Activity of Crotalaria protease inhibitors on melanoma proteases

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Introduction: Melanoma is the most common cancer in Brazil and its incidence increases worldwide. New molecules that induce the selective death of tumor cells have been investigated for the cancer treatment. Proteases contribute to tumor development and their inhibition could represent an important strategy in the chemotherapy. Objectives: Study the protease inhibitor activity of Crotalaria extracts on secreted proteases by SK-Mel-28 and MV3 melanoma cells. Material and Methods: Melanoma cells were grown in DMEM, were harvested by centrifugation and the culture supernatant collected for proteases obtainment. Aqueous extracts using different systems were made from distinct organs of Crotalaria juncea (CJ) and Crotalaria spectabilis (CS). Protein content, SDS-PAGE analysis, protease activity and inhibition studies were performed. Results and Discussion: It was necessary to study the proteolytic activity of melanoma cells before proceed the inhibition with Crotalaria extracts. MV3 proteases hydrolyzed faster than SK-Mel-28 enzymes and all of them were able to hydrolyze peptide and protein substrates. Two proteases about 110 and 80 kDa exhibiting strong gelatinolytic activity were secreted by SK-Mel-28. Otherwise, MV3 exhibited only one active protease against gelatin with about 75 kDa. The pH influence and protease inhibitor assays indicated the presence of serine and metalloproteases in melanoma supernatants. These results suggested that inhibitors of these types of proteases could be more effective in suppressing tumor development. Inhibition tests using different extracts from leaf (CS-P and CJ-P), seed (CS-SP), stem (CS-CPVPP and CJ-CA), flower (CS-FA and CS-FPVPP) and root (CS-RA and CJ-RA) decreased the activity of SK-Mel and MV3 proteases in different ways, being MV3, more aggressive than SK-Mel and more sensible to protease inhibition. It is important to note that these extracts possess trypsin inhibitory activity. Conclusions: Crotalaria species are sources of protease inhibitors with tumoricidal activity, since proteases mediates crucial roles in cancer survival.

Key words: protease inhibitors, Crotalaria, chemotherapeutic agents, melanoma
Financial support: FIOCRUZ and FAPERJ.