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.

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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. 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 15 µM 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.

METHODS AND MATERIALS

***In Vivo* model**

Balb/C mice
8-weeks old

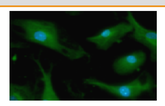
Infected with 500 parasites
RA *T. cruzi* strain

Positive Parasitaemia

Benznidazole was administered orally at 10, 25 or 100 mg/Kg/day, for 30 days.

Parasitaemia and body weight was analyzed. Heart tissue for histological studies, gDNA, RNA/cDNA, proteins extracts and serum were obteined. qPCR, RT-qPCR, Western Blot and enzymatic assays were performed.

***In Vitro* model**



Myocardiocytes : Infected with *T. cruzi (RA) or* stimulated with LPS (10 mg/L)

Benznidazole was administered at 3, 15 or 75 µM

° 30', 60' y 120' to evaluate NF-κB pathway
°° 4 h to evaluate cytokines
°°° 48 h to evaluate NOS2 y NOx
°°°° 96 h to evaluate cellular parasitism

gDNA, RNA/cDNA and proteins extracts were obteined.
qPCR, RT-qPCR, Western Blot and inmunofluorescence assays were performed.

RESULTS

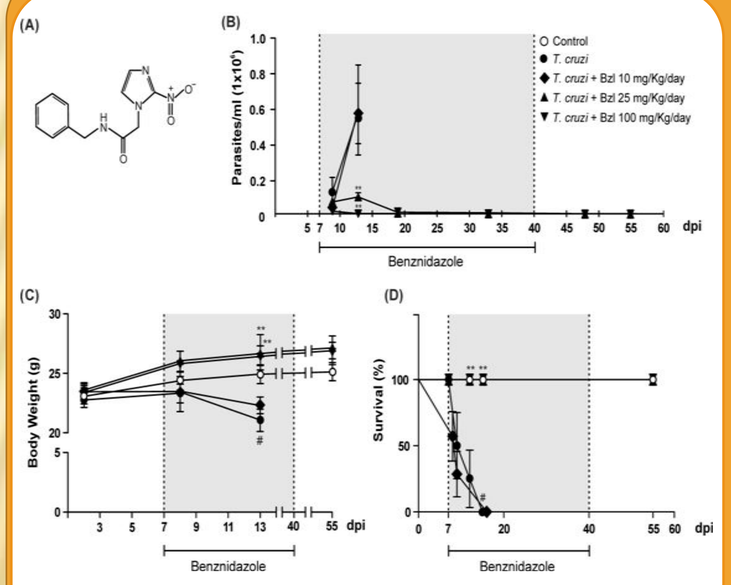
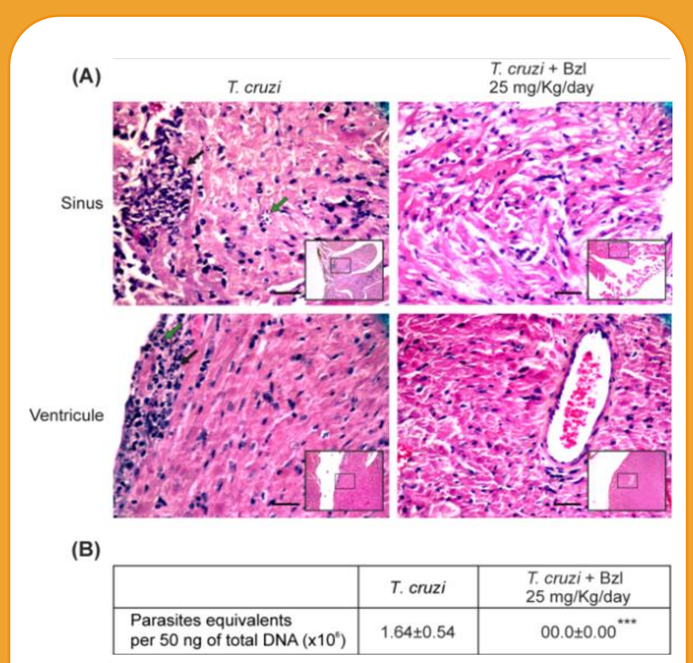


Figure 1. Benznidazole is effective as an antiparasitic drug at a dose lower than the usual one.



	T. cruzi	*T. cruzi* + Bzl 25 mg/Kg/day
Parasites equivalents per 50 ng of total DNA ($x10^6$)	1.64±0.54	00.0±0.00***

Figure 2. Benznidazole clears tissue parasitism and attenuates inflammatory reaction

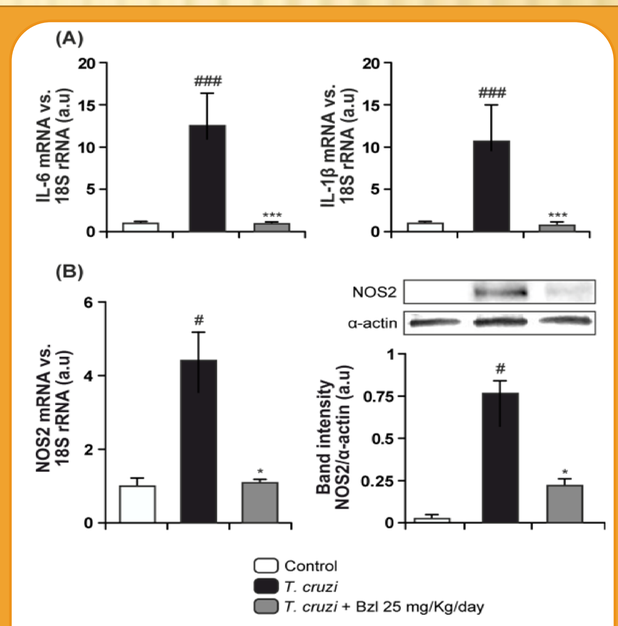


Figure 3. NOS2 and pro-inflammatory cytokines are inhibited by benznidazole in hearts of *T. cruzi*-infected mice

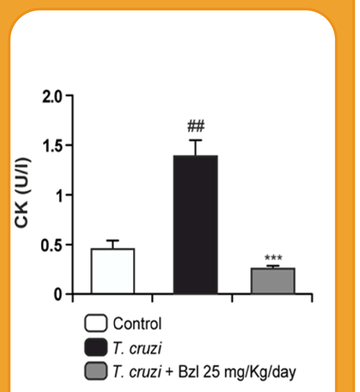


Figure 4. Benznidazole treatment normalizes creatine kinase activity in *T. cruzi*-infected mice

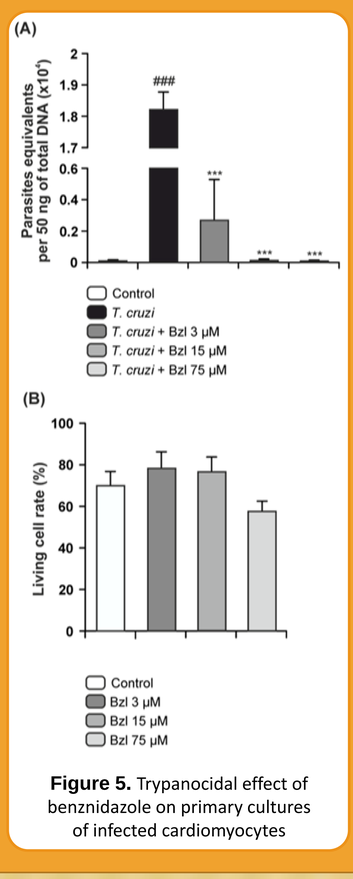


Figure 5. Trypanocidal effect of benznidazole on primary cultures of infected cardiomyocytes

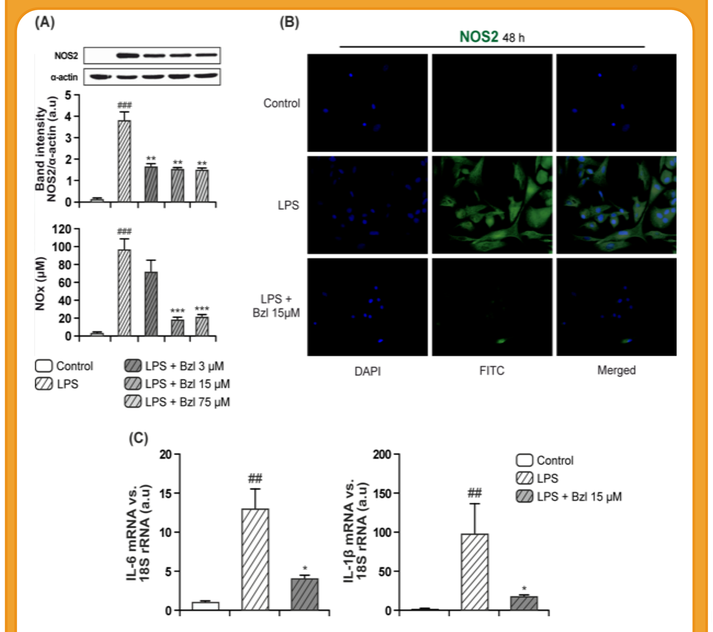


Figure 6. Benznidazole inhibits inflammatory mediators in cultured cardiomyocytes

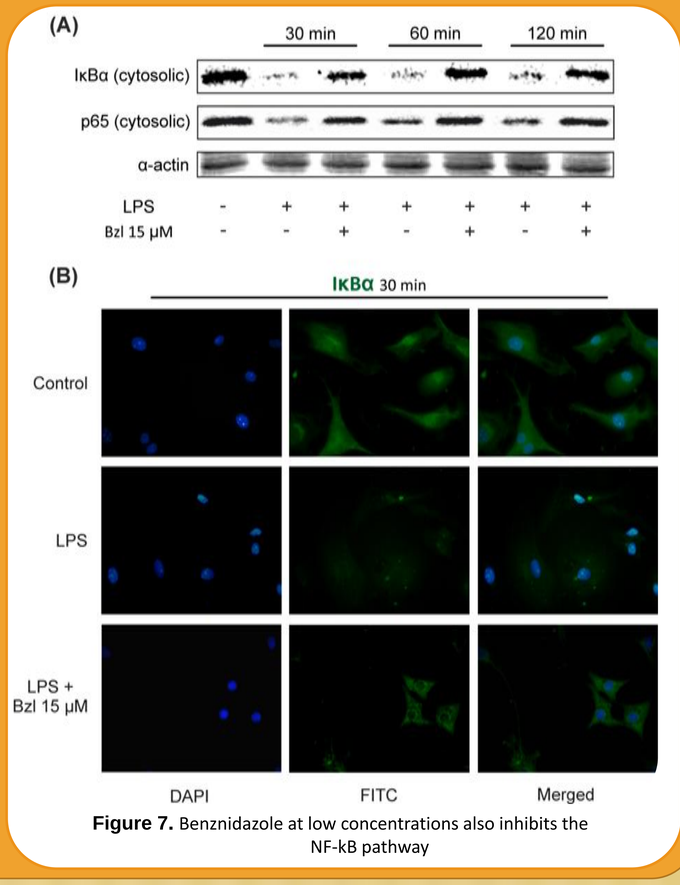


Figure 7. Benznidazole at low concentrations also inhibits the NF-kB pathway

CONTACT

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CONCLUSIONS

In conclusion, this study showed, for the first time, that optimal effects of Bzl can be achieved at doses significantly lower than those usually used to cure experimental Chagas' disease. This may be a relevant finding for dose optimization in the treatment of acute as well as chronic asymptomatic human Chagas' disease. This is especially true if one considers the number and varied severity of adverse effects generated by the use of Bzl, which lead to the abandonment of treatment by a significant proportion of patients.