BIOMARKERS FOR APOPTOSIS IN ARSENIC-TREATED Hepatocellular carcinoma (HEPG2) CELLS

Erika Brown, Clement G. Yedjou and Paul B. Tchounwou

Molecular Toxicology 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 is a well-known toxic and carcinogenic agent associated with various human malignancies, including skin, lung, liver, kidney and bladder cancers. Studies reported by our laboratory show that oxidative stress and DNA damage play a key role in arsenic trioxide (ATO)-induced cytotoxicity in human cancer cells. In the present investigation, we used Hepatocellular carcinoma (HepG2) cells as a model to determine the apoptotic mechanisms involved in arsenic toxicity. To achieve this goal, apoptotic biomarkers were measured by flow cytometry analysis of phosphatidylserine externalization (Annexin V assay) and caspase 3 activity, DNA laddering assay, and western blot analysis of cytochrome c and Bcl-2. In regard to the annexin V assay, annexin V positive cell expression was not detected at 24 hours of ATO exposure. A slight dose-dependent increase \((p<0.05)\) was recorded in ATO-treated cells with regard to caspase 3 activation. These results were confirmed by data of the DNA laddering assay showing clear evidence of nucleosomal DNA fragmentation in ATO-treated cells. Results from the western blot analysis showed increased accumulation of cytochrome c within the treated cells and a down-regulation of Bcl-2. Taken together, our research demonstrated that arsenic-induced apoptosis is mediated by the mitochondria pathway as shown through the inhibition of Bcl-2 and release of cytochrome c followed by initiation of caspase.

Keywords: Arsenic trioxide, HepG2 cells, caspase 3, cytochrome c, Bcl-2, apoptosis

Acknowledgements: This research was financially supported by a grant from the National Institutes of Health (Grant No. 2G12RR013459-11), through the RCMI-Center for Environmental Health at Jackson State University.