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Chapter 12

Crossing Biological Barriers for Leishmaniasis Therapy: From Nanomedicinal Targeting Perspective

Gul Shahnaz, Hafiz Shoaib Sarwar and Masoom Yasinzai

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.75911

Abstract

Despite past 60 years of extensive research in antileishmanial drug development, the successful therapy of this disease cannot be achieved at full potential. The biological barriers encountered by the therapeutic modalities favor the disseminations of the disease like intramacrophage location of parasite, lack of oral bioavailability, permeability across the cutaneous tissue, and active efflux of the drug. Nanomedicines are specifically engineered nano-sized delivery systems. The goal of designing a nanomedicine is to achieve the specific therapeutic objective via targeting the specific cells and intracellular locations, pharmacological receptors, enzymes and proteins, crossing biological barriers, and navigation through endocytic pathways. This chapter will cover various nanomedicinal approaches like targeting the macrophages, pathological organs, efflux pumps, metabolic enzymes, redox biology of *Leishmania* by using polymeric and metal nanocarriers to overcome all the biological barriers thus providing a successful alternative over the conventional therapies.

Keywords: biological barriers, macrophage targeting, nanocarriers, photodynamic therapy, oral bioavailability, leishmaniasis

1. Introduction

The challenges faced by the current antileishmanial therapy include subtherapeutic efficacy, development of resistance, toxicity, and cost-effectiveness [1]. Despite past 60 years of extensive research in antileishmanial drug development, the successful therapy of this disease cannot be achieved at full potential. There is no vaccine available against *Leishmania* and the treatment relies mainly on the chemotherapy. The classic chemotherapeutic agents cannot control the prevalence of *Leishmania* effectively as they encounter various biological barriers

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like the intramacrophage location of *Leishmania* parasite, selective access to the pathological organs, lack of oral bioavailability, permeability across the cutaneous tissue, activity of drug efflux pumps, development of toxicity and serious side effects. *Leishmania* parasite utilizes these barriers in its favor like redox biology of *Leishmania* helps them to survive inside the phagolysosomes of the macrophages, and impermeability of the macrophages for the antileishmanial drugs deprives the free access of drug to the target [2]. The nontargeted nature of current therapeutic modalities results in free circulation of drugs in the blood, and accumulation in the pathological organ at desired concentration cannot be achieved. Permeability glycoprotein (P-gp) efflux pumps present in *Leishmania* actively efflux the drug out of the cell resulting in decreased intracellular accumulation [3]. Lack of oral absorption necessitates the parental formulation of antileishmania drugs, which needs hospitalization of the patients and lack of compliance. These circumstances augment the development of new therapeutic option which can be achieved either by developing the new antileishmanial agents or by changing the drug delivery systems. Traditional new drug development usually takes over 10–12 years and involves extensively high manufacturing cost. Leishmaniasis is a neglected tropical disease and receives very little funding regarding the research and development due to low market turnover [4]. So, switching the research toward the new drug delivery systems such as nanomedicine is a suitable approach in pursuit of successful therapy of leishmaniasis.

Nanomedicine is a specifically engineered nano-sized particulate drug delivery system designed for the improved pharmaceutical formulations. The nanoparticles can achieve the discrete therapeutic objectives which are otherwise impossible with conventional drug delivery systems like targeting a specific cell and organelles, enzymes, proteins, and pharmacological receptors, accumulating in the pathological organs, bypassing the organs prone to the toxic effects, crossing biological membranes, and navigating through endocytic pathways [5]. All these properties of nanoparticles can address the biological barriers encountered in the effective therapy of leishmaniasis. Various polymeric and metal nanocarriers-based strategies like macrophage targeting, organ targeting, improved oral bioavailability, and photodynamic therapy are being explored for their supreme antileishmanial effects.

This chapter will discuss the various biological barriers compromising the effectiveness of antileishmanial therapy and the role of nanomedicine to overcome the problems associated with the conventional therapeutic modalities thus providing a platform for the enhanced antileishmanial therapy.

2. Current medical management of leishmaniasis

For the past six decades, the standard first-line drugs for the treatment of leishmaniasis are antimonial drugs, meglumine antimoniate, and sodium stibogluconate [6]. Antimonial compounds required to be administered IV/IM at the dose of 20 mg/kg of Sb-V for 10 days in case of cutaneous leishmaniasis (CL) and for 28 days in case of visceral leishmaniasis (VL). Antimonial drugs act by inhibiting a thiol metabolic enzyme trypanothione reductase (TR) and thus causing a decreased trypanothione ($T[SH]_2$) levels which results in the decreased ability of the parasite to counteract the oxidative stress [7]. However, the variations in the clinical

response and development of resistance from the past several years are a persistent clinical threat. The activity of aqua glycoproteins, trypanothione reductase/trypanothione (TR/T[SH]$_2$) system, and permeability glycoprotein (P-gp) efflux pumps is involved in the development of resistance resulting in decreased intracellular accumulation of antimony in subtherapeutic concentrations thus jeopardizing the effectiveness [8, 9]. Serious toxic effects associated with antimonial therapy like cardiotoxicity, changes in ECG, renal and liver impairment, muscle pain, and severe fatigue further limit the therapeutic potential of antimonial compounds [10].

Amphotericin B (AmB), a polyene antibiotic, is the second-line standard drug for the leishmaniasis since the 1960s [11]. Whereas, in India, the AmB is the first-line drug approved for VL due to widespread resistance against antimonial compounds. The standard dose of AmB for VL is 1 mg/kg every other day for 20 days via IV route. It has selective activity against the *Leishmania*, *Trypanosoma cruzi*, and fungi due to the presence of ergosterol in the said microbes compared with the mammal cell having cholesterol in their cell membranes. AmB binds with the ergosterol and induces pore formation [12]. Moreover, resistance against the AmB is further related to the change in cell membrane composition and fluidity. *Leishmania donovani*-resistant strains showed a significant change in the sterol profile, in which the ergosterol was replaced by a precursor known as cholesta-5,7,24-trien3β-ol [13, 14]. This is due to the loss in functionality of S-adenosyl-ʟ-methionin-C24Δ-sterol methyltransferase resulting in the impaired C-24 transmethylation [15]. The use of AmB results in nephrotoxicity resulting in renal failure, thrombocytopenia, anemia, anaphylaxis, convulsions, phlebitis, and high fever [16].

Miltefosine (MILT) has been recently tagged as an antileishmanial drug required to be administered at the dose of 50 mg orally three times a day for 28 days. The variation in the clinical response has been observed due to the species variations and the development of resistance [17]. Promastigote-resistant strains of *L. donovani* have been developed in the laboratory that was resistant against the MILT up to 40 μM [18]. The mechanism of resistance was found to be greater than 95% of reduced accumulation indicated by the ^{14}C-labeled MILT. Pérez-Victoria et al. [19] reported the involvement of novel plasma membrane P-type transporters from the aminophospholipid translocase subfamily to be responsible for the reduced accumulation of glycerophospholipid and MILT in the resistant promastigotes [19].

Pentamidine (PTM) is also being used as the second-line therapy against leishmaniasis; however, the use is limited in zoonotic settings. The recommended dose is 2–3 mg/kg, IV or IM once a day for 4–7 doses in case of CL, while for VL, its dose is 2–4 mg/kg administered every other day via IV or IM for up to 15 doses. The use of PTM in the pentavalent antimonial Sb(V) refractory patients in India resulted in decreased efficacy from 95 to 70% within a short duration suggesting the development of resistant against the PTM. Resistance to PTM in *Leishmania* is due to the inhibition of polyamine biosynthetic, and studies suggested that PMT is transported into the cell via polyamine and arginine transporters [20].

From above discussion, it is evident that resistance against the antileishmanial agents is rising, compromising the therapeutic efficacy. Apart from the development of resistance, other factors like associated toxic effects, unavailability of oral dosage forms, longer duration of therapy, and high cost also contributing toward the suboptimal control of leishmaniasis. These limitations arise primarily due to the various biological barriers encountered by the

antileishmanial agents. Considering the development of parasitic resistance and a limited number of effective antileishmanial drugs, there is an imperative demand to revise the standard medical management of leishmaniasis. Administering the available standard drugs with appropriate delivery systems that help to cross the biological barriers seems to be an encouraging strategy, which requires being given serious consideration.

3. Biological barriers to leishmaniasis therapy

3.1. Intramacrophage location

Mononuclear phagocytes (MP; monocytes, macrophages, and dendritic cells) along with eosinophils and neutrophils constitute the first line of defense against the invading pathogens and are involved in detection and elimination of the foreign bodies [21]. When the sand fly takes the blood meal, it inoculates the promastigotes of *Leishmania* along with saliva. The saliva contains immunogenic proteins that trigger the immune response. The promastigotes are immediately taken up by the MP cells like macrophages following a receptor-mediated endocytic event. During initial recognition, the macrophage receptors play a vital role depending upon the *Leishmania* species like scavenger receptors (SRs), mannose receptors (MRs), complement receptors (CRs), and fibronectin receptors (FRs). The binding of the parasite to specific receptors then determines the course of infection [22]. Upon the successful entrapment of *Leishmania* inside macrophages, complex cellular signals are produced like activation of lysosomal enzymes, production of nitric oxide ($NO^{\bullet}$), and initiation of oxidative burst as shown in **Figure 1** [23, 24]. Oxidative burst is a potent antileishmanial response produced by the reactive oxygen species (ROS) namely hydroxyl ion (OH^{-}), hydrogen peroxide (H_2O_2), peroxynitrite ($ONOO^{-}$), and hypochlorous acid (HOCI) [25]. *Leishmania* employs the various mechanisms

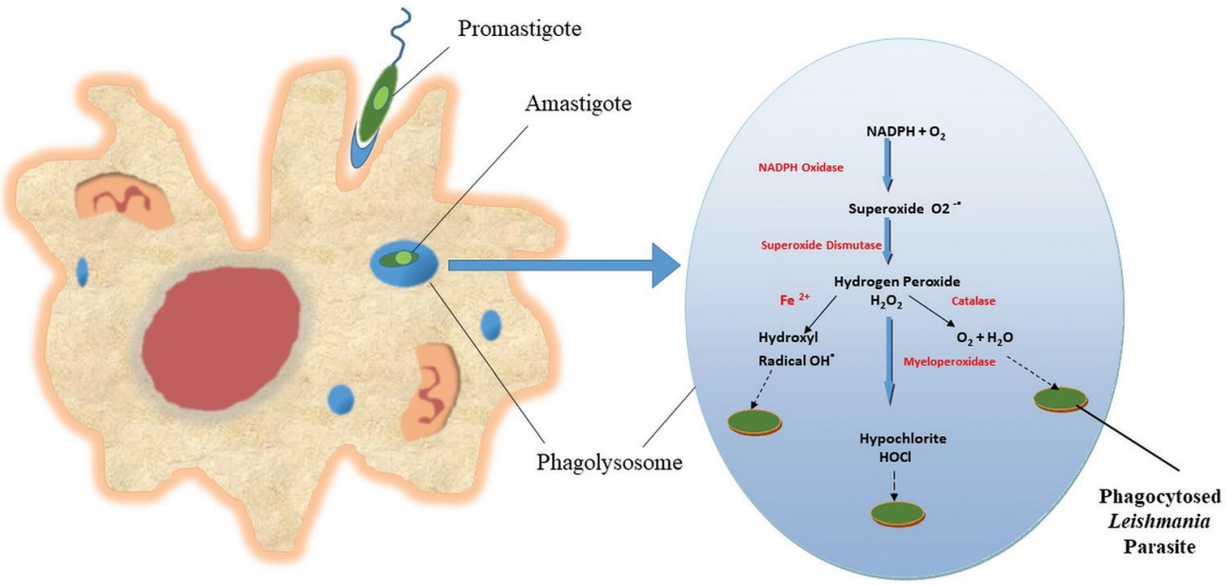


Figure 1. Endocytosis of *Leishmania* parasite and macrophage-induced oxidative burst.

http://dx.doi.org/10.5772/intechopen.75911

to counteract the ROS-like activation of protein tyrosine phosphatase (PTP) and TR/$T[SH]_2$ system [26, 27]. Thus, *Leishmania* parasite survives the oxidative stress induced by the macrophages owing to its unique redox biology where it replicates and utilizes macrophages as a source of propagation of infection. The macrophage cell membrane is not freely permeable to the antileishmanial agents and acts as a barrier against the intracellular accumulation of chemotherapeutic agents at the concentrations required for optimum therapeutic effectiveness.

3.2. Activity of P-gp efflux pumps

The activity of P-gp efflux pumps presents another major barrier to the antileishmanial therapy [28]. Active efflux of the drug via the efflux pumps is one of the most common mechanisms for developing multidrug resistance (MDR) in the microorganism [29]. In fact, MDR mediated through efflux pumps has been described in various organisms like fungi, bacteria, and protozoa including *Leishmania* [30]. P-gp efflux pumps belong to the ATP-binding cassette (ABC) transporters, acting as the physiological barrier by extruding the toxins and xenobiotics out of the cells. The ABC transporters are the largest superfamily of efflux pumps known; being present in all organisms, from archaebacteria to higher eukaryotes. Various types of drugs with a wide range of chemical structure can be recognized by a single P-gp molecule ranging in molecular weight from 250 g/mol (cimetidine) to 1202 g/mol (cyclosporine). P-gp is primarily found in epithelial cells which have the excretory roles including the apical surface of epithelial cells lining the colon, small intestine, where it is involved in the decreased oral bioavailability of drugs [31]. In *Leishmania*, two types of ABC transporter have been reported to be amplified in the laboratory strains when exposed to different drugs: P-gp and multidrug resistance-related protein (MRP) also known as P-gp A [32]. P-gp A is believed to be involved in the decreased intracellular accumulation of antimonial compounds, the first-line therapy against *Leishmania*, resulting in the subtherapeutic response and emergence of resistance. The gene responsible for the P-gp A has been found to be amplified in the laboratory mutant strains of *Leishmania* that were resistant to the antimonial compounds [33]. However, this transporter is not involved in the efflux of antimonial drugs in the form of Sb-III or SB-V rather it confers resistance by sequestration of Sb-III conjugated with $T[SH]_2$ in the form of Sb-III-$T[SH]_2$ adducts as presented in **Figure 2** [34]. $T[SH]_2$ acts as the main reducing agent and is oxidized into its disulfide form $T[S]_2$ which is reduced back to $T[SH]_2$ by the activity of NADPH-dependent enzyme TR [35]. $T[SH]_2$ exerts its protective effect by the reduction of $NO^{\bullet}$, H_2O_2, and $ONOO^{-}$. Sb-V are converted to its trivalent form (Sb-III) inside the cell, and Sb-III has the ability to form a complex with the thiol groups of $T[SH]_2$. This Sb-III-$T[SH]_2$ conjugate is sequestrated by the P-gp A pumps. Thus, in *Leishmania,* the P-gp efflux pumps work in coordination with the activity of TR/$T[SH]_2$ system resulting in decreased intracellular accumulation of SB-III [36].

3.3. Lack of oral bioavailability

Oral administration is the most suitable method of delivering the drugs due to the convenience of dosing, noninvasive nature, and high acceptance at patient levels [37]. Most of the therapeutic agents used for systemic and localized GIT effects are administered orally because of the highly absorptive nature of the intestine that provides a large surface of around 300–400 m^2. The oral administration is successful only in the case where the drugs have sufficient bioavailability.

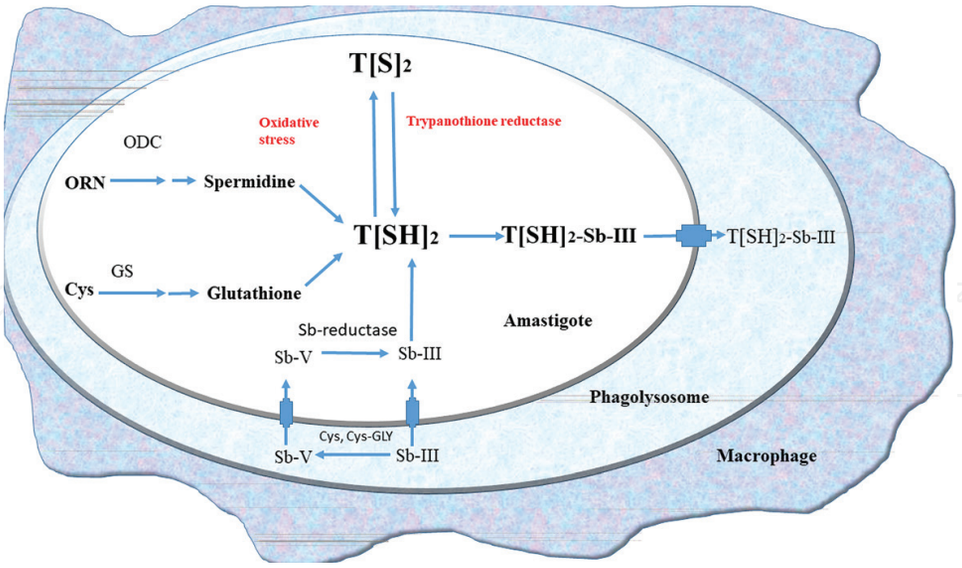


Figure 2. Mechanism of P-gp efflux pumps and TR-mediated drug resistance against antimonial compounds. ORN = ornithine, ODC = ornithine decarboxylase, GS = glutathione synthetase, Cys = cysteine, and Gly = glycine.

Many physiochemical and physiological factors determine the oral bioavailability of drugs like solubility, permeability, a mucus layer, partition coefficient, stability, dissolution, pH, enzymatic degradation, and activity of P-gp efflux pumps. Unfortunately, most of the antileishmanial drugs encounter the above-described barriers and exhibit limited oral bioavailability except for MILT.

In fact, solubility and permeability govern the oral bioavailability. Most of the drugs diffuse across cell membrane via passive transport, and for that purpose, the drug should be lipophilic in nature as the unionized form is better to diffuse across the phospholipid bilayer. However, the drug molecules should not be lipophilic enough to remain soluble in the lipid bilayer suggesting a suitable log P value. To maximize the possibilities of passive diffusion, the ideal log P value is considered to be around 2. The molecular weight of the drug also has a role in the passive diffusion of the drug and molecular mass less than 500 Da is considered to be favorable for the absorption across the small intestine [38]. The effect of solubility, permeability, and molecular weight is better explained in Lipinski' s rule. Lipinski's rule of five is a very useful tool to predict the drug-like characteristics of a compound and is especially applicable to assess whether a drug is orally active or not [39]. Lipinski's rule states that in general, an orally active drug has no more than one violation of the following criteria:

- No more than five hydrogen bond donors (the total number of nitrogen-hydrogen and oxygen-hydrogen bonds);
- No more than 10 hydrogen bond acceptors (all nitrogen or oxygen atoms);
- A molecular mass less than 500 Da;
- An octanol-water partition coefficient log P not greater than 5.

Most of the antileishmanial drugs do not follow the Lipinski's rule of five, therefore, not absorbed orally. According to biopharmaceutics classification system (BCS), AmB is class IV drug with the

Drug	Molecular weight (Da)	Log P	Hydrogen acceptor	Hydrogen donor	Rule of five
Sodium stibogluconate	907.88	−3.4	17	5	No
Amphotericin B	924.079	−0.66	17	12	No
Paromomycin	615.62	−8.3	19	13	No
Pentamidine	340.41	2.32	6	4	Yes
Miltefosine	407.57	2.25	2	0	Yes

Table 1. Physicochemical properties of antileishmanial agents.

aqueous solubility of <1 mg/L at physiological pH, molecular weight of 924 Da, and log P of 0.95, 17 hydrogen bond acceptors and 12 hydrogen bond donors and in this way does not follow the rule of 5. Similarly, sodium stibogluconate possesses a molecular weight of 910.10 Da and log P of −0.34, 17 hydrogen bond acceptors, and 5 hydrogen bond donors, thus violate the rule of 5. The physiochemical properties of the antileishmanial drugs are presented in **Table 1**.

Thus, the lack of oral bioavailability of most of the antileishmanial agents is the major limitation in the cost-effective and optimum therapy of leishmaniasis. Minimal oral absorption below the minimum effective concentration (MEC) necessitates the formulation of antileishmanial drugs for the parenteral administration. The long-term parenteral administration has its own limitations as it requires the patient to be hospitalized, increased cost of the therapy, and patient compliance.

3.4. Skin as barrier to topical therapy

Skin is the largest organ of the body and protects the organism from the external environment. Histologically, the skin is divided into the superficial layer called the epidermis and a deeper layer, the dermis. The several strata make up the epidermis distinguished by the changes in keratinocytes from dermis-epidermis junction to the outer surface of epidermis, the stratum corneum (SC). SC is designated as the main barrier to the transport of substances across the skin and is formed by the corneocytes characterized as densely packed, dead, and keratinized cells. These cells are surrounded by the intracellular lipid matrix composed of nonpolar lipids in lamellar lipid layers, making SC a hydrophobic layer [40]. Although SC is only 10–20 μm in thickness, it acts a barrier and hampers the penetration of microorganisms, drugs, and other chemicals besides being involved in the transepidermal water loss.

Drug delivery across the skin follows three possible pathways: the intracellular route, between the corneocytes sinuously through the lipid layer; the transcellular route, through the corneocytes and lipid matrix; and through the cutaneous appendices (sweat and sebaceous glands, hair follicles). The drug diffusion across the skin is essentially a passive transport; however, it follows either one or combination of the three pathways. However, it must be noted that the intracellular route is considered to be the most suitable for the drug diffusion as it offers less resistance compared to the transcellular route in which the drug molecules have to move

between the intercellular hydrophobic region to intracellular hydrophilic region repeatedly. The skin appendages, although having a small surface area compared to the total skin (0.1%), present an opportunity for the penetration of ions, polar compounds, and large molecules and thus circumvent the low diffusional character of SC [41].

To deliver the drug across the skin, the choice of the adequate molecule in terms of molecular weight and partition coefficient is very important. A molecular weight less than 600 Da, low melting point, and suitable log P are desired. Thus, the drug with high molecular weight and hydrophilic character will face the maximum resistance and their penetration will be limited [40]. The drugs for the CL-like paromomycin (PA), antimonial compounds, face the problem of skin penetration due to their hydrophilicity and high molecular weight. In case of CL, the lesions are developed and parts of epidermis and dermis are lost; therefore, the barriers provided by the SC are absent and almost any type of drug can be absorbed. However, the formation of scar tissues and keratotic nodules during the healing process restores the functionality of SC thus depriving the drug absorption at the end of the treatment, and complete healing of the lesion is difficult [42].

4. Nanodrug delivery system for leishmaniasis

The drug delivery systems are crucial in drug development and design, and many active pharmaceutical ingredients result in serious side effects when administered nonspecifically. The lack of appropriate drug delivery system causes the therapeutic modalities to be accumulated in healthy tissue inciting the adverse effects, lower bioavailability, and inefficient targeting of the desired pathological organs. Most of the latest researches in the field of leishmaniasis are focused on addressing the physiological, biological, and biopharmaceutical aspects of the use of nanotechnology. Nanodrug delivery systems provide an attractive opportunity to resolve the drug delivery problems associated with the therapy of leishmaniasis by crossing the above-demonstrated barriers encountered by the antileishmanial drugs. Examples of nanotechnology progress in pharmaceutical products include liposomes [43], niosomes [44], nanodisks [45], nanoemulsions [46], polymeric nanoparticles [47], solid lipid nanoparticles, and polymer-drug conjugates [48] as described in **Table 2**.

4.1. Liposomes

Liposomes are the lipid bilayer systems described in 1965 and rapidly taken as drug delivery systems [49]. In 1977, Ward and Hanson, first time reported the encapsulation of Sb-V into liposomes for targeted delivery to liver and spleen in VL. After intravenous administration, the Sb levels of liver and spleen were found to be 20-fold higher compared to the free drugs [50]. However, due to the toxic effects in monkeys, the interest in liposomal Sb-V was declined [51]. The same concept was also applied to AmB in order to avoid its toxicity by encapsulating into multilamellar liposomes. The liposomal AmB got a little bit more attention than Sb-V and initiated model for the development of three-lipid-based AmB drug delivery systems licensed for clinical use (Ambisome®, Amphocil®, and Abelcet®) [52]. However, the only true liposomal

Type of nanocarrier	Active moiety	Targeting approach	Strain tested	Model	Ref
Thiolated chitosan NPs	Amphotericin B	Macrophage targeting	*L. donovani*	J774.1 macrophages/BALB/c mice	[69]
Chitosan NPs	Rifampicin		—		[71]
Nanocapsules	Doxorubicin			Wistar rats	[72]
Gelatin nanoparticles	Amphotericin B		*L. donovani*	J774.1 macrophages/ Hamster	[73]
Liposomes	Antimony		*L. chagasi*	Peritoneal macrophages	[74]
GCPQ chitosan	Amphotericin B	Organ targeting via oral route	*L. donovani*		[76]
MT-chitosan	Amphotericin B		*L. donovani*	J774.1 macrophages/BALB/c mice	—
Liposomes	Zinc phthalocyanine	Photodynamic therapy	*L. braziliensis*		[86]
Metal oxide	ZnO		*L. tropica* KHW23		[87]
Liposomes	Paromomycin	Skin permeating nanocarriers	*L. major*	BALB/c mice	[90]
SLN	Amphotericin B		*L. major*		[92]

Table 2. Various types of nanocarriers and different targeting approaches for leishmaniasis.

formulation, Ambisome®, is recommended for treating patients with leishmaniasis who are resistant to antimonials. The efficacy of liposomal AmB was further enhanced by decorating the liposomal surface with specific ligands like polysaccharides, peptides, antibodies, and glycolipids. The decorated liposomes were able to specifically target the macrophages to avoid the exposure of AmB to healthy tissues [53, 54]. The detail of macrophage-targeted liposomes will be discussed under the section macrophage targeting.

4.2. Niosomes

Niosomes are the attractive alternatives over liposomes due to their increased stability, low cost, and biodegradability [55, 56]. Niosomes are the vesicles consisting of nonionic surfactants. Niosomal formulations of sodium stibogluconate were more efficacious compared to the liposomes and free drugs against experimental murine VL [56]. More recently, in vivo studies demonstrated that the niosomes containing autoclaved *L. major* have a significant result in the prevention of CL in BALB/c mice [57]. Niosomes have also found their role in vaccination against leishmaniasis. Purified gp63 entrapped into niosomal formulation provided considerable resistance to the leishmaniasis when used as the subcutaneous vaccine in C57BL/10 mice [58]. Advancement of a commercial antiparasitic vaccine for the human appliance is a central goal that faces modern science. Therefore, further research will be required to investigate immunological pathways, followed after vaccination with *Leishmania* antigens loaded into niosomes, and possible unwanted adverse effects in order to assess the real potential for a vaccination trial in humans.

4.3. Polymeric nanoparticles

Polymeric nanoparticles are very valuable in the treatment of infectious diseases like leishmaniasis owing to the small size and abilities to enhance the cellular uptake, cross the biological barriers, and deliver drugs at the site of infection [59, 60]. The use of polymer for the development of nanocarriers provides us the opportunity of modifying the functional groups with various chemical methods to incorporate the desired ligands for better penetration and enhanced endocytosis by the active or passive targeting. The ability of polymeric nanocarriers to bear the physiological strains and tunable surface properties provides an edge over liposomes and niosomes. While utilizing the polymeric nanoparticles for leishmaniasis, the category of polymers is of considerable importance as the hydrophobicity of the polymer will facilitate the internalization by macrophages, the core target in leishmaniasis. For example, polymethylmethacrylate-based nanoparticles indicated a superior macrophage uptake compared to the polycyanoacrylate [61]. Various studies reported the potential of polymeric nanocarriers in leishmaniasis. Primaquine-loaded polymeric nanoparticles were found to be 21-fold more efficient compared to the free primaquine [62]. β-aescin-loaded polylactide-co-glycolide nanoparticles showed twofold increase in efficacy against J744.1 macrophage-infected *L. donovani* model [63]. In a recent study, PEGylated polylactic acid nanoparticles loaded with bisnaphthalimidopropyl derivatives have been tested against human macrophage and THP-1 murine J744 macrophage model of leishmaniasis [64].

4.4. Polymer drug conjugate

The advances in the field of polymer engineering have opened new dimensions for the drug delivery. One example is the polymer therapeutics in which the drug molecules are attached to the polymer backbone by using a suitable chemical method. In this way, the efficacy of the drug can be increased significantly with the reduction in the toxicity. The hydrophobic drug encounters a problem of free circulation in the blood. The hydrophilicity of these drugs can be increased by conjugation of these hydrophobic drugs with the hydrophilic polymer. These polymer-drug conjugates provide increased plasma half-life and retention in the infectious tissue with the minimum toxicity. The conjugation of AmB with the N-2-(hydroxypropyl) methacrylamide resulted in the increased efficacy as compared to the free drug (fungizone) [65].

4.5. Nanodisks

Nanometer scale, a lipoprotein-stabilized phospholipid bilayer disk complexes termed nanodisks (NDs) are novel transport vehicles different from liposomes because they do not hold an aqueous core and are completely soluble in aqueous phase media [66]. NDs harboring poorly soluble antileishmanial agent AmB-nanodisks demonstrate an effective therapy for experimental CL (*L. major*) infection in BALB/c mice. Surprisingly, AmB-nanodisks were illustrated to have a long-term effect in that parasite burden continued to decrease for more than 100 days subsequent the final treatment. The results shown for intraperitoneal administration are most likely because of the small size of the ND [45].

http://dx.doi.org/10.5772/intechopen.75911

5. Nanomedicinal targeting approaches for leishmaniasis

Paul Ehrlich in 1891 was the first to theorize the concept of "magic bullets" providing the first description of drug targeting paradigm. The aim of drug targeting is delivering the drug at the right concentration at the right time and at the right place. The evolution of this "magic bullet" concept revolutionized the drug delivery systems and provided a vast platform, known as nanomedicine, to achieve the very specific and highly desirable therapeutic outcomes that are otherwise impossible to achieve with conventional drug delivery systems [67]. Their small size at nanoscale dictates the very unique properties like the interaction with the biological entities, penetration across the membrane, intracellular trafficking, accumulation at the target area, improved blood circulation, and biodistribution. For example, in case of VL, the major organs representing the parasitic burden are liver, spleen, and bone marrow, and the drug has to target the parasite inside the macrophage in these organs [68]. In CL, the drug must reach the parasite inside the macrophages at the inner layers of skin by crossing the skin barrier, SC. To maximize the potential of nanocarriers, a suitable strategy is required to target the pathological area via a patient-friendly route of administration while avoiding the healthy tissues. In view of this, various nanomedicinal targeting approaches have been explored for the therapy of leishmaniasis like macrophage targeting, organ targeting via the oral route, use of permeability enhancers, and photodynamic therapy (PDT).

5.1. Macrophage-targeted drug delivery

The niche in which *Leishmania* parasite lives presents challenges to drug delivery, and depending upon the species and the area affected, the drug has to achieve antiparasitic levels at multiple sites. Furthermore, the antileishmanial drug has to cross the multiple membranes before they act on the parasite. Nanoparticle-mediated drug delivery system to overcome the cell membrane barriers, release the drug inside the cells, and specifically target *Leishmania*-infected macrophages is emerged as a promising strategy to overcome resistance [69]. Various phagocytic receptors are expressed on the surface of *Leishmania* like MRs, CRs, SRs, and FRs [22]. These receptors bind with the specific ligands on the surface of *Leishmania* parasite and internalize the parasite. MRs are highly expressed especially on the *Leishmania*-infected macrophages. MRs recognize and bind with mannose and fructose glycoproteins followed by rapid endocytosis of the parasite. The mannose-binding protein belongs to the lectin-like carbohydrate-binding groups and cytoplasmic group that are involved in the remodeling of the cytoskeleton during endocytosis [2]. Scavenger receptors are the glycoproteins and are responsible for recognizing a broad range of ligands like chemically modified proteins, the apoptotic cell, low-density lipoproteins, phosphatidylserine, and various polyanionic molecules [70].

Targeting the macrophages via these receptors with surface-decorated nanocarriers leads to the accumulation of appreciable amounts of drug at the same niche where the parasite resides inside the macrophages. Various studies conducted on the macrophage-targeted drug delivery are summarized in **Table 2**. Recently, our research group utilized the MRs for macrophage-targeted delivery of mannose-anchored thiolated nanocarriers loaded with AmB. The uptake studies by

using the J744.1 macrophages indicated that macrophage-targeted nanocarriers provided AmB concentration of 28.6 ± 1.4 μg/10^6 cells as compared to 0.4 ± 0.01 μg/10^6 cells of the free AmB. These results provided the evidence that macrophage-targeted nanocarriers were 71-fold more efficient than the nontargeted ones. Also, the macrophage-targeted nanocarriers were having superior antileishmanial activities against *L. donovani*-infected macrophage model with 13-fold reduced IC_{50} values compared to the nontargeted ones. *In vivo* efficacy studies against *L. donovani*-infected BALB/c mice model at the dose of 1 mg/kg indicated that mannose-bearing thiolated chitosan (MTC) nanocarriers were significantly more effective in reducing the parasitic burden (89 ± 7%) against the free AmB (17 ± 4%) [69]. In another study, Chaubey and Mishra [71] also targeted the macrophages via mannose-decorated chitosan for the delivery of rifampicin against VL. *Ex vivo* cellular uptake studies indicated 16-fold increased uptake in case of mannosylated chitosan nanoparticles (mCNPs). The pharmacokinetic studies revealed that mCNPs exhibited Cmax of 5.40 ± 1.64 μg/ml with MRT of 58.48 ± 9.1 h compared to Cmax of 279.00 ± 17.71 μg/ml and MRT of 1.82 ± 0.2 h for free rifampicin indicating the long circulating time of the mCNPs. Similarly, very encouraging results were obtained with *in vivo* biodistribution studies conducted at the dose of 12 mg/kg. The maximum accumulation of drug was observed in liver (57.5 ± 1.3%) followed by spleen (14.2 ± 1.5%), achieved with mCNPs when compared with the free rifampicin (6.91 ± 1.3% for liver and 1.1 ± 0.2% for spleen) after 6 h. The drug accumulation is attributed to the fact that macrophage-targeted nanocarriers were rapidly taken up by the MP cells and delivered to the liver, main pathological organ of VL [71]. Similarly, curcumin-loaded mannosylated chitosan-based nanoparticles were reported to be highly efficacious against the free drug. Their study indicated that mannosylated nanoparticles effectively increased the endocytosis with the mean residence time of 39.38 h compared with the 0.30 h of free drug solution [68].

Kansal et al. [72] utilized scavenger receptors for the macrophage-targeted delivery of doxorubicin via phosphatidylserine-decorated nanocapsules (PS-NCs-DOX) for the therapy of leishmaniasis. Flow cytometry analysis indicated 1.75-fold increased uptake of PS-NCs-DOX compared with nonmodified nanocarriers (NCs-DOX). PS-NCs-DOX also accumulated in liver and spleen at higher concentration against NCs-DOX confirmed via *in vivo* biodistribution analysis in Wistar rats. Highly significant antileishmanial activities were observed in *Leishmania*-infected hamster model. PS-NCs-DOX exhibited 85.23 ± 4.49% inhibition of splenic parasitic burden compared with 72.88 ± 3.87 and 42.85 ± 2.11% parasite inhibition for NCs-DOX and free DOX, respectively [72]. Another study reported the development of 1, 2-diacyl-sn-glycero-3-phospho-l-serine (PS)-coated gelatin nanoparticles (GNPs) bearing amphotericin B for the enhanced *in vitro in vivo* antileishmanial efficacy in VL. The nanocarriers decorated with 1, 2-diacyl-sn-glycero-3-phospho-l-serine (PS-AmB-GNPs) were more efficient in terms of uptake by J774A.1 macrophages analyzed via flow cytometry [73]. Also, PS-AmB-GNPs exhibited a very significant reduction in parasitic burden providing 85.3 ± 7.89% inhibition compared to 50.5 ± 5.12% of free AmB in *Leishmania*-infected hamster model. Antimony-loaded liposomes have also been modified with phosphatidylserine (Sb-LP) for the enhanced uptake of macrophages via scavenger receptors. Sb-LP was 16-fold more effective than free drug against *L. chagasi*-infected macrophage model [74].

5.2. Organ targeting via oral route

One of the limitations associated with the conventional antileishmanial therapy is the free systemic circulation of the drug and distribution into different body organs including pathological

and nonpathological. The exposure of nonpathological organs to the drugs is associated with the severe toxicity of the antileishmanial drugs thus limiting its therapeutic potential [17]. In this regard, the specific organ targeting is a promising strategy that reduces the toxic effects by minimizing the exposure to nondesired organs and improves therapeutic efficacy by increasing the drug accumulation at the desired organs. One such example of the nanoliposomal formulation of AmB is Ambisome® for VL, when administered is taken up by MP cells and transported to the liver and spleen via passive targeting [52, 75]. Although this strategy greatly improves the safety of AmB, the macrophage-targeted nanocarriers described above can be of more potential in this regard. The surface modification with the ligands actively targets the infected macrophages because of the high expression of endocytic receptors like MRs. However, one factor, the parenteral delivery of these systems, limits their wide application and acceptance at the patient level due to the hazards and high cost associated with and needs to be addressed yet. In pursuit of the solution to this limitation, Serrano et al. [76] provided the concept of organ targeting via the oral route and introduced nanomedicine in which nanoparticles were taken up by the intestinal epithelia and are transported to liver, spleen, and lungs as shown in **Figure 3**, thus enhancing the bioavailability of these pathological organs of VL and bypassing the organs of potential toxicity [76]. This technique utilized specifically engineered polymeric excipients with the potential to interact with specific proteins in the intestinal epithelium thus enhancing the permeation and absorption of the constituted nanocarriers.

Serrano et al. [76] illustrated this concept by utilizing N-palmitoyl-N-methyl N,N-dimethyl-N,N,N-trimethyl-6-O-glycol chitosan (GCPQ) nanoparticles loaded with AmB. Such modification of chitosan will provide the mucoadhesive character to the nanocarriers. As the mucus is a negatively charged glycoprotein, the positively charged polymer will provide increased electrostatic

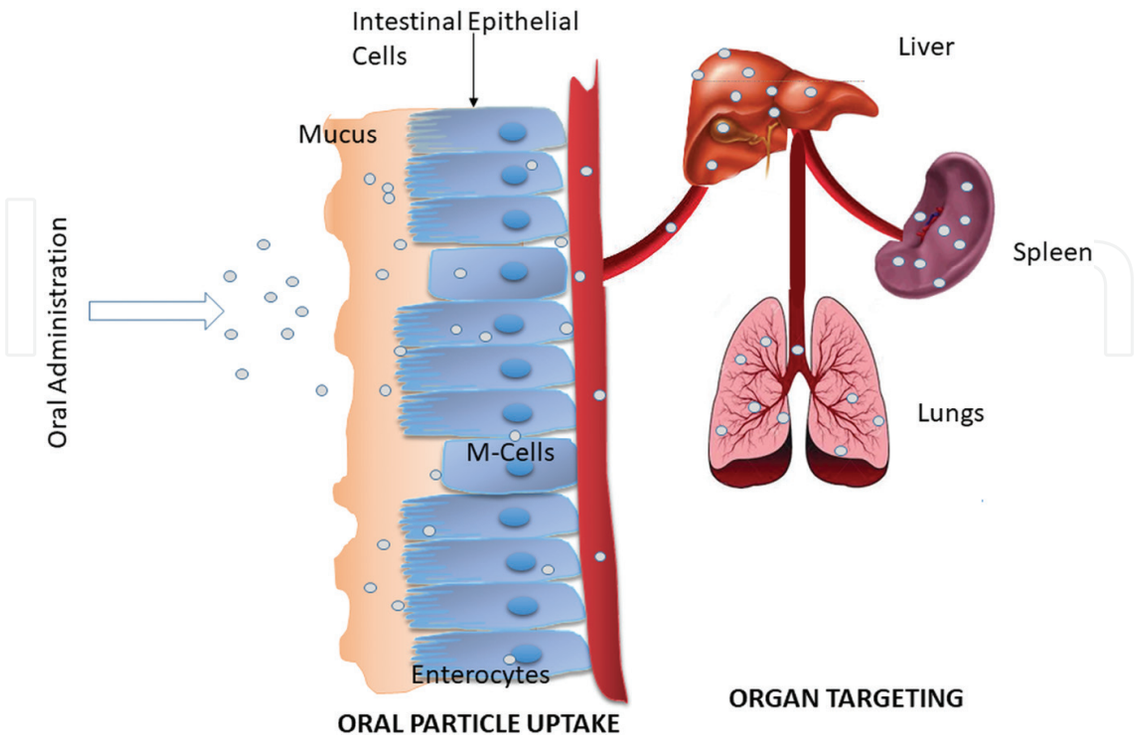


Figure 3. Uptake of nanoparticles via oral route and accumulation in liver, spleen, and lungs.

interaction and bind with proteins thus better chances to be taken up by the enterocytes. Single-dose oral pharmacokinetic studies in CD-1 mice were carried out by utilizing AmB-GCPQ, Amb-sodium deoxycholate (Amb-d), and AmB in dextrose solution at the dose of 5 mg/kg. The nanoparticulate formulations, AmB-GCPQ, and Amb-d exhibited higher plasma drug levels compared to the AmB in dextrose. These results indicate that the particulate formulations were able to cross the intestinal membrane. Furthermore, significantly higher levels of Amb-GCPQ were found in target organs, i.e., liver and spleen as compared to the Amb-d. The target organ to kidney ratio was also determined and provided very encouraging results. As AmB is a nephrotoxic drug, target organ:kidney ratios are crucial. Lung:kidney AUC0-24 ratios for AmB-GCPQ and Amb-d were 1.44 and 0.86, respectively, while the corresponding spleen:kidney ratios were 1.22 and 0.81, respectively, and the corresponding liver:kidney ratios were 0.88 and 0.40, respectively. These data demonstrate that when compared to the deoxycholate micelles, GCPQ nanoparticles delivered relatively more drug to the target organs (liver, lung, and spleen) rather than kidney. These findings were also supported by the low urinary excretion of Amb-GCPQ, while AmB in dextrose delivered most of the drug to the kidney, a fact that contributes to the nephrotoxicity associated with AmB and reduced drug levels in target organs. Also, the oral particle location to major organs was studied by coherent anti-Stokes Raman spectroscopy. The results located the GCPQ nanocarriers within the hepatocytes in the liver, intracellular spaces in the hepatocytes. The reason for their location in the hepatocytes and lungs is their uptake by the intestinal villi from where they are transported to the liver via the hepatic portal vein. GCPQ nanocarriers were also taken up by the M cells of Peyer's patches from where they are carried to the systemic circulation via the lymphatic system. The *in vivo* efficacy studies were carried out in VL murine model. The data indicated oral GCPQ nanocarriers were equal in efficacy to the parenterally administered AmB [76].

Recently, our research group utilized thiolated polymer-based mannose-anchored nanocarriers to target the visceral organs via oral route for the delivery of AmB against VL (unpublished data). Thiolated polymers, the so-called thiomers, are well known for their mucoadhesion, permeation enhancing, and P-gp inhibition properties with great impact on the nanodrug delivery. Thiomer contains thiol group (—SH) covalently attached to the polymer chain, and by the virtue of –SH, the thiolated polymer can interact with the proteins and receptors via disulfide bond formation (—S—S—) in disulfide exchange mechanism [77]. Mucus in the intestine acts as the physical barrier for the diffusion of drugs across the intestinal membrane. The structure of mucus is complex, which arises from the properties of mucins. Mucins are large glycoproteins composed of more than 800 amino acids, also containing cysteine- and disulfide-rich domains. Mucins have long flexible proline, threonine, and serine (PTS) domains that are glycosylated. The glycans terminate with negatively charged carboxylic groups. Diffusion in the mucus structure depends on the charge of the molecules. Mucus contains pores that are 200–400 nm in diameter, thus allowing diffusion of many APIs [78, 79]. If APIs are encapsulated in nano- or microcarriers, the size of the carrier can preclude diffusion in mucus. Thiomer-based nanocarriers will remain adhered to the mucus by making disulfide bond with the cysteine-rich units of mucin, and by the virtue of small size of nanocarriers, they can easily pass through the pores in the mucus. After crossing the mucus barrier, they are taken up by the enterocytes, M-cells of Peyer's patches and also cross the membrane via paracellular route owing to permeation-enhancing capabilities of thiomers. The primary mechanism of the permeation enhancing by thiomers is the inhibition of PTP. The inhibition of PTP is

accomplished by the disulfide (—S—S) bond formation by thiomer with cysteine-rich units and consequently increased phosphorylation of membrane proteins thus leading to the opening of tight junction [80]. Furthermore, the mannose anchoring to the thiolated polymer enables the nanoparticles to target the macrophages via mannose receptors. Thus, the combined effect of mucoadhesion, permeation enhancing, and macrophage targeting successfully target the pathological organs of VL, i.e., liver, spleen, and lungs via the oral route.

5.3. Photodynamic therapy

The survival of *Leishmania* inside macrophages is dependent upon its unique redox biology that neutralizes the ROS produced by the macrophages [81]. PDT targets the redox biology of *Leishmania* by producing the ROS that supersedes neutralizing capabilities of the parasite. The increased ROS thus exert the antileishmanial effects by jeopardizing the reducing potential [82]. PDT involves the delivery of special drug called photosensitizers (PS) to the infectious tissue with nanocarriers and subsequent exposure to a light of specific wavelength. The photosensitizers absorb the light and then transfer the energy to the molecular oxygen which is converted to the free oxygen [$O^{\bullet}$] or $OH^{\bullet}$. These ROS are responsible for the killing of the cells or tissue where the PS is localized. The photooxidation of biomolecules changes the structure and functions of cells. The generation ROS can be classified into two types of reactions. Type I reactions called electron transfer reactions and are responsible for producing various free radicals including highly reactive $OH^{\bullet}$. The type II reaction involves the energy transfer via molecular oxygen leading to the formation [$O^{\bullet}$] [83]. However, the clinical application of PDT is limited to the easily accessible areas where the direct exposure of LASER or incoherent light can be provided. Consequently, the PDT finds its application in the treatment of CL [84]. The most commonly used photosensitizer against CL is a porphyrin precursor, 5-aminolevulinic acid (ALA). ALA is a substrate for heme synthesis and its exogenous delivery results in the accumulation of protoporphyrin IX (PpIX). When exposed to the light of a suitable wavelength like the red or blue light after some specific intervals, cell death occurs due to the apoptosis and necrosis caused by the activated PpIX generating ROS [85]. Zinc phthalocyanine (ZnPc) is another commonly used PS for the PDT. However, one major concern with applying the PS to the skin is that they cannot penetrate to the deeper layers of skin because of the SC barrier of the skin. Due to which their efficacy at full potential is hampered.

Nanoparticles have been extensively explored to improve the efficacy of PDT against CL, due to the ability to penetrate the skin by crossing SC barrier and also protect the PS from aggregation and subsequent inactivation. The current PDT against the CL involves the indirect destruction of the parasites either by enhancing the immune response or by killing the macrophages. Montanari et al. [86] conducted a study, in which they delivered ZnPc, loaded in liposomes, to treat the infection induced by *L. braziliensis*. They indicated ZnPc alone has 20% activity against the promastigotes and amastigotes; however, when incorporated into the liposomes the antileishmanial activity increased up to 100% for promastigotes and 80% for amastigote. Moreover, the penetration studies indicated liposomal ZnPc showed eightfold increased penetration and sevenfold increased accumulation of ZnPc into the deeper layers of skin as compared to the ZnPc alone. Their study provided the proof that targeted PDT with nanocarriers greatly enhances the penetration and accumulation of PS into deeper layers of skin thus enhanced antileishmanial efficacy [86].

Metallic nanoparticles have found their application in the PDT due to their surface localized plasmon response, and they enhance the effectiveness of PDT by producing ROS. Also, they are not involved in the immune system activation. Several studies have been reported in which the effectiveness of the metal nanoparticles in the PDT has been established. PEGylated silver-doped zinc oxide nanoparticles (DSNs) for the PDT of leishmaniasis have been reported by the Nadhman et al. [87]. They indicated DSNs were highly efficacious in providing the photodynamic effect than nondoped zinc oxide nanocarriers (NDSNs). Doping of zinc oxide with silver enhanced the band gap and thus excitation at the visible light source. The IC_{50} of DSNs was in the range of 0.009 (±0.0012) to 0.02 μg/ml (±0.0023), while that of NDSN was 0.1 μg/ml (±0.016). The DSNs were 10 times more active than the NDSN. Free radical scavenger studies indicated 77–83% cell death occurs due to singlet oxygen, while 18–27% due to the production of hydroxyl ions [87].

5.4. Skin-permeating nanocarriers

The role of the skin as barriers to the drug delivery has been discussed above in detail. The nanomedicine is a promising strategy to cross the skin barrier since they offer several advantages over the conventional drug delivery systems, and skin permeation and follicular targeting are the most significant regarding the topical treatment of CL. The nanoparticles larger than 20 nm and lesser than 200 nm can be accumulated in the hair follicles where they are retained for longer period of time for up to 10 days, thus providing the continuous supply of the drug for the absorption [88, 89]. Various types of nanocarriers have been utilized for this purpose but the lipid-based nanocarriers such as liposomes, solid lipid nanoparticles, and nanoemulsions are most extensively studied for skin permeation.

Ferriera et al. [90] first time reported the encapsulation of PA into liposomes and evaluated their permeation across the stripped and intact mouse skin. The results exhibited significantly increased PA penetration into and across the intact skin compared to the PA in solution. However, this model was based on the hairless skin and cannot be extrapolated for human due to the presence of hairs. Topical treatment of *L. major* infected BALB/c mice resulted in a decrease in lesion size in animals treated with PA-loaded liposomes and free PA gel. However, local relapse, characterized by the reappearance of ulcers, occurred faster in animals treated with free PA than in those treated with liposomes. These findings suggest that liposomes represent a promising alternative for the topical treatment of CL using PA [90]. Jaafari et al. [42] reported the efficacy of liposomes loaded with PA at 10 and 15%. Both types of liposomal formulations indicated high retention and permeation profile of PA in the mouse skin. These formulations exhibited 3–4 times better efficacy against *L. major* amastigotes compared to simple PA solution. Significant reduction in the lesion size and parasitic burden in liver and spleen was observed in *L. major*-infected BALB/c mice with topical PA liposomal formulations compared to the control mice. However, in this study, comparison of topical treatment with free PA was not reported [42].

Frankenburg et al. [93] evaluated the effectiveness of AmB-based lipid nanoformulations applied topically to *L. major* experimentally infected mice. The three evaluated formulations (Amphocil, Fungizone, and Abelcet) were ineffective when applied topically, except when Amphocil and Abelcet were dispersed in 5% ethanol. No relapse was observed during the follow-up period after treatment [91]. Subsequently, Amphocil dispersed in 5% ethanol was tested in *L. major*-infected patients in a prospective placebo-controlled study. The results

provided the significant reduction in the lesion size against the placebo-treated lesions. This treatment exhibited complete healing lesions with no evidence of relapse on follow-up visits [92]. This modality was also used for the topical treatment of an infant patient who had not responded to the topical application of a PA ointment, resulting in resolution of the skin lesions and absence of local or systemic side effects [93].

6. Conclusion

The conventional therapy of leishmaniasis failed to provide the satisfactory control over the progression of disease due to the involvement of certain biological barriers. *Leishmania* parasite resides inside macrophages providing a barrier of macrophage cell membrane permeability for the drugs. The activity of P-gp efflux pumps directly related to the decreased intracellular accumulation of antimonial compounds. The physiochemical properties of antileishmanial drugs limit their oral bioavailability making it necessary to deliver drug via IV route. SC provides another barrier to delivery of drugs in CL. The biological barriers encountered by the chemotherapeutic agents leads to the development of resistance, lack of effectiveness and toxicity, thus are the factors jeopardizing the full therapeutic potential of antileishmanial drugs. These biological barriers cannot be tackled with the conventional drug delivery systems, and lack of therapeutic choices necessitates the development of new drug delivery system with the better therapeutic profile. In this area, nanotechnology is a great hope that provided real breakthrough over conventional formulations. The nanotechnology-based pharmaceutical formulations can easily navigate through the biological barriers and enhance the therapeutic effectiveness of antileishmanial drugs. Various types of nanocarriers, like liposomes, niosomes, polymeric nanocarriers, and metal oxide nanoparticles provided very encouraging results regarding leishmaniasis therapy. One of the very promising aspects of nanotechnology is the targeted delivery of nanocarriers to a very specific organ of pathology and avoiding the healthy tissues. In this regard, the targeting approaches like receptor-mediated macrophage targeting, organ targeting via oral route, photodynamic therapy provided a platform for successful therapy of the disease. Various nanotechnology-based formulations of antileishmanial drugs are in different phases of clinical trial. However, a lot of efforts from the scientific community are required to further investigate the targeted delivery of antileishmanial agents to translate the nanomedicinal concepts into first-line gold standard therapy.

Author details

Gul Shahnaz[1], Hafiz Shoaib Sarwar[1] and Masoom Yasinzai[2]*

*Address all correspondence to: rector@iiu.edu.pk

1 Department of Pharmacy, Faculty of Biological Sciences, Quad-I-Azam University, Islamabad, Pakistan

2 Centre for Interdisciplinary Research in Basic Sciences, International Islamic University, Islamabad, Pakistan

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