Journal of Antimicrobial Chemotherapy (2001) **48**, 445–458

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Antileishmanial activity of the violacein extracted from *Chromobacterium violaceum*

J Antimicrob Chemother 2001; **48:** 449–450

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Sir,

Leishmaniasis is a disfiguring and sometimes fatal protozoan disease, affecting more than 12 million people worldwide. The drugs used to treat the disease were developed more than 50 years ago and are potentially toxic. *Leishmania amazonensis* is very common in Brazil and is associated with different forms of the disease, including cutaneous, hyperergic mucocutaneous, anergic diffuse cutaneous and visceral leishmaniasis.[1,2] The violacein {3-[1,2-dihydro-5-(5-hydroxy-1H-indol-3-yl)-2-oxo-3H-pyrrol-3-ylidene]1,3-dihydro-2H-indol-2-1} extracted from *Chromobacterium violaceum* was obtained as described previously.[3,4] Briefly, *C. violaceum* (CCT 3496) was cultivated on cotton in modified 1 L Roux bottles, was extracted with commercial ethanol, and was purified by filtration, Soxhlet extraction, crystallization and high-performance liquid chromatography. The violacein was analysed and identified by NMR spectroscopy, thermogravimetric analysis, mass spectrometry, UV-VIS spectroscopy and infrared spectroscopy.

An *in vitro* assay was performed with promastigote forms from *L. amazonensis* (MHOM/BR/77/LTB0016) in order to determine the ED_{50}. Infective promastigotes containing a high percentage of metacyclic forms were grown at 26°C in Schneider's *Drosophila* medium[5] (Gibco, Paisley, UK) supplemented with 10% (v/v) heat-inactivated fetal calf serum. Cells were harvested in the late log phase, resuspended in fresh medium, counted in a Neubauer chamber and adjusted to a concentration of 4×10^6 cells/mL. The characterization of this strain was performed by molecular techniques such as isoenzyme electrophoresis and indirect radioimmune assay using specific monoclonal antibodies.[6]

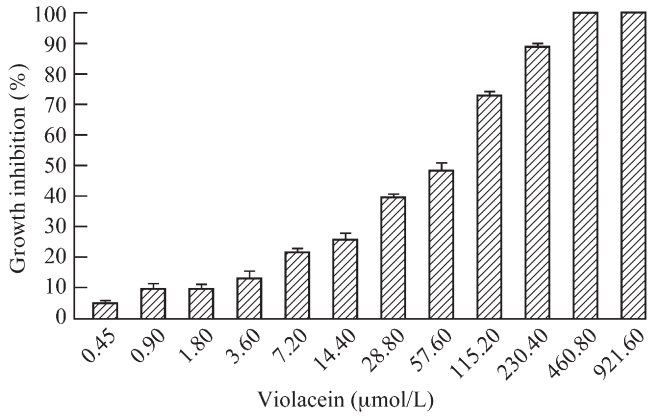

Figure. Effect of different concentration of violacein against *L. amazonensis* promastigotes.

To evaluate the activity of violacein, promastigotes (4×10^6 cells/mL) were incubated in the conditions described above, with the drug at a concentration ranging from 0.45 to 921.6 μmol/L. The violacein was solubilized in DMSO (Sigma) and diluted in Schneider's *Drosophila* medium (the highest concentration of DMSO was 1.4%, which was not toxic to the parasites). Controls were performed using cultures in the presence or absence of DMSO and the parasites alone. After 24 h of incubation, the surviving parasites were counted in a Neubauer chamber and compared with controls. All tests were done in triplicate and pentamidine isothionate (May & Baker Laboratory, UK) was used as a reference drug, in the same concentration range used for violacein.

The antileishmanial activity is expressed as EC_{50}/24 h, which is the concentration that causes 50% of the reduction in survival/viability compared with identical cultures without treatment (medium). Violacein showed a EC_{50}/24 h value of 4.3 ± 1.15 μmol/L (Figure). When compared with pentamidine, which is a second choice drug used in the treatment of leishmaniasis (EC_{50}/24 h = 0.46 μmol/L), violacein was 10 times less active. At 16.8 μmol/L pentamidine inhibited 100% of the growing of the promastigotes forms, while a violacein concentration of 460.8 μmol/L was necessary. Although more effective than violacein, pentamidine is extremely toxic, resulting in renal and hepatic problems, together with changes in the electro-

cardiogram. When male albino mice were treated with violacein (10 μmol/L for seven consecutive days) no side-effects were observed.[3]

Acknowledgements

This work was supported by Brazilian Research Council (CNPq) and the São Paulo State Research Foundation (FAPESP).

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