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Dose-dependent effect and pharmacokinetics of fexinidazole and its metabolites in a mouse model of human African trypanosomiasis

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ABSTRACT

Human African trypanosomiasis (HAT) is a neglected tropical disease, with a population of 70 million at risk. Current treatment options are limited. In the search for new therapeutics, the repurposing of the broad-spectrum antiprotozoal drug fexinidazole has completed Phase III trials with the anticipation that it will be the first oral treatment for HAT.

This study used the recently validated bioluminescence imaging model to assess the dose and rate of kill effect of fexinidazole in infected mice, and the dose-dependent effect of fexinidazole on trypanosome infection. Pharmacokinetics of fexinidazole in plasma and central nervous system (CNS) compartments were similar in both infected and uninfected mice. Drug distribution within the CNS was further examined by microdialysis, showing similar levels in the cortex and hippocampus. However, high variability in drug distribution and exposure was found between mice.

1. Introduction

Human African trypanosomiasis (HAT) is caused by *Trypanosoma brucei* sspp. Manifestation of 'sleeping sickness', or chronic-stage HAT, occurs when the parasites migrate into the central nervous system (CNS). Although fewer than 7000 cases are reported each year, nearly 70 million people remain at risk [1]. Current therapies are limited by toxicity, route of administration and stage-specific activities [2]. Two drugs are currently undergoing clinical trials: fexinidazole and the oxaborole SCYX-7158 [3].

Fexinidazole, which offers a potential new safe oral treatment, was rediscovered during a screening programme against *T. brucei* by the Drugs for Neglected Diseases Initiative in 2005 [4], and completed Phase II/III trials for the treatment of chronic-stage HAT in April 2015 [5,6], with drug registration planned by the end of 2016 [7]. Preclinical studies showed oral effectiveness in curing both chronic and acute stages of the disease in mice [8]. Fexinidazole is metabolized rapidly by a range of cytochrome P450 enzymes, including CYPs 1A2, 2B6, 2C19, 3A4 and 3A5, and flavin-containing mono-oxygenase into two active metabolites: fexinidazole sulfoxide and fexinidazole sulfone [6,8,9].

The recently completed Phase II/III clinical trials performed in Africa compared the safety and efficacy of fexinidazole treatment with that of the combination therapy NECT (nifurtimox and eflornithine) [3]. The 10-d oral fexinidazole treatment regimen currently being implemented is 1800 mg/d (3 × 600 mg taken with food) for 4 d followed by 1200 mg/d for 6 d (2 × 600 mg taken with food). Clinical studies also aim to determine if lower doses and shorter treatment regimens can be used [7].

Studies on fexinidazole in mice were undertaken to: (i) further validate the use of optical imaging [10] in a *T. brucei* mouse model with acute and chronic infection to monitor drug effect in real-time, and measure dose response and time–kill [11]; (ii) determine any differences in pharmacokinetics between infected and uninfected mice; and (iii) expand pharmacokinetic studies to the CNS using microdialysis to provide data on the distribution of fexinidazole and its metabolites. This study aimed to provide further knowledge on the relationship between drug exposure, parasite distribution and rate of kill, as well as to test the full potential of the mouse imaging model.

2. Methods and materials

2.1. Parasites

The red-shifted bioluminescent *T. b. brucei* strain GVR35-VSL-2 [10] was used in all infection studies. This parasite strain produces a chronic infection in mice 21 d post-infection (D21 p.i.), as detailed in previous studies [11].

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2.2. Drug sources

Fexinidazole was kindly provided by Dr Benedict Blayney, Sanofi, France. Fexinidazole sulfone and sulfoxide were generously donated by Dr Stephen Patterson from Drug Discovery Unit, University of Dundee, UK.

2.3. Animal models

The experimental protocol was performed with the approval of the UK Home Office Animals (Scientific Procedures) Act 1986 and the London School of Hygiene and Tropical Medicine (LSHTM) Ethics Committee. Work undertaken at LSHTM was under the project Licence 70/8427 'Biology and control of protozoal infections'. Microdialysis experiments were performed at Pharmidex under the Home Office Project Licence 70/7723 'Discovery ADMET studies for novel therapeutics.'

Female CD1 mice weighing 20–25 g (Charles River Laboratories, Wilmington, MA, USA) were housed in specific-pathogen-free individually vented cages, and fed ad libitum. Mice were infected with 5×10^3 trypanosomes/mL from a donor mouse by the intravenous route, as detailed previously [11], and randomized into three animals per cage (two cages = one experimental group). ARRIVE guidelines were adhered to in this report [12].

2.4. Drug dose effect in vivo

The protocol to assess the drug efficacy of fexinidazole using bioluminescence imaging (BLI) has been described previously [11,13]. In brief, infected mice were grouped and treated on D21 p.i. with 200, 66.7, 22.2 or 7.4 mg/kg fexinidazole in the oral formulation carrier solution methylcellulose (0.5% w/v, Sigma, Gillingham, UK)/Tween 80 (5% v/v, Sigma) in sterile H_2O. Mice were dosed orally (p.o.) daily for 5 d. Two control groups were included in the study: an untreated control and a CNS-positive control in which mice were dosed intraperitoneally (i.p.) with 40 mg/kg diminazene aceturate (Sigma) to clear peripheral parasitaemia alone. Mice were imaged on D21 (prior to dosing), D25 (after last dose), D28 and D35 on an IVIS Lumina II (Perkin Elmer, Wokingham, UK) with the following settings: a set of exposure times; 1, 3, 10, 30, 60 and 180 s, a binning of 4, 1 f/stop, an open filter, and field of view E (12.5 × 12.5 cm) to provide a qualitative representation of rate of kill. Ten minutes prior to imaging, mice were dosed with 150 mg/kg i.p. luciferase substrate, D-Luciferin [Perkin Elmer; diluted in Dulbecco's phosphate buffered saline (PBS)] [13].

At the end of the experiment, blood was collected via cardiac puncture and mixed with a chaotropic salt (guanidine HCl) in a 50/50 solution and stored at 4 °C for DNA extraction using the High Pure polymerase chain reaction (PCR) template according to the manufacturer's instructions (Roche, Welwyn Garden City, UK). For ex-vivo imaging and quantitative PCR analysis of the brains, mice were perfused with PBS to remove blood, and the brains were snap frozen on dry ice and stored at –80 °C for later DNA extraction, also using the Roche High Pure PCR template kit. Using a primer sequence to target the invariant surface glycoprotein (ISG-75), parasite burden was determined by real-time quantitative PCR using SYBR Green (Applied Biosystems, Foster City, CA, USA) incorporation in an ABI Prism 7000 sequence detection system (Applied Biosystems) relative to the standard curve using the methods detailed previously [11].

2.5. Pharmacokinetics

2.5.1. Study details

Plasma and brain concentration profiles of the parent compound fexinidazole and its sulfoxide and sulfone metabolites were determined after oral dosing in both uninfected mice and mice infected with *T. b brucei* GVR35-VSL-2. Infected or uninfected mice were allocated at random into groups of three per sampling time point, and a single dose of fexinidazole 200 mg/kg was administered orally to both groups on D24 p.i. At eight selected time points after dosing (0.25, 0.5, 1, 2, 4, 6, 8 and 12 h), mice were culled and plasma was collected by centrifugation of heparinized blood at 2000 *g* for 10 min. The plasma was then snap frozen on dry ice and stored at –80 °C. Brain samples were also collected after perfusion with PBS via the hepatic portal vein, before being snap frozen on dry ice and stored at –80 °C.

2.5.2. Sample preparation

Brain samples were thawed and homogenized individually with a 1:1 weight:volume ratio of 0.1% v/v aqueous formic acid in a Bullet blender model BBY5E (Next Advance, Averill Park, NY, USA) for 5 min at speed 8. In a clean microcentrifuge tube, 25 µL of homogenized brain was added to 100 µL of extraction solvent (acetonitrile containing 200 ng/mL tolbutamide, an internal standard). The tube was vortex agitated for 1 min and centrifuged at 3000 *g* at 4 °C for 20 min. The supernatant was then collected and stored at –80 °C.

Plasma samples were thawed, and a 50-µL aliquot of each was added to 150 µL of extraction solvent (as used for brain sample preparation) in a clean microcentrifuge tube and vortexed for 1 min. The tube was centrifuged at 3000 *g* at 4 °C for 20 min, and the supernatant was collected and stored at –80 °C.

Calibration standards for quantification of analyte concentrations in plasma were prepared by spiking aliquots of control plasma with specific amounts of analyte to give nominal concentrations over the ranges of 1–5000 ng/mL for fexinidazole and 50–50 000 ng/mL for each of the metabolites. Calibration standards for brain were prepared by spiking control brain homogenate to give nominal concentrations over the ranges of 2–10 000 ng/g for fexinidazole and 10–40 000 ng/g for each of the metabolites. Calibration standards were prepared for analysis alongside study samples using the same method.

2.5.3. Brain and plasma sample analysis

The brain and plasma extracts were analysed for fexinidazole and its sulfoxide and sulfone metabolites using an Agilent 1290 UHPLC and autosampler combined with an Agilent 6550 QTof iFunnel mass spectrometer (Agilent Technologies, Craven Arms, UK). Extracts were thawed and diluted 1:1 with water, and 6-µL aliquots were injected on to an Agilent RRHD C18 column (50 mm × 2.1 mm, 1.8 µm) maintained at 50 °C in a column oven. Sample components were eluted from the column using a mobile phase consisting of aqueous formic acid (0.1% v/v, Channel A) and acetonitrile containing 0.1% v/v formic acid (Channel B); a gradient elution programme increased the proportion of B from 2% to 95% between 0.5 and 1.3 min p.i., maintained at 95% until 2.5 min, before returning to 2% at 2.6 min p.i.

The mass spectrometer was operated in positive ion electrospray mode scanning m/z 100–1000 in 0.3 s. The instrument was calibrated before data acquisition, and internal references were infused continuously to compensate for any drift during the course of the run. Peaks in extracted ion chromatograms [corresponding to the expected mass/charge ratios (accurate to four decimal places) of singly protonated analyte molecules, with windows of ±20 ppm] were integrated and quantified against calibration curves generated using MassHunter v B.05.01 software (Agilent).

2.6. Microdialysis

2.6.1. Surgical procedure

General anaesthesia was induced using isoflurane (5% in O_2) and maintained at 2% during the surgery. Microdialysis guide cannulae