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DOI: https://doi.org/10.1016/j.ijantimicag.2017.01.038

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PII: S0924-8579(17)30169-3
DOI: http://dx.doi.org/doi: 10.1016/j.ijantimicag.2017.01.038
Reference: ANTAGE 5115

To appear in: International Journal of Antimicrobial Agents

Received date: 19-8-2016
Accepted date: 30-1-2017


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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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ARTICLE INFO

Article history:
Received 19 August 2016
Accepted 30 January 2017

Keywords:
Pharmacokinetics
Time-kill
Bioluminescence
Fexinidazole
Microdialysis
Trypanosoma brucei
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 x 600 mg taken with food) for 4 d followed by 1200 mg/d for 6 d (2 x 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].

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 $5 \times 10^3$ 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$_2$O. 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 x 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 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 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 x 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 isofluorane (5% in O₂) and maintained at 2% during the surgery. Microdialysis guide cannulae (MAB8, Microbiotech, Stockholm, Sweden) were stereotaxically implanted simultaneously into the prefrontal cortex (PFC) (AP +2.1, DL −0.5, V −1.0) and the ventral hippocampus (VH) (AP −2.9, DL +2.7, V −1.7) (co-ordinates in mm from bregma/dura) [14] of female CD1 mice (n=3). Guide cannulae were secured with acrylic cement and a tether anchor screw (Instech, Plymouth Meeting, PA, USA) attached. On completion of surgery, animals were administered analgesics and subcutaneous saline, and placed in an incubator until they regained their righting reflex. Mice were used in experiments 48 h after surgery.

2.6.2. Microdialysis procedure

Microdialysis was performed according to methodology described previously [15]. On the day prior to the experiments, animals were placed in microdialysis cages for overnight habituation. The following morning, pins were removed from guide cannulae and replaced with microdialysis probes (2 mm Polyether Sulphone membrane, MAB8.4.2, Microbiotech, Stockholm, Sweden). Probes were perfused with artificial cerebrospinal fluid containing 1% hydroxypropyl-β-cyclodextrin, 145 mM NaCl, 2.7 mM KCl, 1.0 mM MgCl₂, 1.2 mM CaCl₂ and 10.0 mM Na₂HPO₄, pH 7.40 at 1.5 μL/min. After 1 h of equilibration, pre-administration basal microdialysate was collected for 20 min. Animals were dosed (5 mL/kg p.o.) with fexinidazole in aqueous Tween 80 (5% w/v)/methyl cellulose 400 (0.5% w/v) at 200 mg/kg. Microdialysates (45-μL aliquots) were collected in microvials containing acetonitrile (15 μL) using a 30-min sampling regime for 3.5 h.
2.6.3. Analysis of microdialysis samples

Microdialysis samples were analysed using an Agilent 1200 high-performance liquid chromatography system combined with an Agilent 6410A triple quadrupole mass spectrometer. A mobile phase of water/0.1% formic acid (Channel A) and acetonitrile/0.1% formic acid (Channel B) was used to elute sample components from a Kinetex column packed with 5 µm of C$_{18}$ material (2.1 mm x 50 mm at 50°C). The mobile phase composition was held at its initial 5% B for 0.1 min p.i., then programmed to increase linearly to 95% B at 1.2 min; after a further 0.5 min at 95% B, the composition was returned to its initial 5% B at 1.80 min p.i. Fexinidazole was detected monitoring the transition m/z 280.1 → m/z 140; its sulfoxide and sulfone metabolites were detected monitoring the transitions m/z 296.1 → m/z 140 and m/z 312.1 → m/z 140, respectively.

Analyte concentrations were quantified against calibration standards prepared in artificial cerebrospinal fluid/acetonitrile, with 4-µL aliquots of sample and standard being injected.

3. Results

3.1. Drug effect

VSL-2 infected mice were treated with fexinidazole at 7.4, 22.2, 66.7 and 200 mg/kg, six mice per dose [9]. BLI and parasitaemia were assessed prior to treatment on D21 p.i. and then on D25, 28, 30 and 35 p.i. (Fig. 1).

The BLI data showed that by D25, only the 66.7 mg/kg and 200 mg/kg doses were able to reduce the bioluminescence signal (the loss in bioluminescence can be seen
in Fig. 1A). The bioluminescence in the 7.4 mg/kg dose group decreased after treatment (indicated as blue areas in Fig. 1A), but was above background detection, whereas the 22.2 mg/kg dose group exhibited a reduction in bioluminescence, with just the highly vascular areas of the nose and feet having a detectable signal. Three days later on D28, mice treated with the highest doses of 66.7 and 200 mg/kg fexinidazole were still negative for detectable bioluminescence. Relapse of trypanosome infection occurred in the mice treated with 7.4 mg/kg fexinidazole on D28, with disseminated bioluminescence levels similar to D21, while the 22.2 mg/kg dose group also showed increased BLI levels.

By the end of the experiment (D35), three of the four doses had relapsed, with relative levels of bioluminescence indicating a dose-dependent effect (Fig. 1B). The 66.7 mg/kg treatment group, despite showing no detectable bioluminescence 7 d earlier on D28 p.i. (Fig. 1A), had early stages of drug relapse, with bioluminescence being present throughout the entire animal. Mice treated with 200 mg/kg fexinidazole showed no bioluminescence on D35, indicating that the mice were clear of infection.

In order to quantify the dose-dependent effect, the mean bioluminescence of each group was determined and shown in relation to days p.i. in Fig. 1B, demonstrating that BLI can provide both dose and time-kill effect data. All four doses showed an initial drop in bioluminescence from \( \sim 10^{10} \) photons/s to \( 2.8 \times 10^9 \) photons/s (7.4 mg/kg group), \( 8.5 \times 10^7 \) photons/s (22.2 mg/kg group) and to just above background at \( 1 \times 10^6 \) for mice treated with 66.7 mg/kg and 200 mg/kg fexinidazole. However, at the three lowest doses (7.4, 22 and 66.7 mg/kg), bioluminescence increased with the re-appearance of the infection after D28.
A rapid drop in bioluminescence from $10^{10}$ photons/s to below background detection of $6 \times 10^5$ photons/s was seen in the mice treated with 200 mg/kg fexinidazole, where it remained until the end of the experiment. The initial drop in parasitaemia was also detected in blood films (Fig. 1B) after treatment, with all treated groups except 7.4 mg/kg fexinidazole (parasitaemia of nine trypanosomes/10 fields of view) exhibiting undetectable parasitaemia.

On D28, the mice treated with 7.4 mg/kg fexinidazole had a slight increase in parasitaemia to 28 trypanosomes/10 fields of view. Mice treated with 200 mg/kg fexinidazole and diminazene aceturate remained blood-film negative until the end of the experiment.

On D35, the end of the experiment, both the *T. b. brucei* GVR35-VSL-2 and wild-type-infected mice were culled and the perfused brains were removed for quantitative PCR analysis to determine parasite burden. The excised brains of the VSL-2 mice were also imaged prior to freezing (Fig. 1C). The BLI and quantitative PCR data (Fig. 1C) of the excised brains reflected the whole animal imaging; in other words, increasing doses of fexinidazole resulted in lower bioluminescence and a decrease in parasitic burden.

### 3.2. Pharmacokinetics of fexinidazole and its metabolites.

A study to investigate the pharmacokinetics of fexinidazole and its metabolites was performed using both infected and uninfected female CD1 mice. A dose of 200 mg/kg fexinidazole was administered orally, with terminal plasma and brain samples
collected at eight time points (n=3/time point). Plasma and brain samples were extracted and analysed to generate concentration vs time profiles of the parent compound and its two major metabolites.

The profiles are illustrated in Figs 2 (plasma) and 3 (brain), and appeared to be broadly consistent with published data [4,16]. Fexinidazole was the least abundant of the three components in plasma and brain, but demonstrated good brain penetration with brain:plasma concentration ratios of 4.0 for uninfected animals and 1.8 for infected animals (based on AUC$_{0-12}$). Fexinidazole sulfoxide and sulfone plasma concentrations were higher than those of unchanged drug, consistent with its extensive first-pass metabolism. They were also greater in the brain despite both metabolites being considerably less brain penetrant than the parent compound (brain:plasma concentration ratios of 0.20 and 0.18 for sulfoxide and 0.15 and 0.13 for sulfone for uninfected and infected animals, respectively).

The total exposure of fexinidazole and its two main metabolites in plasma and brain were similar between the infected and uninfected groups (Tables 1 and 2). There is perhaps a suggestion of a trend for exposure in the infected group to be lower, but the relatively high variability observed for concentrations at replicate time points means that this cannot be confirmed.

3.3. **Microdialysis**

Recovery of fexinidazole in microdialysates was estimated using the in-vitro recovery method [16]. In the presence of 1% 2-hydroxypropyl-β-cyclodextrin, recovery of fexinidazole was 14.7% at 25 ng/mL and 13.8% at 100 ng/mL. Recoveries for the
sulfoxide and sulfone metabolites were not determined, so only qualitative comparisons between concentrations of fexinidazole and its metabolites are discussed. Concentrations quoted for fexinidazole and its metabolites have all been corrected by a factor corresponding to the mean recovery for fexinidazole at the two concentrations tested. Concentrations quoted for metabolites may therefore be subject to additional error due to the potential for differences in recovery.

The microdialysis studies showed that unbound fexinidazole and its metabolites were present in the interstitial fluid (ISF) of both brain regions investigated in this study (PFC and VH). Plots of concentration against sampling time (Fig. 4) show fairly marked interindividual variability. For example, Mouse 2 showed $C_{\text{max}}$ for fexinidazole of ca. 60 ng/mL in both PFC and VH, with the other two replicates showing $C_{\text{max}}$ of <5 ng/mL. However, concentration vs time profiles within individual animals for the three compounds in ISF sampled from PFC and VH showed consistent trends.

4. Discussion

Previous publications [10,17,18] have shown how the BLI mouse model of infection for HAT can be used to identify compounds with activity against the CNS stage of infection, and significantly shorten the time of the assay. The present study shows how the model can be used to determine: (i) the dose–response effect and rate of kill; (ii) the difference in pharmacokinetics of a drug between infected and uninfected mice; and (iii) the actual free drug concentrations in the CNS compartment using microdialysis.
The use of static pharmacokinetic/pharmacodynamic models to evaluate preclinical drug profiles has not accounted for drug–parasite interactions or how well the drug distributes through both the plasma and tissue [19]. By using a dynamic model that looks at the relationship between drug distributions within the plasma and tissue and the rate of kill of a drug (concentration and/or time dependent), an understanding of drug exposure can be established [20]. By using the bioluminescence model to detect the dose-dependent effect of fexinidazole, the time-kill properties of the drug during a real-time infection were assessed, showing that fexinidazole is dose dependent as relapse occurred early at the lower doses of 22 mg/kg and 66.7 mg/kg.

Comparing the pharmacokinetic profiles of fexinidazole and metabolites between both the infected and uninfected mice, it appears that trypanosome infection did not alter the metabolism and disposition of fexinidazole significantly.

Both fexinidazole and its sulfoxide metabolite showed two maxima in their concentration vs time profiles in both plasma and brain. The first $T_{\text{max}}$ was ca. 0.5–1.0 h post-dose with a secondary maximum in concentration at the 6-h time point. This was seen in all replicates in both plasma and brain, and is perhaps a consequence of a food effect as observed by Tarral et al. [8]. The sulfone metabolite showed a single $T_{\text{max}}$ at 6 h in both plasma and brain, with mean $C_{\text{max}}$ in uninfected animals being approximately twice that in infected animals; this was the only indication of a significant difference in pharmacokinetic profiles between the two groups (plasma $P=0.0012$, brain $P=0.0376$).
The microdialysis data suggest that there is a homogenous distribution of fexinidazole and its metabolites within the grey matter of the brain, with similar levels present in the PFC and VH for individual animals. Large variability in measured concentrations of all three drug components was observed between individuals, frequently up to and sometimes beyond 10-fold (e.g. the sulfoxide concentrations for the 2.0–2.5 h samples were measured as 192, 4093 and 1801 ng/mL in the three individuals). Despite there being low variability of concentration profiles for fexinidazole and metabolites in the ISF from PFC and VH within animals, there was high variability between animals, with $C_{\text{max}}$ values ranging from 9 ng/mL to 372 ng/mL for fexinidazole from the PFC. Interindividual variability was also seen in human plasma concentrations after the administration of a single dose, and was noted to be apparent with the parent and metabolites [8].

Fexinidazole will be used to treat HAT patients with a dose regimen of 1800 mg/d for 4 d followed by 1200 mg/day for 6 d [8]. It is not possible to relate this to the mouse dosing from 200 mg/kg, although there are estimates of allometric scaling of 12 fold between human and mouse [21]. Other factors that have an impact, such as BBB permeation which differs between infected mice and humans [22], parasite distribution and burden, have not been determined.

Fexinidazole has shown promising results in other kinetoplastid diseases, with its efficacy also being assessed against Chagas disease and leishmaniasis [23]. Studies carried out on Chagas disease found that the active metabolites of sulfoxide and sulfone were more effective in curing an acute in-vivo mouse model of *T. cruzi* than the parent drug [23]. In visceral leishmaniasis, both fexinidazole and its
metabolites were shown to be effective orally, with good distribution and absorption of the drug in the target organs of the liver and spleen [24]. These studies suggest that oral fexinidazole has potential as a new treatment for all kinetoplastid diseases.

**Acknowledgements**

The authors wish to thank John Kelly and Martin Taylor (London School of Hygiene and Tropical Medicine) for providing *T. b. brucei* GVR35 VSL-2, and Professor Jeremy Mottram (Glasgow University) for providing the wild-type strain used in this study.

**Funding:** This work was supported by the Bill and Melinda Gates Foundation Global Health Program (Grant Number OPPGH5337).

**Competing interests:** None declared.

**Ethical approval:** This study was approved by the London School of Hygiene and Tropical Medicine Animal Welfare and Ethics Review Board.

**References**


Fig. 1. Bioluminescence imaging to determine dose-dependent effect in vivo. Determining fexinidazole drug efficacy using bioluminescence and quantitative polymerase chain reaction (PCR). Infected CD1 mice were split into groups of six and treated with varying doses of fexinidazole from Day 21 and monitored until Day 35. (A) A single representative mouse (one mouse from group of six) from each dose, showing bioluminescence on a heat map scale (red representing maximum flux) before and after treatment. (B) Quantification of bioluminescence and parasitaemia by averaging (of six mice) total flux and parasite number, respectively. In the top graph, each data point represents the mean ± standard deviation (SD) of the bioluminescence analysis as determined by the ROI of the whole animal as detailed [13]. Red line indicates background bioluminescence determined from bioluminescence imaging of a wild-type-infected mouse. The average microscopic counts from Giemsa-stained blood films, each data point is mean ± SD. (C) Ex-vivo imaging of perfused brain and related quantitative PCR. Parasite burden determined by extraction of DNA from brain homogenates from each of the mice and quantitative PCR analysis. Graph shows mean of six mice ± SD of the number of parasites in 50 ng of DNA. Ex-vivo images were obtained by adding 50 μL of luciferin to perfused brains and imaging using the IVIS Lumina II. The detection limit of the quantitative PCR was 50 trypanosomes/50 ng of DNA.

Fig. 2. Plasma concentration profiles of fexinidazole and metabolites in infected and uninfected mice. Plasma concentration data are presented as the mean (ng/mL) ± standard deviation of three mice. A two-way analysis of variance analysis indicates that there is no significant difference between concentrations in infected and uninfected mice, with the following P values for parent and metabolites; fexinidazole
An unpaired Student’s *t*-test was performed to determine the significance between fexinidazole sulfone concentrations in infected and uninfected mice at 6 h post dose; the analysis showed that there was a significant difference between the two sets of data (*P* = 0.0012). **P ≤ 0.01.

Fig. 3. Brain concentration profiles of fexinidazole and metabolites in infected and uninfected mice. Brain concentration data are presented as the mean (ng/mL) ± standard deviation of three mice. A two-way analysis of variance analysis indicates that there is no significant difference between concentrations in infected and uninfected mice with the following *P* values for parent and metabolites; fexinidazole *P* = 0.9844, fexinidazole sulfoxide *P* = 0.3605, fexinidazole sulfone *P* = 0.0745. An unpaired Student’s *t*-test was performed to determine the significant difference between fexinidazole sulfone concentrations in infected and uninfected mice at 6 h post dose; the analysis showed that there was a significant difference between the two sets of data (*P* = 0.0376). *P ≤ 0.05.

Fig. 4. Assessment of concentrations of fexinidazole and metabolites in brain interstitial fluid (ISF) samples by microdialysis. Two areas of the brain were sampled serially over 3.5 h: the prefrontal cortex and the ventral hippocampus. ISF concentration data (ng/mL) are presented individually for the three mice, with the concentrations of each compound corrected for probe recovery of fexinidazole (values quoted for metabolites are thus subject to error if recoveries were different from that of fexinidazole). (A) Concentration profiles of drug and metabolites within
the prefrontal cortex. (B) Concentration profiles of drug and metabolites within the ventral hippocampus.
Table 1. Total plasma exposure of fexinidazole and metabolites in infected and uninfected mice

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Table 2. Total brain exposure of fexinidazole and metabolites in infected and uninfected mice

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