Pergamon

*Biochemical Pharmacology*, Vol. 49, No. 12, pp. 1721–1727, 1995.

0006-2952/95 $9.50 + 0.00

0006-2952(95)00012-7

# COMMENTARY

# METABOLIC ACTIVATION AND TOXICITY OF SOME CHEMICAL AGENTS TO LUNG TISSUE AND CELLS

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*Key words:* lung toxicity; covalent binding; metabolic activation; chemically reactive metabolites; benzo[*a*]pyrene; 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK); 2-(2-chlorophenyl)-2-(4-chlorophenyl)-1,1-dichloroethane (o,p'-DDD)

In a recent review including over 300 literature citations, Hinson and Roberts [1] wrote: "*Although covalent binding of reactive metabolites to protein correlates with the development of many drug-induced toxicities, the actual sequence of events leading to the development of the toxicities is unknown. Moreover, with many drugs, the reactive metabolite that may covalently bind to protein may also produce oxygen metabolites that may produce the toxicity*" (emphasis added). Similarly, Boyd *et al.* [2] wrote: "*Still relatively little is known about the events intervening between the formation of reactive metabolites and the expression of their adverse biological effects*" (emphasis added). Thus, with 4-ipomeanol-induced Clara cell necrosis and lung damage, the presence of drug-derived covalently bound radiolabel correlates very well with Clara cell destruction, whereas in the case of a drug such as cyclophosphamide [3], whose pneumotoxicity is evoked in large part via reactive oxygen metabolites (superoxide anion radial, $O_2^{\cdot-}$; hydroxyl radical, $^{\cdot}OH$) that have a biological $T_{1/2}$ in the microsecond to nanosecond range, toxicity is observed after all traces of the drug or oxygen radicals are eliminated from the organism. It has been suggested [2] that the mechanisms by which highly reactive metabolic intermediates can initiate organ toxicity are:

1. Covalent binding to structural, catalytic or informational macromolecules including proteins, nucleic acids, and lipids.
2. Generation of $^1O_2$ (singlet oxygen), $H_2O_2$, $^{\cdot}OH$ or other activated oxygen species, which may themselves be toxic.
3. Stimulation of peroxidative decomposition (structure and function) of cellular lipids.

Covalent binding of xenobiotics in lung and its relation to pulmonary toxicities have been demonstrated for PAH†, naphthalenes, furans, 3-methylindole, butylated hydroxytoluene, $CCl_4$, 1,1-dichloroethylene, *O,O,S*-trimethylphosphorothioate, and α-naphthylthiourea and have been reviewed elsewhere [4]. However, it would be dangerously adventurous to propose that covalent binding is a *sine qua non* of pneumotoxicants, since it is well known that substances like paraquat and cyclophosphamide are not significantly covalently bound in lung.

The focus of this commentary will be the metabolic activation of certain xenobiotics in lung, or less commonly, in extrapulmonary tissues, to chemically reactive metabolites that bind and elicit toxicity in mammalian lung [4, 5]. Unlike paraquat, where death is commonly an endpoint, or $CCl_4$ in which serious/lethal compromises in liver function are readily monitored, "toxicity" in this context means necrosis of one or more specific pulmonary cell types that often do not deleteriously influence organ function or animal survival. On the other hand, pulmonary carcinogens may induce adenomas or adenocarcinomas that may be lethal to the host organism.

## *Activation of BP to carcinogenic metabolites in mouse lung*

The basic hypothesis of chemical carcinogenesis is that formation of a covalent bond between a chemical and DNA, RNA, or protein represents the essential first step in the tumor initiation process [6]. Most chemicals require metabolic activation to bind covalently to cellular macromolecules, and the ultimate reactive species, which are electrophilic in character, react with nucleophilic groups of cellular macromolecules.

Among the earliest clear demonstrations of the metabolic activation of a xenobiotic to a chemically reactive intermediate was that by Miller [7] who applied [$^3$H]BP to mouse skin and found covalent binding of its metabolites to skin proteins. Similarly, Brookes and Lawley [8] painted [$^3$H]BP onto the shaved skin of mice and recovered the radiolabel covalently bound to DNA. Later, Grover and Sims [9] reported that rat liver microsomes incubated with exogenous DNA, NADPH and [$^3$H]BP resulted in

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† Abbreviatioins: PAH, polycyclic aromatic hydrocarbons; BP, benzo[*a*]pyrene; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN, *N*-nitrosonornicotine; NAT, *N'*-nitrosoanatabine; $O^6$MG, $O^6$-methylguanine; $O^6$MGMT, $O^6$-methylguanine-DNA methyltransferase; NDMA, nitrosodimethylamine; PEITC, phenylethyl isothiocyanate; and o,p'-DDD, 2-(2-chlorophenyl)-2-(4-chlorophenyl)-1,1-dichloroethane.

Table 1. Pulmonary adenomas in newborn mice treated with benzo[*a*]pyrene (BP) and some of its metabolites

| Compounds | Number of mice with pulmonary adenomas | Number of adenomas | Adenomas/mouse |
|---|---|---|---|
| Control (DMSO) | 8 | 9 | 0.13 |
| BP | 12 | 15 | 0.24 |
| BP 7,8-dihydrodiol | 41 | 110 | 1.77 |
| BP diol epoxide 1 | 2 | 3 | 0.14 |
| BP diol epoxide 2 | 55 | 283 | 4.42 |
| BP tetraols | 1 | 1 | 0.05 |

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covalent binding of radioactivity to DNA. Gelboin [10] confirmed this finding and further reported that pretreatment of rats with 3-methylcholanthrene increased the binding of [$^3$H]BP to DNA by 2- to 4-fold. He also reported that although metabolic activation of [$^3$H]BP was required, the metabolites of BP were relatively stable so that the same level of DNA-bound radioactivity was found whether the DNA was added before or after the incubation. Purification of the DNA in CsCl gradients revealed that the [$^3$H]BP-derived radioactivity was, in fact, bound covalently to DNA.

As BP is a large molecule, the efforts of many workers focussed on the chemical nature of the ultimate carcinogenic species. Kapitulnik *et al.* [11] studied the pulmonary tumorigenic activities of BP, (±)-*trans*-7$\beta$,8$\alpha$-dihydroxy-9$\beta$,10$\beta$-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (diol epoxide 1), (±)-*trans*-7$\beta$,8$\alpha$-dihydroxy-9$\alpha$,10$\alpha$-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (diol epoxide 2), (±)-*trans*-7,8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene (BP 7,8-dihydrodiol), and the tetraols derived from the hydrolysis of diol epoxide 2 in newborn Swiss-Webster mice. Animals were injected i.p. with 4 nmol of each compound on day 1 of life, 8 nmol on day 8 and 16 nmol on day 15, and were killed at 28 weeks of age. Diol epoxide 1 was highly toxic in newborn mice, and most of the animals treated with this compound died before weaning (25 days). Histologic examination failed to reveal the cause of death. Diol epoxide 2 and BP 7,8-dihydrodiol were approximately 10–20 times more active than BP in causing pulmonary adenomas. The tetraols derived from diol epoxide 2 did not induce pulmonary adenomas (Table 1). These data show that BP diol epoxide 2 derived from BP 7,8-dihydrodiol is a highly active pulmonary carcinogen in newborn mice.

Since the studies of Kapitulnik *et al.* [11] were conducted with optical racemates, the next logical step was to study the tumorigenicity of the pure stereoisomers. This study was conducted by Buening *et al.* [12]. BP and each of the enantiomers of the diastereoisomeric BP 7,8-diol-9,10-epoxides derived from *trans*-7,8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene were tested by intraperitoneal administration to mice of 1, 2, and 4 nmol or 2, 4, and 8 nmol of each compound on days 1, 8 and 15 of life. The animals were killed at 34–37 weeks of age. (+)-7$\beta$,8$\alpha$-Dihydroxy-9$\alpha$,10$\alpha$-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (+ epoxide 2; Compound No. 6) had exceptional tumorigenicity, whereas BP and the other optical isomers had little or no activity (Table 2).

More recently, (+)diol epoxide 2 has been found [13] as a major adduct bound to DNA and RNA of bronchial explants after treatment of the cultures with BP. Also, (+)diol epoxide 2 is the major enantiomer formed stereoselectivity *in vivo* from BP [13]. It is concluded that (+)-BP-7$\beta$,8$\alpha$-diol-9$\alpha$,10$\alpha$ epoxide 2 is a major ultimate carcinogenic metabolite of BP in mice (Fig. 1).

It is noteworthy that Cavalieri and Rogan [14] have proposed an alternative mechanism to explain PAH carcinogenesis. They have contended that cytochrome P450 mediates binding of PAH to DNA by two pathways of activation. Monooxygenation to diol epoxides, discussed above, is a minor pathway, whereas one-electron oxidation to form radical cations is the major pathway of activation for the most potent carcinogenic PAH. They have proposed that for BP and other PAH, 80–100% of the DNA-adducts formed by rat liver microsomes or in mouse skin arise via the radical cations.

### *Pulmonary neoplasia associated with the tobacco-specific compound NNK*

In 1982, the Surgeon General of the United States reported that over 3000 specific chemical compounds, many of which are mutagenic or carcinogenic, had been identified and quantified in tobacco smoke [15]. Among these are several nitrosamines, one of which, NNK, has been studied extensively in animals.

Hoffmann *et al.* [16] reported a high incidence of pulmonary tumors associated with the administration of the tobacco-derived nitrosamines NNN and NNK but not NAT administered to rats three times weekly for 20 weeks at total doses of 1, 3, and 9 mmol/kg (Table 3). There was apparently a slight sex difference in lung tumor incidence, males being somewhat more susceptible than females. In addition, in male rats, there was no obvious dose–response relationship in lung tumors, at the doses employed. Although many of the lung tumors were benign adenomas, there were significant numbers of

Table 2. Pulmonary tumors in newborn mice treated with benzo[*a*]pyrene (BP) and stereoisomers of BP-7,8-diol-9,10 epoxides

| Compound number | Treatment | Total doses (nmol) | % Mice with tumors | Tumors/ mouse |
|---|---|---|---|---|
| 1 | Control (DMSO) | 0 | 11 | 0.12 |
| 2 | BP | 7 | 18 | 0.22 |
| 3 | (−)-BP-7$\beta$,8$\alpha$-diol 9$\beta$,10$\beta$ epoxide 1 | 7 | 13 | 0.15 |
| 4 | (+)-BP-7$\alpha$,8$\beta$-diol 9$\alpha$,10$\alpha$ epoxide 1 | 7 | 14 | 0.15 |
| 5 | (−)-BP-7$\alpha$,8$\beta$-diol 9$\beta$,10$\beta$ epoxide 2 | 7 | 9 | 0.09 |
| 6 | (+)-BP-7$\beta$,8$\alpha$-diol 9$\alpha$,10$\alpha$ epoxide 2 | 7 | 71 | 1.72 |
| 2 | BP | 14 | 14 | 0.15 |
| 3 | (−)-BP-7$\beta$,8$\alpha$-diol 9$\beta$,10$\beta$ epoxide 1 | 14 | 22 | 0.25 |
| 4 | (+)-BP-7$\alpha$,8$\beta$-diol 9$\alpha$,10$\alpha$ epoxide 1 | 14 | 15 | 0.34 |
| 5 | (−)-BP-7$\alpha$,8$\beta$-diol 9$\beta$,10$\beta$ epoxide 2 | 14 | 12 | 0.13 |
| 6 | (+)-BP-7$\beta$,8$\alpha$-diol 9$\alpha$,10$\alpha$ epoxide 2 | 14 | 100 | 7.67 |

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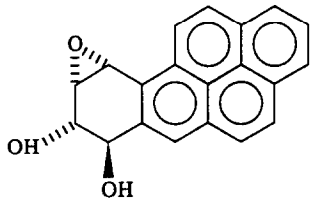


Fig. 1. (+)-Benzo[*a*]pyrene-7$\beta$,8$\alpha$-diol-9$\alpha$,10$\alpha$ epoxide 2, an ultimate carcinogenic metabolite of benzo[*a*]pyrene in mouse lung.

Table 3. Incidence of respiratory tract tumors in Fischer 344 rats receiving subcutaneous injections of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) for 20 weeks

| Dose | % Rats with nasal tumors | % Rats with lung tumors |
|---|---|---|
| 1 mmol/kg | | |
| Males | 74 | 85 |
| Females | 37 | 30 |
| 3 mmol/kg | | |
| Males | 87 | 87 |
| Females | 80 | 47 |
| 9 mmol/kg | | |
| Males | 93 | 93 |
| Females | 93 | 60 |

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adenocarcinomas and squamous cell carcinomas, especially in males.

NNK was administered daily [17] to Fischer 344 male rats for up to 12 days, which resulted in the accumulation and persistence of the promutagenic adduct O$^6$MG in the DNA of the lung. The persistence of this adduct correlated with the inhibition (>95%) of the repair enzyme O$^6$MGMT, which removes the methyl group from O$^6$MG in DNA. These studies demonstrated that although $O^4$-methyldeoxythymidine was also formed, it was removed rapidly from NNK-treated lungs, whereas there was accumulation and persistence of the promutagenic adduct O$^6$MG–DNA in lung during repeated exposure to NNK.

Table 4. Accumulation of $O^6$-methylguanine (O$^6$MG) in rat lung cell types following administration of NNK

| | O$^6$MG (pmol/$\mu$mol guanine) | Alkylation efficiency (O$^6$MG/dose) |
|---|---|---|
| Whole lung | 0.9 | 3.0 |
| Alveolar macrophages | 3.8 | 12.7 |
| Type II cells | 1.1 | 3.7 |
| Clara cells | 28.2 | 93.2 |

Male Fischer 344 rats were injected i.p. with 0.3 mg NNK/kg/day for 4 days and killed 4 hr after the last dose. Data excerpted from Ref. 19. Reprinted with permission from *Cancer Res* **47**: 1143–1148, 1987. Copyright (1987) American Association for Cancer Research, Inc., Philadelphia, PA.

Peterson and Hecht [18] similarly demonstrated a strong correlation ($r = 0.98$) between lung tumor yield and levels of O$^6$MG in A/J mouse lung. They suggested that the formation and persistence of O$^6$MG were critical events in the initiation of lung tumors by NNK. Belinsky *et al.* [19] administered NNK to male Fischer 344 rats in doses ranging from 0.1 to 100 mg/kg, i.p., daily for up to 12 days and found that the ratio of lung O$^6$MG/guanine, when plotted against dose of NNK, an index of alkylation efficiency, decreased as the dose increased. This observation suggested a low-capacity readily saturable uptake mechanism in whole lung. Separation of pulmonary cell types revealed, especially at lower

Table 5. Effect of NNK treatment (10 mg/kg/day for 4 days) on $O^6$-methylguanine-DNA methyltransferase ($O^6$MGMT) activity in lung cells of Fischer 344 rats

| | $O^6$MGMT activity (pmol $O^6$MG/μmol guanine) | |
|---|---|---|
| | Vehicle control | NNK |
| Alveolar macrophages | 31.1 | 28.2 |
| Type II cells | 32.5 | 6.0 |
| Clara cells | 15.2 | Not detectable |

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Table 6. Activation of NNK *in vitro* by pulmonary cell types

| Cell types from rat lung | Methylation index (pmol $O^6$MG/μmol guanine) |
|---|---|
| Macrophages | Not detectable (<0.2) |
| Type II cells | 2.9 |
| Clara cells | 21.7 |

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doses of NNK, a marked concentration of the $O^6$MG adduct in Clara cells (Table 4).

Thus, when corrected for cross-contamination of cell types, the calculated concentration of $O^6$MG in Clara cells was 19–25 times greater than in type II cells and 7.5 times greater than alveolar macrophages. The distribution of NNK within lung was determined in rats treated with 1 mg/kg [$^3$H]NNK and then killed 4 hr later. Autoradiograms revealed that NNK was much more concentrated in Clara cells than in other cell types in lung [19]. Treatment of rats with NNK produced a dose-dependent inhibition of $O^6$MGMT, and this inhibition correlated with $O^6$MG levels in lungs of animals treated with NNK.

Abnormally high steady-state levels of a xenobiotic in a cell type are generally considered to result from high influx, peculiar mechanisms for retention (e.g. covalent binding), or slow efflux. Excepting active or facilitated transport, influx is generally perfusion-related and might account for differences in xenobiotic accumulation between myocardium and bone. Well recognized are differences in efflux, which may reflect diffusion or metabolism (e.g. to glucuronides) and differences in metabolic rates of xenobiotics between cells and organs. Which of these factors account for the disparate distribution of NNK among cell types of the lung?

Male Fischer 344 rats were treated i.p. with NNK (10 mg/kg/day) for 4 days, and pulmonary cell types were purified [20]. One day after the last injection, concentrations of $O^6$MG (pmol $O^6$MG/μmol guanine) in alveolar macrophages were 70, in type II cells 27, and in Clara cells 95. Moreover, the loss of $O^6$MG over a period of 8 days differed markedly among pulmonary cell types. The disappearance of this adduct from DNA of alveolar macrophages was rapid ($T_{1/2}$ about 48 hr) and followed first-order kinetics. In contrast, very little loss of $O^6$MG was observed in Clara cells. The concentration of this adduct only decreased from 100 to 60 pmol $O^6$MG/μmol guanine over the 8-day period. Assuming first-order kinetics, this reflects a $T_{1/2}$ in Clara cells of about 10 days. The disappearance of $O^6$MG from DNA of type II cells was not linear. A rapid decline in adduct concentration was observed in type II cells for the first 3 days after discontinuing treatment (from 25 to 8 pmol $O^6$MG/μmol guanine) followed by a further decline of only 25% over the remaining 5 days. More importantly, as shown previously for whole lung [17], NNK treatment differentially affected $O^6$MGMT among cell types: it had no observable effect on $O^6$MGMT in alveolar macrophages, it reduced $O^6$MGMT activity in type II cells by 82%, and it abolished $O^6$MGMT activity in Clara cells (Table 5).

After a 4-day treatment of rats with NNK, the concentration of the promutagen $O^6$MG was greatest in Clara cells and was 1–10 times greater than in other pulmonary cell types. Thus, Clara cells accumulate and retain preferentially $O^6$MG relative to other pulmonary cell types. In addition, Clara cells clear $O^6$MG much slower than other cells, and this difference correlates well with the selective inhibition by NNK of $O^6$MGMT, the enzyme which converts $O^6$MG to guanine in DNA [20]. The accumulation and persistence of high concentrations of $O^6$MG in Clara cells may result, in part, from saturation, inhibition, or a selectively low rate of re-synthesis of $O^6$MGMT in this cell type during treatment with NNK. Moreover, the accumulation and persistence of high levels of $O^6$MG in Clara cells suggest that this cell type may be the progenitor cell for NNK-induced rat lung tumors.

A study was conducted on the comparative biochemical toxicology of NNK and NDMA in rat lung, since it had been reported [21] that NNK produced a 50% incidence of malignant lung tumors in rats, whereas treatment with equivalent doses of NDMA failed to induce any lung tumors. Male Fischer 344 rats were treated daily with a range of equimolar doses of NNK and NDMA for 4 days and killed 4 hr after the last dose [22]. Purification of pulmonary cell types revealed no cell selectivity for DNA methylation ($O^6$MG) with NDMA, whereas, at equimolar doses of NNK, the $O^6$MG content in Clara cells was about 40 times that of Clara cells from rats treated with NDMA. At lower doses, whole lung DNA methylation was about 4 times greater with NDMA than with NNK, but $O^6$MG content of Clara cells was 50 times higher with NNK than with NDMA. Similarly, pulmonary cells isolated from untreated rats and incubated *in vitro* with NNK revealed approximately 8-fold higher methylation in Clara cells as compared with type II cells (Table 6).

To evaluate the relation between $O^6$MG formation in Clara cells and pulmonary neoplasia [23], Fischer

Table 7. Relation between NNK dose and content of $O^6$MG in specific pulmonary cell types of male Fischer rats

| Dose (mg/kg) | $O^6$MG (pmol/μmol guanine) | | | |
|---|---|---|---|---|
| | Whole lung | Macrophages | Type II cells | Clara cells |
| 0.1 | 0.4 | 1.8 | 0.1 | 3.4 |
| 0.3 | 0.7 | 3.9 | 0.6 | 19.2 |
| 1.0 | 2.2 | 36.2 | 1.1 | 67.4 |
| 10.0 | 6.8 | 60.5 | 5.3 | 85.3 |

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Table 8. Effects of phenylethyl isothiocyanate (PEITC) treatment on NNK-induced pulmonary adenomas in mice

| Pretreatment | Daily dose (μmol) | % Mice with adenomas | Tumors/ mouse |
|---|---|---|---|
| None + NNK | | 100 | 10.7 |
| PEITC + NNK | 5 | 89 | 2.6 |
| PEITC + NNK | 25 | 30 | 0.3 |

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Table 9. Effects of PEITC treatment on NNK-induced $O^6$MG formation in mouse lung

| Pretreatment | Daily dose (μmol) | $O^6$MG (μmol/mol guanine) | |
|---|---|---|---|
| | | 2 hr after NNK | 6 hr after NNK |
| None | | 33.1 | 30.9 |
| PEITC | 5 | 10.6 | 3.9 |
| PEITC | 25 | 12.8 | <1.0 |

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Table 10. Effect of PEITC pretreatment on the metabolism of NNK by mouse lung microsomes

| Pretreatment | % NNK metabolized |
|---|---|
| None | 19 |
| PEITC (5 μmol) | 1.5 |
| PEITC (25 μmol) | 1.5 |

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344 male rats were treated with doses of NNK ranging from 0.1 to 50 mg/kg for 4 weeks. Purification of lung cell types revealed Clara cells to have 10 to 30-fold higher levels of $O^6$MG than other cell types (Table 7).

The earlier finding that there was no detectable $O^6$MG formation *in vitro* by isolated macrophages (see Table 6) suggests that the presence of this adduct in macrophages must derive from its formation in other cells and diffusion into the macrophages. The efficiency of adduct formation was greatest in Clara cells where the content of $O^6$MG increased markedly in the dose range from 0.1 to 1.0 mg/kg NNK. The dose–response curve for tumor incidence revealed a sharp increase in tumorigenicity as the dose of NNK increased from 0.3 to 1.0 mg/kg. When $O^6$MG adduct concentration in specific cell types was plotted against tumor incidence, a linear relationship ($r = 0.99$) was found for Clara cells [23], whereas non-linear plots with poor correlation coefficients were found for other cell types and whole lung. The authors concluded that the $O^6$MG content of Clara cells appears to be an excellent predictor of the carcinogenic potential of NNK in rat lung. The accumulation and persistence of DNA damage in Clara cells are compounded by the low basal activity and sluggish synthesis rate of the repair enzyme $O^6$MGMT in Clara cells (Table 5). It must be remembered that multiple doses of NNK reduced the activity of $O^6$MGMT to below the limits of detection in Clara cells (Table 5) and that there was little loss of $O^6$MG from Clara cells over an 8-day period while this adduct was removed efficiently from type II cells. These factors make Clara cells a sensitive target for NNK-induced neoplasia.

In an amplification of the role of NNK activation *in vivo* with the formation of $O^6$MG and its role in lung tumorigenesis, Morse *et al.* [24] studied the effects of NNK activation on lung tumor formation in mice. PEITC, an inhibitor of NNK activation, was administered by gavage for 4 consecutive days. A single i.p. dose of NNK (10 μmol/mouse) resulted in a 100% incidence of pulmonary adenomas (Table 8), with an accompanying multiplicity of 10.7 tumors per mouse. PEITC clearly reduced both the number of mice with NNK-induced pulmonary tumors as well as the number of tumors per mouse in a dose-dependent manner. Neither dose of PEITC resulted in any overt toxicity. In addition, both doses of PEITC markedly inhibited the formation of $O^6$MG in mouse lung following NNK administration (Table 9).

Based on its ability to inhibit both NNK-induced tumorigenicity and $O^6$MG formation, PEITC was tested for its ability to inhibit pulmonary microsomal activation of NNK *in vitro* (Table 10). The total metabolic activation of NNK was reduced by greater than 90% at two doses of PEITC. This inhibition of

NNK metabolism could readily account for the reduction of NNK-induced $O^6$MG formation and tumor incidence *in vivo* by PEITC.

Thus, pretreatment of mice for 4 days with PEITC prior to the administration of a single dose of NNK markedly reduced the lung tumor incidence, the formation of $O^6$MG, and the metabolic activation of NNK

NNK is known to yield at least four metabolites *in vivo*, most of which are carcinogenic. 4-Ipomeanol, although not carcinogenic, is known to be activated in, and cause necrosis of, Clara cells, thus sharing a cellular site of metabolic activation with NNK. Accordingly, Lin *et al.* [25] studied the effects of 4-ipomeanol and four chemical analogs on NNK metabolism and carcinogenicity in female A/J mice. The analogs reduced metabolite formation from NNK to varying degrees (10–50% reduction *in vitro*) by lung microsomes but had much less or no effect on the formation of the same metabolites by liver microsomes. The same analogs that inhibited NNK metabolism *in vitro* reduced the number of NNK-induced lung tumors by about 50%. The authors concluded that inhibition of NNK metabolism *in vitro* correlated directly with NNK tumorigenesis *in vivo*.

*Selective uptake and covalent binding of o,p'-DDD in mouse and rabbit lung*

Isolated reports have appeared on the covalent binding of o,p'-DDD in mammalian lung, and the published data are consistent with the view that the same principles apply to o,p'-DDD binding in lung as are operative in 4-ipomeanol binding in lung [2] and acetaminophen binding in liver [1].

Autoradiographic studies in mice [26] revealed that following injection of [$^{14}$C]o-p'-DDD, there was an exceptionally high concentration of radioactivity in lung and that preferential labeling occurred in the alveolar region, whereas the tracheobronchial mucosa showed low labeling. The radioactivity in lung was persistent, and 1 and 12 days later the concentration in lung far exceeded (20- to 30-fold that of liver) that in all other organs and tissues. Pretreatment of mice with the P450 inhibitor metyrapone markedly reduced labeling in lung (90%) and in liver (86%). In liver, the highest level of irreversibly bound radioactivity occurred 4 hr after injection of [$^{14}$C]o,p'-DDD, whereas in lung the highest level occurred 24 hr after injection. The level of bound radioactivity in lung was 15, 29, and 28 times higher than in liver at 4 hr, 24 hr, and 4 days after intravenous injection.

The finding that pretreatment with metyrapone virtually abolished covalent binding in lung and liver suggested that a cytochrome P450-catalyzed reactive metabolite mediated binding in these tissues. It is also of interest that pretreatment of mice with a large dose of o,p'-DDD 24 hr before a tracer dose of [$^{14}$C]o,p'-DDD essentially abolished binding of radioactivity in lung but had no effect on binding in liver.

Incubation of [$^{14}$C]o,p'-DDD with microsomes (9000 *g* supernatant) from mouse lung and liver revealed activation [27] and covalent binding *in vitro*. Binding of o,p'-DDD-derived radioactivity to both protein and phospholipid was found: an active role for cytochrome P450 was inferred from the observations that incubation in the presence of metyrapone, carbon monoxide, or sodium dithionite reduced binding by about 90%. The addition of glutathione inhibited covalent binding more potently in liver than in lung. Addition of exogenous DNA to incubates revealed covalent binding of o,p'-DDD-derived radioactivity to DNA.

Clara cells, type II alveolar cells, and pulmonary macrophages were purified (>80% purity) from rabbit lungs and incubated *in vitro* with NADPH and [$^{14}$C]o,p'-DDD [28]. Binding was time and concentration dependent, and was reduced or abolished by the addition of metyrapone, SKF 525-A, a CO atmosphere, or omission of NADPH, thus suggesting an oxidative, cytochrome P450-dependent activation. No detectable binding was found with alveolar macrophages. When corrected for cell viability and purity, binding was about three times higher in Clara cells than in type II cells.

*Conclusions*

Interest in the sequestration of amphiphilic basic amines, which does not rely on covalent binding, and in the covalent binding of an increasing number of chemically unrelated xenobiotics continues to grow among biochemical pharmacologists. Although there is a finite risk of pulmonary malignancy among non-smokers, the incredible increase in risk of pulmonary neoplasia among tobacco smokers propels interest in lung cell biology far beyond basic science into public health. For years, the irrefutable correlation between tobacco smoking and lung cancer has been denied. We now have a confluence of public health interest and interest among scientists in understanding how those 40 or more distinct pulmonary cell types interact beyond the exchange of $O_2$ and $CO_2$. In the next 10 years, let us undertake to understand the cell biology of lung to the same degree as we do the liver, kidney, and myocardium. We owe it to all those individuals who suffer from and die of pulmonary disease.

*Acknowledgement*—The author wishes to express his deep appreciation to Ms. Elaine B. Gram in the preparation of this manuscript.

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