Leishmania and other intracellular pathogens: selectivity, drug distribution and PK–PD

[BSP Autumn Symposium "Microbial protein targets: towards understanding and intervention", 14th–16th September 2016, University of Durham UK]

SIMON L. CROFT*

Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London WC1E 7HT, UK

(*Received 9 August 2017; accepted 25 August 2017; first published online 6 October 2017*)

SUMMARY

New drugs and treatments for diseases caused by intracellular pathogens, such as leishmaniasis and the *Leishmania* species, have proved to be some of the most difficult to discover and develop. The focus of discovery research has been on the identification of potent and selective compounds that inhibit target enzymes (or other essential molecules) or are active against the causative pathogen in phenotypic *in vitro* assays. Although these discovery paradigms remain an essential part of the early stages of the drug R & D pathway, over the past two decades additional emphasis has been given to the challenges needed to ensure that the potential anti-infective drugs distribute to infected tissues, reach the target pathogen within the host cell and exert the appropriate pharmacodynamic effect at these sites. This review will focus on how these challenges are being met in relation to *Leishmania* and the leishmaniases with lessons learned from drug R & D for other intracellular pathogens.

Key words: *Leishmania*, pharmacodynamics, pharmacokinetics, drug distribution.

INTRODUCTION

In 2017, a small collection of drugs and treatments are used and recommended for visceral leishmaniasis (VL) (WHO, 2010; Aronson *et al*. 2017) and even fewer that have proven effective in the treatment of cutaneous leishmaniasis (CL) (González *et al*. 2008, 2009; Aronson *et al*. 2017). Of these, and still widely in use, are the pentavalent antimonials (Pentostam has been in use since the 1940s; Goodwin, 1995), amphotericin B, which was first used for leishmaniasis in 1960 (Sampaio *et al*. 1960) and as a liposomal formulation in 1991 (Davidson *et al*. 1991), paromomycin first used for leishmaniasis in 1963 (Kellina *et al*. 1966), and miltefosine identified as anti-leishmanial in 1984 (Croft *et al*. 1987) and registered for use for VL treatment in 2002 (Sundar *et al*. 2012). The limitations of these drugs and treatments have been reviewed elsewhere (Croft, Olliaro, 2011; Aronson *et al*. 2017). It is worth noting that these drugs providing the standard treatments for VL and CL are mainly re-purposed. To help improve the drug R & D process it is important to ask why it is taking so long to identify purpose-designed anti-leishmanials.

* Corresponding author: Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, UK. E-mail: simon.croft@lshtm.ac.uk

Approaches to the design, discovery and development of anti-infectives have advanced considerably over the past two decades and several unique molecular and biochemical targets of parasites/microbes are the subject of other articles in this volume. For leishmaniasis advances in molecular biology and structural biology have similarly led the elaboration of validated targets and inhibitors (Gilbert, 2013; Horn and Duraisingh, 2014), whilst high-throughput (HTS) and high content (HCS) have led to the identification of novel chemical series (Siqueira-Neto *et al*. 2012; Peña *et al*. 2015), as well as the identification of novel targets (Khare *et al*. 2016). A pragmatic use of screening and extension of chemical series with known anti-kinetoplastid activity has produced lead compounds, and novel chemical entities (NCEs), from oxaboroles, nitroimidazoles, aminopyrazoles; which are all promising candidates in the anti-leishmanial development pipeline (http://www.dndi.org).

The parts played by pharmacokinetics (PK) and medicinal chemistry have also lead to improved design of compounds able to target pathogens in infected tissues, for example in the CNS (Wring *et al*. 2014) and the macrophage (Rajendran *et al*. 2010), tissues that are relevant to the distribution of anti-trypanosomal and anti-leishmanial agents. In addition, the past decade has seen the integration

Parasitology (2018), **145**, 237–247.
doi:10.1017/S0031182017001664

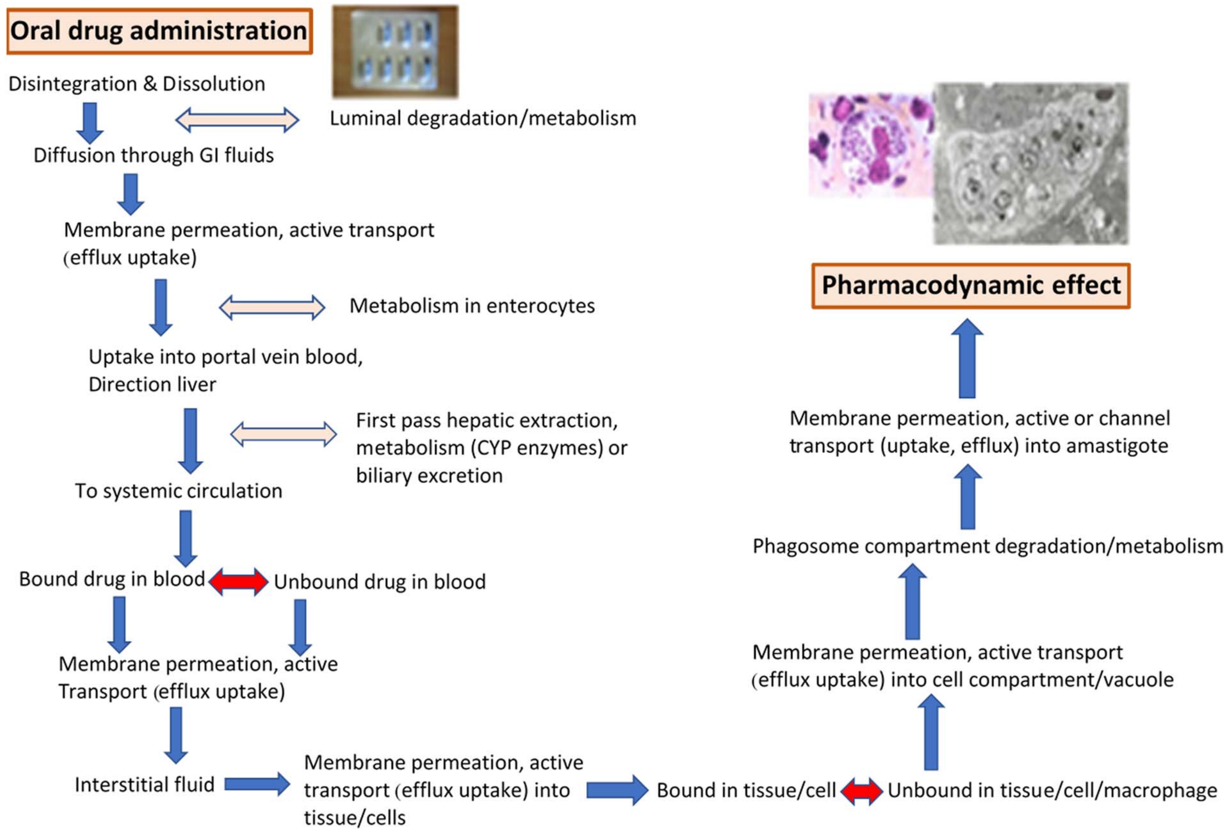

Fig. 1. The physiological barriers and pharmacokinetic requirements for an active compound in an oral drug (miltefosine capsules shown) to reach the *Leishmania* amastigote (adapted from Martinez, Amidon, 2002, *Journal of Clinical Pharmacology*, 620–643). Light and electron micrographs of *L. donovani* amastigotes.

of PK, pharmacodynamics (PD) and physiology-based (PB) modelling, as illustrated in recent reviews on PK–PD analysis (Nielsen and Friberg, 2013) and PB–PK analysis (Edginton *et al*. 2008), into drug design and the prediction of appropriate dosing of novel and current anti-infective drugs. The importance of PK–PD analysis has been well demonstrated for drug design for *Mycobacterium tuberculosis* (Davies and Nuermberger, 2008; Dartois, 2014), a pathogen that occupies a similar intracellular site as *Leishmania*. Another recent approach, using small molecules, has been to target host factors/receptors and nutrient sources, as considered for *M. tuberculosis* (Guler and Brombacher, 2015; Zumla *et al*. 2015), and in a more limited way for leishmaniasis, for example with simvastatin (Parihar *et al*. 2016). Modulation of the host's immune response has been a long-term goal, with some small molecules showing activity through known targets, for example, imiquimod (Buates and Matlashewski, 1999) and tucaresol (Smith *et al*. 2000).

LEISHMANIA, *PHYSIOLOGICAL BARRIERS AND DRUG DISTRIBUTION*

There is nothing new about the concept of selectivity and drug distribution as the basis for the design, discovery and development of anti-infective drugs. Over the past century our work has been framed by the likes of Ehrlich (1913), '…we may speak of magic bullets which aim exclusively at the dangerous intruding parasites, strangers to the organism, but do not touch the organism, itself and its cells…'. Alberts (1985) also described the challenges of achieving drug selective toxicity, comparing them at three levels – whole-body distribution, biochemistry and cytology, while linking the physicochemical properties of compounds/drugs to pharmacodynamic effects.

At the start of the drug discovery process it is essential to consider both: (i) the TPP (target product profile) of the drug needed (see, for example, http://www.dndi.org/diseases-projects/target-product-profiles) and (ii) the required distribution and PK of novel compounds to infected tissues. These considerations have to be integrated into the discovery and development of new treatments for both VL and CL. The design of oral drugs or topical formulations for the treatment of VL and/or CL has to account for the number of physiological barriers that the anti-leishmanial molecule meets before reaching the molecular target. Both the series of membrane barriers and the different protein-binding properties in plasma and tissues (Fig. 1), are challenges to be met before the novel compound reaches the

https://doi.org/10.1017/S0031182017001664 Published online by Cambridge University Press

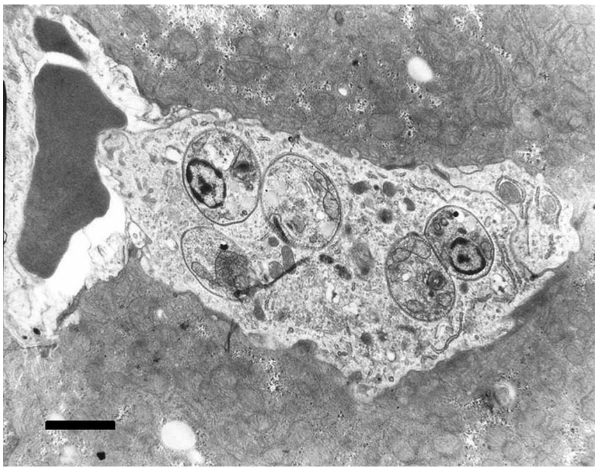

Fig. 2. *Leishmania donovani* amastigotes in mouse liver Kupffer cell, showing blood vessel–host cell interface [bar + 1uM].

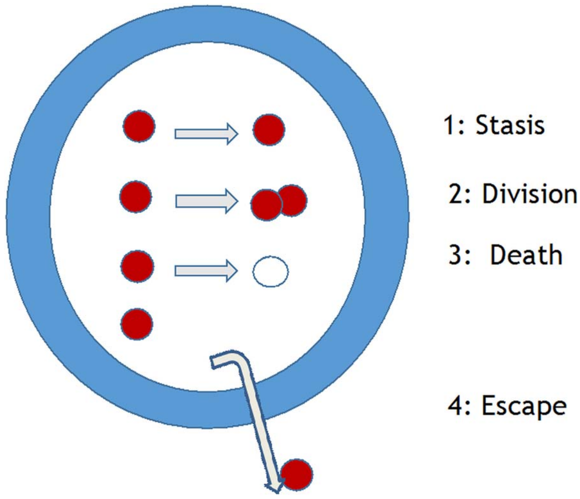

Fig. 3. *Leishmania* amastigotes – potential four fates of the intracellular amastigote in a macrophage [Courtesy of R. Diaz-Gonzalez and S. Croft].

macrophage host cell in which the *Leishmania* amastigote survives and multiplies (Fig. 2). For CL drugs, there are the additional barriers of extravasation and the interstitial fluid compartment, factors often not considered in this complex design. These challenges are not exclusive to those working on drug R & D for *Leishmania*; similar problems arise for intracellular bacteria, in particular *Mycobacteria*, and we can learn much from that research (Dartois, 2014).

So, how can our knowledge of tissue and cellular distribution be exploited to improve drug design? How can our understanding of membrane barriers and protein binding improve our ability to ensure drugs reach the sites where they are needed? How can formulations and drug delivery be best exploited for this purpose?

SELECTIVITY AND PHARMACODYNAMICS

The initial identification of hit and lead compounds is normally determined in *in vitro* assays. Criteria for *in vitro* assays to measure killing of the intracellular *Leishmania* amastigote, were listed several decades ago (Croft, 1986); this list needs to be updated to include: (i) the rate of kill, which has been shown to be important for other protozoa (Sanz *et al*. 2012), (ii) the type of macrophage used (see Seifert *et al*. 2010 below), and (iii) a panel of recent clinical isolates, as there is known strain/species variation in drug sensitivity (Croft *et al*. 2006). The standard phrase to introduce the *Leishmania* parasite in many reviews is '*Leishmania* survive and multiply in host macrophages'. Leaving aside immunological issues of macrophage activation (Kaye and Scott, 2011), measurements of drug or NCE effects should relate to potency against a dividing population of amastigotes in a defined macrophage population. However, there are four possible states for an amastigote in the macrophage (Fig. 3): stasis, division, death or escape. These issues, are also a major concern for *M. tuberculosis* researchers, but have only recently been investigated for *Leishmania*. *In vitro* studies have given some measures of rate of division through ^{3}H-thymidine uptake (Sifontes and Croft, unpublished) and the bromodeoxyuridine analogue (EDU) (Tegazzini *et al*. 2016), both methods indicating incorporation of these indicators of nucleic acid synthesis into about 50% of *Leishmania donovani* amastigotes in macrophage assays. In the steps from *in vitro* assay to rodent model and from rodent model to human, it is essential to know the 'predictivity' of the model. So, are *in vitro* assays with rapid rates of division more predictive of activity in the rodent model than those with slow rates of division? Some *in vivo* studies suggest that the answer is possibly not. Recent studies on *Leishmania major* and *Leishmania mexicana* amastigotes in mouse models have shown that: (i) *L. mexicana* amastigote division rate is slow (12 days) and sub-populations develop that are either semi-quiescent or fast-growing state (Kloehn *et al*. 2015) and (ii) *L. major* amastigotes in lesions similarly show fast and slow replicating populations (Mandell and Beverley, 2017). Similar questions must now be asked about *L. donovani* liver and spleen infections in mouse and hamster models to understand the relevance and predictivity of our current models. This underlines the frailty of the PD parameters currently applied in PK–PD analysis for *Leishmania* models. It would be a great benefit to *Leishmania* research to be able to transfect with reporter genes that reflect *in vivo* growth rates. In studies on *Salmonella* different bacteria cell division rates in different tissues were shown to correlate with different levels of killing by antibiotics (Claudi *et al*. 2014).

In addition to the above concerns about 'predictivity', most amastigote–macrophage *in vitro* assays, the current workhorse of anti-leishmanial drug discovery, over the past three decades, have used either

https://doi.org/10.1017/S0031182017001664 Published online by Cambridge University Press

macrophage cell lines (e.g. THP-1 cells) or primary isolated rodent macrophages (Neal and Croft, 1984; De Rycker *et al*. 2013). These have on the whole been useful in hit and lead identification. Whether there are more appropriate assays for further evaluation and lead identification has been questioned. *Ex vivo* models with splenic and lymph node tissue have been used in drug assays (Osorio *et al*. 2011; Peniche *et al*. 2014) to try to more closely match host physiological conditions. Recently we have developed a medium perfusion/flow system, using interstitial flow rates, with *L. major*-infected macrophages. There is a shift in the dose–response curves of several standard anti-leishmanial drugs in flow *vs* static systems (O'Keeffe, Croft, unpublished), where EC_{50} points were similar but EC_{90} points were significantly lower in the flow model.

MACROPHAGES

The host macrophage is a central consideration in drug design and targeting in addition to its role *in vitro* drug assays, from monocytic cell lines (such as THP-1, J774 lines) used in HTS, to murine peritoneal or bone marrow macrophages used in evaluation and mechanistic studies. The role of the macrophage in the immune response, pathogenesis, and parasite invasion and survival in the phagosomal compartment has been well described elsewhere (Kaye and Scott, 2011). One 'macrophage' factor that is important in therapy and certainly in deriving PD parameters, is the heterogeneity of the macrophage cell population (Gordon *et al*. 2014) with different cell types having different functions and properties in different tissues. Recent *in vitro* studies with *L. donovani* in amastigote macrophage assays has shown that significant differences in amastigote drug susceptibility are macrophage-type dependent (Seifert *et al*. 2010; Koniordou *et al*. 2017). This is not just an issue for standardization of assays, it is also important in understanding differences in host cell drug accumulation and role of transporters, as well as drug metabolism, for example pentavalent antimonials are metabolized by macrophages (Frézard *et al*. 2009) and their activity altered through macrophage activation (Murray *et al*. 1988). The changes in macrophage metabolism with *Leishmania* infection could also impact drug activity and be different between macrophage populations, as has been shown for *M. tuberculosis* using RNA-seq analysis (Andreu *et al*. 2017). Connections between drug uptake to cell type, infection and activation status have long been defined for anti-bacterials (Carlier *et al*. 1990). With new sensitive methods to measure anti-leishmanial drug accumulation into immune cells (Kip *et al*. 2015) further studies to define *Leishmania*–host cell–drug interactions are needed.

There is also opportunity to exploit the physicochemical properties of the phagolysosomal compartment, by manipulating conditions to improve drug activity, as exemplified by doxycline treatment of *Coxiella* infection (Maurin *et al*. 1992). The relationship between the pH of the phagolysosomal vacuole and amastigote survival is well established and pH manipulation with basic drugs, such as chloroquine, leads to parasite death. We have recently shown that in combination with chloroquine the activity of paromomycin can be significantly enhanced against *L. major* and *L. mexicana* amastigotes *in vitro* in mouse peritoneal macrophages (Wijnant *et al*. 2017*a*). The acidic environment of the lysosomal compartment of cells has been studied in relation to the accumulation of charged basic molecules with dicationic molecules such as azithromycin having high accumulation levels (see Van Bambeke *et al*. 2006). The potential for concentration and trapping through protonation of novel molecules was exquisitely exploited by Rabinovitch *et al*. (1986) using amino acid esters to kill *L. amazonensis* amastigotes in mouse peritoneal macrophages. Regulation of the phagosome compartment occurs through a number of membrane enzymes and transporters, including the well-characterized vacuolar proton-ATPase (v-ATPase) involved in vacuole acidification and biogenesis (Vinet *et al*. 2011) and the cation transporter Nramp 1 (Jabado *et al*. 2000). Vacuolar enzyme and pH manipulation is being exploited in anti-cancer therapies, where the actions of known inhibitors synergize with standard drugs, with an impact on chemosensitization and reversal of chemoresistance (Swietach *et al*. 2012). This is an area little studied in relation to *Leishmania*; one report has shown naloxonazine can upregulate V-ATPase in *L. donovani*-infected THP-1 cells (De Muylder *et al*. 2016). Outside the phagolysomal vacuole, the metabolic changes seen in macrophage biology and the numerous pathways altered by *Leishmania* infection, from iron to cholesterol and beyond has been recently reviewed (Duque and Descoteaux, 2015).

Finally, the macrophage cell surface has several defined receptors that have been used in drug targeting to these host cells. 25 years ago Nègre *et al*. (1992), showed that allopurinol riboside linked to mannosylated – poly-L-lysine as the carrier molecule to target the mannose receptor, improved drug activity 50-fold in an *in vitro* macrophage model. There have been several other publications describing tagged liposomes or nanoparticles in experimental models to target and increase drug accumulation by infected macrophages. However, given the added complexity of the synthesis, costs, stability and PK, it is unlikely that this approach will lead to new treatments for leishmaniasis.

PK AND PREDICTIVE MODELS

As part of the effort to reduce the attrition rates that occur at each stage of the drug R & D process – from *in vitro* to animal models, from animal models to

https://doi.org/10.1017/S0031182017001664 Published online by Cambridge University Press

clinical candidate, and then in clinical trials – major efforts have been made to develop more predictive models to advance optimized novel compounds at each stage (for pharmacodynamics see above) and their behaviour in animal models and humans, i.e. PK. PKs normally encompass the properties of a novel compound/drug of absorption, distribution, metabolism and excretion. The anti-infective drug researcher also needs to ensure appropriate distribution of the compound to the infected tissue in the host, as well as retention of the compound within the infected tissue/cells for a period to give sufficient exposure to significantly reduce the parasite load. Over the past two decades there has been an increasing focus on the interrelationship between PDs and PKs, including tissues other than just plasma in the analysis. The PK–PD paradigms were first outlined for anti-bacterials (Craig, 1998) with an initial focus on how this information, which often focuses on defining drug activities in terms of either concentration dependent rate of killing or time-dependent rate of killing, can be used to help determine effective dose regimens. For anti-leishmanials PK–PD analysis has helped to redefine miltefosine dosing in children for both VL and CL (Dorlo *et al*. 2012; Castro *et al*. 2017). However, for most anti-leishmanial drugs we have little data on their time-dependent or concentration-dependent activities, nor how these data can be used to optimize dose regimes. Analytical and mathematical tools are available to simulate PK/PD relationships of anti-leishmanials when there is sufficient and appropriate data. One such system has been developed for anti-malarial pre-clinical development, which has helped to identify the properties of compounds important for clinical performance and, importantly, partner drugs for combination therapy (Patel *et al*. 2015; Aljayyoussi *et al*. 2016).

This analysis of the PK–PD relationship has also become central to drug development. A Pfizer team analysed the role of fundamental PK–PD as part of a process to improve the survival rate of compounds in clinical trials, i.e. what predictive indicators are of most importance to reduce attrition. They defined 'three pillars of survival' for a novel compound as: (i) exposure at the target site of action over a desired period of time; (ii) binding to the pharmacological target as expected for its mode of action; and (iii) expression of pharmacological activity commensurate with the demonstrated target exposure and target binding (Morgan *et al*. 2012). An important element of PK–PD analysis is that *in vivo* only the free (i.e. not bound to protein) drug concentration determines activity (Smith *et al*. 2010); hence for *Leishmania* knowledge of drug concentration in the phagosome vacuole is of importance but so far undetermined. For leishmaniasis, we have limited retrospective data on miltefosine, as summarized by Dorlo *et al*. (2012). More research has been reported on the liposomal formulation of amphotericin B, the unilamellar liposome AmBisome ™. We have shown that in a BALB/c mouse model (with data from liver, spleen and plasma) that following iv dosing of AmBisome parasites in the liver are killed quicker and more effectively than those in the spleen (Voak *et al*. 2017). This can be explained by more extensive drug accumulation by the liver than spleen and the different drug kinetics between the two organs, although drug accumulation in these target organs was higher during the early stages of infection than later stages. Earlier studies with AmBisome in mice showed lower amphotericin B accumulation in infected than uninfected mice and also that the formulation was less effective in the later stage than an early stage infection (Mullen *et al*. 1998; Gershkovich *et al*. 2010). The effectiveness of drugs in different tissues and distribution of drug in the tissues needs to be considered alongside the changes that occur in liver and spleen structure and function during early and late stages on infection (Yurdakul *et al*. 2011; Kaye and Beattie, 2016). Recent studies on *M. tuberculosis* drugs to study their spatial distribution in lung lesions used MALDI mass spectrometry imaging to reveal which TB drugs penetrated the lesions (Prideaux *et al*. 2015); this is an approach which should be applied to help us understand the tissue distribution of anti-leishmanials.

The use of transfected *Leishmania* parasites with either fluorescent or bioluminescent properties have been used in studies on infection, pathology and chemotherapy over the past decade. But in terms of analysing key properties of anti-leishmanial drug action, these methods are only now starting to be fully exploited, in particular in rodent models of several species causing CL where drug efficacy and relapse have been measured (Coelho *et al*. 2016; Caridha *et al*. 2017). However, with good signal and high-resolution imaging it will be important to improve the methodologies to measure: (i) dose response effect, (ii) *in vivo* rate of kill and (iii) any differences in drug efficacy between sites of infection.

Drug combinations, which are presently for leishmaniasis really co-administrations, have proved to be clinically advantageous in the treatment of VL (Sundar *et al*. 2011). Current combinations of standard anti-leishmanial drugs are based solely on doses used in monotherapies. As novel oral compounds are developed over the next 5 years and genuine combinations are discussed, then drug combinations based upon knowledge of PK–PD components of partner drugs and their interactions will be needed, as shown for anti-malarials (Hastings *et al*. 2016). We also know that major challenges for treatment are VL–HIV co-infections (Van Griensven *et al*. 2014). There have been some *in vitro* studies on

https://doi.org/10.1017/S0031182017001664 Published online by Cambridge University Press

efficacy of combined anti-retroviral/anti-leishmanial drug interactions (Costa *et al*. 2016) and indication of anti-leishmanial activity of HIV-protease inhibitors (Van Griensven *et al*. 2013). However, there have been few studies where a rational approach to fully understand interactions between anti-leishmanial and anti-retroviral drugs and how this knowledge could be used to design more effective treatments. In contrast to tuberculosis and malaria where drug-drug interactions have been well characterized (see University of Liverpool, UK website http://www.hiv-druginteractions.org) there is limited analysis on anti-leishmanial drugs.

The collection of more PD data (from HCS screens) and PK data with the need to include specific compartments for tissues infected and uninfected, plus the overriding need to integrate all to inform dosing of the novel drug in humans, has refocused need for the application of computational, modelling and systems biology (Van der Greef and McBurney, 2005; Zhao and Iyengar, 2012). Recent mathematical model of anti-malarials has focused on simulations of PK/PD to predict clinical activity of new compounds (Aljayyoussi *et al*. 2016). For leishmaniasis, a disease caused by a parasite that survives, multiplies and subverts the immune responses of the host macrophage (Kaye and Scott, 2011), the elements of immunity and immunopathology also have to be built into any predictive model. A computational Petri net model of *L. donovani* infection and granulomas in mouse liver (Albergante *et al*. 2013; Moore *et al*. 2013) illustrates another approach to disease simulation, an approach that is being further exploited to understand the relationship between the immune response and the activity and the PKs of anti-leishmanial drugs (http://www.crackit.org.uk/multiscale-model-minimise-animal-usage-leishmaniasis-drug-development and http://www.leishsim.org) .

CL, SKIN AND TOPICAL FORMULATIONS

Considering the variety of clinical manifestations and the impact of CL, there is a notable absence of drugs and treatments that are clinically effective (González *et al*. 2008, 2009); this is an area of research neglect. This does not only apply to the classical forms of CL; there is also a need for improved treatments offering shorter courses, and less toxic drugs for post kala-azar dermal leishmaniasis (PKDL), as cases are infective to sandflies (Hirve *et al*. 2016) and PKDL patients act as a human reservoir for transmission and as such are a threat to elimination and control programmes.

Most research on CL has been focussed on immunological responses to CL in mice and humans (Kaye and Scott, 2011; Scott and Novais, 2016), leading to paradigms on T-cell responses, which in conjunction with developments in knowledge on skin immunity and inflammation (see Pasparakis *et al*. 2014) has provided some new understanding of the pathogenesis of CL. There have been several studies over the past decade showing how this understanding of skin immune response can be exploited for treatment, with a good example being the use of imiquimod in mice and humans (Buates and Matlashewski, 1999; Miranda-Verastegui *et al*. 2009). More recently, other approaches to treatment have resulted from long-term human CL research in South America. Novais *et al*. (2017) showed that NLRP3 inflammasome is activated by CD8+ T cell-mediated cytotoxicity and drives disease progression. This led to experimental studies in mice using a number of small molecule inhibitors of the inflammasome, for example, MCC950 and the diabetes drug glyburide. They showed that treatment with compounds that inhibit NLRP3 inflammasome activation, MCC950 or glyburide, failed to develop the severe disease seen in untreated mice.

Apart from the immunotherapy approaches, the design and delivery of small molecules after oral administration to the skin has to be considered in the context of vascularization (drug gradient and distance between blood vessel and site), an interstitial fluid compartment, the impact of inflammation on drug accumulation and extra-vasation, blood flow rate (slow) and local oxygen tension (low). Improved understanding of PK of anti-leishmanial drugs in the skin has come from clinical studies where Dorlo and colleagues provided data on both PK and PD in human CL (*L. major)* patients treated with miltefosine (Dorlo *et al*. 2008) although establishment of the full relationship between exposure and response has yet to be determined. We are now also beginning to understand how local inflammation at the CL site of infection can lead to specific accumulation of some drugs, for example liposomal amphotericin B, to improve cure (Wijnant *et al*. 2017*b*).

A critical decision in the development pathway for CL treatment is whether to choose systemic or topical administration. Topical formulations have been used for the treatment of CL since the 1920s when stibosan (an early pentavalent antimonial) ointment was used to treat 'oriental sore'. We have come a long way since 1935 when the use of an ointment consisting of '1 part pulverized vegetable charcoal and nine parts of concentrated sulphuric acid' was described. However, the full exploitation of pharmaceutics and knowledge of skin, from utilization of knowledge of skin physiology and PB–PK models, alteration of vasculature, role of protein binding and other factors (Jepps *et al*. 2013), including lymphatic flow to deeper layers (Dancik *et al*. 2012), are only now being applied to the development of new treatments for CL. The renaissance in the topical approach was led by El-On *et al*. (1984)

with paromomycin, using an irritant and transdermal enhancing agent (methyl benzethonium chloride) to increase drug permeation by pore formation, the basis for the product Leishcutan® (Teva, Israel). Another formulation of topical paromomycin, containing 15% paromomycin–0·5% gentamicin and several excipients (called WR 279,396) with known absorption and skin PK (Ravis *et al*. 2013) when applied with an occlusion, has successfully completed phase three trials (Ben Salah *et al*. 2013).

Focus on potency, permeation, and distribution (Jepps *et al*. 2013) is important for both formulation design and the selection of appropriate compounds with both high potency and, through their chemical properties, dermal distribution. As part of our strategy, we : (i) identify novel compounds that work against a panel of clinical isolates of the 15 species, as *Leishmania* species that cause CL vary significantly in their drug susceptibility (Escobar *et al*. 2002; Croft *et al*. 2006); (ii) select active compounds with appropriate medicinal chemistry, toxicity and ADME (absorption, distribution, metabolism, excretion) properties; (iii) test in mouse models of infection (oral and systemic administration); (iv) optimize for compound structure and formulations in relation to skin distribution; (v) decide whether topical administration is appropriate and further optimize the formulations using both mouse and human skin in permeation studies; and (vi) ensure that treatment is effective against early-stage infections with intact skin (prior to ulceration) as the aim is to develop a treatment effective before the patient has developed into a large disfiguring ulcer.

When considering the PK of drugs for CL, it is important to remember that the *Leishmania* are in macrophages in the skin dermis, and that the infection is not superficial like many bacterial or fungal infections. Within the dermis at the site of infection, there is well-characterized inflammation and granuloma development (Scott and Novais, 2017) and amastigotes in macrophages. This is found in both the nodule that precedes ulceration or later in the dermal rim around the ulcer. The aim for a systemic formulation is penetration from the vasculature *via* the interstitial fluid and distribution to the inflammatory site of infection. However, for a topical formulation the aims are permeation of the skin barriers and then distribution to the site of infection. In both cases exposure following distribution and residence of the compound at the site of infection has to be optimized. Some of the practices of pharmaceutical scientists working on skin for cosmetics and other purposes have been adopted for CL studies over the past decade. Using methodologies like the Franz cell, it is possible to measure the rate of diffusion of anti-leishmanial drugs across skin (of animal models and humans) alone and in different formulations, as shown for buparvaquone where the most effective topical formulation *in vivo* proved to be the one that crossed the skin most slowly in the Franz cell model (Garnier *et al*. 2007*a*, *b*). Recently this work has been extended to include *Leishmania*-infected skin. Permeation markers, for example caffeine and ibuprofen, as well as some standard anti-leishmanial drugs have been shown to have different *in vitro* permeation properties through normal mouse skin, compared with mouse skin removed from a nodule of infection (Van Boxclaer *et al*. 2016*a*). Drugs permeate significantly faster through skin taken from the site of infection, possibly due to oedema and the different immunological profile at this site of inflammation. As more extensive exposure in the dermis is critical to formulation design, the permeation properties of formulation excipients alone and together need to be explored. A re-examination of topical formulations of the anti-leishmanial drug miltefosine, using *in vitro* and *in vivo* models already mentioned, and a range of formulations in which the partition of miltefosine was characterized, was unable to identify a formulation with good permeation and efficacy (Van Bocxlaer *et al*. 2016*b*).

CONCLUSIONS FOR LEISHMANIASIS DRUG R & D

There have been several reviews that represent the drug R & D process as a linear diagram from discovery to clinical trial. However, drug R & D is a multidisciplinary iterative process with many decision points, and the involvement of several teams across disciplines (Baxter *et al*. (2013). The parasitologist has key roles within this complex picture and an awareness of the comprehensive list of detailed information that he/she should aim to provide as part of a drug research team – ranging from work on enzyme targets to PK is needed. Although the concept of the 'minimum information about a bioactive entity (MIABE)' (Orchard *et al*. 2011) was established to provide guidance for what and how results should be reported, their review also provides a fundamental list of research information that needs to be gleaned from studies. In the specific area of leishmaniasis and *Leishmania*, where there are a large variety of assays and models involving different species, strains and stages of the *Leishmania* parasite, different host cells and different mammalian hosts, it is hardly surprising that there can be significant differences in data obtained between laboratories resulting in reports of irreproducibility of compound activities. In addition to basic precepts, such inclusion of controls, Fig. 4 is an attempt to summarize the main PD and PK-related factors that must be considered when collecting data during drug discovery and early pre-clinical studies for a novel anti-leishmanial compound. Although there are benefits for standardization, a process necessary when determining

https://doi.org/10.1017/S0031182017001664 Published online by Cambridge University Press

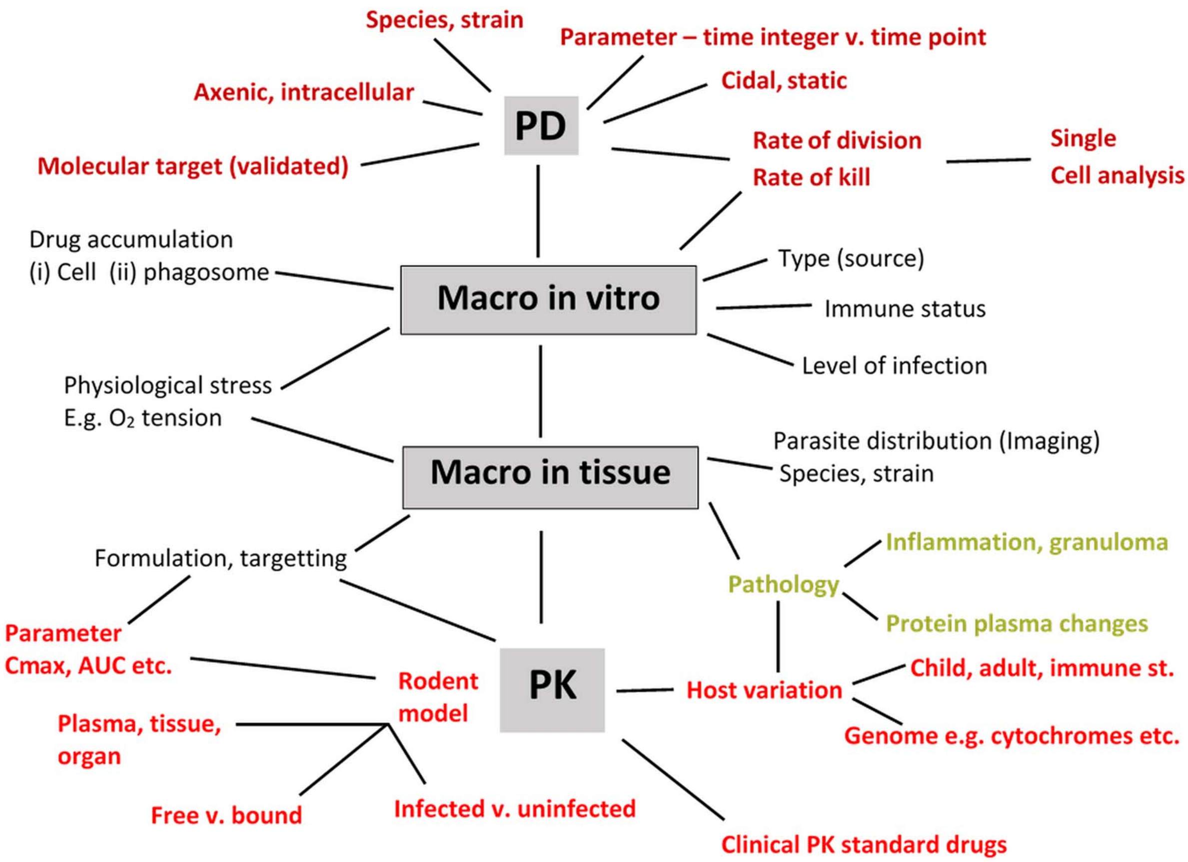

Fig. 4. Information to collect to support discovery and early pre-clinical development of a novel anti-leishmanial compound. PD – pharmacodynamics (axenic, intracellular amastigotes); PK – pharmacokinetics (C_{max}: maximum concentration in plasma; AUC: Area-under-the curve as measure of drug exposure; macro – macrophage; free *vs* bound – protein-binding properties of compound.

drug sensitivity of clinical isolates (Hendrickx *et al*. 2017), it is hardly feasible in the drug R & D process. But it is feasible for all those concerned to provide the levels of information sought and provided (Orchard *et al*. 2011) so that data can be interpreted by all those interested in playing a role in the development of the next drug and treatment for leishmaniasis.

ACKNOWLEDGEMENTS

The author is grateful to innumerable colleagues, partners, collaborators and mentors over the past decades whose advice, comments and input have contributed to this review.

FINANCIAL SUPPORT

The author has been supported recently by UK Medical Research Council (MRC), GSK Open Lab Foundation, NC3Rs CRACK IT, EU FP7 programme, DNDi Geneva and BBSRC for research on leishmaniasis.

REFERENCES

Albergante, L., Timmis, J., Beattie, L. and Kaye, P. M. (2013). A Petri net model of granulomatous inflammation: implications for IL-10 mediated control of *Leishmania donovani* infection. *PLoS Computational Biology* **9**, e1003334.

Alberts, A. (1985). *Selective Toxicity. The Physicochemical Basis of Therapy*, 5th Edn. Chapman & Hall, London.

Aljayyoussi, G., Kay, K., Ward, S. A. and Biagini, G. A. (2016). OptiMal-PK: an internet-based, user-friendly interface for the mathematical-based design of optimized anti-malarial treatment regimens. *Malaria Journal* **15**, 344.

Andreu, N., Phelan, J., de Sessions, P. F., Cliff, J. M., Clark, T. G. and Hibberd, M. L. (2017). Primary macrophages and J774 cells respond differently to infection with *Mycobacterium tuberculosis*. *Scientific Reports* **7**, srep42225.

Aronson, N., Herwaldt, B. L., Libman, M., Pearson, R., Lopez-Velez, R., Weina, P., Carvalho, E., Ephros, M., Jeronimo, S. and Magill, A. (2017). Diagnosis and treatment of Leishmaniasis: clinical practice guidelines by the infectious diseases society of America (IDSA) and the American society of tropical medicine and hygiene (ASTMH). *American Journal of Tropical Medicine and Hygiene* **96**, 24–45.

Baxter, K. L., Horn, E., Gal-Ed, N., Zonno, K., O'Leary, J., Terry, P. F. and Terry, S. F. (2013). An end to the myth: there is no drug development pipeline. *Science Translational Medicine* **5**(171), 171.

Ben Salah, A., Ben Messaoud, N., Guedri, E., Zaatour, A., Ben Alaya, N., Bettaieb, J., Gharbi, A., Belhadj Hamida, N., Boukthir, A., Chlif, S., Abdelhamid, K., El Ahmadi, Z., Louzir, H., Mokni, M., Morizot, G., Buffet, P., Smith, P. L., Kopydlowski, K. M., Kreishman-Deitrick, M., Smith, K. S., Nielsen, C. J., Ullman, D. R., Norwood, J. A., Thorne, G. D., McCarthy, W. F., Adams, R. C., Rice, R. M., Tang, D., Berman, J., Ransom, J., Magill, A. J. and Grogl, M. (2013). Topical paromomycin with or without gentamicin for cutaneous leishmaniasis. *The New England Journal of Medicine* **368**, 524–532.

Buates, S. and Matlashewski, G. (1999). Treatment of experimental leishmaniasis with the immunomodulators imiquimod and S-28463: efficacy and mode of action. *Journal of Infectious Diseases* **179**, 1485–1494.

Caridha, D., Parriot, S., Hudson, T. H., Lang, T., Ngundam, F., Leed, S., Sena, J., Harris, M., O'Neil, M., Sciotti, R., Read, L., Lecoeur, H., Hickman, M. and Grogl, M. (2017). Use of optical imaging technology in the validation of a new, rapid, cost-effective drug screen as part of a tiered *in vivo* screening paradigm for development of drugs to treat cutaneous Leishmaniasis. *Antimicrobial Agents and Chemotherapy* **61**, e02048-16.

Carlier, M.-B., Scorneaux, B., Zenebergh, A., Desnottes, J.-F. and Tulkens, P. M. (1990). Cellular uptake, localization and activity of fluoroquinolones in uninfected and infected macrophages. *Journal of Antimicrobial Chemotherapy* **26**, 27–39.

Castro, M. M., Gomez, M. A., Kip, A. E., Cossio, A., Ortiz, E., Navas, A., Dorlo, T. P. C. and Saravia, N. G. (2017) Pharmacokinetics of miltefosine in children and adults with cutaneous leishmaniasis. *Antimicrobial Agents and Chemotherapy* **61**, e02198–16.

Claudi, B., Spröte, P., Chirkova, A., Personnic, N., Zankl, J., Schürmann, N., Schmidt, A. and Bumann, D. (2014). Phenotypic variation of Salmonella in host tissues delays eradication by antimicrobial chemotherapy. *Cell* **158**, 722–733.

Coelho, A. C., Oliveira, J. C., Espada, C. R., Reimão, J. Q., Trinconi, C. T. and Uliana, S. R. B. (2016) A luciferase-expressing *Leishmania braziliensis* line that leads to sustained skin lesions in BALB/c mice and allows monitoring of miltefosine treatment outcome. *PLoS Neglected Tropical Diseases* **10**(5), e0004660.

Costa, S., Machado, M., Cavadas, C. and do Céu Sousa, M. (2016). Antileishmanial activity of antiretroviral drugs combined with miltefosine. *Parasitology Research* **115**, 3881–3887.

Craig, W. A. (1998). Pharmacokinetic/pharmacodynamic parameters: rationale for antibacterial dosing of mice and men. *Clinical Infectious Diseases* **26**, 1–10.

Croft, S. L. (1986). *In vitro* screens in the experimental chemotherapy of leishmaniasis and trypanosomiasis. *Parasitology Today* **2**, 64–69.

Croft, S. L. and Olliaro, P. (2011). Leishmaniasis chemotherapy – challenges and opportunities. *Clinical Microbiology and Infection* **17**, 1478–1483.

Croft, S. L., Neal, R. A., Pendergast, W. and Chan, J. H. (1987). The activity of alkyl phosphorylcholines and related derivatives against *Leishmania donovani*. *Biochemical Pharmacology* **36**, 2633–2636.

Croft, S. L., Sundar, S. and Fairlamb, A. H. (2006). Drug resistance in leishmaniasis. *Clinical Microbiology Reviews* **19**, 111–126.

Dancik, Y., Anissimov, Y. G., Jepps, O. G. and Roberts, M. S. (2012). Convective transport of highly plasma protein bound drugs facilitates direct penetration into deep tissues after topical application. *British Journal of Clinical Pharmacology* **73**, 564–578.

Dartois, V. (2014). The path of anti-tuberculosis drugs: from blood to lesions to mycobacterial cells. *Nature Reviews. Microbiology* **12**, 159–167.

Davidson, R. N., Scott, A., Maini, M., Bryceson, A. D. M. and Croft, S. L. (1991). Liposomal amphotericin B in drug resistant visceral leishmaniasis. *The Lancet* **337**(8749), 1061–1062.

Davies, G. R. and Nuermberger, E. L. (2008). Pharmacokinetics and pharmacodynamics in the development of anti-tuberculosis drugs. *Tuberculosis* **88**, S65–S74.

De Rycker, M., Hallyburton, I., Thomas, J., Campbell, L., Wyllie, S., Joshi, D., Cameron, S., Gilbert, I. H., Wyatt, P. G., Frearson, J. A., Fairlamb, A. H. and Gray, D. W. (2013) Comparison of a high-throughput high-content intracellular *Leishmania donovani* assay with an axenic amastigote assay. *Antimicrobial Agents and Chemotherapy* **57**, 2913–2922.

Dorlo, T. P. C., van Thiel, P. P. A. M., Huitema, A. D. R., Keizer, R. J., de Vries, H. J. C., Beijnen, J. H. and de Vries, P. J. (2008). Pharmacokinetics of miltefosine in old world cutaneous leishmaniasis patients. *Antimicrobial Agents and Chemotherapy* **52**, 2855–2860.

Dorlo, T. P. C., Balasegaram, M., Beijnen, J. H. and de Vries, P. J. (2012). Miltefosine: a review of its pharmacology and therapeutic efficacy in the treatment of leishmaniasis. *Journal of Antimicrobial Chemotherapy* **67**, 2576–2597.

Duque, G. A. and Descoteaux, A. (2015). Leishmania survival in the macrophage: where the ends justify the means. *Current Opinion in Microbiology* **26**, 32–40.

Edginton, A. N., Theil, F.-P., Schmitt, W. and Willmann, S. (2008). Whole body physiologically-based pharmacokinetic models: their use in clinical drug development. *Expert Opinion on Drug Metabolism & Toxicology* **4**, 1143–1152.

Ehrlich, P. (1913). A lecture on chemotherapeutics. *Lancet* **ii**, 445–451.

El-On, J., Jacobs, G. P., Witztum, E. and Greenblatt, C. L. (1984). Development of topical treatment for cutaneous leishmaniasis caused by *Leishmania major* in experimental animals. *Antimicrobial Agents and Chemotherapy* **26**, 745–751.

Escobar, P., Matu, S., Marques, C. and Croft, S. L. (2002). Sensitivities of *Leishmania* species to hexadecylphosphocholine (miltefosine), ET-18-OCH(3) (edelfosine) and amphotericin B. *Acta Tropica* **81**, 151–157.

Frézard, F., Demicheli, C. and Ribeiro, R. R. (2009). Pentavalent antimonials: new perspectives for old drugs. *Molecules* **14**, 2317–2336.

Garnier, T., Mäntylä, A., Järvinen, T., Lawrence, J., Brown, M. and Croft, S. (2007*a*). *In vivo* studies on the antileishmanial activity of buparvaquone and its prodrugs. *Journal of Antimicrobial Chemotherapy* **60**, 802–810.

Garnier, T., Mäntylä, A., Järvinen, T., Lawrence, M. J., Brown, M. B. and Croft, S. L. (2007*b*). Topical buparvaquone formulations for the treatment of cutaneous leishmaniasis. *Journal of Pharmacy and Pharmacology* **59**, 41–49.

Gershkovich, P., Wasan, E. K., Sivak, O., Li, R., Zhu, X., Werbovetz, K. A., Tidwell, R. R., Clement, J. G., Thornton, S. J. and Wasan, K. M. (2010). Visceral leishmaniasis affects liver and spleen concentrations of amphotericin B following administration to mice. *Journal of Antimicrobial Chemotherapy* **65**, 535–537.

Gilbert, I. H. (2013). Drug discovery for neglected diseases: molecular target-based and phenotypic approaches. *Journal of Medicinal Chemistry* **56**, 7719–7726.

González, U., Pinart, M., Reveiz, L. and Alvar, J. (2008). Interventions for Old World cutaneous leishmaniasis. *Cochrane Database of Systematic Reviews* (4): CD005067.

González, U., Pinart, M., Rengifo-Pardo, M., Macaya, A., Alvar, J. and Tweed, J. A. (2009). Interventions for American cutaneous and mucocutaneous leishmaniasis. *Cochrane Database of Systematic Reviews* (2): CD004834.

Goodwin, L. G. (1995). Pentostam® (sodium stibogluconate); a 50-year personal reminiscence. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **89**, 339–341.

Gordon, S., Plüddemann, A. and Martinez Estrada, F. (2014). Macrophage heterogeneity in tissues: phenotypic diversity and functions. *Immunological Reviews* **262**, 36–55.

Guler, R. and Brombacher, F. (2015). Host-directed drug therapy for tuberculosis. *Nature Chemical Biology* **11**, 748–751.

Hastings, I. M., Hodel, E. M. and Kay, K. (2016). Quantifying the pharmacology of antimalarial drug combination therapy. *Scientific Reports* **6**, srep32762.

Hendrickx, S., Guerin, P. J., Caljon, G., Croft, S. L. and Maes, L. (2017). Evaluating drug resistance in visceral leishmaniasis: the challenges. *Parasitology* online. doi: 10.1017/S0031182016002031.

Hirve, S., Boelaert, M., Matlashewski, G., Mondal, D., Arana, B., Kroeger, A. and Olliaro, P. (2016) Transmission dynamics of visceral Leishmaniasis in the Indian subcontinent – a systematic literature review. *PLoS Neglected Tropical Diseases* **10**(8), e0004896.

Horn, D. and Duraisingh, M. T. (2014). Antiparasitic chemotherapy – from genomes to mechanisms. *Annual Review of Pharmacology and Toxicology* **54**, 71–94.

Jabado, N., Jankowski, A., Dougaparsad, S., Picard, V., Grinstein, S. and Gros, P. (2000). Natural resistance to intracellular infections. *Journal of Experimental Medicine* **192**, 1237–1248.

Jepps, O. G., Dancik, Y., Anissimov, Y. G. and Roberts, M. S. (2013). Modeling the human skin barrier – towards a better understanding of dermal absorption. *Advanced Drug Delivery Reviews* **65**, 152–168.

Kaye, P. and Scott, P. (2011). Leishmaniasis: complexity at the host-pathogen interface. *Nature Reviews. Microbiology* **9**, 604–615.

Kaye, P. M. and Beattie, L. (2016). Lessons from other diseases: granulomatous inflammation in leishmaniasis. *Seminars in Immunopathology* **38**, 249–260.

Kellina, O. I., Iniakhina, A. V. and Iastrebova, R. I. (1966). Treatment of cutaneous leishmaniasis with monomycin. *Med Parazitol (Mosk)* **35**, 283–287.

Khare, S., Nagle, A. S., Biggart, A., Lai, Y. H., Liang, F., Davis, L. C., Barnes, S. W., Mathison, C. J. N., Myburgh, E., Gao, M.-Y., Gillespie, J. R., Liu, X., Tan, J. L., Stinson, M., Rivera, I. C., Ballard, J., Yeh, V., Groessl, T., Federe, G., Koh, H. X. Y., Venable, J. D., Bursulaya, B., Shapiro, M., Mishra, P. K., Spraggon, G., Brock, A., Mottram, J. C., Buckner, F. S., Rao, S. P. S., Wen, B. G., *et al.* (2016). Proteasome inhibition for treatment of leishmaniasis, Chagas disease and sleeping sickness. *Nature* **537**, 229–233.

Kip, A. E., Rosing, H., Hillebrand, M. J. X., Castro, M. M., Gomez, M. A., Schellens, J. H. M., Beijnen, J. H. and Dorlo, T. P. C. (2015). Quantification of miltefosine in peripheral blood mononuclear cells by high-performance liquid chromatography-tandem mass spectrometry. *Journal of Chromatography. B. Analytical Technologies in the Biomedical and Life Sciences* **998–999**, 57–62.

Kloehn, J., Saunders, E. C., O'Callaghan, S., Dagley, M. J. and McConville, M. J. (2015). Characterization of metabolically quiescent Leishmania parasites in murine lesions using heavy water labeling. *PLoS Pathogens* **11**, e1004683.

Koniordou, M., Patterson, S., Wyllie, S. and Seifert, K. (2017). Snapshot profiling of anti-leishmanial potency of lead compounds and drug candidates against intracellular L. donovani amastigotes with focus on human derived host cells. *Antimicrobial Agents and Chemotherapy* **61**, e01228-16.

Mandell, M. A. and Beverley, S. M. (2017). Continual renewal and replication of persistent Leishmania major parasites in concomitantly immune hosts. *Proceedings of the National Academy of Sciences of the United States of America*.

Maurin, M., Benoliel, A. M., Bongrand, P. and Raoult, D. (1992). Phagolysosomal alkalinization and the bactericidal effect of antibiotics: the Coxiella burnetii paradigm. *Journal of Infectious Diseases* **166**, 1097–1102.

Miranda-Verastegui, C., Tulliano, G., Gyorkos, T. W., Calderon, W., Rahme, E., Ward, B., Cruz, M., Llanos-Cuentas, A. and Matlashewski, G. (2009). First-line therapy for human cutaneous Leishmaniasis in Peru using the TLR7 agonist imiquimod in combination with pentavalent antimony. *PLoS Neglected Tropical Diseases* **3**, e491.

Moore, J. W. J., Moyo, D., Beattie, L., Andrews, P. S., Timmis, J. and Kaye, P. M. (2013). Functional complexity of the Leishmania granuloma and the potential of *in silico* modeling. *Frontiers in Immunology* **4**.

Morgan, P., Van Der Graaf, P. H., Arrowsmith, J., Feltner, D. E., Drummond, K. S., Wegner, C. D. and Street, S. D. A. (2012). Can the flow of medicines be improved? Fundamental pharmacokinetic and pharmacological principles toward improving phase II survival. *Drug Discovery Today* **17**(9–10), 419–424.

Mullen, A. B., Baillie, A. J. and Carter, K. C. (1998). Visceral Leishmaniasis in the BALB/c mouse: a comparison of the efficacy of a nonionic surfactant formulation of sodium stibogluconate with those of three proprietary formulations of amphotericin B. *Antimicrobial Agents and Chemotherapy* **42**, 2722–2725.

Murray, H. W., Berman, J. D. and Wright, S. D. (1988). Immunotherapy for intracellular Leishmania donovani infection: gamma interferon plus pentavalent antimony. *Journal of Infectious Diseases* **157**, 973–978.

Muylder, G. D., Vanhollebeke, B., Caljon, G., Wolfe, A. R., McKerrow, J. and Dujardin, J.-C. (2016). Naloxonazine, an amastigote-specific compound, affects Leishmania parasites through modulation of host-encoded functions. *PLoS Neglected Tropical Diseases* **10**, e0005234.

Neal, R. A. and Croft, S. L. (1984). An *in-vitro* system for determining the activity of compounds against the intracellular amastigote form of Leishmania donovani. *Journal of Antimicrobial Chemotherapy* **14**, 463–475.

Nègre, E., Chance, M. L., Hanboula, S. Y., Monsigny, M., Roche, A. C., Mayer, R. M. and Hommel, M. (1992). Antileishmanial drug targeting through glycosylated polymers specifically internalized by macrophage membrane lectins. *Antimicrobial Agents and Chemotherapy* **36**, 2228–2232.

Nielsen, E. I. and Friberg, L. E. (2013). Pharmacokinetic-pharmacodynamic modeling of antibacterial drugs. *Pharmacological Reviews* **65**, 1053–1090.

Novais, F. O., Carvalho, A. M., Clark, M. L., Carvalho, L. P., Beiting, D. P., Brodsky, I. E., Carvalho, E. M. and Scott, P. (2017). CD8+ t cell cytotoxicity mediates pathology in the skin by inflammasome activation and IL-1β production. *PLOS Pathogens* **13**, e1006196.

Orchard, S., Al-Lazikani, B., Bryant, S., Clark, D., Calder, C., Dix, I., Engkvist, O., Forster, M., Gaulton, A., Gilson, M., Glen, R., Grigorov, M., Hammond-Kosack, K., Harland, L., Hopkins, A., Larminie, C., Lynch, N., Mann, R. K., Murray-Rust, P., Lo Piparo, E., Southan, E., Steinbeck, C., Wishart, D., Henning Hermjakob, H., Overington, J. and Thornton, J. (2011) Minimum information about a bioactive entity (MIABE). *Nature Reviews Drug Discovery* **10**, 661–669.

Osorio, Y., Travi, B. L., Renslo, A. R., Peniche, A. G. and Melby, P. C. (2011). Identification of small molecule lead compounds for visceral Leishmaniasis using a novel *ex vivo* splenic explant model system. *PLoS Neglected Tropical Diseases* **5**, e962.

Parihar, S. P., Hartley, M.-A., Hurdayal, R., Guler, R. and Brombacher, F. (2016). Topical Simvastatin as host-directed therapy against severity of cutaneous Leishmaniasis in mice. *Scientific Reports* **6**, srep33458.

Pasparakis, M., Haase, I. and Nestle, F. O. (2014). Mechanisms regulating skin immunity and inflammation. *Nature Reviews*. *Immunology* **14**, 289–301.

Patel, K., Simpson, J. A., Batty, K. T., Zaloumis, S. and Kirkpatrick, C. M. (2015). Modelling the time course of antimalarial parasite killing: a tour of animal and human models, translation and challenges. *British Journal of Clinical Pharmacology* **79**, 97–107.

Peña, I., Manzano, M. P., Cantizani, J., Kessler, A., Alonso-Padilla, J., Bardera, A. I., Alvarez, E., Colmenarejo, G., Cotillo, I., Roquero, I., de Dios-Anton, F., Barroso, V., Rodriguez, A., Gray, D. W., Navarro, M., Kumar, V., Sherstnev, A., Drewry, D. H., Brown, J. R., Fiandor, J. M. and Martin, J. J. (2015). New compound sets identified from high throughput phenotypic screening against three kinetoplastid parasites: an open resource. *Scientific Reports* **5**, srep08771.

Peniche, A. G., Renslo, A. R., Melby, P. C. and Travi, B. L. (2014) Development of an *ex vivo* lymph node explant model for identification of novel molecules active against Leishmania major. *Antimicrobial Agents and Chemotherapy* **58**, 78–87.

Prideaux, B., Via, L. E., Zimmerman, M. D., Eum, S., Sarathy, J., O'Brien, P., Chen, C., Kaya, F., Weiner, D. M., Chen, P.-Y., Song, T., Lee, M., Shim, T. S., Cho, J. S., Kim, W., Cho, S. N., Olivier, K. N., Barry, C. E. and Dartois, V. (2015). The association between sterilizing activity and drug distribution into tuberculosis lesions. *Natural Medicines* **21**, 1223–1227.

Rabinovitch, M., Zilberfarb, V. and Ramazeilles, C. (1986). Destruction of Leishmania mexicana amazonensis amastigotes within macrophages by lysosomotropic amino acid esters. *Journal of Experimental Medicine* **163**, 520–535.

Rajendran, L., Knölker, H.-J. and Simons, K. (2010). Subcellular targeting strategies for drug design and delivery. *Nature Reviews*. *Drug Discovery* **9**, 29–42.

Ravis, W. R., Llanos-Cuentas, A., Sosa, N., Kreishman-Deitrick, M., Kopydlowski, K. M., Nielsen, C., Smith, K. S., Smith, P. L., Ransom, J. H., Lin, Y.-J. and Grogl, M. (2013). Pharmacokinetics and absorption of paromomycin and gentamicin from topical creams used to treat cutaneous leishmaniasis. *Antimicrobial Agents and Chemotherapy* **57**, 4809–4815.

Sampaio, S. A. P., Godoy, J. T., Paiva, L., Dillon, N. L. and de Lacaz, C. S. (1960). The treatment of American (mucocutaneous) leishmaniasis with amphotericin B. *Archive of Dermatology* **82**, 627–635.

Sanz, L. M., Crespo, B., De-Cózar, C., Ding, X. C., Llergo, J. L., Burrows, J. N., García-Bustos, J. F. and Gamo, F.-J. (2012). P. falciparum *in vitro* killing rates allow to discriminate between different antimalarial mode-of-action. *PLoS ONE* **7**, e30949.

Scott, P. and Novais, F. O. (2016). Cutaneous leishmaniasis: immune responses in protection and pathogenesis. *Nature Reviews Immunology* **16**, 581–592.

Seifert, K., Escobar, P. and Croft, S. L. (2010). *In vitro* activity of anti-leishmanial drugs against *Leishmania donovani* is host cell dependent. *Journal of Antimicrobial Chemotherapy* **65**, 508–511.

Siqueira-Neto, J. L., Moon, S., Jang, J., Yang, G., Lee, C., Moon, H. K., Chatelain, E., Genovesio, A., Cechetto, J. and Freitas-Junior, L. H. (2012). An image-based high-content screening assay for compounds targeting intracellular *Leishmania donovani* amastigotes in human macrophages. *PLoS Neglected Tropical Diseases* **6**, e1671.

Smith, A. C., Yardley, V., Rhodes, J. and Croft, S. L. (2000). Activity of the novel immunomodulatory compound tucaresol against experimental visceral leishmaniasis. *Antimicrobial Agents and Chemotherapy* **44**, 1494–1498.

Smith, D. A., Di, L. and Kerns, E. H. (2010). The effect of plasma protein binding on *in vivo* efficacy: misconceptions in drug discovery. *Nature Reviews*. *Drug Discovery* **9**, 929–939.

Sundar, S., Sinha, P. K., Rai, M., Verma, D. K., Nawin, K., Alam, S., Chakravarty, J., Vaillant, M., Verma, N., Pandey, K., Kumari, P., Lal, C. S., Arora, R., Sharma, B., Ellis, S., Strub-Wourgaft, N., Balasegaram, M., Olliaro, P., Das, P. and Modabber, F. (2011). Comparison of short-course multidrug treatment with standard therapy for visceral leishmaniasis in India: an open-label, non-inferiority, randomised controlled trial. *Lancet* **377**(9764), 477–486.

Sundar, S., Singh, A., Rai, M., Prajapati, V. K., Singh, A. K., Ostyn, B., Boelaert, M., Dujardin, J.-C. and Chakravarty, J. (2012). Efficacy of miltefosine in the treatment of visceral leishmaniasis in India after a decade of use. *Clinical Infectious Diseases* **55**, 543–550.

Swietach, P., Hulikova, A., Patiar, S., Vaughan-Jones, R. D. and Harris, A. L. (2012). Importance of intracellular pH in determining the uptake and efficacy of the weakly basic chemotherapeutic drug, doxorubicin. *PLOS ONE* **7**, e35949.

Tegazzini, D., Díaz, R., Aguilar, F., Peña, I., Presa, J. L., Yardley, V., Martin, J. J., Coteron, J. M., Croft, S. L. and Cantizani, J. (2016) A replicative *in vitro* assay for drug discovery against *Leishmania donovani*. *Antimicrobial Agents and Chemotherapy* **60**, 3524–3532.

Van Bambeke, F., Barcia-Macay, M., Lemaire, S. and Tulkens, P. M. (2006). Cellular pharmacodynamics and pharmacokinetics of antibiotics: current views and perspectives. *Current Opinion in Drug Discovery & Development* **9**(2), 218–230.

Van Bocxlaer, K., Yardley, V., Murdan, S. and Croft, S. L. (2016*a*). Drug permeation and barrier damage in Leishmania-infected mouse skin. *Journal of Antimicrobial Chemotherapy* **71**, 1578–1585.

Van Bocxlaer, K., Yardley, V., Murdan, S. and Croft, S. L. (2016*b*). Topical formulations of miltefosine for cutaneous leishmaniasis in a BALB/c mouse model. *Journal of Pharmacy and Pharmacology* **68**, 862–872.

Van der Greef, J. and McBurney, R. N. (2005). Innovation: rescuing drug discovery: *in vivo* systems pathology and systems pharmacology. *Nature Reviews*. *Drug Discovery* **4**, 961–967.

Van Griensven, J., Diro, E., Lopez-Velez, R., Boelaert, M., Lynen, L., Zijlstra, E., Dujardin, J.-C. and Hailu, A. (2013). HIV-1 protease inhibitors for treatment of visceral leishmaniasis in HIV-co-infected individuals. *The Lancet Infectious Diseases* **13**, 251–259.

Van Griensven, J., Zijlstra, E. E. and Hailu, A. (2014). Visceral Leishmaniasis and HIV coinfection: time for concerted action. *PLoS Neglected Tropical Diseases* **8**, e3023.

Vinet, A. F., Jananji, S., Turco, S. J., Fukuda, M. and Descoteaux, A. (2011). Exclusion of synaptotagmin V at the phagocytic cup by Leishmania donovani lipophosphoglycan results in decreased promastigote internalization. *Microbiology* **157**, 2619–2628.

Voak, A., Harris, A., Qaiser, Z., Croft, S. and Seifert, K. (2017). Treatment of experimental visceral leishmaniasis with single-dose liposomal amphotericin B – pharmacodynamics and biodistribution at different stages of disease. *Antimicrobial Agents and Chemotherapy* in press.

Wijnant, G. J., Van Bocxlaer, K., Yardley, V., Murdan, S. and Croft, S. L. (2017*a*). Efficacy of a paromomycin plus chloroquine combination therapy in experimental cutaneous Leishmaniasis. *Antimicrobial Agents and Chemotherapy* in press.

Wijnant, G. J., Van Bocxlaer, K., Yardley, V., Harris, A., Murdan, S. and Croft, S. L. (2017*b*). Accumulation of amphotericin B in lesions and healthy skin areas of L. major infected BALB/c mice after AmBisome treatment. Worldleish 6, poster C-0351. http://worldleish2017.org/documentos/Abstracts_BookWL6_final.pdf.

World Health Organization (2010). Control of Leishmaniasis, *WHO Technical Report Series*, 949.

Wring, S., Gaukel, E., Nare, B., Jacobs, R., Beaudet, B., Bowling, T., Mercer, L., Bacchi, C., Yarlett, N., Randolph, R., Parham, R., Rewerts, C., Platner, J. and Don, R. (2014). Pharmacokinetics and pharmacodynamics utilizing unbound target tissue exposure as part of a disposition-based rationale for lead optimization of benzoxaboroles in the treatment of stage 2 human African trypanosomiasis. *Parasitology* **141**, 104–118.

Yurdakul, P., Dalton, J., Beattie, L., Brown, N., Erguven, S., Maroof, A. and Kaye, P. M. (2011). Compartment-specific remodeling of splenic micro-architecture during experimental visceral Leishmaniasis. *American Journal of Pathology* **179**, 23–29.

Zhao, S. and Iyengar, R. (2012). Systems pharmacology: network analysis to identify multiscale mechanisms of drug action. *Annual Review of Pharmacology and Toxicology* **52**, 505–521.

Zumla, A., *et al*. (2015). Towards host-directed therapies for tuberculosis. *Nature Reviews Drug Discovery* **14**, 511–512.