Inhibition of Benzo(a)pyrene and Benzo(a)pyrene 7,8-Dihydriodiol Metabolism by Benzo(a)pyrene Quinones

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ABSTRACT

Benzo(a)pyrene 1,6-, 3,6-, and 6,12-quinones were found to be noncompetitive inhibitors of mixed-function oxidation of benzo(a)pyrene (BP) and trans-7,8-dihydro-7,8-dihydroxybenzo(a)pyrene (BP 7,8-dihydriodiol). BP 6,12-quinone was the most potent inhibitor of both BP and BP-7,8-dihydriodiol oxidation followed by BP 1,6-quinone and BP 3,6-quinine. The K_i for inhibition of BP mixed-function oxidation by BP 6,12-quinone was 0.35 µM. Metabolism of BP 7,8-dihydriodiol was more sensitive to inhibition by BP quinones; the K_i for BP 6,12-quinine was 0.10 µM. High-pressure liquid chromatographic analysis indicated no effect of BP quinones on the distribution of BP and BP-7,8-dihydriodiol metabolites.

BP quinones were reduced by 3-methylcholanthrene-induced rat liver microsomes in the presence of reduced nicotinamide adenine dinucleotide phosphate. Under anaerobic conditions, where reoxidation was prevented, reduction of BP quinones was rapid and followed first-order kinetics. Under aerobic conditions, steady-state levels of reduced and oxidized quinones were observed; BP 1,6- and 3,6-quinones were 30 to 40% reduced while BP 6,12-quinone was less than 5% reduced. All three quinones stimulated oxygen uptake by microsomes. Rapid reoxidation of the reduced quinones could account for the observed levels of oxidized quinone. Homogeneous rat liver reduced nicotinamide adenine dinucleotide phosphate-cytochrome P-450 reductase catalyzed reduction of the quinones at rates consistent with those for microsomal reduction, and the rate was not enhanced by addition of homogeneous cytochrome P-448.

The strong inhibition of BP and BP 7,8-dihydriodiol metabolism by BP quinones provides an explanation for the stimulatory effect of uridine diphosphoglucuronic acid on BP metabolism and DNA modification by BP metabolites.

INTRODUCTION

Chemical carcinogenesis initiated by BP requires metabolic activation via microsomal mixed-function oxidation. Considerable evidence suggests that anti- and syn-BP 7,8-diol-9,10-epoxides derived from mixed-function oxidation of BP 7,8-dihydriodiol and the most mutagenic and carcinogenic metabolites of BP (12, 14, 17). DNA which is exposed in vitro to metabolites of BP is modified primarily at N-2 of guanine by anti- and syn-BP 7,8-diol-9,10-epoxides (25-27, 29) and at undefined positions by further metabolites of BP phenols (15, 29). Only the former modification is apparent in DNA isolated from mammalian cells. This lack of interaction of phenol metabolites with DNA seems to be due to ready conjugation of BP phenols with glucuronic acid by UDP-glucuronosyltransferase (6, 21, 23) and with sulfate via sulfotransferases (4, 22, 24).

A major category of metabolites produced during BP microsomal mixed-function oxidation are the BP quinones. BP 1,6-, 3,6-, and 6,12-quinones are probably derived from an initial 6-hydroxylation of BP (16, 17, 23, 29, 34). In addition, it has been shown that BP 3,6-quinine may be generated from BP-3-phenol (32). The proportion of each quinone formed does vary with the source of microsomes; however, BP 3,6-quinine makes up the largest proportion of the quinone formed (13, 34). The addition of UDPGA to incubations of BP with rat liver microsomes removes most of the BP quinones from the metabolic products, either by trapping of BP 6-phenol or BP 3-phenol as glucuronides (10, 21, 23) or by conjugation of reduced quinones (3, 18). Other BP phenols, and to a lesser extent BP dihydriodiol, are also decreased (23) while water-soluble glucuronides increase (8). Surprisingly, however, the addition of UDPGA significantly enhances the total rate of mixed-function oxidation of BP (2, 8) and increases the covalent binding of BP 7,8-diol-9,10-epoxides to DNA 2.7-fold (8).

The stimulatory effect of UDPGA on BP metabolism has been attributed to removal of an inhibitory product from the reaction (2, 8). In this paper, we show that BP quinones formed by metabolism of BP are indeed potent inhibitors of the mixed-function oxidation of both BP and BP 7,8-dihydriodiol. The stimulatory effect of UDPGA on the modification of DNA by BP 7,8-diol-9,10-epoxides is explained in terms of removal of this inhibition by glucuronic acid conjugation of BP quinones or their precursors.

MATERIALS AND METHODS

Chemicals. DMSO, hexane, and BP were purchased from Aldrich Chemical Co., Milwaukee, Wis. BP 1.6-, 3.6-, and 6,12-quinones were obtained from the National Cancer Institute Chemical Repository and were dissolved in DMSO. [13C]BP (27 Ci/mmol) was purchased from Amersham Radiochemicals, Arlington Heights, Ill., and purified by high-pressure liquid chromatography shortly before use. [3H]BP 7,8-dihydriodiol was enzymatically synthesized and purified by described procedures (6). Glucose oxidase (Aspergillus niger), glucose, dicoumarol, and other reaction cofactors were purchased from Sigma Chemical Co., St. Louis, Mo.
Instrumentation and Procedures. The induction of male Sprague-Dawley rats (60 to 70 g; Holtzman Co., Madison, Wis.) with 3-methylcholanthrene and the preparation of microsomes were done by techniques previously described (6, 7).

The total oxidation of \([\text{H}]\)BP was measured by the method of Van Cantfort et al. (30), using standard 1-ml incubations containing 0.3 mg MC-microsomes per ml, 15 μM \([\text{H}]\)BP, and reaction cofactors as previously described (6, 8). For analysis of specific BP metabolites, separate incubations were extracted with acetone:ethyl acetate (1:2) and analyzed by high-pressure liquid chromatography (7, 33). \([\text{H}]\)BP 7,8-dihydrodiol metabolism was examined in standard 0.5-ml incubations containing 0.3 mg MC-microsomes per ml and 6 μM \([\text{H}]\)BP 7,8-dihydrodiol. Analysis of tetrahydroxybenzo(a)pyrene and trihydroxybenzo(a)pyrene products by high-pressure liquid chromatography (6) indicated linear product formation through 3-min incubation time. BP quinones were added in DMSO; control incubations received DMSO only. The final concentration of DMSO was 1%, which did not inhibit BP metabolism.

The reduction of BP quinones was monitored as described by Capdevila et al. (3), using an Aminco DW-2 spectrophotometer. Measurements of steady-state levels of reduced BP quinones under aerobic conditions were made using a reaction mixture containing 86 mM potassium phosphate buffer (pH 7.5), 3 mM MgCl₂, 0.06 unit glucose-6-phosphate dehydrogenase per ml, 7 mM glucose 6-phosphate, 0.3 mg MC-microsomes per ml, and 10 μM quinone. After the spectrum of the oxidized quinone was recorded in the split-beam mode, NADPH (240 μM) was added, and repetitive scans between 360 and 660 nm were recorded.

For measurements of the rate of reduction, changes in absorbance of the wavelength pair 460 – 430 nm (BP 1,6-quinone), 485 – 458 nm (BP 3,6-quinone), or 422 – 458 nm (BP 6,12-quinone) were monitored in the dual-wavelength mode.

Anaerobic reaction mixtures contained 75 mM potassium phosphate, pH 7.5, 3 mM MgCl₂, 4 mg glucose per ml, 6.25 units glucose oxidase per ml, 0.06 unit glucose-6-phosphate dehydrogenase per ml, 7 μM glucose 6-phosphate, and 0.08 mg MC-microsomes per ml. The cuvets were sealed, flushed with N₂, evacuated, flushed again with N₂, and stirred for 15 min at room temperature. The N₂ was deoxygenated by passage through a solution of 0.5% sodium dithionite/0.05% 2-anthraquinone sodium sulfonate: 0.4% sodium hydroxide, followed by passage through 1 M potassium phosphate, pH 7.5 (11). Quinone (10 μM) was added with a syringe, and a base line was recorded. Reactions were initiated with NADPH (240 μM). For inhibition experiments, dicoumarol (160 μM) in potassium phosphate buffer was added immediately before the NADPH or reaction mixtures were bubbled with carbon monoxide for 1 min before the addition of NADPH.

The measurement of substrate-dependent oxygen consumption in incubations was done using an Aminco vibrating platinum electrode. The reaction components in the 3-ml incubations were the same as listed above for aerobic quinone reduction except that the microsomal protein concentration was 2 mg/ml and NADPH was 250 μM. Buffer at 37° was bubbled with air for 5 min before use. Incubations containing all reactants except microsomes and substrate were equilibrated for 3 min. Subsequently, total oxygen consumption was measured for the 2 min following the addition of microsomes plus 10 μM quinone or microsomes plus DMSO alone. Oxygen consumption was based on a 100% value of 2.11 × 10⁻⁶ M dissolved oxygen for air-saturated 37° water.

NADPH-cytochrome c reductase activity was measured by the method of Masters et al. (19). One-ml incubations contained 3.1 × 10⁻⁶ M cytochrome c and 0.3 M potassium phosphate buffer, pH 7.5. Reactions were initiated by the addition of 80 nmol NADPH, and the increase in absorbance at 550 nm was measured on a Gilford recording spectrophotometer.

RESULTS

The addition of any one of the 3 BP quinones produced by microsomal metabolism of BP substantially inhibited the mixed-function oxidation of BP by MC-microsomes (Chart 1). The concentration of quinone corresponding to 50% inhibition was dependent upon the specific BP quinone (3,6 > 1,6 > 6,12).

At low concentrations, an equimolar mixture of the 3 quinones provided an intermediate inhibition while, at 10 μM, the mixture was more effective than the most potent individual BP quinone.

Addition of UDPGA reversed the inhibition of BP metabolism caused by 10 μM BP quinone (Table 1). In the presence of UDPGA and quinone, there was a slight stimulation of BP metabolism compared to controls. This stimulation was not as great as that previously observed in the absence of added quinone (8), indicating that some residual inhibition was still present.

<table>
<thead>
<tr>
<th>QUINONE (μM)</th>
<th>BP quinone</th>
<th>UDPGA (0 mm)</th>
<th>UDPGA (2 mm)</th>
<th>UDPGA (5 mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,6-quinone</td>
<td>1.92 (48)</td>
<td>4.02 (101)</td>
<td>4.07 (102)</td>
<td></td>
</tr>
<tr>
<td>3,6-quinone</td>
<td>2.67 (67)</td>
<td>4.13 (104)</td>
<td>4.42 (112)</td>
<td></td>
</tr>
<tr>
<td>6,12-quinone</td>
<td>1.82 (23)</td>
<td>4.36 (110)</td>
<td>4.21 (106)</td>
<td></td>
</tr>
</tbody>
</table>

Table 1
Reversal of quinone inhibition of BP metabolism by UDPGA

Total BP metabolism was assayed as described in “Materials and Methods” with an incubation time of 4 min. Results are expressed as percentages of DMSO controls (9.08 nmol/0.3 mg 4 min) ± S.D. in the presence of varying amounts of BP 1,6 (•), 3,6 (□), or 6,12-quinone (△) or an equimolar mixture of the 3 quinones (○).

Chart 1. Inhibition of BP metabolism by BP quinones. Total BP metabolism was assayed as described in “Materials and Methods” with an incubation time of 4 min. Results are expressed as percentages of DMSO controls (9.08 nmol/0.3 mg 4 min) ± S.D. in the presence of varying amounts of BP 1,6 (■), 3,6 (□), or 6,12-quinone (△) or an equimolar mixture of the 3 quinones (○).
In Chart 2, the metabolism of BP at varying concentrations of substrate is represented as a Lineweaver-Burk plot at 0, 0.5, and 2 μM BP 6,12-quinone. The incubation times were very short so that, even at low concentrations of BP, less than 25% of the substrate was metabolized. At this concentration of microsomal protein (0.3 mg/ml), the K_m for BP metabolism was 0.76 μM, which is similar to that reported by Robie et al. (28). The addition of BP 6,12-quinone markedly decreased the V_max while only slightly increasing the K_m. Inhibition by 2 μM BP 6,12-quinone was 3.8-fold at 1 μM BP and decreased to only 2.5-fold at saturating BP concentrations. Inhibition was, therefore, very close to noncompetitive (1). The K_i for inhibition of BP mixed-function oxidation by BP 6,12-quinone was determined from this to be 0.35 μM. Analysis of the effect of BP 6,12-quinones on the distribution of BP metabolites indicated a completely unselective effect (Table 2).

Analogous experiments were carried out to determine the effect of BP quinones on the formation of anti- and syn-BP 7,8-diol, 9-epoxides. The conversion of BP 7,8-dihydrodiol to stereoisomeric tetrahydroxybenzo(a)pyrenes which are derived from the hydrolysis of the unstable oxides is shown in Table 3 in the presence and absence of 0.5 μM BP 6,12-quinone. Again, the effect of the quinones was unselective with respect to anti- and syn- attack on BP 7,8-dihydrodiol; again, the relative effectiveness of the 3 quinones was the same as for BP metabolism. The 1:1:1 mixture of 3 quinones (3.3 μM concentrations of each quinone) was as effective as 10 μM of the most potent BP 6,12-quinone.

In Chart 3, the concentration dependence of BP 7,8-dihydrodiol mixed-function oxidation is represented as a Lineweaver-

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Table 2

<table>
<thead>
<tr>
<th>Metabolites</th>
<th>Control (nmol/mg/min)</th>
<th>Quinone (nmol/mg/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BP 9,10-diol</td>
<td>0.86 ± 0.10</td>
<td>0.46 ± 0.06</td>
</tr>
<tr>
<td>BP 4,5-diol</td>
<td>0.26 ± 0.02</td>
<td>0.14 ± 0.02</td>
</tr>
<tr>
<td>BP 7,8-diol</td>
<td>0.41 ± 0.03</td>
<td>0.21 ± 0.02</td>
</tr>
<tr>
<td>Quinones</td>
<td>0.36 ± 0.04</td>
<td>0.20 ± 0.03</td>
</tr>
<tr>
<td>9-OH</td>
<td>0.27 ± 0.02</td>
<td>0.14 ± 0.02</td>
</tr>
<tr>
<td>3-OH</td>
<td>1.00 ± 0.11</td>
<td>0.55 ± 0.04</td>
</tr>
<tr>
<td>Total</td>
<td>3.16 ± 0.36</td>
<td>1.68 ± 0.20</td>
</tr>
</tbody>
</table>

* BP 9,10-, 4,5-, and 7,8-diol, trans-dihydrodiol of benzo(a)pyrene; 9-OH, 9-hydroxybenzo(a)pyrene; 3-OH, 3-hydroxybenzo(a)pyrene.

** Numbers in parentheses, percentages of the control metabolite level.

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A recent report by Capdevila et al. (3) indicates that rat liver microsomes contain an enzyme system which catalyzes NADPH reduction of quinones. A ten μM concentration of each BP quinone failed to inhibit NADPH-cytochrome c reductase. NADPH-dependent quinone reductase activity in MC-microsomes was measured under aerobic and anaerobic conditions. Under aerobic conditions, reduction proceeded to a steady-state ratio of reduced/oxidized quinone which depended on the structure of the quinone (Chart 4). At 10 μM quinone concentration, BP 3,6- and 1,6-quinoles were 30 to 40% reduced in the presence of NADPH while the most active inhibitor, BP 6,12-quinone, was <5% reduced in the steady state. At the lower concentrations of 6,12-quinone (2 μM), the steady-state level of reduced 6,12-quinone was slightly higher (15% reduced), probably reflecting the increased percentage of the quinone in solution at this concentration. Anaerobic reduction of all 3 quinones followed first-order kinetics with rate constants for the respective BP quinones in the order 3,6 > 1,6 > 6,12 (Chart 5).

Lind et al. (18) have reported that reduction of BP 3,6-quione by MC-microsomes was inhibited by dicoumarol, a potent inhibitor of DT-diaphorase (16). On the other hand, Capdevila et al. (3) found that reduction of BP 3,6-quione by phenobarbital-induced rat liver microsomes was insensitive to dicoumarol and to CO (3). In this study, we found in our preparations of MC-microsomes that BP quinone reduction was insensitive to dicoumarol. CO did not inhibit reduction of any of the quinones and actually produced a small but reproducible enhancement of the rate of reduction. The dominant role of NADPH-cytochrome P-450 reductase was demonstrated by the reconstitution of full BP quinone reduction activity by cytochrome P-450 reductase alone, with no rate enhancement by cytochrome P-448 from MC-microsomes (Table 4). There was no reduction by NADPH in the absence of cytochrome P-450 reductase. The rate of quinone reduction expressed per unit of cytochrome c reductase activity was similar in MC-microsomes and in the reconstituted P-450 reductase system.
Effect of BP 6,12-quinone addition on the metabolism of BP-7,8-dihydrodiol by MC-microsomes

Standard reaction mixtures (0.5 ml) containing 0.15 mg microsomes and 6 μM dihydrodiol were incubated at 37° for 3.5 min. Subsequent extraction and analysis steps were carried out as described in “Materials and Methods.”

<table>
<thead>
<tr>
<th>Conditions</th>
<th>pmol/mg/min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>anti derivatives</td>
</tr>
<tr>
<td></td>
<td>unidentified</td>
</tr>
<tr>
<td>MC-microsomes</td>
<td>34</td>
</tr>
<tr>
<td>MC-microsomes + 0.5 μM BP 6,12-quinone</td>
<td>28 (82)</td>
</tr>
</tbody>
</table>

a Unidentified derivative eluting immediately before Fraction I.

b Assignment of peak identities I to V is the same as previously described (6).

c The sum of Fractions I, III, and V over Fractions II and IV.

d BP 6,12-quinone (0.25 nmol) was added in 10 μl DMSO to incubations just prior to the 5-min preincubation. Reactions were initiated by adding dihydrodiol in 20 μl acetonitrile.

e Numbers in parentheses, percentages of metabolites formed in control incubations.

DISCUSSION

The conversion of polycyclic hydrocarbon phenols and dihydrodiols to water-soluble metabolites by UDP-glucuronosyltransferase (9, 21, 31) should result in a decrease in the conjugation of BP metabolites to DNA. However, we have recently reported that the addition of UDPGA to microsomal incubations containing BP actually increases the conjugation of BP 7,8-diol-9,10-epoxides to DNA (8). Bock (2) has also reported a stimulatory effect of UDPGA on microsomal BP metabolism which he attributes to removal of product inhibition by conjugation of BP-3 phenol. The strong inhibition of BP metabolism by BP quinones provides an explanation of this effect. We report here that a 3 μM concentration of an equimolar mixture of BP 3,6-, 1,6-, and 6,12-quinones inhibits total BP oxidation by 37% and the subsequent oxidation step to anti- and syn-BP 7,8-diol-9,10-oxides by 85%. Under these incubation conditions, this quantity of BP quinones is generated from BP in about 10 min. In the presence of UDPGA, the level of free BP quinones is decreased by 4- to 5-fold, either via conjugation of the precursors BP 6-phenol and BP 3-phenol (10, 21, 23) or by conjugation of reduced quinones (3, 18).

Thus, the inhibitory action of quinones and their removal via the action of UDP-glucuronosyltransferase provides an explanation of the stimulation by UDPGA of BP metabolism and covalent binding to DNA which we have observed.

The involvement of cytochrome P-450 reductase in quinone reduction is clearly demonstrated by reconstitution of the activity with purified enzyme. The rate of quinone reduction is proportional to the amount of cytochrome c reductase in both microsomes and reconstituted vesicles. The slightly increased rate of quinone reduction in the presence of CO suggests that, in MC-microsomes, reductive turnover of cytochrome P-450 (blocked by CO) competes with reduction of BP quinones by P-450 reductase. Noncompetitive inhibition of BP metabolism by BP 6,12-quinone is also consistent with electron transfer from P-450 reductase to the quinone.

Since BP quinones clearly oxidize reduced cytochrome P-450, the actual participation of the cytochrome in microsomal quinone reduction depends on the relative rates of reduction of BP quinones and cytochrome P-450 by P-450 reductase. The relative rates for flavoprotein reduction of BP 3,6-quinone (0.012 sec⁻¹) and cytochrome P-450 (0.20 sec⁻¹) indicate that, in microsomes, the dominant pathway is via cytochrome
Inhibition of BP and BP 7,8-Dihydrodiol Metabolism

Chart 4. Steady-state ratios of reduced/oxidized BP quinones under aerobic conditions. After addition of NADPH to incubations, repetitive scans from 360 to 660 nm were recorded in the split-beam mode until a steady state was reached. Dithionite was then added to obtain the spectrum of the fully reduced quinone.

P-450 even though both proteins can catalyze reduction. In the presence of CO, only the flavoprotein is likely to be involved. In either case, BP quinones are in effect catalyzing a futile cycle at P-450 reductase or cytochrome P-450 in which electrons are transferred to oxygen rather than effecting mixed-function oxidation.

Chart 5. First-order plot of anaerobic reduction of BP quinones. The rate of reduction was monitored under anaerobic conditions as described in "Materials and Methods" in the presence of 10 μM quinone. First-order rate constants were obtained from slope of the equation

$$-\ln \left(\frac{\text{oxidized}}{\text{oxidized}_0}\right) = kt,$$

where (oxidized)₀ is equal to the initial concentration of oxidized quinone (10 μM), t is time (sec), and k is the first-order rate constant (sec⁻¹).

Table 4

Reduction of BP 3,6-quinone by purified microsomal enzymes

The rate of reduction of BP 3,6-quinone (10 μM) under anaerobic conditions and NADPH-cytochrome c reductase activity were measured as described in "Materials and Methods." Purified cytochrome P-448 and NADPH-cytochrome P-450 reductase were reconstituted with diisoctyl phosphatidylcholine (35 μg/ml).

<table>
<thead>
<tr>
<th>Reductase system</th>
<th>Anaerobic quinone reduction (nmol/min)</th>
<th>Quinone reduction/unit reductase</th>
</tr>
</thead>
<tbody>
<tr>
<td>MC-microsomes</td>
<td>20.9 ± 1.7</td>
<td>0.50 ± 0.04</td>
</tr>
<tr>
<td>NADPH-cytochrome P-450 reductase</td>
<td>25.6 ± 4.2</td>
<td>0.32 ± 0.05</td>
</tr>
<tr>
<td>NADPH-cytochrome P-450 reductase + P-448</td>
<td>27.8 ± 1.13</td>
<td>0.34 ± 0.01</td>
</tr>
</tbody>
</table>

a Purified to homogeneity by the method of Guengerich (10) with detergent removed.
b Purified to homogeneity by the method of Dignam and Strobel (5) with detergent removed.
c Units of NADPH-cytochrome c reductase are nmol cytochrome c reduced per min.
d Mean ± S.D.

The lack of effect of dicoumarol indicates that DT-diaphorase is not important in the microsomal reduction of BP quinones. In this regard, we are in conflict with a recent report of the reduction of BP 3,6-quinone by MC-microsomes (18) but are in agreement with the findings of Capdevila et al. (3) using phenobarbital-induced rat liver microsomes. This conflict may arise from varying degrees of contamination of microsomes by cytosolic DT-diaphorase which may be eliminated by a more extensive washing of the microsomes.

BP 6,12-quinone, which was the most potent inhibitor, was least readily reduced by NADPH and was present under aerobic conditions in an almost fully oxidized state. However, 2 other
Chart 6. Concentration dependence of BP quinone reduction. Quinone reduction was monitored under anaerobic conditions as described in "Materials and Methods" with varying concentrations of BP 3,6-quinone (x) and BP 6,12-quinone (C).

Table 5

Ratios of quinone reduction and quinone-dependent oxygen consumption in MC-microsomes

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Anaerobic quinone reduction (nmol/mg/min)</th>
<th>Oxygen consumptiona (nmol/mg/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BP 1,6-quinone</td>
<td>68.6 ± 6.0</td>
<td>7.2 ± 0.9</td>
</tr>
<tr>
<td>BP 3,6-quinone</td>
<td>87.0 ± 7.1</td>
<td>5.0 ± 0.3</td>
</tr>
<tr>
<td>BP 6,12-quinone</td>
<td>13.86 ± 1.7</td>
<td>7.8 ± 0.8</td>
</tr>
<tr>
<td>Quinone mixture (1:1:1)</td>
<td>8.2 ± 0.9</td>
<td></td>
</tr>
</tbody>
</table>

a Background oxygen consumption (incubations receiving DMSO only) has been subtracted.

b Mean ± S.D.

factors determine the effectiveness of the quinones as inhibitors; the Km for reduction and the proportion of the quinone in the oxidized state under steady-state aerobic conditions. A lower Km for reduction and a higher proportion in the oxidized state both favor BP 6,12-quinone as an inhibitor of mixed-function oxidation over the other 2 quinones.

The role of quinones in BP metabolism is important for 2 reasons. (a) the removal of quinones by UDPGA (either by trapping of BP 6-phenol, BP 3-phenol, or reduced quinones) explains the unexpected stimulatory effect of UDPGA on DNA modification by BP metabolites. This effect of UDPGA may be expected to play a significant role in BP carcinogenesis under conditions where BP quinones reach a concentration of about 1 µM. Cytosolic DT-diaphorase activity may be expected to play an important role in quinone conjugation in whole cells as suggested by Lind et al. (18). These mechanisms may be even more relevant for other polycyclic hydrocarbons, depending on the relative effectiveness of specific quinone derivatives as inhibitors. (b) The fact that quinones may inhibit the metabolism of BP 7,8-dihydrodiol far more than BP offers the possibility of detoxification and excretion of polycyclic hydrocarbon metabolites with a substantial decrease in the carcinogenic modification of DNA by bay-region oxides. The role of BP quinones and UDP-glucuronosyltransferase in regulation of hydrocarbon metabolism is currently being investigated in this laboratory.

REFERENCES

16. Lesko, S., Caspary, W., Lorentzen, R., and Ts'o, P. O. Enzyme formation of 6-oxobenzo(a)pyrene radical in rat liver homogenate from carcinogenic benzo(a)pyrene. Biochemistry, 3978-3984, 1975.
Inhibition of BR and BP 7,8-Dihydrodiol Metabolism


Inhibition of Benzo(a)pyrene and Benzo(a)pyrene 7,8-Dihydrodiol Metabolism by Benzo(a)pyrene Quinones


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