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Evaluation of trypanocidal activity of combinations of anti-sleeping sickness drugs with cysteine protease inhibitors

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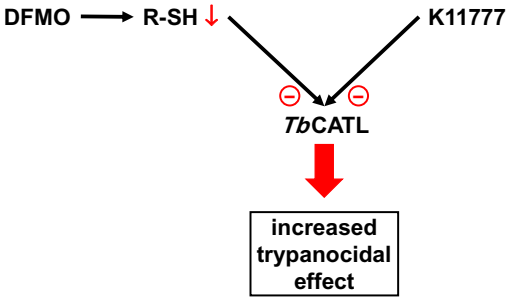
HIGHLIGHTS

- The CP inhibitor K11777 and DFMO show synergistic trypanocidal activity.
- The synergistic interaction of K11777 and DFMO is due to a reduction in thiol levels.
- The K11777/DFMO combination was less toxic to human cells than to trypanosomes.

GRAPHICAL ABSTRACT



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ABSTRACT

Chemotherapy of human African trypanosomiasis (HAT) is unsatisfactory because only a few drugs, with serious side effects and poor efficacy, are available. As drug combination regimes often achieve greater therapeutic efficacy than monotherapies, here the trypanocidal activity of the cysteine protease inhibitor K11777 in combination with current anti-HAT drugs using bloodstream forms of *Trypanosoma brucei* was investigated. Isobolographic analysis was used to determine the interaction between cysteine protease inhibitors (K11777, CA-074Me and CAA0225) and anti-HAT drugs (suramin, pentamidine, melarsoprol and eflornithine). Bloodstream forms of *T. brucei* were incubated in culture medium containing cysteine protease inhibitors or anti-HAT drugs alone or in combination at a 1:1 fixed-dose ratio. After 48 h incubation, live cells were counted, the 50% growth inhibition values determined and combination indices calculated. The general cytotoxicity of drug combinations was evaluated with human leukaemia HL-60 cells. Combinations of K11777 with suramin, pentamidine and melarsoprol showed antagonistic effects while with eflornithine a synergistic effect was observed. Whereas eflornithine antagonises with CA-074Me, an inhibitor inactivating the targeted *Tb*CATL only under reducing conditions, it synergises with CAA0255, an inhibitor structurally related to CA-074Me which inactivates *Tb*CATL independently of thiols. These findings indicate an essential role of thiols for the synergistic interaction between K11777 and eflornithine. Encouragingly, the K11777/eflornithine combination displayed higher trypanocidal than cytotoxic activity. The results of this study suggest that the combination of the cysteine protease inhibitor K11777 and eflornithine display promising synergistic trypanocidal activity that warrants further investigation of the drug combination as possible alternative treatment of HAT.

1. Introduction

Human African trypanosomiasis (HAT) or sleeping sickness is one of the most neglected tropical diseases. HAT affects mainly poor people living in 250 rural foci scattered over 36 sub-Saharan African countries where it is a major cause for morbidity and mortality

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(WHO, 2014). The disease is caused by the flagellated protozoan *Trypanosoma brucei* and transmitted by the bite of infected tsetse flies. The extracellular parasites live first in the blood and lymphatic system and later enter the central nervous system causing the typical symptoms of HAT which are confusion, disturbed sleep pattern, sensory disturbances, extreme lethargy, poor condition and coma (WHO, 2014). If left untreated, HAT patients will ultimately die within months or years (WHO, 2014). Sadly, only a few drugs (suramin, pentamidine, melarsoprol and eflornithine) and one drug combination (nifurtimox/eflornithine) are currently available for chemotherapy of HAT (Steverding, 2010). All these drugs are outdated and have major drawbacks including poor efficacy, significant toxicity and the requirement for parental administration (Fairlamb, 2003; Steverding, 2010). In addition, the compounds are increasingly subject to drug resistance (Delespaux and de Koning, 2007; Matovu et al., 2001). Hence, effective and better tolerated chemotherapies are urgently needed for treatment of HAT.

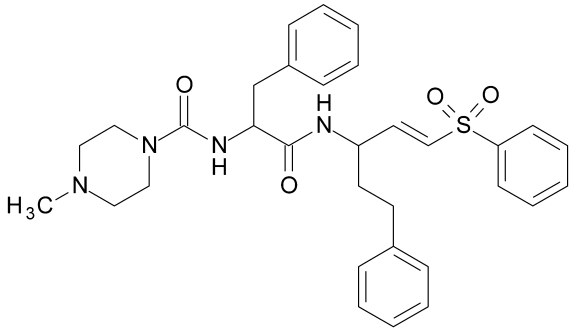
It is well established that drug combination regimes often achieve greater therapeutic efficacy than monotherapies (e.g. in the treatment of cancer, AIDS and tuberculosis). Drug combinations may be less toxic than monotherapy regimes and have the additional advantage of reducing the risk for development of drug resistance. This is because drug combinations may interact synergistically, i.e. their combined effect may be greater than the sum of their separate effects (Chou, 2006). However, additive effects are also desirable as they permit decreased dosage while maintaining efficacy (Chou, 2006). The recently introduced nifurtimox/eflornithine combination therapy as first line treatment of second stage HAT, however, failed to show synergistic trypanocidal activity *in vitro*. Biochemical analysis revealed that the metabolomic changes associated with the combination are the sum of those found in each monotherapy with no indication of additional effects (Vincent et al., 2012). In addition, nifurtimox as a nitro-heterocyclic drug has mutagenic, toxic and carcinogenic side effects. Therefore, other drug combinations should be investigated to find combination therapies with less toxic adverse effects.

Proteases play a key role in the life cycle of *T. brucei* and in the pathogenicity of HAT (Steverding, 2013). A chemically validated drug target is the *T. brucei* cathepsin L-like protease, *Tb*CATL (aka brucipain, rhodesain or trypanopain-Tb) (Steverding et al., 2012), a cysteine protease belonging to the C1 family of papain-like enzymes (Caffrey and Steverding, 2009). *Tb*CATL is found in the lysosome of bloodstream forms of *T. brucei* where it is involved in the degradation of endocytosed host proteins (Scory et al., 1999). Targeting *Tb*CATL with small-molecule inhibitors is trypanocidal both *in vitro* and *in vivo* (Caffrey et al., 2000; Scory et al., 1999; Troeberg et al., 1999). One promising inhibitor that has emerged over the last 15 years is the peptidomimetic vinyl sulphone K11777 (Fig. 1) (Caffrey and Steverding, 2008). This inhibitor has been shown to reduce the parasitaemia and to prolong the survival of *T. brucei*-infected mice (Caffrey et al., 2000). Considering that K11777 has been approved by the FDA to enter a phase I safety trial in the United States (Rodenko and de Koning, 2013), the main aim of this study was to evaluate the trypanocidal efficacy of K11777 and anti-HAT drug combinations on bloodstream forms of *T. brucei in vitro*.

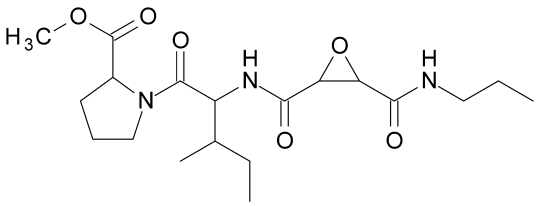
2. Materials and methods

2.1. Drugs

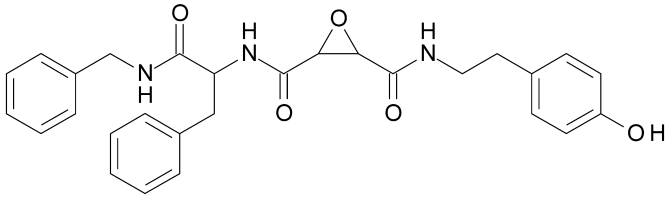
Eflonithine (DL-α-difluoromethylornithine), suramin, pentamidine and CA-074Me ((L-3-*trans*-propylcarbamoyloxirane-2-carbonyl)-L-isoleucyl-L-proline methyl ester) were purchased from Sigma-Aldrich (Gillingham, UK). Z-FR-AMC (benzyloxycarbonyl-phenylalanyl-arginyl-7-amido-4-methyl coumarin) was from Enzo Life Sciences Ltd. (Exeter, UK). Melarsoprol was a gift from



K11777



CA-074Me



CAA0225

Fig. 1. Chemical structures of K11777, CA-074Me and CAA0225.

Sanofi-Aventis Deutschland GmbH (Frankfurt, Germany). K11777 ((N-methyl-piperazinyl-phenylalanyl-homophenylalanyl-vinylsulfone-phenyl)) was kindly provided by Dr Conor R. Caffrey (University of California San Francisco, San Francisco, USA). CAA0225 ((2*S*,3*S*)-oxirane-2,3-dicarboxylic acid 2-[((*S*)-1-benzylcarbamoyl-2-phenyl-ethyl)-amide] 3-{[2-(4-hydroxy-phenyl)-ethyl]-amide}) was synthesised as previously described (Steverding et al., 2012).

2.2. Cell culture

Bloodstream forms of *T. brucei* clone 427-221a (Hirumi et al., 1980) and human myelocytic leukaemia HL-60 cells (Collins et al., 1977) were grown in Baltz medium (Baltz et al., 1985) and RPMI 1640 medium (Moore et al., 1967), respectively. Both media were supplemented with 16.7% heat-inactivated foetal bovine serum. Trypanosomes and HL-60 cells were cultured at 37 °C in a humidified atmosphere containing 5% CO_2.

2.3. Isobolographic analysis

The interaction of drugs was evaluated using the isobolographic method as described previously (Steverding and Wang, 2009). First, the 50% growth inhibition (GI_{50}) value, i.e., the drug concentration