BIOTRANSFORMATION AND CYTOTOXICITY OF BENZO(A)PYRENE IN HT-29 COLON CELLS

Jeremy N. Myers and Aramandla Ramesh

Department of Biochemistry and Cancer Biology, Meharry Medical College, 1005 Dr. D. B. Todd Jr. Blvd., Nashville TN, 37208, USA

Abstract: The majority of sporadic cancer deaths are attributed to environmental factors. Benzo(a)pyrene (BaP), a member of the Polycyclic Aromatic Hydrocarbons (PAH) family of compounds is an ubiquitous environmental toxicant; found in charcoal broiled/smoked meats, cigarette smoke, automobile exhaust, and industrial emissions. Diet makes up a substantial amount of BaP intake (~ 3 - 17 mg/person/day). The magnitude of human exposure to BaP through diet has generated a great deal of interest with regard to the association of ingested BaP with gastrointestinal carcinogenesis. Given the fact that colon cancer ranks 3rd among cancer-related mortalities, and in the United States alone around 60,000 lives are lost every year to colon cancer, it is necessary to understand the mechanisms that underlie BaP-induced colon carcinogenesis. Through metabolic activation, BaP is biotransformed into diol-epoxides that bind with DNA of the colon cells forming adducts, which initiate carcinogenesis by causing errors in DNA replication resulting in mutations. Benzo(a)pyrene biotransformation proceeds through specific biochemical pathways (epoxide and quinone) each one producing specific reactive metabolites. The binding of these metabolites to DNA, subsequent formation of adducts and persistence of these adducts are metabolite-specific in colon cells exposed to this toxicant. The purpose of this study is to determine the exposure concentration-specific biotransformation of BaP and the profiles of metabolites generated in HT-29 human colon cancer cells. The cells were exposed to BaP over the course of 10 days and then counted using a Coulter Counter. The concentrations of BaP used were 1µM, 5µM, 10µM, 25µM, 50µM, and 100µM. Based on a dose – response study 5µM, 10µM, and 25µM of BaP in DMSO (vehicle for BaP; 0.01%); 4 days after exposure were selected as optimal concentrations to analyze the effect of this toxicant on growth, viability, biochemical, and molecular endpoints in HT-29 cells. Analysis of cell cycle via FACS showed a distinct inhibition of the S and G2-phase in cell treated with increasing concentrations of BaP. The expression level of both Phase I and Phase II drug metabolizing enzymes were analyzed by western blot and RT-PCR. An increase in Cytochrome P450 1A1 and 1B1 expression occurred in a BaP concentration-dependent manner. Phase II metabolizing enzymes had equal expression levels irrespective of BaP concentrations used. In summary, our findings suggest the involvement of metabolic activation of BaP and subsequent generation of reactive metabolites contributing to cytotoxicity.

Key words: Benzo(a)pyrene, Polycyclic Aromatic Hydrocarbons, colon, HT-29 cells.

Acknowledgements: This research was supported by grants from the National Institutes of Health (Grant Nos. 1RO1CA142845-O1A1, 1RO3CA130112-01, 5T32HL007735-12) and the Southern Regional Education Board.