Evaluation of the antitumor and antiangiogenic potential of two new analogues of thalidomide

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Introduction: Phthalimides are analogues of thalidomide that have been researched for its antiangiogenic and immunomodulatory properties. Objective: To assess the in vitro cytotoxic and antiangiogenic activities in vivo of thalidomide and two analogues (SC-10 and SC-11). Methods: The in vitro studies were performed with Sarcoma 180-treated and -untreated cells harvested from the intraperitoneal cavity under aseptic conditions after 24 hours exposure (50 and 100µg/mL). Sarcoma cells were evaluated for cellular membrane integrity and internucleosomal DNA fragmentation by flow cytometry (Guava EasyCyte Mine). The cell lines were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 U/mL penicillin and 100 µg/mL streptomycin, at 37°C with 5% CO₂. Negative control was incubated with the vehicle used to dilute the substance (DMSO 1.6%). Doxorubicin (Dox, 0.3 µg/mL) was used as positive control. Data were compared by one-way analysis of variance (ANOVA) followed by the Newman-Keuls test (P < 0.01). The antitumor effect of thalidomide and two analogues was analyzed in mice transplanted with the Sarcoma 180 tumor and treated intraperitoneally (50mg/kg/7 days). Antiangiogenic activity was evaluated by the quantification of microvascular density (DMV) with immunostaining of intratumor endothelial cells with anti-CD-31. The digital images captured (200x) with windows® bitmap format (512 x 384 pixels) were analyzed by Morphometric Analysis System (SAMM), a software developed specifically for this purpose. Data were compared by one-way analysis of variance (ANOVA) followed by the Dunnett test (P < 0.01). Results: The cytometry analyzes of Sarcoma-treated cells showed that the analogues (SC-10 and SC-11) and the both concentrations tested (50 and 100 µg/mL) were capable to reduce cell membrane integrity (68.3 ± 0.5, 68.0 ± 1.7 and 69.6 ± 0.9, 46.1 ± 1.6 %) and to cause dose-dependent internucleosomal DNA fragmentation (24.9 ± 2.5, 48.4 ± 1.2 and 42.0 ± 1.6, 58.4 ± 1.1%, respectively) when compared to the negative control (86.1 ± 1.5 and 8.7 ± 0.5%) (P < 0.01), indicating the presence of cells with late stage apoptotic characteristics or in the process of secondary necrosis. In a similar way, Dox caused membrane disruption (58.4 ± 0.2 %) and DNA fragmentation (50.6 ± 2.0 %) (P < 0.01). The inhibition of the tumor growth was only significant in mice treated with thalidomide (53.5%) and the analogues SC-10 and SC-11 (67.9% and 67.4%, respectively) (P < 0.01), indicating the presence of cells with late stage apoptotic characteristics or in the process of secondary necrosis. In a similar way, Dox caused membrane disruption (58.4 ± 0.2 %) and DNA fragmentation (50.6 ± 2.0 %) (P < 0.01). The inhibition of the tumor growth was only significant in mice treated with thalidomide (53.5%) and the analogues SC-10 and SC-11 (67.9% and 67.4%, respectively) (P < 0.01). The immunohistochemistry analyzes of intratumor endothelial cells showed reduction of DMV in the groups treated with analogues SC-10 and SC-11 (2.7 ± 0.1 and 4.1 ± 1.5%) (P < 0.01), though thalidomide did not present antiangiogenic action (5.4 ± 0.5, P > 0.05), when compared to the negative control (7.8 ± 0.6). Conclusion: These results suggest antitumor activity of the two phthalimides analogues, probably triggered by apoptotic pathways and antiangiogenic action.