In vitro trypanocidal activity of DB745B and other novel arylimidamides against Trypanosoma cruzi

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Objectives: As part of a search for new therapeutic opportunities to treat chagasic patients, in vitro efficacy studies were performed to characterize the activity of five novel arylimidamides (AIAs) against Trypanosoma cruzi.

Methods: The trypanocidal effect against T. cruzi was evaluated by light microscopy through the determination of IC50 values. Cytotoxicity was determined by MTT assays against mouse cardiomyocytes.

Results: Our data demonstrated the trypanocidal efficacy of these new compounds against bloodstream trypanomastigotes and intracellular amastigotes, exhibiting IC50 values ranging from 0.015 to 2.5 and 0.02 to 0.2 μM, respectively. One of the compounds, DB745B, was also highly active against a broad panel of isolates, including those naturally resistant to benznidazole. DB745B showed higher in vitro efficacy than the reference drugs used to treat patients (benznidazole IC50 = 12.94 μM) and to prevent blood bank infection (gentian violet IC50 = 30.6 μM).

Conclusions: AIAs represent promising new chemical entities against T. cruzi and are also potential trypanocidal agents to prevent transfusion-associated Chagas’ disease.

Keywords: Chagas’ disease, chemotherapy, T. cruzi

Introduction

Chagas’ disease (CD) is a neglected disease of poor, rural and forgotten populations, representing one of the main public health problems in 22 developing countries of Latin America.1 Nifurtimox and benznidazole are recommended for all acute, early chronic and reactivated cases, but produce variable results mostly related to the endemic area. Both exhibit considerable undesirable side effects, are administered over 30 or more days and are not very effective against the late chronic phase.2,3 Another challenge is blood prophylaxis in endemic areas, since the only trypanosomicidal agent (gentian violet) has toxicity problems, gives the blood a purple colour and may stain the skin and mucosa of recipients.4

In vitro and in vivo studies have shown the promising efficacy of diamidines and congeners, mainly arylimidamides (AIAs), against Trypanosoma cruzi.2,5,6 Because recent findings also reported the pharmacological properties and biological efficacy of AIAs, such as DB745, against Leishmania in models of in vitro and in vivo infection,1 in this study the trypanocidal activity of five novel AIAs was evaluated in vitro against different strains of T. cruzi.

Methods

Drugs

All amidines (see Figure S1; available as Supplementary data at JAC Online) were synthesized according to published procedures.8 Benznidazole (LAFEPE, Brazil) and gentian violet (Sigma-Aldrich) were used as previously reported.5

Cardiac cell cultures and cytotoxicity assays

To rule out toxic effects against mammalian cells, uninfected primary cultures of embryonic cardiomyocytes (CMs) were incubated at 37°C for 24 and 72 h and LC50 values were determined by MTT colorimetric assays.5
Results

DB667, DB709, DB745B, DB749 and DB946 gave a dose-dependent trypanocidal effect against BTs (Y strain) (see Figure S2; available as Supplementary data at JAC Online). DB709, DB749 and DB946 showed significant activity against BTs and intracellular parasites, regardless of the drug resistance parasite phenotype, giving IC50 values ranging from 0.3 to 0.7 µM, and greater efficacy than gentian violet (Table 2). DB745B and DB667 tested against a broader panel of T. cruzi strains showed that both were more active than benznidazole, while DB745B was less active than DB745B (data not shown).

Discussion

A systematic lead discovery programme performed by the Consortium for Parasitic Drug Development (http://www.thecpdd.org/) demonstrated that novel AIAs such as DB745B and
DB766 are effective against Leishmania infection in vitro and in vivo, do not exhibit mutagenicity, display low acute toxicity, have moderate oral bioavailability, are distributed to different tissues such as the liver and spleen, present large volumes of distribution and have an elimination half-life ranging from 1 to 2 days in mice. As DB766 also presented potent anti-T. cruzi activity, we screened for the trypanocidal effect of five novel AIAIs, including DB745B. All AIAIs exhibited considerable activity against T. cruzi, but DB745B was the most active, even in the presence of blood constituents. The loss of activity exhibited by DB667, DB709, DB749 and DB946 may be related to their association with and/or inactivation by serum components, as reported previously.

The efficacy of DB745B in the presence of blood is a desirable characteristic shared by a few other AIAIs, such as DB766, that was noted during the evaluation of new trypanocidal agents for use in blood banks. Although the transfusion procedures that have been implemented have reduced the number of blood-related new infections, these procedures are not universally followed. Also, the only trypanocidal agent available for chemical prophylaxis of blood in areas of high endemicity is gentian violet, which is a toxic cationic dye that has several limitations.1

DB745B was more active than benznidazole (e.g. DB745B is about 860 and 90 times more effective against BTs and intracellular parasites, respectively). DB745B is effective against a large panel of strains (855, 875, M1523 and RBVIII strains are present in peridomialic and sylvatic ecotopes), including those that express natural resistance to benznidazole.10 and is more active than the diamidines DB569 and DB75, confirming previous studies that revealed the superior activity of AIAIs compared with diamidines against T. cruzi.2,6 Dose-dependent and timepoint studies demonstrated that DB745B is faster acting than DB766, requiring further studies to explore the possibility that different transport mechanisms and/or cellular targets may be operating. Our findings warrant additional in vivo studies with DB745B using acute and chronic experimental models of T. cruzi infection with the goal of identifying novel lead AIAI candidates against this parasite.

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### Transparency declarations
None to declare.

### Supplementary data
Figures S1 and S2 and Table S1 are available as Supplementary data at JAC Online (http://jac.oxfordjournals.org).

### References