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Uptake of Benzo[a]pyrene—Ferric Oxide Particulates by Human Pulmonary Macrophages and Release of Benzo[a]pyrene and Its Metabolites (40536)[1]

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Pulmonary alveolar macrophages (PAM) play an important role in phagocytosis and presumably in detoxification of foreign material reaching the terminal airways. Carcinogenic polynuclear aromatic hydrocarbons (PAH), such as benzo[*a*]pyrene (BP) can be either inhaled directly or more likely in combination with a carrier; e.g., airborne particulates, industrial soots, and tobacco smoke. PAH adsorbed to ferric oxide particulates are retained in the lung of experimental animals for several days, whereas free PAH are cleared very rapidly (1). The incidence of lung tumors in hamsters is increased following BP–ferric oxide treatment when compared to animals receiving BP alone (1, 2). In addition, ferric oxide stimulates the phagocytic rate of PAM as well as stimulates a recruitment of mononuclear leukocytes into PAM (3).

It has been previously shown that cultured human PAM can metabolize BP (4–7). When PAM were incubated with either BP or its proximate carcinogenic form, BP-7,8-diol, a metabolite(s) of BP that was mutagenic for cocultivated Chinese hamster V-79 cells was released (8). The release of mutagenic and/or carcinogenic metabolites from carcinogens adsorbed on phagocytized particulates may play a role in the pathogenesis of lung disease, including bronchogenic carcinoma. During their transport up the respiratory tract, macrophages with engulfed particulates may be temporarily retarded in areas of stasis and turbulance (9) allowing for the release of either procarcinogens or ultimate carcinogens which could then be absorbed by the bronchial epithelial cells, or they could activate particulates which have been deposited in the regions. Histological data suggest that the origin of many lung cancers is in the areas of bronchial branching where macrophages may accumulate due to turbulence. We report here data concerning the uptake of ferric oxide particulates with adsorbed BP by human PAM, the metabolism of BP, and the extracellular release of BP and BP metabolites.

Materials and methods. Human lung specimens were obtained at the time of either surgery or immediate autopsy (10). PAM were prepared by differential adhesion as described previously and cultured for 7 days (4) prior to incubation with [^{3}H]BP–ferric oxide particulates (1 mg per milliliter) for generally 1 hr. [^{3}H]BP–ferric oxide particulates were prepared from [^{3}H]BP (20 Ci/mmole; Amersham–Searle, Arlington Heights, Ill.), diluted with unlabeled BP (NCI, Chemical Repository, Bethesda, Md.), and ferric oxide [type R8089; particle size 93% (by weight) less than 5 μm diameter, and 6.8% (by weight) less than 1 μm diameter; Minerals, Pigments and Metals Division, Chas. Pfizer & Co., Inc., N.Y.] as described by Little *et al.* (11). The concentration of BP was 336 μg per milligram BP–ferric oxide. To determine the uptake of BP–ferric oxide by PAM (approx 500,000 cells), [^{3}H]BP–ferric oxide (1 mg/ml) was added to the PAM and incubated for 1, 5 and 25 hr, respectively. The PAM were washed three times with medium to remove nonengulfed [^{3}H]BP–ferric oxide, scraped from the dish with a rubber policeman, counted with a Coulter counter (Coulter Electronics, Hialeah, Fla.) and the radioactivity was determined in an aliquot. PAM incubated for 1 hr with [^{3}H]BP–ferric oxide were used for the following experiments.

The release of BP and BP-metabolites from PAM (5×10^6 cells after phagocytosis for 1

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hr of BP–ferric oxide) was determined by adding fresh media to the PAM and incubating the cells for 1, 2 or 6 hr. The media was then extracted three times with ethyl acetate/acetone and the metabolites separated using high-pressure liquid chromatography as described previously (12). To determine intracellular metabolites the PAM were scraped from the dish, treated with 0.25% trypsin for 1 hr at 37° followed by homogenization in a Potter–Elvhjem Teflon–glass homogenizer and centrifuged at 800 rpm for 10 min to remove BP–ferric oxide particulates and cell debris. The metabolites were isolated from the supernatant and separated as described above for the media.

For the cell-mediated mutagenesis assay, 5×10^5 V-79 cells were added to each 100-mm culture dish containing PAM that had been previously incubated with BP–ferric oxide for 1 hr. The V-79 cells and PAM were then coincubated for 2 days. The incidence of ouabain-resistant (O^r) mutant V-79 cells, and the cloning efficiency were determined as described by Harris *et al.* (8). For the control, V-79 cells were incubated with BP–ferric oxide without PAM.

The effect of coincubating PAM with human bronchial explants on the binding of BP to bronchial DNA was investigated. Five bronchial explants, cultured for 7 days, were placed on a 100-mm dish containing PAM with phagocytized BP–ferric oxide. (Both explants and PAM were isolated from the same patient.) After either 6 or 24 hr the mucosal layer was removed by scraping and cellular DNA and protein were isolated by the phenol-extraction procedure (13). The level of binding of BP to DNA and to protein was assayed as previously described (4).

Results. Ferric oxide particulates with BP adsorbed to them were phagocytized by cultured human PAM as observed by phase-contrast microscopy (Fig. 1) and by biochemical methods. After 1 hr of coincubation with BP–ferric oxide, the PAM had engulfed 19% (0.848 μmole BP/10^6 cells), 25% after 5 hr (0.965 μmole BP), and 60% after 25 hr (2.907 μmole BP) of the total amount of BP.

After removal of the nonphagocytized BP, the release of BP and BP-metabolites by PAM was determined. Approximately 5% of the BP initially within the PAM was released into the extracellular space either as free BP (82%) or as BP-metabolites (18%) within 1 hr; although only minor amounts of water-solu-

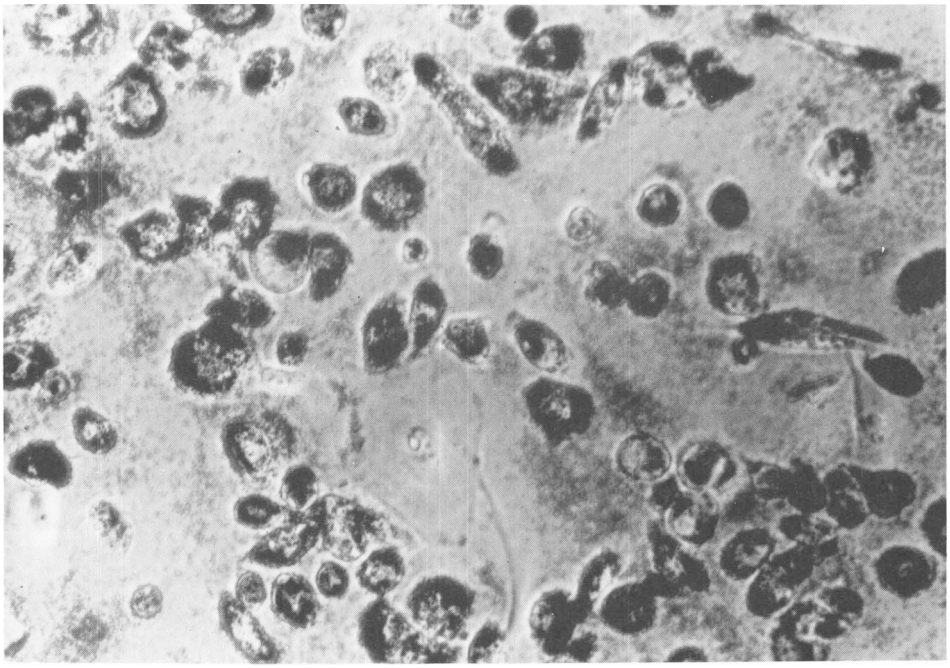

FIG. 1. Ferric oxide particulates coated with BP phagocytized by cultured human PAM.

ble metabolites were detected after 1 hr (Table I), they represented 20% of the total metabolites after 6-hr incubation. The PAM contained both free BP released from ferric oxide and BP-metabolites. While small amounts of diols were released into the media after 1 hr, only phenols and quinones of BP were found within the cells. No tetrols, produced by further metabolism of the primary metabolites, were seen at the earlier time period and were primarily intracellular after 6-hr incubation (4.3% of total metabolism).

To determine if the released BP and/or BP-metabolites could enter other cell types, the PAM containing BP–ferric oxide particulates were coincubated with either Chinese hamster V-79 cells for detection of ouabain-resistant (O^r) mutants or with bronchial explants to determine binding of BP-metabolites to cellular macromolecules. When PAM containing BP–ferric oxide particulates were coincubated with V-79 cells for 2 days, followed by selection of O^r-mutants, an increased O^r-mutation frequency in the V-79 cells was observed (Table II). An increased level of binding of BP to bronchial DNA and protein was also observed when bronchial explants were coincubated with PAM containing BP–ferric oxide (Table III).

TABLE I. RELEASE OF BP AND BP METABOLITES FROM HUMAN PAM CONTAINING BP—FERRIC OXIDE PARTICULATES

Compound released (pmole/10^6 PAM)	1 hr	2 hr	6 hr
BP	288	332	453
BP-Metabolites	107	126	145
Water soluble	5	8	29
Organic solvent extractable	102	118	16
Tetrols[a]	n.d.	n.d.	5
(7/8,9)-Triol and *trans*-9,10-diol	2	1	3
trans-4,5-Diol	3	4	3
trans-7,8-Diol	4	3	5
3-OH	3	9	7
9-OH	4	18	17
1,3-Quinone	19	20	16
3,6-Quinone	22	21	24
6,12-Quinone	21	24	17
Unidentified	27	18	19

[a] Nomenclature for BP compounds: *trans*-4,5-diol; *trans*-4,5-dihydro-4,5-dihydroxybenzo[*a*]pyrene; *trans*-7,8-diol; trans-7,8-dihydro-7,8-dihydroxybenzo[*a*]pyrene; *trans*-9,10-diol, *trans*-9,10-dihydro-9,10-dihydroxybenzo[*a*]pyrene; (7/9,9)-triol, trihydroxytetrahydrobenzo[*a*]pyrene in which the 8-hydroxy and 9-hydroxy are *trans* to the reference 7-hydroxy.

Discussion. Several chemicals and physical agents have been shown to modify the passage of chemicals through the membrane, the intracellular metabolism of the chemicals, and the release of metabolites into the extracellular space, all of which could be important cocarcinogenic events. The presence of asbestos (14) and silica (15, 16) greatly enhanced the passage of PAH through a synthetic bilayer membrane, while mineral dusts altered the permeability of the cell membrane (17). Once the BP-particulates were phagocytized

TABLE II. OUABAIN-RESISTANT O^r MUTATION FREQUENCY IN V-79 CELLS CAUSED BY BENZO[*a*]PYRENE AND MEDIATED BY PAM[a]

Control	Day after coincubation	Cloning efficiency[b]	O^r Mutants per 10^6 survivors per 10^6 PAM
(V-79 + BP–Fe_2O_3)	1	78	<1
	5	62	<1
	7	74	<1
V-79 + PAM + BP–Fe_2O_3	1	27	4
	5	51	7
	7	76	7

[a] 5×10^5 V-79 cells were added to 100-mm culture dishes containing PAM which had previously been incubated with BP–ferric oxide for 1 hr. For the control, V-79 cells were incubated with BP–ferric oxide (1 mg/ml) alone. O^r, ouabain-resistant Chinese hamster V-79 cells.

[b] Cloning efficiency compared with that observed when V-79 cells were incubated without both BP–ferric oxide and PAM. The absolute cloning efficiency for V-79 cells was 81%.

TABLE III. INTERACTION BETWEEN HUMAN PULMONARY MACROPHAGES AND HUMAN BRONCHUS DURING METABOLISM OF BENZO[*a*]PYRENE[a]

Group[b]	Time (hr)	pmole BP bound per 10 mg	
		DNA	Protein
HB and PAM	6	247	1457
HB and PAM	24	4583	9965
HB alone	24	120	883

[a] Macrophages (8×10^6 cells per 100-mm dish) were incubated with 5 ml of culture medium containing [^{3}H]benzo[*a*]pyrene–Fe_2O_3 (1 mg per ml; 336 μg benzo[*a*]pyrene per mg) for 1 hr. The macrophages were washed with culture medium to remove the extracellular [^{3}H]BP–Fe_2O_3. Five explants of human bronchus were then added to each dish for either 6 or 24 hr. In the group with only human bronchial explants, [^{3}H]BP–Fe_2O_3 (1 mg per ml) was added to the culture medium prior to the addition of the explants.

by PAM, the size and the chemical nature of the particulates influenced the dissociation of BP (18). Several chemical compounds, associated with chemicals and physical agents taken up PAM, have been shown to induce the mixed-function oxidases in PAM (5, 19). As these enzyme systems are responsible for the activation/deactivation of chemical carcinogens, an induced level could enhance the formation of ultimate or proximate carcinogens.

Our results indicate: (i) that BP–ferric oxide is phagocytized by PAM even after a short period of incubation; (ii) BP is released from the ferric oxide carrier in the PAM; and (iii) BP and its metabolites are then excreted from the PAM into the extracellular space. Most of the released material was BP but ultimate mutagenic metabolites, as shown by the induction of O^r mutation in V-79 cells were also released; V-79 cells by themselves are not able to activate either BP or its proximate mutagenic form, 7,8-diol (8).

BP and/or BP metabolites released by the PAM were also taken up by bronchial explants and were further metabolized into compounds that can bind DNA. We have shown previously that cultured human bronchus is able to activate both BP and its 7,8-diol into the ultimate carcinogenic form (13, 21), which then reacts with cellular DNA. The binding level of BP to bronchial DNA was higher when the bronchial explants were coincubated with PAM containing BP–ferric oxide, than with BP–ferric oxide alone. This observation could possibly be explained by the fact that the epithelium in the latter case was not in physical contact with the particulates and could not elute BP from them. Kennedy and Little (22) have previously reported the ability of bronchial epithelial cells to elute and to adsorb BP from BP–ferric oxide administered intratracheally. However, in a previous experiment we found a higher level of 7,8-diol, the proximate carcinogenic form of BP, when PAM and bronchial explants were incubated with BP than was formed when BP was either incubated with PAM or bronchial explants alone (4); this should result in a higher binding level of BP to DNA in the coincubated tissues (21).

Our studies suggest that PAM may play a role in the carcinogenic process of the bronchi by virtue of their ability to phagocytize particulates carrying carcinogens, thus concentrating the carcinogen; and during their passage up the respiratory tract by the mucociliary transport system releasing the carcinogen and its activated forms to be taken up by the bronchial epithelium. Interaction with other industrial pollutants such as metals and asbestos might enhance metabolic activation of chemical carcinogens and/or change the permeability of the membrane of PAM (23–25) resulting in release of more carcinogenic metabolites and at a higher level.

Summary. Human pulmonary macrophages were able to phagocytize BP–ferric oxide particulates in a time-dependent manner. BP was eluted from the engulfed particulates and released into the extracellular space either as free BP or as metabolites of BP. Some of the released products were mutagenic for Chinese hamster V-79 cells. A higher binding level of BP to bronchial DNA was found when bronchial explants were coincubated with PAM containing BP–ferric oxide than when incubated with BP–ferric oxide alone.

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