THE ROLE OF HEPATIC METABOLITES OF BENZENE IN BONE MARROW PEROXIDASE-MEDIATED MYELO- AND GENOTOXICITY

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INTRODUCTION

Chronic exposure of humans to benzene causes bone marrow depression leading to pancytopenia and aplastic anemia (Goldstein, B.D., 1983). Benzene also causes genotoxic effects such as structural chromosome aberrations and DNA strand breaks (Dean, B.J., 1985) that might be related to the increased incidence of acute myelogenous leukemia that is associated with chronic exposure (Infante, P.F., White, M.C., 1983; Aksoy, M., 1985; Arp, E.W., et al., 1983).

Benzene metabolism, which is required for toxicity occurs predominantly in the liver via cytochrome P-450 (Sammett, D., et al., 1979; Tunek, A., 1980). Phenol (P), hydroquinone (HQ) and catechol are hepatic metabolites of benzene, and their production appears necessary for benzene-induced myelotoxicity (Arp, E.W., et al., 1983; Sammett, D., et al., 1979) but they show no overt toxicity to the liver. These metabolites are transported from the liver to the bone marrow (Rickert, D.E., et al., 1979; Greenlee, W.P., et al., 1981) where they are bioactivated in a peroxidase-mediated reaction (Sawahata, T., et al., 1985; Irons, R.D., 1985) to biological reactive compounds that bind to macromolecules and which have been implicated in mediating the toxic effects of benzene (Sammett, D., et al., 1979; Rickert, D.E., et al., 1979).

In bone marrow, benzene appears to have a cytotoxic affect on hematopoietic progenitor cells in intermediate stages of differentiation regardless of cell lineage (Irons, R.D., et al., 1979; Lee, E.W., et al., 1974; Tunek, A., et al., 1981). Injury to marrow stromal cells appears to be an important factor in benzene-induced myelosuppression (Frash, V.N., et al., 1976; Gaido, K., et al., 1986). The stromal macrophage, a cell essential in the regulation of hematopoiesis, has been implicated as a target of benzene-induced hematotoxicity (Lewis, J.G., et al., 1988; Thomas, D.J., et al., 1989; MacEachern, L., et al., 1988). Lewis et al. (1988) demonstrated *in vitro* a selective and pronounced inhibition of macrophage function following the addition of various benzene metabolites to the culture medium. Recently, Thomas and Wierda (1989) reported that bone marrow-derived macrophages, exposed to HQ or its oxidation product 1,4-benzoquinone, secreted less interleukin-1, a monokine capable of regulating the synthesis of several hematopoietic factors (Lee, M., et al., 1987). In addition, MacEachern et al. (1988) reported an activation of resident bone marrow macrophages in mice receiving benzene or P and HQ.

Macrophages contain considerable amounts of prostaglandin H synthase (PHS) an enzyme with both cyclooxygenase and peroxidase activities (Scott, W.A., et al., 1980; Ueki, S., et al., 1979). Since phenol and hydroquinone serve as reducing co-substrates

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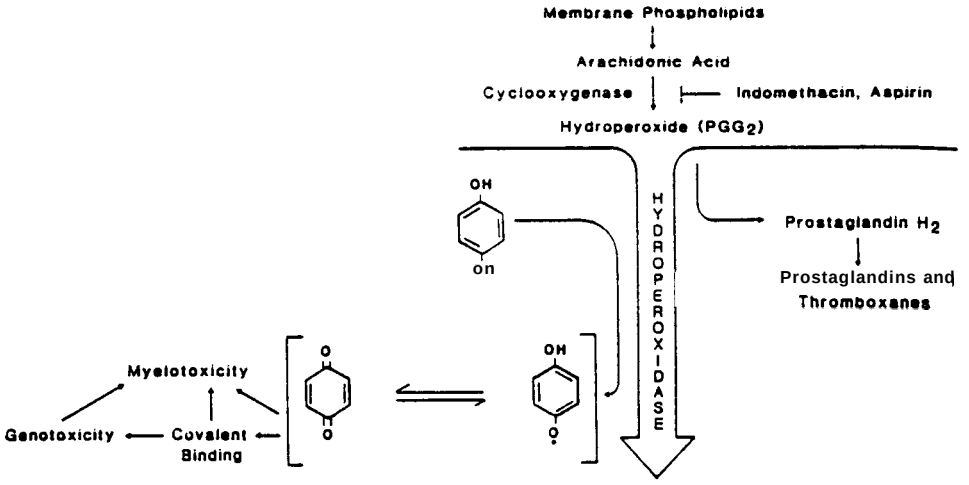

Figure 1. Postulated mechanism for the role of PHS in mediating benzene-induced bone marrow damage. Chronic exposure to benzene causes the release of arachidonic acid from the membrane lipid of marrow stromal cells particularly the resident stromal macrophage resulting in constitutive synthesis of prostaglandins. The oxidation of the co-substrate hydroquinone by PHS-peroxidase converts it to a reactive intermediate which can bind to macromolecules and cause toxicity. Inhibition of PHS-cyclooxygenase with indomethacin inhibits prostaglandin synthesis and prevents bioactivation of hydroquinone.

for the peroxidase of PHS (Markey, C.M., et al., 1987), macrophages have the capacity to oxidize these benzene metabolites to compounds capable of reacting with cellular macromolecules. Indeed, Post et al. have demonstrated that the macrophage can metabolize phenol to protein-binding species (Post, G., et al., 1986).

The role of PHS in benzene-induced myelotoxicity seems particularly relevant, as benzene administration elevates bone marrow levels of prostaglandin E_2 (Gaido, K.W., et al., 1987; Kalf, G.P., et al., 1989), a negative regulator of myelopoiesis (Gentile, P.S., et al., 1987). Nonsteroidal anti-inflammatory drugs, known inhibitors of cyclooxygenase, have been reported not only to inhibit this rise in prostaglandin, but to prevent benzene-induced myelotoxicity as well (Gaido, K.W., et al., 1987; Kalf, G.F., et al., 1989). Because nonsteroidal anti-inflammatory drugs inhibit the cyclooxygenase-catalyzed formation of prostaglandin G_2 (PGG_2), treatment with these agents would eliminate the peroxidase-catalyzed reduction of PGG_2, thereby avoiding the oxidation of phenolic co-substrates, if present, to reactive compounds.

The research to be described was designed to determine whether PHS produces benzene-induced myelo- and genotoxicity by the following proposed mechanism (Figure 1). Benzene is metabolized in the liver and bone marrow to P and HQ. Benzene per se may act on bone marrow cells to effect the constitutive release of arachidonic acid from membrane phospholipids via the activation of protein kinase C which is known to activate the arachidonic acid cascade in macrophages (Pfankuche, H.J., et al., 1986). Arachidonic acid would be converted by the cyclooxygenase component of PHS to the hydroperoxide (PGG_2). The hydroperoxide in turn would drive the endoperoxidase activity of the enzyme. The cooxidation of HQ or P during endoperoxidase conversion of PGG_2 to PGH_2, the immediate precursor molecule for prostaglandins, would result in increased levels of prostaglandins. In the case of HQ, cooxidation would generate p-

benzoquinone which is known to cause genotoxic damage in the form of adducts, strand breaks, SCE and micronucleus formation (Dean, B.J., 1985). The inability of remaining viable stem and/or progenitor cells to proliferate due to the constitutive production of high levels of prostaglandins, known to be negative regulators of hematopoiesis, coupled with genotoxic damage from reactive metabolites such as p-benzoquinone might explain the benzene-induced myelotoxicity.

The experiments described herein demonstrate the arachidonic acid-dependent, indomethacin-sensitive, bioactivation of P and HQ to genotoxic compounds by macrophage peroxidase and purified PHS. Furthermore, we show that inhibition of PHS with indomethacin prevents benzene-induced myelo- and genotoxicity in mice when indomethacin is coadministered with benzene.

RESULTS

Activation of Phenol and Hydroquinone to Biological Reactive Intermediates in Macrophages

The inhibition of the toxic effects of benzene by indomethacin (Kalf, G.F., et al., 1989) suggested that metabolites of benzene such as P and/or HQ might be converted to reactive compounds by PHS-peroxidase. Consequently, we tested the ability of macrophages to effect the arachidonic acid-dependent metabolism of P and HQ to reactive species that bind to macromolecules. In these experiments mouse peritoneal macrophages or $P388D_1$ cells, which morphologically and functionally resemble macrophages, (Koren, H.S., et al., 1975) were used. Similar results can be obtained with both cell types.

Purified peritoneal macrophages incubated with [^{14}C]P in the presence and absence of arachidonic acid metabolize P to reactive compounds that irreversibly bind macromolecules in a reaction that is dependent on the concentration of arachidonic acid (Figure 2). These results suggest that PHS-peroxidase in macrophages might be responsible for the cooxidation of P and/or HQ to reactive species during the benzene-induced formation of prostaglandins. As can be seen in Figure 3, [^{14}C]HQ is also bioactivated by $P388D_1$ cells to species which bind to macromolecules. The addition of TPA causes the further activation of the arachidonic acid cascade which stimulates the oxidation of HQ to reactive species via PHS and this is prevented by indomethacin, a cyclooxygenase inhibitor. Benzene, which has been shown to activate protein kinase C (Roghani, M., et al., 1987) and thus the arachidonic acid cascade (Pfankuche, H.J., et al., 1986), also stimulates the bioactivation of HQ in an indomethacin-sensitive reaction.

Bioactivation of P or HQ to species which bind to macromolecules was shown to occur in a macrophage lysate. The bioactivation of P or HQ occurred in a time and concentration-dependent manner (data not shown). The binding of P or HQ equivalents to protein was decreased significantly, compared to the complete system, for incubations containing the peroxidase inhibitor aminotriazole and for reactions carried out in the absence of either the macrophage lysate or H_2O_2 (Figure 4). The effect of hydroxyl radical scavengers was investigated to determine if the macrophage lysate Indirectly activated P and HQ through the generation of hydroxyl radicals. The addition of dimethylsulfoxide (DMSO) or mannitol to standard incubation mixtures unexpectedly resulted in a slight but significant increase in both P and HQ equivalents bound to TCA-precipitable material (Figure 4). The addition of the cytochrome P-450 Inhibitors, metyrapone and SKF 525A to the macrophage lysate had no affect on the bioactivation of P or HQ (data not shown). These results suggested that adherent macrophages and a macrophage lysate were capable of the bioactivation of P or HQ to covalent binding species and that this was most probably catalyzed by PHS-peroxidase. We, therefore, investigated the PHS-catalyzed oxidation of HQ and examined the DNA-damaging effects of the reactive metabolite generated during the reaction.

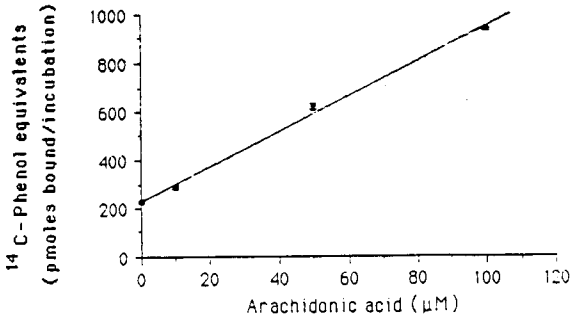

Figure 2. Aracidonic acid-dependent activation of phenol by macrophages. Incubation mixtures contained 2.3 x 10^6 macrophages, 0.25 mM [^{14}C] phenol (8000 dpm/nmole), 0.1 mM arachidonic acid diluted to a final volume of 1 mL with RPMI-1640 buffered to pH 7.3 with 5 mM HEPES. Reactions were terminated after 30 min by the addition of trichloracetic acid. Irreversible binding of [^{14}C] phenol equivalents was assessed by liquid scintillation counting after extensive washes with acetone/hexane/methanol. Reprinted from reference 26.

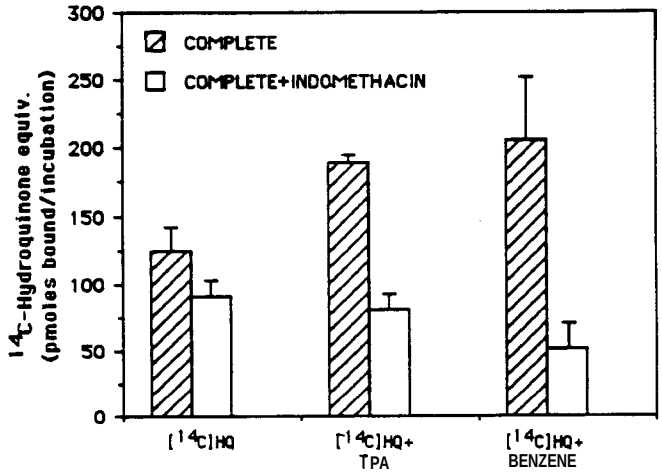

Figure 3. Covalent binding of [^{14}C] hydroquinone to macromolecules in P388D1 cells. P-388 cells (2x106 per incubation) were preincubated for 15 min. with either 100 mM indomethacin in ethanol or ethanol alone (1% final concentration), then treated with (50 μM) [^{14}C]HQ. Some incubations included 100 ng TPA or 170 mM benzene coadministered with the hydroquinone. After a 30 min. incubation at 37°C, 5% CO_2, cells were lysed by freezing and thawing in 10 mM Tris, 1 mM EDTA buffer pH 8.0 containing 2 mm PMSP, 0.1 mM Leupeptin, 0.1 mM pepstatin A and 0.01% Triton X-100. Macromolecules were precipitated in 10% TCA, washed 3 times with acetone followed by two washes in acetone/methanol (1:1, V:V), and radioactive HQ covalently bound to macromolecules was determined by scintillation spectrophotometry. Values represent the mean ± S.D. of three experiments.

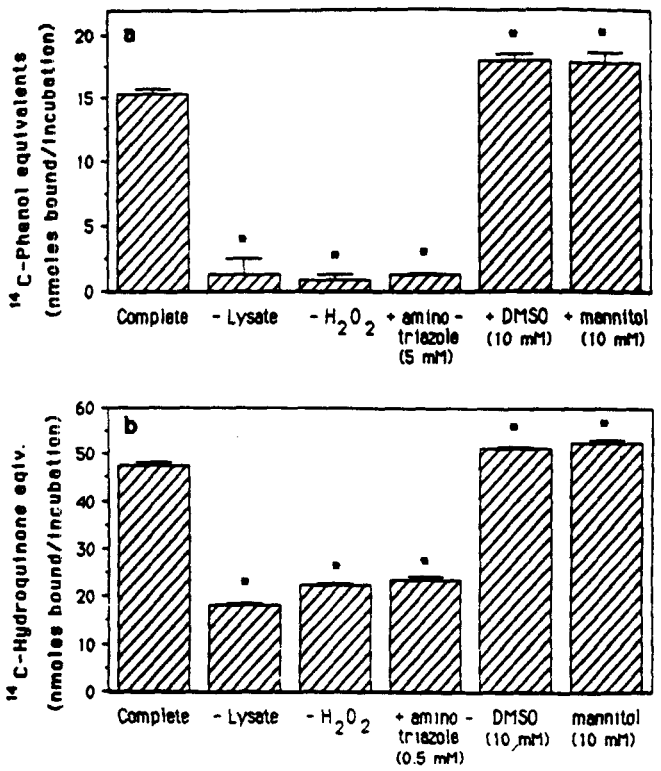

Figure 4. The effect of aminotriazole, DMSO, and mannitol on the H_2O_2-dependent activation of (a) ^{14}C-phenol and (b) ^{14}C-hydroquinone to protein-binding metabolites catalyzed by the macrophage lysate. Reprinted from reference 32.

Activation of Hydroquinone to p-Benzoquinone by PHS-Peroxidase

Purified PHS catalyzed the arachidonic acid-dependent oxidation of HQ to p-benzoquinone which was measured directly by HPLC (Table 1). p-Benzoquinone formation was dependent on the presence of enzyme and arachidonate. Addition of indomethacin significantly inhibited the formation of p-benzoquinone as did the addition of cysteine. The use of H_2O_2 in place of arachidonate also resulted in the formation of p-benzoquinone (data not shown). As expected, indomethacin had no affect on the H_2O_2-dependent oxidation of HQ to p-benzoquinone. When cysteine was added to standard reaction mixtures, p-benzoquinone was not detected (Table 1). A cysteine conjugate resulting from the PHS-catalyzed oxidation of HQ was detected which co-eluted on HPLC with an authentic monocysteine-hydroquinone conjugate synthesized as previously described (Schlosser, M.J., et al., 1989). As with the formation of p-benzoquinone, monocysteine-hydroquinone formation was dependent on the presence of PHS, arachidonate and cysteine (Table 2). Indomethacin inhibited the arachidonate-dependent but not the H_2O_2-dependent formation of monocysteine-hydroquinone.

In order to determine whether PHS can oxidize HQ to a DNA-binding species, calf thymus DNA was incubated with PHS and arachidonic acid for 10 min (Figure 5). Radioactivity bound to calf thymus DNA increased over the 10 min incubation period, whereas reaction mixtures containing heat-inactivated PHS remained constant over

Table 1. Archidonic acid-dependent Formation of p-Benzoquinone by Prostaglandin H Synthase [a]

System	nmol/incubation
complete [b]	17.3 ± 3.6
- PHS	0.9 ± 0.2
- arachidonate	1.0 ± 0.1
+ indomethacin (10 μM)	4.3 ± 0.7
+ cysteine (100 μM)	<0.1

[b] Complete system at 37°C contained hydroquinone (100 μM), PHS (5 μg/mL), hematin (1 μM), and arachidonic acid (100 μM) in 0.1 M Na^+/K^+ phosphate buffer, pH 7.0 in a final volume of 0.5 mL. Reactions were preincubated with indomethacin or 1% ethanol for 10 min. p-Benzoquinone was measured using HPLC with electrochemical detection. Data is expressed as the means ± standard deviations.

time. Similar results were obtained when mtDNA was used in place of calf thymus DNA (Table 3). mtDNA-binding was dependent on enzyme and was inhibited by indomethacin. Thus [^{14}C]HQ, functioning as a co-substrate for PHS-peroxidase, is cooxidized in an arachidonic acid- and time-dependent reaction to compound(s) that bind to mtDNA. [^{14}C]HQ-derived mtDNA adducts were isolated as deoxyribonucleoside adducts by hydrophobic chromatography on an LH-20 column following sequential digestion of radiolabeled mtDNA with nucleases (data not shown). A ^{14}C-labeled adduct was found to co-migrate on a thin layer cellulose chromatographic plate (Figure 6) with a 2'-deoxyguanosine (2'-dG) adduct standard. The structure of the 2'dG adduct, (3'OH) benzetheno (1,N2) deoxyguanosine has been reported (Snyder, R., et al., 1987) (Figure 7).

Table 2. Arachidonic acid-dependent Formation of Monocysteine-hydroquinone by Prostaglandin H Synthase

System	nmol/incubation
complete a	14.6 ± 0.2
- PHS	0.8 ± 0.1
- arachidonate	0.6 ± 0.1
- cysteine	<0.1
+ indomethacin (10 μM)	6.3 ± 0.3

[a] Complete system at 37°C contained hydroquinone (100 μM), PHS (5 μg/mL), hematin (1 μM), cysteine (100 μM) and arachidonic acid (100 μM) in 0.1 M Na^+/K^+ phosphate buffer, pH 7.0 in a final volume of 0.5 mL. Reactions were preincubated with indomethacin or 1% ethanol for 10 min. Monocysteine-hydroquinone was measured using HPLC with electrochemical detection. Data is expressed as the means ± standard deviations.

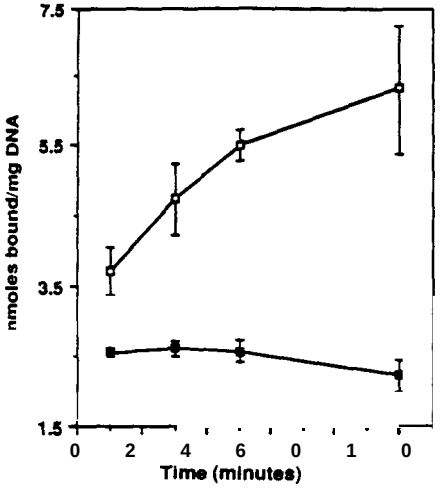

Figure 5. Time course for DNA binding by hydroquinone during PHS-catalyzed reactions: (?) contained calf thymus DNA (100 mg/mL), [^{14}C]hydroquinone (100 mM; 10,000 dpm/nmol), PHS (5 mg/mL) with hematin (1 mM), and arachidonic acid (10 mM); (?) as above except PHS was heat-inactivated. Data points represent the means ± standard deviations of triplicate determinations.

HQ has been reported to produce strand breaks in DNA (Sawahata, T., et al., 1985). Therefore, we tested for the ability of benzoquinone formed from HQ by PHS-peroxidase to cause strand breakage by determining its ability to nick supercoiled Bluescript plasmid DNA, thereby converting it to its open circle form. Bluescript is a small (3.9 Kb) circular plasmid that migrates slower on an agarose gel in its nicked open circular form than its supercoiled form. DNA damage was detected by incubating supercoiled Bluescript plasmid with PHS, arachidonic acid and HQ (Fig. 8). The oxidation of HQ by PHS produced product(s) capable of causing single strand breaks in the plasmid, thereby converting it to a nicked circle that could be isolated on an agarose gel. These PHS-catalyzed DNA strand breaks were found to be dependent on native enzyme and HQ for plasmid nicking, and were inhibited by indomethacin. When the autoradiogram prepared from a blot hybridization of the gel was scanned using an LKB laser scan densitometer, it was found that the intensity of the open circle band in the complete system containing active enzyme (lane 3) was 88% above the complete system that had been inhibited by indomethacin (lane 6).

The Role *of* ***PHS*** in the induction of Myelo- and Genotoxicity in Mice by Benzene: The *Effect of* Indomethacin on *Benzene-Induced* Bone Marrow Depression and **Micronucleus** Formation in ***C57Bl/6*** Mice

The oxidation of HQ to p-benzoquinone, the formation of a [^{14}C] HQ-derived adduct in DNA and the ability of p-benzoquinone to nick supercoiled plasmid DNA were inhibited by indomethacin. These results suggest that PHS-peroxidase plays a role in the oxidation of HQ to p-benzoquinone, a putative genotoxic metabolite of benzene and that, *in vivo*, toxicity to marrow progenitor cells induced by benzene might result from the production by PHS of biological reactive species from P and HQ acting as co-substrates for PHS-peroxidase. If this is indeed the case, then the myelotoxic effects of benzene should be prevented by the coadministration of PHS inhibitors such as indomethacin or other nonsteroidal anti-inflammatory drugs.

Table 3. Prostaglandin H Synthase-Catalyzed Activation of Hydroquinone to DNA-binding Metabolite(s)[a]

System	nmol/incubation
complete	5.11 ± 0.55
- PHS	1.98 ± 0.46
+ indomethacin (10 mM)	2.07 ± 0.45

[a] Complete system contained [^{14}C]hydroquinone (100 μM; 10,000 dpm/nmol), PHS (5 μg/mL), hematin (1 μM), arachidonic acid (10 μM), and mtDNA (100 μg/mL) in 0.1 M K^+ phosphate buffer, pH 7.0 in a final volume of 0.5 mL. Reactions were preincubated with indomethacin or 1% ethanol for 1 min. Radioactivity bound to mtDNA was measured by liquid scintillation spectrometry. Data is expressed as the means ± standard deviations.

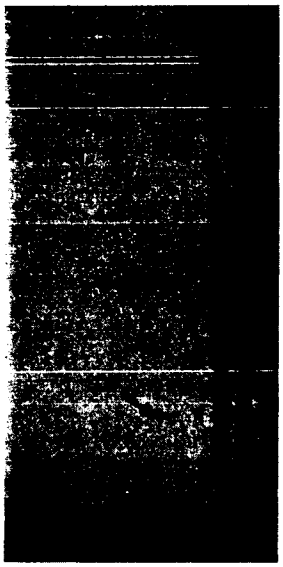

Figure 6. Autoradiogram of the TLC plate. Arrow indicates the position of the radiolabeled [^{14}C] p-benzoquinone-mtDNA deoxynucleoside adduct (Rf=0.22). The [^{14}C] p-benzoquinone-deoxyguanosine adduct standard appears in lane 2 at a Rf of 0.22. Lane 1 represents [^{14}C] hydroquinone alone.

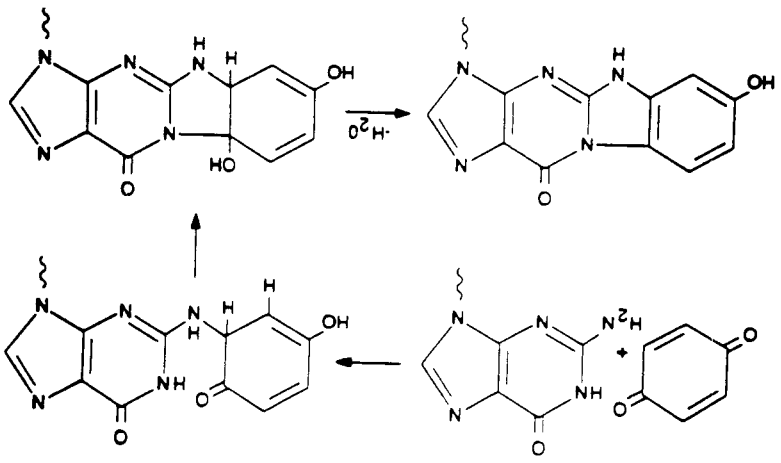

Figure 7. Proposed mechanism for the formation of adduct 2.

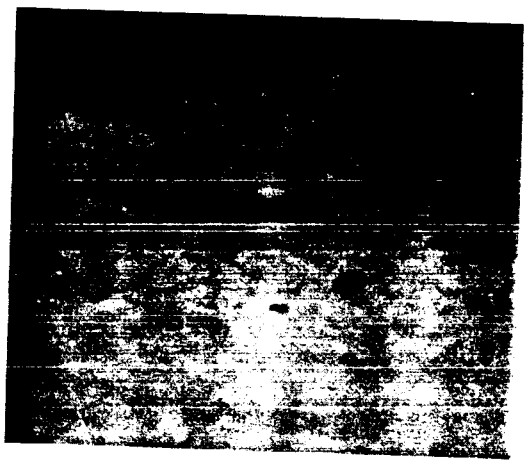

Figure 8. Blot hybridization of 75 ng Bluescript plasmid DNA incubated with the PHS complete system or with heat inactivated PHS in place of active enzyme, in the absence and presence of 10 mM indomethacin. Enzyme incubations and blot hybridizations were carried out routinely done in the lab. Lane 1: Linear Bluescript plasmid, digested with ECO R1. Lane 2: Supercoiled Bluescript plasmid. Lane 3: Supercoiled Bluescript plasmid incubated with the PHS complete system. Lane 4: Supercoiled Bluescript plasmid incubated with the PHS complete system, omitting HQ. Lane 5: Supercoiled Bluescript plasmid and heat inactivated enzyme, incubated with the PHS complete system. Lane 6: Supercoiled Bluescript plasmid and the PHS complete system in the presence of 10 mM indomethacin. Lane 7: Supercoiled Bluescript plasmid and heat inactivated enzyme incubated with the PHS complete system and 10 mM indometnacin.

Depression of bone marrow cellularity is a commonly used parameter to determine benzene toxicity. Administration of benzene (600 mg/kg body weight) to a day for two days caused a significant decrease in bone marrow cellularity measured as a decrease in nucleated cells in the marrow (Table 4). The effect was dose dependent and there was no change in body weight or viability of the cells flushed from the femur (data not presented). Coadministration of indomethacin (2 mg/kg body weight) with benzene completely prevented the depression of bone marrow cellularity. Indomethacin alone had no effect. Meclofenamate (4 mg/kg) or aspirin (50 mg/kg), which inhibit cycloxygenase activity of PHS by mechanisms different from that of indomethacin, also significantly prevented benzene-induced bone marrow depression (data not presented).

Under conditions where benzene causes significant myelotoxicity in the form of decreased bone marrow cellularity, it also causes genotoxicity measured by an increase in the frequency of micronucleus formation in peripheral blood polychromatic erythrocytes (PCE). It can be seen in Table IV that benzene at 600 mg/kg body weight also caused a 5.7-fold increase in the frequency of micronucleus formation. Coadministration of indomethacin with benzene prevented the increased frequency of micronucleus formation in PCE without affecting the division or maturation of nucleated erythroid precursors (NCE) as indicated by no change in the PCE/NCE. The administration of indomethacin alone had no affect on micronucleus formation.

Table 4. Prevention of Benzene-Induced Bone-Marrow Depression and Micronucleus Formation in Mice by Indomethacin

Group [a]	Nucleated bone marrow cells x 10^6/femur	Micronuclei/10^3 PCE	Micronuclei/10^3 NCE	PCE/NCE x 10^3
control [b]	11.4 + 1.6	4.5 + 1.0	1.0 + 0.9	34.0 + 4.5
+ benzene [c]	6.2 + 1.6*	25.5 + 9.2*	0.5 + 0.4	24.0 + 10.4
+ benzene + indo [d]	9.4 + 0.7	10.0 + 5.0	0.75 + 0.3	32.4 + 3.0
+ indo [d]	12.4 + 1.8	5.5 + 3.0	1.7 + 0.8	32.0 + 7.6

[a] Each group consisted of 4 C57Bl/6 male mice.
[b] Control animals received corn oil and/or a solution of 4.2% ethanolic PBSA.
[c] Benzene (600 mg/kg in corn oil) was administred ip twice daily for two days.
[d] Indomethacin (2 mg/kg in 4.2% ethanolic PBSA was administered ip twice daily for two days.
Data are expressed as means ± SD
* $p \leq 0.01$ compared to control and indomethacin-treated groups.

DISCUSSION

Adherent macrophages bioactivate P in an arachidonic acid-dependent reaction to species capable of covalent binding to macromolecules (Figure 2). Similar results are obtained with HQ. The addition of TPA or benzene, which cause the release of arachidonic acid from plasma membrane lipids, stimulate the oxidation of [^{14}C] HQ to reactive species (Figure 3). This is prevented by indomethacin, a cyclooxygenase inhibitor. Similar results can be obtained with a lysate of adherent macrophages. The H_2O_2-dependent activation of P or HQ to biological reactive species is inhibited by aminotriazole, a peroxidase inhibitor (Figure 4), but not by inhibitors of cytochrome P-450 such as metyrapone or SKF 525A, or by hydroxyl radical scavengers (Figure 4). The arachidonic acid-dependent activation of HQ or P by the macrophage lysate was not attempted since the concentration of CTAB used to solubilize the membrane-

bound peroxidase also inhibits the cyclooxygenase activity of PHS (data not shown). These results strongly implicate PHS-peroxidase in the bioactivation of these benzene metabolites to biological reactive compounds in macrophages. Further studies using purified PHS confirmed that the enzyme was capable of the arachidonic acid-dependent and indomethacin-sensitive oxidation of HQ to p-benzoquinone (Table 1) which could be measured directly by HPLC or trapped with cysteine as a monocysteine-hydroquinone conjugate (Schlosser, M.J., et al., 1989) (Table 2). The oxidation product(s) of HQ were also able to covalently bind to DNA (Figure 5).

[^{14}C] HQ, functioning as a cosubstrate for PHS-peroxidase, was also shown to be oxidized in an arachidonic acid- and time-dependent reaction to compound(s) that interact with DNA to form adducts and strand breaks. A ^{14}C-labeled adduct was isolated from mtDNA which was found to co-migrate on a thin layer cellulose chromatographic plate (Fig. 6) with a known 2'-deoxyguanosine adduct standard. The structure of the 2'dG adduct was identified as an adduct of pbenzoquinone with guanine to form (3'OH) benzetheno (1,N$_2$) deoxyguanosine (adduct 2; Fig. 7). Interaction of HQ with PHS-peroxidase and arachidonic acid in the presence of supercoiled Bluescript plasmid resulted in single strand breaks in the supercoiled DNA converting it to an open circle form. The PHS-catalyzed strand breaks were dependent on native enzyme, HQ, and arachidonic acid and were prevented by indomethacin (Fig. 8).

Taken together, these results implicate PHS-peroxidase in the bioactivation of HQ to p-benzoquinone, a genotoxic metabolite of benzene, and suggest that in *vivo* toxicity to marrow progenitor cells might result from the production of reactive species by PHS. That this may be the case is evidenced by the facts that benzene-induced depression of bone marrow cellularity and increased frequency of micronucleus formation in mice can be prevented by the coadministration of PHS inhibitors such as indomethacin (Table 4). Indomethacin does not appear to be modulating an alteration of bone marrow cellularity related to an initial benzene-induced inflammatory reaction since data obtained in a subchronic exposure study (to be published elsewhere) indicate that indomethacin can prevent the myelo- and genotoxic effects induced by benzene over a 3 week period. The amount of indomethacin used in our protocols had no effect on hepatic microsomal P-450 content or benzene hydroxylase activity (Pirozzi, S, et al., 1989) and the effective level of indomethacin was not sufficient to affect dihydrodiol dihydrogenase, phospholipase A2 or myeloperoxidase, enzymes which might be expected to be involved in benzene metabolism.

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