Biochemical and Biophysical Research Communications 399 (2010) 429–433

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Amphotericin B inhibits entry of *Leishmania donovani* into primary macrophages

Yamuna Devi Paila [a], Bhaskar Saha [b], Amitabha Chattopadhyay [a,*]

[a] Centre for Cellular and Molecular Biology, Council of Scientific and Industrial Research, Uppal Road, Hyderabad 500007, India
[b] National Centre for Cell Science, Ganeshkhind, Pune 411007, India

ARTICLE INFO

Article history:
Received 19 July 2010
Available online 3 August 2010

Keywords:
Amphotericin B
Cholesterol
Leishmania donovani
Lipid–receptor interactions
Primary macrophages

ABSTRACT

Visceral leishmaniasis is a vector-borne disease caused by an obligate intra-macrophage protozoan parasite *Leishmania donovani*. The molecular mechanisms involved in internalization of *Leishmania* are still poorly understood. Amphotericin B and its formulations are considered as the best existing drugs against visceral leishmaniasis and are being increasingly used. The reason for its antileishmanial activity is believed to be its ability to bind ergosterol found in parasite membranes. In case of *in vivo* amphotericin B treatment, both host macrophages and parasites are exposed to amphotericin B. The effect of amphotericin B treatment could therefore be due to a combination of its interaction with both sterols *i.e.*, ergosterol of *Leishmania* and cholesterol of host macrophages. We report here that cholesterol complexation by amphotericin B markedly inhibits binding of *L. donovani* promastigotes to macrophages. These results represent one of the first reports on the effect of amphotericin B on the binding of *Leishmania* parasites to host macrophages. Importantly, these results offer the possibility of reevaluating the mechanism behind the effectiveness of current therapeutic strategies that employ sterol-complexing agents such as amphotericin B to treat leishmaniasis.

© 2010 Elsevier Inc. All rights reserved.

1. Introduction

Leishmania are protozoan parasites that are responsible for substantial public health problems, especially in tropical and subtropical regions. Visceral leishmaniasis (VL) is a vector-borne disease caused by obligate intra-macrophage protozoan parasites such as *Leishmania donovani*. VL is a systemic disease that is fatal in the absence of treatment [1]. It is estimated that 88 countries are leishmaniasis-endemic. There are 500,000 new cases of VL and more than 50,000 deaths from the disease every year [2]. The emergence of VL as an important opportunistic infection among people with human immunodeficiency virus-1 (HIV-1) infection [3] has created an urgency to provide treatment for this disease. The lifecycle of *L. donavani* has two distinct forms: an extracellular promastigote flagellar form found in the mid-gut of sandflies and an intracellular amastigote form that resides in phagolysosomes of mammalian (host) macrophages. Only female sandflies transmit the disease during a blood meal through flagellar promastigotes, and parasites are internalized by dendritic cells and macrophages which subsequently transform into amastigotes by losing their flagella [1]. Entry of promastigotes into host macrophages involves multiple parasite–host interactions such as recognition of specific ligands on the parasite cell surface by receptors on the macrophage cell surface. A number of studies toward understanding the molecular mechanisms of parasite entry have led to the identification of several candidate receptors facilitating multiple routes of entry thereby highlighting the redundancy in the entry process [4,5]. These include membrane proteins present on the macrophage cell surface such as the mannose–fucose receptor, receptor for advanced glycosylation end products, the fibronectin receptor, the Fc receptor and complement receptors such as CR1 and CR3. The large number of different receptors responsible for the entry of the parasite into host macrophages makes it difficult to establish a unique therapeutic target for the treatment of leishmaniasis.

The entry of *L. donovani* into host cells involves interaction with the plasma membrane of host cells. Cholesterol is an essential component of higher eukaryotic cellular membranes and is crucial in membrane organization, dynamics, function, and sorting [6]. Cholesterol is often found distributed nonrandomly in domains in biological and model membranes [7,8]. Many of these domains (sometimes termed as 'lipid rafts') are believed to be important for the maintenance of membrane structure and function. The idea of such specialized membrane domains assumes significance since the entry of pathogens [9–11] has been attributed to these domains. Importantly, cholesterol plays a vital role in the function and organization of membrane proteins and receptors [12–14].

Abbreviations: AmB, amphotericin B; FITC, fluorescein isothiocyanate; GPCR, G-protein coupled receptor; TLC, thin layer chromatography; VL, visceral leishmaniasis.

* Corresponding author. Fax: +91 40 2716 0311.
E-mail address: amit@ccmb.res.in (A. Chattopadhyay).

0006-291X/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2010.07.099

Previous work by us [15,16] and others [17] has shown the requirement of host membrane cholesterol in the binding and internalization of *Leishmania* promastigotes into macrophage cells. Amphotericin B (AmB) and its formulations are increasingly being used and are considered as the best existing drugs against VL and have a 97% cure rate with no reported resistance [1,18,19]. AmB is a polyene antibiotic and it is believed to be leishmanicidal due to its capability to bind ergosterol which is a major sterol in *Leishmania*. The interaction of AmB with ergosterol leads to the formation of transmembrane AmB channels which induce altered permeability to cations, water, glucose and affect membrane-bound enzymes [20]. Interestingly, AmB also binds cholesterol with comparable affinity [21,22]. In case of *in vivo* AmB treatment, both host and parasite membranes are exposed to AmB. The effect of AmB treatment could therefore be due to a combination of its interaction with both sterols *i.e.*, ergosterol of *Leishmania* and cholesterol of host macrophages. We propose here that AmB interacts with host membrane cholesterol to sequester it in the membrane, thereby effectively reducing the ability of cholesterol to interact with and exert its effects on other membrane components such as receptors responsible for leishmanial entry. To test this hypothesis, we treated primary macrophages with increasing concentrations of AmB followed by leishmanial infection. Our results show that sequestration of cholesterol in the host macrophage membrane inhibits leishmanial infection. Although AmB has earlier been used on isolated leishmanial parasites [19,23,24], this is the first report describing the effect of AmB treatment of host macrophages on leishmanial infection.

2. Materials and methods

2.1. Materials

M-199 medium, penicillin, streptomycin, amphotericin B and FITC were obtained from Sigma Chemical Co. (St. Louis, MO, USA). RPMI-1640 medium and fetal calf serum was obtained from Gibco/BRL Life Technologies (Grand Island, NY). Radiolabeled [^{3}H] thymidine was obtained from Dupont New England Nuclear (Boston, MA, USA).

2.2. Isolation of mouse peritoneal macrophages

Two month old female BALB/c mice were given 2 ml i.p. injections of thioglycollate medium. Three days postinjection, macrophages were obtained by peritoneal lavage. Cells were washed with sterile Hepes–Hanks-buffered-saline (HBS), resuspended in M-199 supplemented with 15% fetal calf serum and incubated in six-well tissue culture plates for 4 h at 37 °C. After incubation, the nonadherent cells were removed by washing the wells with pre-warmed M-199. Fresh medium was added to the adherent cells, and incubation was continued for 18 h. Primary macrophages were maintained at 37 °C in RPMI-1640 medium supplemented with penicillin–streptomycin (1000 units/ml) and 10% heat-inactivated fetal calf serum.

2.3. Parasite culture

L. donovani strain AG83 (MHOM/IN/1983/AG83) parasites were maintained as promastigotes at 22 °C in modified M-199 medium supplemented with penicillin–streptomycin (1000 units/ml) and 15% heat-inactivated fetal calf serum as described previously [25,26].

2.4. Complexation and estimation of cholesterol

Cholesterol complexation was carried out by incubating primary macrophages with AmB in serum-free RPMI-1640 medium at 37 °C for 30 min followed by wash with PBS before being exposed to parasites. Total lipids were extracted from control and AmB-treated macrophages and separated using thin layer chromatography (TLC) as described previously [27]. Cholesterol was quantitated using densitometric analysis.

2.5. Radiolabeling *L. donovani* promastigotes with tritium or FITC for binding studies

Parasites were metabolically radiolabeled with tritium as described previously [28] with some modifications. Radiolabel incorporation was carried out at a density of 1×10^7 parasites in 2 ml of M-199 medium in the presence of 20 μCi/ml [^{3}H] thymidine (sp. activity = 6.5 Ci/mmol) at 22 °C for 3 h. FITC-labeling of parasites was carried out as described previously [29], except that labeling was carried out at 37 °C in PBS.

2.6. Infectivity assays

Thioglycolate-elicited peritoneal macrophages were plated at a density of 1×10^5 in 35 mm culture dishes and grown for 48 h for radiolabeled or FITC-labeled parasite binding experiments. For Giemsa staining experiments, macrophages were plated at a density of 5×10^5 in 10 mm wells and grown for 24 h. Promastigotes were added onto macrophage monolayers at a parasite to macrophage ratio of 10:1 in RPMI-1640 medium at 37 °C for 90 min. At the end of incubation, monolayers were washed twice with PBS to remove free parasites, solubilized with 1% Triton X-100, and assayed for radioactivity using a Packard Tri-Carb liquid scintillation counter. In flow cytometric experiments, FITC-labeled parasites were used to infect macrophages at 37 °C for 90 min. After infec-

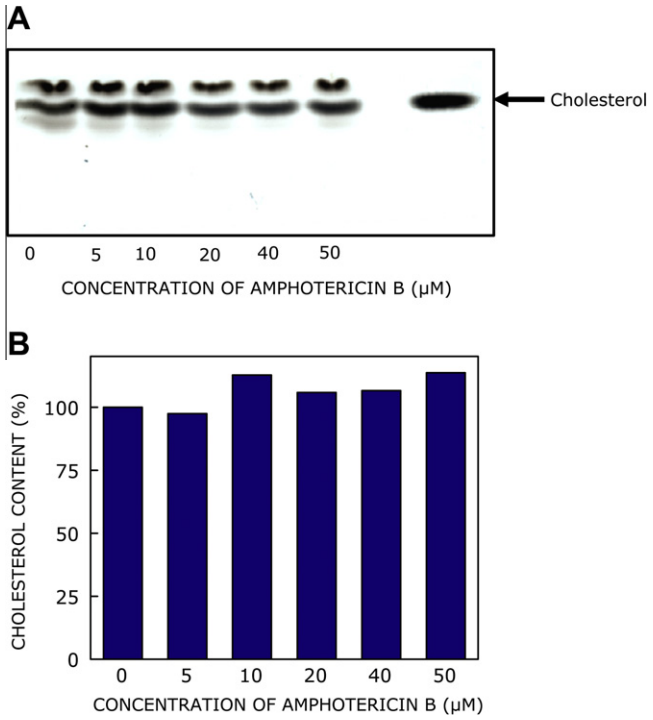

Fig. 1. Effect of amphotericin B (AmB) on host macrophage cholesterol. Total lipids extracted from AmB-treated primary macrophages were separated by thin layer chromatography as shown in (A). The lanes represent lipids extracted from control macrophages (lane 1), and macrophages treated with 5 (lane 2), 10 (lane 3), 20 (lane 4), 40 (lane 5) and 50 (lane 6) μM AmB. The arrow represents position of cholesterol on the thin layer chromatogram identified using a standard in lane 7. Total cholesterol content in the lipid extract was determined by densitometric analysis of the thin layer chromatogram (panel B). Values are expressed as percentages of the cholesterol content in control (without AmB treatment) macrophages. See Section 2 for other details.

tion, macrophages were gently scraped into PBS with 0.1% formaldehyde at 4 °C. The fluorescence from FITC-labeled parasites associated with 5000–10,000 macrophages was analyzed with a CyAn-ADP flow cytometer using SUMMIT software for data analysis. Macrophages treated with increasing concentrations of AmB were exposed to parasites, followed by Giemsa staining. The number of promastigotes in macrophages was visually scored using a Leica microscope with a 100× oil-immersion objective [25,26].

3. Results and discussion

We explored the effect of treatment of thioglycolate-elicited peritoneal host macrophages with AmB on the binding of *Leishmania donavani* promastigotes. As mentioned earlier, AmB interacts with cholesterol to sequester it in the membrane [21,22,30], thereby effectively reducing the ability of cholesterol to interact with other membrane constituents such as receptors. In our experiments, primary macrophages were incubated with increasing concentrations of AmB, followed by a wash to remove any unbound AmB before being exposed to *Leishmania* parasites. We estimated cholesterol content in host macrophages following treatment with AmB. Fig. 1A shows a thin layer chromatogram of lipids extracted from macrophages treated with increasing concentrations of AmB. Quantitative densitometric analysis of cholesterol content on the TLC plate (Fig. 1B) indicates that the treatment with AmB does not physically deplete cholesterol. In other words, cholesterol content of host macrophages remains invariant upon AmB treatment. In order to analyze the effect of AmB treatment on the ability of *Leishmania* to infect primary host macrophages, binding assays with [^{3}H] thymidine-labeled parasites were carried out. As shown in Fig. 2A, treatment of macrophages with increasing concentrations of AmB progressively reduces the binding of parasites to macrophages. For example, a concentration of 50 μM of AmB gives rise to ~60% reduction in macrophage–parasite interaction. In addition, we monitored the binding of *Leishmania donavani* promastigotes to primary macrophages treated with AmB using Giemsa staining. As shown in Fig. 2B, treatment of macrophages with AmB resulted in a concentration-dependent reduction in the number of promastigotes (compared to control cells) with a ~70% reduction when macrophages treated with 50 μM of AmB were used as host.

These results, using radiolabeled parasite binding and Giemsa staining (Fig. 2A and B), were further confirmed by flow cytometric analysis of binding of FITC-labeled promastigotes to macrophages. Fluorescent derivatization of promastigotes with FITC has previously been used as a convenient tool to accurately monitor host–parasite interaction. This method provides a suitable approach for monitoring cell surface interaction phenomena since each cell is analyzed individually for its ability to bind a fluorescent ligand that in this case is the FITC-labeled promastigote [29]. Fig. 2C shows a reduction in fluorescence associated with AmB-treated macrophages as compared to control macrophages when infection is allowed to progress for a period of 90 min. These data support

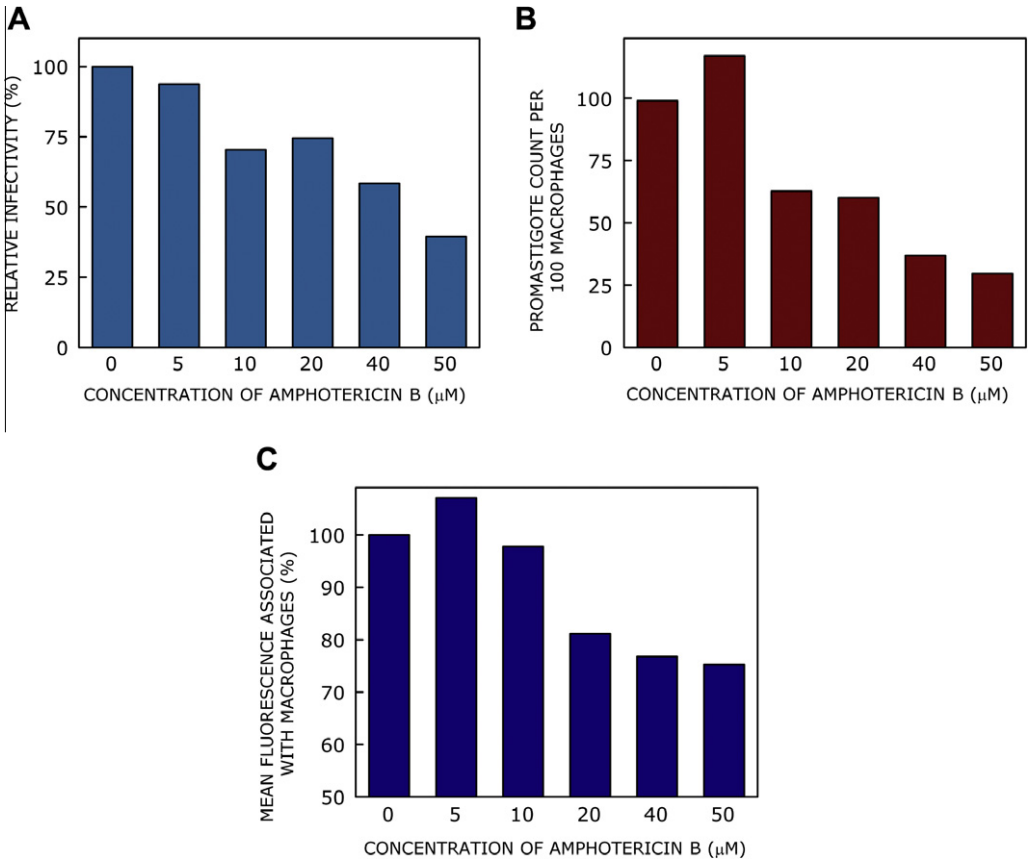

Fig. 2. Effect of cholesterol complexation by amphotericin B (AmB) on the extent of binding of *Leishmania* promastigotes to host primary macrophages. (A) The extent of binding of radiolabeled promastigotes to macrophages with increasing concentrations of AmB is shown. Macrophages were exposed to radiolabeled parasites for 90 min at a parasite to macrophage ratio of 10:1. All values are normalized with respect to the mean counts per minute obtained for control cells. (B) Effect of cholesterol complexation on binding of the parasite assessed by the promastigote count on infected primary macrophages. Macrophages treated with increasing concentrations of AmB were exposed to parasites at multiplicity of infection of 10:1 for 90 min, show a reduction in the number of promastigotes as revealed by Giemsa staining. (C) Flow cytometric analysis of the effect of cholesterol complexation by AmB on the binding of FITC-labeled *Leishmania* promastigotes to host macrophages. Control and AmB-treated primary macrophages were exposed to FITC-labeled promastigotes at a parasite to macrophage ratio of 10:1. Macrophages were treated with increasing concentrations of AmB prior to leishmanial infection. See Section 2 for other details.

our earlier conclusion (Fig. 2A and B) of a reduction in the ability of *Leishmania* promastigotes to interact with the host upon complexation of host membrane cholesterol.

This report represents one of the first studies on the effect of agents that perturb membrane cholesterol on the binding of *Leishmania* parasites to host macrophages. As mentioned earlier, although AmB has previously been reported to possess leishmanicidal property [19,23,24], this is the first report that describes the effect of treatment of host macrophages with AmB on leishmanial infectivity. It was previously shown that physical depletion of cholesterol from macrophages leads to inhibition in binding of *Leishmania* promastigotes into macrophage cells [15,17]. Our present results with the sterol-binding agent AmB suggest that mere sequestration of host plasma membrane cholesterol can inhibit leishmanial infection. Sequestration of membrane cholesterol with AmB could lead to a reduction in the availability of free cholesterol in the host plasma membrane that is essential for parasite entry. Taken together, these results reinforce the crucial requirement of membrane cholesterol in host cells for leishmanial infection.

The molecular mechanism of how cholesterol supports binding of the parasite and its subsequent entry into host macrophages continues to be a key issue. The involvement of multiple membrane-bound receptors in the entry of the parasite into host cells has been mentioned earlier [4,5]. The modulatory role of cholesterol, an essential and representative lipid in the plasma membrane of higher eukaryotic cells, on the signaling of membrane receptors, such as G-protein coupled receptors (GPCRs), has been previously demonstrated [12–14,31]. These results show that perturbing the cholesterol content and/or availability in the membrane may lead to perturbation of receptor–cholesterol interactions leading to loss of receptor function. Interestingly, GPCR signaling has previously been implicated in malarial infection [32].

Membrane cholesterol is believed to influence organization of lipids and proteins in the cell membrane [6–8]. Cholesterol exerts many of its actions by maintaining putative membrane domains in a functional state. Current understanding of the organization of biological membranes therefore involves the concept of lateral heterogeneities in the membrane, collectively termed as membrane domains. Many of these domains (sometimes termed as 'lipid rafts') are thought to be important for the maintenance of membrane structure and function, although characterizing the spatiotemporal resolution of these domains has proven to be challenging [8,33–35]. These specialized regions are believed to be enriched in specific lipids and proteins, and facilitate processes such as trafficking, sorting, and signal transduction. An interesting aspect of these regions (domains) is their ability to aid the entry of pathogens [10,36]. As a result, pathogen entry into cells is believed to depend on the integrity of such regions in the membrane. For example, the entry of *Mycobacteria* [37], *Brucella suis* [38], and *Listeria monocytogenes* [39] has earlier been shown to be dependent on membrane cholesterol. It is possible that the reduction in leishmanial infection upon AmB treatment is due to alterations in the function and membrane organization of one or more of the many receptors on the host cell surface that participate in parasite entry.

On a broader perspective, our results offer the possibility of reevaluating the mechanism behind the effectiveness of current therapeutic strategies to treat leishmaniasis. AmB is the most effective clinically prescribed therapeutic drug to treat VL [1,18,19,40,41]. Although the development of AmB as a therapy against leishmaniasis has its origin in the discovery that it is a potent leishmanicidal agent [42,43], it is possible that its effectiveness *in vivo* is partly based on its ability to sequester cholesterol in the host membrane thereby abrogating macrophage–parasite interaction.

Acknowledgments

This work was supported by the Council of Scientific and Industrial Research, Government of India. A.C. gratefully acknowledges support from J.C. Bose Fellowship (Department of Science and Technology, Govt. of India). Y.D.P. was the recipient of a postdoctoral fellowship from a CSIR Network project on Nanomaterials and Nanodevices (NWP0035). A.C. is an Adjunct Professor at the Special Centre for Molecular Medicine of Jawaharlal Nehru University (New Delhi, India) and Indian Institute of Science Education and Research (Mohali, India), and Honorary Professor of the Jawaharlal Nehru Centre for Advanced Scientific Research (Bangalore, India). We thank members of A.C.'s research group for critically reading the manuscript.

References

[1] F. Chappuis, S. Sundar, A. Hailu, H. Ghalib, S. Rijal, R.W. Peeling, J. Alvar, M. Boelaert, Visceral leishmaniasis: what are the needs for diagnosis, treatment and control? Nat. Rev. Microbiol. 5 (2007) 873–882.
[2] P. Desjeux, Leishmaniasis: current situation and new perspectives, Comp. Immunol. Microbiol. Infect. Dis. 27 (2004) 305–318.
[3] D. Wolday, N. Berhe, H. Akuffo, S. Britton, *Leishmania*–HIV interaction: immunopathogenic mechanisms, Parasitol. Today 15 (1999) 182–187.
[4] J. Alexander, A.R. Satoskar, D.G. Russell, *Leishmania* species: models of intracellular parasitism, J. Cell Sci. 112 (1999) 2993–3002.
[5] M.G. Rittig, C. Bogdan, *Leishmania*–host-cell interaction: complexities and alternative views, Parasitol. Today 16 (2000) 292–297.
[6] O.G. Mouritsen, M.J. Zuckermann, What's so special about cholesterol?, Lipids 39 (2004) 1101–1113
[7] K. Simons, E. Ikonen, How cells handle cholesterol, Science 290 (2000) 1721–1726.
[8] S. Mukherjee, F.R. Maxfield, Membrane domains, Annu. Rev. Cell Dev. Biol. 20 (2004) 839–866.
[9] K. Simons, R. Ehehalt, Cholesterol, lipid rafts, and disease, J. Clin. Invest. 110 (2002) 597–603.
[10] J. Riethmüller, A. Riehle, H. Grassmé, E. Gulbins, Membrane rafts in host–pathogen interactions, Biochim. Biophys. Acta 1758 (2006) 2139–2147.
[11] T.J. Pucadyil, A. Chattopadhyay, Cholesterol: a potential therapeutic target in *Leishmania* infection? Trends Parasitol. 23 (2007) 49–53.
[12] K. Burger, G. Gimpl, F. Fahrenholz, Regulation of receptor function by cholesterol, Cell. Mol. Life Sci. 57 (2000) 1577–1592.
[13] T.J. Pucadyil, A. Chattopadhyay, Role of cholesterol in the function and organization of G-protein coupled receptors, Prog. Lipid Res. 45 (2006) 295–333.
[14] Y.D. Paila, A. Chattopadhyay, Membrane cholesterol in the function and organization of G-protein coupled receptors, Subcell. Biochem. 51 (2010) 439–466.
[15] T.J. Pucadyil, P. Tewary, R. Madhubala, A. Chattopadhyay, Cholesterol is required for *Leishmania donovani* infection: implications in leishmaniasis, Mol. Biochem. Parasitol. 133 (2004) 145–152.
[16] P. Tewary, K. Veena, T.J. Pucadyil, A. Chattopadhyay, R. Madhubala, The sterol-binding antibiotic nystatin inhibits entry of non-opsonized *Leishmania donovani* into macrophages, Biochem. Biophys. Res. Commun. 339 (2006) 661–666.
[17] N.E. Rodríguez, U. Gaur, M.E. Wilson, Role of caveolae in *Leishmania chagasi* phagocytosis and intracellular survival in macrophages, Cell. Microbiol. 8 (2006) 1106–1120.
[18] K.M. Wasan, E.K. Wasan, P. Gershkovich, X. Zhu, R.R. Tidwell, K.A. Werbovetz, J.G. Clement, S.J. Thornton, Highly effective oral amphotericin B formulation against murine visceral leishmaniasis, J. Infect. Dis. 200 (2009) 357–360.
[19] B.E. Cohen, H. Ramos, M. Gamargo, J. Urbina, The water and ionic permeability induced by polyene antibiotics across plasma membrane vesicles from *Leishmania* sp., Biochim. Biophys. Acta 860 (1986) 57–65.
[20] S. Hartsel, J. Bolard, Amphotericin B: new life for an old drug, Trends Pharmacol. Sci. 17 (1996) 445–449.
[21] J.D. Readio, R. Bittman, Equilibrium binding of amphotericin B and its methyl ester and borate complex to sterols, Biochim. Biophys. Acta 685 (1982) 219–224.
[22] R. Mouri, K. Konoki, N. Matsumori, T. Oishi, M. Murata, Complex formation of amphotericin B in sterol-containing membranes as evidenced by surface plasmon resonance, Biochemistry 47 (2008) 7807–7815.
[23] H. Ramos, E. Valdivieso, M. Gamargo, F. Dagger, B.E. Cohen, Amphotericin B kills unicellular leishmanias by forming aqueous pores permeable to small cations and anions, J. Membr. Biol. 152 (1996) 65–75.
[24] M. Vermeersch, R.I. da Luz, K. Toté, J.-P. Timmermans, P. Cos, L. Maes, In vitro susceptibilities of *Leishmania donovani* promastigote and amastigote stages to antileishmanial reference drugs: practical relevance of stage-specific differences, Antimicrob. Agents Chemother. 53 (2009) 3855–3859.
[25] B. Saha, H. Nanda-Roy, A. Pakrashi, R.N. Chakrabarti, S. Roy, Immunobiological studies on experimental visceral leishmaniasis. I.

Changes in lymphoid organs and their possible role in pathogenesis, Eur. J. Immunol. 21 (1991) 577–581.
[26] P. Wadhone, M. Maiti, R. Agarwal, V. Kamat, S. Martin, B. Saha, Miltefosine promotes IFN-gamma-dominated anti-leishmanial immune response, J. Immunol. 182 (2009) 7146–7154.
[27] Y.D. Paila, T.J. Pucadyil, A. Chattopadhyay, The cholesterol-complexing agent digitonin modulates ligand binding of the bovine hippocampal serotonin 1A receptor, Mol. Membr. Biol. 22 (2005) 241–249.
[28] D.M. Mosser, P.J. Edelson, The mouse macrophage receptor for C3bi (CR3) is a major mechanism in the phagocytosis of *Leishmania* promastigotes, J. Immunol. 135 (1985) 2785–2789.
[29] B.A. Butcher, L.A. Sklar, L.C. Seamer, R.H. Glew, Heparin enhances the interaction of infective *Leishmania donovani* promastigotes with mouse peritoneal macrophages. A fluorescence flow cytometric analysis, J. Immunol. 148 (1992) 2879–2886.
[30] J. Bolard, How do the polyene macrolide antibiotics affect the cellular membrane properties?, Biochim Biophys. Acta 864 (1986) 257–304.
[31] G. Gimpl, V. Wiegand, K. Burger, F. Fahrenholz, Cholesterol and steroid hormones: modulators of oxytocin receptor function, Prog. Brain Res. 139 (2002) 43–55.
[32] T. Harrison, B.U. Samuel, T. Akompong, H. Hamm, N. Mohandas, J.W. Lomasney, K. Haldar, Erythrocyte G protein-coupled receptor signaling in malarial infection, Science 301 (2003) 1734–1736.
[33] M. Edidin, Shrinking patches and slippery rafts: scales of domains in the plasma membrane, Trends Cell Biol. 11 (2001) 492–496.
[34] S. Munro, Lipid rafts: elusive or illusive?, Cell 115 (2003) 377–388
[35] K. Jacobson, O.G. Mouritsen, R.G.W. Anderson, Lipid rafts: at a crossroad between cell biology and physics, Nat. Cell Biol. 9 (2007) 7–14.
[36] F.G. van der Goot, T. Harder, Raft membrane domains: from a liquid-ordered membrane phase to a site of pathogen attack, Semin. Immunol. 13 (2001) 89–97.
[37] J. Gatfield, J. Pieters, Essential role for cholesterol in entry of *Mycobacteria* into macrophages, Science 288 (2000) 1647–1650.
[38] A. Naroeni, F. Porte, Role of cholesterol and the ganglioside GM1 in entry and short-term survival of *Brucella suis* in murine macrophages, Infect. Immun. 70 (2002) 1640–1644.
[39] S. Seveau, H. Bierne, S. Giroux, M.C. Prévost, P. Cossart, Role of lipid rafts in E-cadherin- and HGF-R/Met-mediated entry of *Listeria monocytogenes* into host cells, J. Cell Biol. 166 (2004) 743–753.
[40] J. Berman, Current treatment approaches to leishmaniasis, Curr. Opin. Infect. Dis. 16 (2003) 397–401.
[41] H.W. Murray, Treatment of visceral leishmaniasis in 2004, Am. J. Trop. Med. Hyg. 71 (2004) 787–794.
[42] A.K. Saha, T. Mukherjee, A. Bhaduri, Mechanism of action of amphotericin B on *Leishmania donovani* promastigotes, Mol. Biochem. Parasitol. 19 (1986) 195–200.
[43] J.D. Berman, W.L. Hanson, W.L. Chapman, C.R. Alving, G. Lopez-Berestein, Antileishmanial activity of liposome-encapsulated amphotericin B in hamsters and monkeys, Antimicrob. Agents Chemother. 30 (1986) 847–851.