Potent Mammary Carcinogenicity in Female CD Rats of a Fjord Region Diol-Epoxide of Benzo[c]phenanthrene Compared to a Bay Region Diol-Epoxide of Benzo[a]pyrene

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Abstract

Two diol-epoxide metabolites of benzo[c]phenanthrene and benzo[a]pyrene, polynuclear aromatic hydrocarbons which occur in the environment, were tested for carcinogenicity by direct injection into the mammary fat pads of female CD rats. The compounds anti-3,4-dihydroxy-1,2-epoxy-1,2,3,4-tetrahydrobenzo[c]phenanthrene (BcPDE), a fjord region diol-epoxide, and anti-7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene, a bay region diol-epoxide, were applied at total doses of 12.2 µmol. 6-Nitrochrysene was applied at the same dose as a positive control (K. El-Bayoumy, A. Rivenson, P. Upadhyaya, Y-H. Chae, and S. S. Hecht, Cancer Res. 53: 3719–3722, 1993). The sterically hindered fjord region diol-epoxide BcPDE was a powerful mammary tumorigen and carcinogen, rapidly inducing significantly more fibroadenoma and adenocarcinoma than either of the other compounds. Anti-7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene was a weaker mammary tumorigen than BcPDE and 6-nitrochrysene. The results of this study provide the first evidence for mammary tumorigenicity of polynuclear aromatic hydrocarbon diol-epoxides and demonstrate the potent mammary carcinogenicity of BcPDE.

Introduction

Human exposure to carcinogenic PAHs is common, occurring mainly through the diet and by inhalation of polluted air or cigarette smoke (1). PAHs are enzymatically transformed to a variety of metabolites, among which diol-epoxides have been identified in some instances as major ultimate carcinogens (2). Some PAHs such as DMBA are well recognized mammary carcinogens in the rat (3), but the mammary carcinogenicity of diol-epoxide metabolites of PAHs has not been explored. Since PAHs are prominent among the environmental carcinogens which may be involved in causing human breast cancer (4), it is important to evaluate the mammary carcinogenicity of their diol-epoxide metabolites.

Two of the most potent PAH mammary carcinogens in rats are DMBA and DB[a,f]P (Fig. 1; Ref. 5). These compounds have a common structural feature, a sterically hindered bay region enclosed within four rigidly constrained carbon-carbon bonds as illustrated in Fig. 1. When all four bonds are part of six-membered rings, as in DB[a,f]P, BPDE, and BgCDE, the sterically hindered bay region is known as a fjord region. Although the ultimate carcinogens of DB[a,f]P have not as yet been determined, 3,4-dihydroxy-1,2-epoxy-1,2,3,4-tetrahydrobenzo[a]anthracene, in which the epoxide ring is in a sterically hindered bay region, is a suspected major ultimate carcinogen of DMBA in the rat mammary gland (6–8). Other diol-epoxides that have their epoxide rings in sterically hindered bay regions or fjord regions are known to be highly tumorigenic in newborn mice or on mouse skin or mutagenic in various systems, e.g., BcPDE, 5,6-diMeCDE, and BgCDE (9–13). Collectively, these data suggested to us that such sterically hindered bay region diol-epoxides should be potent mammary carcinogens. As an initial test of this hypothesis, we have compared the carcinogenicity of BcPDE with that of BPDE upon direct application into the rat mammary gland. BPDE, a well-known ultimate carcinogen of the extensively studied carcinogen benzo[a]pyrene, is a bay region diol-epoxide which lacks the steric constraints imposed by the fjord region of BcPDE. The recently discovered mammary carcinogen 6-NC was included as a positive control (14).

Materials and Methods

Chemicals. BPDE, BPDE, and 6-NC were synthesized (14–16). Their purities were greater than 99% based on analysis by high performance liquid chromatography, thin layer chromatography, UV, mass spectrometry, and 360-MHz proton nuclear magnetic resonance. DMSO was obtained from Aldrich Chemical Co. (Milwaukee, WI). Solutions of the test compounds in DMSO were prepared immediately prior to injection. All compounds were stable in DMSO for several days at least, as determined by normal phase high performance liquid chromatography analysis.

Bioassay. One hundred twenty 21-day old female CD rats [Crl:CD(SD)BR] were obtained from Charles River Breeding Laboratories (Kingston, NY). They were maintained on water and Teklad Rodent diet (W)8604 ad libitum (Harlan Teklad, Madison, WI). The rats were housed under standard conditions as described previously (14). They were randomly assigned to four treatment groups as summarized in Table 1. The doses of the compounds to be tested were determined based on previous studies (14, 17). At age 30 days, each rat was given three injections of 0.1 ml of DMSO containing 2.04 µmol of test compound (Groups 1–3) or of DMSO alone (Group 4). The solutions were injected into the mammary tissue underlying each of the three left thoracic nipples. The corresponding three right thoracic nipple areas were injected with three 0.1-ml amounts of DMSO only. On the next day, the tissue underlying each of the three left inguinal nipples was treated with test compounds or DMSO as above and the corresponding three right nipple areas were treated with DMSO only. All injections were carried out under light ether anesthesia. Body weights were measured weekly during the first month, then monthly until termination. Rats were inspected and palpated weekly for the presence of mammary tumors beginning 7 weeks after treatment and then every two weeks for the remainder of the experiment. Animals were sacrificed when large or ulcerated tumors developed or when moribund. The experiment was terminated 38 weeks after injection.

At necropsy, all organs and especially the mammary glands were examined macroscopically for any gross lesions or abnormalities. The tissues were fixed in 10% buffered formalin solution, processed for paraffin sections, and stained...
with hematoxylin and eosin. Mammary tumors were classified according to Russo et al. (18), modified to the specifics of our experiment (Table 1). Tumor incidence data were analyzed by the \( \chi^2 \) test; body weight and tumor multiplicity data were analyzed by Student’s \( t \) test.

Results

Fig. 2 summarizes body weights in the four groups. Body weights were significantly lower (\( P < 0.01 \)) in the BcPDE and 6-NC groups than in control rats throughout the experiment, except at week 32 in the 6-NC group. Body weights were also significantly depressed (\( P < 0.01 \)) in the BPDE-treated group from week 7 until termination.

The rats treated with BcPDE developed an inflammatory reaction at the injection site more extensively than the other groups. The inflammation ranged from mild congestion and edema to severe local skin necrosis and crust formation. The necrotic area became umbilicated and surrounded by edema. Scar formation started 3–4 weeks after injection.

The incidence of palpable mammary tumors rose rapidly in the BcPDE group, reaching 50% between 11 and 13 weeks (Fig. 3). Based on the percentage incidence of palpable mammary tumors, BcPDE was significantly more tumorigenic than the next most active compound, 6-NC, through the first 28 weeks of the experiment (\( P < 0.01 \)). The incidence of palpable mammary tumors was less than 10% in the BPDE and vehicle control groups. At sacrifice, additional mammary tumors were found. The final percentage incidences of mammary tumors were: BcPDE, 100; BPDE, 43; 6-NC, 93; and DMSO, 3. BcPDE and 6-NC were significantly more carcinogenic than BPDE (\( P < 0.01 \)).

Large tumors developed in the BcPDE and 6-NC treated groups (mean tumor sizes per rat, 35 and 24 cm\(^3\), respectively). Fifty % of the BcPDE-treated rats died or were sacrificed within 32 weeks after injection; 90% or greater of the rats in the other groups were alive at this point.

Histological findings are summarized in Table 1. Virtually all tumors were found on the left side. Adenocarcinomas and fibroadenomas were the most common tumors. The incidence and multiplicity of adenocarcinomas were significantly higher in the BcPDE group than in any of the other groups (\( P < 0.01 \)). The incidence and multiplicity of fibroadenomas were significantly higher in the rats treated with BcPDE than in the BPDE group (\( P < 0.05 \)).

Discussion

The results of this study provide the first evidence for the carcinogenicity of a PAH diol-epoxide in rat mammary tissue. The significantly higher activity of BcPDE over BPDE provides initial support for our hypothesis that sterically hindered PAH diol-epoxides will be strong mammary carcinogens. This hypothesis is being tested further in ongoing bioassays of other sterically hindered diol-epoxides. The relatively weak activity of BPDE is consistent with previous work which showed that its precursor, 7,8-dihydro-7,8-dihydroxybenzo-[\( \alpha \)]-pyrene, was also a weak mammary tumorigen in the rat (19).

Topical application on mouse skin and injection in newborn mice are the most commonly used bioassay systems for PAH diol-epoxides (20). Some diol-epoxides, including enantiomers of BcPDE, are more active in one system than the other; consequently, the carcinogenicity of a PAH diol-epoxide in any system is not readily predictable (10, 20). Based on our data, the response of rat mammary tissue to diol-epoxides may be similar to that of newborn mouse lung in that BcPDE and 6-NC are both potent tumorigens. However, in the newborn mouse, BPDE is a more potent tumorigen than 6-NC, which contrasts with the results obtained here (21–24).
Inflammation and necrosis at the injection site were more extensive in the rats treated with BcPDE than in the other groups. Apparently, BcPDE had a toxic effect which was not expressed by either BPDE or 6-NC. Cell replication associated with the toxicity of BcPDE together with the formation of BcPDE metabolites may lead to changes in the distribution of this metabolite. On mouse skin, benzo[c]phenanthrene is a weak carcinogen, in contrast to the potent tumor-initiating activity of benzo[c]pyrene (9). Studies of benzo[c]phenanthrene metabolism by rat hepatic cytochromes P-450 and microsomes have shown that the formation of BcPDE is minimal, possibly accounting for the low tumorigenic activity of the parent compound, although the analogous metabolic experiments do not appear to have been performed in mouse skin (26, 27). Humans are exposed to benzo[c]phenanthrene by inhalation of air or by ingestion of food or water contaminated with combustion effluents (28, 29). Its potential role in human breast cancer would depend on its conversion to BcPDE in human tissues, which would likely be different from that in the rat or mouse. No information is available on the metabolism of benzo[c]phenanthrene in human tissues, although benzo[a]pyrene does form DNA adducts in cultured human mammary epithelial cells (30). We are currently developing methods designed to evaluate the presence of BcPDE-DNA adducts in human breast tissue.

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