VITAMIN D₃ POTENTIATES THE ANTITUMOR EFFECTS OF ARSENIC TRIOXIDE IN HUMAN LEUKEMIA (HL-60) CELLS

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Abstract: Recent studies in our laboratory have indicated that oxidative stress plays a key role in ATO-induced cytotoxicity in human leukemia (HL-60) cells. Pro-oxidants have been known to plays a role in free radical-mediated oxidative stress. Medical reports have indicated that Vitamin D₃, a well-known antioxidant works synergistically with tamoxifen to inhibit breast cancer cell proliferation. Therefore, the ultimate goal of this research was to determine whether supplementation of vitamin D₃ increases the activity of ATO toxicity in HL-60 cells. To accomplish this goal, HL-60 cells were treated either with 50% lethal dose of ATO alone or with ATO and different low doses of vitamin D₃. Live and death cells were determined by both trypan blue exclusion test and MTT assay, respectively. The extent of oxidative cell/tissue damage was determined by measuring MDA levels by spectrophotometry. Cell apoptosis was measured by flow cytometry analysis of phosphatidylserine externalization using Annexin V assay kit. The results of MTT assay indicated that vitamin D₃ exposure potentiates the toxic activity of ATO in HL-60 cells in a dose dependent manner. A similar trend was obtained with the trypan blue exclusion test. Co-administration of vitamin D₃ and ATO resulted in a significant (P<0.05) increase in MDA level compared to ATO alone. A statistically significant and dose-dependent increase (p <0.05) was recorded in annexin V positive cells (apoptotic cells) with increasing doses of vitamin D₃ in ATO-treated cells. This finding was confirmed by the result of DNA laddering assay showing clear evidence of DNA fragmentation in ATO and vitamin D₃ treated HL-60 cells. Overall, the present study indicates that vitamin D₃ potentiates the antitumor effects of ATO at least part, via oxidative stress and phosphatidylserine externalization.

Keywords: Vitamin D₃, arsenic trioxide, HL-60 cells, oxidative stress, apoptosis.

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