

Studies on the Effectiveness of Diarylheptanoids Derivatives against *Leishmania amazonensis*

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In a previous work we demonstrated that diarylheptanoids extracted from Centrolobium sclerophyllum are very active against Leishmania amazonensis promastigotes. In order to continue our studies with these class of compounds, we decided to evaluate the activity of several diarylheptanoids derived from curcumin (diferuloyl methane) against the extracellular form (promastigotes) of L. amazonensis. Furthermore, an experiment against the intracellular form of the parasite (amastigotes) was carried out, comparing the most active compound among the curcumin derivatives (the methylcurcumin) with des-O-methylcentrolobine, the most active diarylheptanoid derived from C. sclerophyllum.

Key words: *Leishmania amazonensis* - *Centrolobium sclerophyllum* - diarylheptanoids

Leishmania parasites causes a disease which has been associated with different clinical forms, including cutaneous, hyperergic mucocutaneous and the anergic diffuse leishmaniasis (Leon et al. 1990). The disease is endemic in some geographical areas of Brazil, where it constitutes a serious health problem. *L. amazonensis* has been isolated from patients with visceral disease or with post-kalazar dermal leishmaniasis (Barral et al. 1991, Leon et al. 1992). The drug of choice for the treatment of leishmaniasis are the pentavalent antimonials (Sb^V) which presents renal and cardiac toxicity. Besides, the precise chemical structure and mechanism of action of these drugs are unknown up to date. The second choice for the treatment of the disease is a diamidine (pentamidine isethionate), which also cause serious side effects.

In order to find new drugs against leishmaniasis, we have been studying compounds belonging to the class of diarylheptanoids. In a previous work (Araujo et al. 1998) we described the des-O-methylcentrolobine, a phenolic diarylheptanoid derived from *Centrolobium sclerophyllum*, as highly effective against the extracellular form

(promastigotes) of *L. amazonensis*. In the present work, we are evaluating the effectiveness of phenolic/non-phenolic diarylheptanoids derived from *Curcuma longa* L, obtained through several chemical modifications in the original chemical structure of the curcumin (diferuloyl methane), in order to potentially increase their activity against the extracellular form of *L. amazonensis*. Furthermore, a comparative study were done between the most active compound from *C. sclerophyllum* and the methylated derivative from curcumin against the intracellular form (amastigotes) of *L. amazonensis*.

MATERIALS AND METHODS

Culture and maintenance of the parasite - *L. amazonensis* promastigotes, MHOM/BR/77/LTB0016 strain, were grown at 25°C in LIT medium supplemented with 10% (v/v) heat inactivated fetal calf serum (FCS). Cells were harvested in the late log phase, resuspended in fresh medium, counted in Neubauer's chamber and adjusted to a concentration of 4X10⁶/ml. This strain has been characterized by molecular and immunological techniques (Grimaldi Jr et al. 1987).

Curcumin derivatives - The original curcumin used was from commercial source and several structural modifications were done yielding the compounds showed in Fig. 1. The compounds were fully characterized by IR, ¹H and ¹³C NMR and mass spectrometry (Denise CF Gomes, Marco Edilson F Lima, Catarina AC Araujo, Leonor L Leon and Leila V Alegrio, manusc. in prep.).

In vitro drug assay - To the promastigotes culture in the above concentration, the compounds were added for screening (from 160 µg/ml to 5 µg/

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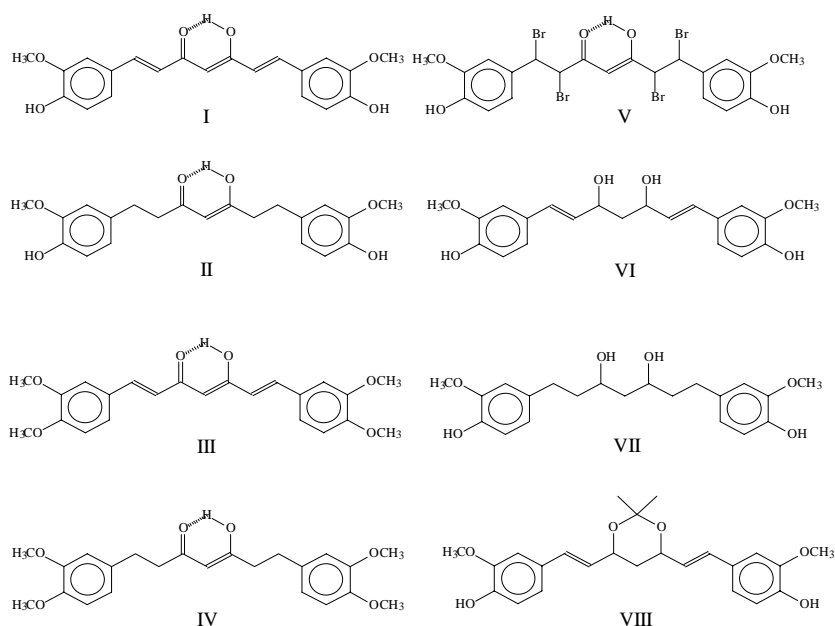


Fig. 1: chemical structures of the curcumin derivatives.

ml), solubilized in dimethylsulfoxide (DMSO) (the highest concentration used was 1.6 %, v/v) and incubated at 25°C. After 24 hr of incubation, the parasites were counted and compared with the controls: DMSO without the compounds and the parasites alone. All tests were done in triplicate and pentamidine isethionate (May & Baker Lab., England) was used as reference drug (Canto-Cavalheiro et al. 1997, Araujo et al. 1998).

In vivo drug assay - The *in vivo* experiment was carried out comparing the methylated curcumin derivative (LD90=35 µM) with des-O-methylcentrolobine (LD50 = 57 µM), a diarylheptanoid derived from *C. sclerophyllum*. *L. amazonensis* promastigotes (3X10⁶/50 µl/animal) and the drugs (20 mg/kg) were injected subcutaneously in the footpad of Balb/c mice (five animals/group). The lesions size (produced by intracellular forms of the parasites, the amastigotes) were measured at each 15 days in a period of 75 days.

RESULTS AND DISCUSSION

Our previous work (Araujo et al. 1998) was the first relate in the literature concerning the anti-*Leishmania* activity of compounds belonging to the class of the diarylheptanoids, being the most active a phenolic derivative, des-O-methylcentrolobine [2- β -(*p*-hydroxyphenyl)-ethyl]-6-(*p*-hydroxyphenyl)-tetrahydropyran] against *L. amazonensis* promastigotes. In the present work, phenolic and non-phenolic diarylheptanoids derivatives were stud-

ied, which had as original molecule a diferuloyl methane (curcumin).

The most active compound among the curcumin derivatives against the *L. amazonensis* promastigotes was the non-phenolic methylated derivative, with a LD50 < 5 µg/ml, LD90=35 µM (Table, Fig. 2). We could observe that in low concentrations, such as 5 µg/ml and 10 µg/ml the differences in the drug's activities were not significant ($p > 0.05$). On the other hand, in a range of concentration from 20 µg/ml to 160 µg/ml, the curcumin, methylated curcumin and dimethylketal curcumin showed a significantly high activity ($p < 0.05$), compared to the others curcuminoids. Also, the diol-derivatives were the less active compounds at all concentrations.

Data from the literature, suggested that the phenol group must be essential for a good biological activity, such as an anti-inflammatory effect (Mukhopadhyay et al. 1982). On the other hand, Cleason et al. (1993) described anti-inflammatory activity of three non-phenolic diarylheptanoids. In our experiments the non-phenolic compound (the methylated curcumin) was much more effective against *L. amazonensis*, than the phenolic derivative (des-O-methylcentrolobine).

Based in our previous *in vitro* results with des-O-methylcentrolobine [2- β -(*p*-hydroxyphenyl)-ethyl]-6-(*p*-hydroxyphenyl)-tetrahydropyran], a diarylheptanoid derived from *C. sclerophyllum*, which showed a LD50 of 57 µM (Araujo et al. 1998),

TABLE
Biological activity of curcumin and its derivatives against *Leishmania amazonensis*

Compounds	Mean of LD50 ± SD	
	µg/ml	µM
Curcumin	9 ± 2	24.4 ± 5
Reduced curcumin	>160	-
Methylcurcumin	< 5	-
Reduced methylcurcumin	48.5 ± 6	121 ± 15
Br-curcumin	90.5 ± 23	131.5 ± 33
Diolcurcumin	>160	-
Reduced diolcurcumin	>160	-
Dimethylketal curcumin	16.5 ± 2	40 ± 5

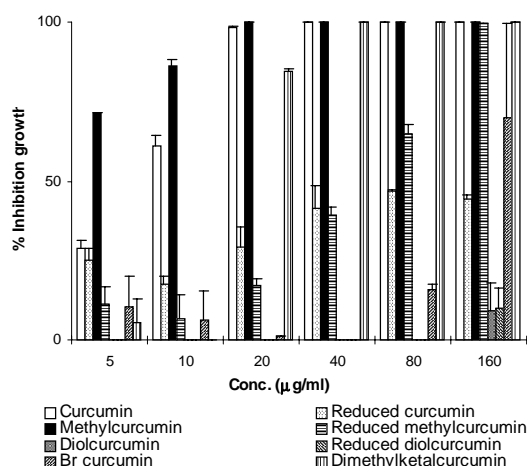


Fig. 2: the *in vitro* effect of the curcumin derivatives on *Leishmania amazonensis*.

an *in vivo* comparative study was carried out with this compound and the methylcurcumin. In this experiment, where only one dose of each compound was applied, the mice treated either with the des-O-methyl centrolobine or methylcurcumin, at the 45th day of following up, showed a decrease of 34.5% and 65.5% in the lesion size, respectively, when compared with the mice inoculated with the parasites alone. However, when we continued to evaluate the lesions of all the groups no difference was observed after that. It is interesting to notice that, in the overall, the difference in the amount of the compounds needed to inhibit about 50% of the promastigotes growth, had no influence in the *in vivo* results obtained.

Our data would suggest that the anti-*Leishmania* activity may be related mainly to others structural factors, such as: the presence of the β-dicarbonylic system; the α, β-non-saturated system associated to aromatic nucleus; and finally the electronic density increasing in the aromatic

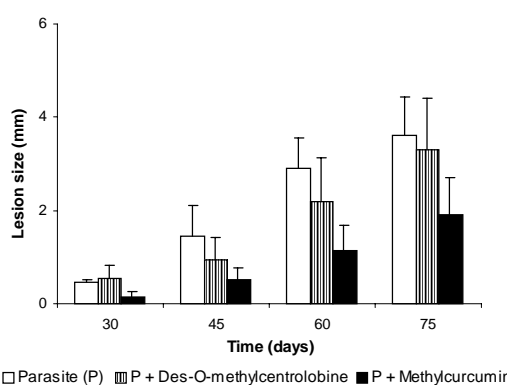


Fig. 3: the *in vivo* effect of the diarylheptanoids derivatives on *Leishmania amazonensis*.

nucleus. Seems that these factors potentialize the effectiveness of these molecules.

It is very hard to make any kind of association between the *in vitro* and the *in vivo* results, since we do not know which kind of change these compounds undergo inside the vertebrate host. However, it is important to say that there was no inflammatory reaction in the area where the drug was injected. Further *in vivo* different protocols, varying the inoculation route, inoculation time, etc, should be done in order to understand the potential mechanism occurring until the drug reach its target, e.g. the parasite.

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