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Abstract (poster session)

Looking for new antiprotozoal drugs: betulin and betulinic acid derivatives induce antiproliferative activity through cell cycle arrest at the G0/G1 phase in Leishmania infantum cells

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Introduction: Leishmaniasis is a disease endemic in 98 countries affecting more than 12 million people. The treatment consists in pentavalent antimonial compounds, amphotericin B, pentamidine and miltefosine, among others. However, these current drugs are limited due to its toxicity, development of resistance, length of the treatment and high cost. Thus it is important continue the search for new effective and less toxic treatments. In the present study we evaluated the effects of new semisynthetic betulin and betulinic acid derivatives on Leishmania infantum promastigotes. Material and Methods: Promastigotes of L. infantum Nicolle (zymodeme MON-1) were maintained at 26ºC with weekly transfers in RPMI 1640 medium enriched with 10% inactivated fetal bovine serum. The anti-Leishmania activity of 16 synthetic derivatives of betulin (BT) and betulinic acid (BA) was evaluated by MTT method. The concentration that inhibited 50% of cell viability was determined via dose-response regression analyses (Graphpad prism 5.5). Effects on promastigotes were also analyzed by flow cytometry in order to study cell cycle and to evaluate phosphatidylserine externalization (apoptosis marker). The alterations on morphology and DNA integrity were also studied. Results and conclusions: Of the sixteen semisynthetic betulin and betulinic acid derivatives, only BT06 and AB13 inhibited significantly promastigotes viability, with IC50 values of 50.8µM and 25.8µM respectively. The higher anti-Leishmania activity of the betulinic acid derivative AB13 could be associated with higher capacity of Alpha-keto-Beta-unsaturated group for accepting electrons from physiologic nucleophiles (Michael addition reaction) compared to the imidazole carbamate group attached to C28 of betulin derivative BT06. The promastigotes exposed to AB13 suffered significant morphological changes, namely decrease of mobility, smaller size and rounder shape. None of the two derivatives showed to induced significant death by apoptosis/necrosis. However, both derivatives promoted the retention of L. infantum promastigotes in G0/G1 phase suggesting an arrest of the cycle at this stage. Inhibition of L. infantum promastigotes proliferation seems to represent a major mechanism of action of derivatives BT06 and AB13. These results thereby show that these two derivatives may be promising molecules for the development of new treatment strategies for leishmaniasis. Acknowledgements: This work was supported FCT POCTI (FEDER).