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Role of diaxial versus diequatorial hydroxyl groups in the tumorigenic activity of a benzo[*a*]pyrene bay-region diol epoxide

(carcinogenesis/polycyclic hydrocarbons)

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ABSTRACT **Tumorigenic activities of the (7*R*,8*S*,9*S*,10*R*)-7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydro derivatives of benzo[*a*]pyrene [(+)-B[*a*]P diol epoxide-2] and 6-fluorobenzo-[*a*]pyrene (6-FB[*a*]P diol epoxide-2) were evaluated in newborn CD-1 mice. A total dose of 14 nmol of either diol epoxide was administered to preweanling mice, and tumorigenic activity was determined when the mice were 32 to 36 weeks old. At the termination of the study, 13% of solvent-treated control mice had developed lung tumors with an average of 0.19 tumor per mouse. No other tumors were observed in control animals. (+)-B[*a*]P diol epoxide-2 induced pulmonary tumors in 60% of the mice with an average of 1.9 tumors per mouse, and 14% of the male mice developed hepatic tumors with an average of 0.18 tumor per mouse. In contrast, 6-FB[*a*]P diol epoxide-2 had no significant tumorigenic activity at the 14-nmol dose. Although both bay-region diol epoxides have the same absolute configuration, (7*R*,8*S*,9*S*,10*R*), the hydroxyl groups of (+)-B[*a*]P diol epoxide-2 prefer the pseudoequatorial conformation whereas the hydroxyl groups of 6-FB[*a*]P diol epoxide-2 prefer the pseudoaxial conformation. The tumorigenicity results reported here are the first direct demonstration that conformation of the hydroxyl groups in a bay-region diol epoxide, in addition to the documented effect of absolute configuration, is an important determinant in the tumorigenic activity of these ultimate carcinogens.**

The bay-region diol epoxides derived from *trans*-7,8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene exist as a pair of diastereomers in which the 7-hydroxyl group is either cis (diol epoxide-1) or trans (diol epoxide-2) to the 9,10-epoxide oxygen. In the absence of specific structural features, the hydroxyl groups of benzo-ring dihydrodiols prefer the pseudoequatorial conformation (1). Although this conformational preference is maintained for the diol epoxide-2 diastereomers, the diol epoxide-1 diastereomers have a slight preference for the conformation in which the hydroxyl groups are pseudoaxial (2–5). Tumor studies with bay-region diol epoxides derived from benzo[*a*]pyrene (B[*a*]P) (6, 7), chrysene (8, 9), benz[*a*]anthracene (10, 11), and benz[*c*]acridine (12, 13) have shown that the diol epoxide-2 diastereomers have high tumorigenic activity relative to the diol epoxide-1 diastereomers, which in some cases are inactive. Both diastereomers of the benzo[*e*]pyrene bay-region 9,10-diol-11,12-epoxides, which have their hydroxyl groups essentially locked in the pseudoaxial conformation due to steric crowding in the bay region (14), have little or no tumorigenic activity (15). These observations have implicated the potential importance of conformational factors in the expression of tumorigenic activity of bay-region diol epoxides.

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In addition to the conformational factors discussed above, absolute configuration of bay-region diol epoxides is an important determinant of tumorigenic activity. Each diastereomeric diol epoxide exists as a pair of enantiomers. In the absence of unusual steric crowding in the bay region, the enantiomer of diol epoxide-2 with (*R,S,S,R*) absolute configuration is uniquely tumorigenic among the optically active bay-region diol epoxide isomers of B[*a*]P (16, 17), chrysene (9), and benz[*a*]anthracene (18). An ideal molecule to evaluate the role of conformation of the hydroxyl groups in the expression of tumorigenic activity would, therefore, be a bay-region diol epoxide-2 isomer from one of these hydrocarbon diol epoxides with an (*R,S,S,R*) absolute configuration whose hydroxyl groups prefer the pseudoaxial rather than pseudoequatorial conformation. This conformational shift for the diol epoxide-2 isomer must be achieved with minimal structural alteration.

In the case of *trans*-7,8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene, the presence of a peri 6-fluoro substituent causes a shift in conformation such that the pseudoaxial conformation is markedly preferred due to adverse electrostatic interaction between the fluorine and the 7-hydroxyl group (19). This conformational preference carries over to the 7,8-diol-9,10-epoxide-2 diastereomer of 6-fluorobenzo[*a*]pyrene (6-FB[*a*]P) that also prefers the unusual pseudoaxial conformation of a diol epoxide-2 diastereomer (20, 21). Since the van der Waals radius of fluorine (1.35 Å) is very close to that of hydrogen (1.2 Å), fluorine substitution at C-6 represents a very minimal steric alteration in the molecular dimensions of the 7,8-diol-9,10-epoxide, yet markedly alters its conformation. In the present study, we have evaluated the tumorigenic activity of the (7*R*,8*S*)-diol-(9*S*,10*R*)-epoxide-2 isomers of B[*a*]P and 6-FB[*a*]P.

MATERIALS AND METHODS

Chemicals. (+)-(7*R*,8*S*,9*S*,10*R*)-7,8-Dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene [(+)-B[*a*]P diol epox-

Abbreviations: B[*a*]P, benzo[*a*]pyrene; (+)-B[*a*]P diol epoxide-2, (+)-(7*R*,8*S*,9*S*,10*R*)-7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene; 6-FB[*a*]P, 6-fluorobenzo[*a*]pyrene; 6-FB[*a*]P diol epoxide-2, (7*R*,8*S*,9*S*,10*R*)-7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydro-6-fluorobenzo[*a*]pyrene; 6-FB[*a*]P diol epoxide-1, (7*R*,8*S*,9*R*,10*S*)-7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydro-6-fluorobenzo[*a*]pyrene; diol epoxide-1 and -2, diastereomers of the bay-region diol epoxides derived from *trans*-7,8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene in which the 7-hydroxyl group is either cis or trans, respectively, to the 9,10-epoxide oxygen.

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ide-2] was prepared as described (22, 23). (7*R*,8*S*,9*S*,10*R*)-7,8-Dihydroxy-9,10-epoxy-7,8,9,10-tetrahydro-6-fluorobenzo[*a*]pyrene (6-FB[*a*]P diol epoxide-2) and (7*R*,8*S*,9*R*,10*S*)-7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydro-6-fluorobenzo-[*a*]pyrene (6-FB[*a*]P diol epoxide-1) were prepared from the metabolically formed (−)-(7*R*,8*R*)-dihydrodiol of 6-FB[*a*]P as described (20, 21). The resulting diol epoxides were >99% chemically as well as enantiomerically pure. Absolute configurations of the diol epoxides are shown in Fig. 1. Because of the small quantities of the fluorinated diol epoxides available, their signs of rotation ($[\alpha]_D$) have not been determined.

Newborn Mouse Tumorigenesis. Pregnant CD-1 mice (Charles River Breeding Laboratories) were housed in plastic cages on corn cob bedding. They delivered their litters from 5 to 8 days after arrival. Within 24 hr of birth, 10 pups in each litter were given an i.p. injection of the first dose of compound. Subsequent injections were given on the 8th and 15th days of life. The mice were administered a total dose of 14 nmol of compound divided into three injections of 2, 4, and 8 nmol, respectively. Control mice were given three injections of dimethyl sulfoxide (5, 10, and 20 μl). The mice were weaned at 25 days of age and killed at 32 to 36 weeks of age. At necropsy, the major organs of each animal were examined, tumors were counted, and tissues were fixed in 10% (vol/vol) formalin in phosphate buffer (Fisher Scientific). All pulmonary and hepatic tumors were examined histologically. Pathology of the lung (24) and liver (25, 26) tumors were the same as has been described. Statistical significance of the newborn mouse tumor data was evaluated by the Fisher 2×2 exact test.

RESULTS

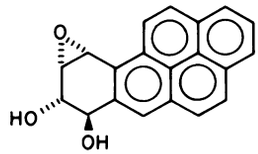
The tumorigenic activities of (+)-B[*a*]P diol epoxide-2, 6-FB[*a*]P diol epoxide-2, and 6-FB[*a*]P diol epoxide-1 in newborn mice are shown in Table 1. At termination of the study, 12.9% of the control mice had developed pulmonary tumors with an average of 0.19 tumor per mouse. Of the bay-region diol epoxides of B[*a*]P and 6-FB[*a*]P used in this study, only (+)-B[*a*]P diol epoxide-2 had high tumorigenic activity. This compound produced a 60% lung tumor incidence with an average of 1.90 lung tumors per mouse, and these values were highly significant ($P < 0.001$) compared to the other treatment groups. [We have shown (8) that, of the four optically active bay-region diol epoxides of B[*a*]P, only (+)-B[*a*]P diol epoxide-2 had significant tumorigenic activity in the newborn mouse tumor model.] In addition, (+)-B[*a*]P diol epoxide-2 produced a significant incidence of hepatic tumors in male mice ($P < 0.05$) compared to control mice or mice treated with either of the two diol epoxide isomers of 6-FB[*a*]P.

When the data for combined male and female mice were analyzed, 6-FB[*a*]P diol epoxide-1 and 6-FB[*a*]P diol epoxide-2 were found to be devoid of any significant tumorigenic activity at the 14-nmol dose. The pulmonary tumor incidence in male mice treated with 6-FB[*a*]P diol epoxide-2 (0.40 tumor per mouse) was not significantly different ($P < 0.20$) from control values but was significantly lower ($P < 0.05$) than the incidence observed in male mice treated with (+)-B[*a*]P diol epoxide-2 (1.68 tumors per mouse).

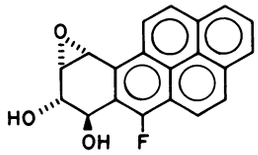
DISCUSSION

The present study was designed to evaluate the role of conformation of the hydroxyl groups (pseudoaxial vs. pseudoequatorial) in the expression of tumorigenic activity of bay-region diol epoxides of polycyclic hydrocarbons. Results with a number of polycyclic hydrocarbon bay-region diol epoxide diastereomers (6–13) had revealed that nearly all of the tumorigenic activity resides in the diol epoxide-2 diastereomers, which have their hydroxyl groups in the pseudoequatorial conformation. [In the case of benzo[*c*]-phenanthrene, the racemic diol epoxide-1 diastereomer has skin tumor-initiating activity equal to that of racemic diol epoxide-2 (27). However, because of steric crowding in the bay region of benzo[*c*]phenanthrene, the hydroxyl groups of both diastereomers prefer the pseduoequatorial conformation (5).] However, interpretation of these results is complicated in that the (*R*,*S*,*S*,*R*) enantiomer of each diol epoxide-2 diastereomer has high tumorigenic activity (9, 16–18), despite the fact that the enantiomer with the (*S*,*R*,*R*,*S*) absolute configuration also has hydroxyl groups that prefer the pseudoequatorial conformation. Clearly, absolute configuration of the bay-region diol epoxide is a critical determinant of tumorigenic activity among these compounds.

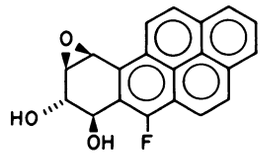
The results of the present study directly implicate a role for conformation of the hydroxyl groups in the tumorigenic activity of bay-region diol epoxides. Both (+)-B[*a*]P diol epoxide-2 (23) and 6-FB[*a*]P diol epoxide-2 (20, 21) have the (7*R*,8*S*,9*S*,10*R*) absolute configuration, and the unfluorinated compound has significantly higher tumorigenic activity than the fluorinated derivative. As a result of the fluorine substituent at C-6, 6-FB[*a*]P diol epoxide-2 has its hydroxyl groups predominantly in the pseudoaxial conformation (20, 21) in contrast to the predominantly pseudoequatorial conformation (23) for the unfluorinated bay-region diol epoxide (Fig. 2). The lack of tumorigenic activity of 6-FB[*a*]P diol epoxide-1 in the present study is consistent with the reported



(+)-BP-(7R,8S,9S,10R)-DIOL EPOXIDE-2



6-FBP-(7R,8S,9S,10R)-DIOL EPOXIDE-2



6-FBP-(7R,8S,9R,10S)-DIOL EPOXIDE-1

FIG. 1. Absolute configuration of bay-region 7,8-diol-9,10-epoxides of B[*a*]P and 6-FB[*a*]P used in the present study.

Table 1. Tumorigenicity of bay-region diol epoxides of B[*a*]P and 6-FB[*a*]P in newborn mice

Compound	Total dose, nmol	Mice weaned at day 25, no.	Mice alive at termination		Pulmonary tumors		Hepatic tumors	
			Sex	No.	Mice with tumors, %	Average no. per mouse	Mice with tumors, %	Average no. per mouse
Solvent	—	78	Female	34	17.6	0.26	0	0
			Male	28	7.1	0.11	0	0
			Total	62	12.9	0.19		
(+)-B[*a*]P diol epoxide-2	14	69	Female	30	76.6	2.10	0	0
			Male	28	42.9	1.68	14.3	0.18
			Total	58	60.3	1.90		
6-FB[*a*]P diol epoxide-1	14	76	Female	30	3.3	0.10	0	0
			Male	40	12.5	0.18	0	0
			Total	70	8.6	0.14		
6-FB[*a*]P diol epoxide-2	14	72	Female	33	0	0	0	0
			Male	35	8.6	0.40	0	0
			Total	68	4.4	0.21		

Groups of 80 CD-1 mice received i.p. injections of 1/7, 2/7, and 4/7 of the total dose of 14 nmol of each compound on days 1, 8, and 15 of life, respectively. Animals were killed at 32–36 weeks of age.

inactivity of (+)-B[*a*]P diol epoxide-1 (16). Both of these compounds have the (7*R*,8*S*,9*R*,10*S*) absolute configuration, and each prefers the pseudoaxial conformation for its hydroxyl groups (20, 21, 23).

Substitution of fluorine for hydrogen in 6-FB[*a*]P diol epoxide-2 introduces the following two major changes in the molecule: (*i*) the conformational shift toward a preference for pseudoaxial hydroxyl groups as described above and (*ii*) an inductive effect that is expected to destabilize the benzylic carbocation derived from the diol epoxide of 6-FB[*a*]P relative to that of B[*a*]P. At pH 7 in aqueous and partly aqueous media, the influences of these two factors on solvolytic reactivity of the 6-FB[*a*]P diol epoxide-2 largely offset each other, with the result that the half-life of the fluorinated diol epoxide is only 2–3 times shorter than that of its unfluorinated counterpart (21). Thus, under physiological conditions the solvolytic lifetimes of the two diol epoxides should be comparable, and more rapid solvolytic destruction of one diol epoxide relative to the other is unlikely to account for the dramatic difference in tumorigenic activity. The influence of the inductive effect of fluorine on the efficiency of reactions of the diol epoxides with cellular target molecules is unknown. The observation by Thakker *et al.* (20) that the 9,10-epoxy-7,8,9,10-tetrahydro derivatives of B[*a*]P and 6-FB[*a*]P, which lack the 7,8-diol substituents, have approximately equal mutagenic activity in several cell systems suggests that a purely inductive effect of a 6-fluoro substituent is small on differential reactivity of the tetrahydroepoxides toward covalent modification of DNA relative to solvolysis or other detoxification processes. In contrast, 6-FB[*a*]P diol epoxide-2 is less mutagenic by a factor of 3 to 6 to strains TA98 and TA100 of *Salmonella typhimurium* and Chinese hamster V79 cells than is (+)-B[*a*]P diol epoxide-2 (20). These results indicate that the effects of fluorine substitution at C-6 are mediated by altered diol conformation resulting from interactions of the fluorine with the hydroxyl group at the adjacent C-7 position. The lower tumorigenic activity of 6-FB[*a*]P diol epoxide-2 compared to (+)-B[*a*]P diol epoxide-2 in newborn mice is consistent with our mutagenicity results (20) and is, to our knowledge, the first direct demonstration of an influence of conformation of hydroxyl groups in bay-region diol epoxides on the expression of tumorigenic activity. The altered conformation of 6-FB[*a*]P diol epoxide could result in inefficient alkylation of the cellular target or more efficient repair once this target is alkylated.

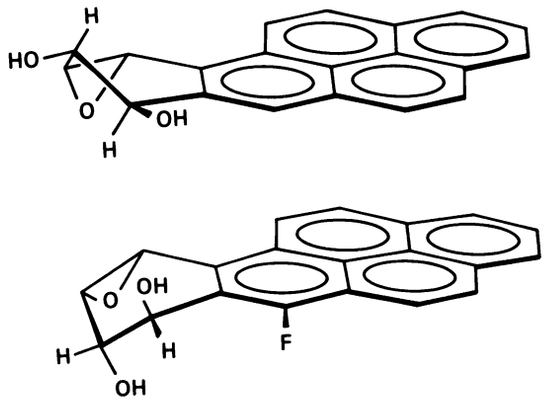


FIG. 2. Preferred conformations of the 7,8-diol-9,10-epoxide diastereomers of B[*a*]P (*Upper*) and 6-FB[*a*]P (*Lower*) with the (7*R*,8*S*,9*S*,10*R*) absolute configuration. The presence of the 6-fluoro substituent causes a decrease in the NMR coupling constant for the carbinol hydrogens ($J_{7,8}$) from 9 Hz to 5 Hz (20, 21).

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