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In vivo SPECT imaging of [^{123}I]-labeled pentamidine pro-drugs for the treatment of human African trypanosomiasis, pharmacokinetics, and bioavailability studies in rats

Björn Cohrs [a,1], Yi Zhao [b,2], Ulf Lützen [b,3], Juraj Culman [c,4], Bernd Clement [a,5], Maaz Zuhayra [b,*]

[a] Institute of Pharmaceutical Chemistry, Christian-Albrechts-University Kiel, Gutenbergstr. 76, 24118 Kiel, Germany
[b] Clinic of Nuclear Medicine, Molecular Imaging und Therapy, UK-SH Campus Kiel, Arnold-Heller-Str. 3, House 50, 24105 Kiel, Germany
[c] Institute of Clinical and Experimental Pharmacology, UK-SH Campus Kiel, Arnold-Heller-Str. 3, House 30, 24105 Kiel, Germany

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ABSTRACT

Pentamidine is an effective antiparasitic agent and approved drug for the treatment of African trypanosomiasis (sleeping sickness). However, pentamidine suffers from poor orally bioavailability and lacks central nervous system (CNS) delivery. Therefore its applicability is limited to intravenous or intramuscular treatment of the first stage of the African trypanosomiasis. For this reason, several new pentamidine pro-drugs have been developed with the aim of providing improved orally availability and CNS penetration.

Aim: this work aims to measure and to compare the distribution, bioavailability, and ability to cross the blood–brain barrier of [^{123}I]-labeled pentamidine and its pro-drugs, *N,N′*-dihydroxypentamidine and *N,N′*-bis(succinyloxy) pentamidine, using SPECT (single photon emission computed tomography) after intravenously and per orally administration in rats.

Methods: a total of 60 male Sprague Dawley rats were examined. Each [^{123}I]-labeled substance ($n=3$) was applied to 12 rats ($n=6$ i.v. and $n=6$ orally). In two additional test series both [^{123}I]iodopentamidine ($n=6$) and *N,N′*-bis(succinyloxy)-[^{123}I]iodopentamidine ($n=6$) were administered orally together with the non-radioactive homologues. To evaluate the *in vivo* stability of the labeled compounds, [^{123}I]NaI solution was administered intravenously ($n=6$) and orally ($n=6$). *In vivo* SPECT images were acquired after 30 min, 4 h, and 24 h and blood samples were taken over 24 h. The SPECT images were fusioned with previously acquired magnetic resonance images. After the last SPECT the rats were perfused, sacrificed and the organ γ-radiation levels were determined with a γ-counter. Analysis and quantification of the reconstructed SPECT images was performed using the region of interest technique.

Results and conclusion: the data showed a highly improved oral bioavailability of the [^{123}I]-labeled pro-drugs compared to [^{123}I]-labeled pentamidine. While [^{123}I]iodopentamidine was mainly renally eliminated the pro-drugs were primarily metabolized in the liver and underwent biliary elimination. Considering pentamidine's nephrotoxicity this feature has to be seen as an advantage of the pro-drug principle. Moreover, a significantly higher concentration in the brain was detected after intravenous injection of *N,N′*-dihydroxy[^{123}I]iodopentamidine compared to [^{123}I]iodopentamidine. The feasibility of an effective treatment of second stage African trypanosomiasis, in which the parasites already infected the brain, with the herein investigated pro-drugs remains to be clarified with infected animals in additional *in vivo* studies.

* Corresponding author. Tel.: +49 430 597 3101; fax: +49 430 597 3065.
E-mail addresses: bjoern.cohrs@adacap.com (B. Cohrs), Yi.Zhao@uksh.de (Y. Zhao), uluetzen@nuc-med.uni-kiel.de (U. Lützen), juraj.culman@pharmakologie.uni-kiel.de (J. Culman), bclement@pharmazie.uni-kiel.de (B. Clement), mzuhayra@nuc-med.uni-kiel.de (M. Zuhayra).
[1] Tel.: +49 228 92588308.
[2] Tel.: +49 431 597 9534; fax: +49 431 597 3065.
[3] Tel.:+49 431 597 3148; fax: +49 431 597 3150.
[4] Tel.: +49 431 597 3519; fax: +49 431 597 3522.
[5] Tel.: +49 431 8801126; fax: +49 431 8801352.

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1. Introduction

The urgency of the development of new drugs for the treatment of African trypanosomiasis (sleeping sickness) is actually very large. The increasing development of resistance to existing therapies and the limited choice of available drugs as well as their severe or sometimes lethal side effects often cause problems in the treatment of patients (Baker et al., 2013). African sleeping sickness is caused by trypanosome infection and is therefore also known as human African trypanosomiasis (HAT). There are two different types of trypanosomes which cause either a slower, chronical (*Trypanosoma brucei gambiense*) or a faster, acute (*Trypanosoma brucei rhodesiense*) course of disease. The trypanosomes are transmitted by the bite of a tsetse fly and infect the patient at first in the periphery causing unspecific symptoms like fever and swollen lymphnodes. If the patient is not treated during this early stage infection, the parasites will move from the periphery across the blood–brain barrier and invade the CNS. When untreated this disease is always fatal. Pentamidine (1) and suramin are available drugs for the treatment of first stage sleeping sickness, but both drugs are neither orally bioavailable nor sufficiently CNS accessible. For the treatment of second stage HAT, in which the trypanosomes have already infected the CNS, the highly toxic melaminophenyl arsenical compound melarsoprol is still used and has to be administered intravenously. A therapy with eflornithine in combination with nifurtimox may be better tolerated, but it is even much more expensive and only effective against *T. brucei gambiense* (Simarro et al., 2011).

For those reasons various pro-drugs of pentamidine (1) have been developed recently with the aim of providing an orally available and CNS penetrating drug in order to improve the treatment of first and especially second stage African sleeping sickness (Kotthaus et al., 2011). Due to the strong basic amidine moieties pentamidine (1) suffers from poor oral bioavailability and lacks CNS delivery. Therefore, its applicability is limited to intravenous (i.v.) or intramuscular (i.m.) treatment of the first stage of the African trypanosomiasis (Barrett et al., 2011; Clement et al., 2006). (Baker et al., 2013) By means of hydroxylation of the amidine moieties basicity could be reduced and lipophilicity increased (Clement, 2002). A further increase in lipophilicity and water solubility was achieved by esterification of hydroxylated amidine moieties (Clement et al., 2006; Kotthaus et al., 2011). The metabolic activation of this double pro-drug to the active drug pentamidine (1) was previously shown *in vitro* and *in vivo* (Clement et al., 1992; Gruenewald et al., 2008; Kotthaus et al., 2011) (Fig. 1). The metabolic activation was so fast, that after a short time pentamidine (1) was the only detectable compound. Among various number of different pentamidine pro-drugs the here examined pro-drugs presented the best physicochemical and pharmacokinetic characteristics in previous studies (Kotthaus et al., 2011). Therefore, they were considered to be the most appropriate pro-drug candidates for a potential treatment of second stage African trypanosomiasis. The issue of their oral bioavailability and in particular, the question about the ability of these new substances to cross the blood–brain barrier as a prerequisite for the treatment of the second stage of the sleeping sickness is of great importance.

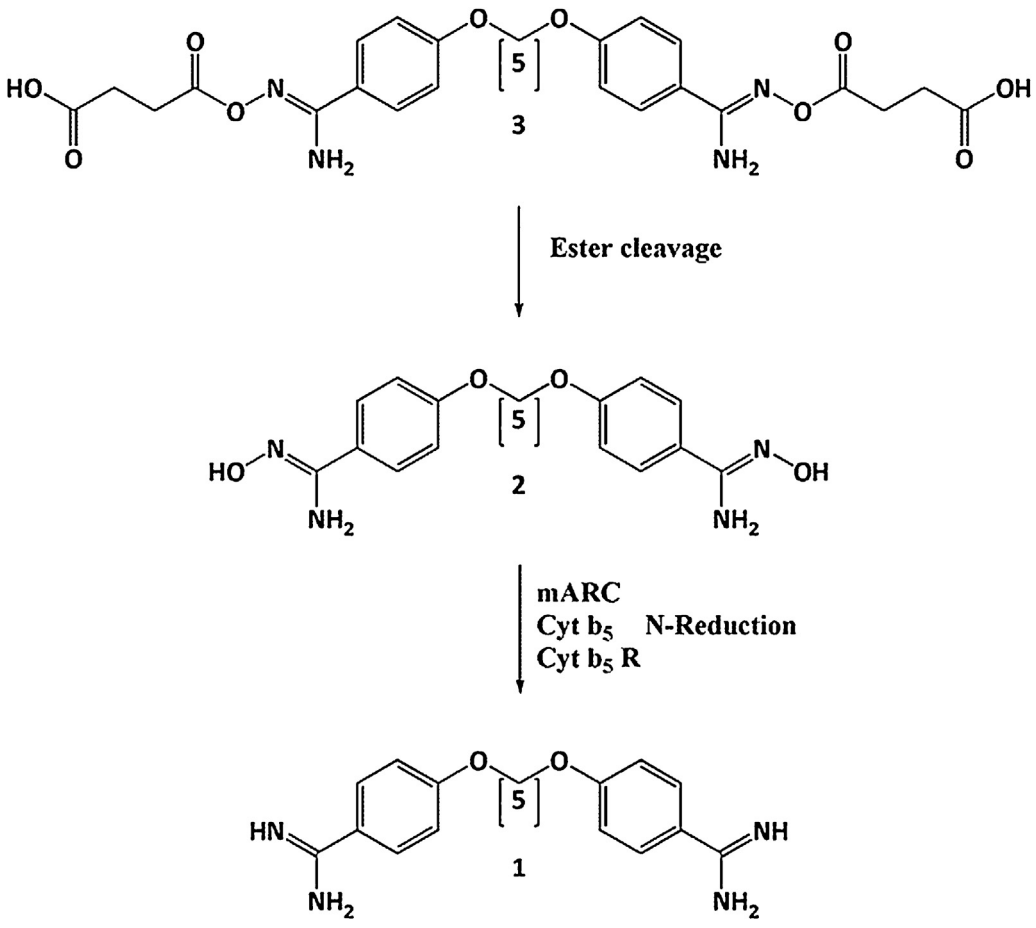

Fig. 1. Simplified metabolic activation of the pentamidine-pro-drugs.