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THE EFFECTS OF A COCARCINOGEN, FERRIC OXIDE, ON THE METABOLISM OF BENZO[a]PYRENE IN THE ISOLATED PERFUSED LUNG

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An isolated perfused New Zealand rabbit lung preparation was used to investigate the effects of a cocarcinogen, ferric oxide (Fe_2O_3), on the metabolism of benzo[a]pyrene (BaP), a ubiquitous potent carcinogen that has been associated with the increased incidence of human bronchiogenic carcinoma in occupational and urban settings. [^{14}C]-BaP was administered intratracheally to an isolated perfused lung (IPL) preparation with and without Fe_2O_3 after intraperitoneal pretreatment of the whole animal with BaP or intratracheal pretreatment of the whole animal with Fe_2O_3 and/or BaP. BaP and its metabolites were isolated from serial blood samples up to 180 min after administration of [^{14}C]BaP to the IPL. BaP and its metabolites were also isolated from lung tissue, washout fluid, macrophage, and trachea bronchi at the end of the perfusion at 180 min. Patterns of BaP metabolites were determined by chromatographic techniques and liquid scintillation counting.

Fe_2O_3 pretreatment to the whole animal or administration of Fe_2O_3 to the IPL altered BaP metabolism by the perfused lung. Fe_2O_3 pretreatment to the whole animal resulted in an increase in the total rate of appearance of metabolites of BaP in the blood (ng/g lung·h), while Fe_2O_3 administration to the IPL resulted in a decrease in the total rate of appearance of BaP metabolites in the blood and inhibited the effect of pretreatment. Administration of Fe_2O_3 with BaP to the IPL with or without Fe_2O_3 pretreatment to the whole animal, or BaP administration to the IPL preceded by Fe_2O_3 pretreatment to the whole animal, enhanced dihydrodiol formation and depressed formation of water-soluble metabolites. Since dihydrodiol formation is considered to be the active pathway of BaP metabolism, these data suggest that pulmonary exposure to a known cocarcinogen, Fe_2O_3, in the presence of BaP results in increased production of dihydrodiols of BaP, which may be further metabolized to the ultimate carcinogenic form(s) of BaP. Therefore, Fe_2O_3 can enhance the metabolic activation of BaP by the lung, as well as act as a carrier for penetration and retention of BaP in the lung.

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INTRODUCTION

In the United States, lung cancer represents the highest single cause of cancer deaths (Silverberg, 1981). Thus, there is an imperative need for extensive studies of causative agents, cofactors, and pathogenic mechanisms responsible for the development of this type of cancer. A whole range of factors unique to the functional, morphological, and biochemical characteristics of the respiratory organs requires a specialized study of carcinogenic mechanisms in the lung.

Epidemiological and experimental evidence (Bingham et al., 1976) indicates that the interplay of multiple environmental factors appears to be responsible for the induction of lung cancer. It has been shown that the lungs are capable of metabolizing and binding foreign substances (Anderson and Eling, 1976; Cohen and Moore, 1976; Lubawy and Issac, 1980a,b; Niemeier and Bingham, 1972; Perera, 1981; Smith et al., 1978; Vainio et al., 1976; Warshawsky et al., 1980; Young, 1976). One such compound is benzo[a]pyrene (BaP), a ubiquitous environmental carcinogenic pollutant (National Academy of Science, 1972; Perera, 1981) formed during processes that involve incomplete combustion of organic materials (Badger, 1962; National Academy of Sciences, 1972).

To understand the mechanism of BaP carcinogenesis in the lung (Perera, 1981), a detailed knowledge of the rate and pattern of formation of its metabolites and the factors controlling their formation is required. Such factors include particulate matter found in the ambient air of both occupational and urban settings, which carries a multitude of chemicals including BaP.

It has been established experimentally that the carcinogenicity of BaP in the respiratory tract (Feron et al., 1980; Stenbäck and Rowland, 1978) has been enhanced in the presence of ferric oxide (Saffiotti et al., 1972) in the hamster. In addition, it has been shown that BaP is resistant to photodegradation under ambient conditions when adsorbed on particulates, such as ferric oxide or soot (Butler and Crossley, 1981; Korfmacher et al., 1980), and therefore BaP can reach the lung as the parent compound. It has been indicated that ferric oxide acts as a carrier for penetration and retention of BaP in the lung tissue (Feron et al., 1976; Henry et al., 1975; Mitchell, 1982; Sellakumar et al., 1976). However, the effects of Fe_2O_3 on the metabolism of BaP in the lung are unknown.

The isolated perfused rabbit lung (IPL) preparation reflects the biochemical events that occur *in vivo* in the organ and therefore is an excellent preparation for investigating pulmonary metabolism of foreign compounds (Mehendale et al., 1981; Niemeier, 1976; Roth, 1979; Smith et al., 1980) adsorbed onto airborne particulate matter (Warshawsky et al., 1983). Comparative metabolism of compounds of varying physical states in lung tissue of different cell types (Lubawy and Perrier, 1980; Warshawsky et al., 1981, 1983; Warshawsky and Myers, 1981) can be studied in the whole lung

preparation. The IPL also permits the exposure of test chemicals to all lung cell types of varying metabolic capacity (Serabjit-Singh et al., 1980) under conditions of blood circulation approximating the *in vivo* situation.

An aspect of the current work has been to assess the effects of Fe_2O_3 exposure on the rate and pattern of formation of BaP metabolites in the lung. To mimic a variety of different exposure regimens, Fe_2O_3 was administered (1) with BaP *in vitro* to the IPL following a variety of *in vivo* pretreatments and (2) as an *in vivo* pretreatment to the whole animal followed by BaP *in vitro* administration to the IPL.

MATERIALS AND METHODS

Chemicals

[7,10-^{14}C]BaP (21 mCi/mmol), 98% pure, was purchased from Amersham (Arlington Heights, Ill.), and unlabeled BaP, 99% pure, was purchased from Aldrich Chemical Co. (Milwaukee, Wis.). BaP was checked for purity by chromatographing the compound on either (1) 0.1 mm silica-gel thin-layer chromatography (TLC) plates (Eastman Kodak 6061) in *n*-hexane or benzene and locating the spot with 254-nm light or (2) a Varian 8500 HPLC using a Hibar II (EM Reagents, Cincinnati, Ohio) reverse-phase C_{18} 24 cm X 4.6 mm column, 10 μm particle size with a methanol–water gradient from 80 to 100% methanol. The HPLC chromatogram was monitored at 268 nm with a flow of 1 ml/min at room temperature (Warshawsky et al., 1980, 1981; Schoeny and Warshawsky, 1983). The labeled BaP was further purified to 99.5% by TLC using benzene as the solvent. The unlabeled BaP was further purified to 99.5% by use of neutral alumina column chromatography with benzene or toluene as the eluant followed by recrystallization in a benzene–isopropanol mixture. The Fe_2O_3 (Fisher Scientific Co., Fairlawn, N.J., Lot 723282) was analyzed by the Analytical Division of Kettering Laboratory for size distribution and found to contain particles sized at 97.5% $\leqslant$ 10 μm, 90.5% $\leqslant$ 5 μm, and 65% $\leqslant$ 2 μm.

BaP metabolite standards were supplied by H. Gelboin of the National Cancer Institute; J. Selkirk of Oak Ridge National Laboratories; R. Harvey of Ben May Cancer Institute; and the Chemical Repository of the National Cancer Institute at Illinois Institute of Technology Research Institutes: 3-hydroxy (3-OH); 3,6-, 6,12-, and 1,6-quinones; 9,10-dihydrodiol (9,10-diol); 7,8-dihydrodiol (7,8-diol); 4,5-dihydrodiol (4,5-diol); 4,5-epoxide; 4,5-quinone; 6-hydroxy (6-OH); 7-hydroxy (7-OH); 9-hydroxy (9-OH). A combination of HPLC techniques, scintillation counting, and ninhydrin spraying (Sigma Chemical Co., St. Louis, Mo.) of the 7,8-diol spot on TLC was used to detect an unidentified metabolite, designated S-metabolite, that chromatographed about 1 min after BaP on HPLC (Warshawsky et al., 1980, 1981). The term nonextractable refers to metabolites including conjugates of BaP that were not extracted from tissues by an organic solvent

mixture of benzene:acetone:isoamyl alcohol (13:10:0.1) (Warshawsky et al., 1981).

The liquid scintillation cocktail, Scintiverse, was supplied by Fisher Scientific (Cincinnati, Ohio) and the corn oil was a commercial product (Mazola). All solvents were HPLC or analytical grade; the HPLC grade methanol was supplied by Fisher Scientific (Fairlawn, N.J.).

Lung Perfusion

Male New Zealand white rabbits weighing up to 5 kg were fed single-batch standard laboratory diets and water *ad libitum* and were allowed to equilibrate with their environment on a 12-h light/dark cycle at 70 ± 2°F with a relative humidity of 45% for at least 3 wk before treatment. No pesticides were used in the housing area (Vesell et al., 1973). At least five animals were used per treatment group, with the exception of phenobarbital and 3-methylocholanthrene (3 MC), where four animals were used.

Intraperitoneal *in vivo* pretreatments were performed to induce lung enzymatic capacity for the metabolism of BaP: BaP or 3MC, 20 mg/kg body weight in corn oil vehicle (3 ml/kg), performed 24 h before sacrifice; or sodium phenobarbital, 50 mg/kg in saline vehicle on 3 successive days with the last dose 24 h before sacrifice. Intratracheal *in vivo* pretreatment with 10-mg/kg dose of Fe_2O_3 and/or BaP in 2 ml physiological saline was performed once a week for 5 wk and the rabbits sacrificed during wk 6. The saline was used merely as a vehicle for administration; neither Fe_2O_3 or BaP was soluble to any extent in saline. The 10-mg/kg dose of Fe_2O_3 or BaP for the intratracheal (i.t.) pretreatments of the rabbits were based on proportional doses used in hamsters (Saffiotti et al., 1972), and the ip doses to the whole animal with BaP, 3MC, and phenobarbital were based on prior standard dose levels used in enzyme induction studies (Grover et al., 1972; Lake et al., 1973). The equipment, perfusion system, preparation of the lung, and the method for intratracheal pretreatment are described elsewhere (Niemeier, 1976; Niemeier and Bingham, 1972; Warshawsky et al., 1980, 1981).

One microcurie of pure [7,10 ^{14}C]BaP was mixed with unlabeled pure BaP to give 312 μg BaP (1.24 μmol, 0.8 μCi/μmol), and this was evaporated gently to dryness under N_2. The 312 μg of BaP was taken up in 0.5 ml ethanol, diluted with 0.5 ml saline (1:1), and injected intratracheally on the IPL. The total rate of appearance of metabolites in the blood is maximal at this dose (unpublished results). Mixtures of BaP adsorbed onto particulate were prepared as follows. Labeled and unlabeled BaP mixtures, as already described, were evaporated with Fe_2O_3 (1 mg/kg body weight; the dose of Fe_2O_3 of 1 mg/kg used in the IPL did not cause the perfusion system to become inoperable, as was the case for 10 mg/kg) under N_2. The total mixture of BaP adsorbed on Fe_2O_3 plus the remaining nonadsorbed BaP was suspended in 1 ml saline and injected intratracheally on the IPL. In both cases the syringe was rinsed once with 1 ml saline and

injected. The residual radioactivity left in the syringe in each case was determined by washing out the ^{14}C with toluene and counting an aliquot in the liquid scintillation counter. Hence, the total substrate delivered was equal to 312 μg minus the residual BaP left in the syringe.

Analyses

Aliquots of 5.5 ml were taken from the perfusion system at each of 6 times during the course of a 3-h perfusion, i.e., 15, 30, 45, 60, 90, 120, and 180 min. The lungs were weighed after removal from the perfusion system and lavaged 3 times with physiological saline (5 ml/g lung tissue). Lung tissue was separated from the trachea bronchi, and each was weighed separately. Extraction procedures using benzene:acetone:isoamyl alcohol (13:10:0.1), preparation of the organic extracts and tissue residues of blood, lung, trachea bronchi (TB), macrophage pellet (MAC), and lavage washout fluid (WO; fluid remaining after centrifuging to obtain the macrophage pellet) and the detailed methods for metabolite analyses using a combination of TLC and HPLC techniques are described elsewhere (Warshawsky et al., 1980, 1981; Schoeny and Warshawsky, 1983). It should be noted that most of the data were obtained using TLC methods before HPLC methods were routinely used in the laboratory.

All samples were processed under nitrogen and subdued yellow lighting to minimize photooxidation. The total rates of appearance of the metabolites in the blood (ng/g lung/hr) were determined from the linear regression analyses of the blood samples collected at designated time points from 15 through at least 90 min. The percentage of each of the metabolites in the blood was based upon the total rate of appearance of metabolites in the blood. The percentage of BaP present at the end of the perfusion was based upon the summation of BaP remaining in all tissues, e.g., blood, TB, MAC, WO, and lung tissue, divided by the dose delivered to the IPL. The amounts of BaP and total metabolite reported as percentages present in each tissue were based on the differences in extraction efficiencies, percentage of dose delivered, total recovery as a percentage of dose, and total dose recovered in each tissue. The total percentages of BaP and its metabolites in all tissues were then normalized at 100%. Statistical analyses using the Student-Neuman-Keuls test were performed on the rates and patterns of metabolism of BaP in the blood for all experiments and on the distribution of BaP and its metabolites in the tissue of those experiments that perfused 180 min.

RESULTS

Influence of Pretreatment of Enzyme Inducers On the Metabolism of BaP in the IPL

Phenobarbital pretreatment to the whole animal in comparison with no pretreatment did not induce the aryl hydrocarbon hydroxylase enzyme

system (Thorgeiorsson and Nebert, 1977), as indicated by the total rate of appearance of metabolites of BaP in the blood (162 ± 22 versus 256 ± 38 ng/g lung•h, ± SE). The changes in the metabolic profile were slight. Both 3MC and BaP intraperitoneal pretreatments to the whole animal in comparison with corn oil pretreatments increased the metabolite production (Lubawy and Isaac, 1980b; Smith et al., 1978; Ueng and Alvares, 1982), as evidenced by the total rate of appearance of metabolites of BaP in the blood (836 ± 343 and 1718 ± 287 versus 466 ± 94 ng/g lung•h, ± SE). The BaP pretreatment induced BaP metabolism in lung to a greater extent ($p = 0.01$) than 3MC pretreatment. The patterns of metabolite formation in the blood were essentially the same from BaP- and 3MC-induced animals; in comparison with corn oil-treated animals, there were increases in the 9,10- and 4,5-diols and phenols and decreases in the nonextractables and the 7,8-diol + S-metabolite. In particular, the 9,10-diol increased from 15.3 ± 4.9% in the corn oil experiments to 33.3 ± 2.0% ($p = 0.05$) and 27.9 ± 4.5% for the 3MC and BaP experiments, respectively; the 4,5-diol increased from 1.2 ± 0.5% to 4.3 ± 0.8% and 3.8 ± 0.9% ($p = 0.05$); and the phenols increased from 2.7 ± 1.1% to 14.0 ± 2.4% ($p = 0.01$) and 13.0 ± 3.5% ($p = 0.05$); while the nonextractables decreased from 56.3 ± 7.8% to 32.6 ± 2.7% and 36.3 ± 4.7%, and the 7,8-diol plus S-metabolite decreased from 17.9 ± 0.9% to 5.4 ± 1.0% ($p = 0.01$) and 8.4 ± 0.7%. BaP given intratracheally as a pretreatment in comparison with no pretreatment (Warshawsky et al., 1980) significantly increased ($p = 0.01$) the total rate of appearance of metabolites in the blood (1290 ± 114 versus 256 ± 38 ng/g lung•h, ± SE) and the formation of the 9,10-diol ($p = 0.05$; 32.8 ± 3.8% versus 14.5 ± 3.4%); there were decreases in phenols from 9.7 ± 1.1% to 5.9 ± 1.0% and quinones ($p = 0.05$) from 10.7 ± 1.8% to 2.8 ± 0.9%, and no change in the nonextractables (51.8 ± 2.8% versus 54.4 ± 5.4%) and the 7,8-diol (5.4 ± 1.8% versus 6.8 ± 0.9%).

Influence of Fe_2O_3 Administration to the IPL on BaP Metabolism

Fe_2O_3 administered concurrently with BaP to the perfusion system with respect to the administration of BaP alone inhibited the rate of BaP metabolism (Table 1); the metabolic patterns showed a marked increase in the 7,8-diol plus S-metabolite and decreases in the phenols and quinones. Results for *in vivo* pretreated saline controls (not shown) are similar to the results for nonpretreated controls.

Fe_2O_3 or BaP pretreatment to the whole animal altered the effects of Fe_2O_3 administered together with BaP to the perfusing system on BaP metabolism (Table 1). Fe_2O_3 pretreatment resulted in increases in the 7,8-diol plus S-metabolite and quinones and decreases in the 4,5-diol ($p = 0.05$) and the nonextractables, whereas BaP pretreatment resulted in a decrease in the 7,8-diol plus S-metabolite and a significant increase in the nonextractables. Additionally, the rates of apperance of metabolites in

TABLE 1. Influence of Fe_2O_3 Administration to the IPL on BaP Metabolism[a]: Rate of Appearance and Pattern of BaP Metabolites in the Blood

Rate and pattern	No pretreatment, BaP[c] on the IPL ($n = 9$)	No pretreatment, (BaP + Fe_2O_3)[c] on the IPL ($n = 5$)	BaP i.t.[b] pretreatment, (BaP + Fe_2O_3)[d] on the IPL ($n = 5$)	Fe_2O_3 i.t.[b] pretreatment, (BaP + Fe_2O_3)[d] on the IPL ($n = 5$)
Total rate of appearance of metabolites in blood (ng/h•g lung, ± SE)	256 ± 38	165 ± 51	252 ± 64	248 ± 59
Metabolic pattern in blood (%, ± SE)[e]				
7,8-Diol + S-metabolite	6.6 ± 0.9[f]	14.0 ± 2.9	7.8 ± 1.4[g]	23.5 ± 5.5
9,10-Diol	15.4 ± 4.0	20.4 ± 1.9	16.5 ± 1.4	28.6 ± 6.7
4,5-Diol	3.3 ± 0.6	6.0 ± 3.1	5.4 ± 1.5	1.6 ± 0.9[f]
Phenols	9.7 ± 1.1[g]	5.4 ± 1.6	4.1 ± 0.8	5.5 ± 2.3
Quinones	10.6 ± 1.8	5.6 ± 1.5	3.5 ± 1.0	10.4 ± 1.5[g]
Nonextractables	54.5 ± 5.4	48.0 ± 4.5	67.9 ± 2.5[h]	30.3 ± 4.4

[a]All metabolites separated by TLC. Column 1 is compared to column 2 to indicate the effect that Fe_2O_3 administration to the IPL has on BaP metabolism. Columns 3 and 4 are compared to column 2 to indicate the effects that coadministration of Fe_2O_3 with BaP to the IPL has on both BaP and Fe_2O_3 pretreatments; n = number of animals; statistics performed by Student-Newman-Keuls test.

[b]10 mg/kg once a week X 5.

[c]312 μg, 1 μCi.

[d]1 mg/kg of Fe_2O_3 plus 312 μg BaP, 1 μCi.

[e]Metabolite pattern values expressed as percent of total rate of appearance of metabolite in blood ± SE.

[f]$p = .05$.

[g]$p = 0.10$.

[h]$p = 0.01$.

TABLE 2. Influence of Fe_2O_3 Administration to the IPL on BaP Metabolism[a]: Percent of BaP and Total Metabolite Remaining in Each Tissue at 180 Minutes (± SE)

Tissue distribution of BaP and its metabolites[c]	No pretreatment, BaP on the IPL ($n = 3$)	No pretreatment,[b] (BaP + Fe_2O_3) on the IPL ($n = 3$)	BaP i.t. pretreatment,[b] (BaP + Fe_2O_3) on the IPL ($n = 3$)	Fe_2O_3 i.t. pretreatment, (BaP + Fe_2O_3) on the IPL ($n = 3$)
% of BaP remaining in the IPL at the end of the perfusion at 180 min	65.3 ± 4.3[d]	37.2 ± 8.7	24.1 ± 5.7	43.9 ± 4.4
% Of total compound as BaP in each tissue				
Blood	12.7 ± 1.4[e]	2.2 ± 0.5	0.4 ± 0.1[d]	1.9 ± 0.3
TB	10.4 ± 2.6[d]	3.5 ± 1.9	2.1 ± 1.6	4.1 ± 1.8
MAC	8.7 ± 7.4	10.0 ± 8.4	5.9 ± 0.9	11.0 ± 7.1
WO	2.5 ± 1.1	0.6 ± 0.2	0.6 ± 0.2	0.9 ± 0.4
Lung	31.0 ± 3.6[d]	15.0 ± 4.0	15.0 ± 4.2	25.9 ± 1.8
% Of total compound as metabolites in each tissue				
Blood	15.5 ± 5.2	18.3 ± 6.8	32.9 ± 15.5	15.7 ± 0.9
TB[c]	3.7 ± 1.2[d]	13.0 ± 2.6	5.3 ± 0.1[f]	2.7 ± 0.1[d]
MAC	0.3 ± 0.1	5.6 ± 3.1	1.7 ± 0.6	11.8 ± 10.8
WO	3.3 ± 2.3	4.8 ± 2.4	6.6 ± 2.4	4.6 ± 0.6
Lung	12.0 ± 1.9	20.9 ± 4.7	18.1 ± 11.1	2.1 ± 0.1[d]

[a]Based on TLC data. Columns 1, 3, and 4 compared to column 2. See footnote in Table 1 for explanation.

[b]Experiments perfused for at least 120 min.

[c]TB = trachea bronchi; MAC = macrophage; WO = lavage washout, supernatant fraction remaining following centrifugation of lavage fluid to obtain macrophage pellet; Lung = lung tissue following lavage.

[d]$p = 0.05$.

[e]$p = 0.01$.

[f]$p = 0.1$.

the blood following Fe_2O_3 or BaP pretreatments to the whole animal, 637 ± 203 and 1290 ± 114 ng/g lung•h (± SE), respectively, were substantially decreased when Fe_2O_3 was administered to the perfusion system (Table 1).

The coadministration of Fe_2O_3 with BaP to the perfusing lung with respect to BaP administration alone resulted in a significant decrease ($p = 0.05$) in the amount of unmetabolized BaP remaining at the end of the perfusion and changes in the metabolite distribution of BaP in the tissue (Table 2). In the presence of Fe_2O_3, the relative amounts of metabolites in TB ($p = 0.05$), MAC, and lung were increased with corresponding significant decreases of BaP in blood ($p = 0.01$), TB ($p = 0.05$), and lung ($p = 0.05$) and decrease of BaP in WO. Of particular note, there was a significant percentage increase in TB ($p = 0.05$) of nonextractables from 12.5 ± 4.1% to 36.4 ± 6.3% based on the total compound present in this tissue. Although not significant, similar trends in terms of increased percentages were observed for the nonextractables and the 7,8-diol plus S-metabolite in the blood, WO, and lung. It should be made clear that these data do not agree with the rate of appearance of metabolites in the blood (Table 1). It would be expected that more BaP would be present at the end of the perfusion. These data suggest that after an equilibration time, BaP adsorbed on Fe_2O_3 becomes more biologically available for metabolism, e.g., that BaP is leached from Fe_2O_3 and/or deposited by the macrophage in other tissues resulting in the enhanced metabolism of BaP (Autrup et al., 1979; Harris et al., 1978).

Pretreatment regimen with Fe_2O_3 or BaP resulted in no change in the amount of unmetabolized BaP remaining at the end of the perfusion as compared to the experiments where no pretreatment was followed by BaP and Fe_2O_3 *in vitro* (Table 2). However, Fe_2O_3 and BaP pretreatments altered the metabolite distribution of BaP. Pretreatment with BaP resulted in decreases of total metabolite in TB and MAC and BaP in the blood ($p = 0.05$), whereas Fe_2O_3 pretreatment resulted in significant decreases of metabolite in TB ($p = 0.05$), and lung ($p = 0.05$) and increases of BaP in the lung. Of particular note, there was a significant decrease following Fe_2O_3 pretreatment in the TB ($p = 0.01$) of the nonextractables from 36.4 ± 6.3% to 4.8 ± 1.1%, based on the total compound present in the TB. Although not significant, a similar trend was observed for the nonextractables in the TB in the BaP pretreatment experiments.

Influence of Fe_2O_3 Pretreatment on BaP Metabolism

Fe_2O_3 pretreatment to the whole animal resulted in a significant increase ($p = 0.05$) in the total rate of appearance of metabolites in the blood with respect to the no-pretreatment experiments (Table 3). This increased rate contributed to a significant increase ($p = 0.01$) of the 7,8-diol + S-metabolite, an increase of the 9,10-diol, a significant

TABLE 3. Influence of Fe_2O_3 Pretreatment on BaP Metabolism[a]: Rate of Appearance and Pattern of BaP Metabolites in the Blood

Rate and pattern	No pretreatment, BaP[d] on the IPL ($n = 9$)	Fe_2O_3 i.t. pretreatment,[b] BaP[d] on the IPL ($n = 5$)	(Fe_2O_3 + BaP) i.t. pretreatment,[c] BaP[d] on the IPL ($n = 5$)
Total rate of appearance of metabolites in blood (ng/h·g lung, ± SE)	256 ± 38[f]	637 ± 203	1745 ± 378[f]
Metabolic pattern in blood (% ± SE)[e]			
7,8-Diol + S-metabolite	6.6 ± 0.9[g]	13.3 ± 2.3	12.9 ± 4.7
9,10-Diol	15.4 ± 4.0	26.3 ± 5.6	29.1 ± 4.0
4,5-Diol	3.3 ± 0.6	2.9 ± 2.0	2.0 ± 0.8
Phenols	9.7 ± 1.1[f]	4.9 ± 0.7	5.2 ± 1.6
Quinones	10.6 ± 1.8	14.3 ± 4.4	8.4 ± 3.7
Nonextractables	54.4 ± 5.4[h]	37.7 ± 5.8	42.4 ± 10.4

[a]All metabolites separated by TLC. Columns 1 and 3 compared to column 2 to indicate the effects of Fe_2O_3 pretreatment on BaP metabolism; n = number of animals; statistics performed by Student-Newman-Keuls test.

[b]10 mg/kg once a week X 5.

[c]10 mg/kg BaP and Fe_2O_3, once a week X 5.

[d]312 μg, 1 μCi.

[e]Metabolite pattern values expressed as percent of total rate of appearance of metabolite in blood ± SE.

[f]$p = 0.05$.

[g]$p = 0.01$.

[h]$p = 0.10$.

decrease ($p = 0.05$) in the phenols, and a decrease in the nonextractables. With the addition of BaP to the Fe_2O_3 pretreatment regimen, the total rate of appearance of metabolites was significantly increased ($p = 0.05$), but there were no changes in the metabolic pattern. BaP intratracheal pretreatment alone (Warshawsky et al., 1980) resulted in a total rate of appearance of metabolites of 1290 ± 114 ng/g lung•h (± SE).

Fe_2O_3 pretreatment to the whole animal resulted in a decreased amount of unmetabolized BaP remaining at the end of perfusion (Table 4) and small changes in the metabolite distribution of BaP in the tissues. In the presence of Fe_2O_3 pretreatment, there were decreased amounts of BaP in the blood with corresponding increases of metabolites in lung and MAC. Increased percentages of the 7,8-diol plus S-metabolite were observed in lung, blood, MAC, and TB.

With the addition of BaP to the Fe_2O_3 pretreatment regimen, there was a further decrease in the amount of unmetabolized BaP remaining at the end of the perfusion and changes in the distribution of metabolites in the tissues. There was an increased percentage of metabolites in the blood ($p = 0.01$), MAC, and WO with corresponding decreases of BaP in the blood ($p = 0.01$), TB, MAC, and lung. However, this type of pretreatment did not enhance the production of the 7,8-diol plus S-metabolite but rather induced changes in quinone and/or nonextractable metabolite distribution in the tissues.

Influence of BaP Pretreatment and Fe_2O_3 Administration to the IPL on BaP Metabolism

Intraperitoneal BaP pretreatment to the whole animal showed a significant increase in the total rate of appearance of metabolites in the blood when compared to the corn oil control (Table 5). This increased rate resulted in a significant increase in the 4,5-diol ($p = 0.05$) and the phenols ($p = 0.05$), increases in the 9,10-diol, a significant decrease in the 7,8-diol plus S-metabolite, and a decrease in the nonextractables. With the addition of Fe_2O_3 to the IPL, the total rate of appearance of metabolites in the blood was significantly decreased. This decrease in the rate was accomplished by a significant increase in the nonextractables ($p = 0.05$), significant decreases in the phenols ($p = 0.05$), and decreases in the 7,8-diol plus S-metabolite, the 9,10-diol, and quinones. These results did not differ in comparison with BaP it pretreatment (Table 1).

Intraperitoneal BaP pretreatment to the whole animal resulted in a significant decrease ($p = 0.01$) in the amount of unmetabolized BaP remaining at the end of the perfusion (Table 6) and an alteration in the metabolite distribution of BaP in the tissues. In the presence of BaP pretreatment, there were decreases in the BaP distribution in the blood, MAC, WO, and lung with corresponding increases of metabolites in blood. Of particular note, there was a significant increased percentage distribution ($p = 0.05$) in the blood of the 7,8-diol plus S-metabolite, from 2.3 ± 0.1%

TABLE 4. Influence of Fe_2O_3 Pretreatment on BaP Metabolism[a]: Percent of BaP and Total Metabolite Remaining in Each Tissue at 180 Minutes (± SE)

Tissue distribution of BaP and its metabolites[b]	No pretreatment, BaP on the IPL ($n = 3$)	Fe_2O_3 i.t. pretreatment, BaP on the IPL ($n = 3$)	(Fe_2O_3 + BaP) i.t. pretreatment, BaP on the IPL ($n = 2$)
% Of BaP remaining in the IPL at the end of the perfusion at 180 min	65.3 ± 4.3[c]	46.4 ± 7.3	27.5 ± 2.4[c]
% Of total compound as BaP in each tissue			
Blood	12.7 ± 1.4[c]	7.9 ± 1.1	1.1 ± 0.8[d]
TB	10.4 ± 2.6	6.6 ± 1.5	2.4 ± 0.6[c]
MAC	8.7 ± 7.4	2.7 ± 0.9	6.2 ± 4.3
WO	2.5 ± 1.1	2.0 ± 0.5	2.4 ± 0.1
Lung	31.0 ± 3.6	27.2 ± 5.0	15.4 ± 2.7
% Of total compound as metabolites in each tissue			
Blood	15.5 ± 5.2	20.7 ± 1.2	31.5 ± 0.4[d]
TB	3.7 ± 1.2	2.5 ± 0.9	4.0 ± 2.0
MAC	0.3 ± 0.1[c]	0.9 ± 0.2	1.5 ± 0.1[c]
WO	3.3 ± 2.3	4.6 ± 2.8	8.3 ± 0.1
Lung	12.0 ± 1.9	24.9 ± 10.3	27.3 ± 4.5

[a]Based on TLC data. Columns 1 and 3 compared to column 2. See footnotes in Table 3 for explanations.

[b]TB = trachea bronchi; MAC = macrophage; WO = lavage washout, supernatant fraction remaining following centrifugation of lavage fluid to obtain macrophage pellet; Lung = lung tissue following lavage.

[c]$p = 0.1$.

[d]$p = 0.01$.

TABLE 5. Influence of BaP Pretreatment and Fe_2O_3 Administration to the IPL on BaP Metabolism[a]: Rate of Appearance and Pattern of BaP Metabolites in the Blood

Rate and pattern	Corn oil ip pretreatment,[b] BaP[d] on the IPL ($n = 9$)	BaP ip pretreatment,[c] BaP[d] on the IPL ($n = 5$)	BaP ip pretreatment,[c] (BaP + Fe_2O_3)[e] on the IPL ($n = 5$)
Total rate of appearance of metabolites in blood (ng/h•g lung, ± SE)	466 ± 94[g]	1718 ± 287	224 ± 68[g]
Metabolic pattern in blood (%, ± SE)[f]			
7,8-Diol + S-metabolite	17.9 ± 0.9[g]	8.4 ± 0.7	3.9 ± 1.5[i]
9,10-Diol	15.3 ± 4.9	27.9 ± 4.5	16.1 ± 2.3
4,5-Diol	1.2 ± 0.5[h]	3.8 ± 0.9	2.8 ± 0.7
Phenols	2.7 ± 1.1[h]	13.0 ± 1.8	2.4 ± 0.6[h]
Quinones	6.5 ± 2.9	10.6 ± 1.8	6.5 ± 1.6[i]
Nonextractables	56.3 ± 7.8	36.3 ± 4.7	70.7 ± 3.0[g]

[a]All metabolites separated by TLC. Columns 1 and 3 compared to column 2 to indicate the effects of Fe_2O_3 administered to the IPL preceded by BaP pretreatment has on BaP metabolism in the IPL; n = number of animals; statistics performed by *Student-Newman-Keuls test.*

[b]3 ml corn oil/kg, 24 h before sacrifice.

[c]20 mg/kg in corn oil, 24 h before sacrifice.

[d]312 μg, 1 μCi.

[e]1 mg/kg of Fe_2O_3 plus 312 μg BaP, 1 μCi.

[f]Metabolite pattern values expressed as percent of total rate of appearance of metabolite in blood ± SE.

[g]$p = 0.01$.

[h]$p = 0.05$.

[i]$p = 0.10$.

TABLE 6. Influence of BaP Pretreatment and Fe_2O_3 Administration to IPL on BaP Metabolism[a]: Percent of BaP and Total Metabolite Remaining in Each Tissue at 180 Minutes (± SE)

Tissue distribution of BaP and its metabolites[b]	Corn oil ip pretreatment, BaP on the IPL ($n = 2$)	BaP ip pretreatment, BaP on the IPL ($n = 2$)	BaP ip pretreatment, BaP + Fe_2O_3 on the IPL ($n = 2$)
% Of BaP remaining in the IPL at the end of the perfusion at 180 min	60.9 ± 3.4[c]	23.5 ± 2.5	36.0 ± 7.1[d]
% Of total compound as BaP in each tissue			
Blood	15.8 ± 5.6	1.5 ± 1.0	1.5 ± 0.2
TB	5.4 ± 1.8	6.3 ± 3.0	1.9 ± 1.5
MAC	3.8 ± 0.4[d]	1.0 ± 0.7	11.5 ± 0.1[c]
WO	5.2 ± 3.9	2.1 ± 0.4	0.6 ± 0.2[d]
Lung	30.6 ± 4.7[d]	12.6 ± 0.7	20.5 ± 6.8
% Of total compound as metabolites in each tissue			
Blood	14.5 ± 2.5	39.7 ± 17.9	25.1 ± 1.8
TB	3.4 ± 0.1	5.9 ± 4.6	3.3 ± 1.8
MAC	0.9 ± 0.3	0.6 ± 0.5	1.7 ± 0.3
WO	2.6 ± 0.4	8.9 ± 7.2	5.2 ± 0.7
Lung	17.6 ± 0.8	21.4 ± 8.0	28.7 ± 4.9

[a]Based on TLC data. Columns 1 and 3 compared to column 2. See footnotes in Table 5 for explanations.

[b]TB = trachea bronchi; MAC = macrophage; WO = lavage washout, supernatant fraction remaining following centrifugation of lavage fluid to obtain macrophage pellet; Lung = lung tissue following lavage.

[c]$p = 0.01$.

[d]$p = 0.1$.

to 6.5 ± 0.6% based on the total compound present in the blood. Similar increased percentages in the blood were observed for the 9,10-diol (2.3 ± 1.1% to 14.8 ± 5.9%), the phenols (2.8 ± 0.7% to 9.1 ± 5.4%) quinones (1.1 ± 0.3% to 8.9 ± 3.4%), and nonextractables (21.9 ± 4.2% to 55.4 ± 16.5%).

With the addition of Fe_2O_3 to the lung there was a slight increase in the amount of unmetabolized BaP remaining at the end of the perfusion as compared to BaP alone, which was accompanied by a significant increase of BaP in MAC ($p = 0.01$), an increase of BaP in lung and a decrease of BaP in WO (Table 6). These data do not agree with the rate of appearance of metabolites in the blood (Table 5). As indicated previously with reference to Table 2, it would be expected that more BaP would be present at the end of the perfusion. These data suggest, as evidenced by the significant amount of BaP present in the MAC, that BaP adsorbed on Fe_2O_3 becomes biologically available for metabolism after a period of time, e.g., that BaP is leached from Fe_2O_3 and/or deposited by the macrophage in other tissues, resulting in an enhanced metabolism of BaP (Autrup et al., 1979; Harris et al., 1978).

DISCUSSION

We have attempted to simulate the pulmonary metabolism of BaP *in vivo* using an IPL preparation in the presence of Fe_2O_3 under a variety of exposure conditions. Our efforts have been directed toward determining the immediate effects of ferric oxide exposure on BaP metabolism in the lung.

BaP ip or BaP intratracheal pretreatment to the whole animal followed by BaP administration to the perfusing lung, in comparison to the appropriate control, significantly increased the rate of appearance of BaP metabolites in the blood, which is attributable to the BaP induction of the P_1-450 (P-448) enzyme system (Thorgeirsson and Nebert, 1977). The metabolic profile showed a marked increase in the 9,10-diol following BaP ip or BaP intratracheal pretreatment, while there was a significant decrease in the phenols and quinones for BaP intratracheal pretreatment in comparison with no pretreatment and BaP ip pretreatment. These data indicate that, even though BaP was given by two different routes of administration, the rates of metabolism were significantly higher than the appropriate controls in both cases and similar enzyme systems were induced.

Similar studies with 3MC indicated that it was not as good an enzyme inducer (P_1-450) as BaP for the lung, as indicated by the total rate of metabolism. 3MC definitely caused an increase in the rate of metabolism compared to corn oil controls, but was not significantly different at the 90% confidence level (Smith et al., 1978). Following 3MC and BaP induction, similar metabolic profiles resulted which were different from corn oil controls; both 3MC and BaP appeared to stimulate 9,10-dihydrodiol

production. These data indicate that the metabolite patterns produced by P_1-450 (P-448) enzyme inducers, such as BaP and 3MC, are similar, and that more nonextractable material is excreted into the blood stream than in the appropriate controls. Therefore, based on the fact that 3MC and BaP produced similar metabolic profiles, BaP pretreatments were used for studying the effect of ferric oxide on BaP metabolism in the lung perfusion system due to the increased enzyme inducing ability as evidenced by the increased rate of appearance of metabolites of BaP in the blood.

The data indicate that Fe_2O_3 interacts with BaP by altering the rate and pattern of metabolism. Fe_2O_3 most probably acts as a physical agent by decreasing the biological availability of BaP when administered together with BaP to the lung. With time, BaP can be readily leached from Fe_2O_3 and metabolized or deposited by macrophage in other tissues such as the TB (Autrup et al., 1979; Harris et al., 1978; Stoner et al., 1978; Yang et al., 1977). This may be of importance due to the fact that BaP produces primarily bronchiogenic carcinomas in laboratory animals (Feron et al., 1980; Saffiotti et al., 1972; Sellakumar et al., 1976; Stenbäck and Rowland, 1978).

In vivo pretreatment with Fe_2O_3 or BaP resulted in an increase in the total rate of metabolism of BaP, while the *in vitro* administration of Fe_2O_3 with BaP led to a decrease in the total metabolic ability. More specifically, when Fe_2O_3 is administered to the perfused lung, the rate of appearance of metabolites in the blood is decreased and can be reflected in enzyme inhibition of catabolism leading to decreased turnover by BaP or Fe_2O_3 pretreatments and a slight depression of catabolism by basal enzymes. Furthermore, Fe_2O_3 administered to the lung *in vitro* with or without Fe_2O_3 pretreatment enhanced dihydrodiol formation, in particular the 7,8-diol plus S-metabolite, with respect to no pretreatment or corn oil or BaP pretreatments. Similar results have been obtained in other IPL studies; acute exposure to cigarette smoke resulted in an inhibition of BaP metabolism (Lubawy and Isaac, 1980a) and an increased formation of the 7,8-diol (Lubawy and Perrier, 1980) while pretreatment with cigarette-smoke condensate increased the rates of pulmonary BaP metabolism in rabbit in a manner similar to that seen in other species (Lubawy and Isaac, 1980b).

The data indicate that Fe_2O_3 enhances diol formation of BaP, in particular the 7,8-diol plus S-metabolites, and depresses nonextractable materials. This is very important due to the fact that diol formation is considered part of the active pathway of BaP leading to the ultimate metabolite, 7,8-diol-9,10-epoxide (Grover et al., 1976; Jeffrey et al., 1976; Meehan et al., 1976; Wislocki et al., 1976). This suggests that Fe_2O_3 enhancement of BaP carcinogenicity (Saffiotti et al., 1972) may be due to the increased production of dihydrodiols of BaP through the active pathway leading to the formation of the ultimate metabolite(s).

This work helps to clarify the findings of a number of investigators

(Creasia et al., 1976; Henry et al., 1975; Kobayashi and Okamoto, 1974; Sellakumar et al., 1976; Stenbäck et al., 1975): (1) particulates used in maintaining BaP in the lung for long periods appear responsible for increased tumorigenic response due to the slow release of BaP from particulates, and (2) particulates appear to influence metabolic pathways. The results presented indicate that both factors may be responsible—i.e., slower release of BaP from particulates as measured by appearance of metabolites in blood and in lung tissue of the IPL, and a significant change in metabolic pathways.

With concomitant administration of particulates and BaP, and therefore a slower release rate, BaP is effectively being administered to the lung tissue in small doses when compared with the same amount of BaP given alone. This type of treatment with particulate appears to be similar to previous observations: a carcinogen is much more effective in increasing the tumorigenic response when given in small divided doses over a period of time, as opposed to a single large equivalent dose (Saffiotti et al., 1972). Thus, it appears that the IPL is a good model system for studying BaP metabolism in the lung in the presence of environmental particulates (Warshawsky et al., 1983).

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