The anti-parasitic drug miltefosine suppresses activation of human eosinophils and ameliorates allergic inflammation in mice

Eva Knuplez1 | Melanie Kienzl1,2 | Athina Trakaki1 | Rudolf Schicho1,2 | Akos Heinemann1,2 | Eva M. Sturm1 | Gunther Marsche1,2

1Division of Pharmacology, Otto Loewi Research Center, Medical University of Graz, Graz, Austria
2BioTechMed-Graz, Graz, Austria

Correspondence
Gunther Marsche, Division of Pharmacology, Otto Loewi Research Center, Medical University of Graz, Universitätspaz 4, 8010 Graz, Austria.
Email: gunther.marsche@medunigraz.at

Funding information
Austrian Science Fund, Grant/Award Numbers: P30144, DK-MOLIN, W1241

Background and Purpose: Miltefosine is an alkylphosphocholine drug with proven effectiveness against various types of parasites and cancer cells. Miltefosine is not only able to induce direct parasite killing but also modulates host immunity, for example by reducing the severity of allergies in patients. To date, there are no reports on the effect of miltefosine on eosinophils, central effector cells involved in allergic inflammation.

Experimental Approach: We tested the effect of miltefosine on the activation of human eosinophils and their effector responses in vitro and in mouse models of eosinophilic migration and ovalbumin-induced allergic lung inflammation.

Key Results: The addition of miltefosine suppressed several eosinophilic effector reactions such as CD11b up-regulation, degranulation, chemotaxis and downstream signalling. Miltefosine significantly reduced the infiltration of immune cells into the respiratory tract of mice in an allergic cell recruitment model. Finally, in a model of allergic inflammation, treatment with miltefosine resulted in an improvement of lung function parameters.

Conclusion and Implications: Our observations suggest a strong modulatory activity of miltefosine in the regulation of eosinophilic inflammation in vitro and in vivo. Our data underline the potential efficacy of miltefosine in the treatment of allergic diseases and other eosinophil-associated disorders and may raise important questions regarding the immunomodulatory effect of miltefosine in patients treated for leishmania infections.

KEYWORDS
allergic inflammation, eosinophils, miltefosine

Abbreviations: BMDE, bone marrow-derived eosinophil; fMLP, N-formylmethionyl-leucyl-phenylalanine; FSC, forward scatter; PI, propidium iodide.

[Correction added on 16 March 2021, after first online publication: Article title has been amended in this current version.]

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2021 The Authors. British Journal of Pharmacology published by John Wiley & Sons Ltd on behalf of British Pharmacological Society.
1 | INTRODUCTION

To date, miltefosine (Impavido®) is the only oral drug approved for the treatment of leishmaniasis with limited mild or moderate side effects (Pijpers et al., 2019). The development of miltefosine is a success story of public–private partnership, a breakthrough in medicine affordability and patient drug adherence, landing it on the World Health Organization (WHO)’s List of Essential Medicines (Berger et al., 2017; Sunyoto et al., 2018). Miltefosine disrupts membrane structures and affects phosphatidylcholine synthesis in susceptible promastigote cells (Pinto-Martinez et al., 2018; Rakotomanga et al., 2007). Due to its detergent-like properties, miltefosine is thought to interact with the mucosa of the gastrointestinal tract during oral use and cause its most commonly listed side effects—nausea, vomiting and diarrhoea (Bhattacharya et al., 2007). During prolonged treatment, the severity of the side effects was reported to decrease over time (8.2% during Week 1 to 3.2% during Week 4) (Bhattacharya et al., 2007).

Miltefosine exerts immunomodulatory effects on human cancer cells by inhibiting the PI3K/Akt signalling pathway (Ruiter et al., 2003), induces IL-12-dependent Th1 responses (Wadhone et al., 2009) and shows anti-inflammatory effects in endothelial cells, suppressing vascular inflammation (Fang et al., 2019). However, the immunomodulatory effects of miltefosine on primary human cells have so far only been described for T cells (Bäumer et al., 2010) and mast cells (Weller et al., 2009).

In plasma, miltefosine is mainly bound to albumin (96–98%) (Kip et al., 2018) and accumulates predominantly in cholesterol-rich microdomains of the cell membranes (lipid rafts) (Malta de Sá et al., 2015). Miltefosine increases membrane fluidity (Moreira et al., 2014), modulates lipid raft-dependent signalling (Weller et al., 2009) and could therefore be an attractive drug candidate for the treatment of diseases characterized by abundant lipid raft activation, such as allergic diseases (Dölle et al., 2010). Miltefosine attenuates allergic inflammation in T cell-dependent mouse models of dermal inflammation (Bäumer et al., 2010), improves local dermatitis in patients with atopic dermatitis (Dölle et al., 2010), inhibits activation and degranulation of mast cells, and significantly reduces allergic disease manifestation in patients (Magerl et al., 2013; Maurer et al., 2013; Rubiková et al., 2018).

Surprisingly, there are no reports on the effects of miltefosine on eosinophils, a key cell type involved in the initiation and propagation of immune responses in allergic diseases (Stone et al., 2010). Here, we studied in detail whether miltefosine exerts immunomodulatory effects on eosinophils in vitro and in mouse models of allergic lung inflammation.

2 | METHODS

2.1 | Materials

Unless otherwise indicated, all purchased reagents were from Sigma (Vienna, Austria). Eotaxin-2 (CCL24) used for in vivo chemotaxis and recombinant human C5a were acquired from R&D Systems (Minneapolis, MN, USA). Eotaxin-1 (CCL11) and eotaxin-2 (CCL24) used in vitro assays were obtained from ImmunoTools (Friesoythe, Germany). Antibody against phospho-Akt (Ser 473) (Cat#9271, RRID:AB_329825) was obtained from Cell Signalling Technology (Danvers, MA, USA), while secondary goat anti-rabbit Alexa Fluor 488 IgG antibody (Cat# A-11008, RRID:AB_143165) was from Life Technologies (Thermo Fisher Scientific, Waltham, MA, USA). Annexin V, propidium iodide (PI) (Cat# 556547), CD63-FITC (Cat# 561924, RRID:AB_10894192), Siglec-F-PE (Cat# 552126, RRID:AB_394341), CD3-PE Cy5 (Cat# 553065, RRID:AB_394598), CD11b-PE-Cy7 (Cat# 552850, RRID:AB_394491) and CD11c-BV421 (Cat# 562782, RRID:AB_2737789) were from BD Biosciences (Vienna, Austria). TruStain fcX CD16/32 (Cat# 101320, RRID:AB_1574975), Ly-6C-FITC (Cat# 128005, RRID:AB_1186134), Ly-6G-APC (Cat# 127613, RRID:AB_1877163), CCR3-BV421 (Cat#144517, RRID:AB_2565743) and I-A/I-E-V510 (Cat# 107635, RRID:AB_2561397) were from BioLegend (San Diego, CA, USA). Aluminium hydroxide gel used as an adjuvant was acquired from InvivoGen (Toulouse, France). CD11b-FITC mouse anti-human antibody (Cat# iMO53OU) used for measuring CD11b up-regulation was obtained from Beckman Coulter (Krefeld, Germany). Miltefosine used in in vivo assays was purchased from Cayman Chemical (Ann Arbor, MI, USA). All functional assays of eosinophils were performed in assay buffer (PBS with Ca²⁺ and Mg²⁺, HEPES 10 mM, glucose 10 mM and bovine serum albumin 0.1%, pH 7.4).

Fixative solution was prepared by adding 9 ml of distilled water and 30 ml of FACS sheath fluid (BD Biosciences) to 1 ml of CellFix (BD Biosciences, Vienna, Austria) as described previously (Knuplez, Curcic, et al., 2020).
2.2 | Mice

Animal studies are reported in compliance with the ARRIVE guidelines (Percie du Sert et al., 2020) and with the recommendations made by the British Journal of Pharmacology (Lilley et al., 2020). All animal experiments were performed in the animal facilities of the Medical University of Graz. The experimental procedure used in this study was approved by the Austrian Federal Ministry of Science, Research and Economy (protocol number: BMWFW-66.010/0207-WF/V/3b/2017); it conforms to Directive 2010/63/EU and was performed in accordance with national and international guidelines. BALB/c mice (RRID:IMSR_CRL:028) were either bred in-house or obtained from Charles River. Δdbl GATA-1 and interleukin-5 (IL-5) transgenic (IL-5Tg) mice (both BALB/c background) were originally obtained from Dr Helene Rosenberg (NIH, Bethesda, MD, USA) and bred in our facilities. IL-5Tg mice were originally generated by Lindsay A. Dent. A 10-kb genomic mouse il5 sequence under the control of the dominant control region (DCR) from human CD2 was used for the transgene. Δdbl GATA mice were originally generated by C. Yu. A high-affinity, palindromic “double” GATA protein binding site in the Gata1 promoter presumed to mediate positive Gata1 autoregulation was replaced by a floxed Pgk-neo cassette; transient Cre recombinase expression in ES cells left a single loxP site flanked by two Not1 sites. The 21-bp deleted segment comprised nucleotides 671 through –691 upstream of the last nucleotide of the first haematopoietically expressed exon of Gata1. Mice were housed in plastic sawdust floor cages at constant temperature (22°C) and a 12:12-h light–dark cycle with free access to standard laboratory chow and water; 8- to 12-week-old male and female mice were included in experiments, where there were randomly divided in three groups (negative control—vehicle; positive control—ovalbumin or eotaxin stimulated and miltefosine pretreated and ovalbumin or eotaxin stimulated group). Experiments, where bronchoalveolar lavage fluid was collected, could not be performed blinded, due to investigator treating the mice prior to fluid collection. Lung function testing was performed blinded, since Investigator 1 treated the mice and Investigator 2 independently performed lung function testing on mice in a random order. For all animal experiments, at least five mice were included in each group and at least two repeat experiments were carried out. Experiments were designed to make sample sizes relatively equal and randomized among comparison groups. Sample sizes were determined according to previous studies with similar analyses (Knuplez, Curcic, et al., 2020; Theiler et al., 2019).

2.3 | Blood sampling and eosinophil isolation

Blood sampling from healthy volunteers was approved by the Institutional Review Board of the Medical University of Graz (17-291 ex 05/06). All participants signed a written informed consent. Polymorphonuclear leukocytes preparations were purified from citrated whole blood as previously described (Curcic et al., 2015). Firstly, platelet-rich plasma was removed by centrifugation. Next, red blood cells and platelets were removed by dextran sedimentation and polymorphonuclear leukocytes preparations were obtained by density gradient separation. Eosinophils were isolated from polymorphonuclear leukocytes by negative magnetic selection using a cocktail of biotin-conjugated antibodies against CD2, CD14, CD16, CD19, CD56 (neural cell adhesion molecule 1), CD123 (interleukin 3 receptor, α subunit) and CD235a (glycoporphin A) as well as Anti-Biotin Micro-Beads from Miltenyi Biotec (Bergisch Gladbach, Germany). Eosinophil purity was determined by morphological analysis of Kimura-stained cells and was typically greater than 97%.

2.4 | Eosinophil shape change

Eosinophil shape change was determined as described previously (Luschinig-Schratl et al., 2011). Approximately 5 × 10^6 eosinophils per sample were suspended in assay buffer with Ca^{2+} and Mg^{2+}, preincubated with miltefosine in different concentrations (15 min, room temperature [RT]) and then stimulated in water bath (4 min, 37°C) with eotaxin-1 (CCL11). Afterwards, cells were transferred to ice and ice-cold fixative solution was added to terminate the reaction and maintain the change in cell shape until analysis. The samples were analysed on a FACS Canto II flow cytometer (Becton Dickinson, Mountain View, CA, USA), where shape change was determined as the increase in the forward scatter (FSC) property of the cell and was normalized to unstimulated vehicle control.

2.5 | CD11b (integrin, alpha M subunit (complement component 3 receptor 3 subunit) up-regulation

CD11b up-regulation on eosinophils was determined as described in detail elsewhere (Knuplez, Curcic, et al., 2020). Briefly, eosinophils were stained with anti-CD11b–FITC, pretreated either with vehicle or miltefosine (20 μM) and stimulated with eotaxin-2 (CCL24) as indicated in the figure legend. Additionally, CD11b up-regulation assay was performed in the polymorphonuclear leukocytes fraction (see the Supporting Information), where cells were pre-stained with anti-CD16-PE to distinguish between eosinophil and neutrophil polymorphonuclear leukocytes fractions. CD11b up-regulation on neutrophils (CD16 + cells) was induced with N-formylmethionyl-leucyl-phenylalanine (fMLP). Eosinophil or neutrophil CD11b expression was determined by flow cytometry as the geometric mean of the fluorescence in the FITC channel and expressed as per cent of unstimulated vehicle response.

2.6 | CD63 expression

Eosinophils were stained with anti-CD63-FITC (1:100) and pre-incubated with vehicle or miltefosine (20 μM) (15 min, RT). Subsequently, cells were primed with cytochalasin B (5 μg·ml\(^{-1}\)) and
stimulated with C5a in two different concentrations (Schratl et al., 2006). Degranulation was analysed by flow cytometry and expressed as fold increase in fluorescence over indicated vehicle mean.

2.7 | Preparation of bone marrow-derived eosinophils (BMDEs)

Mouse eosinophils were derived from bone marrow of BALB/c mice as described before (Dyer et al., 2008; Kienzl et al., 2020; Knuplez, Krier-Burris, et al., 2020). Briefly, following the lysis of erythrocytes in bone marrow, the cells were cultured in RPMI + 20% HyClone FBS (GE Healthcare; # 10309433), 1% P/S, 25-mM HEPES (Thermo Fisher; # 15630–080), 1× non-essential amino acids (Thermo Fisher; # 11140–035), 1-mM sodium pyruvate (Thermo Fisher; # 11360–039) and 50-μM β-mercaptoethanol (Sigma-Aldrich; M3148) supplemented with 100-ng·ml⁻¹ stem cell factor (PreproTech; # 250–03) and 100-ng·ml⁻¹ FLT3L (PreproTech; # 250–31 L). On Day 4, medium was changed to media supplemented with 10-ng·ml⁻¹ IL-5 (Bio-Techne; # 405-MI-005) only, to differentiate progenitors into eosinophils. On Day 14, purity and viability of bone marrow-derived eosinophils (BMDEs) was assessed by flow cytometry staining for mouse eosinophil markers CCR3 (CCR3-BV421) and Siglec-F (Siglec-F-PE), and PI respectively. Cytospins of BMDEs were prepared, stained with a Hemacolor Rapid staining of blood smear and imaged on an Olympus BX41 microscope (Olympus, Vienna, Austria). Day 14 BMDEs were used for further in vitro analyses.

2.8 | Calcium flux

Isolated human or differentiated mouse eosinophils were loaded with 2 μM of Fluo-3 AM in the presence of 0.02% pluronic F-127 for 1 h at RT in the dark. Individual samples were treated as indicated in the figure legend. Changes in [Ca²⁺], were detected as fluorescence in the FL1 channel by a FACSCalibur flow cytometer (Becton Dickinson, Mountain View, CA, USA), as described previously (Heinemann et al., 2003; Knuplez, Curcic, et al., 2020).

2.9 | In vitro chemotaxis

Purified eosinophils were pretreated with either vehicle or miltefosine in different concentrations (15 min, RT) and were allowed to migrate to 10-nM eotaxin-2 (CCL24) in an HTS Transwell 96-well plate with a 5-μm pore size polycarbonate membrane (1 h, 37°C). Eosinophils that have migrated to the lower compartment were enumerated for 1 min by flow cytometric counting on a FACS Canto II (Becton Dickinson, Mountain View, CA, USA) (Knuplez, Curcic, et al., 2020).

2.10 | Flow cytometric analysis of intracellular kinase phosphorylation

Isolated eosinophils were pretreated with either vehicle or miltefosine 20 (μM) (15 min, RT). Following the pretreatment, cells were incubated with 10-nM eotaxin-1 (CCL11) (3 min, 37°C). Subsequently, cells were fixed, permeabilized and stained as described previously (Knuplez, Curcic, et al., 2020). Phosphorylation of Akt residues in fixed eosinophils was quantified as the increase of fluorescence in the FITC fluorescence channel from unstimulated control.

2.11 | Apoptosis

Eosinophil survival after preincubation with vehicle, positive control formaldehyde (3.8%) or miltefosine (5–40 μM) for different time points at 37°C was assessed by annexin V/PI staining, as described previously (Heinemann et al., 2005; Knuplez, Curcic, et al., 2020).

2.12 | In vivo chemotaxis

In vivo eosinophil migration was induced by intranasal application of 4-μg eotaxin-2 CCL24 in 8-week-old male and female heterozygous IL-5 transgenic (IL-5Tg) mice (BALB/c background). The mice and their littermate controls received oral gavages of miltefosine (20 mg·kg⁻¹ in 0.9% NaCl) or vehicle for three consecutive days before CCL24 application. Bronchoalveolar lavage fluid was collected 4 h after experiment had started. Migration of eosinophils was evaluated by flow cytometric counting of highly granular (high side scatter) CD11c⁻/Siglec-F⁻ cells, as described previously (Knuplez, Curcic, et al., 2020). The gating strategy for evaluation of other immune cells was previously published (Knuplez, Curcic, et al., 2020) and was as follows: alveolar macrophages (Siglec-F⁻/CD11c⁺), neutrophils (Siglec-F⁻/CD11b⁻/Ly6G⁻), B cells (CD11c⁻/CD11b⁻/MHCIİ⁺), T cells (CD11c⁻/CD11b⁻/CD3⁻), dendritic cells (Siglec-F⁻/Ly6G⁻/MHCIİ⁻/MHCIİ⁺) and inflammatory monocytes (Siglec-F⁻/Ly6G⁻/MHCIİ⁻/LY6C⁻).

2.13 | Mouse model of allergic lung inflammation

Eight-week-old male and female BALB/c or eosinophil-deficient (Δdbl GATA-1) mice were immunized by intraperitoneal injections of 20 μg of ovalbumin adsorbed to Al(OH)₃ on Days 0 and 7. Mice were challenged by an aerosol of ovalbumin (1 mg·ml⁻¹ in 0.9% NaCl) on Days 14 and 16. During the last 10 days of the model, mice received daily oral gavages of miltefosine (20 mg·kg⁻¹ in 0.9% NaCl) or vehicle. On Day 17, either airway hyperresponsiveness to methacholine was recorded with the FlexiVent system (SCIREQ, Montreal, QC, Canada) or bronchoalveolar lavage fluid was taken and analysed by flow cytometry. Bronchoalveolar lavage fluid supernatants were collected and stored at −70°C for further cytokine assessment. All animal subjects were randomized prior to inclusion in the experiments.
2.14 | Cytokine measurements in bronchoalveolar lavage fluid

Cytokine concentrations in stored bronchoalveolar lavage fluid supernatants from BALB/c and AbdBl GATA-1 mice subjected to ovalbumin/aluminium hydroxide were evaluated using the custom ProcartaPlex™ immunoassay (eBioscience, San Diego, CA, USA) according to the manufacturer's specifications. Fluorescent signals were quantified with the Bio-Plex 200 multiplex suspension array system equipped with Luminex® XMAP® technology combined with the Bio-Plex 5.0 software (Bio-Rad, Hercules, CA, USA). All cytokine concentrations were evaluated in duplicates.

2.15 | Corticosterone measurement in plasma

Corticosterone levels were assessed in plasma of BALB/c mice treated with oral gavages of miltefosine (20 mg·kg⁻¹) once daily for 3 days. A blood sample was collected via cheek bleed 5 h after first miltefosine application on Day 1, as well as 4 h after last treatment on Day 3. Corticosterone levels were determined with a specific enzyme immunoassay kit (Assay Designs, Ann Arbor, MI, USA) with a sensitivity of 0.027 ng·ml⁻¹ as previously described (Farzi et al., 2015) and according to the manufacturer's specifications. The Immuno-related procedures used comply with the recommendations made by the British Journal of Pharmacology (Alexander et al., 2018).

2.16 | Statistical analysis

The data and statistical analysis comply with the recommendations of the British Journal of Pharmacology on experimental design and analysis in pharmacology (Curtis et al., 2018). Statistical analysis was performed using the GraphPad Prism™ 6 software (GraphPad Software, Inc., CA, USA). Data were normalized to baseline (1 or 100%) of the means of negative control in experiments performed with eosinophils isolated from human donors to reduce interindividual source of variation.

Statistical analysis was only performed for groups where n ≥ 5. Additional preliminary data (n = 3) on p-Akt phosphorylation in eosinophils were included in the manuscript to suggest a mechanism previously shown for other cell types (Chugh et al., 2008; Ruiter et al., 2003). The group size given for each experiment is the number of independent values (individual human eosinophil donors or mice). Statistical analysis was performed using these independent values.

Data were tested for normality using D'Agostino and Pearson omnibus normality test. If normality was assumed, comparisons among multiple groups were performed with one-way ANOVA or two-way ANOVA. For these analyses, post hoc pairwise comparisons were performed using Bonferroni’s multiple comparison test (or Dunnett’s multiple comparison test, when comparing samples to the control group), only if a main effect for at least one factor or the interaction between two factors showed statistical significance and if there was no significant variance in homogeneity. Cytokine levels were compared using Mann–Whitney U test. Significance level for the analyses was set to α = 0.05 and significant differences are indicated with the corresponding P value, *P ≤ 0.05.

2.17 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in the IUPHAR/BPS Guide to PHARMACOLOGY http://www.guidetopharmacology.org and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20 (Alexander et al., 2019).

3 | RESULTS

3.1 | Miltefosine suppresses eosinophil activation in vitro

First, we tested the viability of eosinophils after pretreatment with different concentrations of miltefosine. Importantly, miltefosine (up to 20 μM, in the presence of 1-mg·ml⁻¹ bovine serum albumin) showed no toxic effects on eosinophils (Figure S1).

During the state of allergic inflammation, elevated concentrations of cytokines and chemoattractants in the blood activate eosinophils, which leads to a rearrangement of their actin filaments (the so-called “shape change”) (Willetts et al., 2014) and results in an up-regulation of the adhesion molecules integrins (e.g., CD11b/CD18 and Mac-1) on the cell surface (Jia et al., 1999). When human eosinophils were pretreated with miltefosine, we could observe a statistically significant inhibition of their shape change (by approx. 50%) induced by CCL11 stimulation (Figure 1a,b) when using the highest concentration of miltefosine (20 μM). Miltefosine addition did not alter eosinophil shape change in the absence of external stimuli (Figure S2). When isolated eosinophils were pretreated with 20-μM miltefosine, up-regulation of CD11b was reduced by about 50% (Figure 1c,d).

Similarly, miltefosine suppressed CD11b expression of CCL11 activated eosinophils (CD16⁻ cells) in the polymorphonuclear leukocytes fraction (Figure S3A). Notably, miltefosine did not alter CD11b expression of fMLP-stimulated neutrophils (CD16⁺ cells) (Figure S3B).

To determine whether miltefosine has an effect on the chemotaxis of human eosinophils, we performed in vitro chemotaxis assays using eotaxin-2 (CCL24) as chemoattractant. Miltefosine significantly inhibited eosinophilic chemotaxis in a dose-dependent manner (Figure 2a,b).

We next assessed whether miltefosine affects degranulation-associated processes in eosinophils. For that purpose, eosinophils were pretreated with miltefosine and subsequently stimulated with recombinant C5a, a potent inducer of degranulation. Miltefosine effectively suppressed C5a (0.5 nM)-induced CD63 expression, a marker of eosinophilic degranulation (Carmo et al., 2016) (Figure 2c,d).
CD63 expression induced with very high concentrations of C5a (100 nM) was not affected by miltefosine (Figure 2e,f).

Previous studies have shown that miltefosine inhibits PI3K/Akt kinase signalling with an IC50 in the range of 5 to 35 μM, depending on the cell line tested (Kalea et al., 2018; Rybczynska et al., 2001). Our preliminary results show a tendency of miltefosine (20 μM) inhibiting Akt phosphorylation (Figure 3a,b). Moreover, we could demonstrate that intracellular calcium flux in CCL11-stimulated eosinophils was inhibited by about 50% (Figure 3c,d) after only 1 min of miltefosine addition (20 μM).

3.2 | Miltefosine ameliorates ovalbumin-induced lung inflammation

Next, we investigated whether the in vitro results obtained with isolated human eosinophils are also relevant in vivo. We first performed Ca2+ flux assays using mouse bone marrow-derived eosinophils to test whether mouse eosinophils behave similar to human-isolated eosinophils (Figure 4a,b). For that purpose, eosinophils were differentiated from bone marrow cells of BALB/c mice following an established protocol (Kienzl et al., 2020), which yields a pure population of cultured eosinophils as determined by a single population positive for mouse eosinophil markers CCR3 and Siglec-F (Figure S4A). Microscopic analysis of cytospins of BMDEs shows a uniform population of cells exhibiting typical eosinophil staining and granule morphology (Figure S4B). These mature bone marrow eosinophils were used to perform Ca2+ flux assays under similar experimental conditions as isolated human eosinophils (Figure 4a,b). Our data show the level (approximately 50%) and kinetic of Ca2+ flux inhibition in mouse eosinophils resembling that of human eosinophils pretreated with miltefosine and stimulated with CCL24 (Figure 4a,b).

FIGURE 1  Miltefosine concentration-dependently inhibits eosinophil activation. (a, b) Eosinophils were pretreated with miltefosine in different concentrations (0.5–20 μM) (15 min, room temperature [RT]) and stimulated with 10-nM CCL11 (4 min, 37°C). Cells were fixed and the change in cell size (forward scatter [FSC]) was evaluated by flow cytometry. (a) Eosinophil shape change is expressed as per cent of unstimulated vehicle response. Data are shown as mean ± SEM from five individual experiments. *P < 0.05 versus CCL11-stimulated vehicle (one-way ANOVA with Dunnett’s post hoc test). (b) Representative histogram of eosinophil FSC with miltefosine (20 μM) pretreatment and CCL11 stimulation (10 nM). (c, d) Eosinophils were stained with anti-CD11b and treated with either miltefosine (20 μM) or vehicle control (15 min, RT). Subsequently, cells were stimulated with CCL24 (4 min, 37°C) and analysed by flow cytometry. Eosinophil CD11b expression is expressed as per cent of unstimulated vehicle response. Data are shown as mean ± SEM from five individual experiments. *P < 0.05 versus vehicle (two-way ANOVA with Bonferroni post hoc test). (d) Representative histogram of CD11b up-regulation with miltefosine (20 μM) pretreatment and CCL24 (10 nM) stimulation.
significantly suppressed the migration of eosinophils to intranasal CCL24 into the bronchoalveolar lavage of animals (Figure 4d). A trend towards reduced infiltration of immune cells was observed for all detected cell types (Figure 4d). Analysis of the blood immune cell composition revealed an increase in the percentage of neutrophils in blood of IL-5Tg mice treated with miltefosine (Figure S5A); however, when BALB/c mice were treated with miltefosine, no increase in neutrophils was observed (Figure S5B). By testing plasma of BALB/c mice for their corticosterone levels, we observed no significant differences at both of the two tested time points (Figure S6A,B).

We next tested the efficacy of miltefosine in an acute model of allergic lung inflammation. Ovalbumin was used as a model allergen to
reproduce key features of clinical asthma, such as airway hyper-responsiveness to methacholine (Kumar et al., 2008). The treatment protocol of the model is shown in Figure 5a. We observed that daily peroral treatment with miltefosine markedly reduced the number of several infiltrating immune cells into airways of ovalbumin-challenged wild-type mice. Flow cytometric analysis of the composition of immune cells showed that the number of eosinophils as well as infiltrating T cells, B cells and dendritic cells was reduced by 50% upon miltefosine treatment (Figure 5b). Of note, mice treated with miltefosine showed significantly improved lung resistance and a trend towards improved lung compliance (Figure 5c). In order to test whether a decrease in eosinophil numbers was responsible for the reduction of other immune cells, eosinophil-deficient (Δdbl GATA-1) mice were exposed to the same ovalbumin-induced allergic model. In this mouse strain, treatment with miltefosine only had an impact on the number of dendritic cells (Figure 6), while other subgroups of immune cells were not affected.

Supernatants of bronchoalveolar lavage fluid of ovalbumin-stimulated (vehicle) and miltefosine-treated and ovalbumin-stimulated BALB/c and Δdbl GATA-1 mice were further analysed for their cytokine content (Figures S7 and S8). We could observe significantly reduced levels of immunomodulatory cytokine IFN-γ in the bronchoalveolar lavage fluid of miltefosine-treated BALB/c mice (Figure S7A), while no such inhibition was observed in Δdbl GATA-1 mice (Figure S8A). Cytokine content of CCL11, TNF-α, IL-2 and IL-5 in bronchoalveolar lavage fluid was not significantly altered by miltefosine treatment in either BALB/c or Δdbl GATA-1 mice (Figures S7 and S8).

| DISCUSSION |

In the present study, we show for the first time that the Food and Drug Administration (FDA)-approved drug miltefosine inhibits the
Miltefosine inhibits Ca\(^{2+}\) flux in mouse eosinophils and suppresses migration of eosinophils in vivo. (a, b) Bone marrow-derived mouse eosinophils were labelled with Fluo-3 AM and changes in \([\text{Ca}^{2+}]_t\) were detected by flow cytometry. Eosinophils were stimulated with increasing concentrations of CCL24 (0–30 nM) in the presence or absence of miltefosine (20 μM). (a) Results represent fold increase in \([\text{Ca}^{2+}]_t\) over unstimulated vehicle. Data are shown as mean ± SEM from five individual experiments *P < 0.05 miltefosine (20 μM) versus vehicle (two-way ANOVA with Bonferroni post hoc test). (b) Time course of Ca\(^{2+}\) flux in eosinophils. Following baseline measurement (30 s), miltefosine (20 μM) or vehicle was added. After 1 min, CCL24 (10 nM) was added to induce Ca\(^{2+}\) flux. Data are shown as mean ± SEM from five individual experiments. (c, d) Eight-week-old IL-5Tg mice received either miltefosine (20 mg kg\(^{-1}\) day\(^{-1}\)) or vehicle (0.9% NaCl) per os for three consecutive days, followed by intranasal application of CCL24 (4 μg) or vehicle. After 4 h, mice were killed and bronchoalveolar lavage (BAL) fluid was collected. (b) Immune cell composition in BAL fluid was analysed by flow cytometry. Data are shown from six to eight mice from three individual experiments. *P < 0.05 versus eotaxin (CCL24) (Eot) group (one-way ANOVA with Dunnett’s post hoc test). Eot + MF, eotaxin (CCL24) + miltefosine.
activation of human eosinophils. A short pretreatment with miltefosine suppressed human eosinophilic effector responses after stimulation with various agonists in vitro. We were able to transfer our in vitro findings to preclinically relevant endpoints in an in vivo model of eosinophilic migration and allergic inflammation. Most importantly, in a model of ovalbumin-induced allergic lung inflammation, peroral administration of miltefosine significantly reduced the infiltration of immune cells into the lung while improving lung function parameters.

The effects of miltefosine have previously been studied on some other immune cells. Notably, miltefosine was found to inhibit degranulation and antigen-induced chemotaxis of mast cells by modulating lipid rafts and by inhibiting cytosolic PKC (Rubiková et al., 2018). In contrast to our findings with eosinophils, calcium flux in mast cells was apparently not affected by miltefosine pretreatment, indicating cell type-specific differences. However, similar to mast cells, miltefosine led to an inhibition of effector functions and mediator release in eosinophils. In macrophages, miltefosine was found to
increase cholesterol release and phosphorylation of kinases associated with autophagy. Importantly, miltefosine decreased toll-like receptor 4 (TLR-4) recruitment to the cell surface of macrophages and dampened IL-1β release following stimulation with lipopolysaccharide (LPS) (Iacano et al., 2019). Given the fact that TLR-4 stimulation on eosinophils can help polarize macrophages towards pro- or anti-inflammatory phenotypes (Yoon et al., 2019), this finding further supports the evidence that miltefosine may influence the interplay and balance between various immune cell types during the state of inflammation.

It is noteworthy that in all our in vitro experiments, non-toxic concentrations of miltefosine were used to distinguish our results from the non-specific cytolytic effects of the drug. In particular, since homeostatic functions such as tissue remodelling and plasma cell survival (Jacobsen et al., 2012) have recently been attributed to eosinophils, we were mainly interested in inhibiting eosinophil overactivation, to prevent their potential tissue-damaging effector functions. For our in vivo experiments, we used a dosage regimen, comparable with other studies in mice testing miltefosine (Bäumer et al., 2010).

Results from our in vivo experiments show significantly decreased numbers of infiltrating eosinophils in miltefosine-treated animals compared with vehicle-challenged controls. The data correspond to our in vitro experiments in which miltefosine inhibited the activation, migration and up-regulation of adhesion molecules on eosinophils. Interestingly, we additionally discovered a trend towards decreased numbers of other infiltrating immune cells in miltefosine-treated animals, while the numbers of tissue resident alveolar macrophages remained the same across all treatment groups. In order to confirm whether infiltration of other immune cells is directly affected by miltefosine, we performed control experiments in eosinophil-deficient Δdbl GATA-1 mice. We discovered that the decreased infiltration of most immune cells was at least partially due to the decreased eosinophil numbers. This is not unexpected, since activated eosinophils are known to attract and activate other immune cell types such as neutrophils (Yousefi et al., 1995) or B cells (Chu et al., 2011). Moreover, eosinophil-derived CCL17 and CCL22 have proven to be crucial in attracting effector T cells in localized allergic inflammation (Jacobsen et al., 2008). Interestingly, we observed a decrease in dendritic cell (CD11c+/MHCII+/Siglec-F−/Ly6G−/Ly6C−) numbers following miltefosine treatment compared with challenged controls both in wild-type and in eosinophil-deficient mice. It has been reported previously that combination therapy of paromomomycin/miltefosine can influence TLR9 on dendritic cells and therefore modulate Th1 host response.
immune responses in leishmaniasis therapy (Das et al., 2014). As of yet, however, the direct effect of miltefosine on human dendritic cells remains unclear.

To assess whether the observed differences in immune cell count are a consequence of miltefosine directly inhibiting immune cell infiltration or rather indirectly altering the cytokine milieu in the lung, we additionally tested bronchoalveolar lavage supernatants from both BALB/c and Δdbl GATA-1 mice for cytokine expression. Cytokine concentrations of CCL11, TNF-α, IL-2 and IL-5 were not altered in miltefosine-treated and ovalbumin-stimulated mice of both genotypes. Interestingly however, we observed significantly reduced levels of the immunomodulatory cytokine IFN-γ in the miltefosine-treated group of BALB/c mice. These data corroborate previous findings from Verhaar et al. (2013), where they observed similarly reduced levels of IFN-γ in miltefosine-treated animals in a mouse model of inflammatory bowel disease. IFN-γ has on one hand long been considered to be beneficial in allergic inflammation as reviewed by Teixeira et al. (2005), while on the other hand, recent studies recognize its pro-inflammatory functions. Our findings of reduced IFN-γ expression in BALB/c mice and not in Δdbl GATA-1 mice are of particular interest, since it was discovered that eosinophil-derived IFN-γ induces airway hyperresponsiveness and lung inflammation even in the absence of lymphocytes (Kanda et al., 2009). Interestingly, IFN-γ was also found to up-regulate several eosinophil effector functions (Ishihara et al., 1997; Takaku et al., 2011) and promote their survival (Fujisawa et al., 1994).

When we examined the composition of immune cells in mouse blood, miltefosine-treated and CCL24-stimulated IL-5Tg animals showed an increased neutrophil count, yet miltefosine-treated BALB/c animals showed no altered neutrophil numbers at baseline. A previous study showed that patients treated with miltefosine exhibited increased levels of the neutrophilic chemokine IL-8 (CCL8) (Mukhopadhyay et al., 2011). This finding remains to be confirmed in mice. Increased corticosterone levels in mice induced by miltefosine could be another plausible explanation for both increased neutrophil numbers (Liles et al., 1995) and decreased airway inflammation (Suqin et al., 2009). Furthermore, an inverse association between endogenous glucocorticoid and IFN-γ levels was observed in allergic lung inflammation (Suqin et al., 2009). Nonetheless, we observed no significant alterations in corticosterone levels in miltefosine-treated mice.

Fang et al. additionally showed that miltefosine acts on endothelial cells by down-regulating E-selectin, which is important for leukocyte adhesion and infiltration (Leung et al., 2007). Therefore, in our ovalbumin model of allergic inflammation, we cannot neglect additional anti-inflammatory effects of miltefosine. In another point, our work raises important questions regarding the immunomodulatory effect of miltefosine in patients treated for leishmania infections. So far, little has been reported about the drug’s effect on the host responses responsible for fighting the infection. However, some in vitro findings report a strong reversal of T2 responses of leishmania-infected macrophages towards Th1 type following miltefosine treatment (Wadhone et al., 2009). Since eosinophils are one of the primary cells recruited to the sites of leishmania infection (de Oliveira Cardoso et al., 2010) and have been shown to help control parasite load (Watanabe et al., 2004) in mice, it might be of interest to further investigate this issue in patients treated with miltefosine. In line with the present study, we have previously shown that saturated lysophosphatidylcholines, which are structurally similar to miltefosine, inhibit eosinophil effector responses (Knuplez, Curcic, et al., 2020; Knuplez, Krier-Burris, et al., 2020; Trieb et al., 2019).

A limitation of our work needs to be noted. Ovalbumin was used as a model allergen in our in vivo studies, albeit this model fails to completely reflect the aetiology of human asthma and its multi-step developmental process, including environmental factors associated with the disease. Further experiments with other physiological relevant antigens are needed to validate the relevance of our data in human disease setting.

In summary, we demonstrate the inhibitory effect of the orphan drug miltefosine on human eosinophils and its anti-inflammatory effect in vivo in a model of allergic inflammation. Our data highlight the potential efficacy of miltefosine or related molecules in the treatment of allergic diseases and other eosinophil-associated disorders.

FUNDING INFORMATION
This study was supported by the Austrian Science Fund (FWF Grants W1241, DK-MOLIN and P30144).

AUTHOR CONTRIBUTIONS
E.K. designed and performed the experiments, analysed the data, interpreted the results and wrote the manuscript. A.T. and M.K. performed the experiments, analysed the data and edited the manuscript. E.M.S., A.H. and R.S. interpreted the results and edited the manuscript. G.M. designed and supervised the study, interpreted the results and edited the manuscript.

CONFLICT OF INTEREST
A.H. received consultancy fees from AstraZeneca. The other authors declare no conflicts of interest.

DECLARATION OF TRANSPARENCY AND SCIENTIFIC RIGOUR
This Declaration acknowledges that this paper adheres to the principles for transparent reporting and scientific rigour of preclinical research as stated in the BJP guidelines for Design & Analysis, Immunoblotting and Immunochemistry and Animal Experimentation, and as recommended by funding agencies, publishers and other organizations engaged with supporting research.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request. Some data may not be made available because of privacy or ethical restrictions.

ORCID
Eva Knuplez https://orcid.org/0000-0001-6376-5842
Malta de Sá, M., Sresht, V., Rangel-Yagui, C. O., & Blankschtein, D. (2015). Clinical pharmacokinetics of systemically administered antileishmanial drugs. Clinical Pharmacokinetics, 57, 151–176. https://doi.org/10.1007/s40262-017-0570-0

Knuplez, E., Curcic, S., Theiler, A., Bärnthaler, T., Trakaki, A., Trieb, M., ... Marsche, G. (2020). Lyosphosphatidylcholines inhibit human eosinophil activation and suppress eosinophil migration in vivo. Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids, 1865, 158686.

Knuplez, E., Krier-Burris, R., Cao, Y., Marsche, G., O'Sullivan, J., & Bochner, B. S. (2020). Frontline Science: Superior mouse eosinophil depletion in vivo targeting transgenic Siglec-8 instead of endogenous Siglec-F: Mechanisms and pitfalls. Journal of Leukocyte Biology, 108, 43–58. https://doi.org/10.1172/JLB.3HI0120-381R

Kumar, R., Herbert, C., & Foster, P. (2008). Inflammatory bowel disease in asthma mice. Current Drug Targets, 9, 485–494. https://doi.org/10.2174/13894500878453561

Liles, W. C., Dale, D. C., & Klebanoff, S. J. (1995). Glucocorticoids inhibit apoptosis of human neutrophils. Blood, 86(8), 3181–3188.

Lilley, E., Stanford, S. C., Kendall, D. E., Alexander, S. P., Cirino, G., Docherty, J. R., ... Ahluwalia, A. (2020). ARRIVE 2.0 and the British Journal of Pharmacology: Updated guidance for 2020. British Journal of Pharmacology. https://doi.org/10.1111/bph.15178

Luschnig-Schratt, P., Sturm, E. M., Konya, V., Philippose, S., Marsche, G., Fröhlich, E., ... Heinemann, A. (2011). EP4 receptor stimulation down-regulates human eosinophil function. Cellular and Molecular Life Sciences, 68, 3573–3587. https://doi.org/10.1007/s00018-011-0642-5

Magerl, M., Rother, M., Bieber, T., Biedermann, T., Brasch, J., ... Dráber, P. (2018). Mast cell activation and suppress eosinophil migration in vivo. Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids, 1865, 158686.

Mauer, M., Magerl, M., Metz, M., Weller, K., & Siebenhaar, F. (2013). Miltefosine: A novel treatment option for mast cell-mediated diseases. Current Drug Targets, 14, 125–140. https://doi.org/10.1186/1563-9046-14-125

Moreira, R. A., Mendanha, A. S., Fernandes, K. S., Matos, G. G., Alonso, L., Dorta, M. L., & Alonso, A. (2014). Miltefosine increases lipid and protein dynamics in Leishmania amazonensis membranes at concentrations similar to those needed for cytotoxicity activity. Antimicrobial Agents and Chemotherapy, 58, 3021–3028. https://doi.org/10.1128/AAC.01332-13

Mukhopadhyay, D., Das, N. K., Roy, S., Kundu, S., Babhushua, J. N., & Chatterjee, M. (2011). Miltefosine effectively modulates the cytokine milieu in Indian post kala-azar dermal leishmaniasis. The Journal of Infectious Diseases, 204, 1427–1436. https://doi.org/10.1093/infdis/jir551

Ochur, S. L., Jacobsen, E. A., Protheroe, C. A., Biechele, T. L., Pero, R. S., McGarry, M. P., ... Lee, N. A. (2007). Coexpression of IL-5 and eosin-2 in mice creates an eosinophil-dependent model of respiratory inflammation with characteristics of severe asthma. Journal of Immunology, 178, 7879–7889. https://doi.org/10.4049/jimmunol.178.12.7879

Percie du Sert, N., Hurst, V., Ahluwalia, A., Alam, S., Avey, M. T., Baker, M., ... Würbel, H. (2020). The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. PLoS Biology, 18(7), e3000410. https://doi.org/10.1371/journal.pbio.3000410

Piipiers, J., Den Boer, M. L., Essink, D. R., & Ritmeijer, K. (2019). The safety and efficacy of miltefosine in the long-term treatment of post-kala-azar dermal leishmaniasis in South Asia—A review and meta-analysis. PLoS Neglected Tropical Diseases, 13, e0007173. https://doi.org/10.1371/journal.pntd.0007173

Pinto-Martinez, A. K., Rodriguez-Durán, J., Serrano-Martín, X., Hernandez-Rodriguez, V., & Benaim, G. (2018). Mechanism of action of miltefosine on Leishmania donovani involves the impairment of acidocalcisome function and the activation of the sphingosine-dependent plasma membrane Ca2+ channel. Antimicrobial Agents and Chemotherapy, 62, e01614–e01617.

Rakotomanga, M., Blanc, S., Gaudin, K., Chaminade, P., & Loiseau, P. M. (2007). Miltefosine affects lipid metabolism in Leishmania donovani promastigotes. Antimicrobial Agents and Chemotherapy, 51, 1425–1430. https://doi.org/10.1128/AAC.01123-06

Rubiková, Z., Sullmenko, V., Paulinda, T., & Dráber, P. (2018). Mast cell activation and microtubule organization are modulated by miltefosine through protein kinase C inhibition. Frontiers in Immunology, 9, 1563. https://doi.org/10.3389/fimmu.2018.01563

Ruiter, G. A., Zerp, S. F., Bartelink, H., Van Blitterswijk, W. J., & Verheij, M. (2003). Anti-cancer alkyl-lysophospholipids inhibit the phosphatidylinositol 3-kinase–Akt/PKB survival pathway. Anticancer Drugs, 14, 167–173. https://doi.org/10.1097/00001813-20030200-00011

Rybczynska, M., Liu, R., Lu, P., Sharom, F. J., Steinfeis, E., Di Pietro, A., ... Hofmann, J. (2001). MDR1 causes resistance to the antitumour drug miltefosine. British Journal of Cancer, 84, 1405–1411. https://doi.org/10.1054/bjoc.2001.1776

Schratl, P., Sturm, E. M., Royer, J. F., Sturm, G. J., Lippe, I. T., Peskar, B. A., & Heinemann, A. (2006). Hierachy of eosinophil chemoattractants: Role of p38 mitogen-activated protein kinase. European Journal of Immunology, 36, 2401–2409. https://doi.org/10.1002/eji.200535672

Stone, K. D., Prussin, C., & Metcalfe, D. D. (2010). IgE, mast cells, basophils, and eosinophils. The Journal of Allergy and Clinical Immunology, 125, 573–580. https://doi.org/10.1626/jaci.2010.11.017

Sunyoto, T., Potet, J., & Boelaert, M. (2018). Why miltefosine—a life-saving drug for leishmaniasis—is unavailable to people who need it the most. BMJ Global Health, 3, e000709.

Suqin, Z., Ziyin, S., Guorang, H., Runhong, L., & Xinmin, Z. (2009). Effects of miltefosine on Leishmania amazonensis acidocalcisome function and the activation of the sphingosine-dependent protein kinase Cβ. Memorias Do Instituto Oswaldo Cruz (Fundacao Oswaldo Cruz), 100, 137–144.

Theler, A., Bärnthaler, T., Platter, W., Richtig, G., Peihaupt, M., Ritthön, S., ... Heinemann, A. (2019). Butyrate ameliorates allergic airway inflammation by limiting eosinophil trafficking and survival. The Journal of Allergy and Clinical Immunology, 144, 764–776. https://doi.org/10.1016/j.jaci.2019.05.002

Trieb, M., Wolf, P., Knuplez, E., Weger, W., Schuster, C., Peihaupt, M., ... Marsche, G. (2019). Abnormal composition and function of high-density lipoproteins in atopic dermatitis patients. Allergy: European Journal of Allergy and Clinical Immunology, 74, 398–402. https://doi.org/10.1111/all.13620

Verhaar, A. P., Wildenberg, M. E., te Velde, A. A., Meijer, S. L., Vos, A. C. W., Duijvestein, M., ... van den Brink, G. R. (2013). Miltefosine suppresses inflammation in a mouse model of inflammatory bowel disease. Inflammatory Bowel Diseases, 19, 1.
Wadhone, P., Maiti, M., Agarwal, R., Kamat, V., Martin, S., & Saha, B. (2009). Miltefosine promotes IFN-γ-dominated anti-leishmanial immune response. *Journal of Immunology*, 182, 7146–7154. https://doi.org/10.4049/jimmunol.0803859

Watanabe, Y., Hamaguchi-Tsuru, E., Morimoto, N., Nishio, Y., Yagyu, K. I., Konishi, Y., ... Tominaga, A. (2004). IL-5-induced eosinophils suppress the growth of *Leishmania amazonensis* in vivo and kill promastigotes in vitro in response to either IL-4 or IFN-γ. *DNA and Cell Biology*, 23, 412–418. https://doi.org/10.1089/1044549041474805

Weller, K., Artuc, M., Jennings, G., Friedrichson, T., Guhl, S., dos Santos, R. V., ... Maurer, M. (2009). Miltefosine inhibits human mast cell activation and mediator release both in vitro and in vivo. *The Journal of Investigative Dermatology*, 129, 496–498. https://doi.org/10.1038/jid.2008.248

Willetts, L., Ochkur, S. I., Jacobsen, E. A., Lee, J. J., & Lacy, P. (2014). Eosinophil shape change and secretion (pp. 111–128). New York, NY: Humana Press.

Yoon, J., Um, H.-N., Jang, J., Bae, Y.-A., Park, W.-J., Kim, H. J., ... Jung, Y. J. (2019). Eosinophil activation by toll-like receptor 4 ligands regulates macrophage polarization. *Frontiers in Cell and Development Biology*, 7, 329. https://doi.org/10.3389/fcell.2019.00329

Yousefi, S., Hemmann, S., Weber, M., Hölzer, C., Hartung, K., Blaser, K., & Simon, H. U. (1995). IL-8 is expressed by human peripheral blood eosinophils. Evidence for increased secretion in asthma. *The Journal of Immunology*, 154, 5481–5490.

**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section at the end of this article.

---

**How to cite this article:** Knuplez E, Kienzl M, Trakaki A, et al. The anti-parasitic drug miltefosine suppresses activation of human eosinophils and ameliorates allergic inflammation in mice. *Br J Pharmacol*. 2021;178:1234–1248. https://doi.org/10.1111/bph.15368