ABSTRACT

Leishmaniasis currently remains as a public health problem that it has become important, especially in developing countries, where the disease is endemic for most of them. Increased reports on therapeutic failures by the rise of resistant strains as well as the severity of the side effects known for current treatments have promoted an imperative searching for novel antileishmanial agents. Such exploration has established that, in addition to the parasiticide effect, an immunomodulatory action could significantly contribute in therapeutics against leishmaniasis. The crucial observation through findings on immunomodulatory compounds is the non-activity against promastigotes but selective parasiticide effect against macrophage-internalized amastigotes. However, the published examples of this type of action are few but very interesting. Therefore, the present work summarizes some naturally-occurring phenolic-type compounds with antileishmanial activity whose effect has been suggested to be exerted through an immunomodulatory mechanism.

Keywords: Phenolics, Leishmania, immunomodulatory.
La leishmanioisis permanece actualmente como un problema de salud pública que ha cobrado gran relevancia, sobre todo en países en vía de desarrollo, en cuya mayoría, la enfermedad es endémica. El incremento de reportes de fallo terapéutico por la aparición de cepas resistentes así como la severidad de los efectos adversos conocidos para los tratamientos actuales, han hecho imperativa la búsqueda de nuevos agentes antileishmaniales. En esta búsqueda se ha establecido que, adicional al efecto parasitocida, un potencial inmunomodulador podría contribuir significativamente en la terapéutica contra leishmaniosis. Una observación importante, a través de los resultados relacionados con compuestos que poseen efecto inmunomodulador, es la actividad parasitocida selectiva contra la forma amastigote internalizada en macrófagos, ya que la acción contra promastigotes es prácticamente nula. No obstante, en la literatura se encuentran muy pocos ejemplos de este tipo de acciones aunque son muy interesantes. Por lo tanto, el presente trabajo muestra una revisión acerca de compuestos fenólicos con potencial antileishmanial, cuyo mecanismo sugerido es la inmunomodulación.

Palabras clave: Compuestos fenólicos, Leishmania, Inmunomodulación

INTRODUCTION

Since 1988, the term leishmaniosis, instead leishmaniasis, is the name kept by the World Association for the Advancement of Veterinary Parasitology (WA-AVP) and the World Federation of Parasitologists, according to the “Standardized Nomenclature of Animal Parasitic Diseases” (SNOAPAD) (Velez, 1995) in order to refer to the disease caused by parasites of the genus Leishmania. However, the term of leishmaniasis is used more frequently than leishmaniosis (Kassai, 2006).

Leishmaniosis is defined as a group of diseases with high rates of morbidity and mortality in most developing countries (WHO, 2006). The disease is caused by various species of Leishmania (Kinetoplastidae order). 15 species of Leishmania are well known for causing the illness in humans, but 13 of them exhibit zoonotic nature (Myler and Fasel, 2008). Leishmania spp. is transmitted by ca. 30 species of sandflies of the genus Lutzomyia in the New World, and the genus Phlebotomus in the Old World. In Colombia, L. panamensis and L. braziliensis are the etiological agents associated with more widely distributed leishmaniosis (Saravia et al., 2002). However, a recent study has shown an increasing in the prevalence and distribution of L. guyanensis in Colombia, reporting it as the etiologic agent in 94.6% cases in three zones in Tolima (Rodriguez-Barraquer et al., 2008), whose incidence until that report was confined to the Orinoco and Amazon basin regions (Saravia et al., 1998).

EPIDEMIOLOGY AND CLINICAL ASPECTS OF LEISHMANIOSIS

Depending on the parasite species involved into the infection and some host factors (taking into account the symptoms exhibited by individuals after infection) leishmaniosis can be classified in three clinical syndromes: cutaneous leishmaniosis (CL), mucocutaneous
leishmaniosis (MCL) and visceral leishmaniosis (VL). The last one is the most critical form of the disease, which can be lethal if it is untreated (Sharma and Singh, 2009).

Leishmaniosis is contemplated to be prevalent in 88 countries, but 32 of them really consider it as endemic. This condition has led to an underestimation of reality as well as a significant public health problem (Assimina et al., 2008). This disease affects more than 12 million people worldwide. It is considered that 350 million people are at risk of acquiring it, and each year worldwide are reported ca. 2 million new infections (WHO, 2009). In Colombia, the disease is manifested as endemic. In 1994, 1079 municipalities were found to have leishmaniosis reports, predicting a change in the incidence rate within 2.00-6.66 range per 100,000 subjects in the rural population (King et al., 2004). Between 2003 and 2004, 10,000 cases/year were reported, and in 2005 the number of cases was close to 18,000 (Robledo et al., 2006). During 2009, 15,474 cases were reported, 98.9% of them were found to be cutaneous leishmaniosis (Gutierrez-Dueñas, 2010). In 2010, the scenario did not change and the System of Public Health Surveillance (SIVIGILA, Colombia) reported 14,851 cases of leishmaniosis (98.7% cases of cutaneous leishmaniosis) (Gutierrez-Dueñas, 2011), while during 2011, 9241 cases were recorded (98.07% of these were found to exhibit cutaneous type) (Gómez-Romero, 2012). However, it is important to note that these statistics do not consider the underreporting of the disease. For Colombia, 75% cases of CL were reported in Antioquia, Caquetá, Guaviare, Meta, Nariño, Santander, and Tolima Departments. Meanwhile, cases of VL were found to be concentrated in Bolivar, Cordoba, Sucre, and Tolima (PAHO, 2007).

**TREATMENT, SIDE EFFECTS AND DRUG RESISTANCE**

Pentavalent antimonial salts are the first-line treatments, which should be either intramuscularly or intravenously administered during an average of 4 weeks, despite their reported high toxicity (Rodrigues et al., 1999). The cost of such treatment might be US$ 30 (generic sodium stibogluconate), US$ 120 (meglumine antimoniate, Glucantime®), and US$ 150 (sodium stibogluconate, Pentostam®) (Reithinger and Coleman, 2007). In clinical cases where therapeutic failure is reported, the second-line drugs for the control of leishmaniosis are highly toxic and their costs range from US$ 60 (Amphotericin B) and US$ 70 (Pentamidine, Pentacarinat ®). Although the liposomal amphotericin B has not been associated with serious adverse effects, its high cost does not favor the accessing for the most affected population (it can exceed US$ 1500 per treatment) (WHO, 2006).

In addition to the costs and adverse effects-derived drawbacks, the problematic is increased by complications such as long distances for accessing health centers and the transportation difficulties, among other problems which have promoted the occurrence of resistant strains towards treatment (Pérez-Victoria et al., 2006; Rojas et al., 2006; Ribeiro-Gomes et al., 2004). This resistance acquisition (or susceptibility loss) by the parasite, along with the lack of therapeutic options, is reflected in dosage and therapy duration increases (Croft et al., 2006), as so in the incidence of the known adverse effects for these formulations, even until death (Monzote, 2009). The resistance of antileishmanial chemotherapy has been reported in Southern Europe, Iran, Northeast India and South America (Rojas et al., 2006; Mohapatra, 2014).

All above-mentioned facts, joined to the lack of a vaccine, render a requirement based on the imperative searching for new chemotherapeutic alternatives. Understanding the pathogenesis of leishmaniosis, the development and implementation of immune based treatments could offer better opportunities for a successful therapy. Because of that, naturally-occurring compounds possessing leishmanicidal
and immunomodulatory properties would be preferable ones (Singh and Sundar, 2014). There are many considerations on the important role that the regulatory factors from host immune status have on natural resistance or susceptibility against infection (Faleiro et al., 2014).

**IMMUNOMODULATORY ROLES IN LEISHMANIOSIS CONTROL**

The role of the immune system in the development of leishmaniasis has been well-established to be crucial. In this context, some individuals are naturally able for controlling the infection. Thus, following the experimental evidence is exposed remarking the role of the immune system response in the pathology control.

In LC-infected individuals [who respond favorably to the treatment with sodium stibogluconate (Sb)] has been reported a decrease in the expression levels of IL-13 (cytokine of a Th2 profile) in contrast to that observed in individuals who fail treatment, and even, it has been seen that these individuals are characterized by the production of IL-10 in macrophages and T cells (Maurer-Cecchini et al., 2009). This result is consistent with the inhibitory effects of IL-10 on IL-12 (Aste-Amezaga et al., 1998) and IFN-γ (D’Andrea, 1993).

In “knock-out” C57BL/6 mice has shown a marked reduction in the effectiveness of Sb for the IFN-γ gene. In addition, LV-infected patients treated with Sb together with recombinant IFN-γ replied to the treatment faster than those treated only with Sb (Sundar et al., 1995). A study involving L. peruviana-infected patients that exhibited no response towards treatment with meglumine antimoniate indicates that when patients were treated with this pentavalent antimoniate therapy combined with imiquimod (an immunomodulatory drug), 90% patients resolved the infection after 6 months (Arevalo et al., 2001). The foregoing evidence suggests that immunomodulatory activity should be an important feature in the search for antileishmanial agents, which would give advantages by enhancing the activity and/or activate the production of soluble mediators such as cytokines and chemokines. These mediators, in the context of the disease, could be associated with protection (Ji et al., 2003).

**PHENOLIC COMPOUNDS WITH LEISHMANICIDAL AND IMMUNOMODULATORY ACTIVITIES**

Several reports have raised the antileishmanial effects by phenolics could be exerted through immunomodulatory mechanisms. Proanthocyanidins, such as catechin-(4a,8)-catechin and fisetinidol-(4
α,β-catechin 2, obtained from Khaya senegalensis were evaluated for their leishmanicidal potential on intracellular parasites of Leishmania spp. Although they showed no activity on promastigotes of L. donovani, L. major, L. infantum and L. enriettii, these compounds were active against intracellular amastigotes of L. donovani with EC₅₀ ca. 3.9 µg/mL (EC₅₀ of reference drug, Pentostam®, was 7.8 µg/mL) (Kayser and Abreu, 2001). These results open the possibility to speculate that compounds: 1) could act selectively against amastigotes, 2) could be modified within the host cell and the resulted compound has the antileishmanial activity, or 3) could activate the microbiidal machinery of the host cell. Considering the latter fact, it was evaluated the tumor necrosis factor (TNF) production after exposure to test compounds. Interestingly, both compounds were associated with a TNF production very similar to that shown by the positive control (LPS + IFN-γ), supporting the second hypothesis on immunomodulatory activity-mediated antileishmanial effect. These experiments have been extended to other structurally-related compounds. Within the study performed by Kolodziej et al., 2001, twenty-seven phenolics (some hydrolyzable tannins and related compounds such as ellagitannins, gallo-tannins, C-glucosidic ellagitannins, and dehydroellagitannins) were evaluated for its leishmanicidal potential. None showed activity against promastigotes of L. donovani once more, but all phenolics were active against intracellular amastigotes. In addition to these results, other studies have recorded the trend of phenolics (Kolodziej and Kiderlen, 2005; Kiderlen et al., 2001) suggesting common action mechanisms.

In 2003, Radtke et al. published the evaluation of several sage phenolics against a panel of Leishmania parasites. They also found that these compounds exhibited parasiticide activity by stimulating the immune response. The immunomodulatory effects were then evaluated on macrophage’s functions in macrophage-like RAW 264.7, including variation of levels of TNF, interleukin-6 (IL-6), and interferon (IFN), when cells were infected with parasites. Similarly, tested compounds were considered not active against promastigotes but caffeic acid 3, salvianolic acids K 4 and L 5, and the salvianolic acid I methyl ester 6 exhibited noticeable antileishmanial activities against intracellular amastigotes (IC₅₀ 3-23 nM vs. 10-11 nM for the reference Pentostam) and they also showed no cytotoxicity on macrophages (Radtke et al., 2003). However, test phenolics activated Leishmania-infected host cells for releasing TNF ranging 22-117 U/mL and IL-6 ranging 3-42 U/mL. In contrast, their TNF- or IL-6-inducing potential in experiments with non-infected host cells was negligible. The results supported the promising scenario that plant phenolics could be qualified for this mode of action in parasite control.

On this context, extracts, coumarins and phenols obtained from a plant of Geraniaceae family, Pelargonium sidoides, were evaluated for their effects on nonspecific immune functions using an in vitro model for intracellular infection with s parasites (Kayser et al., 2001).
On this context, extracts, coumarins and phenols obtained from a plant of Geraniaceae family, Pelargonium sidoides, were evaluated for their effects on nonspecific immune functions using an in vitro model for intracellular infection with Leishmania parasites (Kayser et al., 2001). Again, test substances exhibited no important activity against promastigotes of L. donovani. Nevertheless, extracts, gallic acid 7 and methyl gallate 8 considerably diminished the amastigotes viability within host cells. These data also indicated that the action of the tested substances probably work across activation of leishmanicidal macrophage functions. This fact was concluded by the levels of both TNF-α and inorganic nitric oxides (iNO) in supernatants of substances-treated macrophage cultures. Due to the large amounts of 7 and 8 in P. sidoides, these metabolites were identified as the prominent immunomodulatory principles (Kayser et al., 2001). Identical trend was recently found by Cabanillas et al., 2014, on evaluating various phenolic compounds (diarylheptanoids 9 and flavonoids 10) isolated from leaves and rhizomes of Renealmia thyrsoides (Zingiberaceae). Test compounds exhibited activity against L. amazonensis axenic amastigotes at different levels but they were also active in the activation of PPARγ nuclear receptor in macrophages. PPARγ is an excellent regulator of specific surface receptors involved in the recognition and
internalization of *Leishmania* parasites, which is well-known to promote the host response (Galès et al., 2010; Chan et al., 2012).

The immunomodulator behavior was also confirmed by Zhu et al., 2013 through the evaluation of extracts and phenolic compounds obtained from rhizomes of *Osmunda japonica*, which exhibited divergent immunomodulatory activity under *in vitro* conditions using the rat peritoneal cells. The extracts stimulated the NO production, and secretion of IFN-γ, TNF-α and IL-1β. While minor NO enhancement was generated by the aldehyde-type phenolics (such as 4-hydroxybenzaldehyde 11 and 3,4-hydroxybenzaldehyde 12), the benzalacetone-type phenolics (such as 4-hydroxybenzalacetone 13 and 3,4-hydroxybenzalacetone 14) inhibited production of immune mediators including cytokines (TNF-α, IL-1β, IL-6), NO, and PGE2. These findings also indicated the excellent potential of some phenolics on immunomodulatory activity.

The phenolic lignan licarin-A 15 inhibited the growth *Leishmania* major promastigotes, by inducing DNA fragmentation (Néris et al., 2013). This compound was more active against intracellular amastigotes (IC50 = 9.6 vs EC50 = 4.7 μg/mL). The activity against amastigotes was associated with immunomodulatory effects, since licarin-A-treated macrophage cultures exhibited a decrease in the interleukin (IL)-6 and IL-10 production. Other lignan, niranthin 16, isolated from the aerial parts of the plant *Phyllanthus amarus*, was found to be a potent anti-leishmanial agent by inhibition of the proliferation of *Leishmania* amastigotes in infected murine macrophages (with no significant cytotoxicity on the host cells) (Chowdhury et al., 2012). The effect of 16 is considered to be conducted through a Th1-type immune response, which causes NO production. On this context, main lignans and phenolics (occurring in fractions of *Myristica fragans*) significantly inhibited, in a dose dependent manner, the production of IL-2,
IL-4 and IFN-γ cytokines in Con A-stimulated lymphocytes (Checker et al., 2008).

Flavonoids have been recognized as active principles against Leishmania amastigotes. Some of them include fisetin 17, 3-hydroxyflavone 18, luteolin 19, quercetin 20 (Tasdemir et al., 2006), hispidulin 21, santin 22 (Sülsen et al., 2007) and flavonoid dimers (Wong et al., 2014). However, their immunomodulatory properties remain unknown. The evaluation of their effects on macrophage immune functions could serve as good information for synergistic antiparasitic compounds with the macrophages as primary effectors (Nahrevanian, 2006). Within this context, Ercil et al., 2005, evaluated 7 flavonoid glicosides (including kaempferol 3-O-(2”',3”'-di-O-galloyl)-β-D-glucopyranoside 23) isolated from Geranium pyrenaicum, against a panel of Leishmania species (L. major, L. donovani and L. amazonensis) on amastigote stage. These compounds also showed appreciable in vitro activities (IC_{50} 4-27 nM) in comparison with Pentostam (IC_{50} 10-11 nM). The results provided evidence that flavonoid glycosides are able to stimulate defense mechanisms (through TNF, IFN-γ and IL-6 levels) in Leishmania-infected RAW 264.7 cells.

In conclusion, the published results indicated the good antileishmanial of some phenolics. An important observation through findings regarding test compounds is the selective parasiticide activity against macrophage-internalized amastigote stage, since the action against promastigotes is practically ineffective. This fact is an indicative of the antileishmanial activity by stimulating the host cell immune response. So far, the results therefore show a suggestive value of phenolics as antileishmanial agents.

However, the evaluations to estimate its immunomodulatory potential are very scarce and insufficient, so further works are required to address in more detail the promising immunomodulatory effect of phenolics and any other secondary metabolites with remarkable antileishmanial effects.
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