METABOLISM OF FOREIGN COMPOUNDS BY ALVEOLAR MACROPHAGES OF RABBITS

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SUMMARY

Alveolar macrophcges of rabbits acetylated p-aminobenzoic acid to the same extent as did lung parenchyma and liver. However, microsomes isolated from macrophages lacked detectable activity of aryl hydrocarbon hydroxylase, even in animals treated with 3-methylcholanthrene, an inducer of this enzyme in liver and lung. Similarly, bromobenzene was metabolized by microsomes prepared from liver and lung but not from alveolar macrophages.

INTRODUCTION

Although the ability of the lungs to metabolize foreign compounds has been described (1-5), it is not known which type of pulmonary cell carries out this important function that may protect the lungs' vast surface area from toxic effects of potentially harmful inhaled substances. Histochemical techniques demonstrate aryl hydrocarbon hydroxylase exclusively in the alveolar walls (6), whereas aniline hydroxylase activity is more readily observed in bronchial epithelial cells (7). Macrophages which reside in the walls and lumens of pulmonary alveoli have long been recognized as a primary defense system against inhaled particulate matter, but the importance of these reticuloendothelial cells in the detoxification of foreign chemicals is unknown. The observations that phagocytic cells of the reticuloendothelial system in the liver contain the microsomal enzyme aromatic hydrocarbon hydroxylase (8) as well as various nonmicrosomal acetylases (9) prompted us to look for drug metabolizing enzymes in alveolar macrophages. The present study compares the metabolism of p-aminobenzoic acid, 7,12-dimethyl-1,2-benzanthracene (DMBA) and bromobenzene in liver, lung parenchyma and alveolar macrophages.

METHODS

Experiments were carried out in male Albino rabbits (NIH) weighing approximately 1.5 kg. In some experiments the animals were treated with 3-methylcholanthrene (25 mg/kg i.p. dissolved in sesame oil) for 3 days prior to killing; control animals received only sesame oil. The rabbits were killed by intravenous injection of air. Alveolar macrophages were collected by 6 pulmonary lavages with 35 ml of 37°C saline (10). The fluid recovered was centrifuged (1,000 RPM for 10 min), and the alveolar cells washed once with 0.02 M Tris in 1.15% KCl (pH 7.4) and then homogenized in 2 ml of this buffer using a high speed blade homogenizer at 4°C. Completeness of homogenization was ascertained by phase contrast microscopy. Liver and lungs were excised, rinsed in cold saline, expressed through a tissue press and homogenized in 3 vol. of the Tris-KCl buffer. Protein concentrations of the various homogenates were determined by Lowry's method (11) and were diluted to 5 mg/ml before the start of enzyme assays. In other experiments the homogenates were centrifuged at 9000 g for 20 min and the supernatant fluid separated and centrifuged for 1 hr at 105,000 g. The microsomes were suspended in 0.1 M sodium phosphate buffer (pH 7.4) so that the concentration of microsomal protein was 2.0 mg/ml in the final reaction mixture.

The metabolism of bromobenzene was determined by incubating ^{14}C-bromobenzene (SA 3.87 mCi/nmoles; 5×10^{-4} M) at 37°C in either a tissue homogenate or microsomal suspension containing (mM) NADH 0.124, nicotinamide 2, NADP 0.2, glucose-6-phosphate 2 and 1 unit/ml glucose-6-phosphate dehydrogenase. The incubation was stopped by the addition of an equal volume of NaOH (1.0 N). The mixture was extracted 3 times with 5 volumes of heptane:isoamyl alcohol (49:1), and an aliquot of the aqueous phase was assayed for polar metabolites by liquid scintillation spectrometry. Previous studies have shown that the extraction procedure removed virtually all the unmetabolized ^{14}C-bromobenzene (12). To separate phenolic metabolites from other polar metabolites the hep-

tane extractions were performed without addition of NaOH so that the phenols were extracted into the heptane phase. Phenols then were determined by shaking the combined heptane extracts with 0.1 volume of NaOH (0.5 N) and counting an aliquot of the NaOH layer. Aryl hydrocarbon hydroxylase activity was determined by the method of Nebert and Gelboin (3,13), using C^{14}-DMBA (SA 5.6 mCi/nmole) as substrate.

Acetylation of p-aminobenzoic acid was measured by incubating ^{14}C-p-aminobenzoic acid (2.3×10^{-4} M; SA 4.26 mCi/mmole) at 37°C with 2 ml of tissue homogenates (10 mg of protein/ml) in a medium similar to that described by Govier (9) containing (mM): acetyl CoA 1.25, NaCl 101, Na Acetate 25, KCl 4.7, K_2HPO_4 1.3, $NaHCO_3$ 3.2, glucose 11, cysteine 10 and sodium citrate 2. At various times 250 µl aliquots were removed and after adjusting the pH to 1.0 by the addition of 50 µl of HCl (1.0 N), 50 µl of 0.2 M HCl-KCl buffer, pH 1.0 (14), and 50 µl of carrier p-acetamidobenzoic acid (5 mg/ml in ethanol) the mixture was extracted with 2 ml of chloroform and centrifuged (2,000 x g for 5 min). The organic phase (1.5 ml) was removed and extracted with 0.5 ml of HCl (0.1 M) before an aliquot of the chloroform was counted by liquid scintillation spectrometry. Recovery of p-acetamidobenzoic acid was quantitative and was checked by adding a known amount of ^{14}C-p-acetamidobenzoic acid to a homogenate just prior to the chloroform extraction.* Blank values were obtained by adding the ^{14}C-p-aminobenzoic acid to a homogenate adjust to pH 1.0 extracting the mixture with 2 ml of chloroform. These values were subtracted from the experimental values before calculating the amount of ^{14}C-p-acetamidobenzoic acid formed. The identity of the ^{14}C-p-acetamidobenzoic acid in the

*The ^{14}C-p-acetamidobenzoic acid was synthesized by mixing 500 µCi of acetic-1-^{14}C-anhydride (SA 118 mCi/mmole), and 700 µmole of unlabeled acetic anhydride to 73 µmoles of p-aminobenzoic acid dissolved in 0.5 ml of pyridine. After incubation at 23° for 3 hrs the excess acetic anhydride and pyridine were evaporated at 40° under vacuum and the residue was dissolved in ethanol. The ^{14}C-p-acetamidobenzoic acid was isolated by electrophoresis on Whatman 3 MM paper in acetic acid (1.5 M) formic acid (0.75 M) buffer (pH 2.0) at 1,000 V for 1 hr. p-Aminobenzoic acid and its acetylated derivatives were identified by spraying the dried electrophoresis paper with dimethylaminobenzaldehyde (1% in 1.0 N HCl)

chloroform extracts was confirmed by paper electrophoresis. Specificity of the method was further confirmed by the virtually identical results obtained when unlabeled p-aminobenzoic acid was acetylated using ^{14}C-labeled acetyl CoA and the ^{14}C-p-acetamidobenzoic acid isolated by paper electrophoresis. Acetylase activity could be detected only in the 100,000 x g supernatant fraction of lung homogenates.

RESULTS

The data in Table 1 indicate that the acetylation of p-aminobenzoic acid was similar in both homogenates and microsomal preparations of liver, lung parenchyma and alveolar macrophages, although the rate of metabolism was slightly greater in lung and in alveolar macrophages than in liver. In contrast, homogenates and microsomal preparations of alveolar macrophages hydroxylated DMBA very slowly (Figs. 1 and 2) and failed to metabolize bromobenzene (Figs. 3 and 4) even in tissues of animals pretreated with 3-methylcholanthrene. These findings suggest that although the macrophages contain certain nonmicrosomal enzymes capable of metabolizing foreign compounds they probably lack cytochrome P-450-dependent drug metabolizing enzymes in microsomes.

Liver and lung possessed different activities of the two microsomal enzymes studied. DMBA was hydroxylated more rapidly by the liver than by the lung (Figs. 1 and 2), whereas bromobenzene metabolism was more rapid in lung than in liver (Figs. 3 and 4). Prior treatment of the animals with 3-methylcholanthrene induced the hydroxylation of DMBA in liver homogenates to a greater extent than in the lung (fig. 2). In contrast, bromobenzene metabolism was decreased both in liver and lung homogenates by pretreatment with 3-methylcholanthrene (fig. 4). Lung microsomes produced considerably more phenolic metabolites of bromobenzene than did microsomes prepared from liver (Table 2). Thus, there appeared to be organ-specific differences in both the rate and the pattern of metabolism of certain foreign compounds.

Table 1

p-Aminobenzoic Acid Acetylation by Rabbit Tissue Homogenates

Tissue	p-Acetamidobenzoic Acid Formed
	(nmoles/mg protein/min)
Alveolar macrophages	.412
Lung parenchyma	.625
Liver	.358

To obtain a sufficient quantity of alveolar macrophages, tissues from 4 rabbits were pooled, homogenized in Tris buffer (0.02M, pH 7.4) containing 1.15% KCl and incubated for 5 min at 37° in the presence of 2.3×10^{-4}M ^{14}C-labeled p-aminobenzoic acid as described in Methods. Protein concentration of the homogenates was 10 mg/ml. Results are typical of 3 separate experiments.

Table 2

Bromobenzene Metabolism in Rabbit Microsomes

Tissue	Polar Metabolites of Bromobenzene	
	(nmoles/mg protein/20 min ± S.E.)	
	PHENOLIC METABOLITES	NON-PHENOLIC METABOLITES
Lung	13.85 ± 1.18	1.63 ± 0.14
Liver	5.32 ± 0.92	4.11 ± 0.77
Significance	$P < .001$	$P < .02$

Values are the means of microsomes prepared from 3 individual rabbits determined in duplicate as described in Methods. Results are typical of 2 separate experiments.

DISCUSSION

Govier (9) demonstrated that in the liver p-aminobenzoic acid and sulfanilamide are acetylated exclusively by the reticuloendothelial cells (Kupffer cells). Our date indicate the phagocytic cells in the lung also contain acet-

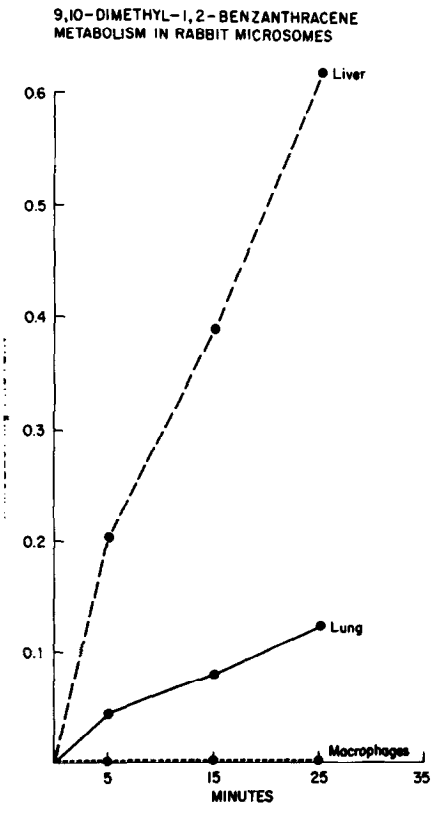

Fig. 1.

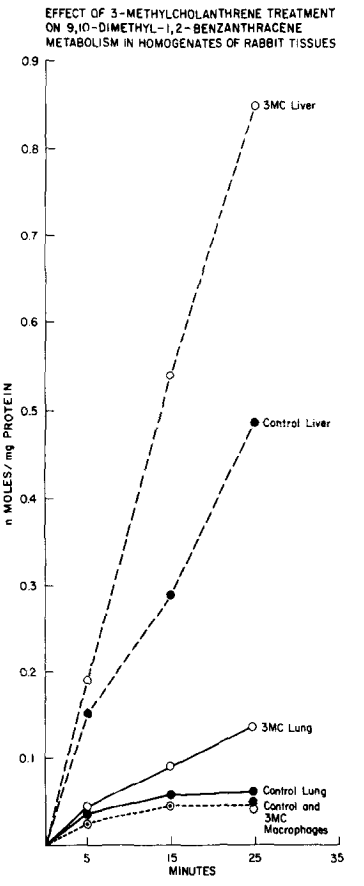

Fig. 2.

Fig. 1. Hydroxylation of 9,10-dimethyl-1,2-benzanthracene in microsomes prepared from alveolar macrophages (dotted line), lung parenchyma (solid line) or liver (hashed line) of rabbits, as described in Methods. Values are for microsomes pooled from 6 rabbits and are typical of 2 experiments.

Fig. 2. Hydroxylation of 9,10-dimethyl-1,2-benzanthracene in homogenates of alveolar macrophages (small-hashed line), lung parenchyma (solid lines) or liver (large-hashed lines), as described in Methods. Closed circles indicate controls, open circles represent rabbits pretreated with 3-methylcholanthrene as described in Methods. Values are for tissues pooled from 4 rabbits and are typical of 2 experiments.

ylase. Since many of these cells are situated within alveolar walls and are not removed during pulmonary lavage it is conceivable that acetylation is carried out exclusively by macrophages. But it is still possible that other types of pulmonary cells may also acetylate p-aminobenzoic acid. In a preliminary experiment (unpublished) we have found that peritoneal macrophages acet-

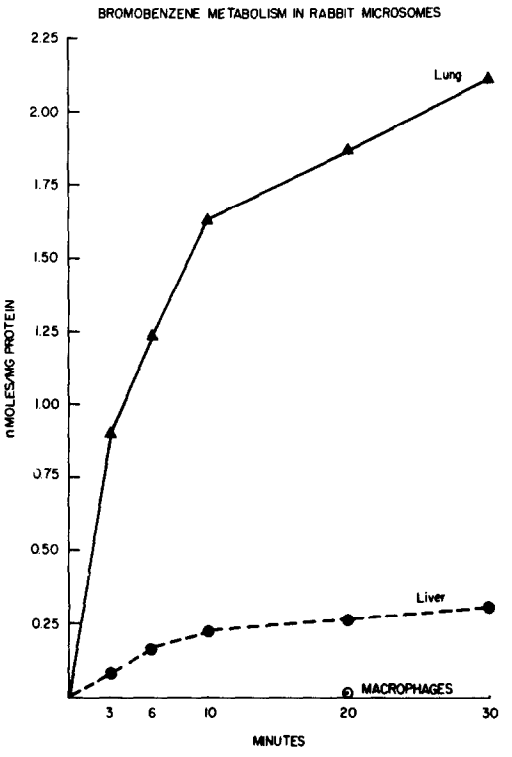

Fig. 3.

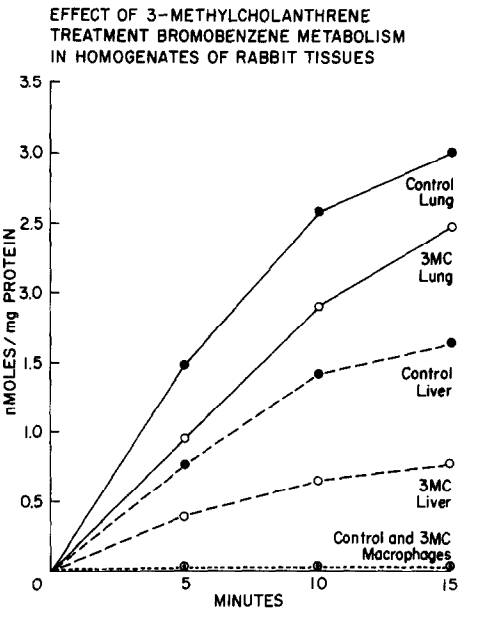

Fig. 4.

Fig. 3. Total metabolism of bromobenzene in microsomes prepared from liver (hashed line), lung parenchyma (solid line) or alveolar macrophages (single point in circle) of rabbits, as described in Methods. Values are for tissues pooled from 6 rabbits and are typical of 2 experiments.

Fig. 4. Total metabolism of bromobenzene in homogenates of alveolar macrophages (small-hashed line), lung parenchyma (solid lines) or liver (large-hashed lines), as described in Methods. Closed circles indicate controls, open circles represent rabbits pretreated with 3-methylcholanthrene as described in Methods. Values are from tissues pooled from 4 rabbits and are typical of 2 experiments.

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ylate p-aminobenzoic at about the same rate as do alveolar macrophages. This finding, together with Govier's data (9), indicates that nonmicrosomal acetylases are present in at least three types of reticuloendothelial cells.

The reticuloendothelial cells of the liver have also been reported to contain an aromatic hydrocarbon hydroxylase which can be induced 17-fold by

pretreatment with 3-methylcholanthrene (8). In contrast, alveolar macrophages do not appear to hydroxylate DMBA to any significant extent even after 3-methylcholanthrene induction (fig. 2). Likewise, alveolar macrophages did not metabolize bromobenzene, a compound which gives a typical type I cytochrome P-450 spectrum in rat liver microsomes (15). Thus, some type of pulmonary cell other than the alveolar macrophage must contain the microsomal enzymes which metabolize these foreign compounds.

Other remarkable findings in bromobenzene metabolism by the liver and lung were the greater amount of phenolic metabolites produced by the lung as compared with the liver (table 2), and the reduction of bromobenzene metabolism by both organs in rabbits pretreated with 3-methylcholanthrene (fig. 4). The latter finding was surprising, since the polycyclic hydrocarbon enhances bromobenzene metabolism by rat liver microsomes (this laboratory, unpublished).

Further knowledge of which cells in the lung metabolize foreign compounds could provide an insight into the pathogenesis of certain chemically-induced pulmonary lesions. For example, necrosis of the bronchial epithelium may be induced by treating mice intraperitoneally with ^{14}C-bromobenzene or other halogenated aromatic hydrocarbons (15,16). This lesion is associated with the selective covalent binding of a metabolite of ^{14}C-bromobenzene to bronchial epithelial cells, and in vitro studies indicate that lung microsomes can metabolize and covalently bind ^{14}C-bromobenzene to proteins. However, preliminary attempts to detect metabolism of bromobenzene in segments of the main bronchi of rabbits have been unsuccessful (unpublished results). Thus, it would be of considerable interest to determine whether a bromobenzene metabolite produced elsewhere in the lung is bound within the bronchial epithelial cells or whether the lesion in those cells results from their ability to produce a toxic metabolite. Similarly, it will be important to learn which pulmonary cells metabolize carcinogenic substances known to produce lung tumors in animals and man.

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