Current Status of Existing and Emerging Chemotherapy and Drug Resistance Mechanisms in Leishmania

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ABSTRACT

Leishmaniasis is a well known fatal disease that is caused by the protozoan species belonging to the genus *Leishmania*. The causative organism is transmitted through female sandflies. It is considered as a neglected tropical disease and targeted for the worldwide elimination by the World Health Organization. It is the major cause of significant morbidity and mortality in several countries of the world. Leishmania parasites cause a wide spectrum of human and animal infections ranging from the life threatening visceral disease to the disfiguring mucosal and cutaneous forms of the disease. Currently, the control of the disease totally relies on chemotherapy, as the vaccine is still under the process of development. Organic pentavalentantimonials [Sb (V)] have been the first-line drugs for the treatment of Leishmaniasis for the last seven decades. Alternatively, Amphotericin B, pentamidine and miltefosine can be used for the treatment of leishmaniasis. However, these drugs have serious limitations, such as high cost, toxicity and resistance has emerged as a major problem.Therefore, the development of new, effective antileishmanial drugs is an urgent need. The new drugs are required in an affordable price in order to control leishmaniasis worldwide. The aim of this article is to review the status of existing and emerging chemotherapy for the prevention and treatment of leishmaniasis and also focuses on the various mechanisms which may lead to antimony resistance in leishmaniasis.

Keyword: - *Leishmaniasis, Drug resistance, Antileishmanial drugs*

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1. Introduction

Leishmaniasis is caused by different species of protozon parasite and belonging to the order kinetoplastida [1, 2]. The *Leishmania* parasite is transmitted by an invertebrate sandfly vector, phlebotomus [3]. These organisms have a digenetic life cycle which includes extracellular, flagellated promastigote satage (molile form) that reside in the gut of the sand fly vector and obligated intracellular amastigote stage (non-motile form), reside and multiply within the phagolysosome of reticulo endothelial cells of mammalian macrophage [4]. Leishmania cause a wide spectrum of diseases (visceral (known as kalaazar), cutaneous and mucosal) in humans. The disease is prevalent in 98 countries with approximately 400 000 new cases per year [5]. 90% cases of Cutaneous Leishmaniasis occur in Afghanistan, Brazil, Iran, Peru, Saudi Arabia and Syria. 90% of Mucocutaneous Leishmaniasis (ML) occurs in Bolivia, Brazil and Peru. Visceral Leishmaniasis (VL) has been reported from 66 countries and 90% of the VL cases occur in Bangladesh, Brazil, India, Napal and Sudan [6]. The primary treatment against Leishmaniasis includes pentavalent antimonials for more than seven decades. Presently, 78% of the recent clinical isolates from the hyperendemic zone of Bihar State still showed *in vitro* resistance to antimonials [7]. The recommended dose is 15-20 mg SbV/kg of body weight per day for 21-28 days through intramuscular or intravenous rout [8]. Low cost is their main advantage. However several disadvantages have decreased the use of antimonial, such as intramuscular administration, prolonged treatment and occasionally life-threatening adverse effects like cardiac arrhythmias, increased hepatic transaminases, pancreatitis and pneumonitis [9, 10, 7].

Second-line drug, such as amphotericin B shows good efficacy. AmB is a polyene antifungal drug often used intravenously for systemic fungal infections [11, 12]. Therapeutic dose of AmB of 1mg/kg by endovenous alternate day for 30 days [13, 14] but recent study in India showed 96% cure rates with a dose of .75mg/kg/day for 15 days [15]. However, serious adverse reactions have been displayed by the treatment with amphotericin B, Its prolonged administration and the frequent side effects, such as fever and chills, nephrotoxicity and hypokalemia, occasional serious toxicities like myocarditis, which necessitate administration in hospital. Lipid formulations of amphotericin B improved highly the safety profile of this drug [15]. In poor countries even short courses of liposomal formulations are unaffordable and the selection of antileishmanial treatment turns more to a question of cost than of efficacy or toxicity [16, 12]. Paromomycin is an aminoglycoside with antileishmanial activity. This drug was associated with 94.6% cure rates, similar to amphotericin B [15, 17]. Many side effect associated with the paromamycin is the ototoxicity, as well as problems in liver function [18], pain at injection site and skin rashes, local pruritus.

Miltefosine (hexadecylphosphocholine), originally developed as a neoplastic agent, is the first orally administered drug for VL and the latest to enter the market [18]. It can be used for both antimony-responding and non-responding patients. The limited use of miltefosine includes its teratogenic potential and it is contraindicated in pregnancy and women of child bearing age group, not observing contraception [19]. Miltefosine long half-life (approximately 150 hours) may facilitate the emergence of resistance. Preliminary data from a phase IV trail in India involving domiciliary treatment with miltefosine and weekly supervision suggests doubling of the relapse rate [20]. This provides warning that drug resistance may develop quickly. This demands an understanding of the molecular and biochemical mechanisms of clinical resistance, which has become a World Health Organization priority [21] (http://www.who.int/tdr/diseases/-leish/strategy.htm).

1.1 Alternative therapy/Strategy

The combination therapy has found new scope in the treatment of leishmaniasis. Paromomycin+sodium stibogluconate administered for 17 days was associated with higher cure and survival rates compared to sodium stibogluconate monothearapy administered for 30 days for VL [22], Oral allopurinol+endovenous pentostam for VL and miltefosin+amphotericinB+paromomycin for VL. The combination of verapamil+diperoxovanadate with sodium antimony gluconate reversed the *in vitro* antileishmanial resistance among clinical *L. donovani* isolates [23, 24]. Some studies are needed to investigate various other factor, such as the identification of effective well-tolerated and short treatment regimen, logistical aspects and the potential risk of developing resistance considering that compliance in field conditions can be low [25, 26].
Sitamaquine is an orally active 8-aminoquinoline Analogue. Animal studies showed very encouraging results against VL, but clinical trials it did not shows high efficacy after treatment during 28 days [27].

2. New Drugs

Currently, the development of both synthetic and natural drugs have relevant importance in the search of new therapeutic alternatives.

2.1 Antileishmanial Synthetic Compounds

The design of new drugs based in know and validation molecular targets in the parasite. The synthetic molecules can display a high toxicity and only a low of compounds have been evaluated in clinical studies (Table -1).

2.2 Antileishmanial Natural Products

The world health Organization (TDR/WHO) with the drug discovery research program has considered a priority the pharmacological investigation of plants [40]. In recent year these has been an intense search for antileishmanial compounds obtained from natural sources, which has led to the identification of several classes of active plant metabolites [41,42]. Advanced studies have been evaluated potential compounds isolated from natural source, which displayed antileishmanial activity (Table -2).

Table -1 Antileishmanial Synthetic Compounds

S.No.	Synthetic Compound	Antileishmanial activity	Year	Ref.

1	Triazole SCH 56592	Exhibition *in vitro* & *in vivo* activity against *L. amazonensis* and *L. donovani*.	1999	[29]
2	9,9-Dimethylxanthene tricyclics	Caused *in vitro* inhibition of amastigotes of *L. donovani*.	2000	[30]
3	Azasterols	Showed *in vitro* activity against promastigotes of *L. donovani* and axenic amastigotes of *L. amazonensis.*	2003	[31]
4	3-substituted quinolones	Antileishmanial *in vitro* effects against *L. chagasi* promastigotes and amastigotes was observed.	2005	[32]
5	Edelfosine and Ilmofosine	Demonstrated high *in vitro* activity against *L. donovani* promastigotes and amastigotes	2005	[33]
6	Nicotinamide	*in vitro* inhibition of *L. infantum* promastigotes and amastigotes	2005	[34]
7	Perifosine new	Significant *in vitro* activity against promastigote of *L. braziliensis, L. amazonensis, L. major and L. infantum*.	2007	[35]
8	N-acetyl-1-cysteine	Showed *in vivo* activity against *L. amazonesis* in BALB/c mice	2008	[36]
9	3,5-disubstituted isoxazole	*In vitro* activity against *L. donovani* promastigotes and amastigotes	2011	[37]
10	Tellurium compound RF07	Exhibited *in vitro* and *in vivo* activity against *L. chagasi*	2012	[38]
11	2,4-dihydroxyben-zophen-one, 4-hydro-xybenzoph-enone and 4,4´-dihydroxy benzophe-none	In Vivo Evaluation of Leishmanicidal Activity	2017	[39]

Table-2 Antileishmanial Compounds obtained from natural sources

S.No.	Natural source	Antileishmanial activity	Year	Ref.
1	Piper aduncum	Exhibited *in vitro* activity against promastigotes amastigotes of *L. amazonensis*.	1999	[43]
2	Holarrhena floribunda	Exhibited *in vitro* activity against promastigote & amastigotes of *L. donovani*	2000	[44]
3	Peschiera australis	Showed *in vitro* activity against promastigotes amastigotes of *L. amazonensis*	2001	[45]
4	Zanthoxylum chiloperone	Demonstrated *in vivo* activity in BALB/c mice infection with *L. amazonensis*	2002	[46]
5	Maesa Balansae	Caused *in vitro* and *in vivo* activity against *L. Donovani.*	2004	[47]
6	Tanacetum parthenium	Displayed activity against promastigotes & amastigotes of *L. amazonensis*.	2005	[48]
7	Ocimum gratissimum	Showed *in vitro* activity against *L. chagasi*	2006	[49]
8	Porophyllum ruderale	Showed *in vitro* activity against promastigote *L. amazonensis.*	2011	[50]
9	Tridax procumbens	Showed *in vitro* activity against promastigote *L. Mexicana.*	2009	[51]
10	Polyathialongifolia	Show significant activity against promastigotes of *L. chagasi, L. braziliensis, L. amazonensis.*	2010	[52]

11	Drimysbrasiliensis miers	Showed *in vitro* activity against promastigote *L. chagasi, L. braziliensis, L. amazonensis*	2011	[53]
12	Valerianawallichii	Shown activity against *L. donovani* promastigotes & amastigotes of *L. major.*	2011	[54]
13	Pentacaliadesiderabilis	Show significant activity against promastigotes of *L. chagasi, L.braziliensis and L.amazonensis.*	2012	[55]
14	Acanthospermumhispidum	Antileishmanial activity against *L. mexicana*	2011	[56]
15	Lantanaukambensis	Showed significant acxtivity against promastigotes of *L. donovani.*	2012	[57]
16	*Ochrosia elliptica* Labill	Showed *in vitro* activity against promastigotes amastigotes of *L. amazonensis Leishmania donovani*	2016	[58]

3. Mechanism of Antimony Resistance

At present the main focus is to study the possible mechanisms responsible for antimony resistance in Leishmania. The primary mechanism of resistance is the decrease in the active drug concentration within the parasite. The parasite may lower the drug level by a variety of mechanisms including reduction of drug concentration within the parasite, either by decreasing drug uptake or by increasing efflux/sequestration of the drug. Other potential resistance mechanisms include inhibition of drug activation and inactivation of active drug [59, 60, 61].

3.1. Mechanism of action and reduction of the metal

According to this Mechanism, pentavalent antimony (SbV) behaves as a prodrug, which undergoes biological reduction to much more active/toxic trivalent form of antimony (Sb(III) that exhibit antileishmanial activity. However, the site of amastigote or macrophage and mechanism of reduction (enzymatic or nonenzymatic) remain controversial. Furthermore, the ability of Leishmania parasites to reduce Sb(V) to Sb(III) is stage-specific. For instance, amastigotes but not promastogotes can reduce Sb(V) to Sb(III) [49]. This explains why amastigotes are more susceptible to Sb(V) but promastigotes are not [62,63,64,65,66]. Other studies have suggested that reduction of Sb(V) to Sb(III) may also take place within macrophages, but level of reduction of Sb(V) to Sb(III) in macrophage cannot be that significant since Sb(III) even at a dose of ~25 μg/ml can kill 50% of the THP1 macrophages [66,67]. Thus, conversion of Sb(V) to Sb(III) may occur at both sites, that is macrophage and parasite. It has been shown that an amount of Sb(V) may be converted to Sb(III) in human [64,65,66,67,68] and animals models [69,70]. The reduction of Sb(V) to Sb(III) requires an active participation of thiol compounds of both mammalian host and parasite origin [71,30,72]. Mammalian thiols, which play important role in this process, include glutathione (GSH), cysteine (Cys) and cysteinyl-glycine (Cys-Gly). The first one is the main thiol present in the cytosol, while the second and third are the predominant thiols within lysosomes of mammalian cells [73, 74]. The parasite-specific thiol compund, trypanothione ($T(SH)_2$) is a complex consisting of glutathione and spermidine, that has been shown to be involved in reduction of Sb(V) to Sb(III) [75]. Compared to GSH, however, the initial rate of reduction of Sb(V) is much higher in the presence of Cys-Gly, Cys, and $T(SH)_2$ [76]. Generally acidic pH and slightly elevated temperature favor reduction of Sb(V) to Sb(III). *In vivo* this process is mediated by $T(SH)_2$ within Leishmania parasites and Cys or Cys-Gly within the acidic compartments of mammalian cells. But the stoichiometry of GSH and Sb(V) required for the reduction of antimony is equal to or more than 5 : 1. As the rate of reduction is very low, the physiological relevance of this conversion is still open to question. Interestingly, promastigotes contain higher intracellular concentrations of $T(SH)_2$ and GSH than amastigotes [77,78] and both stages maintain an intracellular pH value close to neutral [79]. Therefore, nonenzymic reduction of Sb(V) to Sb(III) fails to account for the insensitivity of promastigotes to Sb(V). On the other hand, recent studies have suggested the participation of an parasite-specific enzyme, thiol-dependent reductase (TDR1) in the process of reduction of Sb(V) to Sb(III) [80]. The enzyme TDR1 is a tetramer protein containing domains of the omega class of the glutathione S transferases (GSTs) and using GSH as the reductant. Although TDR1 has been found to be highly abundant in the amastigote stage of the parasite, the enzyme activity and antimony sensitivity in Leishmania amastigotes could not be directly correlated. An arsenate reductase homologue in Leishmania parasite (LmACR2) has also been shown to catalyse the reduction of Sb(V) in *L. major* in presence of GSH. LmACR2 requires glutaredoxin as cofactor for its enzyme activity and is inhibited by As(III), Sb(III) and phenylarsine oxide [81]. In contrast to TDR1, LmACR2 is a monomer. Transfection of LmACR2 in *Leishmania infantum* promastigotes augments pentostam sensitivity in intracellular amastigotes, confirming its physiological significance. It is also possible that more than one mechanism is responsible for the reduction of Sb(V) to Sb(III) [28].

3.2. Uptake of antimony

Involvement of aquaglyceroporin AQP1 has been observed SbIII transport [82]. AQP1s are the members of the aquaporin super family. They are membrane channels that permit transport of small neutral solutes such as glycerol or urea [83]. The MS approaches have been used to demonstrate the accumulation of two forms of antimony i.e. Sb(V) and Sb(III), in both stages of the parasite. In a number of species, the accumulation of Sb(V) is higher in axenic amastigotes than in promastigotes [84,85]. It has been speculated that Sb(V) enters via a protein that recognizes a sugar moiety-like structure shared with gluconate, as gluconate has been shown to inhibit competitively the uptake of Sb(V) in axenic amastigotes [84]. Axenic amastigotes have also been found to be as sensitive to Sb(V) as intracellular parasites [86, 62,87,]. Accumulation of Sb(III) is competitively inhibited by the related metal As(III), whereas the accumulation of Sb(V) is not [83]. This strongly suggests that Sb(III) and As(III) enter the cell by the same route as that in yeasts and mammals [88]. Increased rates of uptake of SbIII correlated with the antimony sensitivity of the wild-type and drug-resistant transfectants of *Leishmania* [16, 12], Transfection of the AQP1 gene in a SAG-resistant field isolate conferred susceptibility to antimony. Overexpression of AQP in *Leishmania* produces hypersusceptibility to SbIII, whereas gene deletion renders the parasite resistant [7, 11]. This has provided a major insight into the uptake mechanism of drugs in *Leishmania* [7, 12]. Downregulation of AQP1 RNA levels seems to be a one of major mechanism of antimony resistance found in field and clinical isolates of *Leishmania* [76, 28].

3.3. Efflux of the drug

Overexpression of the membrane-bound ATP-binding cassette (ABC) transporters on the surfaces of *Leishmania* is another mechanism of antimonial resistance. In addition to *Leishmania*, this transport system modulates the efflux and intracellular accumulation of various drugs and thus resistance in other parasites (e.g., *Plasmodium* spp.) and also in cancer cells. Overexpression of ABC transporters concerns laboratory-derived and in-field resistant parasites [60, 89]. It has been found that, in contrast to infection with Sb-sensitive *L. donovani* isolates, infection with Sb-resistant *L. donovani* isolates upregulates the multidrug resistance-associated protein 1 (MRP1) and the permeability glycoprotein (P-gp) in host cells, thus inhibiting intracellular drug accumulation by decreasing antimony influx [60,89,90]. In animal models, inhibition of the proteins MRP1 and P-gp by lovastatin reverses their action on drug accumulation and allows them to escape a fatal outcome [90]. These results indicate that lovastatin, which can inhibit P-gp and MRP1, might be beneficial for reverting Sb resistance in VL [90]. Flavonoid dimers are also known to reverse antimonial resistance in *Leishmania in vitro* by inhibiting ABC transporters and increasing the intracellular accumulation of the drug [90]. These findings should be confirmed in animal models [92].

3.4. Thiol metabolism

Metabolisms of glutathione, trypanothione and uptake of SbIII respectively [16,12]. Thiol is essential for the survival of parasite. The enzymes that make and use this molecule are targets for the development of new drugs to treat Leishmanial disease [4]. Thiol metabolism possesses a key role in both laboratory and clinical resistant mechanism. Antimony cause the oxidative stress [93], a reducing environment within the cell and the presence of thiol become important for antimony resistance. TSH, the major thiol, is found only in trypanosomatids, and is a conjugate of GSH and spermidine [94]. The syntheses of these two precursors determine the level of TSH. The c-GCS gene, encoding c-glutamylcysteine synthetase, which catalyses the rate-limiting step in GSH biosynthesis [95], suggested that decreasing the intracellular thiol concentration through thiol depletors may increase the leishmanicidal action of drugs and thus reverse parasite resistance [96]. ODC gene encode ornithine decarboxylase, an enzyme involved in the regulation of spermidine biosynthesis, is also overexpressed [97,98]. This suggests that a lowering of intracellular thiol concentration may result in the attenuation of the resistant phenotype. This proposed hypothesis is confirmed by specific inhibitior BSO and DFMO inhibition studies. Overexpression of either ODC or y-GCS in *L. tarentolae* wild-type cells result in increased thiol level, almost equivalent to those of resistant mutant, but the transfectant do not exhibit arsenite resistance [95]. In natural antimonial resistance, the impaired thiol metabolism results in inhibition of SbV activation and decreased uptake of the active form SbIII by amastigotes, these processes are accomplished by the lower expression of the genes γ-glutamylcysteine synthetase, ornithine decarboxylase, and aquaglyceroporin 1, which are involved in the metabolisms of glutathione and trypanothione, and uptake of SbIII, respectively [18, 19, 28]. Interestingly, resistance to Sb(V) in *L. donovani* clinical isolates (India) is also reversed in animal models by treatment with BSO [99,100]. *Leishmania*, upregulation of resistance genes is frequently associated with genomic rearrangement, which leads to gene amplification through homologous recombination between repeated sequences [101,102]. Therefore, either quantification of copy number or expression of genes known to be involved in antimony susceptibility should represent good biomarkers for addressing antimony resistance [98, 103].

4. Conclusions

Drug resistance is a major impediment to successful treatment of Visceral Leishmaniasis. For almost seven decades pentavalent antimonial constituted the standard antileishmanial treatment worldwide, however the last 15 years their clinical value was hampered due to the widespread emergence of resistance of these agents. The last years several mechanisms of in field antileishmanial resistance were identified. Understanding their molecular and biochemical characteristics will lead the design of new drugs and also the molecular surveillance of resistance. In order not to jeopardize the life span of available antileishmanial drugs, their delivery, clinical response, and resistance should be monitored. Overall the development of antileishmanial drugs has been generally slow and new drugs are urgent needed.

6. References

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