ARSENIC TRIOXIDE-INDUCED APOPTOSIS IN HUMAN LEUKEMIA (HL-60) CELLS

Clement G. Yedjou and Paul B. Tchounwou

Cellomics and Toxicogenomics Research Laboratory, NIH-Center for Environmental Health, College of Science, Engineering and Technology, Jackson State University, 1400 Lynch Street, P.O. Box 18540, Jackson, Mississippi, USA.

Abstract: Arsenic trioxide has been used as an effective chemotherapeutic agent in the treatment of acute promyelocytic leukemia (APL) and other type of cancers. Recent studies in our laboratory indicated arsenic trioxide significantly reduced the viability of human leukemia (HL-60) cells and other human cancer cells. However, the mechanisms by which arsenic inhibits growth or induces cancer cell death are still largely unknown. In the present study, we used human leukemia (HL-60) cells as a test model to investigate the in vitro effect of arsenic trioxide on cell growth inhibition and cell death mechanisms. To achieve this goal, we performed the flow cytometric analysis of phosphatidylserine externalization and caspase-3, and agarose gel electrophoresis for DNA fragmentation activity. Human leukemia (HL-60) cells were treated with different doses of arsenic trioxide for 24 h. The flow cytometric assessment (Annexin V) showed a strong dose-response relationship between arsenic trioxide exposure and early stage apoptosis in HL-60 cells. Upon 24 hrs of exposure, the results of annexin V/PI Staining showed that the percentages of early apoptotic cells were 1 ± 0%, 11.5 ± 0.7%, 19 ± 5.7%, 32 ± 4.6, and 18 ± 0% in 0, 2, 4, 6, 8 ug/mL of arsenic trioxide, respectively. The result of caspase-3 activity also indicated a significant increase (p< 0.05) of caspase 3 positive cells undergoing late apoptosis in arsenic trioxide-treated HL-60 cells. These results were further confirmed by the result of DNA laddering assay showing a clear evidence of DNA fragmentation in arsenic trioxide-treated HL-60 cells. In summary, these studies show that arsenic trioxide represents an apoptosis-inducing agent in HL-60 promyelocytic leukemia cells. Findings from these studies indicate that cell death induced by arsenic trioxide is associated through phosphatidylserine externalization and caspase-3 activation, as demonstrated by the results of flow cytometric assessment, and DNA fragmentation analysis.

Keywords: Arsenic trioxide, DNA fragmentation, HL-60 cells, annexin V, caspace-3

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