Tumor-initiating Activity of Benz[c]acridine and Twelve of Its Derivatives on Mouse Skin

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INTRODUCTION

The polynuclear aza heteroaromatic hydrocarbons comprise a group of environmental pollutants (1, 15-17, 22), at least some of which are known to be carcinogenic (3, 8). These N-substituted polycyclic aromatic hydrocarbons are particularly well suited for an evaluation of the influence of the nitrogen heteroatom on the metabolism and tumorigenicity of polycyclic hydrocarbons. Numerous studies during the past several years have indicated that bay-region diol-epoxides are ultimate carcinogenic metabolite of polycyclic aromatic hydrocarbons (cf. Ref. 14). Analogous studies on the aza aromatics have not been performed, although metabolism of B[c]ACR and 7-methylB[c]ACR by microsomal preparations has been reported (4, 5).

The recent synthesis of a large number of potential metabolites of B[c]ACR (7, 9) has permitted an in-depth analysis of the mutagenic activity of these compounds in bacterial and mammalian cells (23). Of the 14 B[c]ACR derivatives tested, only the bay-region epoxides and their potential metabolic precursors showed high mutagenic activity in 2 strains of Salmonella typhimurium and in Chinese hamster V79 cells. These results provided initial evidence for bay-region activation of B[c]ACR to ultimate mutagenic and carcinogenic metabolites (23). The present study was undertaken to evaluate the tumorigenic activity of these derivatives in initiation-promotion experiments on mouse skin.

MATERIALS AND METHODS

Chemicals. Benz[a]anthracene was purchased from Sigma Chemical Co., St. Louis, Mo. Analytically pure benz[a]anthracene 3,4-diol-1,2-epoxide 2 was synthesized as described previously (10). B[c]ACR and the B[c]ACR dihydrodiols and diol-epoxides (7, 9) were obtained by unequivocal chemical synthesis as described. 3,4-H2B[c]ACR was synthesized by a method analogous to that described for the benz(a)-anthracene derivative (11). 4,5,6-H3DB[c,k,l]ACR was isolated in approximately 20% yield by chromatography on silica gel with benzene as developing solvent from the crude reaction products of a 24-hr reaction of 5,6,7-8-tetrahydro-1-naphthylamine (25.3 g), α-naphthol (25.3 g), and iodine (0.5 g) at reflux. Recrystallization from hexane gave 4,5,6-H3DB[c,k,l]ACR as a light yellow solid (m.p. 96-97°). Nuclear magnetic resonance spectrum (δ, CDCl3): 2.2 (2H, apparent quintet, J = 6 Hz); 3.1 (2H, apparent triplet, J = 6 Hz); 3.1 (2H, apparent triplet, J = 6 Hz); 7.2 to 8.0 (6H, m); 8.2 to 8.5 (2H, m); and 9.3 to 9.6 (1H, m). Mass spectrum: M+ (269, base peak). All compounds synthesized for this study were analytically pure as determined by nuclear magnetic resonance spectroscopy. The structures of the B[c]ACR derivatives are shown in Chart 1.

12-O-Tetradecanoylphorbol-13-acetate was purchased from Chemicals for Carcinogenesis Research, Eden Prairie, Minn.

Animals. Female CD-1 mice at 6 weeks of age (Charles River Breeding Laboratories, Inc., Wilmington, Mass.) were housed in polycarbonate boxes with corncob bedding and were fed a commercial diet (Purina Laboratory Chow; Ralston Purina Co., St. Louis, Mo.) and water ad libitum. At 7 weeks of age, the mice were shaved on the dorsal surface with electric clippers. Two days later, 30 mice in each treatment group were given a single topical application of the compounds in 200 μl of 2 The abbreviations used are: B[c]ACR, benz[c]acridine; dihydrodiols, the trans-dihydroxydiene derivatives in which the hydroxyl groups are cis; 3,4-diol-1,2-epoxide, the (±)-3a,4a-dihydroxy-1a,2α-epoxy-1,2,3,4-tetrahydrodiene epoxide diastereomer in which the benzylic hydroxyl and the epoxy oxygen are cis; 3,4-diol-1,2-epoxide, the (±)-3a,4a-dihydroxy-1a,2α-epoxy-1,2,3,4-tetrahydrodiene epoxide diastereomer in which these groups are trans; other diol epoxides are similarly designated; 3,4-H2B[c]ACR, 3,4-dihydrobenz[c]acridine; 4,5,6-H3DB[c,k,l]ACR, 4,5,6-dihydrobenz[c,k,l]acridine. The designations α and β denote relative stereochemistry, and all compounds are racemic mixtures in which enantiomers are possible.

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solvent (5% dimethyl sulfoxide in acetone). Control animals received only solvent. The tumor promoter 12-O-tetradecanoylphorbol-13-acetate (16 nmol/200 µl of acetone) was applied topically twice weekly to each mouse, beginning 12 days after application of the initiator or solvent. Development of skin tumors was recorded every 2 weeks, and papillomas greater than 2 mm in diameter were included in the cumulative total when they persisted for 2 weeks or longer.

RESULTS

The tumor-initiating activity of B[c]ACR and several of its derivatives after 15 to 25 weeks of promotion with 12-O-tetradecanoylphorbol-13-acetate is summarized in Table 1. The tumor-initiating activity of all compounds tested did not show any further increase beyond 22 weeks of promotion. The most potent tumor initiator was 3,4-H₂B[c]ACR, which produced a 97% tumor incidence and 7.90 tumors/mouse at an initiating dose of 0.4 µmol after 15 weeks of promotion. The parent compound B[c]ACR had significant (p < 0.05) tumor-initiating activity only at the 2.5-µmol dose. Of the 5 metabolically possible dihydrodiols of B[c]ACR, only B[c]ACR 3,4-dihydrodiol had significant tumor-initiating activity at the doses tested. At all 3 doses, B[c]ACR 3,4-dihydrodiol, the potential metabolic precursor of a bay-region diol-epoxide, was significantly (p < 0.05) more active than was the parent compound. At 15 weeks of promotion with 12-O-tetradecanoylphorbol-13-acetate, B[c]ACR 3,4-dihydrodiol was 6- to 15-fold more active than was B[c]ACR when the data were expressed as average number of tumors per mouse.

The diastereomeric bay-region diol-epoxides of B[c]ACR, in which the benzylic hydroxyl group and the epoxide oxygen are either cis (isomer 1) or trans (isomer 2), had significant tumor-initiating activity (p < 0.05), although isomer 2 was at least 5-fold more active than was isomer 1. B[c]ACR 3,4-diol-1,2-epoxide 2 had tumor-initiating activity that was equivalent to B[c]ACR 3,4-dihydrodiol, but B[c]ACR 3,4-diol-1,2-epoxide 1 was only one-sixth to one-tenth as active as was the 3,4-dihydrodiol (Table 1). While isomer 1 appeared to have twice the tumor-initiating activity of the parent compound at the 1.0-µmol dose, the difference was not statistically significant (p > 0.2). Interestingly, B[c]ACR 8,9-diol-10,11-epoxide 2 and B[c]ACR 10,11-diol-8,9-epoxide 2 (non-bay-region diol-epoxides), as well as the K-region 5,6-oxide, had no significant tumor-initiating activity at any of the doses tested. 4,5,6-H₃DB[c,k,l]ACR, which has alkyl substitution at C-7 and C-8 in B[c]ACR (Fig. 1) and thus would be expected to undergo less metabolism at the 8,9- as well as at the 5,6-double bonds (2), had tumor-initiating activity that was equivalent to that of B[c]ACR.

DISCUSSION

In the present study, B[c]ACR was found to be a weak tumor-initiator on mouse skin. At initiating doses of 0.4 and 1.0 µmol, the compound was nontumorigenic, while the 2.5-µmol dose of B[c]ACR produced a 37% tumor incidence and 1.33 tumors/mouse after 25 weeks of promotion. Of the 12 derivatives of B[c]ACR tested in the present study, only the bay-region 3,4-diol-1,2-epoxide 2, its potential metabolic precursor (3,4-dihydro-
Tumorigenicity of B[c]ACR Derivatives

Table 1

Tumor-initiating activity of B[c]ACR and B[c]ACR derivatives on mouse skin

Female CD-1 mice (7 weeks old) were treated topically with a single initiating dose of the indicated compounds in 200 μl of 5% dimethyl sulfoxide in acetone. Twelve days later, the mice received twice-weekly applications of 12-O-tetradecanoylphorbol-13-acetate (16 nmol/200 μl of acetone) for 25 weeks as described in "Materials and Methods." Each treatment group consisted of 30 mice, and at least 28 mice in each group survived to termination of the study.

<table>
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<th>Initiator</th>
<th>% of mice with tumors</th>
<th>Tumors/mouse</th>
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<td></td>
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<tr>
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*Mean ± S.E.

p < 0.05 compared to control animals according to the 4-fold contingency test of Mainland and Murray (13).

p < 0.05 compared to control animals (Student's t test).

diol, and 3,4-H₂B[c]ACR had higher tumor-initiating activity than did B[c]ACR. These 3 compounds were from 5- to 80-fold more active than was B[c]ACR.

In an earlier study, we had shown that B[c]ACR 3,4-dihydrodiol must be metabolized by the cytochrome P-450-dependent monooxygenase system before exerting mutagenicity in S. typhimurium, a result consistent with bay-region activation (23). The very high tumorigenic activity of 3,4-H₂B[c]ACR, which was the most active compound tested, provides further support for bay-region activation of B[c]ACR to an ultimate carcinogen. The presumed active tumorigenic metabolite of this aza aromatic compound, 1,2-epoxy-1,2,3,4-tetrahydro-B[c]ACR, has an epoxide in the bay-region and is 4- to 20-fold more mutagenic to strains TA 98 and TA 100 of S. typhimurium and Chinese hamster V79 cells than are the diastereomeric B[c]ACR 3,4-diol-1,2-epoxides (23). Interestingly, isomer 2 of the bay-region diol-epoxide was at least 5-fold more active as a tumor-initiator than was isomer 1, whereas their mutagenic activity in bacteria and mammalian cells differed by no more than a factor of 2. In strain TA 100 of S. typhimurium, isomer 1 was actually more mutagenic than was isomer 2 (23).

With the exception of the lack of correlation of mutagenic with tumorigenic potency of the isomers of the bay-region 3,4-diol-1,2-epoxides, there is a relatively good correlation between mutagenic and tumorigenic responses obtained with the other compounds. Two non-bay-region diol-epoxides (8,9-diol-10,11-epoxide 2 and 10,11-diol-8,9-epoxide 2), which were not tumorigenic in the present study, had less than 5% of the mutagenic activity of the bay-region diol-epoxides in S. typhimurium and Chinese hamster V79 cells (23). Their metabolic precursors (B[c]ACR 8,9- and 10,11-dihydrodiols), as well as the 1,2- and 5,6-dihydrodiols, were nonmutagenic with or without metabolic...
activation (23) and were also nontumorigenic in the present study. The K-region 5,6-oxide had no significant tumor-initiating activity and is also an extremely weak mutagen (23). The results obtained on the metabolism, mutagenicity, and tumorigenicity of B[c]ACR are remarkably similar to those obtained with benz[a]anthracene. Although detailed metabolism studies have not yet been performed on B[c]ACR, the available data would indicate that the K-region 5,6-dihydrodiol is a major metabolite and the 3,4-dihydrodiol is a minor metabolite (5), as has been demonstrated in the metabolism of benz[a]anthracene (18–21). The 3,4-dihydrodiols of benz[a]anthracene (19) and B[c]ACR (5) are further metabolized to bay-region diol-epoxides, although other metabolic pathways predominate. A comparison of the mutagenic activity of 10 tetrahydroepoxides and diol-epoxides of B[c]ACR and benz[a]anthracene has shown significant differences between the N-12-substituted and unsubsti-
tuted compounds, but these differences are small for the bay-
region diol-epoxides and tetrahydroepoxides (23). Likewise, the tumor-initiating activities of benz[a]anthracene and benz[a]

3,4-diol-1,2-epoxide 2 are almost identical to those of the corresponding derivatives of B[c]ACR. In the present tumori-
genicity experiment with the B[c]ACR derivatives, a 2.5-μmol initiating dose of benz[a]anthracene induced a 63% tumor inci-
dence and 1.02 tumors/mouse after 25 weeks of promotion. Benz[a]anthracene 3,4-diol-1,2-epoxide 2 (0.4 μmol) produced a 63% incidence of tumors and 1.77 tumors/mouse. In separate experiments reported previously (12, 24), 3,4-dihydrobenz[a]

anthracene, benz[a]anthracene 3,4-dihydrodiol, and the diaster-
omeric bay-region diol-epoxides of benz[a]anthracene all showed tumor-initiating activity quite similar to that reported here for the corresponding B[c]ACR derivatives. It thus appears that N-12 substitu-
tion of benz[a]anthracene to form B[c]ACR has little or no effect on the tumor-initiating activity of the parent compound, the 3,4-dihydrodiol, or the bay-region diol-epoxides. The present results provide the first example of an aza polycyclic aromatic hydrocarbon which fits the predictions of the bay-region theory of carcinogenesis (6, 14).

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ADDENDUM

After submission of this manuscript, we completed a study comparing the tumor-
initiating activities of benz[a]acridine (N-7-substituted benz[a]anthracene) and B[c]ACR. The experimental methodology was identical to that used in the present study. At tumor-initiating doses of 2.5 and 5 μmol, benz[a]acridine produced skin tumors in 27 and 23% of the mice, respectively, and an average of 0.27 ± 0.08 (S.E.) tumor/mouse at both doses after 25 weeks of promotion with 12-O-tetra-

decanoylphorbol-13-acetate. These values were not significantly different (p <

0.05) from the incidence in control mice (10% incidence; 0.10 ± 0.06 tumor/mouse). B[c]ACR, at an initiating dose of 2.5 μmol, produced a 57% tumor incidence and 0.93 ± 0.18 tumor/mouse. Thus, the N-12-substituted aza aromatic (B[c]ACR) has weak but significant tumor-initiating activity, while the N-7-substituted derivative is inactive at the doses tested. These results are consistent with the very weak mutagenic activity of the bay-region diol-epoxides of benz[a]acridine compared to B[c]ACR (23).

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