Metabolically Activated Carcinogens and Mutations in the p53 Tumor Suppressor Gene in Lung Cancer

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The article by Smith et al. (1) in this issue of the Journal extends the elegant work of this group on mapping reaction sites of polycyclic aromatic hydrocarbon (PAH) diol epoxides and other activated carcinogens in the p53 tumor suppressor gene (also known as TP53) (2–4). Previously, these investigators (4) have shown that diol epoxides of benzo[a]pyrene (B[a]PDE) and benzo[g]chrysene, as well as N-acetoxy-2-acetylaminofluorene and aflatoxin B1 8,9-epoxide, preferentially bind at methylated CpG sequences in the p53 gene. In the present study (1), they mapped the distribution of adducts induced by diol epoxides of five PAH compounds—5-methylchrysene, 6-methylchrysene, 6-methylchrysene, chrysene, benzo[g]chrysene, and benzo[c]phenanthrene—in the nontranscribed strand of p53 in normal human bronchial epithelial cells. The results were consistent with those of their previous studies, in that CpG sequences were commonly adducted (2,4). Moreover, the distribution of adducts corresponded closely to the sites of highest mutation frequency in the p53 gene in lung cancers from smokers, but it was substantially different from that in lung cancers from nonsmokers. These results provide further support for the critical role of metabolically activated cigarette smoke carcinogens as the cause of lung cancer in smokers (5).

PAHs are prominent among the causative agents for lung cancer in smokers and in occupationally exposed workers (5–7). PAHs always occur in complex mixtures and, depending on their structures, have markedly differing carcinogenic activities. Benzo[a]pyrene (B[a]P) is a strong carcinogen and is always found in PAH-containing mixtures. It has frequently been used as a surrogate for PAH exposure and is probably the most extensively studied carcinogen, having been the focus of numerous investigations [reviewed in (8)] beginning in the 1930s. One purpose of the study by Smith et al. (1) was to determine whether the sites of adduct formation of several other PAH diol epoxides were similar to those of B[a]PDE, the major ultimate carcinogen of B[a]P. The authors chose diol epoxides of three chrysene compounds—5-methylchrysene, 6-methylchrysene, and chrysene—which are known to be the ultimate carcinogenic forms of their parent compounds (9,10). 5-Methylchrysene is a potent carcinogen, with activity equal to or greater than that of B[a]P, depending on the test system, while both 6-methylchrysene and chrysene are weakly tumorigenic or inactive (11,12). All three compounds occur in cigarette smoke. Levels of 5-methylchrysene are about 3% as great as those of B[a]P, whereas levels of 6-methylchrysene and chrysene are similar to those of B[a]P (13). The other two compounds used in this study, benzo[c]phenanthrene diol epoxide and benzo[g]chrysene diol epoxide, are ultimate carcinogens of two weakly carcinogenic parents, benzo[g]chrysene and benzo[c]phenanthrene (10,14). Neither parent compound has been routinely quantified in cigarette smoke. The rationale for including these compounds was that benzo[c]phenanthrene diol epoxide and benzo[g]chrysene diol epoxide both have their epoxide rings in sterically congested “fjord regions.” Compounds containing fjord regions have been associated with high DNA reactivity and tumorigenicity, and both of these diol epoxides are strong tumorigens, in contrast to the unmetabolized parent compounds (10,14). The overall pattern of DNA damage in the p53 gene was similar to that previously observed with B[a]PDE, but the relative adduct levels at each reactive site varied among the compounds. The most reactive compounds with the nontranscribed strand of exon 5 were 5-methylchrysene diol epoxide and benzo[g]chrysene diol epoxide, followed by benzo[c]phenanthrene diol epoxide. These results are quite consistent with previous comparative studies of diol epoxide–DNA reactions (15,16). With respect to the role of PAHs from cigarette smoke in human lung cancer, the results with 5-methylchrysene diol epoxide are probably the most important of those presented here because 5-methylchrysene is strongly carcinogenic, although its concentration in cigarette smoke is low.

While the sites of reaction of PAH diol epoxides are similar to the mutational hotspots in the p53 gene in lung cancers from smokers, this observation does not rule out the role of other cigarette smoke carcinogens as causes of these mutations, as Smith et al. (1) discuss. On the basis of their previous work (4), a variety of metabolically activated carcinogens react at these methylated CpG sites. Among carcinogens in cigarette smoke, reactions at these same sites might be expected from pyridyl-oxoybutylating intermediates derived from the tobacco-specific nitrosamines 4-(methylnitrosamino)-1-(3-pyridyl)-1-butane (NNK) and N’-nitrosonornicotine and hydroxylamines derived from aromatic amines. Other reactive agents in cigarette smoke, such as acrolein, crotonaldehyde, and reactive oxygen species, might produce similar damage (5). Further research is necessary to determine whether the sites of reaction identified here are indeed specific to PAHs.

A potential weakness of the study by Smith et al. (1) is the relatively high concentrations of the diol epoxides, 10–50 μM, used in their experiments. These concentrations are far higher than what would be expected in the human lung. Typical levels of the parent PAHs in cigarette smoke are 1–50 ng per cigarette, which translates to a maximum of 20–1000 ng per day uptake of these PAHs in a person who smokes 20 cigarettes. At most, only 1%–10% of these PAHs would be converted to diol epoxides; thus, the exposure to these metabolites would not exceed 2–100 ng (0.006–0.3 nmol per day). Assuming a lung volume of 3 L, the concentration of the diol epoxides in the lung might be estimated to be 0.002–0.1 nM, which is approximately 105–106 less than that used here. Clearly, the concentrations used were dictated by practical considerations. However, it should be noted that Conney and co-workers (17,18) have observed concentra-

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tion-dependent differences in mutational spectra in the hprt gene treated with B[a]PDE and benzo[c]phenanthrene diol epoxide.

In spite of these limitations, the study by Smith et al. (1) provides important additional support for the critical role of metabolically activated carcinogens in the induction of human lung cancer (5). Avoiding exposure to these compounds is the key to decreasing lung cancer incidence. Cigarette smoke, by far, the most common nonoccupational source of exposure to substantial amounts of lung carcinogens, and smoking causes 87% of the lung cancers occurring in the United States (19). The currently observed decrease in lung cancer incidence in males is clearly a result of the decreased prevalence of cigarette smoking (20). Hopefully, these trends will continue and lung cancer will ultimately return to the position as a relatively rare cancer that it had before the mass production and distribution of cigarettes at the beginning of the 20th century.

REFERENCES