Acta Tropica 164 (2016) 280–289

Contents lists available at ScienceDirect

Acta Tropica

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

The effect of *Lucilia sericata*- and *Sarconesiopsis magellanica*-derived larval therapy on *Leishmania panamensis*

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ARTICLE INFO

Article history:
Received 5 March 2016
Received in revised form 28 June 2016
Accepted 24 September 2016
Available online 26 September 2016

Keywords:
Larval therapy
Lucilia sericata
Sarconesiopsis magellanica
Hamster
Larval excretions and secretions
Co-infection
Leishmania panamensis

ABSTRACT

This study's main objective was to evaluate the action of larval therapy derived from *Lucilia sericata* and *Sarconesiopsis magellanica* (blowflies) regarding *Leishmania panamensis* using an *in vivo* model. Eighteen golden hamsters (*Mesocricetus auratus*) were used; they were divided into 6 groups. The first three groups consisted of 4 animals each; these, in turn, were internally distributed into subgroups consisting of 2 hamsters to be used separately in treatments derived from each blowfly species. Group 1 was used in treating leishmanial lesions with larval therapy (LT), whilst the other two groups were used for evaluating the used of larval excretions and secretions (ES) after the ulcers had formed (group 2) and before they appeared (group 3). The three remaining groups (4, 5 and 6), consisting of two animals, were used as controls in the experiments. Biopsies were taken for histopathological and molecular analysis before, during and after the treatments; biopsies and smears were taken for assessing parasite presence and bacterial co-infection. LT and larval ES proved effective in treating the ulcers caused by the parasite. There were no statistically significant differences between the blowfly species regarding the ulcer cicatrisation parameters. There were granulomas in samples taken from lesions at the end of the treatments. The antibacterial action of larval treatment regarding co-infection in lesions caused by the parasite was also verified. These results potentially validate effective LT treatment against cutaneous leishmaniasis aimed at using it with humans in the future.

1. Introduction

Leishmaniasis is a collective term used for describing the diseases caused by 20 pathogenic species of parasites from the genera *Leishmania* (*Kinetoplastida, Trypanosomatidae*) which is transmitted to a vertebrate host by the bite of a female phlebotomine from the genera *Phlebotomus* as vector in the Old World and by *Lutzomyia* in the New World (Alves and Bevilacqua, 2004; Neuber, 2008). Different clinical manifestations develop depending on the interaction between the infecting *Leishmania* species' virulence characteristics and a host's immune response (Pearson and Sousa, 1996). Three clinical syndromes have been recognised to date: cutaneous leishmaniasis (CL), considered to be the most commonly occurring one, mucocutaneous leishmaniasis (MCL), where lesions may extend from the mouth (lips, cheeks and palate) pharynx and larynx and which could lead to ulceration of the nasal mucosa and perforation of the septum (Pearson and Sousa, 1996), and visceral leishmaniasis (VL) where the parasite attacks internal organs, involving high mortality rates (Herwaldt, 1999). The WHO estimates that around 12 million people throughout the world are infected and that 350 million people are at risk of contracting the disease. Between 1.5 and 2 million cases occur annually, of which 1 to 1.5 million are caused by CL and 500,000 by VL (Alves and Bevilacqua, 2004; Tiuman et al., 2011).

Colombia is endemic for leishmaniasis throughout almost the whole country, except for the islands of San Andrés and Providencia; it is also the third country having the greatest amount of

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http://dx.doi.org/10.1016/j.actatropica.2016.09.020
0001-706X/

circulating parasite species infecting humans (Alvar et al., 2012). The Colombian Institute of Health's divulgation and surveillance system (Sivigila-INS, 2014) reported 10,358 cases of leishmaniasis in 2014, 10,195 cases of CL, 139 MCL and 24 VL; these figures were higher than those reported in the previous year (8724). Even though the disease is distributed throughout the whole country, it has greater prevalence in the Antioquia, Meta, Santander, Tolima, Nariño, Guaviare, Córdoba, Bolívar and Putumayo departments (Gomez and Zambrano, 2014). The Tolima, Antioquia, Córdoba, Nariño, Meta, Risaralda and Santander departments had the most cases of CL, greater prevalence occurring amongst males. It is worth noting that single lesions accounted for 61.6% of the cases, the extremities being the areas of the body which were most affected (66% localisation).

Leishmania panamensis is one of the important species causing zoonotic cutaneous leishmaniasis and mucocutaneous leishmaniasis in eight countries in Central and Southern America (Munoz and Davies, 2006). The cutaneous form represents 98% of all cases of leishmaniasis in Colombia; *L. panamensis* is the predominant species isolated from civil patient populations. According to its geographical distribution and the frequency of aetiological agents for cutaneous leishmaniasis in Colombia, *L. panamensis* causes 54–80% of cases and is distributed in the north and south-east of Colombia (Martinez et al., 2010). Treatment against leishmaniasis consists of toxic and poorly-tolerated drugs. Pentavalent antimonial drugs (SbV) (meglumine antimoniate and sodium stibogluconate) have been used during first line attention (Souza et al., 2010); however, some patients are affected by adverse effects and resistance to drugs as the mode of action by which the parasite produces such resistance remains unknown (Ait-Oudhia et al., 2011). Pentamidine and amphotericin B are used as second line, even though their use is restricted due to their costs and toxicity (Ouellette et al., 2004; Singh et al., 2012; Tiuman et al., 2011), causing severe and irreversible effects such as renal insufficiency (Herwaldt, 1999). The liposomal formulation of amphotericin B (ambisome and fungisome) is currently being used in high doses; it is a short-term treatment which is recommended for cutaneous leishmaniasis and visceral leishmaniasis, having few (insignificant) serious adverse effects (Goswami et al., 2016). However, the high cost involved in its formulation and use represents the major obstacle in developing countries like Colombia. The azole group has also been used (ketoconazole, itraconazole and fluconazole), usually in treating fungi; in spite of this, the results have not been clear regarding their anti-parasitic action (Croft and Coombs, 2003). Taking the above-mentioned into account, new anti-leishmanial therapeutic strategies are needed which must be efficient, less toxic for the patients and which do not induce resistance in parasites.

Larval therapy (LT) has been widely used in treating chronic wounds (Valachova et al., 2014), most success having been achieved in cases of necrotising fasciitis, perianal gangrene, surgery, burns, venous and arterial ulcers and those related to diabetic foot (Sherman, 2003; Zarchi and Jemec, 2012). Its mechanism of action consists of initial debridement of tissue and an anti-bacterial effect promoting disinfection (Davydov, 2011; Jaklic et al., 2008); increased granulation tissue production has also been described (Chambers et al., 2003). The larvae of the flies used for such purpose usually come from Diptera from the family Calliphoridae, *Lucilia sericata* (Diptera: Calliphoridae) being the most recognised species (Courtenay et al., 2000; Church, 1996; Namias, 2000). Other fly species have also been shown to be effective, such as *Lucilia caesar, Phornia regina, Calliphour erythrocephala, Cynomyia eachverina* and, more recently, *Lucilia cuprina* and *Lucilia eximia* (Paul et al., 2009; Sherman and Pechter, 1988; Wolff et al., 2010). Linger et al., 2016; have recently managed to produce transgenic *L. sericata* strains using genetic engineering techniques; the larvae expressed and produced human platelet-derived growth factor-BB (PDGF-BB). This represents a promising technology which should have a great impact for eventual use in larval debridement therapy and possibly having a greater range of applications regarding ulcers having differing origins (Linger et al., 2016). The native species *Sarconesiopsis magellanica* has been described in Colombia; it has anti-bacterial properties due to its larval excretions and secretions (ES) (Diaz-Roa et al., 2014) and the action of LT derived from this fly has also been evaluated in an animal model (Diaz-Roa et al., 2016; Gongora et al., 2015).

The effectiveness of LT in treating CL in murine and hamster models has been reported recently. A pilot test on Syrian hamsters (*Mesocricetus aureatus*) where *L. amazonesis* was inoculated into the footpad of one of the animals' rear legs has been carried out (Arrivillaga et al., 2008); reduced ulcer size and cicatrisation were observed, as well as no new symptomatology appearing. Other work tested the effects of larval ES obtained from *L. sericata* stages II and III on *L. tropica* promastigotes and amastigotes, showing that these substances were innocuous against the promastigotes; nevertheless, the appearance of lesions was avoided in laboratory mice which had received 3 doses of the aforementioned substances when administered before the time when clinical manifestations appear. Similarly, an effective reaction regarding the elimination of lesions was recorded in animals treated after the appearance of a lesion (Polat et al., 2012). In a following study by the same group, LT effectiveness was shown in human patients who resisted treatment with meglumine antimoniate, a better response regarding healing cutaneous lesions being obtained in less time (Polat and Kutlubay, 2014).

An experimental animal model which involved using golden hamsters (*Mesocricetus auratus*) was used in the present work for evaluating and comparing the action of LT and the larval ES derived from the blowfly species *L. sericata* and *S. magellanica* against cutaneous lesions caused by infection by the parasite *L. panamensis*. The evolution of the lesions treated with the larvae and their ES was also determined using recognised macroscopic scales; biopsies taken from the ulcers were used for histological and molecular analysis. The *S. magellanica* larvae and the species of parasite selected here were used for the first time in this type of study.

2. Materials and methods

2.1. The animals used for experimentation, infection and treatment

Eighteen 6- to 8-week-old golden hamsters (*Mesocricetus auratus*) weighing 110–140 g were used; they were divided into 6 groups for evaluating LT treatment derived from the two blowfly species (*L. sericata* and *S. magellanica*) and their respective ES (before and after the appearance of lesions). Table 1 summarises each group of hamsters used in the experiments (infected and uninfected), the treatments used and days post-infection (PI) when they were begun. The hamsters from each group (except for 6) were infected with 1×10^7 *L. panamensis* promastigotes in a 200 μL volume (MHOM/CO/87/UA140 strain), kindly donated by Dr Sara Robledo (PECET Group, Universidad de Antioquia, Medellín, Colombia). The dose was injected via intradermic puncture in the lumbosacral region of the back of the animals (Robledo et al., 2012). Before inoculating the parasites, the hamsters were anaesthetised with an 80 mg/mL concentration of ketamine and 10 mg/mL of xylazine, calculated for Kg of weight, to anesthetise and immobilise them for depilation and infection with the pathogens. Parasite concentration was estimated in a Neubauer chamber from a culture of the strain in stationary phase.

LT treatment derived from each fly species was used with group 1 after the appearance of a lesion in the lumbosacral region; this