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Letter to the Editor

Clinical isolates of New World *Leishmania* from cutaneous and visceral leishmaniasis patients are uniformly sensitive to tamoxifen

Sir,

Leishmaniasis affects 12 million people worldwide. Chemotherapy of leishmaniasis relies mainly on expensive and toxic drugs, and great efforts are being directed towards identifying new candidate drugs for the treatment of this neglected disease. We have recently described the activity of tamoxifen, a selective oestrogen receptor modulator, as an antileishmanial agent. Tamoxifen has been shown to possess in vitro activity against visceralising and cutaneous strains of *Leishmania* such as *Leishmania donovani*, *Leishmania infantum chagasi*, *Leishmania braziliensis*, *Leishmania amazonensis* and *Leishmania major*. Half maximal effective concentrations (EC_{50}) of tamoxifen for these parasites range from 9.3 ± 0.3 μM to 19.9 ± 0.3 μM in vitro and the drug is active against promastigotes and intracellular amastigotes at the same range of effective doses [1–3]. We have also demonstrated that treatment with tamoxifen for 15 days in rodent models of cutaneous and visceral leishmaniasis is able to control the disease with equal or better effectiveness as the standard antimonial treatment [2,3]. The aim of this study was to determine whether the antileishmanial activity of tamoxifen previously reported holds true for recent *Leishmania* isolates obtained from cutaneous and visceral leishmaniasis patients in Brazil.

Thirteen isolates of *Leishmania* spp. were obtained from patients attending the Anuar Auad Hospital for Tropical Diseases in Goiânia (Goiás, Brazil) and six isolates were from patients from Natal, Rio Grande do Norte, Brazil. Parasite isolation was obtained from lesion biopsies or bone marrow aspirates performed as part of the diagnostic procedure. Parasites were typed by isoenzyme electrophoresis at Instituto Oswaldo Cruz (Rio de Janeiro, Brazil) or by polymerase chain reaction (PCR) [4,5]. Promastigotes of *L.* (*L.*) *infantum chagasi* (MHOM/BR/1972/LD), *L.* (*Viannia*) *braziliensis* (MHOM/BR/75/M2903) and *L.* (*L.*) *amazonensis* (MHOM/BR/73/M2269) reference strains as well as of the clinical isolates were grown as described previously [1,3]. Promastigote drug susceptibility assays were performed by incubating parasites in the presence of increasing concentrations of tamoxifen (2.5–30 μM) or the control drug amphotericin B (AmB) (0.05–3 μM) for 24 h. Viability was assessed by MTT cleavage as described previously [5]. Drug activity against intracellular amastigotes was tested using BALB/c bone marrow-derived macrophages (BMDMs) as described previously [5]. Briefly, infected BMDMs were treated with increasing concentrations of tamoxifen (3–12 μM) or AmB. Half maximal cytotoxic concentrations of tamoxifen for BMDMs ranged from 20 μM to 30 μM. EC_{50} and EC_{90} (effective concentrations needed to control 50% and 90% of the parasites, respectively) values were determined from sigmoidal regression of the concentration–response curves using Origin 7.5 software (OriginLab Corp., Northampton, MA). Experiments were repeated at least twice using triplicate samples.

Table 1 shows the results of sensitivity testing for the 19 clinical isolates as well as the corresponding type strains included as test controls. Amongst six *L. infantum chagasi* isolates the EC_{50} for promastigotes ranged from 6.2 μM to 10.4 μM, whilst for *L. braziliensis* the EC_{50} varied from 6.0 μM to 10.9 μM. The highest EC_{50} (14.8 μM) was detected in a *L. amazonensis* isolate, well within the expected range based on data previously obtained with the type strains. Values determined for the EC_{90} against promastigotes of all cultured parasites were also uniform, with a mean of 16.95 μM [95% confidence interval (CI) 15.07–18.82 μM]. These findings are consistent with homogeneous sensitivity to the drug in the field, supported by the lack of significant differences between the isolates ($P = 0.6479$, Kruskal–Wallis test).

The sensitivity of amastigotes of 12 field isolates was also tested. In all cases but one, the EC_{50} for amastigotes was lower than the EC_{50} for promastigotes of the same isolate by a factor of 2–3 (Table 1). One isolate of *L. amazonensis* exhibited the same EC_{50} for promastigotes and amastigotes. We had previously observed with type strains that the sensitivity to tamoxifen of promastigotes and amastigotes of a given species was in the same order of magnitude, with amastigotes being slightly more sensitive. This early finding was corroborated now by comparing the EC_{50} means for promastigotes and amastigotes of 12 field isolates (promastigotes, mean $EC_{50} = 9.16$ μM, 95% CI 7.93–10.40 μM; and amastigotes, mean $EC_{50} = 4.51$ μM, 95% CI 3.58–5.43 μM). We therefore confirmed that promastigote testing can be used to evaluate *Leishmania* sensitivity to tamoxifen.

Of the 19 isolates tested in this work, 13 had been studied previously and their sensitivity to pentavalent antimony and AmB determined [5]. Interestingly, whilst their sensitivity to AmB was also uniform, pentavalent antimony effectiveness was less regular, with EC_{50} values against intracellular amastigotes varying up to 8-fold [5].

This study offers support and justification for considering tamoxifen as a candidate for further development as an antileishmanial agent.

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Competing interests: None declared.

Ethical approval: This study was approved by the Ethics Committee for Animal Experimentation of the Instituto de Ciências Biomédicas da Universidade de São Paulo (approval certificate 033/07). The study was also approved by the Ethical Committees on Human and Animal Research of the Universidade

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Table 1
Susceptibility of *Leishmania* isolates to tamoxifen and the control drug amphotericin B (AmB).

Species	Isolate	Tamoxifen (μM)				AmB (ng/ml)	
		Promastigotes		Amastigotes		Promastigotes	
		EC_{50} ± S.D.	EC_{90}	EC_{50} ± S.D.	EC_{90}	EC_{50} ± S.D.	EC_{90}
L. (*L.*) *infantum chagasi*	MHOM/BR/1972/LD	7.8 ± 0.97	26.2	2.8 ± 0.09	5.9	27.2 ± 0.04	110.0
L. (*L.*) *infantum chagasi*	MHOM/BR/2005/3050	9.2 ± 0.41	17.2	3.3 ± 0.01	4.9	27.6 ± 0.30	59.0
L. (*L.*) *infantum chagasi*	MHOM/BR/2005/3052	10.4 ± 0.40	14.8	3.9 ± 0.01	6.4	36.8 ± 0.50	64.4
L. (*L.*) *infantum chagasi*	MHOM/BR/2005/3071	7.4 ± 0.70	14.8	2.6 ± 0.17	6.2	18.4 ± 1.20	60.7
L. (*L.*) *infantum chagasi*	MHOM/BR/2009/340BM	9.4 ± 0.47	12.5	3.7 ± 0.26	7.9	26.6 ± 0.40	46.9
L. (*L.*) *infantum chagasi*	MHOM/BR/2009/340SK	6.2 ± 0.17	11.3	3.1 ± 0.08	6.1	14.6 ± 1.60	33.2
L. (*L.*) *infantum chagasi*	MHOM/BR/2009/341	9.7 ± 0.22	13.9	3.9 ± 0.49	8.0	21.8 ± 0.80	39.7
L. (*L.*) *amazonensis*	MHOM/BR/73/M2269	11.4 ± 2.53	21.9	6.8 ± 0.15	11.0	114.8 ± 0.67	227.8
L. (*L.*) *amazonensis*	MHOM/BR/2004/EGS4	14.8 ± 0.99	22.7	6.8 ± 0.37	8.6	68.0 ± 3.80	213.2
L. (*L.*) *amazonensis*	MHOM/BR/2001/JRS1	9.0 ± 2.93	24.6	4.5 ± 1.17	9.8	58.9 ± 0.14	304.5
L. (*L.*) *amazonensis*	MHOM/BR/2006/JSC6	7.2 ± 0.47	14.8	7.2 ± 0.88	11.3	75.8 ± 1.20	175
L. (*Viannia*) *braziliensis*	MHOM/BR/75/M2903	10.8 ± 3.70	19.7	5.6 ± 0.3	9.0	93.0 ± 12.27	355
L. (*V.*) *braziliensis*	MHOM/BR/2006/BES6	6.0 ± 0.93	12.2	3.5 ± 0.15	7.3	53.9 ± 1.10	141
L. (*V.*) *braziliensis*	MHOM/BR/2006/EFSF6	9.3 ± 0.80	18.3	6.9 ± 0.02	9.8	41.5 ± 1.12	494
L. (*V.*) *braziliensis*	MHOM/BR/2006/GDL6	8.9 ± 1.05	14.0	3.0 ± 0.03	7.9	52.1 ± 2.38	237
L. (*V.*) *braziliensis*	MHOM/BR/2006/HPV6	10.9 ± 0.41	16.9	N/D	N/D	56.5 ± 0.98	170
L. (*V.*) *braziliensis*	MHOM/BR/2003/IMG3	9.2 ± 0.36	14.8	N/D	N/D	77.8 ± 0.53	253
L. (*V.*) *braziliensis*	MHOM/BR/2006/PPS6m	9.1 ± 0.95	21.5	N/D	N/D	37.9 ± 8.59	430
L. (*V.*) *braziliensis*	MHOM/BR//2005/RPL5	8.4 ± 0.41	15.6	N/D	N/D	54.8 ± 0.02	87
L. (*V.*) *braziliensis*	MHOM/BR/2006/TMB6	8.4 ± 0.20	14.1	N/D	N/D	49.5 ± 0.43	100
L. (*V.*) *braziliensis*	MHOM/BR/2006/UAF6	9.1 ± 0.05	14.6	N/D	N/D	86.3 ± 1.30	154
L. (*V.*) *braziliensis*	MHOM/BR/2005/WSS5	6.5 ± 0.57	12.4	N/D	N/D	50.7 ± 1.06	81

EC_{50}, half maximal (50%) effective concentration; S.D., standard deviation; EC_{90}, effective concentration needed to control 90% of the parasites; N/D, not determined.

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