *T. cruzi* that lodge in the cells of the mononuclear phagocyte system.

3.5. Effect of treatment with BZ and DFO on hemoglobin levels

No differences were observed in hemoglobin levels determined prior to infection and on the 10th day after infection (data not shown). Animals of the BZ/DFO-35 group exhibited increased hemoglobin levels on the 16th dpi compared with all other experimental groups, whilst decreased hemoglobin levels were observed in the DFO-35 group animals compared with those of the BZ and BZ/DFO-21 groups (Fig. 2C).

Changes in hemoglobin levels have been reported following infection with various trypanosomes, including *T. cruzi* (Esievo et al., 1982; Igbokwe and Anosa, 1989), and are associated with a reduction in the number of platelets (Cardoso and Brener, 1980; Ruiz et al., 1989), erythrocytes (Cardoso and Brener, 1980) and hematocrit values (Lalonde and Holbein, 1984). Moreover, Marcondes et al. (2000) observed that the acute phase of experimental infection by *T. cruzi* is characterized by anemia, thrombocytopenia, leucopoenia and bone marrow hypoplasia. However, the mechanisms responsible for these hematological alterations are not fully understood.

Pedrosa et al. (1990) have evaluated iron deficiency in mice and correlated the effect of this deficiency with the evolution of CD. These authors showed that the hypohemoglobinemia presented by the host was permanent in animals fed on an iron-free diet, probably because the low supplies of the element were insufficient to compensate for the erythropoiesis that follows anemia. In animals infected with CL and Y strain and treated with DFO, hemoglobin levels recovered suggesting that this treatment does not interfere with the supplies required for erythropoiesis. Hence, the reduction in hemoglobin levels observed in the DFO-35 group may be explained by the presence of infection by *T. cruzi* in association with the action of DFO in the dose employed in the present study.

In comparison with infected animals of other groups, those of the BZ/DFO-35 group exhibited increased levels of serum iron and hemoglobin but similar concentrations of iron in the liver. This shows that the effect of DFO in combination seems to depend on the modification of iron status in the host, since, the BZ/DFO-35 group presented reduced mortality and lower parasitemia levels. Even though similar amounts of hepatic iron were detected in animals of all infected groups, the possibility of a redistribution of iron supplies entering different cells types in the liver cannot be disregarded, and such a process could lead to differential responses regarding the infection. Additionally, the experimental results obtained in the present study reinforce the message that other mechanisms, including oxidative stress, could be involved in animals treated with DFO. Santos et al. (2005) observed that repetitive stress causes a significant increase in the number of parasites during the acute phase of *T. cruzi* infection in the rat, suggesting that stress may enhance pathogenesis through suppression of the immune system of the host. It seems reasonable, therefore, to propose an effect of stress on the immune response to *T. cruzi* infection (Santos et al., 2007). In this context, Hernández et al. (2006) have pointed out a further role of *T. cruzi* in the protection of biological membranes against lipoperoxidative damage. Such an antioxidant action might contribute to cell survival and proliferation by protecting the parasites from toxic oxygen metabolites to which they are continuously exposed.

In conclusion, the data reported in this paper suggest that prolonged treatment of *T. cruzi* infected mice with DFO reduces the availability of iron in the infecting parasites, leading to a reduction in parasitemia and mortality. Furthermore, treatment with DFO in association with BZ produced a mortality rate of 0% showing that modification in iron stores increases the efficacy of BZ. However, the cure rate following BZ and BZ/DFO-35 treatment was only 18% whereas that in mice of the BZ/DFO-21 group was 42% indicating that DFO should be administrated for only a short period of time when used in combination with BZ. The fact that the DFO-35 group showed a 67% cure rate represents strong evidence of the protective effect of DFO in the surviving animals. The results

presented here show that the iron status of the host influences the efficacy of therapy with BZ and can improve the percentage of survival of infected animals.

Acknowledgments

This work was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Rede Mineira de Bioterismo, Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) and Universidade Federal de Ouro Preto.

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