PLOS NEGLECTED TROPICAL DISEASES

RESEARCH ARTICLE

Enantiospecific antitrypanosomal *in vitro* activity of eflornithine

Mikael Boberg[1], Monica Cal[2,3], Marcel Kaiser[2,3], Rasmus Jansson-Löfmark[1,4], Pascal Mäser[2,3], Michael Ashton[1]*

1 Unit for Pharmacokinetics and Drug Metabolism, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden, **2** Parasite Chemotherapy Unit, Department of Medical Parasitology and Infection Biology, Swiss Tropical and Public Health Institute, Basel, Switzerland, **3** University of Basel, Basel, Switzerland, **4** DMPK, Research and Early Development Cardiovascular, Renal and Metabolism, BioPharmaceuticals R&D, AstraZeneca, Gothenburg, Sweden

* michael.ashton@gu.se

OPEN ACCESS

Citation: Boberg M, Cal M, Kaiser M, Jansson-Löfmark R, Mäser P, Ashton M (2021) Enantiospecific antitrypanosomal *in vitro* activity of eflornithine. PLoS Negl Trop Dis 15(7): e0009583. https://doi.org/10.1371/journal.pntd.0009583

Editor: Margaret A. Phillips, University of Texas Southwestern Medical School, UNITED STATES

Received: March 30, 2021

Accepted: June 21, 2021

Published: July 12, 2021

Data Availability Statement: Data are available from the Swedish National Data Service database with SND-ID: 2021-45, https://snd.gu.se/en/catalogue/study/2021-45.

Funding: The Swedish Research Council (2016-05780), www.vr.se/english.html (MA), and Swiss Tropical and Public Health Institute, www.swisstph.ch/en/ (PM), funded the project. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Abstract

The polyamine synthesis inhibitor eflornithine is a recommended treatment for the neglected tropical disease Gambian human African trypanosomiasis in late stage. This parasitic disease, transmitted by the tsetse fly, is lethal unless treated. Eflornithine is administered by repeated intravenous infusions as a racemic mixture of L-eflornithine and D-eflornithine. The study compared the *in vitro* antitrypanosomal activity of the two enantiomers with the racemic mixture against three *Trypanosoma brucei gambiense* strains. Antitrypanosomal *in vitro* activity at varying drug concentrations was analysed by non-linear mixed effects modelling. For all three strains, L-eflornithine was more potent than D-eflornithine. Estimated 50% inhibitory concentrations of the three strains combined were 9.1 μM (95% confidence interval [8.1; 10]), 5.5 μM [4.5; 6.6], and 50 μM [42; 57] for racemic eflornithine, L-eflornithine and D-eflornithine, respectively. The higher *in vitro* potency of L-eflornithine warrants further studies to assess its potential for improving the treatment of late-stage Gambian human African trypanosomiasis.

Author summary

The neglected tropical disease human African trypanosomiasis is lethal unless treated. One of the treatments for the late stage–i.e. when parasites have invaded the central nervous system–of Gambian human African trypanosomiasis is the drug eflornithine, which is dosed as 50:50 racemic mixture of the two enantiomers L-eflornithine and D-eflornithine. This study showed that L-eflornithine was better than D-eflornithine at inhibiting the growth of parasites *in vitro*. The 50% inhibitory concentration for L-eflornithine was 5.5 μM in comparison to 50 μM for D-eflornithine. This higher *in vitro* potency for L-eflornithine warrants further studies to assess its potential as an improved treatment for late-stage Gambian human African trypanosomiasis.

Competing interests: The authors have declared that no competing interests exist.

Introduction

The neglected tropical disease human African trypanosomiasis (HAT), also known as sleeping sickness, is fatal unless treated. The amino acid analogue DL-alpha-difluoromethylornithine, known as eflornithine, was first developed for oncological use [1] and later discovered to have antitrypanosomal activity [2]. Eflornithine, included in the World Health Organization (WHO) model list of essential medicines [3], is dosed intravenously, commonly together with oral nifurtimox, to treat the late stage of Gambian HAT [4–7], which account for 98% of the total HAT cases [8]. The intravenous administration of eflornithine requires hospital-like settings. Treatment accessibility in rural areas would increase if an oral eflornithine treatment was available with easier and less costly logistics [9]. However, clinical trials with oral racemic eflornithine have failed to achieve sufficiently high systemic exposure, most likely due to poor bioavailability at maximum tolerated oral dose [9,10]. The two enantiomers, L- and D-eflornithine, both inhibited the target enzyme ornithine decarboxylase (ODC) in a cell free assay with human ODC [11]. However, the potential difference in antitrypanosomal efficacy on a parasite level may limit the possibility for oral treatment since the enantiomers differ in their oral bioavailability [12]. This study aimed to investigate the antitrypanosomal *in vitro* activities of racemic eflornithine, L-eflornithine and D-eflornithine against three *Trypanosoma brucei* (*T.b.*) *gambiense* strains to support whether a future late-stage Gambian HAT treatment with a potentially more active enantiomer would be feasible or not.

Materials and methods

Compounds

Eflornithine hydrochloride was donated by the UNICEF/UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Disease ([TDR], Geneva, Switzerland). L-eflornithine and D-eflornithine were separated from the racemic mixture by semi-preparative liquid chromatography [13]. Racemic eflornithine, L-eflornithine and D-eflornithine were dissolved in sterile water for the *in vitro* activity assay and diluted in culture medium before incubation of *T.b. gambiense* parasites in 96-well plates.

Parasites and cell culture conditions

The *T.b. gambiense* strain STIB930 is a derivative of the strain TH1/78E (031), which was isolated in 1978 from a patient in Côte d'Ivoire [14]. The K03048 strain was isolated from a patient in South Sudan in 2003 [15]. The 130R strain was isolated 2003 from a patient in the Democratic Republic of the Congo [16]. Parasite incubation conditions were 37˚C, 5% CO_2 atmosphere, in HMI-9 medium [17] with fetal bovine serum and human serum, 15% and 5%, respectively. Parasites were subcultured at appropriate dilutions every two to three days to ensure maintenance in exponential growth phase.

In vitro growth inhibition assays

Racemic eflornithine, L-eflornithine and D-eflornithine were tested in an AlamarBlue serial drug dilution assay, described in detail elsewhere [18], in order to quantify parasite growth inhibition. In brief, serial drug dilutions were prepared in 96-well microtiter plates containing HMI-9 medium. Pre-experimental parasites counts were obtained using a CASY cell counter (OLS OMNI Life Science, Bremen, Germany) before the wells were inoculated with 100,000 *T. b. gambiense* parasites and incubated for 72 hours. The fluorescent agent resazurin was added before the plates were incubated for another four to six hours. SpectraMax Gemini XS microplate fluorescence scanner was used to read the plates at the excitation and emission

wavelengths 536 nm and 588 nm, respectively. To determine the *in vitro* growth inhibition, the study was conducted with five independent experiments for the STIB930 *T.b. gambiense* strain with racemic eflornithine and seven with L-eflornithine or D-eflornithine, respectively. Four independent experiments were performed for the K03048 and 130R *T.b. gambiense* strains with racemic eflornithine and six with L-eflornithine or D-eflornithine, respectively. Time-dependence for the drug exposure was studied for racemic eflornithine, L-eflornithine and D-eflornithine in a series of *in vitro* growth inhibition assays where the *T.b. gambiense* strain STIB930 was under drug exposure for 24, 48 or 72 hours. All other parts of the experiment followed a similar protocol as the AlamarBlue serial drug dilution assay and plate readings as previously described. Racemic eflornithine, L-eflornithine and D-eflornithine were tested in an *in vitro* cytotoxicity assay with L6 rat skeletal myoblast cells using a protocol described in full elsewhere [19]. The positive control in the cytotoxicity *in vitro* assay was podophyllotoxin with a known 50% inhibitory concentration (IC_{50}) of 0.02 μM (0.007 μg/mL).

Data and statistical analyses

Eq 1 was fitted to the antitrypanosomal *in vitro* activity data using non-linear mixed effects modelling as implemented in Phoenix software (Version 8.2, Certara, Princeton, NJ, USA). Firstly, each combination of compound and parasite strain was fitted separately by naïve pooled data analysis to estimate IC_{50}, sigmoidicity factor gamma (γ) that characterizes the concentration-inhibition relationship steepness and maximum inhibition (I_{max}) where I_0 represents the baseline effect without drug exposure according to:

$$\text{Inhibition} = I_0 - \frac{I_{max} \times \text{Concentration}^{\gamma}}{IC_{50}^{\gamma} + \text{Concentration}^{\gamma}} \quad (1)$$

In a second step, each compound was separately fitted to pooled data for all strains. For model validation, parameter estimate plausibility was assessed and bootstrap (n = 1000) using the first-order conditional estimate-extended least square method was performed. The bootstrap estimates were used to establish the 5^{th} and 95^{th} percentiles for the model predictions. Differences in parameter estimates from the bootstrap were assessed as statistically significant for 95% confidence intervals (95% CI) without overlap. For discrimination between nested models with $\gamma = 1$ or estimated γ in non-linear mixed effects modelling, a decrease in -2 log likelihood over 3.84 for the more complex model was regarded as statistically significant ($P < 0.05$) with an assumed χ^2 distribution for the difference in -2 log likelihood. Plots and statistical analysis were made using Rstudio (Version 1.3.1093) with the R software (Version 4.0.3, 2020, The R foundation for Statistical Computing).

Results

Antitrypanosomal *in vitro* activity against STIB930, K03048 and 130R

All compounds inhibited the growth of the three *T.b. gambiense* strains in a concentration-dependent manner (Fig 1). L-eflornithine had the lowest IC_{50} estimates throughout, with 4.1 μM (95% CI 3.1; 5.0), 8.9 μM (7.0; 11) and 7.7 μM (6.8; 8.5) for strains STIB930, K03048 and 130R, respectively. D-eflornithine was less potent with IC_{50} estimates of 39 μM (29; 49), 73 μM (62; 85) and 76 μM (66; 86) for the same strains. IC_{50} values for racemic eflornithine were 6.4 μM (5.2; 7.7), 17 μM (15; 18) and 14 μM (12; 17) for STIB930, K03048 and 130R, respectively (Table 1).

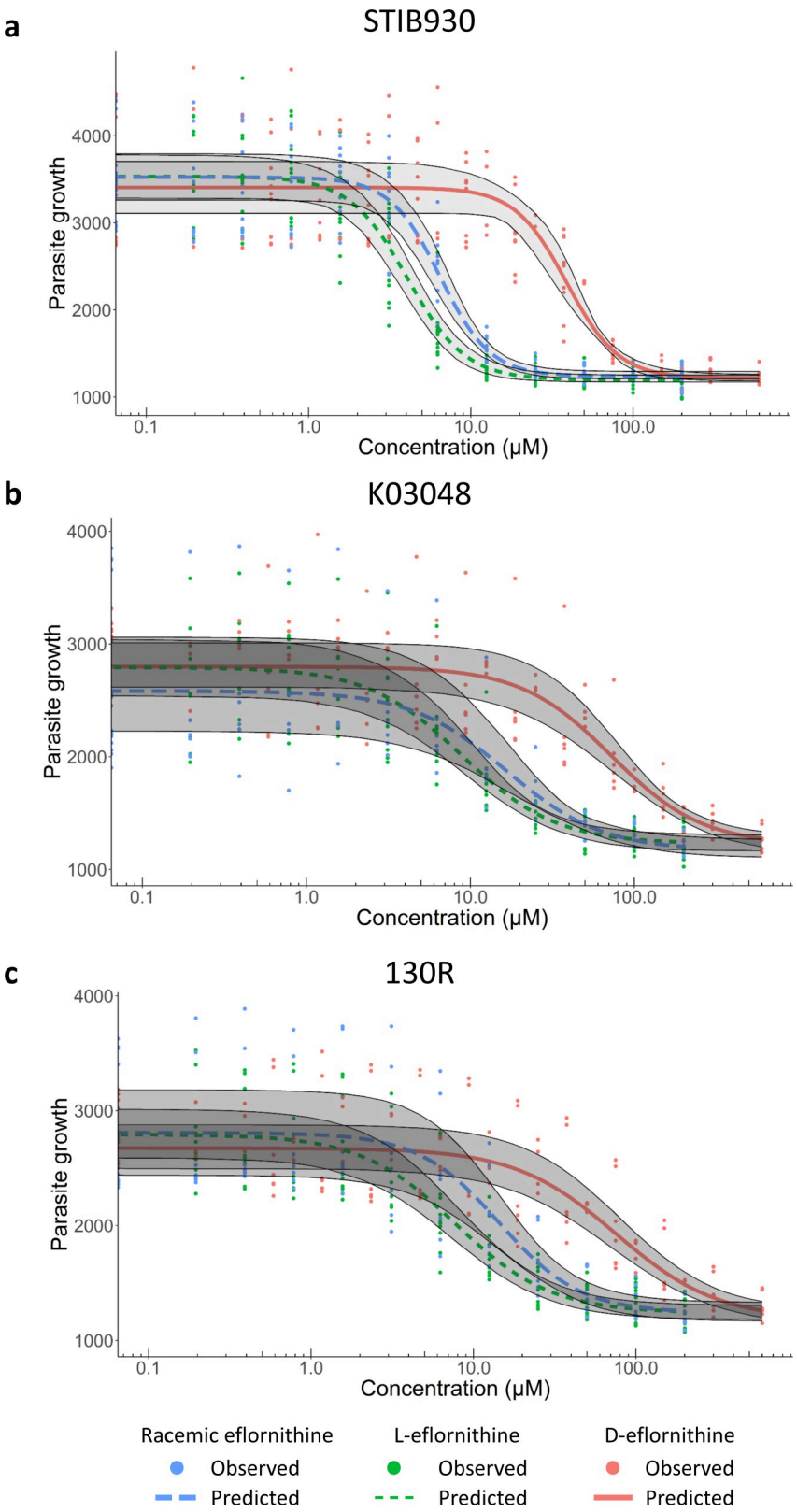

Fig 1. Antitrypanosomal *in vitro* activity for racemic eflornithine and its enantiomers against three different *T.b. gambiense* strains. *In vitro* activity for racemic eflornithine (blue), L-eflornithine (green) and D-eflornithine (red) against *T.b. gambiense* strains a) STIB930, b) K03048 and c) 130R. Parasite growth values are shown as relative

fluorescence in the AlamarBlue serial drug dilution assay. Dots represent observed experimental data, lines the model predictions and grey areas the 5th to 95th percentiles of the model prediction central values.

https://doi.org/10.1371/journal.pntd.0009583.g001

Growth inhibition analysis for all strains pooled

Pooling the data for the three strains resulted in IC_{50} estimates (95% CI) of 9.1 μM (8.1; 10), 5.5 μM (4.5; 6.6), and 50 μM (42; 57) for racemic eflornithine, L-eflornithine and D-eflornithine, respectively. The sigmoidicity factor γ and I_{max} values were similar for racemic eflornithine, L-eflornithine and D-eflornithine (Table 1). The 5th to 95th percentiles for model predictions did not overlap at concentrations close to IC_{50} values for the three treatments (Fig 2). The overall *in vitro* 90% inhibitory concentration (IC_{90}) for racemic eflornithine, L-eflornithine and D-eflornithine were 25 μM, 17 μM and 166 μM, respectively. The growth inhibition was time-dependent as the concentration antitrypanosomal *in vitro* activity relationship after 72 h was steeper, and with a lower IC_{50} estimate compared to the IC_{50} estimates for 24 h and 48 h drug exposure times (S1 Fig and S1 Table). No observations of *in vitro* cytotoxicity were made in the L6 cell assay at relevant *in vitro* concentrations for racemic eflornithine, L-eflornithine and D-eflornithine whereas the positive control podophyllotoxin was cytotoxic with an expected IC_{50} at approximately 0.02 μM (S2 Fig).

Discussion

The enantiospecific eflornithine antitrypanosomal activity is to the best of our knowledge documented herein for the first time. The overall *in vitro* potency of L-eflornithine was about 9-fold higher than D-eflornithine against three *T.b. gambiense* strains. As a result, the IC_{50} estimate for racemic eflornithine was approximately twice that of L-eflornithine due to 1:1 inclusions of much less potent D-eflornithine. The difference in antitrypanosomal activity could possibly be due to an enantioselective eflornithine transport into the *T.b. gambiense* parasites since the enantiomers appear to have similar inactivation properties on an enzyme level [11]. Clinically, total eflornithine concentrations in cerebrospinal fluid over 50 μM, equating to approximately 5.5 times the overall *in vitro* IC_{50} in the present study, have been associated with efficient parasite eradication in late-stage Gambian HAT patients after intravenous

Table 1. IC_{50}, gamma and I_{max} estimates for racemic eflornithine, L-eflornithine and D-eflornithine in three different *T.b. gambiense* strains and overall across all strains.

Parameter	Drug	STIB930 Estimate (95% CI)	K03048 Estimate (95% CI)	130R Estimate (95% CI)	Overall Estimate (95% CI)
IC_{50} (μM)	Racemic eflornithine	6.4 (5.2 to 7.7)	17 (15 to 18)	14 (12 to 17)	9.1 (8.1 to 10)
	L-eflornithine	4.1 (3.1 to 5.0)	8.9 (7.0 to 11)	7.7 (6.8 to 8.5)	5.5 (4.5 to 6.6)
	D-eflornithine	39 (29 to 49)	73 (62 to 85)	76 (66 to 86)	50 (42 to 57)
Gamma	Racemic eflornithine	2.8 (2.4 to 3.4)	1.5 (1.3 to 1.6)	1.7 (1.4 to 1.9)	1.7 (1.5 to 2.1)
	L-eflornithine	2.5 (1.9 to 3.2)	1.5 (1.3 to 1.7)	1.5 (1.4 to 1.6)	1.6 (1.3 to 1.8)
	D-eflornithine	2.8 (2.0 to 4.0)	1.6 (1.2 to 1.8)	1.4 (1.1 to 1.7)	1.7 (1.3 to 2.2)
I_{max}	Racemic eflornithine	0.95 (0.92 to 0.97)	0.93 (0.88 to 0.98)	0.94 (0.91 to 1.0)	0.94 (0.91 to 0.98)
	L-eflornithine	0.96 (0.94 to 0.98)	0.92 (0.87 to 0.95)	0.95 (0.92 to 0.98)	0.94 (0.92 to 0.97)
	D-eflornithine	1.0 (0.96 to 1.0)	0.93 (0.88 to 0.99)	0.93 (0.89 to 0.98)	0.97 (0.93 to 1.0)
Residual variability	Racemic eflornithine	0.12 (0.090 to 0.14)	0.15 (0.054 to 0.22)	0.15 (0.068 to 0.18)	0.18 (0.15 to 0.19)
	L-eflornithine	0.13 (0.11 to 0.15)	0.13 (0.092 to 0.17)	0.12 (0.082 to 0.14)	0.16 (0.14 to 0.17)
	D-eflornithine	0.14 (0.11 to 0.17)	0.14 (0.089 to 0.18)	0.14 (0.085 to 0.17)	0.17 (0.15 to 0.19)

Parameters were estimated with bootstrap (n = 1000), 95% CI– 95% confidence interval

https://doi.org/10.1371/journal.pntd.0009583.t001

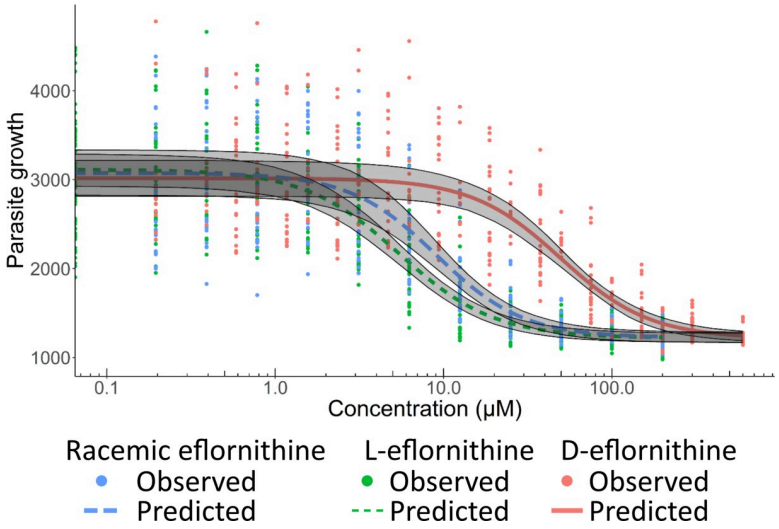

Fig 2. The overall antitrypanosomal *in vitro* activity was elicited by the more active L-eflornithine enantiomer. Antitrypanosomal activity for racemic eflornithine (blue), L-eflornithine (green) and D-eflornithine (red) against three *T.b. gambiense* strains collectively. Parasite growth values are shown as relative fluorescence in the AlamarBlue serial drug dilution assay. Dots represent observed experimental data, lines the model predictions and grey areas the 5th to 95th percentiles of the model prediction central values.

https://doi.org/10.1371/journal.pntd.0009583.g002

infusions of racemic eflornithine [9,20]. The higher potency for L-eflornithine observed in the present study suggests that this threshold value could potentially be decreased by approximately 50% if pure L-eflornithine were administered. Supporting this hypothesis, cerebrospinal fluid concentrations over 23 μM for L-eflornithine, equating to approximately 4 times the overall *in vitro* IC_{50} in the present study, were associated, however not statistically significant, with probability of cure in a clinical study of 25 patients when treated with racemic eflornithine orally [12]. A prospective clinical trial investigating the clinical efficacy of L-eflornithine dosed intravenously and orally at appropriate, tolerated doses could elucidate the clinical potential for L-eflornithine. The pharmacological effect of eflornithine in late-stage Gambian HAT may be expected to depend predominantly on unbound L-eflornithine concentration in the systemic circulation and central nervous system. The plasma protein binding for racemic eflornithine has been reported as negligible [21]. Total eflornithine concentrations are in such case expected to be identical to unbound concentrations and available to target the *T.b. gambiense* parasites. The IC_{50} values for the antitrypanosomal *in vitro* activity in the present study could therefore, with more confidence, be translated to *in vivo* relevant concentrations. The pharmacodynamic effect and cure can be seen as conditioned by critical interactions between the drug, the patient and the *T.b. gambiense* parasite as discussed for other antimicrobial agents [22].

In a more pharmacological and dose-finding oriented perspective, as discussed for antimalarial treatments, the *in vitro* IC_{90} can be used as a free drug minimum inhibitory concentration surrogate [23]. This approach has been successful when, for instance, translating *in vitro* findings to clinically relevant minimum inhibitory concentration proxy [24]. For Gambian HAT, the IC_{90} values in the present study for L-eflornithine and racemic eflornithine at 17 and 25 μM, respectively, were exceeded in serum and cerebrospinal fluid after fourteen days of racemic eflornithine treatment with two-hour intravenous infusions at 100 mg/kg four times per day [25]. Currently, the clinical posology for racemic eflornithine is 200 mg/kg twice daily when combined with nifurtimox [26]. Extrapolation of *in vitro* IC_{50} or IC_{90} to *in vivo* relevant

values of efficacious unbound drug concentration in plasma may be fraught with error since effects also depend on whether the drug reaches its target tissue and on the role of the immune system *in vivo* [27]. Uptake of eflornithine into the central nervous system is low leading to a poor partitioning between plasma and brain or cerebrospinal fluid [28,29]. The reported clinical cerebrospinal fluid to plasma or serum ratios range from 0.1 to 0.5 [9,12,25]. Eflornithine partitioning from plasma to cerebrospinal fluid appears to be non-stereoselective when administered as a racemate orally [12]. Additionally, it is important to take the factors of target occupancy, target turnover and active metabolites into account in *in vitro–in vivo* extrapolation. For eflornithine, no metabolites have been identified, hence can not contribute to pharmacological effects [30]. Moreover, since eflornithine can be seen as a slow acting compound [21], and trypanostatic rather than trypanocidal [31], the pharmacokinetic/pharmacodynamic relationship is important to consider as drug transporters in the body and/or *T.b. gambiense* parasites involved in the drug disposition could affect the clinical efficacy of eflornithine.

Only three *T.b. gambiense* strains were tested in the study which is a limitation. Granted, an analysis with more strains would render more generalizable approximations when extrapolating from the *in vitro* results to the clinic. Eflornithine resistance has been associated with non-expression of the *TbAAT6* transporter gene [32]. This TbAAT6-dependent eflornithine transport into *T.b. gambiense* parasites has been investigated further where lines of trypanosomes showed lower sensitivity to eflornithine when the *TbAAT6* transporter gene was silenced [33]. If the uptake by this amino acid transporter disfavours D-eflornithine, it might contribute to the observed higher *in vitro* activity for L-eflornithine in the present study. Radiolabelled compound could be used to decouple the potentially enantioselective transport of eflornithine into *T.b. gambiense* parasites. *In vivo* studies with L-eflornithine would potentially increase the confidence in the presented findings; however, the experiments mentioned above were assessed as outside of the study scope.

To achieve and sustain global elimination of HAT [34], it is imperative to design, make, test and analyse results for novel compounds in the pipeline. For both patients and care givers, an oral route of administration of drugs would be much preferred. Oral administration of racemic eflornithine has been investigated in clinical [9,20,21,25,35–38] and preclinical [12,39,40] studies but the antitrypanosomal efficacy and tolerability of enantiopure L-eflornithine is still to be investigated. The mechanisms and the potential enantioselectivity of the noted gastrointestinal side effects in the clinical studies with oral racemic eflornithine remain so far unknown. An oral alternative HAT treatment, fexinidazole, has been approved [41,42] and is first line treatment for patients with a cerebrospinal fluid leucocyte count less than 100 per μL. Acoziborole is currently in clinical trials [43]. Overall, these advances are important to achieve global elimination of HAT.

In conclusion, the present study showed that the L-eflornithine enantiomer elicited higher antitrypanosomal *in vitro* activity, as it was more effective than D-eflornithine against three different *T. b. gambiense* strains *in vitro*. This knowledge could be used in the future to predict *in vivo* efficacious doses of the more active L-eflornithine enantiomer using pharmacokinetic/pharmacodynamic models to assess the feasibility of L-eflornithine treatment for late-stage Gambian HAT.

Supporting information

S1 Fig. Time-dependent antitrypanosomal *in vitro* activity for eflornithine and its enantiomers. Time-dependent *in vitro* activity for a) racemic eflornithine (blue dashed lines), L-eflornithine (green small dashed lines) and D-eflornithine (red full lines) after 24 h (thick lines), 48 h (medium lines) and 72 h (thin lines) of drug exposure. Parasite growth values are shown as

relative fluorescence in the AlamarBlue serial drug dilution assay. Dots represent observed experimental data and lines the model predictions. b) Mean IC_{50} values with error bars showing the standard error of the estimates for racemic eflornithine (blue dashed line), L-eflornithine (green dotted line) and D-eflornithine (red full line) after different drug exposure times. Please note the log_{10} scale on the y-axis in S1b Fig.
(TIFF)

S2 Fig. *In vitro* cytotoxicity assay for racemic eflornithine, its enantiomers and podophyllotoxin. a) *In vitro* activity against L6 cells for racemic eflornithine (blue), L-eflornithine (green) and D-eflornithine (red) and b) *in vitro* activity for the positive control podophyllotoxin (dark blue). L6 cell growth values are shown as relative fluorescence in the assay. Dots represent observed experimental data and the coloured lines the model predictions.
(TIFF)

S1 Table. IC_{50}, gamma, I_{max} and residual variability estimates in the time-dependent assay for racemic eflornithine, L-eflornithine and D-eflornithine.
(DOCX)

Acknowledgments

The authors would like to acknowledge Professor Reto Brun for his support.

Author Contributions

Conceptualization: Mikael Boberg, Marcel Kaiser, Rasmus Jansson-Löfmark, Pascal Mäser, Michael Ashton.

Data curation: Mikael Boberg, Monica Cal, Marcel Kaiser, Rasmus Jansson-Löfmark, Pascal Mäser, Michael Ashton.

Formal analysis: Mikael Boberg, Monica Cal, Marcel Kaiser, Rasmus Jansson-Löfmark, Pascal Mäser, Michael Ashton.

Funding acquisition: Pascal Mäser, Michael Ashton.

Investigation: Mikael Boberg, Monica Cal, Marcel Kaiser, Rasmus Jansson-Löfmark, Pascal Mäser, Michael Ashton.

Methodology: Mikael Boberg, Monica Cal, Marcel Kaiser, Rasmus Jansson-Löfmark, Pascal Mäser, Michael Ashton.

Project administration: Mikael Boberg, Monica Cal, Marcel Kaiser, Pascal Mäser, Michael Ashton.

Resources: Marcel Kaiser, Pascal Mäser, Michael Ashton.

Software: Mikael Boberg, Rasmus Jansson-Löfmark.

Supervision: Monica Cal, Marcel Kaiser, Rasmus Jansson-Löfmark, Pascal Mäser, Michael Ashton.

Validation: Mikael Boberg, Monica Cal, Marcel Kaiser, Rasmus Jansson-Löfmark, Pascal Mäser, Michael Ashton.

Visualization: Mikael Boberg, Monica Cal, Marcel Kaiser, Rasmus Jansson-Löfmark, Pascal Mäser, Michael Ashton.

Writing – original draft: Mikael Boberg, Monica Cal, Marcel Kaiser, Rasmus Jansson-Löfmark, Pascal Mäser, Michael Ashton.

Writing – review & editing: Mikael Boberg, Monica Cal, Marcel Kaiser, Rasmus Jansson-Löfmark, Pascal Mäser, Michael Ashton.

References

1. Coyne PE Jr. The eflornithine story. J Am Acad Dermatol. 2001; 45(5):784–6. Epub 2001/10/19. https://doi.org/10.1067/mjd.2001.117853 PMID: 11606936.
2. Bacchi CJ, Nathan HC, Hutner SH, McCann PP, Sjoerdsma A. Polyamine metabolism: a potential therapeutic target in trypanosomes. Science (New York, NY). 1980; 210(4467):332–4. Epub 1980/10/17. https://doi.org/10.1126/science.6775372 PMID: 6775372.
3. WHO. WHO model list of essential medicines, 16th list March 2009. 2009;Available from www.who.int/medicines/publications/essentialmedicines/en/, Accessed Feb 8, 2021.
4. Priotto G, Kasparian S, Mutombo W, Ngouama D, Ghorashian S, Arnold U, et al. Nifurtimox-eflornithine combination therapy for second-stage African Trypanosoma brucei gambiense trypanosomiasis: a multicentre, randomised, phase III, non-inferiority trial. Lancet. 2009; 374(9683):56–64. https://doi.org/10.1016/S0140-6736(09)61117-X PMID: 19559476.
5. Checchi F, Piola P, Ayikoru H, Thomas F, Legros D, Priotto G. Nifurtimox plus Eflornithine for late-stage sleeping sickness in Uganda: a case series. PLoS Negl Trop Dis. 2007; 1(2):e64. Epub 2007/12/07. https://doi.org/10.1371/journal.pntd.0000064 PMID: 18060083; PubMed Central PMCID: PMC2100371.
6. Priotto G, Kasparian S, Ngouama D, Ghorashian S, Arnold U, Ghabri S, et al. Nifurtimox-eflornithine combination therapy for second-stage Trypanosoma brucei gambiense sleeping sickness: a randomized clinical trial in Congo. Clin Infect Dis. 2007; 45(11):1435–42. https://doi.org/10.1086/522982 PMID: 17990225.
7. Nightingale SL. From the Food and Drug Administration. Jama. 1991; 265(10):1229. Epub 1991/03/13. PMID: 1995961.
8. Franco JR, Cecchi G, Priotto G, Paone M, Diarra A, Grout L, et al. Monitoring the elimination of human African trypanosomiasis at continental and country level: Update to 2018. PLoS Negl Trop Dis. 2020; 14(5):e0008261. Epub 2020/05/22. https://doi.org/10.1371/journal.pntd.0008261 PMID: 32437391.
9. Na-Bangchang K, Doua F, Konsil J, Hanpitakpong W, Kamanikom B, Kuzoe F. The pharmacokinetics of eflornithine (alpha-difluoromethylornithine) in patients with late-stage T.b. gambiense sleeping sickness. Eur J Clin Pharmacol. 2004; 60(4):269–78. https://doi.org/10.1007/s00228-004-0759-7 PMID: 15141331.
10. Griffin CA, Slavik M, Chien SC, Hermann J, Thompson G, Blanc O, et al. Phase I trial and pharmacokinetic study of intravenous and oral alpha-difluoromethylornithine. Invest New Drugs. 1987; 5(2):177–86. https://doi.org/10.1007/BF00203544 PMID: 3115911.
11. Qu N, Ignatenko NA, Yamauchi P, Stringer DE, Levenson C, Shannon P, et al. Inhibition of human ornithine decarboxylase activity by enantiomers of difluoromethylornithine. Biochem J. 2003; 375(Pt 2):465–70. Epub 2003/07/16. https://doi.org/10.1042/BJ20030382 PMID: 12859253; PubMed Central PMCID: PMC1223689.
12. Jansson-Lofmark R, Na-Bangchang K, Bjorkman S, Doua F, Ashton M. Enantiospecific reassessment of the pharmacokinetics and pharmacodynamics of oral eflornithine against late-stage Trypanosoma brucei gambiense sleeping sickness. Antimicrob Agents Chemother. 2015; 59(2):1299–307. Epub 2014/12/17. https://doi.org/10.1128/AAC.04101-14 PMID: 25512417; PubMed Central PMCID: PMC4335853.
13. Boberg M, Jonson AC, Leek H, Jansson-Lofmark R, Ashton M. Chiral chromatographic isolation on milligram scale of the human African trypanosomiasis treatment D- and L-eflornithine. ACS Omega. 2020; 5(37):23885–91. Epub 2020/09/29. https://doi.org/10.1021/acsomega.0c03121 PMID: 32984708; PubMed Central PMCID: PMC7513348.
14. Felgner P, Brinkmann U, Zillmann U, Mehlitz D, Abu-Ishira S. Epidemiological studies on the animal reservoir of gambiense sleeping sickness. Part II. Parasitological and immunodiagnostic examination of the human population. Tropenmedizin und Parasitologie. 1981; 32(3):134–40. Epub 1981/09/01. PMID: 6285560.
15. Maina N, Maina KJ, Maser P, Brun R. Genotypic and phenotypic characterization of Trypanosoma brucei gambiense isolates from Ibba, South Sudan, an area of high melarsoprol treatment failure rate. Acta tropica. 2007; 104(2–3):84–90. Epub 2007/09/04. https://doi.org/10.1016/j.actatropica.2007.07.007 PMID: 17765860.

16. Pyana PP, Ngay Lukusa I, Mumba Ngoyi D, Van Reet N, Kaiser M, Karhemere Bin Shamamba S, et al. Isolation of Trypanosoma brucei gambiense from cured and relapsed sleeping sickness patients and adaptation to laboratory mice. PLoS Negl Trop Dis. 2011; 5(4):e1025. Epub 2011/04/29. https://doi.org/10.1371/journal.pntd.0001025 PMID: 21526217; PubMed Central PMCID: PMC3079580.

17. Hirumi H, Hirumi K. Continuous cultivation of Trypanosoma brucei blood stream forms in a medium containing a low concentration of serum protein without feeder cell layers. J Parasitol. 1989; 75(6):985–9. Epub 1989/12/01. PMID: 2614608.

18. Raz B, Iten M, Grether-Buhler Y, Kaminsky R, Brun R. The Alamar Blue assay to determine drug sensitivity of African trypanosomes (T.b. rhodesiense and T.b. gambiense) in vitro. Acta tropica. 1997; 68 (2):139–47. Epub 1997/12/05. https://doi.org/10.1016/s0001-706x(97)00079-x PMID: 9386789.

19. Orhan I, Sener B, Kaiser M, Brun R, Tasdemir D. Inhibitory activity of marine sponge-derived natural products against parasitic protozoa. Mar Drugs. 2010; 8(1):47–58. Epub 2010/02/18. https://doi.org/10.3390/md8010047 PMID: 20161970; PubMed Central PMCID: PMC2817922.

20. Milord F, Loko L, Ethier L, Mpia B, Pepin J. Eflornithine concentrations in serum and cerebrospinal fluid of 63 patients treated for Trypanosoma brucei gambiense sleeping sickness. Trans R Soc Trop Med Hyg. 1993; 87(4):473–7. https://doi.org/10.1016/0035-9203(93)90044-q PMID: 8249087.

21. Burri C, Brun R. Eflornithine for the treatment of human African trypanosomiasis. Parasitol Res. 2003; 90:S49–52. https://doi.org/10.1007/s00436-002-0766-5 PMID: 12811548.

22. Asin-Prieto E, Rodriguez-Gascon A, Isla A. Applications of the pharmacokinetic/pharmacodynamic (PK/PD) analysis of antimicrobial agents. J Infect Chemother. 2015; 21(5):319–29. Epub 2015/03/05. https://doi.org/10.1016/j.jiac.2015.02.001 PMID: 25737147.

23. White NJ. Pharmacokinetic and pharmacodynamic considerations in antimalarial dose optimization. Antimicrob Agents Chemother. 2013; 57(12):5792–807. Epub 2013/09/05. https://doi.org/10.1128/AAC.00287-13 PMID: 24002099; PubMed Central PMCID: PMC3837842.

24. Walz A, Leroy D, Andenmatten N, Maser P, Wittlin S. Anti-malarial ozonides OZ439 and OZ609 tested at clinically relevant compound exposure parameters in a novel ring-stage survival assay. Malar J. 2019; 18(1):427. Epub 2019/12/19. https://doi.org/10.1186/s12936-019-3056-8 PMID: 31849323; PubMed Central PMCID: PMC6918666.

25. Doua F, Boa FY, Schechter PJ, Miezan TW, Diai D, Sanon SR, et al. Treatment of human late stage gambiense trypanosomiasis with alpha-difluoromethylornithine (eflornithine): efficacy and tolerance in 14 cases in Cote d'Ivoire. Am J Trop Med Hyg. 1987; 37(3):525–33. https://doi.org/10.4269/ajtmh.1987.37.525 PMID: 3120607.

26. WHO. WHO interim guidelines for the treatment of gambiense human African trypanosomiasis. 2019.

27. Jansson-Lofmark R, Hjorth S, Gabrielsson J. Does in vitro potency predict clinically efficacious concentrations? Clin Pharmacol Ther. 2020; 108(2):298–305. Epub 2020/04/11. https://doi.org/10.1002/cpt.1846 PMID: 32275768; PubMed Central PMCID: PMC7484912.

28. Sanderson L, Dogruel M, Rodgers J, Bradley B, Thomas SA. The blood-brain barrier significantly limits eflornithine entry into Trypanosoma brucei brucei infected mouse brain. J Neurochem. 2008; 107 (4):1136–46. https://doi.org/10.1111/j.1471-4159.2008.05706.x PMID: 18823367; PubMed Central PMCID: PMC2695853.

29. Levin VA, Csejtey J, Byrd DJ. Brain, CSF, and tumor pharmacokinetics of alpha-difluoromethylornithine in rats and dogs. Cancer Chemother Pharmacol. 1983; 10(3):196–9. Epub 1983/01/01. https://doi.org/10.1007/BF00255762 PMID: 6407769.

30. Furet Y, Duong TH, Combescot C, Breteau M. Une molécule nouvelle en thérapeutique antiparasitaire: L'alpha-difluorométhylonrithine [A new molecule in antiparasitic therapy: alpha-difluoromethylornithine]. Pathologie-biologie. 1987; 35(4):398–404. Epub 1987/04/01. PMID: 3108836.

31. Vincent IM, Creek DJ, Burgess K, Woods DJ, Burchmore RJ, Barrett MP. Untargeted metabolomics reveals a lack of synergy between nifurtimox and eflornithine against Trypanosoma brucei. PLoS Negl Trop Dis. 2012; 6(5):e1618. Epub 2012/05/09. https://doi.org/10.1371/journal.pntd.0001618 PMID: 22563508; PubMed Central PMCID: PMC3341325.

32. Vincent IM, Creek D, Watson DG, Kamleh MA, Woods DJ, Wong PE, et al. A molecular mechanism for eflornithine resistance in African trypanosomes. PLoS Pathog. 2010; 6(11):e1001204. https://doi.org/10.1371/journal.ppat.1001204 PMID: 21124824; PubMed Central PMCID: PMC2991269.

33. Mathieu C, Gonzalez Salgado A, Wirdnam C, Meier S, Grotemeyer MS, Inbar E, et al. Trypanosoma brucei eflornithine transporter AAT6 is a low-affinity low-selective transporter for neutral amino acids. Biochem J. 2014; 463(1):9–18. Epub 2014/07/06. https://doi.org/10.1042/BJ20140719 PMID: 24988048.

34. WHO. Accelerating Work to overcome the global impact of neglected tropical diseases: A roadmap for implementation. WHO Executive Summary [Internet]. 2012:[1–22 pp.]. Available from: http://whqlibdoc.who.int/hq/2012/WHO_HTM_NTD_2012.1_eng.pdf, Accessed on 8 Feb, 2021.

35. Milord F, Pepin J, Loko L, Ethier L, Mpia B. Efficacy and toxicity of eflornithine for treatment of Trypanosoma brucei gambiense sleeping sickness. Lancet. 1992; 340(8820):652–5. https://doi.org/10.1016/0140-6736(92)92180-n PMID: 1355219

36. Van Nieuwenhove S, Schechter PJ, Declercq J, Bone G, Burke J, Sjoerdsma A. Treatment of gambiense sleeping sickness in the Sudan with oral DFMO (DL-alpha-difluoromethylornithine), an inhibitor of ornithine decarboxylase; first field trial. Trans R Soc Trop Med Hyg. 1985; 79(5):692–8. https://doi.org/10.1016/0035-9203(85)90195-6 PMID: 3938090.

37. Pepin J, Milord F, Guern C, Schechter PJ. Difluoromethylornithine for arseno-resistant Trypanosoma brucei gambiense sleeping sickness. Lancet. 1987; 2(8573):1431–3. https://doi.org/10.1016/s0140-6736(87)91131-7 PMID: 2891995.

38. Taelman H, Schechter PJ, Marcelis L, Sonnet J, Kazyumba G, Dasnoy J, et al. Difluoromethylornithine, an effective new treatment of Gambian trypanosomiasis. Results in five patients. Am J Med. 1987; 82(3 Spec No):607–14. https://doi.org/10.1016/0002-9343(87)90107-0 PMID: 3103442.

39. Johansson CC, Gennemark P, Artursson P, Abelo A, Ashton M, Jansson-Lofmark R. Population pharmacokinetic modeling and deconvolution of enantioselective absorption of eflornithine in the rat. J Pharmacokinet Pharmacodyn. 2013; 40(1):117–28. Epub 2013/01/12. https://doi.org/10.1007/s10928-012-9293-x PMID: 23307171.

40. Jansson R, Malm M, Roth C, Ashton M. Enantioselective and nonlinear intestinal absorption of eflornithine in the rat. Antimicrob Agents Chemother. 2008; 52(8):2842–8. Epub 2008/06/04. https://doi.org/10.1128/AAC.00050-08 PMID: 18519728; PubMed Central PMCID: PMC2493103.

41. Mesu V, Kalonji WM, Bardonneau C, Mordt OV, Blesson S, Simon F, et al. Oral fexinidazole for late-stage African Trypanosoma brucei gambiense trypanosomiasis: a pivotal multicentre, randomised, non-inferiority trial. Lancet. 2018; 391(10116):144–54. https://doi.org/10.1016/S0140-6736(17)32758-7 PMID: 29113731.

42. Lindner AK, Lejon V, Chappuis F, Seixas J, Kazumba L, Barrett MP, et al. New WHO guidelines for treatment of gambiense human African trypanosomiasis including fexinidazole: substantial changes for clinical practice. Lancet Infect Dis. 2019. Epub 2019/12/28. https://doi.org/10.1016/S1473-3099(19)30612-7 PMID: 31879061.

43. Dickie EA, Giordani F, Gould MK, Maser P, Burri C, Mottram JC, et al. New Drugs for Human African Trypanosomiasis: A Twenty First Century Success Story. Trop Med Infect Dis. 2020; 5(1). Epub 2020/02/26. https://doi.org/10.3390/tropicalmed5010029 PMID: 32092897; PubMed Central PMCID: PMC7157223.