ARISTOLOCHIC ACID INDUCED OXIDATIVE STRESS AND MODULATION OF Bcl2 IN LLC-PK1 CELLS

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Abstract: Aristolochic acid is an active ingredient responsible for the hepatotoxicity and renal toxicity of the Oriental herb Aristolochic fangchi. Consumption of products containing aristolochic acid has been associated with permanent kidney damage, sometimes resulting in severe kidney failure. Furthermore, some patients have developed certain types of cancers, most often occurring in the urinary tract. In this research, we hypothesized that oxidative stress plays a key role in aristolochic acid-induced toxicity and modulation of Bcl2 expression in LLC-PK1 cells. To test this hypothesis, we performed the MTT assay for cell viability, thiobarbituric acid test for lipid peroxidation, and western blot analysis for Bcl2 expression. Data generated from the MTT assay indicated that aristolochic acid significantly decreases the viability of LLC-PK1 cells in a dose-dependent manner. Upon 48 h of exposure, the cell viability was computed to be (100 ± 5)%, (97.8 ± 6)%, (89.2 ± 9.2)%, (87.2 ± 6.3)%, (77.7 ± 5.4)%, (68.9 ± 11.2)% and (19.6 ± 3)% in 0, 0.6, 1.25, 2.5, 5, 10, and 20 μM of aristolochic acid, respectively. Our result of thiobarbituric acid test resulted in a significant (p < 0.05) increase in malondiadehyde production with increasing doses of aristolochic acid. The result of Western Blot and the densitometric analyses demonstrated a strong dose-relationship with regard to Bcl2 expression in aristolochic acid-treated LLC-PK cells which was up-regulated at 6 μM following by a down-regulation between 9 and 12 μM. In summary, findings from this study demonstrated that aristolochia acid is cytotoxic to LLC-PK cells. This cytotoxicity is found to be associated with oxidative stress. Taken together, these data indicated that aristolochia acid is able to cause oxidative stress and probably cell cycle arrest through the expression of Bcl2 as confirm by our result of lipid peroxidation assay and western blot analysis.

Keywords: Aristolochic acid, MTT assay, lipid peroxidation, Bcl2 expression

Acknowledgements: This research was financially supported in part by a grant from the National Institutes of Health (Grant No. 1G12RR13459), through the RCMI Center for Environmental Health, and in part by the Department of Medicine at the University of Mississippi Medical Center. Jackson, Mississippi, USA.