Phosphoramidon treatment improves the consequences of chagasic heart disease in mice

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A B S T R A C T

Chagas’ disease, caused by the protozoan parasite Trypanosoma cruzi, is an important cause of cardiomyopathy. Microvascular spasm and matrix dissolution, modulated by endothelin-1 (ET-1), is implicated in the pathogenesis of chagasic heart disease. To further elucidate the role of ET-1 in murine chagasic heart disease, C57BL/6 †129sv mice were infected with T. cruzi (103 trypomastigotes of the Brazil strain). These mice are resistant to death during the acute phase but progress to chronic cardiomyopathy. Infected mice were compared with infected mice treated with phosphoramidon, a non-specific metalloprotease inhibitor that is also a potent inhibitor of endothelin-converting enzyme, at a dose of 10 mg/kg. Mice were treated with phosphoramidon for the initial 15 days post infection (PI). All mice were evaluated 200 days PI. Examination of infected, untreated mice revealed marked inflammation, vasculitis and fibrosis. The hearts of infected treated mice had significantly less pathology. Cardiac magnetic resonance imaging (MRI) revealed that right ventricular internal diameter (RVID) was significantly greater (P<0.05) in the infected untreated group (2.9 ± 0.22 mm) as compared with the infected treated group (1.73 ± 0.35 mm). In another experiment phosphoramidon treatment was also tested in CD1 mice, which have a high mortality during the acute phase of infection with 5 × 104 trypomastigotes of the Brazil strain. One group of CD1 mice was untreated while the other group received phosphoramidon for the initial 15 days PI. The mortality rate in untreated mice was 70% by day 35 PI, while all treated infected mice lived. The parasitemia in both groups was similar. The cardiac pathology was more severe in untreated mice. MRI revealed the RVID to be significantly greater in the untreated infected group as compared with the phosphoramidon-treated infected mice (2.74 ± 0.03 mm versus 1.64 ± 0.4 mm P < 0.05). Transthoracic echocardiography revealed that the percentage fractional shortening was reduced in infected CD1 mice but not in those infected mice treated with phosphoramidon. There was no effect of phosphoramidon in uninfected mice. Phosphoramidon (100 µg/ml) had no effect on parasites

Key words: endothelin, phosphoramidon, endothelin-converting enzyme, Trypanosoma cruzi, Chagas’ disease, cardiac magnetic resonance imaging, echocardiography.

Abbreviations: ECE, endothelin-converting enzyme; EDD, end diastolic diameter; ESD, endsystolic diameter; ET-1, endothelin-1; %FS, percentage fractional shortening; LV, left ventricular; RVID, right ventricular internal diameter.

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in vitro. These data are consistent with the hypothesis that ET-1 contributes to the pathogenesis of murine chagasic cardiomyopathy and suggests that interventions targeting ET-1 would improve the outcome in chagasic heart disease.

INTRODUCTION

Chagas’ disease caused by *Trypanosoma cruzi*, causes acute myocarditis and chronic cardiomyopathy. Chagasic heart disease is the result of infection of the various cellular components including the cells of the endothelium (endothelial and vascular smooth muscle cells) cardiac myocytes and fibroblasts. We believe that an early event in the development of chagasic heart disease is infection of the cells of the endothelium. In fact, evidence has been presented that underlying chagasic heart disease is, in part, a vasculopathy [1].

*T. cruzi* infection of the cardiovascular system results in both ischaemia and inflammation. Examination of the heart in human and experimental acute chagasic myocarditis reveals inflammation, myonecrosis, vasculitis and numerous parasite pseudocysts. Chronic chagasic cardiomyopathy is characterized by fibrosis, myocyteolysis, minimal inflammation and a virtual absence of parasites. We have demonstrated that *T. cruzi* infection of endothelial cells causes an increase in the synthesis of biologically active endothelin-1 (ET-1) [1]. The expression of ET-1 was found to be increased in the myocardium of *T. cruzi*-infected mice [2,3] suggesting that ET-1 contributes to chagasic cardiomyopathy. ET-1 has several physiological properties that influence vascular tone in vessels, myocardial function and cardiovascular remodelling [4,5]. The final step in processing of big ET-1 to the active 21 amino acid products is carried out by endothelin-converting enzyme (ECE), a membrane-bound zinc metallopeptidase. The conversion of big ET-1 to ET-1 is essential for biological activity, since the pressor action of big ET-1 is almost completely inhibited by a relatively large dose of phosphoramidon that inhibits ECE [6,7]. Phosphoramidon is a dual ECE and neutral endopeptidase inhibitor that inhibits ECE-1 activity in the myocardium as does another specific inhibitor, FR901533 [8].

In the present report we treated *T. cruzi*-infected mice with phosphoramidon. We found that this compound dramatically improved the structural and functional abnormalities caused by infection with *T. cruzi*. This suggests a role for ET-1 in the remodelling that accompanies chagasic heart disease.

MATERIALS AND METHODS

Infection and treatment of mice

Nine-week-old C57BL/6 x 129sv male mice (bred in our own facility) were infected with 1 x 10⁶ trypomastigotes of the Brazil strain of *T. cruzi*. Nine-week-old CD1 male mice (Jackson Laboratories, Bar Harbor, ME, U.S.A.) were infected with 5 x 10⁹ trypomastigotes of the Brazil strain. In both cases mice were treated with phosphoramidon (Sigma, St Louis, MO, U.S.A.; 10 mg/kg) intraperitoneally, from the day of infection for the initial 15 days post infection (PI).

The Institutional Animal Use Committee of the Albert Einstein College of Medicine approved this work.

Exposure of parasites to phosphoramidon

Cultures of epimastigotes were exposed to phosphoramidon (100 µg/ml) in liver infusion tryptose medium. In addition, cultured myoblasts that had been previously infected with trypomastigotes were treated with phosphoramidon (100 µg/ml).

Histopathology

Hearts were fixed in 10% (v/v) buffered formalin and stained with haematoxylin and eosin.

Cardiac magnetic resonance imaging (MRI)

Mice were anaesthetized with ketamine (150 mg/kg)/xylazine (10 mg/kg) solution. Once anaesthetized, a set of standard electrocardiographic (ECG) leads was attached to their limbs. The ECG signal was fed to a Gould ECG amplifier associated with a Gould 2200s recorder and interfaced to a PC running Ponemah Physiology Suite software. Mice were wrapped in a small blanket and positioned head up in a home-built 35 mm (inner diameter) MRI coil. Gating of the MRI data acquisition was accomplished, as described [9–11], by using the rising phase of the QRS complex to trigger a standard 5-V square-wave gating signal. This signal was fed from the Gould 2200s recorder to the cardiac gating box associated with the 9.4 T vertical wide-bore GE Omega spectrometer (operating at a ¹H frequency of 400 MHz). This spectrometer is equipped with S50 shielded gradients and the temperature within the gradient coils was maintained at 30 °C with a Neslab water cooling/heating unit to reduce the likelihood of a drop in body temperature during the imaging experiment. Prior to each acquisition heart rate was determined from the ECG and the gating delay was set to acquire diastolic images or systolic images. Cardiac gated multi-slice spin echo imaging was employed. Slice selection was achieved with a 90° sinc pulse. A 40-mm field of view was used. The 128 x 256 pixel image was interpolated to 256 x 256 pixels. The separation between slices was 0.5 mm. An echo time of 18 ms and repetition time of approx. 100–200 ms (varying with heart rate) was used as described previously [12].

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Phosphoramidon and Chagas’ disease

Figure 1  Representative myocardial sections of C57BL/6 × 129sv mice 200 days PI with the Brazil strain of T. cruzi. (A) Infected untreated and (B) infected phosphoramidon-treated. There is a marked reduction in inflammation and fibrosis in mice treated with phosphoramidon. Photomicrograph in (A) was taken at 16 × and panel B at 6.3 × magnification.

Measurements of chamber dimensions were made from long and short axis images.

Transthoracic echocardiography
Echocardiography was performed in control and infected mice. We measured left ventricular (LV) end-diastolic diameter (EDD), LV end-systolic diameter (ESD) as described previously. LV percentage fractional shortening (%FS) was determined as \[ \frac{[(EDD - ESD)]}{EDD} \times 100 \] [12].

Statistical analysis
Statistical analysis was performed with non-parametric methods employing one-way ANOVA of ranks (SIGMA STAT, SPSS Science, Chicago, IL, U.S.A.).

RESULTS

Mortality and parasitaemia
Infected C57BL/6 × 129sv mice did not die acutely. They developed a transient parasitemia of \( 1 \times 10^4 \). Infected CD1 mice experienced a 70% mortality by day 35 PI. Those mice that survived the acute phase were studied at 200 days PI. The parasitemia in infected untreated CD1 mice was similar to that observed in phosphoramidon-treated infected mice (4.53 ± 1.86 × 10^4 (n = 4) versus 3.6 ± 0.95 × 10^4 (n = 5).

Effect of phosphoramidon on parasites in vitro
Phosphoramidon had no effect on epimastigote growth in liver infusion tryptose medium. There was no significant difference in the number of tryptomastigotes in the supernatant of infected cultured myoblasts treated or untreated with phosphoramidon. This was evaluated at 24, 48 and 72 h PI.

Histopathology
The myocardium of infected C57BL/6 × 129sv mice revealed marked inflammation, fibrosis and vasculitis. There was a marked attenuation of these pathological findings in the phosphoramidon-treated mice (Figure 1).

Cardiac MRI
RVID was significantly greater \( (P < 0.05) \) in infected untreated C57BL/6 × 129sv mice (2.9 ± 0.22 mm) compared with the treated infected mice (1.73 ± 0.35 mm). RVID of uninfected phosphoramidon-treated mice (1.67 ± 0.07 mm) was not significantly different from RVID of uninfected, untreated mice (1.47 ± 0.27 mm; Figure 2). In another experiment CD1 mice were infected with \( 5 \times 10^4 \) Brazil strain. CD1 mice are susceptible to mortality during the acute phase of infection. One group of CD1 mice was untreated, while another group was treated with phosphoramidon for the initial 15 days PI. The mortality rate in untreated mice was 70%, while all treated infected mice lived. The parasitaemia in both

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Figure 3 RVID of CD1 mice determined by cardiac-gated MRI of infected (Inf) mice (n = 4) and phosphoramidon-treated (Inf + P) infected mice (n = 4).
These mice were examined 200 days PI (Inf versus Inf + P is significant at P < 0.05). There is no significant difference between Inf + P and uninfected control (results not shown).

Figure 4 Percentage fractional shortening in CD1 mice as determined by echocardiography.
There is a significant reduction in percentage fractional shortening in infected (Inf; n = 13) compared with uninfected control mice (n = 8) and to phosphoramidon-treated infected mice (Inf + P; n = 8). Inf versus control and Inf versus Inf + P are significant at P < 0.05. There was no significant difference between uninfected control versus Inf + P mice.

The RVID of CD1 mice was significantly (P = 0.012) reduced in phosphoramidon-treated infected CD1 mice (1.67 ± 0.04 mm) compared with the RVID in untreated infected CD1 mice (2.74 ± 0.03 mm; Figure 3). There was no significant effect of phosphoramidon treatment on RVID in untreated infected CD1 mice compared with that in untreated uninfected CD1 mice (RVID 1.97 ± 0.07 versus 1.95 ± 0.08, P = 0.86).

DISCUSSION
During the course of T. cruzi infection, inflammation, fibrosis and vasculitis are most severe in the right atrium and ventricle. Indeed, the present study confirms the results of our previous cardiac MRI studies demonstrating that right ventricular pathology and enlargement is commonly observed in chronically infected C57BL/6 × 129sv and CD1 mice [10,11]. Previous reports from our laboratory suggested that ET-1 contributes to the pathogenesis of chagasic heart disease. These studies revealed that plasma ET-1 levels were elevated. In addition, mRNAs for preproET-1, ECE and ET-1 were induced in the myocardium of T. cruzi-infected mice. Phosphoramidon, an inhibitor of ECE, was administered to C57BL/6 × 129sv mice that ordinarily do not die of acute infection but gradually develop cardiomyopathy. The treated mice had significantly less inflammation and fibrosis and the RVID was smaller than untreated infected mice.

Phosphoramidon reduced the mortality of infected CD1 mice. In addition, the inflammation and fibrosis were ameliorated and the RVID was markedly decreased compared with infected untreated CD1 mice. Echocardiography studies demonstrated that in chronically infected CD1 mice, the EDD was larger and the %FS was reduced. In phosphoramidon-treated infected CD1 mice these findings were significantly diminished. Phosphoramidon, in the absence of infection, had no effect on any of the functional parameters, as determined by cardiac MRI or echocardiography. Importantly, phosphoramidon had no effect on parasites in vitro.

Although phosphoramidon is not a specific inhibitor of ECE-1, it has been shown to markedly inhibit its activity in the myocardium [8]. The results in this study are consistent with studies demonstrating a role for ET-1 in cardiovascular remodelling. It is also possible that some of the effect of phosphoramidon on cardiac remodelling may be due to effects on other proteases. The finding that phosphoramidon treatment for an initial period of 2 weeks after infection resulted in reduction of pathology suggests that ET-1 antagonists and ECE inhibitors have therapeutic potential in attenuating the pathology and myocardial dysfunction in chagasic heart disease.

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