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PULMONARY MICROSOMAL METABOLISM OF BENZO[a]PYRENE FOLLOWING EXPOSURE OF RATS TO SILICA

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Because some evidence suggests that there may be an increased incidence of lung cancer in silicosis and because previous studies have shown that exposure of rats to silica alters the pulmonary cytochrome P-450 system, we studied the effects of exposing rats to silica on the lung microsomal metabolism of benzo[a]pyrene (BaP). Rats were exposed to silica by intratracheal administration, lung microsomes were obtained 2 wk later from untreated and silica-treated animals, and the amounts of microsomal tissue and metabolites formed during the in vitro microsomal metabolism of BaP were measured. When the formation of BaP metabolites in equal amounts of lung microsomal tissue from the 2 treatment groups is compared, 3-OH BaP, BaP 4,5-diol, and BaP 9,10-diol are reduced by 45–70%, but the formation of BaP 7,8-diol or the BaP-quinones is not significantly altered following exposure to silica. In fact, the ratio of the BaP diols and BaP quinones, potentially toxic metabolites, to the relatively nontoxic 3-OH BaP produced by equal amounts of lung microsomal tissue is increased more than threefold following exposure of rats to silica. Since exposure of rats to silica leads to increased levels of lung microsomal protein, the amounts of BaP metabolites that could be produced by all microsomal tissue in the lungs were calculated. In silica-treated animals, the calculated total lung production of 3-OH BaP, BaP 4,5-diol, and BaP 9,10-diol tends to be increased by 1.2- to 2.0-fold, but BaP 7,8-diol and the BaP quinones are increased by 3.5-fold. These results demonstrate that exposure of rats to silica may alter the capacity of the lungs to metabolize benzo[a]pyrene, and the greatest effect seems to be enhanced accumulation of BaP 7,8-diol and the BaP quinones.

Inhalation of crystalline silica in various forms may lead to silicosis, an important occupational lung disease. Although silicosis is best known as a chronic inflammatory and fibrotic lung disease, there is some evidence that suggests a possible link between this disease and lung cancer. Epidemiological studies in this regard have lead to con-

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flicting conclusions. For example, Goldsmith et al. (1982) have summarized some data that support a relationship between silicosis and lung cancer, but Heppleston (1985) and McDonald (1989) have done epidemiological studies to suggest that the evidence for such a relationship is not conclusive. Experiments performed with animals have shown that silica may be a carcinogen or a cocarcinogen (Stenbäck & Rowland, 1979; Wagner et al., 1980). Although these studies are certainly not conclusive, the International Agency for Research on Cancer (1987) has recommended that there is sufficient evidence from animal research and limited human evidence for an association between silica and lung cancer.

There are several different mechanisms by which exposure to silica may increase the incidence of lung cancer. One possible mechanism is that inhalation of silica may alter the activity of the pulmonary cytochrome P-450 system. This system is located in the endoplasmic reticulum of some lung cells and is involved in the metabolism of lipid-soluble foreign compounds that enter the lungs through the circulation or via inhalation (Philpot & Smith, 1984; Guengerich, 1988, 1991). In general, the foreign compounds are converted to more water-soluble metabolites, which are more readily excreted in the urine. However, metabolism may also result in the formation of toxic or carcinogenic products. In this regard, one of the most important foreign compounds to be metabolized in lungs is benzo[a]pyrene (BaP), a ubiquitous pollutant present in emissions from combustion of tobacco products and fossil fuels (Phillips, 1983). Benzo[a]pyrene is known to be involved in the induction of cancer, and the lungs appear to be the most susceptible tissue. During the metabolism of BaP by the cytochrome P-450 system, several different metabolites and intermediate metabolites are produced. Some of these compounds have been identified as ultimate carcinogens and/or mutagens (Phillips, 1983; Depierre & Ernster, 1978). Therefore, alterations in the pulmonary cytochrome P-450 mediated metabolism of BaP may be a factor in susceptibility to BaP-induced carcinogenesis.

Recently, we reported that intratracheal administration of silica to rats increases the total lung content of cytochrome P-450 and activity of NADPH cytochrome P-450 reductase, two of the major components of the cytochrome P-450 system (Miles et al., 1993). Furthermore, the metabolism of two foreign compounds, 7-ethoxycoumarin and 7-ethoxyresorufin, is enhanced following exposure to silica, suggesting that similar effects may occur with other xenobiotics. Therefore, the objective of our current study was to investigate the effects of administration of silica to rats on the pulmonary microsomal metabolism of benzo[a]pyrene. Rats were exposed to silica via intratracheal instillation. Two weeks later, the animals were sacrificed and microsomes were prepared from the lungs. Comparisons between tissue from silica-

treated and untreated animals were made by measuring (1) the amounts of microsomal tissue obtained from the lungs and (2) the amounts of various metabolites formed following the in vitro pulmonary microsomal metabolism of benzo[a]pyrene.

METHODS

Treatment of Animals and Preparation of Lung Microsomes

The treatment of animals with silica and preparation of lung microsomes were done as we reported previously (Miles et al., 1993). The silica (Min-U-Sil; <5 μm in diameter; Pennsylvania Glass Sand Corp., Pittsburgh, PA) was boiled in 1.0 *M* HCl to remove contaminating Fe_2O_3, washed extensively with water, dried in an oven at 110°C, and then sterilized by heating at 200°C for at least 2 h (Dethloff et al., 1986). Suspensions of silica in sterile saline were sonicated just prior to administration. Male Sprague-Dawley rats (200–250 g; Hilltop Labs, Scotsdale, PA) were lightly anesthetized with sodium methohexital (35 mg/kg body weight) and placed in a vertical position. A curved 18-gauge cannula with a ball on the end was then inserted into the trachea and positioned just above the tracheal bifurcation. Silica-treated rats received a dose of 20 mg in 0.5 ml sterile saline through the cannula, and untreated animals received 0.5 ml sterile saline only. The rats were returned to their cages and sacrificed 2 wk later. The animals were anesthetized with sodium pentobarbital (150 mg/kg body weight), the heart and lungs were removed, and the lungs were perfused with 0.16 *M* NaCl to remove blood. The lungs and trachea were then trimmed free of the heart, weighed, and saved for preparation of microsomes.

In order to obtain the pulmonary microsomal fraction from untreated and silica-treated animals, the trachea and connective tissue were first removed from the lungs. The tissue was then minced by chopping it 4 times with a McIlwain tissue chopper (Mickle Engineering Co., Gomshall, Surrey, UK) set at a slice thickness of 5 mm, and the mince was resuspended in ice-cold incubation medium (145 m*M* KCl, 30 m*M* Tris-HCl, 1.9 m*M* KH_2PO_4, 8.1 m*M* K_2HPO_4, and 3 m*M* $MgCl_2$, pH 7.4). A 25% (w/v) tissue homogenate was prepared with a Teflon–glass Potter-Elvehjem homogenizer by using 16 complete passes with the pestle as described by Hook et al. (1972). The homogenate was then subjected to differential centrifugation (0–4°C) to obtain the microsomal fraction. Debris and cell nuclei were removed by centrifugation at 1000 × g for 10 min in a refrigerated centrifuge (model RC2-B, Ivan Sorvall Co., Norwalk, CT). Mitochondria were removed by slow sedimentation with 3 sequential centrifugations for 20 min each at 3000, 10,000, and 15,000 × g to minimize contamination of the

microsomes with mitochondria. The microsomal fraction was obtained by centrifugation of the postmitochondrial supernatant at 105,000 × g for 75 min in an ultracentrifuge (model LS-50, Beckman Instruments, Palo Alto, CA). The microsomal pellets were resuspended in buffer, frozen quickly with liquid nitrogen, and stored at –80°C until used in experiments. Protein content was measured by the method of Lowry et al. (1951), and the microsomal fractions were shown to be free of mitochondrial contamination by the absence of succinate cytochrome *c* reductase activity (Singer & Kearney, 1957).

Measurements of Benzo[a]pyrene Metabolism

The in vitro metabolism of benzo[a]pyrene (BaP) in lung microsomes from untreated and silica-treated animals was measured by using two different techniques, that is, a fluorescence method and high-performance liquid chromatography (HPLC). The fluorescence technique, which measures BaP hydroxylase activity, provides a measure of the production of the two hydroxylated products, 3-OH BaP, the major BaP metabolite, and 9-OH BaP. The method we used was described previously by Nebert and Gelboin (1968). Each reaction mixture (final volume of 5.5 ml) contained lung microsomes from untreated or silica-treated rats (100 µg microsomal protein/ml), bovine serum albumin (0.5 mg/ml), and NADPH (0.4 mg/ml). The reaction was initiated by adding BaP (final concentration = 100 µ*M*) in 55 µl of methanol. Incubations were done at 37°C under air. One-milliliter samples were removed from the incubation mixture for analysis at 0, 5, 10, 20, and 30 min. The samples were added to 1 ml ice-cold acetone to stop the reaction. Metabolites were then extracted in 3.25 ml hexane, and 1 ml of the organic phase was extracted in 3 ml NaOH (1 *M*). The metabolites in the base-extractable fraction were determined by measuring fluorescence with a model LS-50 luminescence spectrophotometer (Perkin-Elmer Corp., Norwalk, CT) set at an excitation wavelength of 396 nm and an emission wavelength of 522 nm. Since these measurements were made primarily to determine time courses and not to quantitate the amounts of metabolites, which is better done with HPLC, the results were expressed in fluorescence units.

The production of several BaP metabolites, including BaP phenols, BaP dihydrodiols, and BaP quinones, was measured by using HPLC analysis. For these experiments, the reaction mixture (final volume = 5 ml) contained lung microsomes from untreated or silica-treated animals suspended in incubation medium (1.5 mg protein/ml), bovine serum albumin (0.5 mg/ml), and NADPH (0.83 mg/ml). The reactions were initiated by the addition of BaP (final concentration = 100 µ*M*) and 6 µCi of [7,10-^{14}C]benzo[a]pyrene (specific activity = 59 mCi/mmol; Amersham Corp., Arlington Heights, IL) in 50 µl methanol. Incubations were carried out under air at 37°C for 30 min, a time during which

production of metabolites was linear. In each experiment, a zero-time control was done. The reactions were stopped by the addition of 2.5 ml ice-cold acetone (HPLC grade). Reaction mixtures were then extracted twice with 5 ml HPLC-grade ethyl acetate each time. $MgSO_4$ (250 mg) was then added to the ethyl acetate extract and the extract was evaporated under nitrogen to dryness. The samples were resuspended in 1–3 ml HPLC-grade methanol, filtered through a 0.45-μm Metricel membrane filter, and again evaporated to dryness under nitrogen. Finally, samples were reconstituted in 170 μl HPLC-grade methanol for subsequent HPLC analyses.

The separation of BaP metabolites was carried out by using an HPLC system obtained from Shimadzu Scientific Instruments, Inc. (Columbia, MD). The system was equipped with two model LC-600 pumps, an automatic sample injector (model SIL-6B), and a spectrophotometric detector (ultraviolet-visible, UV-VIS; SPD-6AV). Control of the system was by a Shimadzu Diamond Scan computer with Microsoft Windows 3.0 software (Microsoft Corp., Redmond, WA). Compounds were separated with a μBondapak C_{18} column (3.9 × 300 mm; particle size = 10 μm; pore size = 125 Å; Waters Associates, Milford, MA) by injecting a sample volume of 150 μl and using a 40-min linear gradient of 60–100% methanol : water. The solvent flow rate was 1 ml/min and the system was operated at room temperature. Detection of the metabolites was achieved by monitoring radioactivity. The eluate leaving the UV detector of the HPLC system entered a β-RAM flow-through monitor (In/Us Systems, Inc., Tampa, FL). The β-RAM liquid scintillation detector system was equipped with a cell for scintillation counting (volume = 1.8 ml) and a module for adding scintillation fluid to the eluate. This system was controlled by a computer equipped with Scintflow-Scintco software (In/Us). Scintillation cocktail (In-Flow 3; In/Us) was added so that the final ratio of fluid entering the detector was 3 : 1 (scintillation fluid : eluate). The radioactivity was obtained for the BaP peak and each metabolite peak from computer integration of the peak areas (cpm). The amounts of each BaP metabolite formed were calculated from the cpm of the BaP peak obtained from the sample prior to incubation. The peak for BaP and each metabolite was identified from retention times obtained from spectrophotometric analyses (254 nm) of nine authentic standards. The standards include BaP 9,10-diol, BaP 7,8-diol, BaP 4,5-diol, 3-OH BaP, 9-OH BaP, BaP 1,6-dione, BaP 3,6-dione, BaP 6,12-dione, and BaP (NCI Chemical Carcinogen Respository, Midwest Research Institute, Kansas City, MO).

Statistical Analyses

For all comparisons, we determined that the variances of the two populations were equal by using the *F*-test. Therefore, all comparisons

of statistical signifiance were made by using the unpaired Student's *t*-test. A value of $p < .05$ was taken as the limit to indicate significance.

RESULTS

Microsomal Protein Levels, Lung Weights, and Body Weights

In a previous study (Miles et al., 1993), we found that exposure of rats to silica alters the amount of microsomal tissue which can be obtained from the lungs. Because the total amounts of benzo[a]pyrene metabolites produced in lungs are related to microsomal tissue levels, these measurements were repeated in our current study (Table 1). Although there is no difference in body weights following exposure of the rats to silica, there is a greater than twofold increase in lung weights, confirming our earlier studies (Miles et al., 1993). If the microsomal protein is expressed as milligrams per gram lung weight, there is a 1.6-fold increase in the concentration of microsomal tissue in lungs from silica-treated animals. Furthermore, if lung weights are used to calculate the total amount of microsomal protein that can be obtained from the lungs, 3.6-fold more microsomal tissue can be obtained from lungs of silica-treated animals than from untreated animals. Since exposure of rats to silica leads to increases in microsomal tissue, these results must be taken into account when determining the amounts of metabolites produced during the in vitro lung microsomal metabolism of benzo[a]pyrene.

Benzo[a]pyrene Hydroxylase Activity

Experiments were carried out to measure the production of hydroxylated metabolites of BaP during its in vitro metabolism by equal amounts of microsomes from lungs of untreated and silica-treated rats. The results are shown in Figure 1. The production of these

TABLE 1. Lung weights, body weights, and pulmonary microsomal protein levels in untreated and silica-treated animals

Measurement (units)	Untreated animals	Silica-treated animals
Body weight (g)	319 (±4)	312 (±5)
Lung weight (g)	1.73 (±0.03)	3.86 (±0.15)[b]
Microsomal protein (mg/g lung)	2.19 (±0.12)	3.62 (±0.23)[b]
Microsomal protein (mg/lungs)[a]	3.85 (±0.21)	13.87 (±0.94)[b]

Note. Values are means ± SEM from seven different animals in each treatment group.

[a]Total microsomal protein obtained from the lungs. Each value was obtained by multiplying (mg microsomal protein/g lung) × lung weight.

[b]Significantly different from untreated animals ($p < .05$).

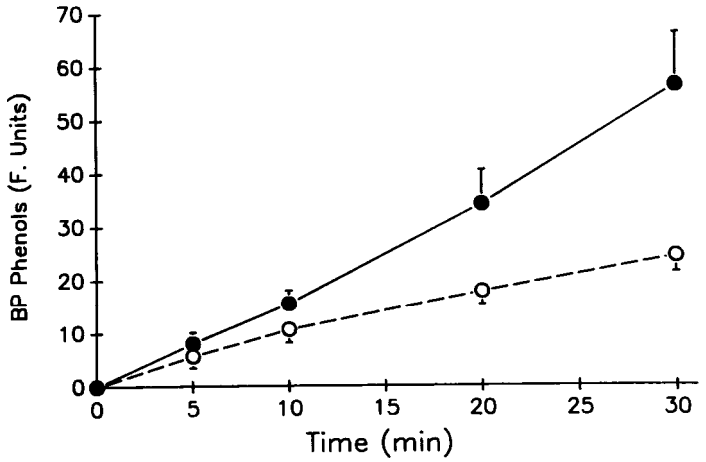

FIGURE 1. Time courses for the formation of BaP phenols during the in vitro metabolism of BaP by equal amounts of lung microsomes from untreated (solid circles) and silica-treated (open circles) rats. The animals were given silica (20 mg in 0.5 ml saline) or saline alone (untreated group) by intratracheal administration, and lung microsomes were obtained 2 wk later. Equal amounts of microsomal suspensions from each treatment group (100 μg protein/ml) were incubated at 37°C in medium containing BSA (0.5 mg/ml) and NADPH (0.4 mg/ml). Reactions were initiated by addition of BaP (final concentration = 100 μ*M*) in methanol. At various times, samples were taken and extractions performed as described in the Methods. The BaP phenols were measured with a fluorimetric method, and the results are expressed in fluorescence units. Points are mean values ± SEM for five different animals in each treatment group.

metabolites is approximately linear for 30 min, a result that was consistent for production of all BaP metabolites that we measured. The rate of production of hydroxylated metabolites is approximately 50% less in lung microsomes from silica-treated animals than in an equal amount of microsomes from untreated animals. These experiments were performed as an initial screening to determine if exposure to silica alters BaP metabolism. A more complete assessment of BaP metabolism was therefore undertaken.

Measurement of Other Benzo[a]pyrene Metabolites

The formation of several different compounds produced during the in vitro metabolism of BaP was measured by HPLC analysis. The results obtained from measurements using equal amounts of lung microsomes from untreated and silica-treated animals are shown in Table 2. It should be pointed out that the distribution of BaP metabolites we measured in microsomes from untreated animals is similar to that reported previously by Prough et al. (1979) for rat lung microsomes. There are significant reductions in the amounts of two BaP metabolites, BaP 4,5-diol (reduced by 60%) and 3-OH BaP (reduced by 70%), formed by lung microsomes from silica-treated rats. The production of BaP 9,10-diol is also decreased by about 45%, but this

TABLE 2. Formation of benzo[a]pyrene (BaP) metabolites by equal amounts of lung microsomes from untreated and silica-treated animals

Metabolite	Untreated animals	Silica-treated animals
BaP 9,10-diol	11.9 (±4.3)	6.6 (±2.1)
BaP 4,5-diol	22.5 (±3.0)	9.6 (±2.0)[a]
BaP 7,8-diol	11.6 (±3.0)	9.6 (±2.4)
BaP quinones	120.4 (±13.4)	146.2 (±11.9)
3-OH BaP	204.0 (±24.5)	62.6 (±5.4)[a]

Note. Values are expressed as pmol metabolite formed/mg microsomal protein in 30 min. The values shown are means ± SEM from seven different animals in each treatment group.

[a]Significantly different from untreated animals ($p < .05$).

difference is not statistically significant. On the other hand, the amounts of the three BaP quinones, which we measured together, are not significantly altered, although the tendency is for them to be increased in silica-treated microsomes. Exposure to silica does not seem to cause much difference in the production of BaP 7,8-diol. Thus, a comparison of measurements made with equal amounts of lung microsomes from untreated and silica-treated animals shows that the formation of 3-OH BaP, BaP 4,5-diol, and BaP 9,10-diol are reduced while the levels of the BaP quinones and BaP 7,8-diol are not significantly altered following exposure to silica.

These results obtained with equal amounts of microsomal tissue suggest that the metabolism of BaP is modified in a differential manner following exposure to silica. It appears as though the production of the relatively nontoxic 3-OH BaP is reduced while the formation of the potentially toxic BaP diols and BaP quinones is not affected as much. Therefore, we expressed our results as the ratio of BaP diols and BaP quinones to 3-OH BaP. The results, which are shown in Figure 2, demonstrate that exposure to silica leads to a greater than threefold increase in this ratio. Therefore, these results suggest that silica exposure may lead to a redistribution of BaP metabolites such that there is an increase in the potential for accumulation of the more toxic metabolites.

Because exposure of rats to silica leads to an increase in the level of microsomal tissue in the lungs, the total amounts of metabolites that could be formed during the metabolism of BaP by all microsomal tissue in the lungs were calculated. This is done by multiplying the amount of metabolite formed per milligram microsomal protein by the total amount of microsomal protein in the lungs. The results are shown in Table 3. The total amounts of BaP 9,10-diol, BaP 4,5-diol, and 3-OH BaP tend to be increased by 1.2- to 2.0-fold in

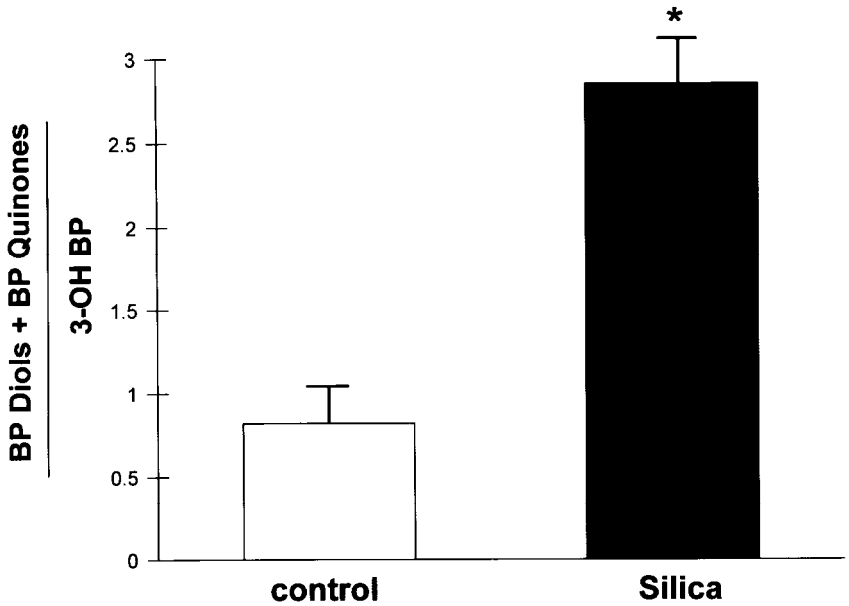

FIGURE 2. Effects of exposure to silica on the ratio of BaP diols and BaP quinones to 3-OH BaP produced during the in vitro metabolism of BaP. Animals were given silica (20 mg in 0.5 ml saline) or saline alone (control) by intratracheal administration, and lung microsomes were obtained 2 wk later. Equal amounts of lung microsomal suspensions (1.5 mg protein/ml) from each treatment group were incubated with BSA (0.5 mg/ml) and NADPH (0.83 mg/ml). The reactions were initiated by the addition of BaP (final concentration = 100 μ*M*) and [^{14}C]BaP. Metabolites of BaP were isolated by HPLC analysis as described in the Methods. The results shown here are mean values ± SE for seven different animals in each treatment group. Asterisk indicates values are significantly different from control animals ($p < .05$).

TABLE 3. Benzo[a]pyrene (BaP) metabolites formed by all lung microsomes obtained from untreated and silica-treated animals

Metabolite	Untreated animals	Silica-treated animals
BaP 9,10-diol	49 (±17)	98 (±35)
BaP 4,5-diol	88 (±13)	132 (±35)
BaP 7,8-diol	47 (±18)	163 (±38)[a]
BaP quinones	479 (±67)	1654 (±270)[a]
3-OH BaP	789 (±108)	967 (±186)

Note. Values are expressed as pmol metabolite formed in 30 min by all microsomes obtained from the lungs of untreated and silica-treated animals. The values shown are means ± SEM from seven different animals in each treatment group.

[a]Significantly different from untreated animals ($p < .05$).

lungs from silica-treated animals, although none of these differences is significant. The major effect of exposing animals to silica is a 3.5-fold increase in the production of both BaP 7,8-diol and the BaP quinones. It is not possible to separate the individual quinones to the point that each can be accurately measured with HPLC analysis. However, it does appear that BaP 6,12 quinone is the quinone that is most consistently increased in lungs from silica-treated animals (data not shown). These results suggest that following exposure of rats to silica, the potential exists for the total lung production of increased amounts of some benzo[a]pyrene metabolites, especially BaP 7,8-diol and the BaP quinones.

DISCUSSION

The results of these experiments demonstrate that exposure of rats to silica leads to alterations in the pulmonary microsomal metabolism of benzo[a]pyrene (BaP). These changes are evident, even when the data are viewed from different perspectives. If the production of BaP metabolites by equal amounts of lung microsomal protein from control and silica-treated rats is compared, there is an increase in the ratio of the BaP diols and BaP quinones, potentially toxic BaP metabolites, to 3-OH BaP, a relatively nontoxic metabolite. On the other hand, if you consider the total number of BaP metabolites that can be produced by all lung microsomal tissue, the major effects are 3.5-fold increases in the amounts of BaP 7,8-diol and the BaP quinones produced by lungs from silica-exposed animals. This latter effect is due to a combination of silica effects on the cytochrome P-450 system and on the total amounts of lung microsomal tissue. In either case, exposure to silica appears to lead to an increase in the accumulation of potentially toxic BaP metabolites, BaP 7,8-diol and the BaP quinones.

Although our results indicate that the pulmonary microsomal metabolism of benzo[a]pyrene may be altered following exposure of rats to silica, it is not yet known if these alterations are involved in increasing susceptibility to lung cancer. It is known that BaP is present in tobacco smoke (Phillips, 1983) and that there may be an increased incidence of lung cancer in silicotics who have smoked cigarettes (Goldsmith & Guidotti, 1986). In this regard, it is known that chemical carcinogens, such as BaP, are converted by metabolism into electrophilic substances that react with DNA as a probable precursor to tumor formation. The metabolism of BaP by the cytochrome P-450 system and its relationship with cancer has been reviewed by Phillips (1983) and Cohen (1990). In our experiments, the major effect of exposure to silica on lung BaP metabolism is a greatly enhanced total lung microsomal production of BaP 7,8-diol and the BaP quinones. The toxicity of BaP 7,8-diol has been studied extensively. During the

microsomal metabolism of BaP, BaP 7,8-diol is formed via the action of microsomal enzymes, including the cytochrome P-450 system and epoxide hydrolase (Gelboin, 1980). BaP 7,8-diol is further metabolized by mixed-function oxidases to 7,8-dihydro-7,8-dihydroxybenzo[a]pyrene-9,10-oxide (BaP 7,8-diol 9,10-oxide). It is this diol epoxide that binds to DNA when intact tissues are exposed to BaP (Borgen et al., 1973; Sims et al., 1974). Thus, these studies suggest that BaP 7,8-diol is the proximate carcinogen and that BaP 7,8-diol 9,10-oxide is the ultimate carcinogen. Our studies suggest that exposure to silica may lead to an increased ability of the lungs to produce the 7,8-diol from BaP.

We have previously studied the effects of exposing rats to silica on the pulmonary cytochrome P-450 system (Miles et al., 1993) and found that there is a differential effect on two cytochrome P-450 isozymes. Cytochrome P-4502B1 levels are reduced by 50% in comparisons of equal amounts of microsomal tissue and increased by 1.5- to 2.5-fold in comparisons of total lung microsomal tissue following exposure of animals to silica. On the other hand, cytochrome P-4501A1 levels are not altered in comparisons of equal amounts of lung tissue but are increased by almost fourfold in comparisons of total amounts of lung microsomal tissue following exposure to silica. The results from our current study suggest that exposure to silica may have effects on BaP metabolism that are similar to its effects on cytochrome P-450 isozymes. For example, the effects of silica on the formation of 3-OH BaP, BaP 4,5-diol, and BaP 9,10-diol are similar to effects on cytochrome P-4502B1, and the effects of silica on the production of BaP 7,8-diol and the BaP quinones are similar to its effects on cytochrome P-4501A1. To the best of our knowledge, the isozymes responsible for the formation of different BaP metabolites in rat lungs are not known. However, it has been reported that both cytochromes P-4502B1 and P-4501A1 are responsible for BaP metabolism in rabbit lungs (Devereux et al., 1989; Domin & Philpot, 1986) and that the formation of BaP 7,8-diol in these tissues is due primarily to cytochrome P-4501A1 (Cheung et al., 1984).

One metabolite of benzo[a]pyrene, 9-OH BaP, has been reported to be formed by lung microsomal metabolism in rats, hamsters, and humans (Prough et al., 1979). In our experiments, we were not able to measure production of 9-OH BaP. This is probably due to the use of methanol as the organic solvent vehicle to deliver BaP to the microsomes. In a previous study (Kontir et al., 1986), we demonstrated that various organic solvent vehicles have differential effects on the formation of BaP metabolites by rabbit lung microsomes. When rat lung microsomes are used, there are relatively more BaP diols and quinones formed, but no 9-OH BaP is produced when methanol is used as the vehicle. Conversely, when dimethyl sulfoxide (DMSO) is used as the vehicle, 9-OH BaP is formed, but less of the diols and

quinones are produced. Methanol was used in our experiments because we were particularly interested in measuring the benzo[a]-pyrene quinones and diols.

The increase in total lung microsomal protein levels following exposure to silica suggests an increase in the number and/or size of some types of cells. However, the identities of these cell types are not known. One possibility is that greater numbers of inflammatory cells or alveolar macrophages are responsible for the increased microsomal tissue levels. Driscoll et al. (1990) investigated the effects of intratracheal instillations of silica on pulmonary recruitment of inflammatory cells and alveolar macrophages in rat lungs. Calculations based on their data suggest that these cell types do not account for much of the silica-induced increase in microsomal protein seen in our studies. Another possibility is that the hypertrophy and hyperplasia of alveolar type II cells, reported by Miller et al. (1987), is responsible for the increased microsomal levels. It is well known that these cells contain more cytochrome P-450 than all other lung cells, except for Clara cells (Devereux et al., 1989). In this regard, we are doing experiments using immunocytochemistry in an attempt to localize cellular changes in cytochrome P-450 isozyme levels in the lungs.

The objective of our current study was to determine the effects of exposing rats to silica on the pulmonary microsomal metabolism of BaP. There are at least two potential criticisms of our approach. First, we used intratracheal instillations of silica rather than inhalation, which represents a more physiologically relevant means of exposure. Second, we used a single high dose of silica and sacrificed the animals after 2 wk rather than doing a low chronic exposure. Undoubtedly, the latter is more like human exposures that result in silicosis. In this regard, experiments are now being performed in our laboratory in which rats are exposed to several different doses of silica via inhalation for much longer periods of time. Although some of these potential criticisms are valid and results obtained from inhalation exposures may prove to be valuable, the results from our previous work (Miles et al., 1993) and current study should not be underestimated. These results do suggest that exposure to silica has the ability to alter the pulmonary microsomal cytochrome P-450 system in a manner that may lead to the formation of increased amounts of BaP 7,8-diol, a proximate carcinogen.

Finally, in our experiments, we measured only the pulmonary metabolism of BaP. No attempts were made to measure BaP metabolism in other organs. For example, hepatic metabolism may play a major role in the breakdown of this xenobiotic. Therefore, it is not possible to know from our experiments how exposure to silica affects the pharmacokinetics of BaP in vivo. However, our results clearly demonstrate that exposure of rats to silica affects the ability of the

lungs to metabolize BaP, and this may be important, given the possible link between silicosis and lung cancer.

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