Oxygen radical formation during bioactivation of benzo[a]pyrene by rat liver and lung microsomes

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Benzo[a]pyrene (B[a]P) is a carcinogenic compound formed during combustion processes and is found in e.g. car exhaust, cigarette smoke etc. The molecule requires metabolic activation to exert its mutagenic, DNA damaging effects. One pathway of activation by cytochrome P450 is independent of free radical formation and results in stable B[a]P-7,8-dihydrodiol-9,10-epoxide (BPDE) DNA adducts. Another metabolic pathway is peroxidation via formation of a B[a]P-derived carbon-centered radical, which could result in oxygen radical production and oxidative DNA damage. In order to elucidate the contribution of both pathways in an organ-specific setting and to analyze the metabolic enzymes involved, ESR spectroscopy was used to detect radical formation at 37° C in rat liver and lung microsomes, utilizing different enzyme co-factors, scavengers and inhibitors in the presence of the spin traps DMPO or POBN. Bioactivation of B[a]P resulted in increased free radical formation in NADPH-supported rat lung microsomes, but not in NADPH-supported rat liver microsomes and in CuOOH-supported lung or liver microsomes. Both the values of the hyperfine constants of the POBN spin adduct as well as the characteristic 6-lines spectrum of the DMPO spin adduct showed that the metabolic activation of B[a]P resulted in solvent-derived carbon-centered radical formation. Experiments with scavengers and inhibitors revealed that the production of these radicals was caused by oxygen radicals produced during NADPH-supported rat lung microsomal B[a]P metabolism, and could be prevented by cytochrome P450 inhibition. Altogether, utilizing ESR spectroscopy and spin trapping techniques, it is shown that rat lung cytochrome P450-mediated B[a]P metabolism, but not rat liver mediated B[a]P metabolism, results in the production of reactive oxygen species.