FUNCTIONALIZED PLGA NANOPARTICLES TRIGGER APOPTOSIS BY RECEPTOR-MEDIATED PATHWAY

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Tumoral cells are known to have a higher ascorbic acid uptake by a family of integral membrane glycoproteins, called glucose transporters (GLUTs), than normal ones. Drug delivery systems are being broadly studied in current pharmaceutical research since they have several advantages in comparison with conventional therapies. Nanoparticles (NPs) can accumulate in tumors cells after administration, and their biodistribution is largely determined by their surface biochemical properties. Dehydrocrotonin (DHC, diterpene lactone) from *Croton cajucara*, Brazilian medicinal plant, has antitumoral properties. Previous results of our group showed that functionalized NP with L-ascorbic acid 6-stearate (AAS), NP-AAS-DHC suspension, was more effective as antitumoral than free DHC or NP-DHC in HL-60 cells. The aim of this study was to reach further insights of the nature of apoptosis mechanisms induced by DHC and its nanoparticles forms, evaluating caspase-8 and -9 activitie. In extrinsic (receptor dependent) and intrinsic (mitochondrial dependent) pathways of apoptosis, caspase-8 and –9, respectivelly, have been found to play a major role as initiator mediating apoptosis and thus allowing a sensitive detection of ongoing apoptotic activity. Caspase-9 activity in HL-60 cells was significantly increased by complexes NP-DHC and NP-AAS-DHC, compared to free DHC. However, there were no significant statistical differences between caspase-9 activation comparing NP-DHC and NP-AAS-DHC systems. Similar phenomena was observed in caspase-8 assay, but caspase-8 activities were slightly higher after treating with NP-AAS-DHC as compared to free DHC and NP-DHC, suggesting that NP-AAS-DHC promotes an increased activation of receptor-mediated apoptosis pathway and, therefore, a distinct cell-death mechanism. This study shows a promising application for the NP-AAS-DHC system for antitumoral treatment, but future *in vivo* studies will be necessary to comprove this improved efficiency.

Supported by FAPESP, CNPq
Key words: Drug delivery, Functionalization, Leukemia