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Exploiting knowledge on pharmacodynamics-pharmacokinetics for accelerated anti-leishmanial drug discovery/development

Shyam Sundar^{*,1}, Neha Agrawal², and Bhawana Singh^{1,3}

¹Department of Medicine, Institute of Medical Sciences, Banaras Hindu University, Varanasi-221 005, UP, India.

²Department of Hepatology, Temple University, Philadelphia, PA 19140, USA

³ Department of Pathology, Wexner Medical Center, The Ohio State University, Columbus-43210, Ohio, USA.

Abstract

Introduction—Being on the top list of neglected tropical diseases, leishmaniasis has been marked for elimination by 2020. In the light of small armamentarium of drugs and their associated drawbacks, the understanding of pharmacodynamics and/or pharmacokinetics becomes priority to achieve disease elimination and sustaining it.

Areas covered—We have looked into pharmacological aspects of existing and emerging drugs for treatment of leishmaniasis. An in depth understanding of pharmacodynamics and pharmacokinetics (PKPD) provides rational for drug designing and optimizing the treatment strategies. It forms a key to prevent the drug resistance and avoid drug associated adverse effects. We have compiled the researches on understanding the PKPD of different anti-leishmanial formulations, that holds hopes for improved and/or effective disease intervention.

Expert opinion—Understanding the pharmacological aspect of drug forms the basis for the clinical application of novel drugs. Tailoring drugs dosage and individualized treatment can avoid the adverse events and bridge gap between the *in vitro* models and their clinical application. An integrated approach, with pragmatic use of technological advances can improve phenotypic screening, physiochemical properties of novel drugs. Concomitantly, this can serve to improve clinical efficacies, reduce incidence of relapse and accelerate the drug discovery/development process for leishmaniasis elimination.

Keywords

pharmacology; nano formulations; host directed therapy; combination therapy

*Corresponding Author: Dr. Shyam Sundar, Infectious Disease Research Laboratory, Department of Medicine, Institute of Medical Sciences, Banaras Hindu University, Varanasi-221005.

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1. Introduction

Leishmaniasis is amongst the infectious diseases, caused by a trypanosomatid, *Leishmania*. The disease is transmitted by the bite of infected phlebotomine vector. The parasite has digenetic mode of life cycle with flagellated promastigote forms, in the sandflies gut which are transferred into mammalian host upon the sandfly's bite where promastigotes differentiate into non-flagellated amastigotes in the phagolysosome of host macrophages. The disease ranges from self-healing cutaneous (CL) to disfiguring mucocutaneous (MCL), and to life threatening visceral leishmaniasis (VL) and its sequel post kala-azar dermal leishmaniasis (PKDL). The disease is prevalent in 98 countries worldwide, with 0.2 to 0.4 and 0.7 to 1.2 million new VL and CL cases respectively, each year. With overall case fatality rate of 10%, leishmaniasis causes 20,000 to 40,000 deaths per year worldwide with major VL foci (90% cases) in India, Bangladesh, Sudan, South Sudan, Ethiopia and Brazil. In 2005, India, Nepal and Bangladesh signed a treaty for disease elimination, which was renewed in 2014 to eliminate the disease by 2020, and address the unmet issues in leishmaniasis treatment [1].

The mainstay of disease control relies on the early case detection, effective treatment and vector control strategies. However, the poor socio-economic status of afflicted population remains the biggest hurdle for disease control contributing towards ignorance of the disease by the policymakers, financial bodies and pharmaceutical industries. This has resulted in significant lag in the effective management and control of the disease. Lack of an effective vaccine means sole dependence of the current disease management on chemotherapy[2]. In the current scenario, limited chemotherapeutic options are available for leishmaniasis, and out of them, only few have proven efficacies. However, technological advancements in the field of medicine have accelerated the research for drug development which has elaborated the list of validated drug targets[3].

Indeed, varying chemotherapeutic responses within and/or different geographical regions, in addition to the conventional drug related issues, calls upon for better understanding of drug pharmacodynamics and/or pharmacokinetics (PKPD). This would facilitate the scientists in deciding the minimal inhibitory dosage for clearing infection and pharmacological analyses. Thus, the understanding of PKPD remains an essential criterion for efficient drug designing and therapeutically targeting the disease. For VL elimination to be a realistic goal, a high jump, superseding the issues associated with drug efficacies, resistance, toxicities, asymptomatic carriers and vector management, is urgently required. This review sheds light on the PKPD of the current treatment options as well as the emerging treatment options for leishmaniasis.

2. Current chemotherapies for leishmaniasis

In the absence of vaccine, the current therapeutic regimens rely solely on chemotherapeutics. In the current scenario, limited chemotherapeutic options are recommended for clinical cure of leishmaniasis, out of which only few have proven efficacies. Table 1, highlights the treatment options for leishmaniasis and their associated drawbacks.

2.1 VL

Pentavalent antimonials were developed in 1930s, used for the treatment of leishmaniasis. The drug is commercially available as meglumine antimoniate (Glucantime, Aventis) and sodium stibogluconate (SSG; Pentostam, GlaxoSmithKline), and has remained the first choice of treatment for more than 70 years. Subsequently, the high costs of treatment, parental drug administration (for 30 days), serious drug related adverse events and high incidence of drug resistance limited its utility[4]. These drawbacks led to the abandonment of the use of SSG for treatment of VL in the Indian subcontinent, bringing pentamidine (Aventis) on the center stage in 1980s, for treating SSG drug resistant cases with VL[5]. High cost of treatment made the drug unaffordable to poor community in developing countries. Later, from 1990s, anti-fungal amphotericin B (AmB; Fungizone, Bristol-Myers Squibb, Montreal, Canada) has been used for treating leishmaniasis in India; however, the need for multiple dosing, prolonged hospitalization, high cost and frequent adverse events led to the introduction of liposomal formulation of AmB (AmBisome, Gilead Sciences, Foster City, USA). It was approved by Food and Drug Administration (FDA) in 1997, for treating leishmaniasis[6]. The affinity of drug towards the membrane ergosterol rationalized its use for treating thousands of cases. Thus far, no oral medication to treat leishmaniasis until miltefosine became the first registered oral drug for leishmaniasis, in 2002[7]. Later, in 2005, it gained the center stage in VL elimination program; however, a decade of miltefosine use reduced its effectiveness and increased number of relapsed cases. In 2007, injectable paromomycin (aminoglycoside antibiotic) came up as another drug after successful phase-III trial[8]. However, 21 intramuscular injections and poor availability hindered its use, later, sitamaquine (primaquine analogue), emerged as another oral drug [9], unfortunately low efficacy and significant nephrotoxicity resulted in cessation of its clinical development. Later, single dose liposomal AmB (AmBisome) emerged as the treatment of choice in the India subcontinent for its excellent safety and efficacy [10].

2.2 CL, MCL and PKDL

Pentavalent antimonials (Pentostam, GlaxoSmithKline, London, UK) and meglumine antimoniate (Glucantime) (MA) remained the mainstay for the treatment of CL, while better tolerated AmBisome (LAmB; Gilead, UK) has been the second line drug. Recent researches have explored the different treatment regimens with a total dose of 20–25mg/kg for the treatment of CL and MCL[11]. Cure rates have been variable with varying success rates, depending upon the *Leishmania* species involved in disease pathogenesis and geographical location[12]. The use of conventional AmB deoxycholate formulation, was associated with infusion-related side effects (fever, rigor and toxicity)[13]. Liposomal AmB has recently been reported to cure multi-focal presentation of CL in the form of ulcerated nodules due to *L. infantum* infection[14]. CL treatment with L-AmB requires multiple dosing (3mg/kg per day achieving a total dosage of 21mg/kg, intravenously)[15]. Recent report from murine CL model has reported that liposomal AmB at dosage of 6.25 and 12.5 mg/kg, resulted in significant reduction in parasite loads and lesion size was observed [16]. Conversely, the analysis of pharmacokinetics of liposomal formulation of AmB revealed for better drug efficacy and correlation between drug dose, intralesional drug accumulation and reductions in parasitic burdens as well as lesion sizes[16]. The elevated levels of drugs were also associated with the increased vascular permeability and macrophage infiltration that

attributed towards the tissue inflammation[17]. Other drugs used in the treatment of CL, MCL and PKDL included pentamidine, ketoconazole and miltefosine [18].

3. Drugs PKPD and leishmaniasis

Pharmacodynamics deals with the understanding of the drug concentrations and their effects on biological system. In the case of intracellular infections, pharmacodynamics provides rational basis for determining the optimal dosage especially, to address the issues associated with drug resistance. In the light of increasing number of drug resistance [5], there remains a need to carefully monitor the drug dosage, understand drug characteristics, host factors (epidemiological, immunological and genetic) and the parasite (susceptible and resistant phenotypes) in question. Different approaches for improving the drug efficacy and minimizing the associated toxicities, have significantly improved the drug development and some have progressed to clinical trials. Similarly, drug pharmacokinetics also remains a crucial component of drug discovery and/or development where study of time course of the drug absorption, distribution metabolism and excretion is done. The application of pharmacokinetic knowledge serves to provide safe and efficacious therapeutic management of drugs for disease intervention.

Thus, better understanding of pharmacokinetics and pharmacodynamics (PKPD) will open ways for the new interventions and their translational potential.

3.1 PKPD of SSG and its formulations

Pentavalent antimonials remained to be the mainstay in the treatment of VL unless, it met with severe clinical limitations related to toxicities, *in vivo* pharmacokinetics issues, drug resistance etc. Liposomal AmB has been the reference drug in Europe with better tolerance than antimonials. However, recent study reported for the use of antimonials for the treatment of relapsed cases from liposomal AmB with sustained cure in transplantation, HIV cases and in immunocompromised infants can be attributed to other factors rather than just antimicrobial resistance[19]. Another study from Turkey reported for improved biochemical and hematological parameters upon pentavalent antimonial treatment as compared to intravenous liposomal AmB, irrespective of their costs for treating VL in children [20].

3.1.1 SSG formulations—A non-ionic surfactant vesicle formulation of SSG (NIV-SSG) elicited significant immunological responses, and had comparative efficacies to vesicular formulation of AmB during acute and chronic infections [21]. Unfortunately, emergence of drug resistant phenotypes and inability of this formulation to confer protection against subsequent infections halted the research in this field[22]. However, a recent study, reported for good clinical efficacies of NIV-SSG against different clinical isolates of *Leishmania*[23], reignited the scientific interest. Later, in 2003, different vesicular formulation of SSG (SSG-NIV and SSG-NIV-dextran) reported for the improved SSG pharmacokinetics, by increasing targeted drug delivery, elimination half-lives and distribution volumes thereby decreasing renal clearance and improving drug efficacies with negligible toxicities [24].

Later in 2008, liposomal formulation of SSG (LMA), caused suppressed parasite growth and reduced infectivity to sand flies in canine model at twenty-fold lower dose than the conventional drug[25]. Another formulation of SSG in phosphatidylcholine-stearylamine (PC-SA)-bearing liposome possessed improved drug retention properties and was effective against SSG-resistant *L.donovani*. The formulation exhibited minimal toxicity, in the liposomal formulation as compared to the conventional SSG[26].

All cationic liposomes possessed potent anti-leishmanial properties however, in 2015, it was shown that phosphatidylcholine-dimethyl dioctadecyl ammonium bromide (PC-DDAB) vesicle was most potent. This can be attributed to the enhanced entrapment of SSG in PC-DDAB that induced ultrastructural changes in the parasite. This formulation was potent enough to alleviate SSG-sensitive and/or resistant parasites by disintegrating amastigotes in liver, spleen and bone marrow at minimal dose of 3mg/kg body weight. The formulation was non-cytotoxic with normal levels of serum ALP and SGPT (essential enzymes of liver functions) as well as serum urea and creatinine levels (markers of kidney function) at therapeutic doses [23, 27].

Recently, Azevedo *et al.* have tested the pharmacokinetics of the mixture of conventional and pegylated liposome complexes with MA that effectively decreased inflammation, granuloma formation, increased IFN- γ /IL-10 ratio in T-cells and decreased parasite loads without any marked changes in the hepatic and/or renal functions[28]. Considering the pharmacodynamic parameter of accelerated blood clearance (ABC), which potentially interfere with the targeted delivery of drug to macrophages Lopes *et al.* have used the drug tartar emetic (TE) in liposomal formulation for the treating leishmaniasis. Earlier, the drug toxicity stopped its use however, the liposomal formulation was shown to have no effect on erythrocyte counts, liver and/or spleen weight and histology. The drug induced the ABC process, thereby increasing the antimony concentration in the liver without toxicity to liver and other organs [29].

3.2 PKPD of AmB and its formulations

AmB (a regimen of 15 doses of 1mg/kg on alternate days) was the first-line drug after the failure of SSG. The leishmanicidal activity of AmB can be attributed to its pore-forming ability in the plasma membrane. Being fundamentally a fungicide, it selectively attacks the fungal ergosterol [30], similar mechanism supposedly works as its anti-leishmanial action. However, poor drug solubility in aqueous medium (at physiological pH) limits its parenteral administration and oral delivery. This led to the development of micellar formulation of drug in the form of Fungizone, however, the drug associated renal and severe other toxicities limited its use[31]. This stimulated the research for second generation of AmB, in 1990s, in the form of AmBisome (small unilamellar liposome, Gilead Sciences, USA), Abelcet (ribbon-like AmB-phospholipid complexes, Lediant Biosciences Inc, USA) and Amphocil (colloidal dispersion of AmB with sodium cholesteryl sulfate, Liposome Technology, USA) [32]. These formulations still possessed the threat of non-specific tissue distribution that subverted their reach to the infected macrophages. Thus, accentuating the dose regimen required for achieving the desired therapeutic effects eventually caused serious adverse effects by increasing the drug exposure. In comparison to AmB deoxycholate, liposomal

formulation had higher plasma exposure and higher levels in the tissues[33–36], and therefore, significantly altered the excretion and mass balance of drug. This can be attributed to the packaging of the AmB within the liposome that retains the drug and permits its access to the different tissues in the body[37].

3.2.1 AmB liposomal formulation—Earlier studies have already established the pre-clinical pharmacokinetics of L-AmB (liposomal AmB) in mice, rats, canine and rabbits[38, 39]. Additionally, single time-point bio-distribution studies have revealed for significantly lower concentrations of AmB in livers and spleens of infected mice in intravenous administrations as compared to the oral administrations of L-AmB[40]. L-AmB, as a single dose drug was less effective at chronic stage as compared to the acute stage[21], however, intermittent dose proved efficacious in clearing visceral infection[41]. Since the drug efficacy usually depends upon its concentration at infection site that was observed in terms of decreased potency of L-AmB during chronic infection was significant in spleens as compared to the livers, and the changes the bio-distribution of drug was not observed in blood[42]. Other lipid formulations of AmB, used in past included- Abelcet and Amphocil. However, these drugs were effective in clearing the infection with less toxicity as compared to their conventional counterpart but these formulations were expensive[5] with significantly lower safety profile thus, limiting their usage. Research for scaling up the production of AmB by formulating the nanodrug significantly improved the drug efficacy, with reduced parasitic burdens in livers[43].

Liposomal formulation has been a good choice for clinicians across Europe, United States and South Asia. It was developed with the aim to improve the efficacy and widen the therapeutic window for treatment. The pharmacokinetic studies have established for the low toxicity at the organ level which facilitates intravenous administration of high dosage of drug [39]. Liposomal AmB at 10 mg/kg dosage has been shown to be effective in clearing 90% parasites within two days of administration (intravenous). The liposomal formulation exhibited higher plasma exposure, lower distribution and excretion rates with significantly improved drug efficacies. So, liposome based formulation provides safer therapeutic option [34, 37, 44, 45]. However, a recent research has established the utility of fungisome in CL despite of its less safety and efficacy profiles, it still holds potential as pharmacotherapeutic option[46]. Though these formulations provide an efficacious, minimal toxic and shorter therapeutic regimen (3–5 days) however, high costs of treatment limit their reach in low income countries.

3.2.2 Nanoformulations of AmB—The aforementioned issues fostered the need for oral treatment options, which could potentially improve the patients' compliance and address the pharmacological issues. An ideal delivery system needs to fulfill the two criteria- first, overcome the adverse effects associated with consumption and secondly, improved drug efficacies. There is plethora of formulations designed for safe and improved delivery of AmB including the nanosuspensions, nanodisks and co-chelates lipid formulations etc.

3.2.2.1 Nanosuspensions and co-chelates: The nanosuspensions of AmB are designed with the aim to improve the drug solubility and reported to effectively reduce the parasitic burdens in mice. Later, in 2003, self-emulsifying drug delivery system used peceol

as excipient for oral delivery of AmB. This formulation markedly improved the oral absorption of AmB but was limited due to high dosage volumes of lipids administered each time (1.33 gm/kg) making the drug administration procedure a cumbersome task[47, 48]. This brought co-chelates of AmB into the scene subsequently, high costs associated with daily dosing called upon for alternative strategies. This led to the use of polymeric nanoparticles with the aim to potentially reduce doses, toxicities and improve drug bioavailability. Thus, these researches led the foundation for the use of nanocarriers as excipients to enhance the drug bioavailability and minimize the drug associated adverse effects.

3.2.2.2 Polymeric nanoformulations: The bioavailability, low patient compliance, nephrotoxicity and technical issues associated with AmB led to the emergence of formulations with improved bioavailability and drug entrapment efficiencies. Polymeric nanoformulations can be categorized into two types: synthetic and natural nanoformulations.

Amongst **synthetic nanoformulations**, nanoprecipitation based AmB preparation with vitamin E-TPGS (α -tocopheryl polyethylene glycol 1000 succinate) as stabilizer exhibited biphasic drug release characteristics i.e. initial rapid drug release was followed by a sustained release. The biphasic drug release property significantly improved bioavailability (800% higher than AmB), lowered hemolytic and nephrotoxic effects in rats [49]. The complex of AmB with poly (α -glutamic acid) (PGA) has been proved to have better uptake potency, better plasma clearance and low distribution in kidney than liposomal AmB[50]. In 2015, Asthana *et al.* with the aim to improve the drug efficacy used the cationic stearylamine based nanoparticle that possessed the dual characteristic of polymeric nanoparticle as well as liposomes. The formulation significantly reduced the parasite loads and was found to exhibit rapid plasma clearance rates, minimal kidney distribution with low creatinine and BUN levels[51].

3.2.2.3 Natural nanoformulations: Protein (albumin and gelatin) and polysaccharide (chitosan, alginate etc.) based polymers have been mostly used in for developing antileishmanials. Covalently functionalized lipo-polymerosome based nanoformulation of AmB with self-assembled glycol chitosan-stearic acid polymer, significantly reduced the AmB toxicity and improved anti-parasitic activity by stimulating T_{H1} responses and NO production. The formulation offered low cost, stable and less toxic therapeutic alternative to conventional or liposomal AmB formulations (Fungizone and Ambisome) [52]. Lipo-polymerosome based AmB nanoformulation also exhibited high internalization efficacies in macrophages. Conversely, the PKPD analyses revealed high rates of drug distribution in liver and spleen with minimal nephrotoxicity and high efficacy for lowering parasitic burden [53]. Another report for the efficacy of the polyester nanoformulation of AmB exhibited significantly lowered the parasitic burden than the conventional drug with the added advantage of oral administration [43].

Gelatin-based nanoparticles (AmB loaded onto lecithin and gelatin hybrid nanoparticle) exhibited increased intestinal permeability and increased *in vivo* bioavailability with negligible hematological or renal toxicities (normal BUN and plasma creatinine levels) [32]. Conversely, nanocapsular formulation of doxorubicin with phosphotidylserine (PS) coating

was shown to possess excellent cellular uptake and parasite clearance rates in *L. donovani* infected hamsters without cytotoxic and hemolytic effects. Thus, nano-capsular coating with macrophage targeted signal (PS), improved the drug delivery and reduced the minimal dose for parasite clearance[54].

The targeted approach for intracellular *Leishmania* led to the emergence of antigen-presenting cells (APCs)-specific AmB nanocarrier (pan-DR-binding epitope-derivatized-dendrimer) in order to reduce the dosage of the drug, needed for treatment. Nanocarrier loaded AmB had significantly shortened the treatment duration, toxicity and enhanced the T-cell specific immune responses in murine CL [55]. Akin, study from Brazil used PLGA and dimercaptosuccinic acid nanoparticles (PLGA-DMSA-AmB) and suggested for the improved cell viability, reductions in parasitic burden and lower dose frequencies to achieve therapeutic levels similar to conventional AmB[56]. In 2014, another modification of gelatin nanoformulation in phosphatidylserine coating (PS-AmB-GNP) was directed towards the macrophages. The drug showed polarized distribution in macrophage- rich organs (liver and spleen) with potential anti-parasitic activity, improved macrophage effector functions in terms of NO production with negligible hemolytic toxicities[57].

Another PLGA based nanoformulation of oleanolic acid in combination with TPGS was efficacious in *L. donovani* infection with negligible nephrotoxicity and non-significant changes in biochemical parameters (ALT, AST and BUN) [58]. The excellent stability, biocompatibility, biodegradability, immune adjunctive effects, easy availability in dry as well as liquid forms made chitosan nanoparticles a stable alternative for liposome[59].

Chitosan-based AmB-nanoformulation was less toxic alternative than commercially available formulations (Fungizone and AmBisome). It exhibited enhanced internalization potencies for macrophage uptake, immune stimulatory potential, normal blood urea nitrogen (BUN) and plasma creatinine levels without any histopathological complications. The treatment was marked by elevated levels of TNF- α , IL-12 and NO production, while dampened anti-inflammatory responses suggesting that this nanoformulation is cost effective and safe treatment option for VL [60]. Chitosan in the form of solid-lipid nanoparticles (SLN) was a better substitute for polymeric nanoformulation as it exhibited heightened macrophage activation and effector responses with negligible toxicities [61]. In 2015, another chitosan formulation with cross-linked AmB in sodium alginate-glycol chitosan stearate nanoparticles (AmB-SA-GCS-NP) exhibited improved pharmacokinetic properties. The drug exhibited enhanced efficacy, bio-distribution in spleen and liver with negligible *in vitro* and *in vivo* toxicities in VL model[62].

3.2.2.4 Polymeric cores based nanoformulation: Poloxamer 407 based AmB formulation for treatment of *L. amazonensis* infection, showed significantly reduced lesion sizes, parasitic burden and high levels of IFN- γ , IL-12, GM-CSF and nitrite accompanied with lower levels of IL-4, IL-10 and IgG1 without any signs of renal or hepatic toxicities as compared to the conventional AmB[63].

The calcium phosphate nanoparticle amalgamation with AmB (CaP-AmB-NP) exhibited safe pharmacokinetic values, in terms of negligible toxicities (creatinine and BUN),

hemolytic effects(<3 percent). The formulation possessed excellent cellular uptake and tissue bio-distribution in experimental *L.donovani* infection[64].

In 2017, micellar formulation of AmB (AmB coated with chitosan), improved the drug efficacy (*in vitro* and *in vivo*) and exhibited markedly lower hemolytic and cytotoxic properties as compared to conventional AmB formulation. The formulation demonstrated no abnormal signs in histopathology, normal BUN and plasma creatinine levels and was found to instigate T_{H1} responses without any nephrotoxic effects [65]. There were no evidences for skin distribution and AmB kinetics until recent trial for skin pharmacokinetics of liposomal AmB and the deoxycholate formulations. This trial revealed for higher drug concentration in the lesions that improved the efficacy of liposomal over conventional deoxycholate formulation with potential therapeutic benefits in *L.major* infection[16].

Chitosan based betulinic acid formulation has also shown to be a non-toxic alternative for CL as documented from *in vitro* and *in vivo* drug trials [66]. Recently, bovine serum albumin (BSA) based nanoparticles loaded with AmB has shown lower cell and tissue toxicities, with potential benefit for treating CL [67].

3.3 PKPD of miltefosine and its formulations

Miltefosine, an alkylphosphocholine analogue, originally developed as anti-cancer agent was licensed for treatment of VL in 2002 and for CL in 2004. Although there are plethora of evidences about the mechanism of action including induction of apoptosis[68], perturbation of lipid metabolism [69], cellular signaling pathway, alteration in membrane composition [70] and mitochondrial dysfunction[71].

After the success of phase III trial (cure rates of 94%), a phase IV trial was conducted in India and Bangladesh that epitomized miltefosine as a part of VL elimination program [72]. Although it was well-tolerated but was often associated with gastrointestinal adversity[70]. The commercial formulations of miltefosine are available as – Impavido (Knight Therapeutics, Toronto, Canada).

The drug possessed the anti-leishmanial properties however, not all *Leishmania* species were equally susceptible to the drug. Variable infectivity of parasite to host cell and susceptibility of the clinical versus the laboratory strains are some factors which led to the lack of consensus for the use of drug.

3.3.1 Miltefosine formulations—Different formulations of miltefosine have been reported to improve the anti-parasitic activities of miltefosine. The use of oral miltefosine (25mg/kg/day) with topical PM (10% gel), significantly reduced the lesion sizes, parasitic burden in infected mice as compared to monotherapy in CL [73, 74]. The cocktail of CpG-oligonucleotide with miltefosine activated T_{H1} cytokine responses, lymphocyte proliferation, ROS/RNS production and phagocytic ability of macrophages leading to parasite elimination [75]. In order to combat the toxicity and drug resistance issues, therapeutic switching using miltefosine with fluconazole (anti-fungal agent) and picroliv (immunomodulator) enhanced antileishmanial potency (77 to 88%), cell-mediated immune responses, ROS/RNS production and phagocytic index [76]. Akin, miltefosine in combination with ketoconazole

and picroliv (immunomodulator) improved the drug efficacy from 72 to 82% in hamsters. The combination triggered ROS/RNS production and cell-mediated immune responses thereby served as less toxic and cost-effective treatment option [77]. Likewise, picroliv and miltefosine in combination with PM acted similarly for eliminating *L. donovani* [78]. Another combination of miltefosine (200 mg/kg/day) with pentoxifylline (an immunomodulator with NF- κ B inhibitory potential) exhibited potential antileishmanial properties and reduced the *L. amazonensis* burden [79].

Lipid nanoformulation of miltefosine with cholesterol exhibited improved *in vivo* drug efficacies and safe biochemical profiles with no gastrointestinal complication. The formulation significantly dampened the hemolytic potential of miltefosine without affecting the anti-parasitic activity [80]. The amalgamation of miltefosine with silver nanoparticles has also shown to improve the antileishmanial efficacy due to its biocompatibility with the macrophage. The formulation caused morphological aberration and DNA fragmentation in parasites [81].

The synergistic effect of miltefosine with AmB and prostaglandin lipid mediator improved the parasitic clearance (>95%) and reduced the synthesis of T_H2 cytokines (IL-10 and TGF- β). Furthermore, the formulation induced ROS-dependent apoptosis in *L. donovani* by triggering mitochondrial damage thereby served as adjunctive therapy for VL [82].

The side effects (vomiting and diarrhea) associated with oral treatment in CL cases, led scientists to use the drug, as topical formulation for CL. Unfortunately, the low permeation rates and skin irritability associated with its application, failed to treat CL [83]. However, a study from Ribeiro *et al.* reported for the improved *in vivo* efficacy of photodynamic therapy using liposomal chloroaluminiumphthalocyanine (AICIPC) in combination with systemic miltefosine. The combination significantly reduced the parasitic burden and viable parasite counts in CL model [84]. Nanoformulation of miltefosine with curcumin has shown increased *in vivo* drug efficacies for parasite clearance by triggering lymphocyte proliferation, the ROS/RNS production as well as improved phagocytic ability of macrophages [85]. Miltefosine and ketoconazole- loaded nanoniosomes have shown potent activity against CL [86]. Acknowledging the oxidative potential of miltefosine, recently, intraperitoneal treatment with miltefosine and ascorbic acid proved efficacious in abrogating the damaging effects caused by miltefosine. Thus, ascorbic acid mitigated the oxidative damages by miltefosine and efficiently reduced the parasitic burden [87].

3.4 PKPD of Paromomycin and its formulations

Paromomycin (PM) is a broad-spectrum antibiotic, derived from *Streptococcus* spp. Originally developed for the treatment of intestinal parasites in 1960s, later in 1990, the sulfate formulation of drug was evaluated for the treatment of leishmaniasis. The low cost, better efficacy and short treatment times made it a potential candidate for first line treatment of VL [88, 89].

Of many possible intervention strategies, topical formulation of PM has attracted particular interest, in the treatment of CL. PM with 10% methylbezethonium chloride (MBCL) in paraffin base formed the basis for the CL treatment in 1985–1986 [90] with 39% cure rates

and high concentration of MBCL limited its use [91, 92]. However, later studies evaluated different combinations of PM, which are discussed below. In 1990s, London School of Hygiene and Tropical Medicine developed 15% PM with 10% urea in paraffin, which was not successful [93, 94]. After this trial, many researches fished out different formulations for their efficacies against leishmaniasis. In this context, PM with MBCL and pentavalent antimony led to re-epithelialization of lesions and 84–90% cure rates in Colombian cases with American CL[95, 96]. The combination of PM (12 to 18mg/kg daily) (intramuscular) with the standard SSG(20mg/kg daily) (intravenous) was significantly more effective than SSG alone for the treatment of VL with minimal toxicities in India and Sudan [97, 98]. Non-ionic surfactant vesicular formulation of PM exhibited significant decline in the parasitic burdens in the bone marrow and improved the macrophage associated effector functions[99].

To counteract the problem of treatment failure and long treatment time, combination of PM and miltefosine was safe, efficacious and affordable option with successful treatment of VL. It was shown to instigate TNF- α and NO production in TLR-4 dependent manner, which served to clear off the *L.donovani* infection [100]. Later in 2014, the combination was shown to induce TLR9-dependent NF- κ B signaling that enhanced in the DC maturation, antigen presentation and T-cell stimulation to produce IFN- γ , IL-12 while dampening the IL-10 production [101].

The transferosomal formulation of PM with phosphatidylcholine, sodium cholate and ethanol yielded no cytotoxic effects and was able to reduce lesion sizes in CL at half the effective drug concentration as compared to monotherapeutic regimen of PM alone [102]. With the aim to enhance the targeted delivery of PM to macrophages, PM was loaded in albumin nanospheres (PM-MS). The formulation yielded no cytotoxic effects, improved drug efficacies and sustained drug release to clear off promastigotes as well as amastigotes[103]. The formulation exhibited targeted delivery to the macrophages and safe biochemical profiles with no symptoms of toxic effects (normal blood urea and BUN)[104].

In the search for efficacious treatment, topical formulation of PM with gentamycin was found to have better efficacy than with other formulation (PM with MBCL and/or urea) with 100% cure of lesions due to *L.major/L.mexicana/L.amazonensis/L.panamensis* in murine models without any toxic effects [105].

The search for PM based topical formulation led to phase III trial of topical cocktail of PM (15%) and gentamycin (0.5%) with no significant differences in the cure rates as compared to monotherapy with PM alone in ulcerative *L.major* infection [106]. The pharmacokinetics of the two regimens revealed for limited absorption of the combination with the aim to avoid drug accumulation and toxicity[107]. The hepato- and nephrotoxicity associated with conventional drug regimens, even as topical formulation led to formation of nanoliposomal PM. The topical treatment effectively cured the lesions without marked alterations in the liver and kidney histopathology as well as the hepatic enzymes. However, long term use of drug was associated with mild necrosis of liver and kidney [108].

Micellar nanogel formulation of PM with poloxamer 407 possessed excellent *in vivo* tolerance as well as improved anti-leishmanial efficacy with negligible cytotoxic effects

during *in vitro* and *in vivo* trials [109]. Nanoparticle based formulation of PM significantly improved the *in vivo* drug efficacy against *L.tropica* infection. The drug possessed no toxic effects and boosted T_{H1} immune cells effector responses in terms of high IFN- γ to IL-4 ratio and NO production thereby inhibited the parasite growth [110, 111]. In CL model, 15% PM with aquaphilic base exhibited well-tolerable potency and effective in diminution of the lesion sizes but it was associated with adverse effects - erythema, swelling, hard edema, superficial necrosis, pruritis and pain however, adverse events were of minimal grades only in 5–10% of cases [112].

4. PKPD of emerging antileishmanials

In the light of shortcomings associated with the conventional chemotherapeutic options, recent decades have experienced plethora of research on identifying new therapeutic targets [4]. Therefore, the search for potential therapeutic options has become the need of the hour. In this context, there is an array of compounds and pathways that have gained significant attention in recent years. This section describes the PKPD of potential candidates thus, emphasizes the need for streamlined approach for developing and bringing these candidates at field level.

4.1 Local therapy

Local therapeutic approach has been attractive option for reducing the systemic toxicities associated with the parental chemotherapeutic options. Although the use of topical treatment alone needs further research however, they have been used with variable success rates in different geographical regions. The topical therapies are associated with minimal toxicity, promote wound healing, re-epithelization and reduce scar formation. It included the physical therapy (thermos- and cryotherapy), photodynamic therapy and topical drug applications.

4.1.1 Thermotherapy—Failure of parasites to grow at temperatures >39°C led to the idea of thermotherapy-based approach for curing leishmaniasis. The treatment was started in various forms usually, radiofrequency waves remained the common mode of therapy for CL. A cohort study from Afghanistan, reported for the higher thermotherapy cure rates (69.4%) as compared to intramuscular SSG (44.8%). It came up as inexpensive, effective, well-tolerated and safe therapeutic option for CL as compared to SSG [113]. The thermosensitivity of parasites expanded the arena for treatment when another trial reported for the success of thermotherapy at 50°C for 30 seconds (once weekly for 4 weeks) as compared to intralesional SSG treatment. The treatment yielded complete cure rates of 80.7% as compared to 55.3% from intralesional SSG treatment without any reported relapse cases, after six months of treatment [114].

Another trial validated the previous trial findings, where thermotherapy at 50°C for 30 seconds (one session) was more efficacious for re-epithelialization without any systemic toxicities [115]. In 2012, another study from Afghanistan, reported for the efficacy of single session of thermotherapy as compared to the five doses of SSG for treating CL, and thus providing a cost-effective, non-toxic alternative [116]. In 2013, radiofrequency induced heat therapy (RFHT) was employed for treating CL cases (98%) from Indian cohort and was established as durable and safe cure for CL cases [117]. Thermotherapy from Colombian

cohort yielded similar efficacies to miltefosine however, gastrointestinal adverse effects superseded the pain associated with thermotherapy [118]. Another landmark trial was reported in Sri Lanka, where RFHT was used for treating *L.donovani* induced CL. The single session of thermotherapy was quite effective than standard intralesional SSG and evolved as cost effective and safe therapeutic option [119]. Recently, thermotherapy was found effective for treating localized CL lesions in Brazilian population as compared to conventional SSG[120]. Thus, thermotherapy now forms a crucial treatment option for treating CL cases with minimal toxic side effects.

4.1.2 Cryotherapy—Cryotherapy involves the use of liquid nitrogen (-195°C) on or around the lesion, the treatment has yielded promising results in different ethnic groups. The therapy was first tried in 1986 for curing CL cases, where 100% cases were clinically cured in 3–8 weeks without any scarring. The therapy yielded no adverse effects and no reported cases of relapse, thus, came up as safe and effective treatment option [121]. This finding gained significant attention for using the therapy in different ethnic groups. Liquid nitrogen cryo-surgery reported for significant clinical improvement without any systemic adversities however, mild cutaneous complications were observed in the form of hypo/hyper-pigmentation and scarring [122]. Another study in 2000, reported for the cryotherapy as an alternative to intralesional pentostam injection with satisfactory tolerance rates in patients without any adverse events [123]. Later in 2003, study from Iran reported that the combination of cryotherapy with MA as better alternative than either of monotherapeutic approaches[124], the combination therapy came upon as safe, efficacious, well-tolerated, inexpensive treatment option for treating CL in children[125]. However, study from Ethiopian cohort reported for better efficacy of cryotherapy than pentostam treatment [126]. The cryotherapeutic approach further gained validation in Greece and Jordan, where the treatment yielded excellent results without any reported relapses[127, 128]. Thus, the treatment was simple and inexpensive option with rare side effects.

In 2012, study in Yemen reported alternative cryotherapy with carbon dioxide (CO_2) for resource poor settings. CO_2 slush treatment was used since 2005, in regions where liquid nitrogen was unavailable, with treatment costs of 20USD and minimal side effect[129]. In spite of being a safe and simple treatment option, variable success rates of cryotherapy can be attributed to inappropriate application, lesion sizes and duration of illness.

4.1.3 Photodynamic therapy (PDT)—Biophysical method of photodynamic therapy (PDT) is a recently emerging therapeutic approach for the treatment of CL. The approach has gained significance as it is safe and inexpensive option as compared to the chemotherapy, with added advantage of shorter regimen sessions and preventing the emergence resistant strains [130]. The first clinical use of anti-parasitic PDT came into being for the treatment of leishmaniasis in 2003, in combination with aminolevulinic acid (ALA) in a water-in-oil emulsion led to effectively reduce the lesion sizes in one week [131]. In 2006, it was further validated from another clinical study from Iran, which suggested for satisfactory healing and cosmetic outcomes, with no amastigotes in lesion smears. Although the treatment was associated with mild local inflammatory reaction however, no clinical signs of recurrence were noticed [132]. AICIPC photosensitized *L.amazonensis* underwent

cytolysis, due to generation of ROS, however, the study did not gained much attention due to its lytic effect on host cell[133]. In 2010, sunlight triggered photodynamic ultra-deformable liposome with zinc phthalocyanine exerted leishmanicidal potential, with uniform distribution in stratum corneum, deeper penetration potential and higher drug carrying capacities [134].

Indeed, the topical application of photosensitizer can potentially limit the efficacy of the formulation therefore, targeted approach to enhance the drug penetration was of interest for the scientific community. This led to the use of dimethylsulfoxide (DMSO), iron chelator ethylenediaminetetraacetic acid sodium and glycolic acid to enhance penetration efficacy for the treatment of nodular carcinomas, keratosis etc.[135]. This idea from carcinoma model, using the photosensitizer in intralesional formulation inspired the usage of intralesional ALA-PDT therapy for CL. Indeed, the initial trial yielded promising results in clinical cure of recurrent CL. However, intralesional ALA-PDT was more efficacious than topical therapy as reported previously [136]. Another study for the use of combination of photosensitizer (urea and aluminium phthalate) based PDT rendered the parasite more susceptible to photolysis with enhanced efficacies and without any adverse effects on host cell [137]. The use of PDT with phenothiazine photosensitizer, toluidine blue O yielded lethal effects on parasites (at 10 µg/ml concentrations) reducing the parasitic burdens within a short duration of time[138].

Later PDT with zinc phthalocyanines against *L.braziliensis*/*L.amazonensis* yielded promising results at minimal concentration of 10µM, however, further studies about the mechanism of action still needed attention [139]. Likewise, PDT in combination with curcumin as photosensitizer significantly reduced the parasite viability and morphology *in vitro*[140].PDT against *L.braziliensis* revealed for the up to 71% reductions in the infection rates [141]. The use of nanoemulsions significantly improved the solubility of zinc-phthalate and in turn their efficacies for *Leishmania* clearance[142].Later in 2018, innovation in PDT approach involved the use of zinc porphyrin-based photosensitizer (zinc-mesotetrakis (N-ethylpyridinium-2-yl) porphyrin) which led to approximately 40% reduction in the parasitic burden with loss in their cytoplasmic material. Ultrastructural studies reported for the wrinkled plasma membrane and reduced cellular volumes with respect to the parasite while no considerable toxic effects were observed for mammalian cells [143]. Similarly, the use of ultra-deformable liposomes with sodium cholate improved skin-drug delivery of AICIPC during PDT. It exhibited anti-leishmanial properties against *L.braziliensis* by exerting excellent penetration potential in the dermis, ROS generation, DNA damaging effects however, limited selectivity of the formulation limited its healing ability during CL[144]. Thus, this field remains an exciting venture for future research to develop an inexpensive, non-toxic therapeutic approach.

4.1.4 Topical formulations—CL, being a skin disease called upon for research for topical formulations due to the systemic toxicities associated with the conventional chemotherapeutics. Topical formulation of chlorpromazine was used successfully for treating diffuse CL in humans[145] however, another trial using imidazole derivative failed to treat CL [146]. In 1986, topical application of 15% PM with 12% MBCL was used for topical therapeutic option in CL [147]. Later, gentamycin formulation of PM [105, 148]was

used successfully to treat CL, however, intralesional SSG therapy was seen to exhibit better efficacy than topical PM [149]. In 2005, another trial in Peru reported the efficacy of topical formulation of SSG with 5% imiquimod as well tolerated, and was reported to be a safe option for healing the lesions with minimal erythema [150].

Different formulation of AmB as nanosphers [151], microemulsion [152], nanoemulsion [153], oleic acid emulgel [154] and microtubes [155] came up as safe, easy to use and painless therapeutic options. Unfortunately, the physiochemical properties limited their skin permeation, reformulation and usage as topical drugs. This led to the search for other lead compounds with potential topical application. In this context, NO-generating topical formulation improved clinical condition however, was not successful in curing the lesions [156] while liposomal PM (10 or 15%) was the choice for treating *L. major* infection [157]. In another study, a hydrophilic formulation of 10% PM clinically cured CL with mild and local adverse effects [158]. Later, micellar nanogel formulation of PM with was safe and efficacious option for treating CL in mice model [109].

Topical liposomal formulation of SSG with/without oleic acid effectively reduced the lesion sizes and spleen parasitic burdens with minimal toxicities [159]. Recently, the topical formulation of SSG in the form of nano-deformable liposomes has proved safe and efficacious treatment option for treating CL [160]. Another study reported for self-emulsifying drug delivery system (SEDDS) for curcumin was capable of clearing 100% *L. tropica* from infected macrophages with minimal hemolytic potential [161]. Bezoxaboroles derivative exhibited high skin permeation potential and suppressed the lesion growth in *L. major* infection [162].

4.2 Immunomodulatory and host directed therapies

Pre-clinical models of leishmaniasis have issued statutory concerns about the limitations associated with current chemotherapeutics and combination therapies are also not an exception.

Host-directed immunomodulatory therapies is expected to fill the gap between the conventional parasite directed chemotherapeutics and host immunity in synergistic way (chemotherapy-based reduction in parasitic burdens and concomitantly boosting the host immunity to avoid reinfections). Recombinant protein like IFN- γ alone [163] or with antimony, failed to improve the cure rate significantly, however, the combination was safe, well-tolerated with no reported adverse events [163, 164]. In a small study from Brazil, granulocyte-monocyte colony stimulating factor (GM-CSF) (5 μ g/kg daily) in combination with SSG exhibited minimal adverse events and led to disease resolution, reduced secondary infections and reversed the neutropenia [165].

In experimental studies, monoclonal antibody (MAb)-based blockade of IL-10 receptor came up as another therapeutic option in combination with SSG, instigated the IFN- γ dependent pathways and accelerated the SSG mediated parasite clearance. The anti-IL-10R treatment induced dose sparing effect and reduced the SSG dose required for parasite clearance thereby reduced the SSG associated adverse effects [166].

Research with a number of mammalian protein kinase inhibitors are still underway, the most notable examples included- sunitib, sorafenib, lapatinib and imatinib. These kinase inhibitors possessed anti-leishmanial properties by efficiently controlling the parasitic burdens and lesion sizes in mice [167, 168]. Recently, Nandan *et al.* have reported for oral therapy with Akt inhibitor, miransertib, that selectively promoted mTOR-dependent autophagy of parasite without toxic effects on macrophages in VL and CL [169].

In 2014, the periodic immunomodulation with domperidone (dopamine receptor antagonist) enhanced the phagocytic polymorphonuclear cell responses with good safety profiles and minimal side effects [170]. Imiquimod is a FDA approved immunomodulatory agent, activated T_H1 responses and activated macrophages for killing *Leishmania*; the drug alone or in combination with MA cured SSG non-responder during CL [171, 172].

TLR agonists have important role in determining the immune responses. The combination of TLR agonist (imiquimod) with antimony was safe and effective in treating CL cases as compared to antimony alone [173]. The stimulatory regimen of TLR agonists alone or in combination with *L.infantum* antigen boosted pro-inflammatory responses (IFN- γ , TNF- α and IL-6 production) that resulted in disease resolution [174].

4.3 Repurposed drugs

Drug repurposing has emerged as inexpensive option that surpasses the array of research and clinical steps required for drug development. It has given rise to 30% of the FDA approved drugs and vaccines. Indeed, repurposed drugs are inexpensive and safe therapeutic option due to already known pharmacokinetic and safety profiles of the available drugs. The list of repurposed drugs has been extending each day in search for the effective anti-leishmanial agents. In spite of the ongoing research, only a few of the repurposed drugs have reached the arena of clinical trials (Table 2).

In 1962, the first repurposed drug licensed for the treatment of leishmaniasis came as AmB. Later, many antifungal azoles were observed to possess anti-leishmanial properties due to their ability to inhibit ergosterol biosynthetic enzyme, C14 α -demethylase. Ergosterol biosynthetic inhibitors- fluconazole, itraconazole, ketoconazole and terbinafine have been studied since decades. The safety profile of oral fluconazole moved it towards the clinical trials however, high therapeutic dosage and low cure rates limited its use [175]. In CL, ketoconazole demonstrated cure rate of 89%, while its combination with SSG rendered the ketoconazole more effective, easy to use and with minimal side effects [176, 177].

Readily available and inexpensive, xanthine oxidase inhibitor, allopurinol, went for trial in canine CL and significantly improved the manifestation of lymphadenopathy, splenomegaly and lesions. The regimen significantly reduced the parasitic burdens in the lymph nodes and bone marrow (in murine model) however, human trial reported for conflicting results in humans *L.brazieliensis* and *L.panamensis* infections [178, 179].

Anti-microbials have potency against *Leishmania*, the best example came up as PM (aminoglycoside antibiotic), which was first used in 1990s for treating leishmaniasis [88, 98]. Later many other antibiotics were tested for their anti-leishmanial potential (Table 2). In

2006, another trial using the combination of rifampicin with omeprazole emerged as highly efficacious (approx. 80%), safe (no side effects) and inexpensive treatment option for the management of CL [180]. Azithromycin regimen for treating leishmaniasis has reported for variable efficacies for different geographical regions [181, 182]. The combination of azithromycin with miltefosine induced significant disease improvement however, it was associated with relapses[183]. Conversely, azithromycin combination with SSG was not an effective therapeutic choice[184]. Anti-plasmodial tafenuquine, impaired the bioenergetics of *Leishmania* by depleting the ATP levels and plasma membrane permeability. Drug induced mitochondrial dysfunctions, ROS production and concomitantly, DNA fragmentation in parasite without any toxic side effects on host cells [185].

Amongst anti-cancer agents hydroxamate or benzamide-based small lysine deacetylase inhibitors possessed high potency in clearing off parasitic infection, with limited host toxicity[186]. Similarly, estrogen receptor modulators- tamoxifen and raloxifen, have shown potencies against different species of *Leishmania*. Tamoxifen, induced apoptotic cell death in parasites by inducing mitochondrial damage and alterations in the intravacuolar pH, whereas raloxifene causes appearance of autophagosomes and alterations in mitochondrial membrane potential leading to apoptosis in parasites[187, 188]. Tamoxifen has also been used in combination with conventional chemotherapeutics [189–191], thereby holding promises for future therapeutics.

Anti-depressants have crucial role in clearing *Leishmania* infection. In this context, imipramine, clomipramine and amitriptyline are worth mentioning. Imipramine has been known to inhibit trypanothione reductase and trigger apoptosis. It also exhibits host-directed immunomodulatory properties by inducing T_H1 cytokines while decreasing the expression of disease exacerbating IL-10 and TGF- β [192]. Conversely, clomipramine severs the ATP levels in parasites; while amitriptyline, less efficiently affects ATP levels. Amitriptyline related compound, cyclobenzaprine instigates the ROS induced parasite death[193]. Mianserin, targeted 3-hydroxy-3-methylglutaryl coenzyme A (HMGR), rate limiting sterol biosynthetic enzyme, similar to AmB was efficacious against *L. donovani*[194]. Imipramine has shown to alter the sterol synthesis in parasite while, in combination with miconazole, acted synergistically to eliminate the parasite[195]. Recently, sertraline (serotonin reuptake inhibitor), induced perturbation in parasite bioenergetics and alterations in polyamine metabolism served to disarray the parasite virulence[196].

Likewise, pentoxifylline, initially used for treating vascular diseases by inhibiting TNF- α mediated immune inflammation, alone or combination with SSG was successful in treating refractory MCL/CL cases[197, 198]. However, another trial of CL reported adverse events associated with the pentoxifylline monotherapy and less efficacy of the combined therapy than SSG monotherapy [199].

The success of phase I trial of fexinidazole in trypanosomiasis raised hopes for its trial in leishmaniasis. The study of pharmacological behavior in murine model revealed for its oxidation into sulfoxide and sulfone, with anti-parasitic potential. Single dosing cleared 99% of parasites within 24 hours while, a regimen of 200mg/kg daily, resulted in 98.4% suppression of VL infection. These drugs undergo activation by NADH-dependent

nitroreductase therefore, enzyme overexpression is directly associated with parasite's fexinidazole sensitivity [200]. However, a Phase II proof-of-concept study assessed the safety and efficacy of fexinidazole for the treatment of primary VL adult patients in Sudan. Fourteen patients were treated with fexinidazole 1800mg PO for 4 days, followed by 1200mg PO for 6 days. All patients showed clinical improvement during treatment and the majority had parasite clearance (by microscopy) at the end of treatment. However, the response was not sustained, and relapses were observed during the follow-up period. Due to low efficacy rate, this study was terminated prematurely (F Alves, Personal Communication).

Besides, above mentioned compounds, recent research holds promises for utilizing the repurposed drugs as anti-leishmanial agents with well tolerated PKPD profiles. In 2014, nitroimidazole-oxazole oral formulation (DNDI-VL-2098) has shown preclinical efficacy[201]. In 2016, Novartis foundation reported for the unprecedented efficacy of proteasome inhibitor, GNF6702, in kinetoplastid diseases (leishmaniasis, chagas disease and sleeping sickness)[202]. Recently, a novel preclinical entity (7, DDD853651/GSK3186899) has yielded substantial pharmacological and toxicological success in mice model, by inhibiting parasite cdc-2-related kinase 12 (CRK 12)[203].

Although the list of repurposed drugs has been extending each day, there remains a need for choosing the compounds with established pharmacological datasets.

Conclusions

The set target of leishmaniasis elimination can be achieved by scaling up the implementation of intervention strategies, this need coordinated partnership of all stakeholders. The understanding of pharmacodynamic potential of emerging drugs remains the mainstay in their journey from preclinical evaluation to their translational implications. Further, it is also necessary for sustaining the elimination upon post-elimination, in order to develop optimal treatment strategy. Understanding of drug PKPD by different *in vitro* and *in vivo* infection models, simulating human infection can guide the therapeutic advancement of human diseases.

Recent decade has experienced significant progress in PKPD, paving the path for fighting against the crisis of drug resistance and develop new therapeutic strategies. Further, the integration of PKPD with pharmacokinetics, physiology with genomic, metabolomic and proteomic tools can create landmark in disease intervention. Thus, need for integrated approach can provide comprehensive understanding of host-pathogen interactions and accelerate the drug discovery/development process bringing it from bench side to bedside.

Expert opinion

Drug development remains a sturdy process, with low chances of success from target discovery till the point of clinical trial. One of the major gaps in the drug development process is the pharmacological understanding of drug interaction with the host system. Although the technological advances in biomedical research have extended the list of druggable targets with therapeutic benefits, careful stratification of druggable targets is

desirable. The process of stringent drug screening and optimization involves the pragmatic use of array of analytical approaches from whole genome sequencing to metabolomics and proteomics. Additionally, thorough understanding of the PKPD of the test compound remains prerequisite for the drug discovery.

The goal of achieving the disease cure, with minimal toxicities to the patients in different cohort studies suggest for the equal importance of drug selection and dosage. In the light of the varied immunological and genetic makeup of individuals, tailoring the dosage can potentially benefit the patient and avoid adverse drug reactions. The careful understanding of pharmacological principles can potentially reduce the incidence of drug resistance, thereby complementing the elimination and post-elimination perspectives.

Understanding of drug PKPD also calls upon for considering the inter-individual variability of drug tolerance. Furthermore, pharmacodynamic considerations of prior drug exposures, disease chronicity and type of infection (visceral/cutaneous) plays vital role in successful treatment. Indeed, variability in drug PKPD can also compromise the therapeutic drug concentrations. Thus, it may contribute for poor therapeutic efficacies and adverse events. Thus, patient centered approach, assessing the drug PKPD to optimize the dosing for programmatic cure of disease can accelerate the pace of disease elimination program. Drug PKPD has surely helped the clinicians in making better choices for currently available chemotherapeutic options additionally, it has fostered the development of new antimicrobials. Likewise, integration of PKPD with pharmacokinetics and physiology can help in drug designing and dosing for optimal disease intervention with minimal adverse effects.

Currently, novel drug candidates are evaluated through non-inferiority trials to measure treatment outcomes, but the uncertainty associated with clinical endpoints limits the evaluation of treatment outcomes[204]. Furthermore, high costs and time of development process restricts the drug development program. These issues undermine the need for more accurate PKPD assays for comparing the potencies of novel therapeutic agents with conventional chemotherapy and exploring their translational potential in human.

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**Considerable interest, * Special interest

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Article Highlights

- The drawbacks of current chemotherapeutics include the toxic side effects and drug resistance which call for thorough understanding of the pharmacodynamics and/or pharmacokinetics (PKPD).
- Current drug development process focuses only on the drug screening and lead optimization that yields extended list of drug targets and anti-parasitic drugs.
- Individual variabilities and drug susceptibility of pathogenic species makes PKPD an important factor in successful cure of disease.
- Understanding the drug's PKPD can provide an approach for tailoring drug dosage, to clear off the infection.
- Better understanding of PKPD will simplify the drug discovery, selection and improve the success rates of treatment options.

Table1:

Anti-leishmanial drugs (used till date) and their characteristics.

Generic name of drugs (Marketed as)	Chemical formula	Mechanism of action	Administration route	Adverse effects	References
Pentavalent antimonials (Pentostam) Sodium stibogluconate (Glucantime)	$C_7H_{18}NO_8Sb$ $C_{12}H_{38}Na_3O_{26}Sb_2$	Attains active form in amastigotes, inhibits trypanothione reductase in parasite, increases ROS production.	Intravenous/intramuscular	Painful injections, drug resistance and cardiotoxic	[4]
Pentamidine (Pentacarinat)	$C_{19}H_{24}N_4O_2$	Binds to kinetoplast DNA, inhibits topoisomerase II	Intramuscular	Gastrointestinal problems, and diabetes mellitus	[6]
Amphotericin B (Fungitericin,Fungisone) Liposomal AmB and lipid associated AmB (AmBisome, Abelcet,Amphocil, Amphomul,Kalsome)	$C_{47}H_{73}NO_{17}$	Complexes with parasite membrane ergosterol, pore formation and cell death	Intravenous	Infusion-related,nephrotoxicity, hypokalemia, weakness azotemia, anemia	[6]
Miltefosine (Impavido)	$C_{21}H_{46}NO_4P$	Induces apoptosis, inhibits cytochrome c oxidase,phosphatidylcholine synthesis.	Oral	Nephrotoxic, diarrhea, raised creatinine and potentially teratogenic	[79]
Paramomycin (Humatin, Paromycin)	$C_{23}H_{45}N_5O_{14}$	Binds to 30S subunit of ribosome and inhibits translation	Intramuscular (VL) Topical (CL)	Nephrotoxic and ototoxic	[8]

Table 2:

Repurposed drugs for treating leishmaniasis (under clinical trial)

Drugs	Disease model/Species tested	Experimental model	Outcome	References
Allopurinol	<i>L. infantum</i>	Canine	Failed to confer protection	[178]
	<i>L. panamensis, L. braziliensis</i>	Human	Failed to confer protection	[179]
Domperidone	<i>L. infantum</i>	Canine	Prophylactic option, reduced the risk of disease development	[170]
Rifampicin and omeprazole	<i>L. tropica</i>	Human	Highly effective in management of CL	[180]
Delamanid	<i>L. donovani</i>	Mice	Sterile cure of VL	[205]
Azithromycin	<i>L. vianniabraziliensis</i> is <i>L. major</i>	Human	Successful Failed	[181] [206, 207]
Itraconazole	<i>L. tropica</i>	Human	Not effective	[208]
Imiquimod (topical)	<i>L. peruviana, L. braziliensis</i>	Human	Failed to cure CL	[209]
Imiquimod+Meglumine antimoniate	CL	Human	Successful	[150]
Ketoconazole	CL	Human	Successful	[176]
Fluconazole	<i>L. braziliensis</i> (CL)	Human	Successful	[210]
Pentoxifylline+SSG	<i>L. braziliensis</i> (CL)	Human	Less effective than SSG monotherapy	[211]
Terbinafine	<i>L. tropica</i> (CL)	Human	Successful	[212]
Terbinafine+Glucantime				
Fexinidazole + Nitafoxanide	<i>L. donovani</i>	Human	Successful	[200]