International Journal for Parasitology: Drugs and Drug Resistance 6 (2016) 12–22

Contents lists available at ScienceDirect

International Journal for Parasitology: Drugs and Drug Resistance

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

Low-dose benznidazole treatment results in parasite clearance and attenuates heart inflammatory reaction in an experimental model of infection with a highly virulent *Trypanosoma cruzi* strain

Ágata Carolina Cevey, Gerardo Ariel Mirkin, Federico Nicolás Penas, Nora Beatriz Goren*

Instituto de Investigaciones en Microbiología y Parasitología Médica (IMPaM — UBA, CONICET), Argentina

ARTICLE INFO

Article history:
Received 29 September 2015
Received in revised form
3 December 2015
Accepted 7 December 2015
Available online 12 December 2015

Keywords:
Trypanosoma cruzi
Chemotherapy
Inflammatory mediators
NF-κB

ABSTRACT

Chagas disease, caused by *Trypanosoma cruzi*, is the main cause of dilated cardiomyopathy in the Americas. Antiparasitic treatment mostly relies on benznidazole (Bzl) due to Nifurtimox shortage or unavailability. Both induce adverse drug effects (ADE) of varied severity in many patients, leading to treatment discontinuation or abandonment. Since dosage may influence ADE, we aimed to assess Bzl efficacy in terms of parasiticidal and anti-inflammatory activity, using doses lower than those previously reported. BALB/c mice infected with the *T. cruzi* RA strain were treated with different doses of Bzl. Parasitaemia, mortality and weight change were assessed. Parasite load, tissue infiltrates and inflammatory mediators were studied in the heart. Serum creatine kinase (CK) activity was determined as a marker of heart damage. The infection-independent anti-inflammatory properties of Bzl were studied in an *in vitro* model of LPS-treated cardiomyocyte culture. Treatment with 25 mg/kg/day Bzl turned negative the parasitological parameters, induced a significant decrease in IL-1β, IL-6 and NOS2 in the heart and CK activity in serum, to normal levels. No mortality was observed in infected treated mice. Primary cultured cardiomyocytes treated with Bzl showed that inflammatory mediators were reduced via inhibition of the NF-κB pathway. A Bzl dose lower than that previously reported for treatment of experimental Chagas disease exerts adequate antiparasitic and anti-inflammatory effects leading to parasite clearance and tissue healing. This may be relevant to reassess the dose currently used for the treatment of human Chagas disease, aiming to minimize ADE.

1. Introduction

Chagas disease is caused by infection with the obligate intracellular protozoan parasite *Trypanosoma cruzi*. This disease is endemic throughout Central and South America, representing a major public health problem. The disease is characterized by an acute phase with high parasitaemia and variable symptoms, including acute myocarditis, meningoencephalitis or generalized infection symptoms (hepatosplenomegaly). This is followed by a chronic phase that may remain asymptomatic during the whole life or develop into serious digestive or cardiac alterations, which are found in about 30% of infected individuals and may lead to dilated cardiomyopathy (Teixeira et al., 2002).

The disease is currently treated with benznidazole (Bzl) (N-benzyl-2-nitroimidazole acetamide), a drug known to reduce parasite burden during acute and early chronic infection (Coura, 2009). During the chronic phase, the effect of Bzl is more controversial, although some reports have shown that individuals treated with Bzl and evaluated decades after the initial infection acquire significant protection from progression of heart pathology (Viotti et al., 2006; Fabbro et al., 2007). Although Bzl has been used in clinical settings, its mechanisms of action have not been fully elucidated yet (Maya et al., 2007). However, several studies have suggested that Bzl treatment should still be recommended at late phases of Chagas disease to prevent progression, regardless of the lack of complete parasite clearance (Garcia et al., 2005; Sosa-Estani and Segura, 2006; Viotti et al., 2006). Indeed, there is a general premise that etiological treatment contributes to reducing parasite load and rearranging the host immune response, leading to a balanced inflammatory response, which is crucial to control Chagas disease morbidity (Garcia et al., 2005; Viotti et al., 2006).

* Corresponding author. IMPaM-UBA, CONICET, Paraguay 2155, piso 12, Buenos Aires 1121, Argentina.
E-mail address: ngoren@fmed.uba.ar (N.B. Goren).

http://dx.doi.org/10.1016/j.ijpddr.2015.12.001

Campi-Azevedo et al. (2015) have recently characterized the phagocytic capacity and cytokine profile of leukocytes from Chagas disease patients in the indeterminate and cardiac phases, both before and one year after Bzl treatment. Their findings highlighted that Bzl treatment contributes to an overall immunomodulation in the indeterminate phase and induces a broad change of the immune response in patients in the cardiac phase, eliciting an intricate phenotypic/functional network compatible with beneficial and protective immunological events. However, Bzl contains a nitro group linked to an imidazole whereby unwanted side effects are common. The most important adverse reactions observed with Bzl are cutaneous reactions (allergic dermatitis) (Pérez-Molina et al., 2009), digestive intolerance, polyneuritis, bone marrow depression, toxic hepatitis (Viotti et al., 2009), peripheral neuropathy and angioedema (Miller et al., 2015). These side effects, which force about 10% of patients to suspend the treatment, represent the main disadvantage of Bzl treatment. In addition, Hasslocher-Moreno et al. (2012) showed that up to 26% of patients treated with Bzl during the chronic phase develop skin reactions and that some show gastrointestinal (10%) and/or neurological (around 5%) disorders. Nevertheless, the incidence of adverse reactions has been insufficiently reported, making it difficult to interpret the safety profile of Bzl. Current evidence supports the treatment of adults without advanced cardiac disease or significant morbidity using either Bzl or nifurtimox, the other drug available (Jackson et al., 2010). Nifurtimox is associated with gastrointestinal and neuropsychiatric side effects in nearly all patients, only half of whom can tolerate the full treatment course (Priotto et al., 2009). Bzl is better tolerated, but due to intermittent medication shortages and drug-induced rash including Stevens–Johnson syndrome, many patients fail to complete the treatment. Although, as stated above, it has been proposed that Bzl is usually better tolerated than Nifurtimox, Rojo et al. (2014) have recently reported about the controversial toxicity of both drugs. However, Maya et al. (2007) found no significant adverse drug effects in a large series of patients treated with these drugs. Upon infection, the parasite is able to invade and multiply within diverse cell types, including macrophages. The acute phase of infection is characterized by the presence of parasites in the host bloodstream and diverse tissues. A crucial step in cardiomyopathy is the infiltration of monocytes and their differentiation into macrophages. These cells may either inhibit *T. cruzi* multiplication or provide a favourable environment in which it can divide and be disseminated to other sites within the body (Tanowitz et al., 1992; Penas et al., 2015). Besides, there is substantial evidence showing that cardiac tissue, an important target of *T. cruzi* infection, produces marked amounts of pro-inflammatory cytokines, chemokines and enzymes, including inducible nitric oxide synthase (NOS2) and metalloproteinases, resulting in inflammation and cardiac remodelling in response to parasite infection (Penas et al., 2013).

In addition to its antiparasitic activity, Bzl exerts immunomodulatory effects in macrophages stimulated with lipopolysaccharide (LPS) and treated with a high concentration of Bzl (1 mM) (Piaggio et al., 2001). These immunomodulatory effects of Bzl have also been described in LPS-challenged mice pre-treated with high doses of Bzl, showing the ability of Bzl to increase survival and decrease serum levels of IL-6 and TNF-α in C57BL/6 mice (Pascutti et al., 2004). The fact that components of *T. cruzi* as glycosylphosphatidylinositol-anchored mucin-type glycoproteins and glycoinositolphospholipids through the toll-like receptors TLR2/TLR6 and TLR4, respectively, induce proinflammatory cytokines (Junqueira et al., 2010) validates the use of LPS in studies that explore the mechanism of action of Bzl.

In the present study, we considered the administration of doses of Bzl lower than those usually used in experimental models of *T. cruzi* infection, to evaluate its parasiticidal as well as immunomodulatory effects, to minimize the adverse side reactions that usually lead to cessation of therapy.

2. Materials and methods

2.1. In vivo model: mice and infection

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) 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. Eight-weeks old BALB/c male mice (7 per group) were infected intraperitoneally with 500 bloodstream trypomastigotes of the lethal RA (pantropic/reticulotropic) strain of *T. cruzi*, (DTU VI) as previously described (Celentano and González Cappa, 1993; Zingales et al., 2009). Benznidazole (Abarax®, ELEA, Argentina. PubChem Compound Database CID = 31593), suspended in corn oil, was administered orally at 10, 25 and 100 mg/kg/day, for 30 consecutive days. The groups received the following treatments: Group 1 = Corn oil, Group 2 = Bzl 10 mg/kg/day, Group 3 = Bzl 25 mg/kg/day, Group 4 = Bzl 100 mg/kg/day, Group 5 = Uninfected, untreated. Treatment started soon after the detection of parasites in blood, which occurred at day 7 post-infection (p.i.).

Parasitaemia of infected untreated mice (Group 1) peaks between day 10 and 13. Therefore, they were sacrificed at the time when they began to show signs of cachexia, since they did not survive after 15 days. Mice of Groups 2 through 5 were sacrificed at day 55 p.i. Each experiment was carried out three times.

2.2. Parasitaemia and survival

Presence of parasites in blood was evaluated by microhematocrit method (Feilij et al., 1983). Parasitaemia was analysed using Pizzi's technique modified by Brener (1962) every three to seven days, and survival was observed daily, until the end of the experiment. Parasitaemia was expressed as parasites per millilitre of blood.

2.3. Histopathological studies

Hearts from *T. cruzi* infected untreated and benznidazole-treated infected mice (25 mg/kg/day), were fixed in formalin and embedded in paraffin. Six non-contiguous sections (5 μm) were cut and stained with haematoxylin-eosin. Cellular infiltrates and the presence of amastigote nests were examined using a Nikon Eclipse E600 microscope (Nikon Inc.). Images were captured with a Spot RT digital camera. At least thirty random microscopic fields (400×) were analysed in each microscopic section, using the open source Image J software (NIH, USA).

2.4. Creatine kinase activity

The activity of CK as a marker heart injury was determined using commercially available assay kits according to manufacturer's instructions (Wiener Lab, Rosario, Argentina). The kit relies on the reduction of $NADP^+$ and the increase of absorbance measured at 340 nm.