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CASE REPORT

TWO CASES OF VISCERAL LEISHMANIASIS IN COLOMBIA RESISTANT TO MEGLUMINE ANTIMONIAL TREATMENT

Iván Darío VÉLEZ(1), Lina María COLMENARES(1) & Carlos Aguirre MUÑOZ(2)

SUMMARY

Visceral leishmaniasis (VL) affects over 500 000 people worldwide each year. The disease occurs in the Mediterranean basin, Central and South America and is caused by *Leishmania infantum* (syn *L. chagasi*). VL is an endemic disease in Colombia, particularly along the Caribbean coast and the Magdalena River Valley and 90% of VL cases occur in children under the age of five. The first line of treatment is chemotherapy with pentavalent antimonial compounds, including sodium stibogluconate (Pentostam®) and meglumine antimoniate (Glucantime®). These compounds are the ones most used in Colombia, at a dose of 20 mg/kg/day for 28 days. Nevertheless resistance of *L. infantum* to pentavalent antimonials is becoming an important problem.

No cases of VL resistant to pentavalent antimonial compounds have previously been reported from Colombia. This report describes the two cases of VL resistance to antimonial compounds in a girl and a boy who did not respond to previous treatment with Pentacarinat® and Glucantime® regimens but were treated successfully with liposomal amphotericin B. Based on our findings, we recommend liposomal amphotericin B as the first line of treatment for VL due to its low toxicity, shorter administration period and the low price obtained by WHO.

KEYWORDS: Visceral leishmaniasis; Pentavalent antimonial; Therapeutic failure; Liposomal Amphotericin B.

INTRODUCTION

Visceral leishmaniasis (VL) caused by *L. donovani* and *L. infantum* affects over 500 000 people annually around the world[12,23,25]. VL due to *L. donovani* is an anthroponosis that mainly affects young adults in eastern Africa and India and typically produces post-kala azar dermal leishmaniasis (PKDL) after treatment. By contrast VL due to *L. infantum* mainly affects children and is a zoonosis whose reservoir is the dog. Its geographical distribution includes the Mediterranean basin as well as South and Central America[13,22,23].

VL is endemic to Colombia, particularly along the Caribbean coast and the Valley of Magdalena River. It is caused by *L. infantum* transmitted by the bite of the phlebotomine sand flies *Lutzomia evansi* (Caribbean coast) and *Lu. longipalpis* (Magdalena River Valley)[4,8,31].

The amastigotes of these *Leishmania* species replicate in macrophages from the liver, spleen, bone marrow and lymph nodes, causing severe and ultimately lethal lesions[13]. VL is usually fatal in the absence of effective treatment[13,23].

Active VL may also represent relapse (recurrence 6-12 months after apparently successful treatment) or late reactivation (recrudescence) of subclinical or previously treated infection. Reactivation can be spontaneous but is often provoked by an intercurrent insult to T (CD4+) cell number or function-corticosteroid or cytotoxic therapy, antirejection treatment in transplant recipients, or advanced HIV disease[13].

The first line of treatment involves the use of pentavalent antimonial compounds (SbV) such as sodium stibogluconate (Pentostam®) and meglumine antimoniate (N-metil-glucamine) (Glucantime®)[5,9,13]. Common side effects with sodium stibogluconate and meglumine antimoniate include abdominal pain, anorexia, vomiting, nausea, myalgia, arthralgia, headache, and malaise. Elevated amylase and lipase levels occur in most patients, but only a minority manifests clinically apparent pancreatitis. Persons with renal insufficiency seem to be at increased risk of this complication. Electrocardiographic changes are dose-dependent and include T-wave inversion and a prolonged QT interval. Arrhythmias and sudden death have been reported with doses greater than 20 mg of SbV/kg body weight/day. Pentavalent antimonials should be used with caution in elderly patients and those with heart disease, nephropathy and in pregnancy[5,11,17].

In Colombia, Glucantime® is currently used for VL at a dose of 20 mg/kg/day during 28 days, according to guidelines from the Ministry of Health[16].

(1)Programa de Estudio y Control de Enfermedades Tropicales, PECET. Universidad de Antioquia, Calle 62 No 52-59, SIU Laboratorio 632, Medellín-Colombia, Tel. 574 219 6502, Fax. 574 219 6511, E mails: idvelez@udea.edu.co, linaco80@yahoo.com.ar

(2)Departamento de Pediatría y Puericultura, Facultad de Medicina, Universidad de Antioquia, Calle 67 No 51-37, Medellín-Colombia, Tel. 574-263 7885, E mail: ceaeme@une.net.co

Correspondence to: Dr. Iván Darío Vélez, Programa de Estudio y Control de Enfermedades Tropicales, PECET, Universidad de Antioquia, Calle 62 No 52-59, SIU Laboratorio 632, Medellín-Colombia. Tel. 574 219 6502. Fax. 574 219 6511, E mail: idvelez@udea.edu.co

Although Colombia had not reported cases of VL resistant to pentavalent antimonials until now, this is already an important problem in Africa and India[2,20,23,29]. Primary resistance to SbV is seen in approximately 1% of cases in Africa and up to 60% in certain regions of India[2,5]. In India the recommended treatment for VL produced by *L. donovani* is Miltefosine®[12,13,23,24,25], although there are no reports of the effectiveness on this medication in treating VL produced by *L. infantum*.

Several other compounds have been used successfully to treat VL patients. Amphotericin B deoxycholate (Fungisone®) is effective but requires parenteral administration over prolonged periods and is associated with nephrotoxicity and other side effects. Various doses and durations of therapy have been used, 1.0 mg per kg body weight per day for 15 days or 1.0 mg per kg body weight every other day for 30 days being two alternatives[13,14,17,21]. Pentamidine isethionate (Pentacarinat®) at 2-4 mg/kg body weight/day for up to 15 days is an effective but potentially toxic option. Pentamidine has a number of side effects including hypotension, life-threatening hypoglycemia caused by pancreatic β-cell injury, and later insulin-dependent diabetes mellitus[5,13,14,17,21]. Aminosidine (Paromomycin), a parenterally administered aminoglycoside, (11 mg/kg daily for 21 days) has shown promise in some studies[12,14,25,26,29]. The imidazoles ketoconazole and itraconazole have been used successfully in some cases, but primary failures occur and they are not recommended for general use[21,25]. Allopurinol was ineffective for cutaneous leishmaniasis (CL) in Colombia[30]. Combined therapies of allopurinol and antimonials with interferon have also been tried in limited studies[12,14,25].

Amphotericin B causes secondary effects such as renal disturbances, anemia, fever, malaise and hypokalemia[5,14,17,25]. During the past years, liposomes have been used to transport chemotherapeutic agents into macrophages, which are the target cells for *Leishmania* amastigotes, improving the efficacy of the treatment and reducing its toxicity[5,17,22,25]. Liposomal amphotericin B (Ambisome®) is highly effective for treating VL and has low toxicity, as different studies have demonstrated[1,12,13,14,20,22,25].

Reports on the use of chemotherapy associated with immunotherapy have shown considerable efficacy in some cases of diffuse leishmaniasis using immunotherapy in American CL patients for whom antimonial drugs are restricted (elderly patients and those with other conditions, such as cardiopathy, nephropathy and pregnancy), an immunological stimulus associated with a chemotherapy treatment leading to a reduction in antimony volume required and treatment period. This complex provides substantial benefits to the patient, including the reduction of outpatient visits, and ambulatory expenses, over standard therapy[7,11,28].

In South America immunostimulation by injections of killed promastigotes plus BCG is used in the treatment of cutaneous leishmaniasis. This appears to promote healing even in cases of diffuse cutaneous and mucosal leishmaniasis[13]. Three doses of killed *L. amazonensis* preparation alone provided no protection against CL in Colombian soldiers[13,32]. Current laboratory efforts are focused on novel antigens and adjuvants, live-attenuated vaccine, recombinant purified and subunit proteins, naked DNA, bacteria expressing *Leishmania* antigens and the targeting of dendritic cells[13].

In VL the focus is on the combined administration of a therapeutic vaccine with antileishmanial drugs for treatment of PKDL. Use of therapeutic vaccine could reduce the dose and duration of chemotherapy[29].

PKDL is a source of the human infection for dissemination of VL so the ability to treat this condition could be critical to elimination of the disease. Clinical trial guidelines are being generated and vaccine candidates and drug regimens are under selection for a clinical trial for PKDL in Sudan, for which the estimated completion date is 2009[29].

Safe, effective and affordable treatments for VL are urgently needed in regions where the disease is endemic, incorporated in formulations compatible with administration in rural settings[14,24,26,29].

This report describes the successful treatment with liposomal amphotericin B (Ambisome ®) of Colombian girl and boy with VL who had previously received pentamidine and N-methyl glucamine regimens in the Caribbean coast of Colombia.

Case 1: The patient was a 16 month-old female from the rural area of the municipality of Ovejas (department of Sucre, Colombia), consulted for the first time on January 9th 1994, having developed clinical manifestations during the previous 20 days. These consisted of high intermittent fever, chills, sweating, generalized mucocutaneous pallor, adynamia, hyporexia, and progressive abdominal distention. She was conscious and hydrated, weighing 6.8 kg, with a heart rate of 100/min, respiratory rate of 36/min and body temperature of 37 ºC. She showed a profound mucocutaneous pallor and a globulous abdomen with splenomegaly of 5 cm below the left costal margin.

The laboratory test showed anemia, normal leukocyte and platelet count, normal urinalysis and coagulation test, negative seroaglutination for febrile antigens, with marrow and splenic aspirations being negative for *Leishmania* or any other hemoparasites.

A diagnosis of anemic syndrome was made. Despite having negative laboratory tests, the clinical suspicion of leishmaniasis persisted and the patient was hospitalized in order to confirm the diagnosis and to allow treatment.

The patient was treated with Glucantime® at a dose of 20 mg/kg/day for a period of 21 days. There was an evident recovery on the fifth day of treatment, with diminished fever and pallor. Bone marrow and splenic aspirates remained negative until the treatment was completed. The patient was released from the hospital in good health.

The patient returned four months later with the same clinical manifestations. The splenomegaly had increased to 8 cm below the left costal margin; hemoglobin was 4.5 g/dL, hematocrit 13%, and leukocyte count 3.3 x 10^9/L with 40% neutrophils and 60% lymphocytes. Total serum proteins, albumin and globulins were normal. Bone marrow and splenic aspirates showed abundant amastigotes of *Leishmania.* The patient was hospitalized again and started a new scheme with N-methyl glucamine antimoniate at the same dosage. A blood transfusion was performed. The patient apparently recovered and the anemia disappeared although splenomegaly persisted. The splenic aspirate was negative for *Leishmania* at the end of the treatment.

Six months later, the patient returned having suffered for two months from similar manifestations and growth retardation. A splenomegaly of 10

Table 1
Blood tests (case 1)

Date	Hemoglobin (g%)	Hematocrit (%)	Leukocytes	Differential leukocyte count (%)						Platelet count (mm)	Sedimentation (mm/hour)
				N	L	AL	M	E	B		
1-18-95	3.5	11	2900	12	82	6	0	0	0	63000	160
1-20-95	10.5	33.8	3000	14	80	6	0	0	0	68000	ND
1-25-95	9.4	30.8	2400	10	78	43	3	0	5	54000	117
2-2-95	ND	31	4500	30	60	0	1	3	0	ND	115
2-7-95	ND	31	3200	30	60	4	ND	ND	ND	ND	80
Normal values	11-14	33-42	5000-12000	21 ▼ 66	19 ▼ 63		4.6 ▼ 11.2	0 ▼ 7	0	150000-450000	0-10

N = neutrophils; L = lymphocytes; M = monocytes; E = eosinophils; B = bands; AL = atypical lymphocytes; ND = not done.

cm below the left costal margin was found. The patient was diagnosed with antimonial-resistant VL. Laboratory tests revealed anemia and a splenic aspirate contained abundant amastigotes of *Leishmania.* The patient was hospitalized again and treated with Glucantime® at a dose of 28 mg/kg/day for 28 days, no improvement being achieved.

The treatment was changed to Pentacarinat® at a dose of 4 mg/kg/day over 15 days. The febrile syndrome disappeared but pallor and splenomegaly persisted. At the end of the treatment, abundant amastigotes of *Leishmania* were observed in the splenic aspirate and culture results were positive. The patient continued to have febrile episodes, with persistent pallor and splenomegaly. She was then transferred to a tertiary level hospital in Medellin.

Treatment with intravenous liposomal Amphotericin B (Ambisome®) was initiated at a dose of 2 mg/kg/day for 21 days. Blood tests (Table 1) showed that the patient had pancytopenia as a result of VL. The patient showed improvement on the fourth day of treatment. Secondary effects included hyperkalemia (6.1 mEq/L) on the seventh day of therapy and hypothermia, for which she received treatment. Renal and hepatic functions were normal during the treatment (Table 2-3). Pancytopenia was corrected after 10 days of treatment, and the patient showed a satisfactory improvement. She was released from the hospital in good condition, asymptomatic apart form splenomegaly. The splenic aspirate was negative for *Leishmania*

Table 2
Hepatic tests (case 1)

Date	Alkaline phosphatase (UA/L)	Transaminases	
		GOT UL	GPT UL
1-25-95	ND	16	8
2-2-95	166	22	18
2-8-95	137	34	26
Normal values	100-300 (1-9 years)	15-55 (1-9 years)	5-45 (1-9 years)

ND = not done

Table 3
Renal function tests (case1)

Date	Urea nitrogen (mg/dL)	Urea (mg/dL)	Serum creatinine (mg/dL)
1-25-95	ND	ND	0.6
2-1-95	8.4	17.98	0.7
2-8-95	13.3	28.57	0.5
Normal values	5-18	10-45	0.3-0.7

ND = not done

at the end of the treatment. The patient was followed up for one year and remained in good health.

CASE 2: A 3 year-old male from the rural area of Sincelejo (Colombia), consulted for first time in March 1995, with clinical manifestations developed during the last month, consisting of intermittent fever, paleness, adynamia, chills, anorexia, weight loss, increased abdominal perimeter, cough, rinorrhea and tachypnea.

VL was diagnosed based on a positive spleen aspirate. Treatment with Glucantime® at a dose 20 mg/kg/day during 20 days was initiated. The patient received two schemes of such treatment divided by an interval of three months. Only partial recovery was obtained and the abdominal distension persisted.

One month later, the fever and respiratory signs reappeared and a third scheme of Glucantime® at the same dose was administered, without a total recovery.

Five months later the paleness, fever, and anorexia returned and he was transferred to a third level hospital in Medellin, Colombia.

The bone marrow aspirate was positive for *Leishmania*. The treatment was changed for Pentacarinat® at a dose of 4 mg/kg/ every other day during 18 days.

Table 4
Laboratory test (case 2)

Date	TEST									
	Hb	Hct	L	N	Lym	E	Plat	Na	K	Cr
29-01-96	7.5	24	5600	40	55	5	250000	136	4.3	0.5
02-03-96	10.4	31	6600	34	56	10	202000	ND	ND	ND
05-03-96	11.2	34	11600	39	50	11	286000	139	3.6	0.5
Normal values	11-14	33-42	5000-12000	21-66	19-63	0-7	150000-450000	139-146	3-6	0.3-0.7

Hb = Hemoglobin (g/100mL); Hct = Hematocrit (%); L = Leukocytes; N = Neutrophils (%); Lym = Lymphocytes (%); E = Eosinophils %; Plat = Platelet count; Na = Sodium (mE/L); K = Potassium (mE/L); Cr = Serum Creatinine (mg/dL); ND = Not done.

Five months later he returned in poor condition with growth retardation, paleness, weakness, splenomegaly (under the left iliac fosse), hepatomegaly (3 cm below the right costal margin), and cervical lymphadenopathy; a new marrow aspirate was done and it was positive again. The patient was diagnosed as having resistant VL.

Treatment with Ambisome® was administered at a daily dose of 0.6 mg/kg/day until reaching a total of 15 mg/kg (180 mg). No renal, hepatic or blood toxicity was developed and no signs of immunodeficiency were found. The fever disappeared on the 4th day of therapy, the visceromegalies dimished and the patient recovered.

He was released from the hospital in good condition, asymptomatic but with a splenomegaly of 4 cm below the left costal margin (Table 4).

DISCUSSION

VL caused by *L. infantum* mainly affects young children and is almost always fatal if untreated[13,22,23]. Effectiveness of sodium stibogluconate for VL and CL has been declining during the last two decades, with 37-64% of the patients with VL in India currently failing to be cured by antimony treatment[13,14,22]. This drug is highly toxic and requires long-term parenteral treatment[12,13,14,24,25].

Alternative chemotherapy treatments are pentamidine and amphotericin B, both of which are also toxic. However the declining efficacy of pentamidine leaves amphotericin B as the only alternative for these Sbv-refractory patients[12,13,24,25].

Immunochemotherapy (vaccine with antileishmanial drugs) is maybe an alternative treatment for those patients for whom SbV compounds are not indicated, clinical trial guidelines are being generate[7,11,16,28].

Amphotericin B is a polyene macrolide antibiotic that binds to sterols in cell membranes. It is the most active antileishmanial agent in use. Its infusion-related and renal toxicity may be reduced by lipid-based delivery. Liposomal amphotericin B (Ambisome®), a complex of amphotericin B and distearopalmitoglicerol bound to a liposome of cholesterol and phosfatidylcoline, seems to be less toxic than other amphotericin B lipid formulations such as Amphocil® and Amphotec®[5,12,14,17].

Liposomal Amphotericin B (Ambisome®) is approved for treatment of leishmaniasis in studies coordinated by WHO/TDR[16]. Its principal advantage is its high effectiveness, mitigated somewhat by its high price. In India it is currently available at $ 4 dollar 1 /mg, almost 3000 times higher that the unit cost of sodium stibogluconate price and 900 times more than that of paromomycin. A preferentially low price of $ 22.3 per ampoule (approximately $ 0.47mg, or one-tenth of the official price) has been secured by Médecins Sans Frontières, although this is currently not applicable to India[14]. Liposomal Amphotericin B was selected as the first choice of treatment for VL in the outbreak of the disease that appeared in Ethiopia in 2006 (MSF, personal communication).

The only major restriction on the current use of Amphotericin B is its cost, but this can be compensated by benefits of low toxicity and reduced time required for hospital interment[12,13,22]. However in 2007 the WHO signed an agreement with the manufacturer to produce the drug at $20 per vial for VL treatment[14].

Failure with antimonial compounds treatment can be generated by different causes, among them, host factors such as the immune response, physiological disorders that alter the tissue deposits of the antimoniate and genetic predisposition for not responding to antimoniate therapy. Resistance of *Leishmania* against a given drug may be either natural, or may be acquired when the parasites are exposed to sub-optimal drug doses[20]. In our patients, the therapeutic scheme was that recommended by experts so the resistance cannot be attributed to a resistant strain that survived a low initial dose.

In the absence of effective vaccines and vector control measures, the main line of defense against the disease is chemotherapy. Organic pentavalent antimonials have been the first-line drugs for the treatment of leishmaniasis for the last six decades, and clinical resistance to these drugs has emerged as a primary obstacle to successful treatment and control[2,20,23,29]. A multiplicity of resistance mechanisms has been described:[1,2,20] *Leishmania* infection is classically associated with a depression of T helper type 1 (Th1) cells and preferential expansion of T helper type 2 (Th2) cells and accordingly, skewing of T helper cells towards a Th1 response is considered as a promising therapeutic strategy[32]. In the Th1 response, T cells activate macrophages by releasing the cytokines interferon (IFN)-γ and interleukin (IL)-2. In the Th2 response, T cells release cytokines IL-4, IL-5, IL-10 and transforming growth factor (TGF)-β which inhibit macrophages from killing *Leishmania* spp.[5].

Although the macrophage has effective mechanisms to decimate intracellular pathogens by generating toxic metabolites like nitric oxides and reactive oxygen species for which their activation by interferon-gamma (IFN-γ), released by Th1 cells is mandatory, *Leishmania* is a devious pathogen that evades the immune response by selectively attenuating pro-inflammatory signalling pathways[25].

VL patients require an IFN-γ dominant Th1 pro-inflammatory response for clearing the parasite. IFN-γ switches on a number of immune effector pathways to activate lytic mechanisms within the macrophage, thus facilitating immune containment of the parasite load. On the other hand, induction of an IL-4-biased (Th2 response) antagonizes Th1 protective, and inhibits the immune control *in vivo* leading to severe disseminated forms of the disease. The IL-4 excess production in susceptible hosts probably causes drug unresponsiveness[27].

Progressive non-healing infection (eg, VL, PKDL, and chronic cutaneous leishmaniasis) seems more likely to indicate a net suppressive-type response (eg, Th2 > Th1) rather than an inert Th1 response; under either condition, cell-mediated immunity would be ineffective[13].

The importance of the Thl-Th2 paradigm in the pathogenesis of leishmaniasis has been an area of intense investigation. The susceptibility, resistance, and immunopathogenesis of *L. donovani* infection largely depends on the cytokine profiles elicited after infection[27].

Metalloid containing drugs (arsenic and antimony) are used to combat infectious diseases caused by pathogenic parasites, to survive in such a hostile habitat, it is crucial to develop strategies to exclude toxic substances from the cell and to acquire tolerance. Cells remove metalloids from the cytosol either by active efflux or by sequestration in an integral organelle. Controlling the influx appears to be another way of maintaining a low intracellular metalloid content. The emergence of resistance to metalloid-containing drugs is a serious threat to effective medical treatment[20].

SbV is reduced to trivalent antimony SbIII, which constitutes the active form of the drug against the parasite. Once SbIII is within the cell, it would be conjugated to trypanothione, the parasite- specific spermidine glutathione conjugate. Trypanothione is found to be increased in arsenite and antimoniate resistant cells. This Sb trypanothione conjugate could then be sequestered inside a vacuole by the ATP binding cassette (ABC) transporter PGPA or extruded from the cell by a thiol-X pump. Altered transport of metals avoids the normal function of the antimonials[6,20].

In VL or "kala-azar" caused by *L. donovani*, man is the main reservoir, and selection of resistant strains as a consequence of improper dosages allows their expansion in the human population by means of bloodffeding by sand flies[15]. It has not been demonstrated that immunocompetent individuals infected with *L. infantum* can play a role as reservoirs.

A different situation occurs in immunocompromised host, in which cases of coinfection with HIV and *L. infantum* are more frequent. The parasite is usually found in peripheral blood and skin, which enables humans to infect vectors[3,18,19]. This exposes a new and more dramatic problem since these patients showed a poor response due to host factors and therapeutic regimens; they would have select for resistant strains and also act as reservoirs to disseminate them[2].

In Colombia in 2005 18097 cases of leishmaniasis were reported, of which 17983 were CL (99.4%), 60 (0.3%) were mucocutaneous, and 54 (0.3%) VL. The age group most affected was that of patients aged 15-44 years old (81.3%)[13]. In 2006 16238 cases of leishmaniasis were reported, 11.2% less than in 2005[10].

In other countries where *L. infantum* is endemic, this parasite can cause two clinical syndromes: VL and CL. CL has been treated with low dosages of pentavalent antimoniate, which would select for resistant strains[2]. Furthermore, epidemiological studies have demonstrated that humans can infect sand flies when they bite cutaneous lesions. This could represent a mechanism for the spread of resistant strains of *L. infantum* that previously caused cutaneous lesions but then become able to produce VL in other patients[15,19].

In Colombia 50 cases of VL are diagnosed every year, 90% of them in children under the age of five[9]. These two cases of resistance to treatment provide a warning of the possible appearance of resistance to antimonials in this region, where it has been also found that *L. infantum* together with *L. braziliensis* causes cutaneous lesions[21].

The first reported case of abnormal response to SbV treatment in a Colombian patient with CL produced by *L. panamensis* was in 1989. This patient was treated with 3 Sbv schemes, each of 10 days over a 14 month period and received more than 92 g of SbV in a wide variety of schemes without reaching clinical cure[21].

This report is important because it describes the first two Colombian cases of VL by *L. infantum* with no clinical response to the antimonial compound, under the recommended scheme of treatment. As can be seen, Liposomal Amphotericin B produced an excellent clinical and parasitological response, which persisted for a follow-up period of over 12 months.

The mechanisms that precluded a good clinical response in these patients is not clear, because they did not manifest any of the clinical conditions that could normally result in relative resistance. These include such as immunodeficiency, severe malnutrition or severe infections (tuberculosis or others).

RESUMO

Dois casos de leishmaniose visceral da Colômbia, resistentes ao tratamento com antimoniato de meglumina

A leishmaniose visceral (VL) afeta aproximadamente 500000 pessoas anualmente no mundo. A doença ocorre no mediterrâneo, na América Central e na América do Sul, sendo causada por *Leishmania infantum* (syn. *L. chagasi*). Na Colômbia VL é uma doença endêmica, presente no litoral do Caribe e no Vale do rio Magdalena sendo que 90% de casos de VL ocorrem em crianças menores de cinco anos. O principal tratamento é a quimioterapia com compostos de antimoniais pentavalentes, incluindo stibogluconato de sódio (Pentostam®) e antimoniato de meglumina (Glucantime®). Estes compostos são os mais usados na Colômbia em dosagem de 20 mg/kg/dia durante 28 dias. Entretanto, a resistência de *L. infantum* aos antimoniais pentavalentes está se tornando problema importante. Na Colômbia não existiam relatos de casos de VL resistentes aos antimoniais pentavalentes. Este trabalho descreve os dois primeiros casos colombianos de VL resistentes aos compostos antimoniais em uma

menina e um menino, que foram tratados com regime de Pentamidina e Glucantime®, e demonstra o sucesso obtido no tratamento com anfotericina B liposomal.

Em conclusão, sugerimos como primeira opção de tratamento a anfotericina B liposomal porque é altamente efetiva no tratamento da VL, dada sua baixa toxicidade, curtos períodos de administração e o baixo preço obtido pela organização Médicos Sem Fronteiras.

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