Environmental Health Perspectives
Vol. 55, pp. 47-51, 1984

Metabolic Activation and Lung Toxicity: A Basis for Cell-Selective Pulmonary Damage by Foreign Chemicals

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The lungs may be exposed to potentially toxic metabolites that are either formed *in situ* or which are present in the circulation. Therefore, pulmonary injury may be a prominent effect of certain classes of chemicals that undergo bioactivation in the body. The specific types of lung cells damaged may depend upon factors such as preferential exposure or accumulation of parent compounds and/or metabolites, differences in cellular defense mechanisms, or the specific mechanism of activation of the toxicant. Prior knowledge about the metabolism, disposition and mechanism of bioactivation of a particular compound may allow prediction of the type of lung cell damage it is likely to produce. Conversely, morphological observations of characteristic types of cell-specific injury in the lung may suggest a likely biochemical mechanism of toxicity for the particular chemical involved.

Metabolic Activation and Pulmonary Toxicity

The lung can metabolize certain chemicals via enzyme systems very similar to those in the liver. The lung may be a site of many different kinds of biotransformations, including oxidations, reductions and conjugations. As in the liver, metabolism not only may result in the detoxification or removal of a chemical from the body, but also can lead to products that are more toxic than the parent chemical. Drug detoxification reactions occurring in the lung may be very important in protecting the lung locally against toxic materials produced *in situ* or reaching the lung in the bloodstream, but negligible when considered in terms of the overall *in vivo* disposition of the agents. On the other hand, metabolites that cause irreversible or cumulative changes in the lung may be of major consequence.

The general area of metabolic activation and pulmonary injury already has been reviewed in detail elsewhere (*1*). Therefore, in the present review some conceptual models are presented briefly, and the major emphasis is aimed at the implications these models may have for chemical-induced damage to specific types of cells in the lungs.

Based on available experimental data from a number of laboratories, three different mechanistic models of lung injury involving metabolic activation may be described (*1*). In one instance the protoxin may be activated primarily in the liver, and the ultimate toxic product is carried to the lungs via the bloodstream (Fig. 1). A likely example of a toxicity occurring in this fashion is the severe pulmonary damage caused by certain pyrrolizidine alkaloids, such as monocrotaline (*1,2*). This substance apparently is metabolized exclusively in the liver to a highly reactive pyrrolic derivative that can irreversibly bind to the liver and the lung, leading to damage of both of these tissues. The toxic pyrrolic metabolite, although very reactive, does have sufficient stability that a portion of the amount formed can escape from the liver into the circulation.

In another toxicity model, the cyclic reduction/oxidation of the parent compound leads to high rates of consumption of cellular cofactors such as NADPH and the production of large amounts of superoxide (Fig. 2) (*1,3*). Conceivably, either the intense depletion of essential cellular reducing equivalents and/or the generation of highly reactive oxygen radicals could lead directly to the expression of pulmonary injury and/or could contribute to a severe compromise of pulmonary antioxidant defense mechanisms. Pulmonary injury in this manner may be exemplified by the herbicide paraquat and also possibly by nitrofurantoin. Some typical features in the *in vivo* toxicity shared by these two compounds include its enhancement by dietary deficiencies of antioxidants (e.g., vitamin E and selenium) or by exposure to oxygen-enriched atmospheres. These toxicological similarities suggest these agents may be capable of injuring the lung through common mechanisms. This hypothesis seems further supported by *in vitro* observations showing that pulmonary microsomes promoted high rates of NADPH oxidation and superoxide generation by both compounds (presumably involving a transient free-

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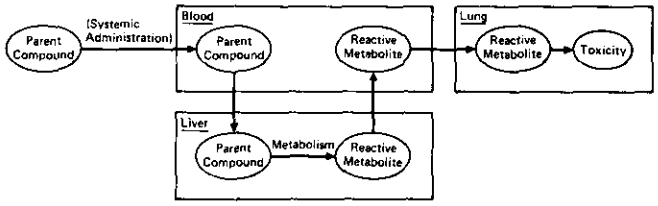


FIGURE 1. Pulmonary toxicity model involving reactive metabolites formed primarily in liver.

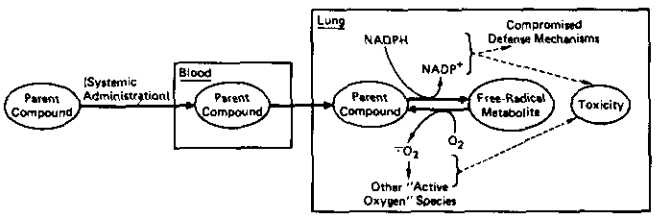


FIGURE 2. Pulmonary toxicity model involving "oxygen activation."

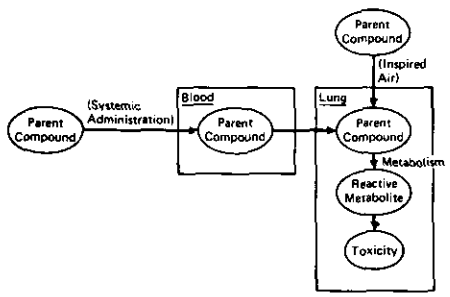
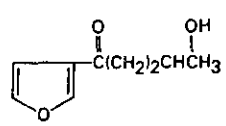


FIGURE 3. Pulmonary toxicity model involving *in situ* metabolism to reactive metabolite.

radical metabolite), even though the chemical structures of the two parent compounds are very different. The superoxide produced can be shown to lead also to other oxygen metabolites, such as singlet oxygen, hydrogen peroxide, and hydroxyl radical. Any, or all, of these products conceivably could contribute to lung injury, possibly by stimulating the peroxidative decomposition of essential cellular lipids, although the ultimate mode of lung damage by such products is yet to be fully defined. Studies concerning the mechanism of lung injury are reviewed in detail by Bus and Gibson (*3*).

A third model for pulmonary injury involving metabolic activation involves the generation of the ultimate toxic metabolite(s) directly within the lung itself (Fig. 3). This mechanism may apply equally well to compounds entering the lung either from the inhaled air or from the circulation. A prototypical example of this type of toxicity is that caused by ingestion or injection of 4-ipomeanol (*1*). This compound,



4-Ipomeanol

a furan derivative, was originally isolated and shown to be the major toxic substance in moldy sweet potatoes which had been responsible for severe lung injury and death in cattle. Indeed, it was the unusually severe, and remarkably selective, pulmonary injury and its association with the ingestion of a foreign substance that led to the interest in the chemical and biological properties of this agent, and its mechanism of toxicity.

4-Ipomeanol causes severe pulmonary damage not only in cattle but also in numerous other animal species that have been tested. An extensive series of experimental studies over the past few years have shown that pulmonary injury by 4-ipomeanol is caused not by the parent compound itself but by a highly reactive, alkylating metabolite of 4-ipomeanol produced by a cytochrome P-450-mediated oxidative activation of the parent compound. The formation of the ultimate lung-toxic metabolite(s) of 4-ipomeanol occurs within the lung itself (*1*).

Although 4-ipomeanol is the most extensively investigated compound that is activated *in situ* to a lung-toxic product, this same kind of mechanism may also be applicable to a variety of other compounds (*1*). For instance, it appears that many other toxic furan derivatives in addition to 4-ipomeanol may also act similarly (*4*). Examples of other lung-toxic furans include the synthetic furan carbamate derivative CMF (*5*), the atmospheric contaminant 3-methylfuran (*6*), and another natural product, perilla ketone (*7,8*). There is evidence that hydrocarbons such as bromobenzene (*9*) and naphthalene (*10,11*) may be metabolized to toxic products in the lungs. Haloalkanes, exemplified by carbon tetrachloride (*12*), may also damage the lungs, apparently due to the *in situ* generation of toxic metabolites. There are some data, too, that suggest thiourea (*13*) and derivatives such as α-naphthylthiourea (*14*) require *in situ* metabolic activation to produce their lung-damaging effects. Other compounds for which there is some evidence that metabolism may play a key role in the expression of toxicity include 3-methylindole (*15*) and butylated hydroxytoluene (*16*).

Metabolic Basis for the Pulmonary Clara Cell as a Target for Environmental Toxicants

There has been considerable interest in the pulmonary drug-metabolizing enzymes, particularly the cytochrome P-450 monooxygenase system and its localization in specific lung cell populations. The basis for this emphasis resides in the likely importance of this system in mediating the formation of reactive toxic metabolites from numerous different structural classes of chemicals, including many cytotoxic or carcinogenic agents that affect the lungs. Such chemicals may be inhaled, ingested, or injected, and they may come from many sources,

including industrial products or by-products, naturally occurring substances, and drugs. Metabolic activation of toxins in specific lung cell types could result in especially high susceptibility of these cells to the adverse effects. This might, in turn, lead to serious perturbations both in the normal pulmonary cell-cell relationships and in nonrespiratory metabolic and other functions of specific lung cell families.

Cellular Location of Cytochrome P-450 Monooxygenase Activity in Lung: Studies with 4-Ipomeanol

The pulmonary toxin 4-ipomeanol provided a useful tool to investigate the location(s) of the cytochrome P-450 system *in vivo* in lung cells for several reasons. (1) The compound is metabolized to a highly reactive alkylating agent that covalently binds to macromolecules immediately at its site of formation. (2) Tissue sections can be washed free of unbound 4-ipomeanol or metabolites, leaving behind only the covalently bound products. (3) Autoradiography can be used to visualize the covalently bound products in fixed lung tissue from animals dosed with radiolabeled 4-ipomeanol.

When the lung sections of such animals were examined by light microscopic autoradiography, the localization of silver grains showed that the covalently bound ipomeanol metabolite was located predominantly in the nonciliated pulmonary bronchiolar (Clara) cells of the terminal bronchioles (*17*). Relatively little or no silver grains were found in the ciliated bronchiolar cells or in parenchymal cells such as the Types I or II pneumocytes.

The above studies provided the basis for our conclusion that Clara cells are an important cellular site of cytochrome P-450 enzyme activity in lung (*17*). Further substantiation of this view was provided by subsequent investigations by Devereux et al. (*18*) on monooxygenase activities in isolated lung cell preparations, and by immunohistochemical studies by Serabjit-Singh et al. (*19*) using antibodies against a purified lung cytochrome P-450.

Recently the metabolic activation of 4-ipomeanol was studied in isolated rabbit lung cells (*20*). The highest rates of P-450-mediated metabolism of 4-ipomeanol were observed in isolated Clara cells. A significant amount of activity, albeit much less than in Clara cells, also was seen in isolated alveolar Type II cells.

Other recent studies compared the metabolism of 4-ipomeanol in rabbit hepatic and pulmonary microsomes and in reconstituted systems containing purified cytochromes P-450 (*21*). These results have helped clarify the basis for the high efficiency of metabolism of 4-ipomeanol by the lungs. Although it is clear that the two major isozymes of P-450 found in rabbit lung are also present in rabbit liver, the relative concentrations of these isozymes (with respect to the total P-450 content) in the two tissues are vastly different. As a consequence of this, the P-450 systems of lung and liver have distinct substrate specificities. The difference in specificity between the P-450 systems of lung and liver and the highly localized nature of the pulmonary system are important factors in the tissue and cell-specific toxicity of 4-ipomeanol. The two major pulmonary P-450 isozymes (P-450I and P-450II) both metabolize 4-ipomeanol and, per nanomole P-450, the lung is much more active than the liver for this reaction. In addition, these isozymes appear to be highly concentrated in the cell type that is most affected by 4-ipomeanol. The pulmonary P-450 system is also present in the alveolar Type II cell, but immunohistochemical, electrophoretic and metabolic evidence shows that it is not nearly as concentrated in these cells as it is in the Clara cell. In the liver, P-450I and P-450II appear to be somewhat evenly distributed throughout the tissue, thereby lessening the chances of critical concentrations of toxic metabolites of 4-ipomeanol being produced in a given hepatocyte. On the other hand, conditions that favor the metabolism of 4-ipomeanol appear to be maximized in the Clara cell of the lung.

Toxicological Significance of the Cytochrome P-450 Enzyme System in Clara Cells

The presence of a cytochrome P-450 system in Clara cells is of potentially broad toxicological significance. Many cytotoxic and/or carcinogenic chemicals require activation by this enzyme system. Thus, the Clara cells would be likely primary targets for the adverse effects of such chemicals and/or they may serve as major sites for production of proximate or ultimate toxic/carcinogenic metabolites that act on other cells. This is vividly illustrated by the severe and preferential damage of pulmonary Clara cells *in vivo* following injection of 4-ipomeanol (*17*). Other studies showing preferential damage or destruction of Clara cells by 3-methylfuran, carbon tetrachloride and a variety of other agents have further supported the importance of the drug-metabolizing capacity of Clara cells in acute chemical-induced lung injury (*1*). The possible importance of Clara cells in environment-associated chronic lung disease also is a question needing further attention. The high xenobiotic-metabolizing capacity and the high susceptibility of Clara cells to environmental chemicals conceivably could be important factors in the pathogenesis of chronic obstructive small airway disease, a problem of major public health significance. Finally, investigations such as those reported by Reznik-Schuller and Lijinsky (*22*) and by Kauffman et al. (*23*) with certain nitroso derivatives provide examples of the induction of tumors of apparent Clara cell origin, which, at least in some instances, may result from the preferential activation of the carcinogens in the Clara cells.

Other Lung Cells as Potential Targets for Toxic Chemicals Requiring Metabolic Activation

Other Bronchiolar Cells

Autoradiographic studies with relatively high doses of 4-ipomeanol and 3-methylfuran show some covalent binding of these compounds to ciliated cells, albeit to a much smaller extent than to nonciliated (Clara) cells (*1*); further studies are needed to determine whether the smaller, but significant, amounts of covalent binding of these toxins to ciliated cells is mediated by enzyme activities present in the ciliated cells or whether it is due to metabolites formed in adjacent Clara cells. Because the ciliated cells apparently are derived from Clara cells (*24*), it would not be surprising if they possessed at least some metabolic activity toward xenobiotic substrates.

The xenobiotic-metabolizing potential of the APUD (amine precursor uptake and decarboxylation) cells present in the bronchial epithelium is not known. This metabolic potential is of interest because the APUD cells have been suggested as the site of origin of small-cell tumors of lung. However, APUD cells are relatively difficult to study in this respect because they occur only rarely in the airway epithelium of adult animals.

Alveolar Epithelium Cells

Histochemical studies (*25*) suggested the presence of some xenobiotic-metabolizing activity in pulmonary alveolar epithelium. Isolated pulmonary Type II cells also have been shown to have some drug-metabolizing capability (*18*). Carbon tetrachloride, which probably requires metabolic activation to damage lung cells, is capable of damaging Type II pneumocytes in addition to pulmonary Clara cells (*12*). It is of interest that bronchiolar cells and alveolar epithelial cells share a common embryologic origin (*26*); perhaps they likewise might share similarities in their capacity to metabolize certain foreign compounds.

Another toxic agent of potential relevance is 3-methylindole. In certain species (ruminants) this agent is capable of damaging both bronchial epithelium and Type I pneumocytes, and it has been speculated that the compound might require metabolic activation to exert its toxic effect (*15*). Unfortunately, 3-methylindole does not seem to reproducibly cause lung damage in species more convenient for laboratory studies, such as mice and rats.

Butylated hydroxytoluene (BHT) causes damage to Type I pneumocytes in mice but apparently not in rats. There is evidence that BHT may undergo metabolism-related covalent binding to lung tissue and that this may be related to its pulmonary toxicity *in vivo* (*16*).

Vascular Endothelium

By virtue of its location at a site for maximum exposure to reactive metabolites reaching the lung by way of the bloodstream, the pulmonary vascular endothelium would be expected to be a primary site for toxic lung damage from preformed reactive products or metabolites reaching the lungs from the bloodstream. Studies showing lung vascular damage by the pyrrolizidine alkaloids and their pyrrolic metabolites are consistent with this view. At present, however, there are no clear examples of pulmonary endothelial cell toxicities occurring by a mechanism involving *in situ* activation. However, the toxic thioureas may merit further investigation in this regard, since these compounds damage the vascular endothelium, and there is some evidence that they need to be activated by metabolism in the lung (*1*). Interestingly, the activation of these compounds may occur by a cytochrome P-450-independent system (*14*).

Other Pulmonary Cells

The available data are insufficient to evaluate the possibility that other types of lung cells are targets for primary damage by toxic metabolites. The only other specific cell type for which relevant metabolic data presently are available is the pulmonary alveolar macrophage. Hook et al. (*27*) found that several xenobiotic-metabolizing activities were extremely low or not detectable in rabbit alveolar macrophages. On the other hand, Harris et al. (*28*) speculated that the alveolar macrophages could be an important source of diffusible carcinogenic metabolites from phagocytized foreign particulates containing adsorbed procarcinogens; the bronchial epithelium and the macrophages in intimate contact with its surface might act in concert in metabolic activation reactions leading to carcinogenesis.

It likewise can be speculated that, if the necessary xenobiotic-metabolizing activities were sufficiently high, the metabolic activation of certain foreign compounds by alveolar macrophages conceivably could lead directly to acute damage of these cells and might thereby compromise the efficiency of an important defense mechanism of the lung. Such a possibility may be worthy of further investigation if suitable models can be identified for study.

Use of Cell-Specific Toxins in Experimental Studies

Toxins which cause cell-specific pulmonary damage (e.g., by virtue of their *in situ* metabolic activation in target cells) may provide useful tools to study the physiologic functions of individual lung cell types. Cell-specific toxins also may be useful for the study of pulmonary defense mechanisms involving cellular regeneration. For example, Type II pneumocytes proliferate, repopulate the alveolar walls, and differentiate into

Type I alveolar cells after primary damage or destruction of Type I cells (*29*). In a similar way, pulmonary Clara cells appear to be responsible for repopulation and repair of damaged bronchiolar epithelium (*24*). It will be of interest to explore further the pathophysiologic consequences of damage to these proliferative cell populations. Simultaneous environmental exposures to multiple agents conceivably could lead to damage both to highly susceptible cell populations, such as Type I alveolar cells or the ciliated bronchiolar cells, and to reparative cell populations, such as the Type II pneumocytes or the Clara cells, and might thereby result in especially disastrous toxicologic consequences.

REFERENCES

1. Boyd, M. R. Biochemical mechanisms in chemical-induced lung injury: roles of metabolic activation. CRC Crit. Rev. Toxicol. 7: 103–176 (1980).
2. Mattocks, A. R. Mechanisms of pyrrolizidine alkaloid toxicity. In: Phytochemical Ecology (J. B. Harborne, Ed.), Academic Press, New York, 1973, pp. 179–199.
3. Bus, J. S., and Gibson, J. E. Paraquat: a model for oxidant-initiated toxicity. Environ. Health Perspect. 55: 37–46 (1984).
4. Boyd, M. R. Biochemical mechanisms in pulmonary toxicity of furan derivatives. In: Reviews in Biochemical Toxicology (E. Hodgson, J. R. Bend and R. M. Philpot, Eds.), Elsevier/North Holland, New York, 1980, pp. 71–102.
5. Guengerich, F. P. Studies on the activation of a model furan compound—toxicity and covalent binding of 2-(N-ethylcarbamoylhydroxymethyl)furan. Biochem. Pharmacol. 26: 1909–1915 (1977).
6. Boyd, M., Statham, C., Franklin, R., and Mitchell, J. Pulmonary bronchiolar alkylation and necrosis by 3-methylfuran, a naturally occurring potential atmospheric contaminant. Nature 272: 270–271 (1978).
7. Wilson, B. J., Garst, J. E., Linnabary, R. D., and Channel, R. B. Perilla ketone: a potent lung toxin from the mint plant, *Perilla frutescens*. Science 197: 573–574 (1977).
8. Dutcher, J. S., and Boyd, M. R. Studies of the *in vivo* metabolic activation and covalent binding of the lung-toxic furan derivative, perilla ketone. In: Microsomes, Drug Oxidations, and Drug Toxicity. Japan Scientific Press, Tokyo, 1982, pp. 557–558.
9. Reid, W. D., Illett, K. F., Glick, J. M., and Krishna, G. Metabolism and binding of aromatic hydrocarbons in the lung. Am. Rev. Respir. Dis. 107: 539–551 (1973).
10. Mahvi, D., Bank, H., and Harley, R. Morphology of a naphthalene-induced bronchiolar lesion. Am. J. Pathol. 86: 559–566 (1977).
11. Tong, S. S., Hirokata, Y., Trush, M. A., Mimnaugh, E. G., Ginsburg, E., Lowe, M. C., and Gram, T. E. Clara cell damage and inhibition of pulmonary mixed-function oxidase activity by naphthalene. Biochem. Biophys. Res. Commun. 100: 944–950 (1981).
12. Boyd, M. R., Statham, C., and Longo, N. The pulmonary Clara cell as a target for toxic chemicals requiring metabolic activation; studies with carbon tetrachloride. J. Pharmacol. Exptl. Therap. 212: 109–115 (1980).
13. Hollinger, M. A., Giri, S. N., and Hwang, F. Binding of radioactivity from ^{14}C-thiourea to rat lung protein. Drug Metab. Disp. 4: 119–123 (1976).
14. Boyd, M. R., and Neal, R. A. Studies on the mechanism of toxicity and development of tolerance to the pulmonary toxin alpha-naphthylthiourea (ANTU). Drug Metab. Disp. 4: 314–322 (1976).
15. Huang, T. W., Carlson, J. R., Bray, T. M., and Bradley, B. J. 3-Methylindole-induced pulmonary injury in goats. Am. J. Pathol. 87: 647–666 (1977).
16. Kehrer, J. R., and Witschi, H. Effects of drug metabolism inhibitors on butylated hydroxytoluene-induced pulmonary toxicity in mice. Toxicol. Appl. Pharmacol. 53: 333–342 (1980).
17. Boyd, M. R. Evidence for the Clara cell as a site of cytochrome P-450-dependent mixed-function oxidase activity in lung. Nature 269: 713–715 (1977).
18. Devereux, T. R., Hook, G., and Fouts, J. R. Foreign compound metabolism by isolated cells from rabbit lung. Drug Metab. Disp. 7: 70–75 (1979).
19. Serabjit-Singh, C. J., Wolf, C. R., Philpot, R. M., and Plopper, C. G. Cytochrome P-450: localization in rabbit lung. Science 207:1469–1470 (1980).
20. Devereux, T. R., Jones, K. G., Bend, J. R., Fouts, J. R., Statham, C. N., and Boyd, M. R. *In vitro* metabolic activation of the pulmonary toxin, 4-ipomeanol, in nonciliated bronchiolar epithelial (Clara) and alveolar Type II cells isolated from rabbit lung. J. Pharmacol. Exptl. Therap. 220: 223–227 (1981).
21. Philpot, R. M., Wolf, C. R., Slaughter, S. R., Bend, J. R., Robertson, I. G. C., Zeiger, E., Statham, C. N., and Boyd, M. R. The role of the cytochrome P-450-dependent monooxygenase system in pulmonary-specific toxic effects of xenobiotics. In: Microsomes, Drug Oxidations, and Drug Toxicity. Japan Scientific Press, Tokyo, 1982, pp. 487–494.
22. Reznik-Schuller, H., and Lijinsky, W. *In vivo* autoradiography and nitrosoheptamethyleneimine carcinogenesis in hamsters. Cancer Res. 39: 72–75 (1979).
23. Kauffman, S. L., Alexander, C., and Sass, L. Histologic and ultrastructural features of the Clara cell adenoma of the mouse lung. Lab. Invest. 40: 708–716 (1979).
24. Evans, M. J., Cabral-Anderson, L. J., and Freeman, G. Role of the Clara cell in renewal of the bronchiolar epithelium. Lab. Invest. 38: 648–655 (1978).
25. Wattenberg, L. W., and Leong, J. L. Histochemical demonstration of reduced pyridine nucleotide-dependent polycyclic hydrocarbon metabolizing systems. J. Histochem. Cytochem. 10: 412–420 (1962).
26. O'Hare, K. H. and Sheridan, M. N. Electron microscopic observations on the morphogenesis of the albino rat lung with special reference to the pulmonary epithelial cells. Am. J. Anat. 127: 181–210 (1970).
27. Hook, G. E. R., Bend, J. R., and Fouts, J. R. Mixed-function oxidases and the alveolar macrophage. Biochem. Pharmacol. 21: 3267–3277 (1972).
28. Harris, C. C., Hsu, I. C., Stoner, G. D., Trump, B. F., and Selkirk, J. K. Human pulmonary alveolar macrophages metabolize benzo(a)pyrene to proximate and ultimate mutagens. Nature 272: 633–634 (1978).
29. Witschi, H. Proliferation of Type II alveolar cells: a review of common responses to lung injury. Toxicology 5: 267–277 (1976).