Karolinska Institutet

Institutionen för mikrobiologi, tumör-och cellbiologi

NOVEL TREATMENT OF AFRICAN TRYPANOSOMIASIS

AKADEMISK AVHANDLING
som för avläggande av medicine doktorsexamen vid Karolinska Institutet offentligen försvaras i MTC, Föreläsningssalen, Theorells väg 1

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av

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ABSTRACT

Human African Trypanosomiasis (HAT) or Sleeping Sickness is fatal if untreated. Current drugs used for the treatment of HAT have difficult treatment regimens and unacceptable toxicity related issues. The effective drugs are few and with no alternatives available, there is an urgent need for the development of new medicines, which are safe, affordable and have no toxic effects. Here we describe different series of lead compounds that can be used for the development of drugs to treat HAT.

In vivo imaging provides a fast non-invasive method to evaluate parasite distribution and therapeutic efficacy of drugs in real time. We generated monomorphic and pleomorphic recombinant *Trypanosoma brucei* parasites expressing the *Renilla* luciferase. Interestingly, a preferential testis tropism was observed with both the monomorphic and pleomorphic recombinants. Our data indicate that preferential testis tropism must be considered during drug development, since parasites might be protected from many drugs by the blood-testis barrier **(Paper I)**.

In contrast to most mammalian cells, trypanosomes cannot synthesize purines *de novo*. Instead they depend on the host to salvage purines from the body fluids. The inability of trypanosomes to engage in de novo purine synthesis has been exploited as a therapeutic target by using nucleoside analogues. We showed that adenosine analogue, cordycepin in combination with deoxycoformycin cures murine late stage models of African trypanosomiasis **(Paper II)**.

Since deoxycoformycin was shown to be a teratogen, we aimed for developing deoxycoformycin independent deaminase resistant cordycepin analogues that could be used as standalone drugs in treatment for African trypanosomiasis. We synthesized and characterized several deaminase resistant cordycepin analogues. 2-Fluorocordycepin (2-fCy) showed selective trypanotoxicity and resistance against adenosine deaminase. 2-fCy showed good in vitro preclinical profile and is a promising new lead for development of treatment against African trypanosomiasis **(Paper III)**.

In order to identify new drugs for the treatment of HAT we performed a focused screen of 5,500 compounds for *Trypanosoma brucei* subsp. A number of 2-aminopyrazines/2-aminopyridines were identified as promising leads. Specifically, CBK201352 a 2-aminopyrazine compound was trypanotoxic for *T. brucei*. In vitro preclinical assays predicted that CBK201352 has promising pharmacokinetic parameters. Mice treated with CBK201352 showed complete clearance of parasites for more than 90 days. Thus, we show that CBK201352 and related analogs are promising leads for the development of novel treatments for HAT **(Paper IV)**.

The intracellular reducing environment of trypanosomes is maintained by a unique thiol system. In trypanosomes the trypanothione and trypanothione reductase (TryR) replace glutathione and glutathione reductase found in most other organisms. TryR is an essential enzyme for the parasite and is absent in mammalian cells making it an attractive drug target. We showed that ebsulfur (EbS) a small sulfur-containing molecule is a NADPH, concentration and time-dependent irreversible inhibitor of the *T. brucei* TryR. We demonstrated that EbS or analogues disrupt the trypanothione system with a novel mechanism and are promising lead compounds for the development of drugs to treat HAT **(Paper V)**.