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Review Article

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Chemotherapy Used in the Treatment of Visceral Leishmaniasis

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Abstract

Visceral leishmaniasis (VL) is a disease caused by an obligate intracellular protozoan belonging to the *Leishmania donovani* complex, which affects the reticuloendothelial system of mammals. Transmission is done through the bite of female *Phlebotomus* in the Old World and *Lutzomyia* in the New World contaminated with *Leishmania*. Worldwide there are an estimated incidence of 50,000-90,000 new cases of VL in the year 2017, located mainly in India, Sudan, Kenya, Somalia, Ethiopia, Brazil and South Sudan, with an estimated mortality rate of 20.000 individuals per year. The Global Burden of Disease Study (GBD) (2016) determined that the disability-adjusted life year (DALY) was 61.1% for VL. This infection has been treated for many years with various chemotherapeutic compounds such as: pentavalent antimonials (PA) denominated sodium stibogluconate (SSG) Pentostam® and meglumine antimoniate Glucantime® (MA), amphotericin B (AB), liposomal amphotericin B (L-AB), miltefosine (MIL) and paromomycin (PM), but generally present several drawbacks: a long period of intravenous administration (IV) or intramuscular (IM) injections, drug resistance has been detected in close relation to the high percentage of inefficient treatments, produce serious side effects in individuals and some have high costs, which makes inaccessible the treatments for people with low economic resources that constitute a large part of the population affected by VL. The SSG has been used during decades, but also present a high toxicity and problems with your effectiveness caused by the resistance of the parasites.

The use of a specific treatment depends on the results obtained in the clinical studies and its effectiveness in each geographical area in particular, for example, the only drug whose use is suggested in India is L-AB administered at a dose of 10mg/kg, a treatment that has an acceptable degree of safety and presents good activity against *Leishmania*. Multiple treatment with several mixed drugs is another option to improve the therapeutic quality, whose implementation has many advantages: the administration time is short, less toxics, the activity of the combined drugs shows greater effect than the sum of its individuals effects (synergistic effect), due to the inhibitory action in different points of the same metabolic pathway or in differents metabolics pathways, besides decreasing the generation of resistance. WHO recommended the following drugs according to the geographical area of origin of the patient:

A) VL caused by *L. donovani* in Bangladesh, Bhutan, India and Nepal 1) liposomal amphotericin plus miltefosine, 2) liposomal amphotericin or 3) miltefosine plus paromomycin; B) VL caused by *L. donovani* in East Africa (Ethiopia, Eritrea, Kenya, Somalia, Sudan and Uganda) and Yemen 1) pentavalent antimonials plus paromomycin; C) VL caused by *L. infantum* in Mediterranean Basin, Middle East, Central Asia, South America 1) Liposomal amphotericin B, 2) Pentavalent antimonials or 3) Amphotericin B deoxycholate.

The research and development for the discovery of new drugs must continue with the purpose of obtain highly effective therapeutic compounds to attack the potential killer: parasites of the *Leishmania donovani* complex.

Introduction

Visceral Leishmaniasis (VL) is a parasitosis caused by flagellate protozoan of the *Leishmania donovani* complex: *L. donovani* in the Indian and Africa; *L. infantum* (syn *L. chagasic*) in the Mediterranean basin, Central and South America [1]. The transmission is produced by vector insect hematophagous of the genus *Lutzomyia* genus in the New World and genus *Plebotomus* in the Old World [2]. *Leishmania* promastigotes reproduce in the digestive system of the insect and are transmitted to mammals during a blood intake of the vector, once inside the vertebrate host, they transform into amastigotes (multiplicative forms) within the macrophages [3].

VL is a potentially fatal disease with worldwide distribution in 76 countries, being endemic in 12 countries of the Americas. For the year 2013 approximately 96% of the cases registered in the Region are concentrated in Brazil (incidence 4.35/100.000) and Paraguay (3.85/100.000) [4], with 229 deaths during that year (6.7%); however, there has been a geographic expansion in Argentina, Colombia and Venezuela [5]. In the World the majority of cases (90%) are concentrated in India, Sudan, Kenya, Somalia, Ethiopia, Brazil and South Sudan [6]. The highest percentage of cases (60%) are in India, in the state of Bihar have been detected 90% of the infected individuals in this country [7]. The World Health Organization (WHO) has included VL in the group of neglected tropical diseases (NTD) because the global impact of this disease is very high, it has spread in regions with low socioeconomic resources worldwide, causing high levels of mortality (up to more than 20,000 deaths/year) with an annual incidence of around 50.000 to 90.000 cases in 2017 [8] and the

aggravating circumstances that it receives minimal attention from the governments and causes a serious state of vulnerability [9,10]. The emergence of co-infection HIV/VL increases the problems associated with chemotherapy such as the resistance of parasites to drugs. Individuals with HIV/VL have been detected in 35 countries, in areas such as south-western Europe, South Asia, Ethiopia and Brazil [11]. The largest parasitic burden in VL/HIV coinfection worldwide is found in North-West Ethiopia, for this reason Abongomera *et al* (2018) [12] decided to conduct a study in this region to evaluate the effectiveness (radical parasitological cure) of L-AB (AmBisome) 30mg/kg in combination with MIL 100mg/day for a period of 28 days, administered to 173 individuals with VL/HIV infection. The results obtained show that the initial cure rate was 83.8% and the mortality rate 12.7%. The use of drug mixtures allows reducing treatment administration times, minimizing costs and decreasing the appearance of resistance [13].

The pathogenesis produced by *L. donovani* complex begins with the penetration of the cells of the mononuclear phagocyte system of the spleen, liver, the lymph nodes and the bone marrow. The hallmark of this infection is the persistence of amastigotes within the macrophages due to a set of mechanisms of evasion of the immune response. VL during its initial period has no apparent symptoms (asymptomatic) in most cases, due to the appearance of an effective immune response of the mammal. The development towards a symptomatic disease is more likely to appear in people with HIV, immunocompromised, in a state of malnutrition and in children under 1 year [14]. VL presents clinical manifestations such as fever, hepatosplenomegaly, anemia, weight loss, fatigue, can lead to death due to the appearance of opportunistic infections that are caused by the depression of the immune system [15]. *Leishmania* escapes the action of the immune system by invading phagolysosomes within macrophages, thus using these cells as transport vehicles to disseminate through the vascular and lymphatic systems, eventually infecting the reticuloendothelial system, that causes damage to bone marrow, hepatosplenomegaly and lymphadenopathy [16].

Drugs for Treatment of VL

The chemotherapeutic compounds used for the treatment of VL are shown below:

Pentavalent Antimonials

Pentavalent antimonials (PA) have two pharmacological presentations called sodium stibogluconate (SSG) Pentostam® and meglumine antimoniate Glucantime® (MA). These were the first drugs tested against VL in the 1940s and continue to be today the main agents to treat this disease due to its low cost and its great effectiveness. Research developed in recent years has shown that fluctuations in the response of patients to treatments in the different geographical regions evaluated. PA are the drugs with better effectiveness in Africa, South America, Bangladesh, Nepal and India (except North Bihar) at a dose of 20mg/kg/day parenterally for 28-30 days [17]. These compounds inhibit glycolytic enzymes, the oxidation of fatty acids and ADP phosphorylation in *Leishmania* amastigotes [18], has been for over 60 years the drug of choice to attack VL in many countries of the world and it has been well tolerated by a large majority of affected individuals. However, it shows multiple disadvantages: a) parenteral administration, b) long periods of treatment, c) its major side effects are cardiac arrhythmias, prolonged QT interval, ventricular premature beats, rashes, ventricular tachycardia, ventricular fibrillation arthralgia, nausea, hepatotoxicity, reversible pancreatitis, vomiting, nephrotoxicity, and even death in African HIV/VL coinfection [19, 20].

Patients leave treatment due to pronounced side effects, which leads to the emergence of drug-resistant parasites, as has occurred in Indian subcontinent. Even in spite of these serious effects and having developed empirically many years ago, they are still used because of their partial efficacy, but they do not meet all the requirements of an ideal chemotherapy for VL [21].

Amphotericin B Deoxycholate

Amphotericin B desoxycholate (AB) is a macrolide polyene antifungal antibiotic agent discovered in 1956, obtained from *Streptomyces nodosus* present in the soil of Orinoco River in Venezuela, is considered the first option treatment for VL in the north of Bihad (India) a locality hyperendemic for VL, because a great antimonial resistance has been detected [22,23]. In this country the dose of administration is 1mg/kg for 20 consecutive days or during alternate days with 15 injections for 30 days [24,25]. This compound has a good *in vitro* activity on *Leishmania*, but shows the great disadvantage of presenting high levels of toxicity. AB binds specifically to ergosterol, which is an essential part of the *Leishmania* membrane and produces pores that increase the permeability, destabilize it and induce the death of parasites. This drug generates serious side effects, for example, fever, chills and thrombophlebitis, hypokalemia, nephrotoxicity, myocarditis that can cause death, for these reasons, intrahospital care and strict medical surveillance are required, which greatly increases the costs associated with the administration of this treatment.

Liposomal Amphotericin B Deoxycholate

L-AB has been recommended by WHO as treatment of choice in the Indian subcontinent [26,27], in addition to being one of the drugs selected for the VL elimination project in India [28]. U.S. regulatory agencies recommend 3mg/kg on days 1 to 5, 14 and 21 and a total dose of 21mg/kg [29]. The improvement of this drug has increased its effectiveness and safety in relation to AB, since it can be administered at high doses with minimal side effects. But on the other hand, it has increased production costs and decreases accessibility in a large number of endemic countries due to the fact that many of the infected people have low economic resources [30]. The high prices of this drug represent a great disadvantage, besides that its administration is intravenously. In L-AB solutions the deoxycholate is replaced by other lipids, which protects the organs from exposure to the free drug. The principle for the development of these compounds is based on their direct action on the macrophages of the liver, spleen and bone marrow. The decrease in toxic effects allows administering higher concentrations of the drug in shorter periods of time, this implies better tolerance and reduction of side effects. There are three formulations: L-AB (AmBisome, Gilead Sciences), lipid complex of AB (ABLC, Abelcet®, Enzon Pharmaceuticals), and cholesterol dispersion of AB (ABCD, Amphotec ™, InterMune Corp.), all these presentations show a decrease in nephrotoxicity in treated individuals [31].

Sundar *et al* (2004) [32] conducted a series of investigations in the city of Bihar, where they compared the effectiveness of AB (1mg/kg by 30 days alternate) and L-AB (2mg/kg by five days) for the treatment of individuals with VL and detected in both study groups a high percentage of cure (96%). L-AB is excellent for administration in the populations undergoing organ transplantation (especially renal) because it is a safe choice and has few interactions with other drugs [33].

L-AB presents some advantages with respect to AB: Better range of tolerance because the use of lipid formulations that reduces substantially the development of side reactions [34], exhibits large plasma exposures with low volumes of distribution, in addition to which in urine and feces very little release of the intact drug is detected [35]. This lipid formulation favors an efficient entry to the infected cells where there is a high persistence; it presents a great capacity of interaction with ergosterol and its precursor molecules that promote the destruction of the parasites and has a high transition temperature that leads to stability in macrophages, tissues and blood [36].

Miltefosine

MIL constitutes the first oral drug available for the chemotherapy effective of LV, which facilitates the treatment of patients. This compound originally was developed as an antineoplastic drug in 1980. Sundar *et al* (2006) [37] performed a trial where they included a total of 249 patients treated with oral MIL, 100-150mg/day for a period of 28 days, 90% of the individuals were cured. Subsequently, in two pediatric studies with 119 patients aged between 2 and 11 years, MIL was administered at 2.5mg/kg daily for 28 days; the results indicate a 94% cure. This drug has a good level of safety and efectivity for its administration in patients with VL, but *Leishmania* has shown resistance in a very short period of time, which causes low efficacy, these factors rule out this compound as a single therapy for VL. In addition, some researchers have proposed that the resistance of *Leishmania* against MIL occurs by decrease in intracellular drug accumulation [38, 39] or by changes in membrane permeability [40,41]. The inhibition of 2 plasma membrane proteins (LdMT and LdRos3) is thought to prevent the internalization of MIL [42]. It is also assumed that the exacerbated expression of ATP-binding cassette P-glycoprotein/MDR1 and ATP-binding cassette subfamily G members, that favors the release of the drug out of the parasite [41].

In India and Nepal there were serious disappointments, one study showed that 86.6% of people were cured but there was 12.8% relapses at 12 months for every 1000 patients treated. The problems associated with MIL are due to the lack of persistence in the plasma over time, which has also been observed in children [43]. Although these drawbacks have been detected, this drug is being widely evaluated as a combination therapy [44].

Highlights of the drugs used in VL chemotherapy are shown in Table 1.

Table 1: *Advantages and disadvantages of the chemotherapy employed in the treatment of VL.*

Drug	Advantages	Disadvantages
Pentavalent antimonials (PA)	First drugs tested against VL in the 1940s and continue to be today the main agents to treat this disease due to its low cost and its great effectiveness [45] Distribution in high concentration in the plasma, liver and spleen [46]	Very toxic Emergence of high level of resistance in India Variable efficacy [47] The treatment with antimonials has been caused several side effects, such as: nausea, abdominal pain, myalgia, pancreatic inflammation, cardiac arrhythmia and hepatitis, leading to the reduction or cessation of treatment [48]
Amphotericin B (AB)	AMB-B has excellent cure rates (~100%) at a dose of 0.75-1mg/kg for 15-20 daily or alternate days intravenous infusions [49] Clinical resistance is rare	Most of the patients experience infusion reactions (e.g., fever, chills, thrombophlebitis) and, occasionally, serious toxic episodes (e.g., hypokalemia, nephrotoxicity, myocarditis, and even death). The major limitation to using this drug is the necessity for prolonged hospitalization and close monitoring due to its high nephrotoxicity [50]
Liposomal Amphotericin B (L-AB)	Safe and effective Reduced toxicity, shorter duration of treatment, high cure rates, efficacy in resistant cases [51]	High cost [52] In some cases, side effects have been detected such as: acute kidney injury [53]
Miltefosine (MIL)	This is the first leading oral drug which is used to treat visceral leishmaniasis First orally effective antileishmanial drug, which is uniformly active in both naïve as well as SbV refractory patients [54]	Teratogenic [55] Abortifacient (limits its use during pregnancy) high cost, need for monitoring for gastrointestinal side effects, and occasional hepatic toxicity and nephrotoxicity [56]

Multidrug Therapy

Studies of combination terapies are the strategy used to solve the inconveniences caused by the use of drugs in the form of monotherapy. These strategies aim to minimize the time of administration of the chemotherapy up to about 11 days, reducing costs of hospitalization and treatment in order to achieve accessibility of people from all social strata, high efficacy in the treatments, decreases the toxic effects on the treated individuals and reduces the probability of resistance of the parasites [13].

Rahman *et al* (2017) [57] evaluated 601 individuals with VL between 2010 and 2013 using: AmBisome monotherapy (n = 158), AmBisome + paromomycin (PMM) (n = 159), AmBisome + MIL (n = 142) or PMM + MIL (n = 142). The percentages of cure after six months of treatment were: AmBisome 98.1%, AmBisome + paromomycin 99.4%, AmBisome + MIL 94.4% and for PMM + MIL 97.9%. All combinations were similar to AmBisome in terms of their ability to produce cure, showed a high level of safety and tolerance, indicating that they can be used to treat VL in Bangladesh.

Mahajan *et al* (2015) [58] performed an analysis with 102 patients presenting coinfection with confirmed HIV-VL who received combination treatment for VL at a treatment center of Médecins Sans Frontières between July 2012 and September 2014. These individuals were treated with 30mg/kg body weight L-AB (AmBisome) distributed in divided into 6 injections of equal doses combined with 14 days of 100mg/day oral MIL (Impavido), all the treated population was suggested to start or continue with antiretroviral therapy (ART). Cumulative incidence of all-cause mortality and VL relapse at 6, 12, and 18 months was 11.7%, 14.5%, 16.6% and 2.5%, 6.0%, 13.9%, respectively. Cumulative incidence of poor outcome at 6, 12, and 18 months was 13.9%, 18.4%, and 27.2%, respectively. In these tests conducted in Bihar, the combination therapy had a good tolerance, in addition to safe, effective, indicating that these drugs can be considered as an option for the treatment of VL in patients coinfected with HIV. The drawback of this combination is that the MIL has a long half-life of almost 152 hours and is therefore responsible for long-term residence and teratogenicity. This characteristic originates the development of resistance, together with the fact that this compound is abortifacient and teratogenic and therefore its use during pregnancy is ruled out. Hendrickx *et al* (2017) [59] evaluated the activity of combined therapy MIL-PMM against a *Leishmania* species closely related to those detected in the Indian subcontinent, using experimental models for *in vitro* and *in vivo* studies with the aim to select multidrug-resistant species by simultaneous exposure to PMM and MIL. The combined use of these compounds in hamster demonstrate a cumulative efficacy but did not lead to a significant susceptibility decrease, indicating that PMM-MIL combination therapy may represent a safe and affordable treatment option for VL. This suggests that the use of PMM-MIL in the form of multidrug therapy combination therapy solves the resistance problem observed with PMM monotherapy, can provide safe and reliable results for the treatment of *L. donovani* VL.

WHO (2012) [60] recommended the following mixtures of drugs according to the geographical area of origin of the patient: A) VL caused by *L. donovani* in Bangladesh, Bhutan, India and Nepal 1) L-AB (5mg/kg by infusion, single dose) plus MIL (daily for 7 days, for children aged 2-11 years, 2.5mg/kg per day; for people aged ≥12 years and < 25kg body weight, 50mg/day; 25-50kg body weight, 100mg/day; > 50kg body weight, 150mg/day), 2) L-AB (5mg/kg by infusion, single dose) plus PMM (daily for 10 days, 15mg (11mg base) per kg body weight per day) 3) MIL (daily for 10 days, for children aged 2-11 years, 2.5mg/kg per day; for people aged ≥12 years and < 25kg body weight, 50mg/day; 25-50kg body weight, 100mg/day; > 50kg body weight, 150mg/day) plus PMM (daily for 10 days, 15mg (11mg base) per kg body weight per day).

Diagnosis of VL

The diagnosis of VL can be made through of studies clinical, epidemiological, parasitological, molecular and immunologicals. Detection is complicated because its symptoms can be confused with other diseases such as: typhoid fever, tropical splenomegaly, schistosomiasis or cirrhosis with portal hypertension, African trypanosomiasis, miliary brucellosis, bacterial endocarditis, histoplasmosis, malnutrition, lymphoma and leukemia [61].

The lack of an efficient and rapid clinical diagnosis causes a loss of valuable time for the early administration of the treatment.

The classical diagnosis is carried out through demonstration of the presence of parasites by aspiration in tissues relevant to VL and subsequent microscopic observation of the stained samples to locate the amastigotes, que are round or oval bodies, 1-4 μm in diameter, with a typical rod-shaped kinetoplast and circular nucleus. The observed sensitivity is 95% in the splenic aspirate, about 76% in liver biopsy, bone marrow aspirate and bloof buffy coat, the lowest in lymph node aspirates which is positive only in about 50% of the cases. These techniques are very invasive and present high risks of harming the patient's health, as in the case of spleen biopsy that shows a risk of hemorrhage of 1/1000 patients [62], in addition they are difficult to perform in the prevailing field conditions.

Direct agglutination test (DAT) and immunochromatographic test using rK39 (ICT-rK39), are the two Gold Standard tests for the serological detection of anti-*Leishmania* antibodies, because they have several advantages: they are high sensitivity and specificity, besides being easy to use in urban and rural health centers, for this reason, numerous investigations have been carried out worldwide in order to evaluate, compare and validate them. Chappuis *et al* (2006) [63] developed a large-scale investigation to evaluate the usefulness of DAT and ICT-rK39 in the diagnosis of VL. DAT provides a sensitivity of 94.8% and a specificity of 97.1%, the results obtained were independent of the region evaluated or of the species that originate the infection, despite the benefits of this test, it has several drawbacks such as: they require prolonged times to obtain the results, the proteins used must have rigorous and regular quality controls. ICT-rK39 is based on the use of rK39, a 39-amino acid repeat of a kinesin-related protein in *L. chagasi* and found in all members of the *L. donovani* complex [64], it is an assay fast, simple to interpret, uses standardized recombinant antigen, easy to use because it has a very simple format (usually dipstick), shows reliable and reproducible results, with sensitivity between 71 and 97% and specificity between 82 and 100% [65]. Despite showing such good results, variations between different regions have been detected and it is less reliable in East Africa, because the kinesin-related gene fragment was obtained from a Brazilian strain of *L. infantum*, and there is a genetic diversity of the kinesin among strains of *L. donovani* in East Africa and Asia [66].

Immunodiagnostic tests, although showing many advantages, also present some drawbacks because the antibodies remain in the blood for many years after parasitological cure, which indicates that VL relapse can not be detected by these tests [67]. It has also been observed that some serological tests do not allow the detection of anti-*Leishmania* antibodies in more than 40% of the HIV-coinfected population, indicating that they present a low sensitivity in the diagnosis of these cases [68]. Studies to develop better recombinant antigens should continue, in order to obtain diagnostic tests with better values of sensitivity and specificity and achieve a faster diagnosis that approximates the real result to administer the treatments more quickly.

Conclusions

The history of chemotherapy against VL begins in the 40s with the administration of PA, which at first was thought to be the solution to cure this infection, but its use has presented many obstacles such as the emergence of resistance on the part of *L. donovani* complex mainly due to the lack of compliance in the programming of the treatment, in addition to the high levels of toxicity observed. For these reasons, amphotericin B deoxycholate has been used in later years, a compound that has been improved in lipid formulations, which shows little toxicity and great efficacy, even though it has the disadvantage of its high cost and its intravenous administration.

An alternative to improve chemotherapy has been the use of drugs in the form of combination therapies, which show multiple advantages with respect to monotherapy: greater clinical efficacy, lower rates of toxicity and resistance, greater cost effectiveness because they require lower concentrations of chemotherapeutic compounds. The efforts continue in the search for a drug against VL that is administered for a short time, very effective, easy to administer in a short period of time, which presents high levels of safety, active against *L. infantum* complex without generating resistance, low cost to allow the treatment of a large number of poor patients, active against all species of the *L. donovani* complex, presenting minimal side effects and it is crucial to allow the healing of individuals in areas where other diseases coexist (eg, tuberculosis, HIV, dysentery). Unfortunately the development of new drugs to attack neglected diseases is an almost impossible process, due to the lack of commercial interest on the part of pharmaceutical companies, as well as a lack of investment in research and development by governments. In addition, the discovery of ideal serological diagnostic kits is essential, with the following characteristics: sensitive and specific, that are efficient, easy to manipulate, that provide results quickly to detect the presence of VL, as well as, that allow to evaluate the treatment chemotherapeutic and determine the existence of radical parasitological cure.

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