Skin Tumor-initiating Activities of the Twelve Isomeric Phenols of Benzo(a)pyrene


Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830 [T. J. S., W. M. B., S. D.]; Department of Biochemistry and Drug Metabolism, Hoffmann-La Roche Inc., Nutley, New Jersey 07110 [W. L., A. H. C.]; and Laboratory of Chemistry, National Institute of Arthritis, Metabolism, and Digestive Diseases, NIH, Bethesda, Maryland 20014 [H. Y., D. M. J.]

ABSTRACT

The skin tumor-initiating activities of the 12 isomeric phenols of benzo(a)pyrene (BP) were determined in mice by use of a two-stage system of tumorigenesis. 11-Hydroxybenzo(a)pyrene was moderately active, whereas 2-hydroxybenzo(a)pyrene and BP were strong tumor initiators when applied topically to CD-1 mice and followed by twice-weekly applications of the promoter 12-O-tetradecanoylphorbol-13-acetate. 1-, 3-, 4-, 5-, 6-, 7-, 8-, 9-, 10-, and 12-hydroxybenzo(a)pyrene had less than 5% of the tumor-initiating activity of BP when the data were expressed as papillomas per mouse. After 30 weeks of promotion, the number of papillomas per mouse was 8.4, 8.5, and 2.8, respectively, for the animals treated with BP, 2-hydroxybenzo(a)pyrene, and 11-hydroxybenzo(a)pyrene. A 5-week latency period before the appearance of the first tumor was observed after the application of either 2-hydroxybenzo(a)pyrene or BP, whereas a slightly longer latency period of 7 weeks was observed following application of 11-hydroxybenzo(a)pyrene. The time required for 50% of the animals to develop tumors was 13 weeks for animals treated with BP and 15 weeks for animals treated with 2- or 11-hydroxybenzo(a)pyrene.

INTRODUCTION

The ubiquitous PAHs are thought to play a significant role in the etiology of human cancer (5, 34). Current information indicates that PAHs exert their toxic, mutagenic, and carcinogenic activities after they have been metabolically activated by microsomal enzymes to reactive metabolites (cf. Refs. 1, 7, 16, and 21). Of the very large number of potential metabolites of the environmental pollutant BP that may be responsible for the carcinogenic activity of this PAH, 19 have been tested as complete carcinogens on mouse skin (9, 13-15, 29). A comparison of the carcinogenic potency of BP with the BP derivatives tested revealed that BP 7,8-diol and 2-OHBp were at least as active as the parent hydrocarbon; benzo(a)pyrene 7,8-oxide was moderately active; and benzo(a)pyrene 4,5-oxide, 11-OHBP, and BP 7β,8α-diol-9α,10α-epoxide had weak carcinogenic activity. The other 10 isomeric phenols of BP, 2 arene oxides (benzo(a)pyrene 9, 10- and 11,12-oxides), and BP 7β,8α-diol-9β,10β-epoxide were inactive at the doses tested (9, 13-15, 29). However, when a PAH is tested as a complete carcinogen, one cannot assume that all derivatives of that compound are equally effective in supplying both initiating and promoting stimuli. In a 2-stage mouse skin system, initiation is the only stage that requires the presence of the carcinogen, and the measured carcinogenic potency of a chemical reflects its capacity for tumor initiation. Thus, it is possible that a carcinogen that lacks promoting ability would not be detected when tested as a complete carcinogen. Because of these considerations, we have undertaken the testing of known and potential metabolites of BP for tumor-initiating activity (12, 23-25). We have previously reported that BP 7,8-diol was approximately as potent and benzo(a)pyrene 7,8-oxide and BP 7β,8α-diol-9α,10α-epoxide were about one-third as active as BP (24). (±)-7β,8α-dihydroxy-9β,10β-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene, benzo(a)pyrene 9,10-oxide, and 11,12-oxide possessed about 1.2, 10%, respectively, of the tumor-initiating activity of BP (23).

In this study, we have compared the tumor-initiating activities of the 12 isomeric phenols of BP. 2-OHBp was found to be equipotent to BP as a tumor initiator at the single dose tested, while 11-OHBp was one-third as active and the other 10 isomeric phenols had 0 to 4% of the activity of BP.

MATERIALS AND METHODS

Female CD-1 mice were purchased from Charles River Mouse Farms, North Wilmington, Mass. Mice 7 to 9 weeks old were shaved with surgical clippers 2 days before treatment, and only those mice in the resting phase of the hair cycle were used. Groups of 30 mice were used in the tumor experiments. The incidence of both papillomas and carcinomas was recorded weekly, and papillomas and carcinomas were removed at random for histological verification. BP was purchased from Aldrich Chemical Company, Inc., Milwaukee, Wis., and was greater than 99% pure. TPA was obtained from Dr. Peter Borchert, University of Minnesota, Minneapolis, Minn.

The 12 isomeric phenols of BP were synthesized as previously described (35). All compounds used in this study were free of detectable contaminants as judged by mass spectrometry, nuclear magnetic resonance spectroscopy, and...
combustion analysis, and high-pressure liquid chromatography (>99% pure). BP and its phenols were dissolved in spectroquality acetone to give a concentration of 400 nmoles of compound per 0.2 ml of solvent. Solutions of the hydrocarbons were prepared under subdued light immediately before use. The mice were treated with the above compounds at a dose of 400 nmoles, also under subdued light. The time lapse between preparation of the above solutions and animal treatment was less than 0.5 hr. TPA was prepared in stock solutions and kept in a freezer until use. Mice received twice-weekly applications of 10 μg of TPA 1 week after treatment with BP or BP phenols.

RESULTS

The skin tumor-initiating activities of the 12 isomeric phenols of BP are shown in Table 1. 2-OHBP was as potent a tumor initiator as BP, whereas 11-OHBP was about one-third as active when the data were expressed as papillomas per mouse. The remaining phenols of BP had less than 5% of the tumor-initiating activity of BP (papillomas per mouse). 1-, 4-, and 8-OHBP were essentially inactive as tumor initiators since solvent-vehicle controls can sometimes give rise to 1 tumor in a group of 30 mice although none were observed in the present study. 3-, 5-, 6-, 7-, and 10-OHBP would be considered very weak or borderline initiators, whereas 9-OHBP and 12-OHBP are definitely tumor initiators with weak activity.

Chart 1 shows the tumor-initiating ability of 2-OHBP, 11-OHBP, and BP in greater detail than does Table 1. 2-OHBP was approximately equipotent to BP as a tumor initiator for the duration of the experiment. This was true in terms of time to the appearance of the first tumor as well as the number of papillomas per mouse. The only difference between the initiating activity of 2-OHBP and BP was that BP caused a slightly higher percentage of the mice to develop papillomas. Also shown in Chart 1 is the moderate tumor-initiating ability of 11-OHBP.

DISCUSSION

This study indicates that 2-OHBP is a strong tumor initiator in mouse skin, with activity comparable to BP at the 1 dose tested. 11-OHBP possessed about one-third the tumor-initiating activity of BP, and 9- and 12-OHBP were weakly active. The remaining 8 isomeric phenols of BP had little or no initiating activity. These results are in good agreement with the high carcinogenic activity of 2-OHBP, the weaker activity of 11-OHBP, and the inactivity of the other 10 phenols of BP as complete carcinogens on mouse skin (9, 29). The slight tumor-initiating activity of 9- and 12-OHBP in the present study suggests that the 2-stage system of skin tumorigenesis may be a more sensitive assay for tumorigenesis than are the systems for complete carcinogenicity described earlier (9, 13-15, 29).

Bresnick et al. (3) recently found that application of 2- or 9-OHBP to mouse skin caused marked epidermal hyperplasia resembling that caused by tumor promoters, but the other 10 isomeric hydroxybenzo(a)pyrenes were either less active or completely inactive. They also reported that BP was less active than 2- or 9-OHBP in causing epidermal hyperplasia (3). The reason that 2-OHBP is a complete carcinogen may be related to the fact that it both initiates tumors and induces hyperplasia, whereas 9-OHBP may be a good promoter but is a poor initiator, thereby causing it to lack complete carcinogenic activity. If the "bay region" diol-epoxide of BP is the ultimate tumor-initiating form of BP, then one would not expect 9-OHBP to have tumor-initiating activity.
2- and 11-OHBP were found to be inactive as mutagens in *Salmonella typhimurium* and in Chinese hamster V-79 cells without a metabolic activating system (30). Incubation of 2-OHBP with a highly purified hepatic cytochrome P-448 monooxygenase system from 3-methylcholanthrene-treated rats indicated metabolism of 2-OHBP to compound(s) that were mutagenic to *S. typhimurium* strain TA 98 (32). Under similar incubation conditions, BP was metabolically activated to a greater extent than was 2-OHBP, whereas 11-OHBP was not metabolized to mutagens (32). The use of an S-9 fraction from Aroclor 1254- or 3-methylcholanthrene-pretreated rats indicated that 2-OHBP was metabolically activated to mutagenic metabolites to a greater extent than was BP (29). The data indicate that 2-OHBP must be metabolically activated to display biological activity.

Studies on the metabolism of BP by rat liver indicated the formation of 1-, 3-, 6-, 7-, and 9-OHBP (11, 17, 19). 3- and 9-OHBP were found to be inactive as mutagens in *Salmonella typhimurium* and in Chinese hamster V-79 cells without a metabolic activating system (30). Incubation of 2-OHBP with a highly purified hepatic cytochrome P-448 monooxygenase system from 3-methylcholanthrene-treated rats indicated metabolism of 2-OHBP to compound(s) that were mutagenic to *S. typhimurium* strain TA 98 (32). Under similar incubation conditions, BP was metabolically activated to a greater extent than was 2-OHBP, whereas 11-OHBP was not metabolized to mutagens (32). The use of an S-9 fraction from Aroclor 1254- or 3-methylcholanthrene-pretreated rats indicated that 2-OHBP was metabolically activated to mutagenic metabolites to a greater extent than was BP (29). The data indicate that 2-OHBP must be metabolically activated to display biological activity.


Skin Tumor-initiating Activities of the Twelve Isomeric Phenols of Benzo(a)pyrene

T. J. Slaga, W. M. Bracken, S. Dresner, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/38/3/678

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.