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1,4-Diamino-2-butanone, a putrescine analogue, promotes redox imbalance in *Trypanosoma cruzi* and mammalian cells

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ABSTRACT

The putrescine analogue 1,4-diamino-2-butanone (DAB) is highly toxic to various microorganisms, including *Trypanosoma cruzi*. Similar to other α-aminocarbonyl metabolites, DAB exhibits pro-oxidant properties. DAB undergoes metal-catalyzed oxidation yielding H_2O_2, NH_4^+ ion, and a highly toxic α-oxo-aldehyde. *In vitro*, DAB decreases mammalian cell viability associated with changes in redox balance. Here, we aim to clarify the DAB pro-oxidant effects on trypomastigotes and on intracellular *T. cruzi* amastigotes. DAB (0.05–5 mM) exposure in trypomastigotes, the infective stage of *T. cruzi*, leads to a decline in parasite viability (IC_{50} *c.a.* 0.2 mM DAB; 4 h incubation), changes in morphology, thiol redox imbalance, and increased TcSOD activity. Medium supplementation with catalase (2.5 μM) protects trypomastigotes against DAB toxicity, while host cell invasion by trypomastigotes is hampered by DAB. Additionally, intracellular amastigotes are susceptible to DAB toxicity. Furthermore, pre-treatment with 100–500 μM buthionine sulfoximine (BSO) of LLC-MK2 potentiates DAB cytotoxicity, whereas 5 mM *N*-acetyl-cysteine (NAC) protects cells from oxidative stress. Together, these data support the hypothesis that redox imbalance contributes to DAB cytotoxicity in both *T. cruzi* and mammalian host cells.

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Introduction

α-Aminocarbonyl metabolites such as 5-aminolevulinic acid [1,2] and aminoacetone [3,4], as well as 1,4-diamino-2-butanone (DAB)[1] [5], a large spectrum microbicide, exhibit pro-oxidant properties. These compounds were shown to undergo phosphate-catalyzed enolization and subsequent aerobic oxidation propagated by superoxide ions yielding oxyradicals and a highly cytotoxic α-oxoaldehyde [6], leading to oxidative damage of proteins, lipids, and DNA [7–10].

DAB is reported to be a potent microbicide to a variety of pathogenic microorganisms, including *Trypanosoma cruzi* [11], *Leishmania amazonensis* [12], *Giardia lamblia* [13], *Entamoeba invadens* [14], *Trichomonas foetus* [15] and *Candida albicans* [16]. The microbicidal mechanism of DAB was first attributed to competitive inhibition of ornithine decarboxylase (ODC) [17], the key enzyme in polyamine biosynthesis [18]. More recently, we reported that DAB cytotoxicity in mammalian cells is related to redox imbalance [5]. Notably, *T. cruzi* is susceptible to DAB toxicity [11] even though it lacks a functional ODC gene [19].

T. cruzi is the etiological agent of Chagas disease and can be found as free forms in the mammalian host as trypomastigotes circulating in the blood, a flagellate form characteristically found in the acute phase of the disease, and as amastigotes in the cytoplasm of the host cells, a non-flagellate and dividing stage. Chagas disease is recognized by the WHO as a neglected tropical disease, with 15–16 million people infected in Latin America and another 75–90 million at risk of infection [20]. The current therapeutic drugs available are benznidazole and nifurtimox, discovered more than three decades ago, that have limited efficacy for chronic treatment and elicit undesirable side-effects [21,22]. In the past few decades, a variety of trypanocidal compounds have been screened based on natural or synthetic cyclic compounds with well-known biological properties, such as antiproliferative, microbicidal or parasiticidal activities [23,24]. More recently, metabolic biochemical pathways and specific enzymes have been explored as targets for drug development, including cruzipain, a cysteine proteinase, which plays a crucial role in all stages of the parasite life cycle [25], and sterol C14 α-demethylase (CYP51), an enzyme essential for sterol biosynthesis, which is potentially inhibited by posaconazole and has

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[1] *Abbreviations used:* BSO, L-buthionine-(*S*,*R*)-sulfoximine; DAB, 1,4-diamino-2-butanone; DAPI, 4′,6-diamidino-2-phenylindole dichloride; DCFH-DA, 2′,7′-dichlorofluorescein diacetate; MEM, modified Eagle's médium; mBBr, monobromobimane; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NAC, *N*-acetyl-cysteine; NBT, nitroblue tetrazolium; ODC, ornithine decarboxilase; oxoDAB, 4-amino-2-oxobutanal; PFA, paraformaldehyde; PMS, phenazinemetasulphate; ROS, reactive oxygen species; TcSOD, *Trypanosoma cruzi* superoxide dismutase.

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already been tested *in vitro* and in animal models [26,27]. Despite intensive effort for the development of new drugs to treat Chagas disease, challenges still exist due to high costs, the limited efficacy of these treatments during the chronic stage of the disease, and the lack of practical protocols to determine a successful treatment in humans [28,29].

We previously elucidated that the chemical mechanism of DAB oxidation to 4-amino-2-oxobutanal (oxoDAB), a reactive α-oxoaldehyde, NH_4^+ ion, and H_2O_2 is mediated by oxygen- and carbon-centered radicals [5]. Additionally, we demonstrated the molecular mechanisms underlying the toxic, pro-oxidant effects of DAB in mammalian cell cultures. Here, we extend these studies to clarify the molecular bases of DAB-induced ultra-structural alterations and death of *T. cruzi* in both cultured trypomastigote cells and monkey kidney epithelial LLC-MK2 cells infected with the amastigote form of *T. cruzi*.

Materials and methods

Reagents and cell cultures

A 100 mM stock solution of 1,4-diamino-2-butanone (DAB) (Sigma, St. Louis, MO) was prepared fresh in ultra-pure distilled water. Stock solutions of catalase, putrescine, aminoguanidine, *N*-acetylcysteine (NAC). All cell treatments were performed in phenol red-free modified Eagle's medium (MEM) (Vitrocell, Campinas, SP, Brazil) supplemented with 2% fetal bovine serum (FBS) (Cultilab, Campinas, SP, Brazil) at 37 °C and in an atmosphere of 5% CO_2.

LLC-MK2 cell cultures

LLC-MK2, a monkey kidney epithelial cell line, was maintained in MEM (Invitrogen, Carlsbad, CA) supplemented with 10% FBS (Cultilab) at 37 °C and in an atmosphere of 5% CO_2. For cell viability assays, LLC-MK2 cells were seeded in a 24-well plate at a density of 5×10^4 cells/well 24 h prior to treatments. Cell viability was determined using the classic MTT method (Sigma) that determines mitochondrial activity; the formazan generated was measured at 570 nm. Cell viability was expressed as percentage of formazan generated, using the control as a reference.

Trypomastigotes

Trypomastigotes of *T. cruzi* (Y strain) were obtained from LLC-MK2 cells 5–6 days post infection with the parasite, as previously described [30]. Parasites (1×10^7/mL) were treated with varying concentrations of DAB for different lengths of time. After the incubations, the motility of trypomastigotes was quantified by observation under light microscopy using a Neubauer chamber. Parasite viability was measured by a colorimetric assay using WST-1 (Roche, Indianapolis, IN) according to the manufacturer's protocol and expressed as the percentage of reduced WST-1, measured at 450 nm compared to the control.

To study the effects of DAB on the parasite invasion process, trypomastigotes (1×10^7/mL) were incubated with different concentrations of DAB for one hour in the presence or absence of 2.5 μM catalase or 5 mM NAC in phenol red-free medium. After washing with PBS, the parasites were resuspended in fresh medium and added to previously seeded LLC-MK2 cells (1:100 host cell to parasite ratio; 5×10^4/13 mm cover slips in 24-well plates). After 4 h incubation, free parasites were removed by washing the plates with PBS and the cells were cultured for an additional 48 h. The cells were then fixed with 4% paraformaldehyde (PFA) for 15 min and the population of intracellular parasites was estimated by randomly counting nuclei stained with 20 μg/mL 4′,6-diamidino-2-phenylindole dichloride (DAPI) (Invitrogen, Carlsbad, CA) using a Nikon Eclipse E600 epifluorescence microscope. At least 100 host cells were counted per field and 4 fields were analyzed per cover slip.

Intracellular amastigotes

To analyze the effect of DAB on intracellular amastigotes, LLC-MK2 cultured cells (5×10^4/13 mm cover slips in 24well plates) were incubated with trypomastigotes (1:100 cell to parasite ratio) for 4 h. Free parasites were removed by washing the cell culture with PBS and the cells transferred to fresh medium for 24 h. Under these conditions, the infected cells contain amastigotes, the intracellular stage of *T. cruzi*. The infected cells were then treated with different concentrations of DAB for 6 or 24 h. Subsequently, cell cultures were fixed with 4% PFA for 15 min, and the amount of intracellular parasites was estimated by randomly counting the nuclei stained by 20 μg/mL DAPI (Invitrogen). At least 100 host cells were counted per field, and 4 fields were analyzed per cover slip.

Assay for thiols

Total thiols were estimated with monobromobimane (mBBr), which produces a stable fluorescent adduct [30,31]. The cells were incubated with 0.8 mM mBBr in PBS for 10 min at room temperature in the absence of light. After centrifugation at 5000*g* for 5 min, the cell pellet was rinsed twice with PBS and incubated with lysis buffer (50 mM Tris at pH 8.0 containing 150 mM NaCl, 0.5% Triton X-100 and a protease inhibitor cocktail). After 30 min at 4 °C, the material was centrifuged at 14,000*g* for 10 min at 4 °C in an Eppendorf 5417F microcentrifuge (Hamburg, Germany). The supernatant was used to determine total thiols. The fluorescence of the bimane-adducts was measured using a SpectraMax multiwell plate reader (Molecular Devices) at $\lambda_{exc} = 394$ nm and $\lambda_{em} = 480$ nm. Protein concentrations were used to normalize the results.

Superoxide dismutase activity

The *T. cruzi* activity of superoxide dismutase (TcSOD) was determined according to the microplate method described by Ewing and Janero [32]. Trypomastigote cell pellets were re-suspended in a 50 mM phosphate buffer at pH 7.4 containing 0.10 M EDTA and a protease inhibitor cocktail and subjected to ultrasonic disruption in an ice bath for 15 min. The suspension was separated by centrifugation at 14,000*g* for 10 min at 4 °C. The supernatant was mixed with a freshly prepared solution of 320 μM NBT and 100 μM NADH in a 50 mM phosphate buffer at pH 7.4 containing 0.10 M EDTA. The reaction was initiated by the addition of 16 μM phenazine-metasulphate (PMS) in a pH 7.4 phosphate buffer containing 0.10 M EDTA. NBT reduction was monitored at 560 nm for 2 min at 25 °C using the SpectraMax multiwell plate reader. The activity of SOD was expressed in units of SOD/mg protein, where one unit is the amount of SOD required to give 50% maximal inhibition of the initial rate of NBT reduction.

Statistical analysis

Data represent at least 3 independent experiments expressed as means ± SD. Student's *t*-test was used for statistical analysis with a significance level of $p < 0.05$.

Results

Microbicidal activity of DAB in T. cruzi trypomastigotes

DAB cytotoxicity in trypomastigotes was first evaluated by determining parasite motility and conducting a mitochondrial

dehydrogenase activity-based viability assay (Fig. 1A and B). The observed decrease in parasite motility was associated with DAB concentration and length of incubation. After a 2 h incubation, high concentrations of DAB (0.5 or 1.0 mM) impaired parasite motility, which was determined by counting the number of motile parasites. In contrast, incubation with lower concentrations of DAB (0.05 or 0.1 mM) for 4 h affected only 45% of the parasite population. Four h incubation was chosen after a preliminary screening test to determine the DAB IC_{50} for trypomastigotes (data not shown). Under DAB treatment *T. cruzi* suffers morphological distortions and the population of parasite round forms increases in a concentration and time dependent fashion. The parasites were treated with different concentrations of DAB (0.05–5.0 mM) and viability was evaluated by a colorimetric assay using WST-1. As expected, trypomastigote viability was lessened after DAB exposure in a dose-dependent manner, resulting in a DAB IC_{50} of approximately 0.2 mM (Fig. 1B). Although DAB-treated parasites lost motility in the first 2 h of incubation, reduction in cell viability was detected only after longer incubations (approximately 4 h).

We further investigated the role of oxidative stress on DAB cytotoxicity in trypomastigotes by evaluating the levels of total thiols and activity of TcSOD (Fig. 2). The observed decline of thiols suggests that DAB elicited changes in the parasite redox balance. Specifically, the treatment of trypomastigotes with 0.10–1.0 mM DAB leads to a 52% decrease of thiols after one hour incubation (Fig. 2A). In parallel, the parasites responded to the oxidative assault of DAB exposure by increasing TcSOD activity, which was dependent on both DAB concentration (0.2 or 0.5 mM) and

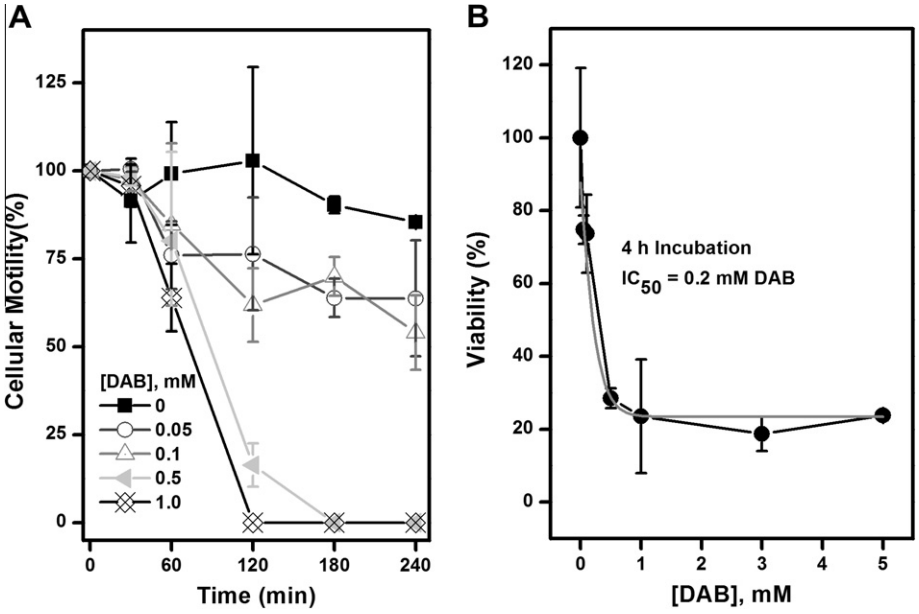

Fig. 1. The motility and viability of trypomastigotes from *T. cruzi* exposed to DAB. Parasites (1×10^7/mL) were treated with different concentrations of DAB in phenol red-free MEM supplemented with 2% FBS at 37 °C and under 5% CO_2. The motility was determined by direct quantification of motile parasites using light microscopy (A). The viability of trypomastigotes was measured by a colorimetric assay using WST-1 (B).

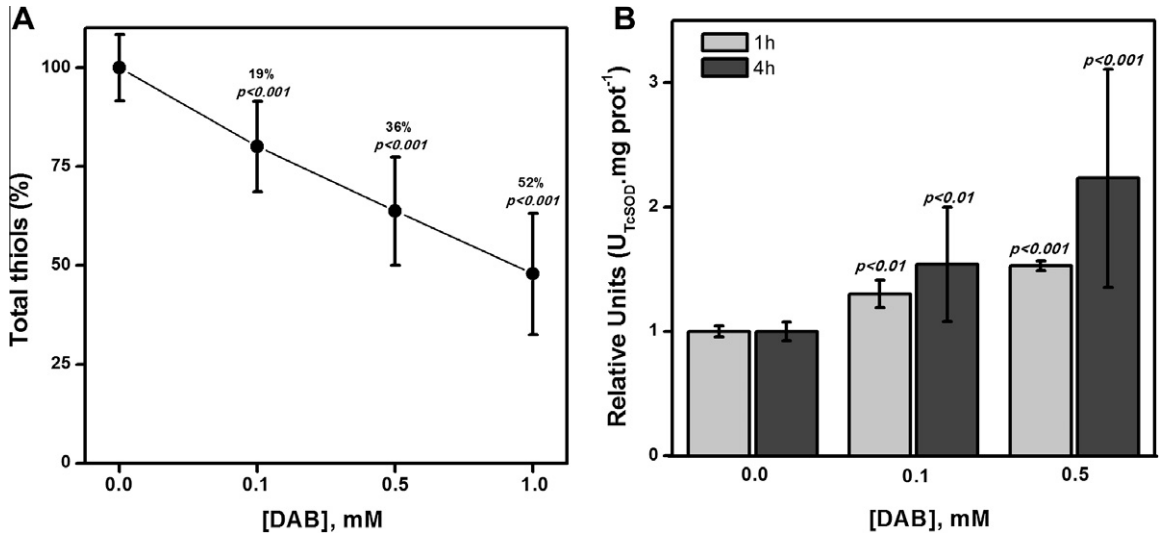

Fig. 2. Trypomastigote TcSOD activity and thiol levels in response to DAB treatment. Parasites (1×10^7/mL) were treated with different concentrations of DAB in phenol red-free MEM supplemented with 2% FBS at 37 °C and under 5% CO_2. The total reduced thiol groups were determined by a reaction with mBBr after parasites were exposed to DAB for 1 h (A). TcSOD activity was determined using the NBT/PMS method after 1 or 4 h of treatment (B). Student's t-test *p-values* for the comparisons between control and DAB-treated samples are shown.

Table 1
The effects of catalase, putrescine and aminoguanidine on DAB toxicity in trypomastigotes.

	Viability (%)	
	0.2 mM	0.5 mM
Control	54 ± 4.6	36 ± 5.6
Catalase (2.5 μM)	84 ± 7.1*	76 ± 9.2*
Putrescine (5 mM)	60 ± 5.9	45 ± 7.7
Aminoguanidine (25 mM)	65 ± 11.1	36 ± 3.8

Parasites (1×10^7/mL) were incubated for 1 h with DAB in phenol red-free MEM supplemented with 2% FBS. Viability was measured by a colorimetric assay using WST-1. *According Student's t-test ($p < 0.01$), the samples are significantly different from control.

incubation time (1 or 4 h) (Fig. 2B). In addition, viability assays in the presence of catalase (2.5 μM) exerted a protective effect against DAB toxicity (Table 1) whereas a trend for protection was attained with putrescine (5 mM), a polyamine precursor for *T. cruzi*, or aminoguanidine (25 mM), an α-oxoaldehyde Schiff scavenger of compounds such oxoDAB. This experiment supports the hypothesis that DAB cytotoxicity may be attributed to oxidative stress.

DAB impairs host cell invasion by trypomastigotes and growth of intracellular amastigotes

Because DAB dramatically affected trypomastigote viability and catalase exerted partial protection, we decided to investigate whether DAB-mediated redox imbalance in trypomastigotes could affect host cell invasion. Trypomastigotes were incubated with different concentrations of DAB (0.1, 0.2 and 0.5 mM) for 1 h before parasite addition to seeded mammalian cells. After 48 h, the infection was evaluated by measuring the population of amastigotes, the intracellular parasite stage. A dose-dependent decrease in the infection was observed with increasing DAB concentrations, reaching 40%, 60% and 85% less amastigotes per host cell after 0.5, 0.2 and 0.1 mM DAB exposure, respectively, as compared to untreated parasites (Fig 3A). To elucidate the oxidative role of DAB on trypomastigotes, reflected by their lower ability to invade host cells, parasites were treated with DAB (0.2 mM) in the presence of catalase (2.5 μM) or NAC (5 mM) (Fig. 3B). As expected, catalase supplementation partially protected the parasites against the effects of DAB, resulting in an approximately 25% lower infection than that of the control and a 30% higher than DAB-treated parasites in the absence of catalase. Conversely, NAC exhibited no protection against DAB toxicity. Light micrographs illustrate these data (Fig. 3C).

Additionally, the effect of DAB on the intracellular stages of *T. cruzi* was studied because the internalization of *T. cruzi* in mammalian host cells is critical for parasite survival. Cultured cells were infected with the parasite and after 24 h were treated with different concentrations of DAB (0.1, 0.5 and 1.0 mM) for either 6 or 24 h (Fig. 4A). Significant dose and time-dependent trypanocidal activity was observed after DAB treatment. For example, the number of intracellular amastigotes was abated to 60% of the original population when infected cells were treated with 0.5 mM DAB for 24 h. Although no significant differences were observed in the amount of intracellular parasites after the 6 or 24 h DAB-incubation, the morphology of the cultured cells was significantly altered after the 24 h incubation. This observation was supported by the dramatic decrease in infected cell population and altered structure observed in the cells treated with 1.0 mM DAB (Fig. 4B).

DAB promotes redox imbalance in epithelial cells

To confirm the protective role of thiols in the cytotoxicity of DAB in LLC-MK2 cells, GSH synthesis was inhibited using BSO, an L-glutamate analog that inhibits the synthesis of glutathionylcysteine, and NAC as a positive control. Pre-treatment of cell cultures with of BSO exacerbated DAB toxicity (Table 2). The cell viability diminished approximately 40% in the presence of BSO (100 or 500 μM) in comparison to cells treated with only DAB. As expected, the addition of NAC mediated robust protection against DAB cytotoxicity, resulting in cellular viability of 90%. Additionally, the BSO treatment resulted in significant decrease (20%) of total thiols. Furthermore, BSO treatment of cell cultures followed by DAB exposure resulted in drastic loss of thiols; cell treated with DAB only had approximately twice the amount of thiols. In contrast, NAC treatment prevented the decrease of thiols induced by the pro-oxidant activity of DAB (Table 2). Light micrographs revealed morphological changes in LLC-MK2 cells caused by DAB in a dose and time-dependent manner that were prevented by NAC supplementation and enhanced by pre-treatment with BSO, thereby confirming the protective role of thiols in the cell redox balance (not shown).

Discussion

Oxyradicals and α-oxoaldehydes such as methylglyoxal and 4-amino-2-oxobutanal, produced during DAB oxidation likely damage lipids and proteins, disrupt mammalian cell viability and activate chemical and enzymatic cell responses against oxidative stress [6,4,5]. DAB toxicity in several pathogenic microorganisms was first attributed to the competitive inhibition of ODC compromising the biosynthesis of polyamines [17,15,13,12]. More recently, we found that DAB causes redox imbalance in RKO cells, a human colon carcinoma cell line, which is mediated by a more general oxidative mechanism of toxicity (unpublished results). However, *T. cruzi*, which is also susceptible to DAB treatment, lacks a functional gene for ODC [19], and Menezes et al. [11] reported ultrastructural changes in DAB-treated epimastigotes mediated to oxidative stress.

Here, we confirm that *T. cruzi* trypomastigotes are highly vulnerable to DAB treatment (Fig. 1AB). Exposure to DAB leads to the decrease of cell motility and viability, two parameters often used for testing drug effectiveness [33–36], similar to DAB-treated epimastigotes, a non-infective stage of *T. cruzi* present in the insect vector [11]. Trypomastigotes exhibited up-regulation of antioxidant defenses, a critical characteristic of virulence [37], and were approximately 6 times more susceptible to DAB treatment with an approximate IC_{50} of 0.2 mM after 4 h than epimastigotes with an apparent IC_{50} of 1.18 mM after 3 days according to Menezes et al. [11].

Interestingly, the trypanocidal drugs that lead to morphological changes in the parasite [38,39] act through redox cycling systems that yields ROS which are likely responsible for the cytotoxicity of these compounds [38,39]. Accordingly, the DAB-mediated depletion of thiols in epimastigotes was accompanied by an increase in TcSOD activity (Fig. 2B), which represents the first line of antioxidant defense in *T. cruzi* [40]. Free thiols in *T. cruzi* are provided mainly by dithiol trypanothione, where two GSH molecules are conjugated to a spermidine moiety, which are more reactive than a single GSH molecule [41]. The crucial role of trypanothione in the parasite oxidative stress defense is attested by the variety of trypanothione-dependent enzymes [42]. Trypanothione is critical for cell redox homeostasis because it delivers reducing equivalents for synthesis of parasite deoxyribonucleotides, and performs other oxy-reduction activities [43]. Additionally, oxidative stress triggered by nifurtimox and other *N*-oxide-containing heterocyclic trypanosomicides in *T. cruzi* is accompanied by a dramatic reduction of low-molecular-weight thiols [42]. Interestingly, amastigotes and trypomastigotes only contain one-third and one-half of the total thiols present in epimastigotes [22]. Furthermore, when the parasites are exposed to high H_2O_2 concentrations, detoxifying en-

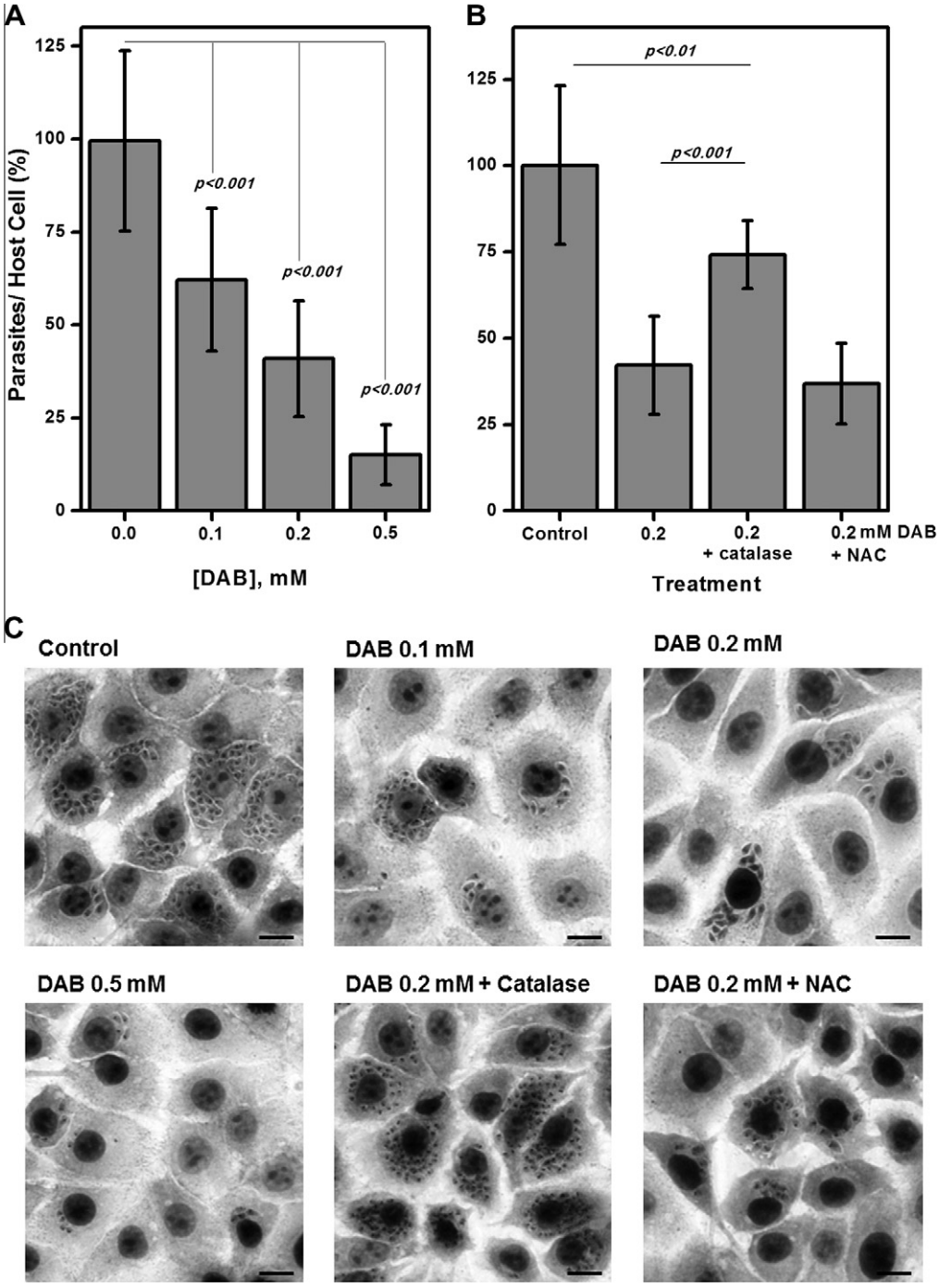

Fig. 3. The effects of DAB on host cell infection by *T. cruzi* trypomastigotes. Parasites (1×10^7/mL) were incubated for one hour with different concentrations of DAB in phenol red-free MEM supplemented with 2% FBS. After washing, the parasites were resuspended in fresh medium and incubated with host cells for 4 h at cell to parasite ratio of 1:100. After 48 h, the cells were fixed with 4% PFA, and the number of intracellular parasites was estimated by randomly counting the nuclei stained with DAPI (A). The infectivity of trypomastigotes was measured after 0.2 mM DAB treatment in the presence of 2.5 μM catalase or 5 mM NAC(B). Control samples were set up as 100%, which contain approximately two parasites per host cell. The bars represent 20 μm in Giemsa-stained infected host cells (C). According to an ANOVA, with Tukey's test, all means are significantly different at a *p*-value of 0.05. Student's t-test *p*-values for the comparisons between control and DAB-treated samples are shown.

zymes such as tryparedoxin peroxidase are up-regulated [44]. Additionally, TcSOD overexpression increases the resistance against oxidative stimuli in the parasites [45]. Here, catalase provided a protective effect on DAB toxicity in trypomastigotes (Table 1), which strongly suggests that H_2O_2 generated by DAB oxidation plays important role in the oxidative injury observed in the trypomastigotes. A similar trend was observed when mammalian cells were treated with DAB in the presence of catalase [5].

In this study, DAB impaired trypomastigote viability and host cell invasion, which were prevented by the addition of catalase, confirming the role of DAB-generated H_2O_2 in the oxidative injury mechanism. However, no protection was verified when the parasites were incubated with NAC (Fig. 3B and C). NAC is a highly permeable molecule that mediates redox protection in cells and can directly act as a reductant [46] or be converted into metabolites capable of stimulating GSH biosynthesis. During short-time incu-

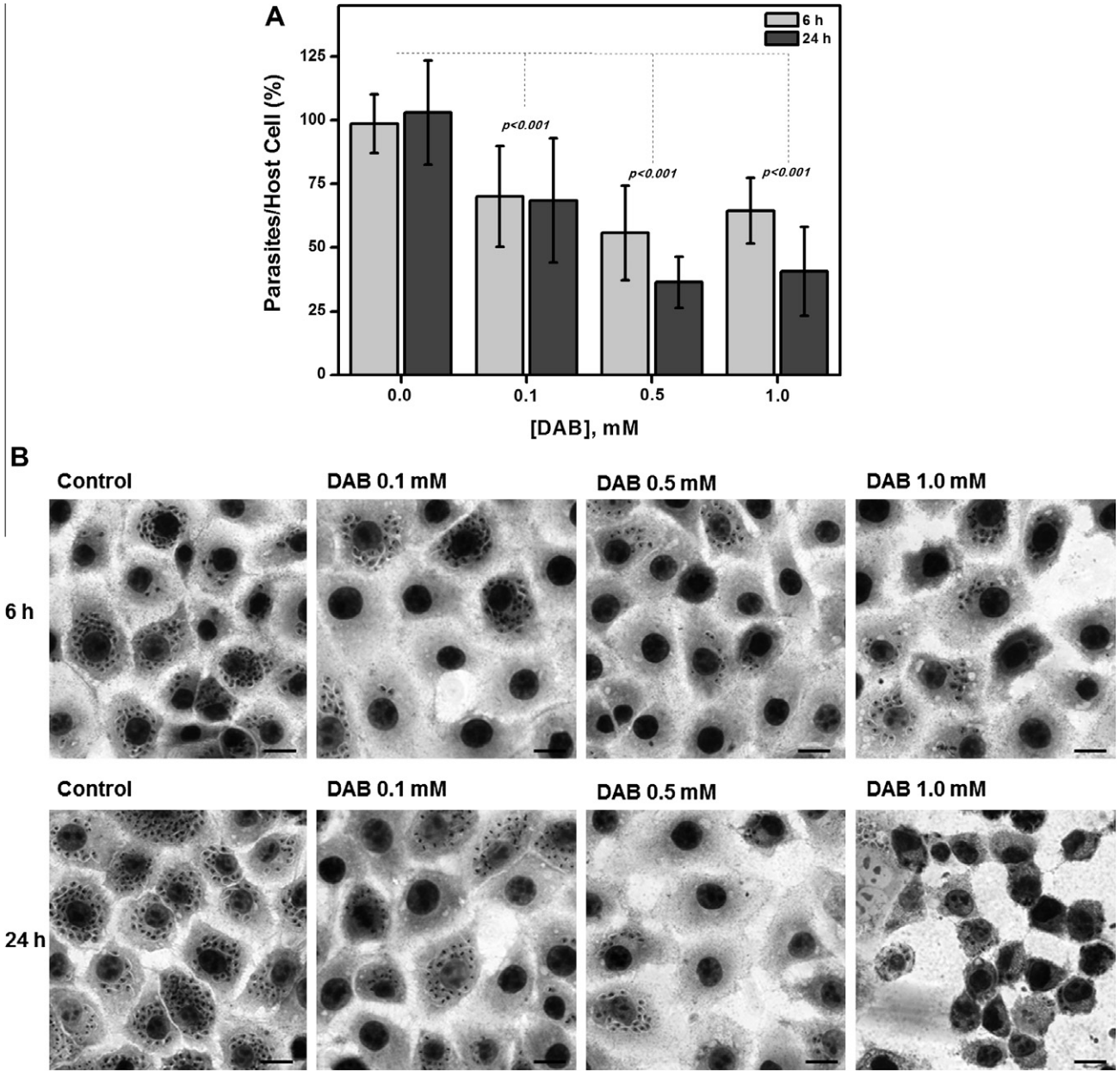

Fig. 4. The effects of DAB on intracellular amastigotes. LLC-MK2 cells were incubated with trypomastigotes for 4 h using a cell to parasite ratio of 1:100 in MEM supplemented with 2% FBS at 37 °C and under 5% CO_2. After 24 h, the infected host cells were treated with different concentrations of DAB for 6 or 24 h. After DAB treatment, cell cultures were fixed with 4% PFA and the amount of intracellular parasites was estimated by randomly counting nuclei stained with DAPI (A). Control samples were set up as 100%, which contain approximately three parasites per host cell. The bars represent 20 μm in Giemsa-stained infected host cells (B). According to an ANOVA Tukey's test, all means are significantly different at a *p*-value of 0.05. Student's t-test *p*-values for the comparisons between control and DAB-treated samples are shown.

Table 2
The protective role of GSH on DAB-induced oxidative damage to LLC-MK2 cells.

	1.5 mM DAB	
	Viability (%)	Total thiols (%)
Control	58 ± 3.0¤	59 ± 7.0¤
NAC (5 mM)	91 ± 5.4*	108 ± 4.3*
BSO (100 μM)	18 ± 8.9¤*	20 ± 9.8¤*
BSO (500 μM)	25 ± 5.7¤*	17 ± 6.2¤*

LLC-MK2 cells (5×10^4) were pre-treated with NAC or BSO in phenol red-free MEM supplemented with 2% FBS for 24 h at 37 °C. Subsequently, cells were treated with 1.5 mM DAB and their viability evaluated after 24 h. LLC-MK2 cells (3.5×10^5) were treated under the same conditions and total thiols were measured using mBBr. ¤The means are significantly different from respective controls in absence of DAB ($p < 0.001$Student's t-test). *The means are significantly different from control in presence of DAB at p-value 0.01 according to Student's t-test.

bations, catalase may exert a higher protective effect than NAC. In addition, LLC-MK2 cells pre-treatment with 5 mM NAC for 24 h followed by DAB exposure resulted in substantial maintenance of viability (Table 2). However, NAC has been reported to both protect host cells from parasite infection [47] and improve the parasite ability to invade host cells [48]. Therefore, NAC might boost the antioxidant response in both host and parasite cells, thus improving host cell protection against parasite infection while concurrently allowing parasites to evade the host cell defense mechanisms.

Recently, Soares et al. [5] reported an approximate IC_{50} = 1.5 - mM for DAB in LLC-MK2 epithelial cell line, which is about 7 times higher than the IC_{50} determined for *T. cruzi* trypomastigotes in this study (Fig. 1B). DAB was also observed to induce redox imbalance

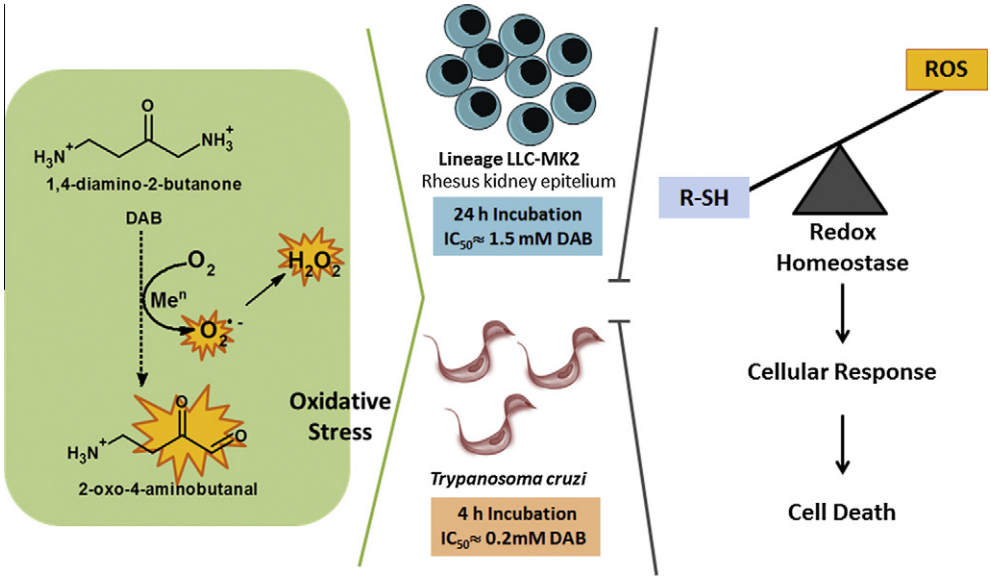

Fig. 5. Proposed mechanism for DAB cytotoxicity to *Trypanosoma cruzi* and mammalian host cell. DAB undergoes metal-catalyzed aerobic oxidation yielding ROS, ammonium ion, an intermediate α-oxoaldehyde (oxoDAB) [Error! Bookmark not defined.]. This reaction takes place mainly in the extracellular environment, resulting in redox imbalance of both LLC-MK_2 cells and *T. cruzi* cells.

in both intra- and extracellular environment (not shown). Accordingly, pre-treatment of cells with BSO aiming to inhibit the synthesis of GSH resulted in a considerable increase of DAB cytotoxicity (Table 2). Cellular changes in the redox state of thiol pools are known to reflect oxidative assault [49]. Considering the features of parasites and mammalian host cells stress defenses [44], several authors [50,51] investigated the potential synergistic effects of BSO with nifurtimox and benznidazole at different life stages of *T. cruzi*, and concluded that BSO potentiates the effects of these drugs in the trypomastigote and amastigote forms.

Finally, DAB treatment of epithelial cells infected with *T. cruzi* leads to a significant dose- and time-dependent attenuation of intracellular parasites (Fig. 4A and B). Similar results were reported using *L. amazonensis*-infected macrophages treated with DAB [12]. The inhibitory effect of DAB on the proliferation of intracellular amastigotes may be related to the impairment of nutrients sustained by the pool of thiols, as observed here when LLC-MK2 cells were treated with DAB (Table. 2). Indeed, it is well known that oxidative stress triggers antioxidant responses that may consume the cellular stock reserves [52]. DAB displayed notable deleterious effects on both intracellular *T. cruzi* amastigotes and host cells, especially at high concentrations (Fig. 4B). This is a frequent outcome in the search for new therapeutic drugs that act on a variety of parasites. Significant lethal effects in free parasites and host cells often appear in parallel when testing new drugs [38,53,54].

In conclusion, as sketched in Fig. 5, DAB efficiently decreases trypomastigote viability and *T. cruzi* infection of host mammalian cells probably by mechanisms linked to oxidative stress, however DAB displays significant lethal effects to host cells as well.

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