ESR AND SPIN TRAPPING STUDIES OF TWO NEW POTENTIAL ANTITRYPANOSOMAL DRUGS

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ABSTRACT

The Electron Spin Resonance (ESR) spectra of radicals obtained from two new potential antitrypanosomal drugs by Trypanosoma cruzi reduction were analyzed. DMPO Spin Trapping was used to investigate the possible formation of free radicals in the trypanosome microsomal system. The Nitro 2 (4-(n-butyl)-1-(5-nitrofurfurylidene)semicarbazide) analogue of Nifurtimox showed better antiparasitic activity than N-oxide 1 (4-(n-butyl)-1-[(7-bromo-N1-oxidebenzo[1,2-c]1,2,5-oxadiazole-5-yl)methylidene]semicarbazide). Only Nitro 2 could produce oxygen redox cycling in T. cruzi epimastigotes. The ESR signal intensities were consistent with the trapping of hydroxyl radical. These results are in agreement with the biological observation that Nitro 2 showed antichagasic activity by an oxidative stress mechanism.

Keywords: Nitrofuran derivatives, N-Oxide derivatives, ROS scavenging, ESR spin trapping, T. cruzi, oxidative stress

1. INTRODUCTION

Parasitic diseases in tropical and subtropical areas constitute a major health and economic problem. Chagas’ disease, produced by several strains of Trypanosoma cruzi, affects approximately 24 million people from Southern California to Argentina and Chile [1]. Nifurtimox and benznidazole are currently used to treat this disease [2]. A characteristic of nifurtimox is that it has a low therapeutic index, making its use limited by side effects. Recently we have explored 5-nitro-2-furaldehyde derivatives to find new substances with fewer side effects than Nifurtimox [8-12]. We have also reported studies on the 1,2,5-oxadiazole N-oxide family in order to determine their antiparasomal activities, tested in vitro against the epimastigote form of T. cruzi. Moreover, we have shown some ESR spectra that prove the facile electronation of the N-oxide moiety. Beside, these new structures were based on the conjunction of N-oxide systems and the semicarbazide moieties (“spermidine-mimetic”) [18]. In addition, we recently reported the electrochemical studies and the evidence of microsomal production of free radicals for 1,2,5-Oxadiazole N-Oxide suggesting its potential antiprotozoal activity [19]. All N-oxide studied showed similar E1/2 to nifurtimox. Additionally, the side chains assessed in this work did not modify the E1/2, an aspect that might be important for the selectivity of these compounds towards trypanothione reductase. Stable free radicals generated using a microsomal system showed hyperfine coupling constants identical to those of the radicals obtained by electrochemical reduction. The ESR spectra also proved that the N-oxide group is protonated, as suggested by the reduction mechanism proposed from the cyclic voltammetric results.

In the present study, we report the ESR and spin trapping results of two new potential antiparasomal drugs: 4-(n-butyl)-1-[(7-bromo-N'-oxidebenzo[1,2-c]1,2,5-oxadiazole-5-yl)methylidene]semicarbazide (N-oxide I) and 4-(n-butyl)-1-(5-nitrofurfurylidene)semicarbazide (Nitro2) (Figure 1). Both molecules have the same side chain but with different groups generating free radical species such as the nitro and N-oxide groups. In this paper we have characterized the free radical species generated by T. cruzi reduction that correlate with the percentage of growth inhibition of T. cruzi epimastigotes in order to suggest a possible mechanism.
2. EXPERIMENTAL SECTION AND THEORETICAL METHODS

2.1. Samples
The N-oxide 1 and Nitro 2 were synthesized according to methods described earlier [9, 20].

2.2. Reagents
Dimethylsulfoxide (DMSO) (spectroscopy grade), glutathione (GSH), 5,5-dimethyl-1-pyrroline N-oxide (DMPO), reduced Nicotinamide Adenine Dinucleotide Phosphate (NADPH), ethylenediaminetetraacetic acid (EDTA) were obtained from Sigma Aldrich Co., St. Louis, MO. Tetrabutylammonium perchlorate (TBAP) used as supporting electrolyte was obtained from Fluka.

2.3. ESR Spectroscopy.
ESR spectra were recorded in the X band (9.85 GHz) using a Bruker ECS 106 spectrometer with a rectangular cavity and 50 kHz field modulation. The hyperfine splitting constants were estimated to be accurate within 0.05 G. ESR spectra of the anion radical drugs and the radical oxygen species were produced using a microsomal fraction (4 mg protein/mL) obtained from \textit{T. cruzi}, in a reaction medium containing 1mM NADPH, 1mM EDTA and 100 mM DMPO, in 20mM phosphate buffer, pH 7.4. The ESR spectra were simulated using the program WINEPR Simphonia 1.25 version.

2.5. Parasites
\textit{Trypanosoma cruzi} epimastigotes (Tulahuen strain), from our collection, were grown at 28 °C in Diamond’s monophasic medium as reported earlier [21,22], with blood replaced by 4 mM hemin. Fetal calf serum was added to a final concentration of 4%. Parasites: 8 x 10^7 cells correspond to 1 mg protein or 12 mg of fresh weight.

3.0 RESULTS AND DISCUSSION

3.1 ESR
We have obtained a well resolved ESR spectra for both anion radical derivatives when they are prepared \textit{in situ} by electrochemical reductions in DMSO, applying a potential corresponding to the first wave as obtained from the cyclic voltammetric experiments (data not shown). However, when \textit{T. cruzi} microsomes are incubated with both compounds, after a 10 min induction period to become the microsomes anaerobic, gave a not well resolved ESR spectra (data not shown), probably attributable to both radical species.

3.2 Effect of Nitro 2 and N-Oxide 1 upon culture growth in \textit{Trypanosoma cruzi} epimastigotes
Several drug concentrations were used in order to determine the respective IC_{50} Nitro 2 produced significant inhibition of epimastigote culture growth (IC_{50} of 7.4±0.5 µM). N-oxide 1 showed a much lower effect upon epimastigote growth (IC_{50} > 30 µM).

3.3 ESR spectra of DMPO-OH- and DMPO-N-Oxide adducts obtained with \textit{T. cruzi} extracts
In order to analyse the antitypanosomal mechanism of these drugs, we incubated both compounds with \textit{T. cruzi} homogenates in the presence of NADPH and EDTA and DMPO (figures 2 and 3). A well-resolved ESR spectrum appeared when DMPO was added to the \textit{T. cruzi}-Nitro 2 system. The ESR signal intensity was consistent with the trapping of the hydroxyl radical (DMPO-OH spin adduct a_{N}=a_{H}= 14.7 G) (Figure 2). These hyperfine constants are in agreement with the splitting constants of other DMPO-OH adducts by DMPO [23]. These results are in agreement with the above-mentioned activity for this compound. However, when the N-oxide 1 compound was incubated with \textit{T. cruzi} in presence of DMPO (figure 3), six ESR line appeared. According with this hyperfine pattern and the hyperfine constants (a_{N} = 15.6 G and a_{H} = 21.6 G ) this spectrum was consistent with the trapping of the N-oxide radical (Figure 3), which can not produce radical oxygen species. These results agree with the low activity of these compounds. On the other hand, N-oxide-produced low growth inhibition of \textit{T. cruzi} occurs at concentrations that do not stimulate hidroxyl radical generation. As seen in their structures, both molecules have the same side chain, which could indicate that the nitro group generated radical species more efficiently than N-oxide group.

Finally, for Nitro 2 the main toxic mechanism seems to be the production of oxidative stress because of the extensive redox cycling that Nitro 2 undergoes.
system indicate that Nitro was consistent with the trapping of the mechanism. Molecule; N-oxide oxidative stress throughout the increase in redox recycling of the mechanisms of toxicity. While Nitro trapping of the hydroxyl radical, and for the N-oxide system. For Nitro resolved ESR spectra were obtained when DMPO was added to the constant 0.25 s number scans: 10.

Spectrometer conditions: microwave frequency 9.68 GHz microwave 7,4, DMPO (100 mM), N-oxide (1 mM in acetonitrile 10 v/v). EDTA (1 mM), NADPH (1 mM), in phosphate buffer (20 mM), pH 7.4, , NADPH (1 mM). Specrometer conditions: microwave frequency 9.68 GHz microwave power 20 mW, modulation amplitude 0.4 G, scan rate 0.83 G/s, time constant 0.25 s number scans: 10.

CONCLUDING REMARKS

The ESR spectra of the anion radicals for N-oxide 1 and Nitro 2 generated by T. cruzi system showed low resolution. However, well resolved ESR spectra were obtained when DMPO was added to the system. For Nitro 2, the ESR signal intensity was consistent with the trapping of the hydroxyl radical, and for the N-oxide 1, its spectrum was consistent with the trapping of the N-oxide radical.

The biological studies and the ESR experiment with the T. cruzi system showed low resolution. However, well resolved ESR spectra were obtained when DMPO was added to the system. For Nitro 2, the ESR signal intensity was consistent with the trapping of the hydroxyl radical, and for the N-oxide 1, its spectrum was consistent with the trapping of the N-oxide radical.

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