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Short Communication

Novel cruzipain inhibitors for the chemotherapy of chronic Chagas disease

María L. Sbaraglini [a,*], Carolina L. Bellera [a], Laura Fraccaroli [b], Luciana Larocca [b], Carolina Carrillo [b], Alan Talevi [a,1], Catalina D. Alba Soto [c,1]

[a] Laboratory of Bioactive Compounds Research and Development (LIDeB), Medicinal Chemistry, Department of Biological Science, Exact Sciences College, National University of La Plata (UNLP), 47 & 115 (B1900AJI) La Plata, Buenos Aires, Argentina
[b] Institute of Sciences and Technology Dr César Milstein (ICT Milstein), Argentinean National Council of Scientific and Technical Research (CONICET), Buenos Aires, Argentina
[c] Department of Microbiology, School of Medicine, University of Buenos Aires (UBA), Instituto de Microbiología y Parasitología Médica (IMPaM-UBA CONICET), Buenos Aires, Argentina

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ABSTRACT

Despite current efforts worldwide to develop new medications against Chagas disease, only two drugs are available, nifurtimox and benznidazole. Both drugs require prolonged treatment and have multiple side effects and limited efficacy on adult patients chronically infected with *Trypanosoma cruzi*. Recently, computer-guided drug repositioning led to the discovery of the trypanocidal effects of clofazimine and benidipine. These compounds showed inhibitory effects on cruzipain, the major cysteine protease of *T. cruzi*, of different parasite stages and in a murine model of acute Chagas disease. The aim of this work was to determine the efficacy of these novel cruzipain inhibitors when administered in a murine model of chronic Chagas disease. Benidipine and clofazimine were able to reduce the parasite burden in cardiac and skeletal muscles of chronically infected mice compared with untreated mice as well as diminish the inflammatory process in these tissues. Further studies should be performed to study the synergism with benznidazole and nifurtimox in view of combined therapies.

1. Introduction

Chagas disease is a potentially life-threatening illness caused by the protozoan parasite *Trypanosoma cruzi*. Almost 8 million people are infected worldwide, mostly in Latin America. However, due to human migration, infected patients have become increasingly detected in the USA and Canada as well as in many European and some Western Pacific countries [1].

Chagas disease presents in two phases: an acute phase and a chronic phase. Years to decades after the onset of the infection, 30% of chronically infected patients will suffer from cardiac disorders and 10% from digestive or mixed alterations [1]. Infection triggers an immune response committed to parasite control that, during the lifelong infection, should keep parasites at bay with minimal tissue damage [2]. In this scenario, it is worthwhile to find drugs that target parasite viability and also alleviate symptoms of the chronic disease [3].

Despite efforts in drug discovery, only two drugs are currently used to treat Chagas disease, namely, benznidazole and nifurtimox. Both require long treatments, display severe side effects and have controversial efficacy in chronically *T. cruzi*-infected adults [3,4]. Therefore, the need for safe and efficacious treatment alternatives against Chagas disease is renewed. Drug repurposing has emerged as an attractive approach for the development of novel therapeutics for neglected diseases. This strategy implies expanding the utility of existing drugs to other therapeutic indications [5], facilitating rapid and cost-effective drug development. On the other hand, combination treatments are increasingly recommended in chemotherapy against other parasitic diseases [6].

Recently, Bellera et al published promising results of two repurposed candidates, benidipine and clofazimine, for the treatment of Chagas disease [7]. Benidipine is a calcium channel-blocking agent currently used for the treatment of hypertension and angina pectoris [8], and clofazimine is an antibiotic mainly indicated for the treatment of leprosy [9]. They were selected in silico for their predicted activity against cruzipain, the major cysteine protease of *T. cruzi*, and their inhibitory effects were confirmed in vitro and in vivo in a murine model of acute Chagas infection [7].

* Corresponding author. Laboratory of Bioactive Compounds Research and Development (LIDeB), Medicinal Chemistry, Department of Biological Science, Exact Sciences College, National University of La Plata (UNLP), 47 & 115 (B1900AJI) La Plata, Buenos Aires, Argentina. Tel.: +54 221 423 5333 x41.
E-mail addresses: mlsbaraglini@biol.unlp.edu.ar; mariasbara@gmail.com (M.L. Sbaraglini).
[1]These two authors contributed equally to this work.

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In this work, the efficacy of benidipine and clofazimine was tested in a murine model of chronic Chagas disease infection. C3H/HeN mice infected with a myotropic *T. cruzi* strain were treated at the chronic phase. The parasite load in peripheral blood and heart and skeletal muscle as well as target tissue inflammation were assessed 60 days after the end of treatment.

2. Materials and methods

2.1. Animals and T. cruzi infection

C3H/HeN mice were bred and housed at the animal facilities of the Instituto de Microbiología y Parasitología Médica (IMPaM-UBA CONICET, Buenos Aires, Argentina). Mice were kept under standard conditions on a 12-h light : dark cycle in a temperature-controlled room (25 ± 2 °C) with food and water ad libitum. All animal procedures were approved by institutional regulations of the Committee for the Care and Use of Laboratory Animals of the Universidad de Buenos Aires (Buenos Aires, Argentina) in accordance with government regulations. All efforts were made to minimise the number of animals used and their suffering. Six-week-old male mice were infected by the intraperitoneal route with 1×10^5 bloodstream forms of the *T. cruzi* K98 strain [a Unidades Discretas de Tipificación (UDT) TcI clone of the CA-I isolate obtained from an Argentinean patient with chronic cardiomyopathy] [10].

Clinical evidence of disease was monitored and parasitaemia levels were measured by counting the number of circulating trypomastigotes from blood samples in a Neubauer chamber as described previously [11].

2.2. Pharmacologic treatment

Benidipine hydrochloride, clofazimine and benznidazole were acquired from Sigma-Aldrich (St. Louis, MO). Benidipine and benznidazole were suspended in 1% carboxymethyl cellulose (Sigma-Aldrich) and clofazimine was suspended in corn oil. Three groups of mice ($n = 4$ per group) were treated with either benznidazole 100 mg/kg/day, benidipine 15 mg/kg/day or clofazimine 30 mg/kg/day administered orally by gavage (0.1 mL) once a day during 30 consecutive days, starting 90 days post infection (dpi) with *T. cruzi*. The untreated infected control group ($n = 4$) was administered vehicle alone. Weight was recorded periodically and blood samples were obtained at different time points. Peripheral blood parasitaemia was measured by counting in a Neubauer chamber the number of parasites obtained from the tail vein blood diluted (1/10) in red blood cell lysis solution (0.83% Tris–NH_4Cl, pH 7.2). Sixty days after the end

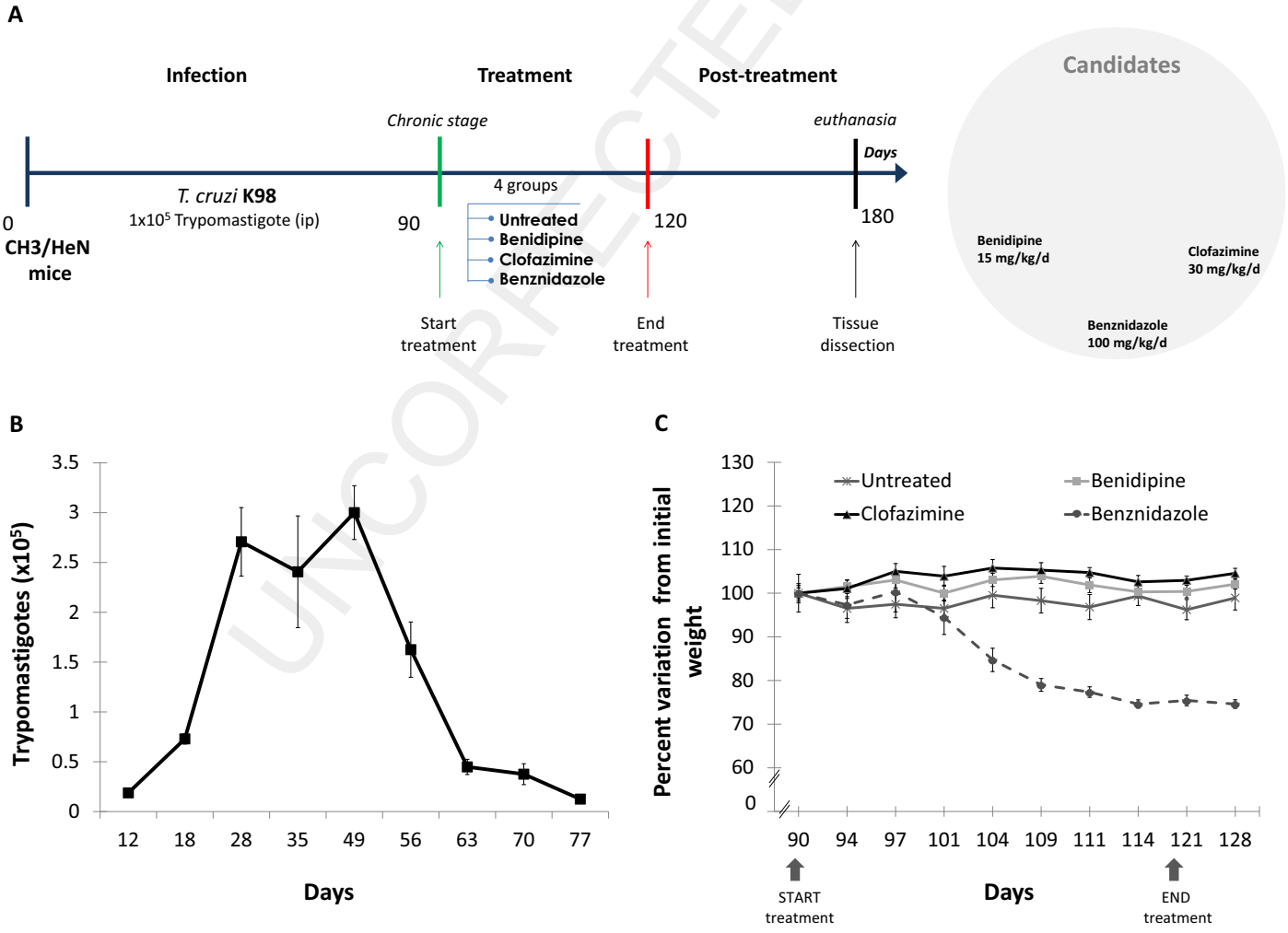

Fig. 1. Acute-phase parasitaemia levels and weight kinetics following treatment of mice chronically infected with *Trypanosoma cruzi* K98 strain. (A) Experimental design. (B) Number of bloodstream parasites quantified by conventional microscopy as described in Section 2.2. (C) Percent variation from initial weight (90 days post-infection) starting from the first day of treatment until the end of treatment. Results are shown as the mean ± standard error of the mean.

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