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Research paper

Targeting polyamine transport in *Trypanosoma cruzi*

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ABSTRACT

Polyamines play critical roles as regulators of cell growth and differentiation. In contrast with other protozoa, the human parasite *Trypanosoma cruzi*, the etiological agent of Chagas disease, is auxotrophic for polyamines. Therefore, their intracellular availability depends exclusively on polyamine transport and inhibition of these uptake processes can alter the viability of the parasite. The polyamine analogues used in this work were successfully tested as antiproliferative agents in cancer cells, bacteria, fungi and also showed a potent antiplasmodial effect. We evaluated the activity of these compounds on polyamine transport in *T. cruzi* and assessed the effects on parasite viability. Three polyamine derivatives, AMXT1501, Ant4 and Ant44, inhibited the putrescine transport in epimastigotes (the insect stage *of T. cruzi*) with calculated IC_{50} values of 2.43, 5.02 and 3.98 μM, respectively. In addition, only Ant4 and Ant44 inhibited spermidine transport with IC_{50} of 8.78 μM and 13.34 μM, respectively. The Ant4 analogue showed a high trypanocidal effect on trypomastigotes (the bloodstream stage of *T. cruzi*) with an IC_{50} of 460 nM, (SI = 12.7) while in epimastigotes the IC_{50} was significantly higher (16.97 μM). In addition, we studied the effect of the combination of benznidazole, a drug used in treating Chagas disease, with Ant4 on the viability of epimastigotes. The combined treatment produced a significant increase on the inhibition of parasites growth compared with individual treatments. In summary, these results suggest that Ant4, a putrescine conjugate, is a promising compound for the treatment of Chagas disease because it showed a potent trypanocidal effect via its inhibition of polyamine import.

1. Introduction

Chagas disease, or American trypanosomiasis, is a neglected tropical infection caused by the hemoflagellate protozoan parasite *Trypanosoma cruzi* [1]. According to the World Health Organization, around 6–7 million people worldwide are estimated to be infected with the parasite, mostly in Latin America, and over 10,000 deaths per year are caused by the disease. Moreover, Chagas disease is a global health problem largely due to population movements from endemic countries to the rest of the world [2,3]. Benznidazole (BZN) and nifurtimox (NFX) are the only drugs approved for the treatment of Chagas disease. These drugs often have serious side-effects and very limited efficacy [4]. In addition, the "Benznidazole Evaluation for Interrupting Trypanosomiasis" (BENEFIT) in patients with chronic Chagas' cardiomyopathy did not significantly reduce cardiac clinical progression through 5 years of follow-up [5]. Thus, the development of new therapeutic alternatives and the identification of novel drug targets in *T. cruzi* are necessary.

Polyamines (putrescine, spermidine and spermine; Fig. 1, compounds **1**–**3**) are polycationic compounds essential for the growth and function of cells [6]. In trypanosomes, they are involved in crucial cellular processes including the synthesis of trypanothione (Fig. 1, compound **4**), a bis-glutathionyl conjugate of spermidine, which is necessary for protection against oxidative damage [7]. Most organisms synthesize their own polyamines from ornithine and in some cases from arginine by the action of ornithine decarboxylase (ODC) and arginine decarboxylase (ADC) [8,9]. *T. cruzi* cannot synthesize putrescine *de novo* due to the lack of both enzymes and, therefore, the intracellular availability of these metabolites relies exclusively on transport processes. Thus, the inhibition of this important uptake process can alter the viability of the parasite. This is the case of the trypanocidal drug, pentamidine (Fig. 1, compound **5**), that blocks polyamine transport in *T. cruzi*

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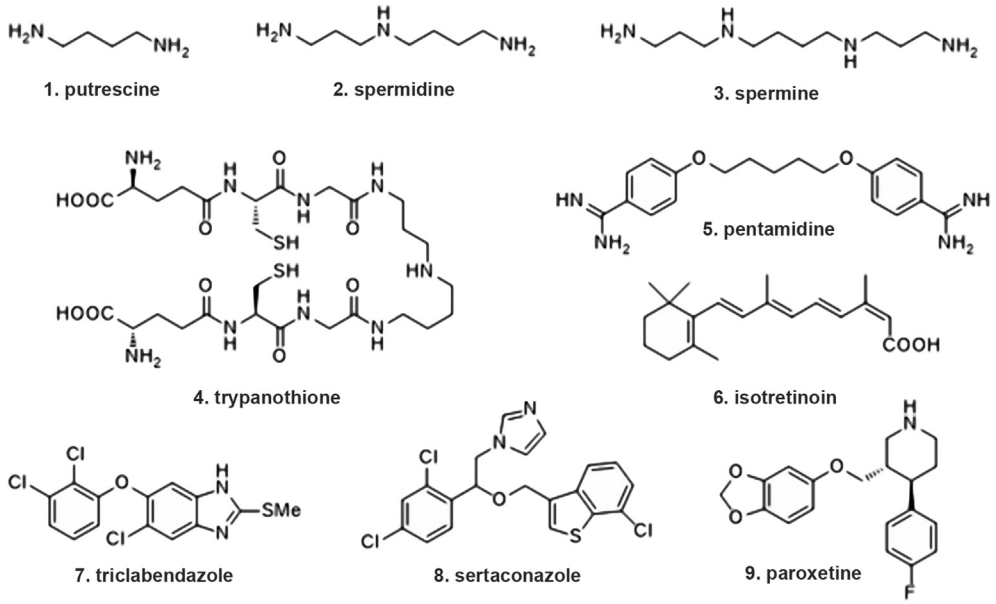

Fig. 1. Chemical structure of polyamines, related metabolites and transport inhibitors. Structures of the native polyamines putrescine, spermidine and spermine (**1**–**3**), the reduced form of trypanothione (**4**) and the previously reported polyamine transport inhibitors pentamidine, isotretinoin, triclabendazole, sertaconazole and paroxetine (**5**–**9**).

[10,11]. In addition, recent studies have shown that isotretinoin (Fig. 1, compound **6**), a retinol derivative drug, has a potent trypanocidal effect by inhibiting polyamine and amino acid uptake in this parasite [12]. Moreover, the polyamine analogues triclabendazole, sertaconazole and paroxetine (Fig. 1, compounds **7**–**9**), discovered by computational simulation, had inhibitory effects on the proliferation of the parasite and also inhibited putrescine transport [13]. Furthermore, different polyamine-based analogs, e.g., diamine, triamine and tetramine derivatives, also had antikinetoplastid activity [14].

Polyamine analogues tested in this work (Fig. 2) have been successfully used as inhibitors of intraerythrocytic *Plasmodium falciparum* proliferation, as polyamine transport inhibitors for cancer treatment, and for bacterial (*Proteus mirabilis*) infections. For example, the anthracene-putrescine conjugate **15** (Ant4) and the anthracene-homospermidine conjugate **16** (Ant44) inhibited the uptake of putrescine and spermidine in the trophozoite stage of *P. falciparum* parasites and showed a potent antiplasmodial effect [15]. The combination of α-difluoromethylornithine (DFMO), an inhibitor of ODC, with polyamine transport inhibitors results in intracellular polyamine depletion and cell death, providing a method to target cancers with high polyamine requirements [16].

Muth et al. have shown that the polyamine derivatives **10** (Trimer44) and **11** (Trimer44NMe) blocked spermidine transport in DFMO-treated human pancreatic cancer cells [17]. An additional study has reported that the polyamine transport inhibitor **17** (AMXT1501) in combination with DFMO also blocked tumor growth by targeting tumor polyamines [18]. The putrescine analogue **12** (Triamide44) has been tested on *Proteus mirabilis*, a bacterium that causes urinary tract infections in humans. This compound inhibited putrescine transport and consequently decreased putrescine-stimulated swarming and urothelial cell invasion in bacteria. In addition, other polyamine derivatives were tested against the fungus *Pneumocystis carinii* that causes pneumonia in immunocompromised hosts. The compounds reduced organism burden, decreased lung inflammation and prolonged the survival of treated *Pneumocystis* pneumonia (PCP) rats [19,20].

Considering the effects of these polyamine-transport-targeting compounds in protozoan organisms, cancer cells and in bacterial and fungi systems, in this work, we evaluated these polyamine analogues as transport inhibitors and trypanocidal drugs in *T. cruzi* via several *in vitro* assays.

2. Results

2.1. Inhibition of putrescine and spermidine transport

The eight polyamine derivatives (**10**–**17**) were tested for polyamine transport inhibition. *Trypanosoma cruzi* epimastigotes were used for putrescine and spermidine transport assays (5 μM and 15 μM, respectively), in the presence of the polyamine derivatives over a wide concentration range (0–30 μM). Those compounds that showed transport inhibition activity were re-evaluated adjusting the concentration range according to the inhibition values obtained in the first assay. The calculated IC_{50} value denotes the concentration of the compound needed to inhibit 50% of uptake of radiolabeled putrescine or spermidine. Only compounds **15**–**17** presented significant putrescine transport inhibition activity with calculated IC_{50} values of 5.02 μM (±0.39), 3.98 μM (±0.24) and 2.43 μM (±0.15), respectively (Table 1 and Supplementary Material, Fig. S1A). In the case of spermidine transport only **15** (Ant4) and **16** (Ant44), but not **17** (AMXT 1501), produced a significant inhibition. IC_{50} values for spermidine transport inhibition were of 8.78 μM (±1.04) and 13.34 μM (±0.94) for **15** and **16**, respectively (Table 1 and Supplementary Material, Fig. S1B).

2.2. Trypanocidal effect of polyamine conjugates

All compounds that showed a significant inhibition of polyamine transport (**15**–**17**) were also examined for trypanocidal activity. First, the effect was evaluated on the epimastigote stage. Cells were incubated with different concentrations of each compound, between 0 and 100 μM, over a 48 h incubation period. The IC_{50} values were then determined, where the IC_{50} value is the concentration of compound which gave 50% relative viability of parasites compared to the untreated control. Among the three compounds analyzed, only conjugate **15** presented a detectable trypanocidal activity with a calculated IC_{50} of 16.97 μM (±1.16) (Table 1 and