Mutagenicity and Tumorigenicity of Dihydrodiols, Diol Epoxides, and Other Derivatives of Benzo(f)quinoline and Benzo(h)quinoline

Subodh Kumar, Harish C. Sikka, Sushil K. Dubey, Anna Czech, Nora Geddie, Chung-Xiou Wang, and Edmond J. LaVoie

Great Lakes Laboratory, State University of New York College at Buffalo, Buffalo, New York 14222; and the Division of Environmental Carcinogenesis, Naylor Dana Institute for Disease Prevention, American Health Foundation, Valhalla, New York 10595.

ABSTRACT

The mutagenic activities of benzo(f)quinoline, benzo[h]quinoline, and a number of their derivatives, including dihydrodiols, K-region oxides, diol epoxides, and tetrahydroepoxides, were assessed in strain TA 100 of Salmonella typhimurium. The dihydrodiol derivatives of benzo(f)quinoline and benzo[h]quinoline were also tested for tumorigenic activity in newborn mice. Benzo(f)quinoline was metabolically activated in the presence of rat liver S-9 preparation to products mutagenic to the bacterial system to a greater extent than was benzo[h]quinoline. However, trans-7,8-dihydro-7,8-dihydroxybenzo[f]quinoline was less mutagenic compared to trans-7,8-dihydroxy-7,8-dihydrobenzo[h]quinoline in the presence of rat liver homogenate. The data on the mutagenic activity of the dihydrodiol derivatives of benzoquinolines were consistent with the intrinsic mutagenicity of the corresponding epoxide derivatives, in that the bay-region diol epoxides and tetrahydroepoxide of benzo[f]quinoline exhibited considerably higher mutagenic activities compared to those of the corresponding derivatives of benzo[f]quinoline at equivalent doses. The K-region oxides of benzo[f]quinoline and benzo[h]quinoline were significantly less mutagenic than their corresponding bay-region diol epoxide and tetrahydroepoxide derivatives. The demonstration that benzo[f]quinoline is significantly more mutagenic than trans-7,8-dihydro-7,8-dihydroxybenzo[f]quinoline, a precursor to the weakly mutagenic bay-region diol epoxide, suggests that the bay-region diol epoxide formation is not the principal pathway for the metabolic activation of benzo[f]quinoline to a mutagen. On the other hand, the isomeric benzo[h]quinoline appears to exert its mutagenic effect via the formation of its bay-region diol epoxide. These results indicate that the position of a nitrogen heteroatom in phenanthrene (the analogous carbocyclic aromatic hydrocarbon) not only has a marked effect on the mutagenic activities of the diol epoxide derivatives, but also can alter the metabolic activation pathways of the parent hydrocarbons. Benzo[f]quinoline, benzo[h]quinoline, and their dihydrodiol derivatives were not tumorigenic in newborn mice.

INTRODUCTION

The aza-phenanthrenes BfQ and BhQ (Fig. 1) are among the major constituents of the basic fractions of urban air particulates, automobile exhaust and cigarette smoke (1–3). While bioassays on the carcinogenic activity of BfQ in either mice or rats have not demonstrated significant carcinogenic activity (4, 5), both BfQ and BhQ are mutagenic in Salmonella typhimurium TA 100 and BfQ is mutagenic in Salmonella typhimurium TM 677 when assayed in the presence of rat liver homogenate (6–12). While there have been limited studies on the metabolism of these aza-arenes, the mechanism(s) by which these chemicals exert their mutagenic activity has not been elucidated (10, 12–14). Recent studies performed with other aza-arenes have shown that bay-region diol epoxides are the major metabolites responsible for their mutagenic and carcinogenic properties (15–20). The precursor dihydrodiols to these bay-region diol epoxides, i.e., BfQ-7,8-dihydrodiol and BhQ-7,8-dihydrodiol, have been shown to be major metabolites formed from incubations with rat liver homogenate and microsomes (13, 14). Therefore, the bay-region diol epoxides of BfQ and BhQ were considered likely candidates responsible for the mutagenic activities of their parent aza-PAHs.

Based on qualitative resonance interpretation (15) the bay-region epoxide of BfQ should be significantly less electrophilic and, consequently, less mutagenic than the bay-region epoxide of BhQ. If benzoquinolines produce mutagenicity through the generation of the bay-region epoxide, BhQ should be more mutagenic than BfQ which is contrary to the available experimental data on the mutagenicity of the two aza-arenes. We postulate on the basis of these data that pathways other than the bay-region diol epoxide formation are also involved in the metabolic activation of BfQ. In an effort to elucidate the mechanism(s) involved in the mutagenesis of BfQ and BhQ, we have assessed the mutagenic activities of K-region oxides, trans-dihydrodiols, bay-region diol epoxides, and the bay-region and non-bay-region tetrahydroepoxides of the two aza-arenes. We also evaluated the mutagenic activities of the corresponding derivatives of phenanthrene, the carbon-analogue of benzoquinolines to examine the influence of the presence and position of nitrogen heteroatom (a) on the metabolic activation of the dihydrodiol derivatives of BfQ and BhQ to mutagenic metabolites, and (b) on the intrinsic mutagenic activities of possible electrophilic metabolites of these aza-arenes. While neither BfQ nor BhQ exhibited significant tumorigenic activity when assayed in newborn mice or rats (4, 5), the potential of some of the major metabolites of these aza-arenes to be genotoxic was also evaluated in a newborn mouse bioassay.

MATERIALS AND METHODS

Chemicals. BfQ and BhQ were purchased from Aldrich Chemicals. Pure samples of BfQ-9,10-dihydrodiol, H-epoxides and syn- and anti-diol epoxides used in the present studies were obtained by unequivocal...
Mutagens. The ability of BfQ and BhQ, and their non-K-region dihydrodiols to generate products mutagenic to *S. typhimurium* TA 100 in the presence of rat liver homogenate at doses ranging from 0 to 200 μg/plate is shown in Fig. 2. None of these compounds was active in the absence of the rat liver homogenate. As reported earlier (10), BfQ was substantially more mutagenic than BhQ. BfQ-7,8-dihydrodiol, the immediate metabolic precursor of the bay-region diol epoxides of BfQ was significantly more active than other dihydrodiols of BfQ. However, it exhibited two- to 6-fold less mutagenic activity than BfQ (Fig. 2). While both the 7,8- and 9,10-dihydrodiols of BfQ were detected as metabolites upon incubation of BfQ with the rat liver microsomes (13, 14), neither of the dihydrodiols exhibited mutagenic activity comparable to the parent compound. In contrast, BhQ-7,8-dihydrodiol, the precursor of the BhQ bay-region diol epoxide, was more mutagenic than BhQ (Fig. 2). At dose levels ranging from 5 to 100 μg/plate BhQ-7,8-dihydrodiol was 2-7-fold more mutagenic than BhQ in the presence of S-9. However, at a higher dose of 200 μg/plate BhQ was extremely toxic, whereas BhQ-7,8-dihydrodiol showed significant mutagenic activity to *S. typhimurium*. Furthermore, BhQ-7,8-dihydrodiol was as mutagenic as its carbon analogue, namely, phenanthrene-1,2-dihydriol, and about fourfold more mutagenic than BfQ-7,8-dihydrodiol (Fig. 2). The 5,6-dihydrodiols (K-region diols) of BfQ and BhQ were inactive in this assay (data not shown).

Intrinsic Mutagenicity of Epoxide Derivatives of BfQ, BhQ, and Phenanthrene. The dose-response relationship for the inherent mutagenicity of the bay-region diol epoxides of phenanthrene, BfQ and BhQ, and tetrahydroepoxides of BfQ and BhQ in strain TA100 of *S. typhimurium* is shown in Figs. 3 and 4. Although large differences in mutagenic potency are

### RESULTS

#### Metabolic Activation of Phenanthrene, Benzoquinolines, and their K-Region and Non-K-Region Dihydrodiols to Bacterial Mutagens

The ability of BfQ and BhQ, and their non-K-region dihydrodiols to generate products mutagenic to *S. typhimurium* TA 100 in the presence of rat liver homogenate at doses ranging from 0 to 200 μg/plate is shown in Fig. 2. None of these compounds was active in the absence of the rat liver homogenate. As reported earlier (10), BfQ was substantially more mutagenic than BhQ. BfQ-7,8-dihydrodiol, the immediate metabolic precursor of the bay-region diol epoxides of BfQ was significantly more active than other dihydrodiols of BfQ. However, it exhibited two- to 6-fold less mutagenic activity than BfQ (Fig. 2). While both the 7,8- and 9,10-dihydrodiols of BfQ were detected as metabolites upon incubation of BfQ with the rat liver microsomes (13, 14), neither of the dihydrodiols exhibited mutagenic activity comparable to the parent compound. In contrast, BhQ-7,8-dihydrodiol, the precursor of the BhQ bay-region diol epoxide, was more mutagenic than BhQ (Fig. 2). At dose levels ranging from 5 to 100 μg/plate BhQ-7,8-dihydrodiol was 2-7-fold more mutagenic than BhQ in the presence of S-9. However, at a higher dose of 200 μg/plate BhQ was extremely toxic, whereas BhQ-7,8-dihydrodiol showed significant mutagenic activity to *S. typhimurium*. Furthermore, BhQ-7,8-dihydrodiol was as mutagenic as its carbon analogue, namely, phenanthrene-1,2-dihydriol, and about fourfold more mutagenic than BfQ-7,8-dihydrodiol (Fig. 2). The 5,6-dihydrodiols (K-region diols) of BfQ and BhQ were inactive in this assay (data not shown).

Intrinsic Mutagenicity of Epoxide Derivatives of BfQ, BhQ, and Phenanthrene. The dose-response relationship for the inherent mutagenicity of the bay-region diol epoxides of phenanthrene, BfQ and BhQ, and tetrahydroepoxides of BfQ and BhQ in strain TA100 of *S. typhimurium* is shown in Figs. 3 and 4. Although large differences in mutagenic potency are

---

**Fig. 1.** Structures of BfQ, BhQ, and phenanthrene.

**Fig. 2.** Metabolic activation of BfQ, BhQ, their dihydrodiol derivatives, and phenanthrene-1,2-dihydriol by hepatic microsomes from Aroclor 1254-treated rats. Experiments were performed as described in "Materials and Methods." Points, average of two separate experiments each with two plates. □, BfQ; ○, BhQ; △, BfQ-7,8-dihydriol; □, BhQ-9,10-dihydriol; △, BhQ-7,8-dihydriol; X, phenanthrene-1,2-dihydriol.
apparent, all the compounds produced a linear or nearly linear dose-response relationship. Mutation frequencies expressed as His* revertants/nmol of epoxide are obtained from these data, as well as additional experiments at higher doses with these epoxides. The results are summarized in Table 1.

Among a total of nine epoxides of these benzoquinolines, the bay-region tetrahydroepoxides were the more mutagenic derivatives of BfQ and BhQ, with the bay-region BhQ-H4-9,10-epoxide being the most active (Table 1). Comparison of the mutagenic activities associated with the bay-region diol epoxides of phenanthrene, BfQ, and BhQ indicates that the position of the nitrogen heteroatom at position 4 of these benzoquinolines diminishes mutagenic activity about 20-fold as compared to when the nitrogen is in the 1 position. Furthermore the difference in the mutagenic activities between bay-region and non-bay-region tetrahydroepoxides was even more pronounced among the related isomers of BfQ-H4-epoxides and BhQ-H4-epoxides than those of phenanthrene-H4-epoxides. Thus, while phenanthrene-H4-2,3-epoxide had 12% of the activity of phenanthrene-H4-3,4-epoxide (28), BfQ-H4-7,8-epoxide and BhQ-H4-7,8-epoxide had only 2.5 and 2.1% of the activities of BfQ-H4-9,10-epoxide and BhQ-H4-9,10-epoxide, respectively.

Among the diol epoxides of BfQ and BhQ, and phenanthrene, only phenanthrene-1,2-diol-3,4-epoxide (syn and anti) and BfQ-7,8-diol-9,10 epoxide (syn and anti) showed significant mutagenic activities (Fig. 3). These two compounds, however, are far less mutagenic than the corresponding derivatives of benz[a]anthracene (15). As observed for the syn and anti isomers of the bay-region diol epoxides of phenanthrene, the anti isomer of the BfQ-7,8-diol-9,10-epoxide exhibited greater mutagenic activity than the syn isomer. None of the diol epoxides of BfQ including anti-BfQ-7,8-diol-9,10-epoxide-N-oxide showed any significant mutagenic activity at doses ranging from 0 to 50 μg/plate. However, the dose-response curve for syn-BfQ-7,8-diol-9,10-epoxide was linear up to 200 μg (data not shown). The tetrahydroepoxides of both BfQ and BhQ were 14–28-fold more mutagenic than their diol epoxides. Both BfQ-5,6-oxide and BhQ-5,6-oxide (K region oxides) were virtually inactive (Table 1).

Comparison of the mutagenic activities associated with the bay-region diol epoxides of phenanthrene, BfQ, and BhQ indicates that the presence and position of the nitrogen heteroatom resulted in a diminished mutagenic response. This is particularly apparent in the case of BfQ. Syn-BfQ-7,8-diol-9,10-epoxide had only 2% of the activity of syn-phenanthrene-1,2-diol-3,4-epoxide and anti-BfQ-7,8-diol-9,10-epoxide-N-oxide had <1% of the activity of anti-phenanthrene-1,2-diol-3,4-epoxide. Anti-BfQ-7,8-diol-9,10-epoxide was not available for comparison. In the case of BhQ, the syn- and anti- isomers of the bay-region diol epoxide had approximately 18% of the activity of the respective anti- and syn- isomers of phenanthrene-1,2-diol-3,4-epoxide.

Table 1 Mutagenic activity of diol epoxides and tetrahydroepoxides of BfQ, BhQ, and phenanthrene in strain TA100 of S. typhimurium

| Derivatives | BfQ | BhQ | Phenanthrene | *
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>5,6-Oxide</td>
<td>0.7</td>
<td>0.3</td>
<td>ND* (19,000)</td>
</tr>
<tr>
<td>H4-7,8-Epoxide</td>
<td>0.07</td>
<td>6.0</td>
<td>ND (3,800)</td>
</tr>
<tr>
<td>H4-9,10-Epoxide</td>
<td>14.0</td>
<td>280.0</td>
<td>ND</td>
</tr>
<tr>
<td>syn-7,8-Diol-9,10-epoxide</td>
<td>1.1</td>
<td>10.0</td>
<td>57.0 (9,600)</td>
</tr>
<tr>
<td>anti-7,8-Diol-9,10-epoxide</td>
<td>ND</td>
<td>33.0</td>
<td>177 (11,000)</td>
</tr>
<tr>
<td>anti-7,8-Diol-9,10-epoxide-N-oxide</td>
<td>1.2</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>anti-9,10-Diol-7,8-epoxide</td>
<td>0.6</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

* Values in parentheses represent the mutagenic activities of the corresponding epoxide derivatives of benz[a]anthracene (15).

---

**Fig. 3.** Intrinsic mutagenic activity of the angular benzo-ring diol epoxides of BfQ, BhQ, and phenanthrene in strain TA100 of S. typhimurium. Experiments were performed as described in "Materials and Methods." Points, average of two separate experiments each with two plates. A, syn-BfQ-7,8-diol-9,10-epoxide; O, anti-BfQ-7,8-diol-9,10-epoxide-N-oxide; X, syn-BhQ-7,8-diol-9,10-epoxide; X, anti-BhQ-7,8-diol-9,10-epoxide; H, syn-phenanthrene-1,2-diol-3,4-epoxide; D, anti-phenanthrene-1,2-diol-3,4-epoxide.

**Fig. 4.** Intrinsic mutagenic activity of the tetrahydro epoxides of BfQ and BhQ in strain TA100 of S. typhimurium. Experiments were performed as described in "Materials and Methods." Points, average of two separate experiments each with two plates. A, BfQ-H4-7,8-epoxide; X, BfQ-H4-9,10-epoxide; O, BhQ-H4-7,8-epoxide; H, BhQ-H4-9,10-epoxide.
Tumorigenicity Studies with Metabolites of BfQ, BhQ, and Phenanthridine in Newborn Mice. The results of this newborn mouse bioassay performed with the major metabolites of BfQ and BhQ are summarized in Table 2. Phenanthridone, which is a major mutagenic metabolite of phenanthridine (benzo[c]quinoline), was also included in this bioassay. Quinoline served as the positive control in this assay (4, 5), and was administered at equimolar doses using the same dosage regime outlined in the “Materials and Methods.” While quinoline was a potent hepatocarcinogen in the male newborn mice in this assay, none of the derivatives of BfQ and BhQ exhibited tumorogenic activity in either male or female newborn mice.

DISCUSSION

The data on mutagenic activities of BhQ-7,8-diol and anti-BhQ-7,8-diol-9,10-epoxide suggest that these derivatives could be the major proximate and ultimate mutagenic metabolites, respectively, of BhQ. Since the 7,8-dihydrodiol of BhQ has been identified as a major metabolite of BhQ (13) and is readily metabolically activated to a mutagen, it appears that the bay-region theory of the metabolic activation of polynuclear aromatic hydrocarbons can be extended to the mutagenesis of BhQ.

The anti-isomer of the bay-region diol epoxide of BfQ was not available for direct evaluation. The weak intrinsic mutagenic activity of syn-BfQ-7,8-diol-9,10-epoxide as well as BfQ-H4-9,10-epoxide, however, indicates that the lack of significant mutagenic activity of BfQ-7,8-dihydrodiol is associated with the inherent weak mutagenic potency of its bay-region diol epoxide(s). Since BfQ is a more potent mutagen than BfQ-7,8-dihydrodiol, a precursor to BfQ bay-region diol epoxide, one may conclude that despite the fact that BfQ is extensively metabolized to BfQ-7,8-dihydrodiol (13, 14), the formation of this dihydrodiol and its conversion to the bay-region diol epoxide may not be the principal mechanism by which BfQ exerts its mutagenic activity. It is the first example among aza-PAHs where it has been shown that the parent hydrocarbon is significantly more mutagenic than the dihydrodiol precursor to its bay-region dihydrodiol epoxide. These results suggest that the bay-region theory proposed for the mutagenesis/carcinogenesis of PAHs and other aza-PAHs (29) may not be applicable to BfQ. In S. typhimurium TA100 in the presence of rat liver homogenate, BfQ is a more potent mutagen than BhQ. The mutagenesis of BfQ may be the result of exceptional mutagenic activity of metabolites other than those formed through the intermediary of the 7,8- or 9,10-dihydrodiol. It is also possible that the arene oxide precursors to either of these dihydrodiols may contribute appreciably to the mutagenic activity of BfQ. Our findings that isomeric BfQ and BhQ are metabolically activated via different pathways suggest the possibility that the position of nitrogen substitution in a polynuclear aromatic hydrocarbon can alter the metabolic pathway(s) by which aza-polyaromatic aromatic hydrocarbons are activated.

Our studies show that the presence of a nitrogen heteroatom has a profound effect on the biological activity of the epoxides of benzoquinolines. Similar results were noted for the bay-region diol epoxides and tetrahydroepoxides of benzacridines (15). Our data on the relative mutagenic activities of the epoxide derivatives of BfQ and BhQ are consistent with qualitative resonance analysis and with Hückel and pertubational molecular orbital calculations used to predict the relative ease of carbonium ion formation from diol epoxides and tetrahydroepoxides (30). The reactivity and, consequently, the biological activity of the epoxide are attributed to the ease of carbonium ion formation from an epoxide. This ease is facilitated by the capacity of the hydrocarbon to delocalize the positive charge of the carbonium ion throughout the π-electron system of the remaining aromatic nucleus of the hydrocarbon. The resonance of the C-10 carbonium ion formed by the ring opening of a bay-region diol epoxide or tetrahydroepoxide places a positive charge on the nitrogen heteroatom of BfQ-7,8-diol-9,10-epoxide, but not of BhQ-7,8-diol-9,10-epoxide. Since delocalization of the positive charge on the electronegative nitrogen heteroatom is energetically destabilizing, one would expect less mutagenicity for the bay-region diol epoxide and tetrahydroepoxide of BfQ than for the corresponding derivatives of BhQ. A significant loss in the mutagenic activities of the H4-epoxides of BfQ and BhQ as a result of the substitution of trans-diol group at the 7,8-position of the molecules is consistent with the observation made with the similar derivatives of other PAHs and aza-PAHs (29). Most likely, the hydroxyl groups increase the bulk of the cyclohexane ring system, which in turn inhibits the interaction of these diol epoxides with the bacterial DNAs.

The bay-region diol epoxide of BhQ was considerably less mutagenic than the phenanthrene bay-region diol epoxide. Yet, BhQ-7,8-dihydrodiol and its carbon analogue phenanthrene-1,2-dihydrodiol were metabolically activated to mutagenic products, presumably the BhQ-7,8-diol-9,10-epoxide and phenanthrene-1,2-diol-3,4-epoxide, respectively, to similar extents (see Fig. 2). These data suggest that BhQ-7,8-dihydrodiol, compared to phenanthrene-1,2-dihydrodiol, is converted more efficiently to the bay-region diol epoxide, implying that the presence of nitrogen heteroatom at position 1 enhances the oxidative metabolism at the 9,10-position of BhQ-7,8-dihydrodiol. These findings differ from the results of a previous study which suggested that the presence of nitrogen atom at position 12 of analogous benz[c]acridine-3,4-dihydrodiol retains oxidative metabolism or shifts it away from the 1,2-position (15).

Bioassays performed with both BfQ and BhQ in newborn mice did not demonstrate a significant tumorigenic effect in either males or females (4, 5). There is a possibility that in newborn mice the dihydrodiols of BfQ and BhQ, the metabolic
precursors of the reactive diol epoxides, may not be formed and/or available in vivo in quantities sufficient to elicit tumorigenic response. Therefore, in order to test whether or not dihydrodiols of BFQ and BHQ are tumorigenic intermediates of the parent hydrocarbons, we assessed the tumorigenic activities of these dihydrodiols directly in newborn mice. A major mutagenic metabolite of phenanthridine, phenanthridone, was also evaluated for tumorigenic activity. As was observed for their parent compounds, these metabolites did not exhibit significant tumorigenic activity in this assay (see Table 2). In contrast to the results obtained for these benzoquinolines and several of their major metabolites, quinoline, which was the positive control in this assay, was a potent hepatocarcinogen in the male newborn mice. These results parallel similar results observed for bioassays performed on the skin of SENCAR mice (12). The lack of tumorigenicity of BHQ in newborn mice may be explained by the low tumorigenic activity of its 7,8-dihydrodiol. However, based upon the mutagenicity data (vide supra), a lack of tumorigenicity of BFQ in newborn mice may be attributed to an extremely low, if any, formation of a reactive and tumorigenic metabolite(s) of BFQ other than that derived from BFQ-7,8-dihydrodiol, a metabolic precursor to the bay-region diol epoxide. In studies in which quinoline, BFQ, BbQ, and phenanthridine were assayed as tumor initiators, only quinoline exhibited significant tumorigenic activity. The data in this study suggest that benzoquinolines, as well as several of their major metabolites identified from in vitro studies, are less tumorigenic than quinoline.

REFERENCES

Mutagenicity and Tumorigenicity of Dihydrodiols, Diol Epoxides, and Other Derivatives of Benzo(\(h\))quinoline and Benzo(\(h\))quinoline

Subodh Kumar, Harish C. Sikka, Sushil K. Dubey, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/49/1/20