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Aryl Hydrocarbon Hydroxylase, Epoxide Hydrase, and 7,12-Dimethylbenz[a]anthracene-Produced Skin Tumorigenesis in the Mouse

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SUMMARY

Mouse skin tumorigenesis initiated by 7,12-dimethylbenz[*a*]anthracene and promoted by repeated applications of phorbol ester is unrelated to genetic differences in the extent of aryl hydrocarbon hydroxylase induction by polycyclic hydrocarbons in inbred or hybrid C57BL 6N and DBA 2N mice. Thus, if aryl metabolism of 7,12-dimethylbenz[*a*] anthracene is a necessary step in the initiation of chemical carcinogenesis, the constitutive level of the hydroxylase activity in the skin of these mice is sufficient. Levels of hepatic epoxide hydrase activity are the same among inbred and hybrid C57BL 6N and DBA 2N mice and, in contrast to the hydroxylase, are not inducible by 3-methylcholanthrene. Epoxide hydrase activity could not be detected in the skin of these mice.

The initial event in the oxidative metabolism of polycyclic hydrocarbons is catalyzed by aryl hydrocarbon hydroxylase (1, 2), a substrate-inducible mono-oxygenase found mainly in mammalian liver microsomes but also present in other tissues (2–5), including skin (6, 7). The initial products formed by aryl hydroxylases such as aryl hydrocarbon hydroxylase from mono- (8), bi- (9), and polycyclic (10) hydrocarbons are reactive epoxides (arene oxides), which can (a) be converted to *trans*-dihydrodiols by the action of microsomal epoxide hydrase(s) (9, 13), (b) rearrange spontaneously to phenols (9, 14, 15), (c) conjugate with glutathione (9, 10, 11), or (d) combine covalently with cellular nucleic acids, histones (16), and other proteins (17). In cell culture, K-region epoxides are many times more active than the parent polycyclic hydrocarbons and the corresponding phenols and *cis*- and *trans*-dihydrodiols in producing malignant transformation (18, 19). Moreover, the magnitude of mutagenicity produced by K-region epoxides correlates well with the known carcinogenicity of the parent aromatic hydrocarbons *in vivo* (20). Induction of aryl hydrocarbon hydroxylase activity by aromatic hydrocarbons occurs in cell culture (1) and in many tissues of different animal species (1, 2, 21). Epoxide hydrase is inducible by 3-methylcholanthrene in Sprague-Dawley rats (13). Therefore the constitutive and inducible levels of either aryl hydrocarbon hydroxylase or epoxide hydrase for both present in any tissue may be im-

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portant for the magnitude of tumorigenesis evoked by epoxide intermediates.

Recently we have reported (22–25) that aryl hydrocarbon hydroxylase induction by aromatic hydrocarbons occurs in some inbred strains of mice but not in other strains. This expressed function segregates as a single autosomal dominant gene, for which we have proposed the *Ah* locus (23–25). Hence, in any mouse which is homozygous or heterozygous for the allele *Ah*, the aryl hydrocarbon hydroxylase system is inducible generally as an all-or-none response in all tissues regularly containing the inducible enzyme (23–25). It is therefore possible within the same hybrid litter to study mice possessing the inducible aryl hydrocarbon hydroxylase and mice that do not have it. The question is then raised: Can one find a relationship between 7,12-dimethylbenz[*a*]-anthracene-produced skin tumor formation, epoxide hydrase activity, and genetic differences in the extent of aromatic hydrocarbon-inducible aryl hydrocarbon hydroxylase activity among *AhAh*, *Ahah*, and *ahah* mice? The pH optima of the control hepatic aryl hydrocarbon hydroxylase activity from *ahah* mice and from *AhAh* mice are distinctly different (23–25). Other dissimilarities in aromatic hydrocarbon metabolism between these strains of mice—such as the position or amount of epoxide formation or the amount of covalent interaction with cellular macromolecules—may also exist and influence the rate of tumor formation.

The experiments were carried out with C57BL 6N mice, which have the inducible aryl hydrocarbon hydroxylase in various tissues, DBA 2N mice, in which the enzyme is relatively nonresponsive to aromatic hydrocarbons (22–25), and the appropriate hybrids. The skin of 3-week-old weanlings was treated once with 20 μg of DMBA,[1] and phorbol ester was applied three times a week as described in the legend to Table 1. We arbitrarily chose a relatively low dose of carcinogen, since a larger dose would be likely to cause tumors in all animals. The constitutive and inducible aryl hydrocarbon hydroxylase (23–25) and epoxide hydrase activities generally are maximal in these mice at about 3 weeks of age[2], and at 4 months of age are 80–90% of these maximal values. After no more tumors appeared, which was at about 4 months of age, each mouse was killed 24 hr after an intraperitoneal dose of MC, and the aryl hydrocarbon hydroxylase and epoxide hydrase specific activities were determined in the liver microsomes. By measuring the hepatic microsomal aryl hydrocarbon hydroxylase activity 24 hr after administration of MC, we could determine with certainty (23–25) whether this enzyme system was genetically inducible or noninducible in many other tissues of that mouse, such as kidney, bowel, lung, or skin.

Table 1 shows that DMBA did not produce skin tumors in any of the C57BL 6N (i.e., B6) inbred mice and caused tumors in 19 out of 21 inbred DBA 2N (i.e., D2) mice. The hepatic aryl hydrocarbon hydroxylase system was inducible by MC about 4–5-fold in the inbred B6 mice and was not inducible in the inbred D2 mice. The skin aryl hydrocarbon hydroxylase activity was induced by MC about 8-fold in the B6 mice and less than 2-fold in D2 mice. Thus no tumors were found in B6 mice even though their skin aryl hydrocarbon hydroxylase activity was about 5 times more inducible by polycyclic hydrocarbons than in the D2 mice. These data suggest that low or noninducible enzyme activity may be correlated with skin tumor formation caused by DMBA. However, inflammation produced by the repeated applications of phorbol ester was considerably more marked in the D2 inbred strain than in the B6 strain; thus tumorigenesis related to phorbol ester-produced skin irritation may be a more important factor than the aryl hydrocarbon hydrox-

[1] The abbreviations used are: DMBA, 7,12-dimethylbenz[*a*]anthracene; MC, 3-methylcholanthrene.

[2] Total epoxide hydrase activity was measured with [^{3}H]styrene oxide as substrate. At least in liver, the same epoxide hydrase appears to be responsible for hydration of styrene oxide and polycyclic arene oxides such as phenanthrene 9,10-oxide (11, 12). However, such an assay provides no information on the levels of epoxide hydrase possibly present as part of a tightly coupled aryl hydrocarbon hydroxylase-epoxide hydrase system (see the text).

[3] Unpublished observations.