ASCORBIC ACID POTENTIATES ARSENIC TRIOXIDE-MEDIATED OXIDATIVE STRESS AND ACTIVATION OF P53 LEVELS IN CULTURED HUMAN LEUKEMIA (HL-60) CELLS

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Abstract: Acute promyelocytic leukemia (APL) is a subtype of acute myelogenous leukemia (AML), defined by the World Health Organization classification. Recent study in our laboratory indicated that ascorbic acid (AA) may improve the clinical outcome of arsenic trioxide (As$_2$O$_3$) for APL patients. However, the mechanism of chemotherapy agents that initiate tumor cell apoptosis through the involvement of oxidative stress is poorly understood. Therefore, the aim of the present investigation was to use human leukemia (HL-60) APL-cells as an in vitro test model to evaluate whether ascorbic acid (AA) modulates oxidative stress associated with As$_2$O$_3$ toxicity. To achieve this goal, we performed the trypan blue exclusion test for cell viability, lipid hydroperoxide assay for assessing the levels of the degradation products of polyunsatured fatty acid (PUFA) hydroperode generation in co-treated cells, and western blot analysis for p53 expression in cells treated with As$_2$O$_3$ alone and/or AA plus As$_2$O$_3$. The results of the trypan blue exclusion test indicated that AA treatment potentiates the cytotoxicity of As$_2$O$_3$ in HL-60 cells, as evidenced by a gradual increase in lipid hydroperoxide levels with increasing doses of AA. Western Blot analysis also showed a strong dose-response relationship with regard to p53 expression within the dose range tested. Taken together, these results indicate that the addition of AA to As$_2$O$_3$-treated HL-60 cells enhances the formation of reactive oxygen species (ROS). Our western blot analysis data demonstrates that As$_2$O$_3$ is able to induce cell cycle arrest through activation of the 53-kDa tumor suppressor protein. Based on these direct in vitro findings, our studies provide evidence that AA may extend the therapeutic spectrum of As$_2$O$_3$ and thereby improve the treatment of APL patients.

Keywords: Arsenic trioxide, HL-60 cells, MDA, ascorbic acid, lipid peroxidation

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