ARSENIC TRIOXIDE CAUSES CELL CYCLE ARREST AND INDUCES INTRINSIC PATHWAY OF APOPTOSIS IN HUMAN LEUKEMIA (HL-60) CELLS

Sanjay Kumar, Clement Yedjou and Paul B. Tchounwou

Molecular Toxicology Research Laboratory, NIH RCMI-Center for Environmental Health School of Science and Technology, Jackson State University, 1400 Lynch Street, Box 18540, Jackson, MS 39217, USA

Abstract: Arsenic trioxide (ATO) has recently been successfully used to treat all-trans retinoic acid resistant relapsing acute promyelocytic leukemia (APL). Its use as induction and consolidation therapy has resulted in complete remission rate of both de novo and relapsed APL patients. However, the detailed molecular mechanisms of its therapeutic action are poorly known. We have used human leukemia (HL-60) cells as a model to elucidate the anti-cancer properties of arsenic trioxide. We hypothesized that ATO arrests cell cycle progression of HL-60 cells at S – phase and leading to cell death by intrinsic pathway of apoptotic signaling. To test the hypothesis, we used western blotting, confocal imaging and spectrofluorometric techniques to identify detailed cellular and molecular mechanisms of ATO action in HL-60 cells. We found that the expression levels of pro-apoptotic molecules (Bax) and cytochrome C were up-regulated, while that of anti-apoptotic Bcl2 protein was down-regulated at 4 and 6µg/mL ATO doses. We also found that ATO stimulates signaling molecules like P38, JNK in a dose dependent manner in HL-60 cells. Oxidative stress and related imbalance of pro- and anti-apoptotic molecules lead to change of mitochondrial membrane potential and opening of the mitochondrial membrane transition pores. Our confocal imaging data shows a clear translocation of Bax from cytosol to mitochondria and of cytochrome c from mitochondria to cytosol in ATO-treated cells. A dose-dependent decrease/drop-down in mitochondrial membrane potential was also observed in ATO-treated cells, as evidenced by JC-1 leveling and spectrofluorometric potential recording. On the basis of these findings, we conclude that mitochondrial pathway of apoptosis plays a key role in ATO pharmacology and should be further assessed as a drug target in APL chemotherapy.

Keywords: Arsenic trioxide, HL-60 cells, cell cycle arrest, apoptosis, mitochondrial pathway, APL treatment.

Acknowledgements: This research was financially supported by National Institutes of Health NCRR Grant No. 5G12RR013459 and MIMHD Grant No. 8G12MD007581, through the RCMI-Center for Environmental Health at Jackson State University.