CHARACTERIZATION OF EPOXIDE ADDUCTS OF POLYCYCLIC AROMATIC HYDROCARBONS (PAH) WITH HEMOGLOBIN (HB)

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We investigated the binding of selected PAH epoxides (benzo(a)pyrene, benzo(b)fluoranthene, dibenz (a,hanthracene, fluoranthene, benzo(ghi)perylene, and benz(a)anthracene) to Hb in order to determine the overall extent of binding kinetics of the PAH to Hb. The following experiments were carried out using mouse (C57BL/6 male) and human Hb in vitro. Packed red cells were resuspended in isotonic saline (2 mls) and 100 l aliquots were used in reactions. PAH epoxide stock solutions were dissolved in tetrahydrofuran (1 mg/ml) and 10 l PAH stock was added to reactions which were carried out at 370 for various times. Reactions were stopped by addition of ice cold water and globin precipitated by addition of Hb to acidified acetone. PAH epoxides were released by incubation with pronase and PAH tetrols extracted using of liquid and solid phase extraction and analyzed spectrophotometrically by HPLC. Similar kinetics were found in both mouse and human suggesting that similar nucleophilic sites of attack of the epoxide are present in both species. In addition, these results point out that the mouse model is appropriate for carrying out investigational studies using carcinogens which potentially can be applied to human biomarker studies. The results demonstrated that that those carcinogens that are weakly active had only a slight degree of binding to hemoglobin, while those that are stronger, such as BPDE, had a larger degree of protein binding. All of the epoxide metabolites yielded detectable levels of Hb adducts that were in proportion to the known carcinogenicity of the parent PAH. These results suggest that the formation of hemoglobin adducts to PAH carcinogens may serve as reliable biomarkers of exposure as well as carcinogenicity.