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Abstract

Background: Benznidazole is an effective drug in the trypanocidal treatment of acute and chronic indeterminate Chagas disease. However, adverse drug reactions (ADR) are common and frequently cause patients to discontinue treatment.

Objectives: We hypothesized that antioxidant supplementation could mitigate benznidazole-induced toxicity.

Methods: We co-supplemented an adult traveler with chronic indeterminate Chagas disease who experienced benznidazole ADR with ascorbic acid (AA), 1000 mg/day. We measured selected serum biomarkers of oxidative stress (TAS, TOS, Nrf2, MDA, GPx3, CAT, T-SOD) at timepoints before and throughout benznidazole treatment, and after AA co-supplementation.

Results: AA co-supplementation effectively mitigated benznidazole-induced ADR during the etiologic treatment of chronic indeterminate CD. The kinetics of serum biomarkers of oxidative stress suggested significantly decreased oxidative insult in our patient.

Conclusions: We hypothesize that the key pathophysiological mechanism to benznidazole-associated toxicity is oxidative stress, rather than hypersensitivity. AA co-supplementation may improve adherence to benznidazole treatment of chronic indeterminate (or acute) Chagas disease. Oxidative stress biomarkers have potential to guide the clinical management of Chagas disease. Prospective studies are needed to establish the benefit of antioxidant co-supplementation to benznidazole treatment of Chagas disease in reducing benznidazole toxicity, parasite clearance and the prevention of end-organ damage.

Introduction

Chagas disease (CD) is caused by infection with the protozoon *Trypanosoma cruzi*. [1] In non-endemic countries, CD is an emerging concern because of migration of infected individuals from endemic areas, transfusion-transmitted infections, organ transplantation and mother-to-child transmission. [1] After the acute phase, chronic asymptomatic infection persists. [1] Approximately one-third of these indeterminate CD cases will eventually develop cardiomyopathy, gastro-intestinal or neurological disease. [1]

The 2019 PAHO guidelines recommend benznidazole (or nifurtimox) as first-line treatment for patients with acute or chronic indeterminate CD. [2] Treatment effectively reduces the number of circulating parasites but it does not prevent disease progression in patients with established heart disease. [3] The recommended daily dose for adults is 5-7 mg/kg PO q12h, for 60 days. Adverse drug reactions (ADR) affect up to 86% of patients, [4] and cause 15-20% to discontinue treatment. [5,6] The most common ADR include nausea, vomiting, allergic dermatitis, peripheral polyneuropathy and myelotoxicity. [6] Strategies to reduce benznidazole toxicity include co-administration of corticosteroids and anti-histaminics, lower dosing and shorter treatment duration. [7–9]

We report on the mitigation of benznidazole toxicity after ascorbic acid (AA) supplementation in a traveler with chronic indeterminate CD. We retrospectively measured serum markers of oxidative stress (OS) in this patient.

Case description and Methods

In October 2020, a 38-year-old Belgian woman who had been a regular blood donor was identified at risk for CD at the Institute of Tropical Medicine in Antwerp, Belgium. She reported multiple stays in Mexico, Brazil, and Peru between 1995 and 2019, that included camping in forested areas. She had a positive ELISA result (ratio 1.50) in an assay targeting recombinant *T. cruzi* antigens (Bioelisa Chagas, Biokit). Antibody detection using an ELISA that targets trypomastigote extract, [10] as well as indirect immune-fluorescence antibody-testing (Chagas IFA, Vircell Microbiologists) was negative, but a

positive PCR-result was obtained in an assay that uses a *T. cruzi* specific primer set (Tcz1–Tcz2) and detection of the amplicons by gel electrophoresis (for details, see the Supplementary Material). [11]

The patient did not recall any acute travel-associated illness. Her past medical history was unremarkable. Her physical examination was normal, she had a body weight of 66 kg. During workup no cardiac or digestive complications of CD were detected.

She was treated with benznidazole (Abarax®, Laboratorio Elea) at 7.6 mg/kg daily in two doses (200 mg and 300 mg). After nine days, she developed a generalized macular, erythematous, pruritic rash, acral edema and painful peripheral neuropathy. The dose was reduced to 4.5 mg/kg (100 mg and 200 mg). However, the symptoms persisted. She had elevated liver enzymes (alanine transaminase (ALT) 157 U/L [< 35]; aspartate transaminase (AST) 108 U/L [14 – 36]) (Table 1). After discontinuation of benznidazole the rash and edema disappeared, and the transaminase levels normalized. After two weeks, benznidazole (4.5 mg/kg) with concomitant administration of prednisolone 8 mg and cetirizine 10 mg daily was attempted. Rash and swelling of hands and feet reappeared the same day and became intolerable after 5 days (see Figure 1S in Supplementary Material).

We then prescribed L-ascorbic acid 1000 mg by mouth, once daily. The patient reported a spectacular alleviation of symptoms; the edema disappeared within an hour and the rash disappeared over the following days. Transaminase levels remained within normal range. Prednisolone and cetirizine were discontinued, and she completed a 60-day course of benznidazole supplemented by daily AA without recurrence of ADR. Two months after completion of treatment a real-time PCR result for *T. cruzi* was negative. [12]

We retrospectively determined the levels of selected markers of OS *i.e.,* an imbalance between the generation of reactive oxygen species (ROS) and antioxidant defenses. Measurements were done in duplicate in serum samples obtained before treatment (Sample A), 4 days after benznidazole dose reduction to 4.5 mg/kg (Sample B), and 2 weeks before completing treatment with benznidazole and ascorbic acid (Sample C) (see Supplementary Material). The samples had been stored at -80°C until analysis.

Briefly, total oxidative status (TOS) and total antioxidant status (TAS) levels were measured spectrophotometrically (Rel Assay Diagnostics). The Oxidative Stress Index (OSI) values, defined as the ratio of TOS to TAS, were calculated. Extracellular glutathione peroxidase (GPx3; AdipoGen Life Sciences), nuclear factor erythroid 2-related factor 2 (Nrf2; MyBioSource) and Malondialdehyde (MDA; MyBioSource) were measured using ELISA. The catalase activity (CAT; Caymanchem) and total superoxide dismutase (T-SOD; Elabscience) activity was determined by colorimetric analysis. OS markers in Samples B and C were compared with pretreatment values and values obtained in healthy controls (HC) (Table 1 and Figure 1).

We measured 2-fold higher (p=0.21) TOS and 0.5-fold lower TAS (p=0.005) in the serum of our patient (before treatment) than in HC sera, indicative of increased OS in *T. cruzi* infection. Compared to HC, TAS remained 0.5 to 0.6-fold lower during benznidazole treatment and after AA co-supplementation. In line with this observation, we measured significantly reduced circulatory concentrations of Nrf2, an important mediator of antioxidant signaling during inflammation. Some of its downstream targets, antioxidant enzymes GPx3, CAT and T-SOD, were also reduced compared to HC throughout the reported clinical course. Following a significant, 9.7-fold increase of TOS (Sample B), the OSI increased from 8.8 to 41 during benznidazole treatment. After AA co-supplementation (sample C), TOS and the OSI (6.3) decreased to the pre-treatment range. Serum MDA concentrations, a marker of lipid peroxidation, were elevated at the time of diagnosis and continued to increase to reach 3.6-fold levels compared to HC towards the end of treatment.

Discussion

Dose reduction and anti-allergic treatment did not alleviate ADR to benznidazole in our patient with indeterminate CD. AA supplementation for the duration of treatment was followed by prompt and sustained relief of the benznidazole-induced dermatitis, acral edema, peripheral neuropathy, and toxic hepatitis. Our patient was able to complete a 60-day benznidazole course.

ADR to benznidazole have often been attributed to hypersensitivity reactions. [6] In our patient, the absence of a clinical response to corticosteroids and the rapid relief following administration of AA suggest that the ADR were not mediated by allergic reactions, but by OS.

After AA supplementation, TOS and TAS levels of our patient were in the pre-treatment range, although the OSI remained higher than in HC. Circulatory Nrf2 concentrations were low. Studies in HeLa cells and AC16 human cell lines have shown that the initial Nrf2 activation in response to *T. cruzi* infection is not sustained and that, to the benefit of intracellular parasitic reproduction, Nrf2 levels decline. [13] Also, exogenous expression of Nrf2 or treatment with several antioxidants, including Nrf2 activators, was found to reduce parasite burden in macrophages. [14]

Antioxidant enzyme concentrations of GPX3 and activities of CAT and T-SOD remained low as well and were not restored by AA supplementation. Reduced antioxidant enzyme concentrations could result from an exhausted adaptive response to OS.

Both *T. cruzi* infection and benznidazole treatment of CD are associated with oxidative stress. [13,14] Evidence from murine experimental studies suggests that persistent OS is an important factor contributing to Chagas cardiomyopathy. [15,17] Supplementation with antioxidants vitamins E and C after benznidazole treatment attenuated cardiac dysfunction in humans with established Chagas' heart disease. [17-19] The trypanocidal effects of benznidazole occur in an oxygen insensitive fashion and are not mediated by drug-induced OS. [15]

Best known for its potent antioxidant action, AA can induce ROS in the presence of electron donors. [20] Puente *et al.* observed an antiparasitic effect of AA *in vitro* and in mice, which they attributed to a lethal pro-oxidant effect of AA on *T. cruzi*. [21] In combination with benznidazole, AA did not reduce the trypanocidal activity on trypomastigotes, but the cytotoxicity of benznidazole was mitigated. [21] Another murine experimental study demonstrated that combined benznidazole and AA treatment reduced *T. cruzi* parasitemia more effectively than either compound alone. [22]

Benznidazole alone, but not in combination with AA, increased intracellular ROS and lipid

peroxidation in cardiac tissue. Combined treatment reduced cardiac parasite loads and inflammatory infiltrates, and prevented elevation of transaminase levels. [22]

Cited evidence informed our decision to empirically administer AA to our patient. The ensuing clinical improvement exceeded our expectations, and the pattern of OS parameters offers a plausible conceptual explanation. Yet, important limitations apply. First, single biomarkers of OS do not exist and various optimized and validated assays are required to measure OS *in vivo*. [23] Our hypothesis should ideally be tested in a panel of markers that covers all aspects of *in vivo* oxidative damage to lipids, proteins, and DNA. Second, assessment of OS biomarkers is subject to preanalytical variation resulting from differences in collection, storage, and processing of samples. For our case, the duration of storage differed for the samples we analyzed. Finally, we acknowledge the limitations of a single case report, and we caution against overinterpretation of our findings.

Conclusions

This case study suggests that AA supplementation effectively mitigates benznidazole-induced oxidative insult during the etiologic treatment of chronic indeterminate CD. Our observations challenge the hypothesis that hypersensitivity is the central pathophysiological mechanism to benznidazole-associated toxicity. [5] In addition, the potential of OS markers as biomarkers to guide CD treatment warrants further investigation. Prospective studies should establish the benefit of AA supplementation to benznidazole treatment of chronic indeterminate CD in reducing benznidazole toxicity, parasite clearance and most importantly, the prevention of end-organ damage of *T. cruzi* infection.

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Tess De Bruyne: Formal analysis, Investigation, Methodology, Writing – review & editing, Data curation; Nina Hermans: Methodology, Supervision, Visualization, Writing – review & editing, Funding acquisition, Project administration; Ralph Huits: Formal analysis, Investigation, Methodology, Supervision, Visualization, Writing – original draft, Writing – review & editing, Data curation, Funding acquisition, Project administration

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Table 1. Levels of oxidative stress markers and transaminases in an indeterminate CD patient before and after treatment with benznidazole, and after ascorbic acid co-supplementation compared to healthy controls (n=9).

marker		units	HC (SD)/ Reference range	Sample A (SD)	p-value *	Sample B (SD)	p-value *	Sample C (SD)	p-value *
(OX)	**TOS**	(µmol H2O2 equiv./L)	4.0 (0.7)	8.8 (2.3)	0.21	38.7 (0.2)	<0.001 ***	7.5 (0.4)	<0.001 ***
(AO)	**TAS**	(mmol Trolox equiv./L)	2.0 (0.7)	1.0 (0.1)	0.005 **	0.9 (0.02)	0.003 **	1.2 (0.1)	0.015 *
index	**OSI**	-	2.0	8.8	-	41	-	6.3	-
(AO)	**GPx3**	ng/mL	9882.3 (1445.3)	1311.0 (60.3)	<0.001 ***	1817.9 (260.5)	<0.001 ***	3699.0 (138.4)	<0.001 ***
(AO)	**CAT**	nmol/min/mL	47.1 (18.3)	7.5 (3.7)	0.017 *	19.0 (6.0)	0.069	11.1 (3.4)	0.026 *
(AO)	**T-SOD**	U/mL	87.4 (13.6)	42.1 (4.0)	0.002 **	46.8 (2.7)	0.003 **	46.1 (2.0)	0.003 **
(AO)	**Nrf2**	(pg/mL)	914.8 (587.9)	339.2 (28.0)	0.019 *	328.8 (18.5)	0.017 *	198.5 (0)	0.006 **
(OX)	**MDA**	(ng/mL)	219.5 (108.9)	512.6 (26.4)	0.005 **	548.4 (0)	0.003 **	787.8 (84.7)	<0.001 ***
Liver enzyme	**ALT**	U/L	[< 35]	14	-	157	-	28	-
Liver enzyme	**AST**	U/L	[14-36]	25	-	108	-	29	-

Table 1. (Legend) Sample A: before benznidazole treatment; Sample B: 4 days after benznidazole dose reduction to 4.5 mg/kg; Sample C: treatment with benznidazole and ascorbic acid. TOS = total oxidative status, expressed in micromolar hydrogen peroxide equivalent to oxidize ferrous to ferric ion per liter, TAS = total antioxidant status expressed in millimolar Trolox equivalent to reduce the stable radical cation 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical to colourless ABTS, OSI = Oxidative Stress Index (TOS/TAS ratio), GPx3 = extracellular glutathione peroxidase, CAT = catalase activity, T-SOD = total superoxide dismutase, Nrf2 = nuclear factor erythroid 2-related factor 2, MDA = malondialdehyde, ALT = alanine transaminase, AST = aspartate transaminase, OX = oxidant, AO = antioxidant. HC = mean value of duplicate measurements in healthy controls (n=9). FC = fold-change compared to HC. SD = standard deviation.

* A T-test was used to analyze the difference between the mean values observed in the patient's samples and in HC (under the assumption of a normal distribution). P-values <0.05 were considered statistically significant (p<0.05 *, p<0.01 **, p<0.001 ***).

Figure 1. Fold Change relative to values in healthy controls of oxidative stress parameters in serum of a patient with chronic indeterminate Chagas disease

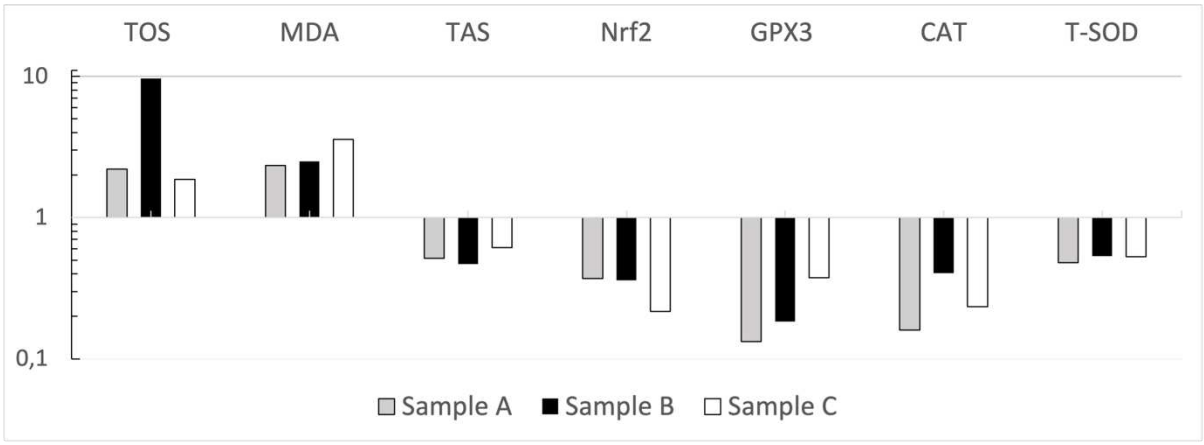

Figure 1. (Legend) The Y-axis (logarithmic scale) represents the Fold Change of oxidative stress parameters in serum of a patient with chronic indeterminate Chagas disease, relative to values measured in healthy controls (n= 9). TOS = total oxidative status, MDA = malondialdehyde, TAS = total antioxidant status, Nrf2 = nuclear factor erythroid 2-related factor 2, GPx3 = extracellular glutathione peroxidase, CAT = catalase, T-SOD = total superoxide dismutase. The oxidative stress parameters were measured in sera obtained two months before benznidazole treatment (Sample A), during benznidazole treatment (Sample B), and 6 weeks after co-supplementation of ascorbic acid (Sample C).