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Treatment with Fenofibrate plus a low dose of Benznidazole attenuates cardiac dysfunction in experimental Chagas disease

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ABSTRACT

Trypanosoma cruzi induces serious cardiac alterations during the chronic infection. Intense inflammatory response observed from the beginning of infection, is critical for the control of parasite proliferation and evolution of Chagas disease. Peroxisome proliferator-activated receptors (PPAR)-α, are known to modulate inflammation.

In this study we investigated whether a PPAR-α agonist, Fenofibrate, improves cardiac function and inflammatory parameters in a murine model of *T. cruzi* infection. BALB/c mice were sequentially infected with two *T. cruzi* strains of different genetic background. Benznidazole, commonly used as trypanocidal drug, cleared parasites but did not preclude cardiac pathology, resembling what is found in human chronic chagasic cardiomyopathy. Fenofibrate treatment restored to normal values the ejection and shortening fractions, left ventricular end-diastolic, left ventricular end-systolic diameter, and isovolumic relaxation time. Moreover, it reduced cardiac inflammation and fibrosis, decreased the expression of pro-inflammatory (IL-6, TNF-α and NOS2) and heart remodeling mediators (MMP-9 and CTGF), and reduced serum creatine kinase activity. The fact that Fenofibrate partially inhibited NOS2 expression and NO release in the presence of a PPAR-α non-competitive inhibitor, suggested it also acted through PPAR-α-independent pathways. Since IκBα cytosolic degradation was inhibited by Fenofibrate, it can be concluded that the NFκB pathway has a role in its effects. Thus, we demonstrate that Fenofibrate acts through PPAR-α-dependent and -independent pathways.

Our study shows that combined treatment with Fenofibrate plus Benznidazole is able both to reverse the cardiac dysfunction associated with the ongoing inflammatory response and fibrosis and to attain parasite clearance in an experimental model of Chagas disease.

1. Introduction

Almost 7 million people worldwide are estimated to be infected with *Trypanosoma cruzi,* the etiological agent of Chagas disease (WHO - World Health Organization, 2016). This disease is endemic throughout Central and South America, representing a major public health problem. The acute phase of infection is characterized by the presence of parasites in the host bloodstream and other tissues, promoting a severe inflammatory response (Teixeira et al., 2002). After the acute phase, generally asymptomatic, the infection evolves to a silent chronic phase. However, after a variable period of time (10–30 years after the onset of infection), 30–40% of the patients develop symptomatic cardiac alterations, including heart failure, arrhythmias, and thromboembolism, which cause major disabilities with high economic and social impact (Benziger et al., 2017). Diverse factors contribute to the development of chagasic dilated cardiomyopathy. Disruptions of the capillary network, due to the inflammatory infiltrate, induce focal myocytolysis, generating microvascular injury and myocardial remodeling. Substantial evidence has shown that the cardiac tissue, an important target of *T. cruzi* infection, induces the production of marked amounts of pro-inflammatory cytokines, chemokines and enzymes, including inducible nitric oxide synthase (NOS2) and metalloproteinases (MMPs), resulting in inflammation and cardiac remodeling due to the parasite infection (Penas et al., 2013). In response, fibroblasts proliferate and the interstitial collagen matrix is increased (Mitelman and Argentinian Society of Cardiology, 2011).

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Benznidazole (N-benzyl-2-nitroimidazole acetamide, Bzl) is the trypanocidal drug of choice in the majority of the endemic countries, including Argentina, since has less severe side effects than Nifurtimox (Maguire, 2015). Although this drug is effective reducing the parasite load during the acute and the chronic phases of the disease, treatment during the latter does not correlate with a better outcome, since both Bzl-treated and placebo-treated patients have similar clinical progression in terms of development of chronic chagasic cardiomyopathy (Morillo et al., 2015). Besides, unwanted side effects are common at the currently used doses (Pérez-Molina et al., 2009; Viotti et al., 2009; Miller et al., 2015; Noguerado-Mellado et al., 2016). These side effects force about 10% of patients to abandon the treatment being the main disadvantage of its use.

Peroxisome proliferator-activated receptors (PPARs), members of the steroid hormone receptor superfamily, are ligand-dependent nuclear transcription factors. Fenofibrate (Fen), a PPAR-α ligand, is a third-generation fibric acid derivative currently used clinically as a hypolipidemic agent to lessen the risk of atherosclerosis (Ling et al., 2013).

Growing evidence has demonstrated the efficacy of PPAR agonists, including Fen, as regulators of inflammation and extracellular matrix remodeling of the heart (Lockyer et al., 2010). Fen can prevent myocardial inflammation and fibrosis in diabetic mice (Zhang et al., 2016). Moreover, Fen has been shown to exert cardioprotective effects against various cardiac disorders in *in vivo* and *in vitro* rat models (Zou et al., 2013; Cheng et al., 2016) and in patients (Yin et al., 2013).

We have previously reported that PPAR-γ ligands reduce the inflammatory reaction using *in vivo* and *in vitro* models of *T. cruzi* infection (Penas et al., 2013) and LPS-stimulated (Hovsepian et al., 2010) or *T. cruzi*-stimulated cardiomyocytes (Hovsepian et al., 2011). Other authors have shown that PPAR-α can inhibit the cardiovascular inflammatory response by reducing NF-kB activity, and through regulation of cytokine-receptor and growth-factor receptor signaling (Lockyer et al., 2010). However, the role of PPAR-α ligands in Chagas disease is currently unknown.

Based on the premise of the Drugs for Neglected Diseases Initiative (DNDi), to develop new therapeutical strategies for the treatment of neglected tropical diseases, relying on drugs already available in the pharmaceutical market, we sought to determine whether the administration of Fen, as an anti-inflammatory drug, improves the cardiac outcome in an experimental model of *T. cruzi* infection in the context of low-dose Bzl treatment.

Our results show, for the first time, that Fen treatment restores echocardiographic parameters to normal, and reduces tissue inflammation and fibrosis in a murine model of mixed *T. cruzi* infection, while a low dose of Bzl is capable to clear blood parasitaemia and heart parasite load.

2. Methods

2.1. Ethics statement

Mice used in this study were bred and maintained in the animal facility at the Instituto de Investigaciones en Microbiología y Parasitología Médica, Universidad de Buenos Aires – CONICET. All procedures carried out with mice were approved by the Institutional Committee for the Care and Use of Laboratory Animals (CICUAL, Facultad de Medicina de la Universidad de Buenos Aires, CD No 2271/2014) and are in accordance with guidelines of the Argentinean National Administration of Medicines, Food and Medical Technology (ANMAT), Argentinean National Service of Sanity and Agrifoods Quality (SENASA) and also based on the US NIH Guide for the Care and Use of Laboratory Animals.

2.2. Mice and infection

Eight-week-old BALB/c mice (7 *per* group) were infected by intraperitoneal route with $1x10^5$ bloodstream trypomastigotes of the non-lethal K-98 clone of *T. cruzi* (TcI) for 6 weeks, followed by re-infection with 100 bloodstream trypomastigotes of the lethal RA (pantropic/reticulotropic) strain of *T. cruzi* (TcVI) for 4 weeks (Celentano and González Cappa, 1993; Zingales et al., 2009).

2.3. In vitro model: neonatal mouse primary cardiomyocytes culture and infection

One-to 3-day old neonatal outbred CF-1 strain mice were euthanized by decapitation after CO_2 exposure, and cardiomyocytes were obtained as previously described (Hovsepian et al., 2013). Cardiomyocytes were cultured in FBS 10%-DMEM-M199 medium at 37 °C under 5% CO_2 atmosphere, and infected at a 5:1 (parasite: cell) ratio in six well polystyrene plates. After 3 h, the infected cultures were washed five times with fresh 1% FBS-DMEM: M-199 medium to remove free parasites. Each experiment was carried out 3 times with 5 replicates *per* group.

2.4. Treatments

2.4.1. In vivo treatments

Mice were treated by oral gavage with Benznidazole (Abarax®, ELEA, Argentina. PubChem Compound Database CID = 31593, Bzl) and/or Fenofibrate (Daunlip®, Montpellier S.A, Argentina. PubChem Compound Database CID = 3339, Fen) suspended in corn oil. Fen dose optimization was carried out using 50, 100, 200 or 300 mg/kg/day for 30 consecutive days. The chosen doses were 25 mg/kg/day for Bzl (Cevey et al., 2016) for 15 consecutive days and 100 mg/kg/day for Fen treatment, for 30 consecutive days. Each experiment was carried out three times.

2.4.2. In vitro treatments

Fen was suspended in PBS. According to the experiment, cells were treated with 50, 100 or 150 μM Fen for 30 min before *T. cruzi* infection. In some experiments cells were pre-treated for 30 min with the non-competitive antagonist of PPAR-α (MK886, 10 μM) before the addition of Fen.

2.5. Parasitaemia and survival

Parasitaemia was evaluated by microhematocrit (Feilij et al., 1983) or the method of Pizzi modified by Brener (1962) every three to seven days. Survival was registered daily until the end of the experiment.

2.6. Doppler echocardiography

Transthoracic echocardiography was performed using an Acuson Sequoia C 512 ultrasound system with a 14-MHz linear transducer. Echocardiographic experiments were performed under light anesthesia (287.5 mg/kg of 2.5% filtered 2,2,2-Tribromoethanol; Sigma-Aldrich). The two-dimensional parasternal short-axis imaging plane was used to obtain M-mode tracings at the level of the papillary muscles. Left ventricular (LV) internal dimensions and LV wall thickness (LVWT) were determined at systole and diastole using leading-edge methods and guidelines of the American Society of Echocardiography (Sahn et al., 1978). End-diastolic measurements were taken at the maximal LV diastolic dimension, and end systole was defined as the time of the most anterior systolic excursion of the posterior wall. Measurements were taken from three consecutive beats for each mouse. Ejection fraction (EF) and shortening fraction (SF) were calculated and used as ejective indexes of systolic function. EF was estimated from LV dimensions by the cubed method as follows: EF (%) = [(LVEDD3 − LVESD3)/