

Role of Cytochrome P4501B1 in Benzo[a]pyrene Bioactivation to DNA-Binding Metabolites in Mouse Vascular Smooth Muscle Cells: Evidence from ³²P-Postlabeling for Formation of 3-Hydroxybenzo[a]pyrene and Benzo[a]pyrene-3,6-quinone as Major Proximate Genotoxic Intermediates

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Received September 9, 2002; accepted December 31, 2002

ABSTRACT

Benzo[a]pyrene (BP), a polycyclic aromatic hydrocarbon (PAH), is a potent atherogen and carcinogen in laboratory animals. Since genotoxic mechanisms may contribute to the development of atherosclerosis by PAHs, we have tested the hypotheses that: 1) BP induces DNA adducts in mouse aortic smooth muscle cells (SMCs); 2) 3-hydroxybenzo[a]pyrene (3-OH-BP) and benzo[a]pyrene-3,6-quinone (BPQ) are proximate genotoxic metabolites; and 3) cytochrome P4501B1 (CYP1B1) mediates the activation of BP and its metabolites to ultimate genotoxic intermediates. Cultured mouse aortic SMCs were treated with BP, 3-OH-BP, or BPQ for 24 h, and DNA adduct formation was analyzed by ³²P-postlabeling. In some experiments, cells were pretreated with the CYP1B1 inhibitor 1-ethynylpyrene (EP) prior to exposure to BP or its metabolites. BP, 3-OH-BP, and BPQ induced formation of several DNA adducts

that were not observed in dimethylsulfoxide-treated cells. Re- and cochromatography experiments indicated that 3-OH-BP and BPQ were proximate genotoxic metabolites of BP. DNA adduct formation was strongly inhibited by EP, a specific inhibitor of CYP1B1. BP treatment of SMCs resulted in induction of aryl hydrocarbon hydroxylase (AHH) activity and CYP1B1, but not CYP1A1, apoprotein. EP also blocked AHH induction by BP. In conclusion, the results of this study support the hypothesis that in SMCs, which are target sites for the development of atherosclerosis, the major bioactivation pathway of BP entails CYP1B1-mediated formation of the 3-OH-BP and BPQ, which are proximate genotoxic metabolites that may in turn get transformed to ultimate DNA-binding metabolites, which may contribute to atherogenesis by PAHs.