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Research brief

In vitro trypanocidal activity of the anti-helminthic drug niclosamide

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Abstract

Only a few drugs are available for chemotherapy of African trypanosomiasis and there is an urgent need for the development of new anti-trypanosomal agents. In this study, the anti-helminthic drug niclosamide was tested for its trypanocidal activity *in vitro* using culture-adapted bloodstream forms of *Trypanosoma brucei brucei* and *Trypanosoma congolense*. The concentrations of niclosamide to reduce the growth rate by 50% and to kill all cells were in the low- and mid micromolar ranges for *T. b. brucei* and *T. congolense*, respectively. The very low toxicity of niclosamide for mammals makes the compound interesting for drug development for African trypanosomiasis.

Index Descriptors and Abbreviations: GI_{50}, 50% growth inhibition value; MIC, minimum inhibitory concentration; Chemotherapy; Drug screening; Niclosamide; *Trypanosoma brucei*; *Trypanosoma congolense*

African trypanosomes are the causative agents of sleeping sickness in man and nagana disease in cattle. The protozoan parasites live extracellularly in the blood and tissue fluids of their mammalian hosts and are transmitted by the bite of infected tsetse flies (*Glossina* sp.). *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense* give rise to sleeping sickness while *T. b. brucei*, *T. congolense* and *T. vivax* are responsible for nagana disease. Since the 1970s, sleeping sickness has re-emerged and currently over 60 million people living in 36 sub-Saharan countries are at risk of contracting the disease (Barrett et al., 2003). At present, the estimated number of cases of human African trypanosomiasis is between 50,000 and 70,000 (WHO, 2006). Approximately 46 million cattle are threatened with nagana and the disease costs livestock producers and consumers an estimated $US1.34 billion per year (Kristjanson et al., 1999). Chemotherapy of African trypanosomiasis relies on just a few drugs, some of which display toxic side effects (Croft, 1997; Fairlamb, 2003). In addition, drug resistance in African trypanosomes is an increasing problem (Ross and Sutherland, 1997; Geerts et al., 2001; Matovu et al., 2001). Moreover, the production of the currently available drugs for treatment of sleeping sickness was recently under threat (Wickware, 2002). Taken together, new strategies to treat African trypanosomiasis are urgently needed.

One strategy to identify new chemotherapies for treatment of African trypanosomiasis is the screening of existing drugs for trypanocidal activity. For instance, DNA topoisomerase inhibitors approved for cancer chemotherapy have been shown to display promising anti-trypanosomal activities (Deterding et al., 2005). If licensed drugs are proved to be useful against trypanosomes, a more rapid application for treatment of African trypanosomiasis with less extensive clinical trials might be possible as their *in vivo* toxicities may be already well established. In this study we investigated the trypanocidal activity of the anti-helminthic drug niclosamide (Fig. 1) and compared this with the

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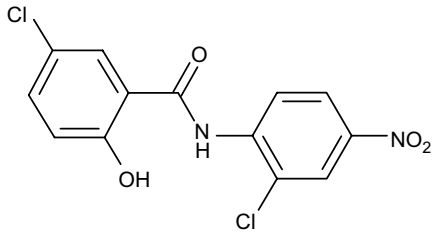

Fig. 1. The structure of niclosamide (2′,5-dichloro-4′-nitrosalicylanilide).

anti-trypanosomal activities of some common drugs to treat African trypanosomiasis.

The trypanocidal activities of niclosamide and some common anti-trypanosomiasis drugs were evaluated *in vitro* with bloodstream forms of *T. b. brucei* 427–221 (Hirumi et al., 1980) and *T. congolense* STIB 910 (Obexer et al., 1995) using the Alamar Blue® assay as described previously (Räz et al., 1997; Merschjohann et al., 2001). For each reagent, the 50% growth inhibition (GI_{50}) value, i.e. the concentration of a compound necessary to reduce the growth rate of the cells by 50% of that of controls, and the minimum inhibitory concentration (MIC), i.e. the concentration of a compound at which all cells were killed, were determined. Niclosamide showed a dose-dependent effect on the growth of both trypanosome species with GI_{50} and MIC values in the micromolar range (Table 1). However, *T. congolense* was about 10 times less susceptible to the compound than *T. brucei*. A similar observation was recently made for the anti-trypanosomal activities of alkaloids and iron chelators (Merschjohann et al., 2001; Merschjohann and Steverding, 2006). Niclosamide was found to posses a greater trypanocidal activity against *T. b. brucei* than the anti-sleeping sickness drug eflornithine (DFMO) (Table 1). However, it should be borne in mind that *T. b. brucei* like *T. b. rhodesiense* is not very susceptible to eflornithine (Zweygarth and Kaminsky, 1991; Iten et al., 1997). This is because eflornithine has trypanostatic rather than trypanocidal activity (Burri and Brun, 2003). In contrast, *T. b. gambiense* shows a higher sensitivity towards eflornithine ($GI_{50} = 1.8$ μM, MIC = 18 μM) (Räz et al., 1997) which is similar to the trypanocidal activity of niclosamide against *T. b. brucei*. Compared with the anti-sleeping sickness drug melarsoprol, niclosamide was 30–90 times less active against *T. b. brucei* (Table 1). Likewise, the trypanocidal activity of niclosamide against *T. congolense* was found to be 10–50 times lower than the anti-nagana disease drug berenil (Table 1).

The general cytotoxicities of niclosamide and the anti-trypanosomiasis drugs were determined with leukemia HL-60 cells using the Alamar Blue® tests (Merschjohann et al., 2001). Niclosamide was also active against HL-60 cells but to a lesser extent with GI_{50} and MIC values in the high micromolar range (Table 1). The GI_{50} and MIC ratio of cytotoxic/trypanocidal activity (selectivity indices) for *T. b. brucei* were found to be greater than 100 (Table 2). Encouragingly, the GI_{50} ratio approaches the selectivity index of the anti-sleeping sickness drug melarsoprol (Table 2). In contrast, the selectivity indices for *T. congolense* were found to be in a modest range and were much lower than those of the anti-nagana disease drug berenil (Table 2).

Taken together, with a GI_{50} value of 0.36 μg/ml (=1.1 μM) and a GI_{50} selectivity index of >100, niclosamide meets almost the hit activity criterion for *T. b. rhodesiense* which are 0.2 μg/ml and >100 (Nwaka and Hudson, 2006).

Niclosamide is of very low toxicity to mammals with an oral LD_{50} of >5000 mg/kg in rats (WHO, 2002). The no-observed adverse effect level of niclosamide is 2000 mg/kg/day of repeated daily oral administration over a period of 4 weeks (WHO, 2002). A single 5-mg/kg oral dose of niclosamide given to rats was shown to result in a maximum plasma concentration of 1.08 μM (Chang et al., 2006). Although this plasma concentration is approaching the MIC value of niclosamide determined for *T. b. brucei*

Table 2
GI_{50} and MIC ratios of cytotoxic to trypanocidal activities of niclosamide and anti-trypanosomiasis drugs[a]

Compound	*T. b. brucei*		*T. congolense*	
	GI_{50} ratio	MIC ratio	GI_{50} ratio	MIC ratio
Niclosamide	147	131	6	12
Eflornithine	>870	1	—	—
Melarsoprol	288	>1000	—	—
Berenil	—	—	692	>333

[a] GI_{50} ratio, $GI_{50(HL\text{-}60)}/GI_{50(Trypanosoma\ sp.)}$; MIC ratio, $MIC_{(HL\text{-}60)}/MIC_{(Trypanosoma\ sp.)}$. GI_{50} and MIC ratios were calculated from GI_{50} and MIC values shown in Table 1.

Table 1
GI_{50} and MIC values of niclosamide and anti-trypanosomiasis drugs for bloodstream forms of *T. b. brucei*, *T. congolense* and HL-60 cells[a]

Compound	*T. b. brucei*		*T. congolense*		HL-60	
	GI_{50} (μM)	MIC (μM)	GI_{50} (μM)	MIC (μM)	GI_{50} (μM)	MIC (μM)
Niclosamide	1.1	3.4	26.8	37.0	162	447
Eflornithine	11.5	>10,000	—	—	>10,000	>10,000
Melarsoprol[b]	0.013	0.1	—	—	3.74	>100
Berenil	—	—	0.53	3.0	367	>1000

[a] The toxicity assay was performed in the presence of 1% DMSO as solvent to increase the solubility of the drugs. GI_{50} values were determined by linear interpolation (Huber and Koella, 1993). MIC values were determined microscopically. Each value represents the mean of three experiments set up in duplicate. The standard deviation was typically around 10%.

[b] The toxic activity of melarsoprol was determined by counting live cells using a Neubauer hemocytometer.