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LOCALIZATION OF METABOLIC ACTIVATION AND DEACTIVATION SYSTEMS IN THE LUNG: Significance to the Pulmonary Toxicity of Xenobiotics

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INTRODUCTION

In recent years, the pulmonary toxicity of drugs and chemicals has been the subject of numerous investigations. A major advance in understanding how certain relatively inert compounds initiate cellular toxicity in the lungs was the realization that many such chemicals undergo metabolism to more reactive intermediates and that lung tissue is capable of catalyzing such reactions. The lungs are a prominent target organ for numerous types of chemically-induced pathological changes (1). They are situated in a primary site for exposure to xenobiotics both externally by inhalation and internally by the circulation. It has been well demonstrated that pulmonary tissue contains enzymatic systems capable of metabolizing drugs and other chemicals (for review see 2–4).

Since the original work by Miller & Miller (5, 6), who first proposed metabolic "activation" to explain chemical hepatocarcinogenic activity, numerous studies in various organs including the lung have shown that metabolism of certain compounds is critical for the onset of cellular damage (for review see 7, 8). The series of events linking metabolic activation to cell damage is still not well understood. However, it would appear that no single mechanism is common to all reactive metabolites. Certain structures, such

as the activated furans and the epoxides of polycyclic aromatic hydrocarbons, react directly with cellular components, typically leading to alkylation of macromolecules (Figure 1*A*). Other activated species such as those of the quaternary bipyridyl compounds, nitroaromatic, and semiquinone free radicals are able to reduce molecular oxygen to activated forms which in turn may initiate cellular damage (Figure 1*B*). Many of the compounds in this

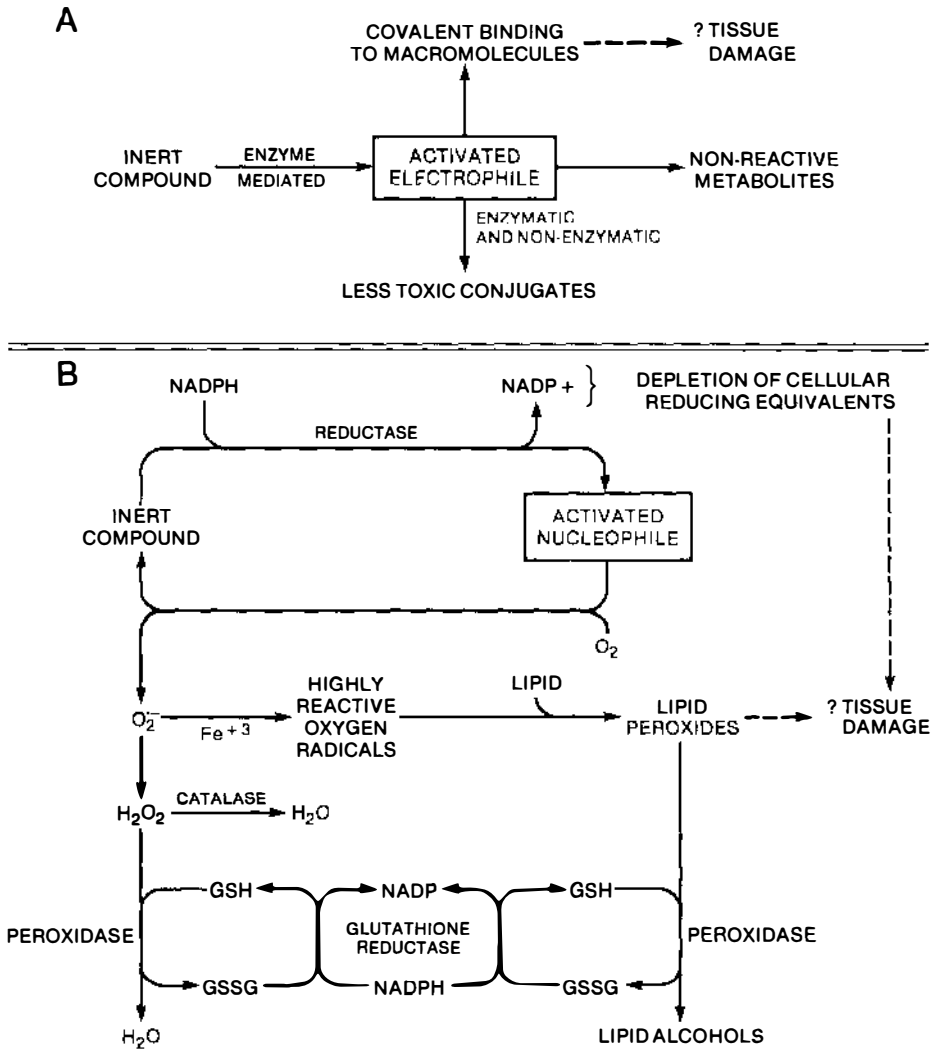

Figure 1 Proposed mechanisms that may be involved in the activation of inert chemicals to reactive intermediates within the lung.

latter category do not appear to react directly with cellular constituents under normal physiological conditions.

While it is well established that the pulmonary toxicity of certain drugs and chemicals is mediated through metabolism, other studies emphasize that the lungs also contain defense mechanisms against reactive intermediates (e.g. 9–11). Ultimate toxicity could be expected to reflect the balance between activation and deactivation within the lung cells. Environmental factors that alter the activity of pulmonary activating and deactivating systems can markedly influence the extent of chemically induced injury (12–16).

This article briefly discusses some recent advances concerning the activation of chemicals in the lung and explores mechanisms by which the lung protects itself against reactive intermediates. Several comprehensive reviews on pulmonary injury induced by drugs and chemicals are available (1, 7, 12). Therefore, this review focuses particularly on less investigated but potentially important mechanisms and factors that may contribute to maintaining or compromising the balance between activation and deactivation of chemicals in the lung. Of particular interest is the cellular localization of pulmonary enzymes responsible for chemical activation and deactivation, and the potential consequences of this heterogeneous enzyme distribution.

GENERAL CONSIDERATIONS

Although a complete understanding of how reactive intermediates precipitate cell damage is lacking, considerable evidence from numerous experiments in both pulmonary and extrapulmonary tissues have led to two generalized hypotheses that show contrasting roles for metabolism (Figure 1). Firstly, an inert substrate may be converted to a reactive intermediate that is electrophilic. Such electrophiles may be capable of interacting with nucleophilic centers within the cell. Secondly, reduction of an inert chemical can produce an electron-rich nucleophile which may in turn reduce critical cellular components.

Reactive Electrophiles

In the lung, several toxins have been shown to undergo metabolic oxidation to highly reactive electrophiles. This reaction is usually cytochrome P-450 mediated requiring NADPH and molecular oxygen. Detailed studies using furans such as 4-ipomeanol and 3-methylfuran (7, 12), bromobenzene (7), naphthalene (17), 2-methylnaphthalene (18), 3-methylindole (19), and butylated hydroxytoluene (20) suggest that reactive intermediates of these chemicals are unstable electrophiles that rapidly react with nucleophilic centers on macromolecules. Covalent binding to tissue constituents is a common feature of this group of chemicals.

Activated electrophiles can also conjugate with glutathione. Significant levels of glutathione-S-transferases in lung imply that this enzymatic activity could be important in the detoxification of unstable electrophiles. Certain highly reactive intermediates, such as those formed from 4-ipomeanol, are capable of reacting with glutathione nonenzymatically (21). Moreover, electrophiles also appear to conjugate with other sulfhydryl compounds, such as cysteine and cysteamine, which may be administered exogenously (9).

Reactive Nucleophiles

Nucleophilic intermediates may result from the catalytic reduction of inert drugs and other chemicals with an appropriate cofactor such as NADPH (Figure 1). Depending upon the redox potential of the nucleophile produced, subsequent reduction of endogenous molecules may occur. Of potentially major importance is the activation of molecular oxygen to superoxide anion by reactive nucleophiles. Such reactions can occur under physiological conditions because of the low redox potential of the $O_2^{\cdot -}/O_2$ coupling [$E^{o'} = 0.31$ V (22)]. Superoxide anion can act as a one electron reductant or a one electron oxidant. While the latter is thermodynamically favorable [$O_2^{\cdot -}/H_2O_2$ coupling, $E^{o'} = 0.87$ V (22)], such reactions appear to be limited kinetically. In biological systems, the ability of $O_2^{\cdot -}$ or products formed therefrom, such as hydroxyl radical, to initiate lipid peroxidation has been an area of intense research (for review see 23). It is conceivable that the peroxidation of membrane lipids by activated oxygen may account for ultimate cellular damage. Superoxide is also capable of reacting with sulfhydryl compounds, ascorbic acid, α-tocopherol, and presumably SH-containing proteins (24).

Apart from low molecular weight compounds, cells contain a complex system of enzymes designed to remove activated oxygen species. Catalase, superoxide dismutase, and glutathione peroxidase each play a crucial role in maintaining nontoxic cellular levels of reactive oxygen. These enzymes generally are ubiquitously distributed and have been shown to be inducible under oxygen stress (10, 25). Since the oxygen tension is highest in the lungs, one would expect that this organ has evolved efficient protective mechanisms against oxygen toxicity. The pulmonary activity and cellular location of these enzymes are discussed in detail below.

In the lungs, paraquat (26, 27) and nitrofurantoin (28, 29) are well-investigated examples of pulmonary toxins that are believed to initiate cell damage through the reduction of molecular oxygen. Other moieties that potentially can activate oxygen include semiquinones and azo anion free radicals (30).

Figure 1*B* illustrates that inert chemicals can act as catalysts for the conversion of O_2 to O_2^- at the expense of reduced pyridine nucleotide. It has been suggested that excessive redox cycling can compromise the cellular reducing equivalents and lead to toxicity (31, 32). The ability of paraquat to stimulate the pentose pathway supports NADPH depletion as a possible mechanism of cell damage (33).

Certain inert chemicals may be activated by enzymatic reduction to products that may act as either reactive nucleophiles or electrophiles. Evidence for this is seen with the nitrofurans and several other nitroaromatic compounds. Reductive activation of nitro-containing compounds may occur initially by a one-electron reduction of the nitro moiety. For example, under aerobic conditions in vitro, an activated intermediate of nitrofurantoin may act as a reactive nucleophile undergoing redox cycling and leading to the formation of reactive oxygen species (Figure 2). In the absence of O_2, reduction of the nitro group results in the accumulation of anion free radicals, which have been detected by electron spin resonance (34). Under anaerobic conditions, further reduction can lead to products that may act as reactive electrophiles and bind to biological macromolecules. It has been proposed that reduction of the nitro free radical to a hydroxylamine produces the ultimate species responsible for covalent binding (7), although direct evidence supporting this hypothesis is not available. Whereas nitrofurantoin can be shown either to promote NADPH oxidation and reduction of O_2 or to bind covalently under the appropriate in vitro conditions, the mechanism of its pulmonary toxicity in vivo appears more likely related to its ability to promote redox cycling. Although some covalent binding of nitrofurantoin is seen in vivo, it does not appear to correlate with lung injury (14).

PULMONARY METABOLIC ACTIVATION SYSTEMS

Cytochrome P-450-Dependent Monooxygenase

Pulmonary cytochrome-P-450-dependent monooxygenase has been shown to metabolize a wide variety of compounds including furans (12), bromobenzene (7), and polycyclic hydrocarbons (17) to highly reactive electrophiles. Experiments in microsomal preparations utilizing metabolic inhibitors such as piperonyl butoxide, SKF-525A, and cobaltous chloride have implicated the obligatory role of the cytochrome P-450 system in the activation of certain pulmonary toxins. Dependence on O_2 and NADPH as well as inhibition by CO are also criteria for establishing that in vitro activation is cytochrome P-450-dependent. Work by Boyd and coworkers (35, 36) using the pulmonary alkylating furan 4-ipomeanol, and by Devereux and colleagues (37) using isolated lung cells, and by Serabjit-Singh et

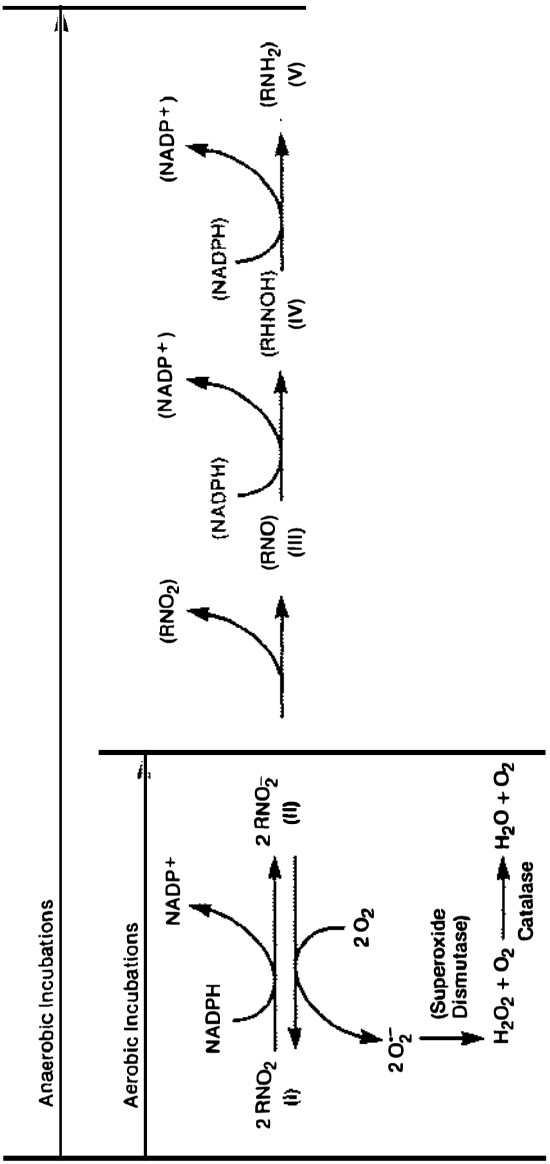

Figure 2 Schematic representation of some features of the in vitro metabolism of nitrofurantoin.

al (38) using immunohistochemical techniques has shown that the nonciliated bronchiolar cells (Clara) are a major site of pulmonary cytochrome P-450. Consequently, many potent alkylating agents specifically damage the terminal bronchioles in vivo (7, 17).

While the localization of cytochrome P-450 to a relatively small lung cell population such as the Claras has been suggested as a possible cause of lung-specific toxicity (7, 38), recent experiments with 4-ipomeanol in purified pulmonary P-450 systems have indicated that kinetic considerations may also be important (39). Wolf and coworkers (40, 44) have shown that the majority of rabbit pulmonary P-450 is comprised of two isoenzymes, $P\text{-}450_I$ and $P\text{-}450_{II}$. Each isoenzyme is capable of activating 4-ipomeanol and both are present in rabbit liver but only at very low concentrations (39, 39a). Antibodies to each cytochrome substantially inhibit 4-ipomeanol metabolism in both liver and lung microsomes. However, the majority of hepatic cytochrome P-450 appears to be relatively inactive in the metabolism of 4-ipomeanol indicating that the pulmonary specificity of 4-ipomeanol toxicity is at least in part due to the kinetics of the cytochrome P-450 species present in lung.

Further evidence indicating that enzyme kinetics are important in determining organ-specific toxicity is seen when 4-ipomeanol activation is examined in different organs of the quail (41). Since avian lung tissue contains relatively little or no cytochrome P-450-dependent monooxygenase activity, it was of interest to examine whether alkylation of avian tissue by 4-ipomeanol differed from mammalian species. Results indicated that, in Japanese quail, hepatic cytochrome P-450 content was fivefold greater than in lung. Furthermore, covalent binding of 4-ipomeanol to isolated microsomes was five to six times greater in quail liver than in lung. Kinetic analysis of 4-ipomeanol covalent binding indicated that, compared with lung microsomes, avian hepatic microsomes demonstrated a greater affinity for the substrate as reflected in the smaller Km (Table 1). Administration of 4-ipomeanol to Japanese quail or Rhode Island Red roosters in vivo produced severe necrosis of the liver with no evidence of pulmonary damage (42). In the rat, where the primary site of 4-ipomeanol toxicity is the lungs, pulmonary microsomal Km for this furan is more than ten times lower than that seen in rat liver (Table 1) (12). Thus, not only the localization of activating enzymes but also their relative affinity for the chemical toxin may be important in determining organ specific toxicity.

Using immunofluorescence and peroxidase-antiperoxidase techniques, Serabjit-Singh and coworkers (38) found that rabbit pulmonary cytochrome $P\text{-}450_I$ was primarily localized in the apices of nonciliated epithelial cells. This finding agrees with the observed localization of 4-ipomeanol covalent binding in lung (35). The same group has reported that cytochrome

Table 1 4-Ipomeanol kinetics in different microsomal preparations[a]

Species	Tissue	K_m (× 10^{-4}M)	V_{max} (nmol bound/min/ nmol P-450)
Rat	Lung	0.29	4.85
	Liver	4.20	0.48
Quail	Lung	2.75	0.23
	Liver	0.72	1.89
Rabbit	Lung	0.5	8.44
	Lung P-450_I	0.35	2.56
	Lung P-450_{II}	1.50	1.99
	Liver	0.90	0.65

[a] For details of methods see (12, 39a, 41).

P-450_{II} is similarly located in the pulmonary Clara cell, although immunofluorescence due to both isoenzymes was detectable in isolated aveolar type II cells (43). Specificity of the lung P-450 isoenzymes for potential lung toxins vary. For example, benzo(a)pyrene is metabolised differently by the two isoenzymes with only P-450_I initiating benzo(a)pyrene-derived covalent binding to DNA (44). The potent mutagens 2-aminoanthracene, 2-aminofluorene, and 2-acetylaminofluorene were activated by pulmonary P-450_{II} only (45). In contrast, aflatoxin B_1 appeared to be activated solely by P-450_I in reconstituted monooxygenase systems but by both in pulmonary microsomal preparations. The significance of isoenzyme specificity toward the activation of pulmonary toxins and localization of cellular damage is only beginning to be understood. Further experimentation characterizing the kinetics of chemical activation in lung is needed.

Pulmonary Prostaglandin Synthetase

Following the original work by Marnett et al (46) showing that prostaglandin synthetase may be responsible for the activation of several carcinogens, Sivarajah et al (47) reported that benzo(a)pyrene could be activated by lung preparations via a pathway dependent on the presence of arachidonic acid. Using guinea pig lung microsomes, it was established that prostaglandin intermediates, most likely the hydroperoxy endoperoxide G_2, could cooxidize benzo(a)pyrene and its 7,8-diol to reactive epoxides capable of covalently binding to macromolecules. This reaction was independent of NADPH and inhibited by indomethacin, a specific blocker of prostaglandin cyclooxygenase. Recently, Sivanajah et al (48) reported that several mammalian tissues including mouse skin, rat intestine, and guinea pig lung, all of which are known target organs for benzo(a)pyrene-mediated toxicity, can activate this compound via the prostaglandin synthetase pathway. Signifi-

cant activity was seen in rabbit kidney and human lung. Other potentially toxic chemicals such as benzidine (49), and N-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide (50) have also been shown to undergo arachidonic acid-dependent activation.

As with P-450-dependent activation, unstable and highly reactive intermediates resulting from cooxidation with prostaglandin precursors could be expected to damage specific cell-types should prostaglandin synthetase be localized in discrete cell populations within the lungs. Early experiments by Ryan & Ryan (51) suggested that the pulmonary distribution of this enzyme system was indeed heterogenous, being located mainly in the alveolar type II cells and pulmonary macrophages. More recently, the biosynthesis of prostaglandins has been shown to occur in isolated bovine endothelial cells (52). Autoradiography using tritiated acetyl-salicylate, which reportedly binds irreversibly to prostaglandin synthetase, indicated that the enzyme was associated with the smooth and rough endoplasmic reticulum and, to a lesser extent, nuclear membrane of endothelial cells. It was also reported that, following intravenous infusion with tritiated acetyl-salicylate, all cells of the alveolar-capillary unit bind radioactivity, but the type II cells are most prominent.

The implications of these findings with respect to pulmonary toxicology may be substantial considering the known existence of prostaglandin biosynthesis pathways in the lungs of many animal species (48). However, very little is known of the activation role of this pathway in vivo. Fisher and coworkers reportedly (Winter Prostaglandin Meeting, Snowbird, Utah, 1980) have reduced the occurrence of benzo(a)pyrene and benzo(a)pyrene-7,8-diol-mediated skin tumors in laboratory animals by concurrent treatment with indomethacin or ETYA, both prostaglandin synthetase inhibitors. Apart from these experiments, no other whole animal data presently implicate this pathway in organ-specific toxicity.

Flavin-Containing Monooxygenase

In addition to cytochrome P-450 oxidases, the lungs have been shown to contain a flavoprotein capable of oxidizing amines and organic sulfur compounds. Flavin-containing monooxygenase was first described by Ziegler and associates in the early 1960s. The physical properties, enzyme activity, and tissue distribution of this enzyme have been described in detail (53). The enzyme generally is not inducible with most drugs, prednisolone being an exception. Furthermore, flavin-containing monooxygenases are not sensitive to carbon dioxide or SKF-525A. Oxidation of known substrates such as tertiary and secondary amines, thiono-sulfur compounds, and alkylhydrazines requires NADPH and molecular oxygen (53).

In most mammalian species, pulmonary activity of flavin-containing monooxygenase ranges from about 50–100% of hepatic activity in rabbit (54) and hamster (53) to almost 200% in the male mouse (55). Notable exceptions are hog and human where pulmonary activity is less than 20% of that seen in liver (53).

Little information regarding the involvement of flavin-dependent monooxygenase in the activation of lung toxins is available. However, considering the ability of this enzyme to activate potentially toxic chemicals in the liver, it is reasonable to suspect that reactive intermediates could be formed in the lung by this route. Indeed, a number of lung-toxic thiocarbamides such as α-naphthylthiourea (56) and thiourea (57) are metabolized by flavin-dependent monooxygenase. Further experimentation is required to clearly define the role, if any, of this enzyme system in the pulmonary damage by reactive intermediates.

In the liver, flavin-dependent monooxygenase is associated with the rough endoplasmic reticulum and nuclear fractions (53). The subcellular location of the enzyme in the lung appears to be microsomal although a detailed study of its distribution has, to our knowledge, not yet been reported. No information on the cellular location of flavin-dependent monooxygenase in the lung is available.

NADPH-Cytochrome P-450 Reductase

Enzymatic reduction of drugs and other compounds represents a major route of chemical activation ultimately leading to pulmonary toxicity. Reductases are commonly flavoproteins with a diverse range of functions and substrates. With respect to xenobiotics, microsomal NADPH-dependent cytochrome P-450 reductase has been the most extensively studied to date and is thought to be responsible, at least in part, for the activation of the pulmonary toxins nitrofurantoin (29), mitomycin C (58), and possibly paraquat (26). The one-electron reduction system of cytochrome P-450 reductase has been well defined in purified systems and the kinetics of one such reaction, nitrofurantoin activation to nitrofurantoin anion free radical, has been investigated in detail (29). Pulmonary NADPH cytochrome P-450 reductase is capable of reducing several moieties: quinone to semiquinone as exemplified by mitomycin activation, nitrofuran to nitrofuran free radical as exemplified by nitrofurantoin, and bipyridylium cation to its respective free radical as exemplified by paraquat. All these reactions have been shown in vitro to result in redox cycling and the formation of superoxide.

The possible importance of this enzyme to in vivo activation and subsequent toxicity in the lung is subject to the same criteria discussed earlier for the P-450 cytochrome. That is, activity and organ-selective damage can be expected to be a function of both the localization and kinetics of the enzyme

toward the substrate. Several laboratories have purified and characterized pulmonary P-450 reductase from different species (59–61). The enzyme isolated from rabbit lung has a molecular weight of about 72,000 and contains 1 mol of flavin mononucleotide and 1 mol of flavin adenine dinucleotide per mol of enzyme (59). NADPH cytochrome P-450 reductase from rabbit lung was spectrally, catalytically, and immunochemically identical to hepatic reductase. Comparative results have been reported for rat lung P-450 reductase (61).

The similar catalytic activity of pulmonary and hepatic NADPH-cytochrome P-450 reductase raises the question of why certain chemicals that are reduced by this enzyme in vitro exert their toxic effect primarily in the lung. Chemical activation by one-electron reduction is most often associated with redox cycling and the activation of molecular oxygen. For these types of toxins, the ultimate cellular damage is most probably mediated through the activated oxygen species or, as mentioned earlier, a critical reduction in cellular reducing equivalents. In either case, the presence of molecular oxygen is important for toxicity. Since pulmonary tissue contains the highest oxygen tensions in the body, the process described above could be expected to be potentiated in that organ. This hypothesis might explain lung-selective toxicity of a number of compounds. However, not all chemicals that undergo NADPH cytochrome P-450-mediated redox cycling primarily damage the lungs. The anthracycline doxorubicin covalently binds to isolated perfused rat lung (62) and causes significant damage to perfused dog lung (R. F. Minchin et al, unpublished data). Doxorubicin is reduced to a semiquinone free radical in the presence of NADPH and cytochrome P-450 reductase (63). However, in vivo, the prime sight of doxorubicin toxicity apparently is the heart (64). Pharmacokinetic considerations seem unimportant as doxorubicin concentrations in the lung are generally higher than in the heart (65).

Preliminary studies aimed at identifying the pulmonary sites where oxygen radicals are produced suggest a principal involvement of type II cells (R. F. Minchin et al, unpublished data). Using an adaptation of the histochemical methods devised by Karnovsky and coworkers (66), lung slices were incubated with 1 mM cerium and 0.7 mM nitrofurantoin. Cerium has been shown to react with reduced molecular oxygen, possible H_2O_2 or $O_2^{\cdot -}$, forming electron-dense areas that can be visualized with the electron microscope. These experiments showed that nitrofurantoin produced cerium-derived electron dense areas concentrated around the alveolar type II cells. Further experiments are required to extend these observations and determine if nitrofurantoin and other related pulmonary toxins affect specific cell-types. It has been reported that paraquat selectively damages alveolar type I and II cells at low doses (67) and has been suggested that

the reason for these observations involves a specific transport mechanism for paraquat into specific lung cell-types (68). Thus, the site-specific toxicity of some pulmonary toxins may be the consequence of localized accumulation in the lungs as opposed to localized activating enzyme systems.

Dees et al (69) found that, in rats, pulmonary cytochrome P-450 reductase was located in the pulmonary bronchi and bronchioles when stained by immunofluorescence. Occasional staining within the lung parenchyma was also seen. Similarly, Serabjit-Singh et al (43) have reported the presence of this enzyme in the terminal bronchioles of rabbit lung using immunohistochemical techniques. Significant cytochrome P-450 reductase activity, measured by the reduction of cytochrome C, has been reported in isolated guinea pig alveolar macrophages (70). The subcellular distribution was 59, 15, and 26% of the total cellular activity in the 16,000g pellet, 100,000g pellet, and 100,000g supernatant respectively.

Xanthine Oxidase

The possible involvement of xanthine oxidase in the activation of potential toxins in the lungs has been suggested for both nitrofurantoin and mitomycin C. In rat lung cytosol, nitrofurantoin undergoes reduction that is dependent on the presence of NADH or hypoxanthine but not NADPH or N-methylnicotinamide (28). Reduction was strongly inhibited by allopurinol, which indicates that activation was primarily due to a soluble enzyme with the characteristics of xanthine oxidase. Microsomal activation of this drug, on the other hand, was due to NADPH-cytochrome P-450 reductase (28). Maximum activity in rat lung microsomes was 6–9 nmol bound/mg protein/5 min when activity was quantified as covalent binding under anaerobic conditions. In the cytosol, activity was 5 nmol bound/mg protein/5 min.

Mitomycin C is an antitumor antibiotic capable of inducing extensive lung damage (71) and has been shown to stimulate oxygen-dependent lipid peroxidation in rat lung microsomes (27). Activation is due to the reduction of its quinone moiety to a semiquinone free radical; this reaction can be catalyzed by NADPH cytochrome P-450 reductase as well as xanthine oxidase (58). Other nitroaromatic and quinone drugs will also undergo xanthine oxidase-mediated reduction.

In vivo, the relative contribution of either enzyme may depend on the distribution of each within the lungs. However, no information to date is available on the cellular localization of xanthine oxidase within this organ. The significance of the xanthine oxidase pathway to in vivo cytotoxicity of pulmonary toxins is yet largely unexplored. However, the in vitro data in lung subcellular fractions and purified enzyme systems indicates that this pathway deserves further attention.

DETOXIFICATION OF ACTIVATED METABOLITES IN THE LUNG

Glutathione

An important substance for the protection of lung and other organs against reactive intermediates is reduced glutathione. This endogenous sulfhydryl compound, which is involved in the modification of toxicity initiated by either reactive electrophiles or activated nucleophiles, is ubiquitously distributed throughout virtually all mammalian tissues and is served by a system of specialized enzymes responsible for the synthesis and regeneration of its reduced form.

The characteristics and properties of glutathione that enable the compound to be such an important link in the deactivation pathways of reactive intermediates have been well reviewed (72, 73). Its role in protecting the lung against both electrophiles and nucleophiles are discussed here with particular emphasis on the changes in pulmonary glutathione status during enzymatic activation of potential toxins.

As can be seen in Figure 1, glutathione may assist in deactivating electrophiles by direct conjugation; these reactions can be enzymatically driven or occur spontaneously. A family of enzymes, glutathione transferases, are responsible for the enzyme-mediated pathways. Transferases have been identified in lung tissue from several species. Work from Bend's laboratory has shown that substrates such as styrene oxide and benzo(a)-pyrene-4,5-oxide form conjugates with glutathione in perfused lung preparations (74, 75). Experiments with isolated rabbit lung cells showed that, using benzo(a)pyrene-4,5-oxide and 5mM glutathione, glutathione-S-transferase activity in intact cells was only 30% of that in sonicated cells (76). These data suggest that intracellular glutathione is insufficient to support maximal transferase activity and that glutathione availability may be rate-limiting.

Many electrophiles that undergo conjugation with glutathione are capable of reacting with the sulfhydryl compound nonenzymatically. Highly reactive metabolites such as those derived from 4-ipomeanol can readily conjugate with gluthathione, and little or no transferase activity appears to be involved in the reaction (21). In vitro experiments with isolated pneumocytes and lung microsomes indicated that 4-ipomeanol forms two glutathione conjugates. Ipomeanol has been shown to decrease lung total glutathione content, a phenomenon that can be prevented by pretreatment with piperonyl butoxide (9).

Conjugation with glutathione could be expected to protect against lung damage initiated by alkylating agents. If glutathione represents a preferred site of interaction, a threshold level for the onset of tissue alkylation, equivalent to some critical reduction in the cellular glutathione, would be expected. Such is not the case with 4-ipomeanol: no threshold effect was

observed over a wide dosage range (7). Glutathione appears to provide an alternative binding site for the activated furan rather than a preferred site. This phenomenon is probably a function of the extremely reactive nature of 4-ipomeanol, which alkylates macromolecules so rapidly that little, if any, escapes the site of activation.

Several recent studies have shown that pulmonary toxins that generate reactive oxygen species also perturb the glutathione balance within the organ. As can be seen from Figure 1, glutathione is active in disposing of peroxides formed from the reduction of molecular oxygen. In these reactions, the disulfide form of glutathione ("oxidized glutathione") is produced and can be subsequently reduced back to free glutathione at the expense of NADPH. The oxidation-reduction turnover of glutathione may produce additional stress on the reducing equivalents of the cell which, as discussed above, represents a possible mode of toxicity.

Dunbar et al (11) have shown that perfusion of rabbit lungs with nitrofurantoin results in a marked decrease in the ratio of reduced to oxidized glutathione in the tissue and a significant leakage of glutathione into the perfusion medium. Similarly, paraquat has been reported both to decrease the tissue ratio of reduced to oxidized glutathione and to increase total efflux of glutathione from perfused rabbit lungs (77). Ventilation of the lungs with 95% oxygen potentiated the effects of paraquat on pulmonary glutathione status. The results with both alkylating agents and pulmonary toxins capable of activating molecular oxygen indicate that both electrophilic and nucleophilic intermediates may decrease total lung glutathione content. Alkylating agents can bind directly with the peptide forming conjugates, whereas reduced intermediates such as those that may result from the activation of nitrofurantoin or paraquat can cause glutathione leakage from the cell. Unlike alkylating agents, however, activated nucleophiles seem able to alter the cellular ratio of reduced to oxidized glutathione.

Lung concentrations of glutathione range from 2–4 mM in rats (9) and rabbits (R. F. Minchin and M. R. Boyd, unpublished observations), which are somewhat lower than hepatic concentrations (6–8 mM) (9, 78). Within the lung itself, glutathione may be hetrogeneously distributed. Devereux and associates (36) found that isolated rabbit type II cells contain about 5.5 nmol/mg protein while isolated rabbit Clara cells contain only 2.6 nmol/mg protein. However, the method used to quantify glutathione also measured other low molecular weight sulfhydryl compounds. Moreover, it is not known whether the procedures for isolation of the different cell-types affected the final glutathione content.

Ascorbic Acid

As noted previously, ascorbic acid reacts with activated oxygen species, rendering these intermediates nontoxic. Furthermore, ascorbate readily

reacts with activated electrophiles such as the N-acetylimidoquinone of acetaminophen (79) and various other alkylating agents (80). The apparent high reactivity of ascorbic acid is due to the low redox potential of the ascorbate radical/ascorbic acid couple which, at pH 7.5, is about 0.31 V (81).

Several studies have shown that ascorbic acid may be an important component of the pulmonary defense system against reactive intermediates. As early as 1964, ascorbate was reported to protect against oxygen toxicity in vivo (82). In microsomal suspensions isolated from guinea pig lung, ascorbate (0.25 mM) was shown to cause a 52% decrease in Fe^{+2}- initiated lipid peroxidation, the result of superoxide formation via the Weiss reaction (83). Furthermore, the spontaneous formation of malonaldehyde in neonatal rat lung can be significantly inhibited by ascorbic acid (84). Pulmonary toxicity of paraquat is reportedly diminished by ascorbate (15) in mice although other investigators have illustrated a synergistic toxic response in rats to these two chemicals (85). The cause of the apparent discrepancies in these findings is still unknown.

Lung contains a specific energy-dependent transport process for ascorbic acid capable of accumulating the vitamin to tissue concentration over 16 times that in the plasma (86). In whole lung, approximately 30% of the pulmonary ascorbate is associated with the lining of the alveolar space (87). In isolated single-cell suspensions, rat lung accumulated ascorbic acid linearly for up to 60 min (86). Similar results were seen in isolated perfused rat lungs, and it was further shown that oxidized ascorbate was not accumulated (88). Recent work with isolated lung cells has shown that the type II cells and pulmonary macrophages are a major site of ascorbic acid uptake (89). These results and others concerning the in vitro and in vivo antioxidant effects of ascorbic acid indicate that this chemical might be an important component of the lung's defense against metabolically activated toxins. Evidence that ascorbic acid is involved in modifying covalent binding of electrophiles to lung tissue is lacking. However, extrapolating data obtained with acetaminophen in hepatic microsomes (79) and ascorbic acid effects on benzo(a)pyrene carcinogenesis (90) in rats strongly suggests that its role in the protection of lung against reactive electrophiles warrants investigation.

Pulmonary Enzymes Protecting Against Activated Oxygen

Several recent studies have attempted to identify reduced oxygen species as the primary cause of toxicity of various chemicals in lung. While the production of superoxide anions and hydroxyl radicals by known lung toxins can be easily shown in vitro, their production and cellular toxicity in vivo have not been definitively demonstrated. A number of studies, investigating activated molecular oxygen in the lungs, have documented that this organ,

like most in the body, contains specific enzymes designed to protect the cell against reactive oxygen species. Of these, superoxide dismutase, catalase, and glutathione peroxidase have received particular attention. As shown in Figure 1*B*, all three enzymes are involved in the removal of potentially toxic forms of oxygen from the cell.

Superoxide dismutase is found in both the mitochondrial and cytosolic fractions of homogenized rat lung (10); the cytosolic enzyme is approximately 5-fold higher in activity than that in the mitochondria. Dismutase activity in lung tissue is inducible by exposure of the lungs to high oxygen tensions either in cultured cells or in vivo. Stevens and coworkers (91) found that treatment of neonatal rats with 95% O_2 for 24 h resulted in specific induction of dismutase activity in the pulmonary macrophages. Almost all of the increase was due to changes in the mitochondrial enzyme. Type I and II alveolar cells and interstitial lung cells showed no change in activity. No induction in the dismutase activity of any cell-type was seen in adult rats treated in a similar manner. More recently, Forman & Fisher (10) found that exposure of adult rats to 85% O_2 for 2–7 days caused a marked increase in the mitochondrial-associated superoxide dismutase activity in the alveolar type II cells. Little change was seen in the dismutase activity of pulmonary macrophages.

The distribution of superoxide dismutase has been shown to vary considerably within the lung (91). In neonatal rats, pulmonary macrophage activity is 1.5–2 times that in the type II or interstitial cells. Type I pneumocytes exhibited minimal activity (<10% of type II activity). In adult rats, superoxide dismutase activity was similar in macrophages, type II, and interstitial lung cells, but remained low in the type I cells. Whether compared in terms of protein or DNA content, rat alveolar type II cells and pulmonary macrophages possessed significantly greater dismutase activity than whole lung (10). Ody and colleagues (92) have reported that porcine pulmonary endothelial cells, isolated from the pulmonary artery, contained superoxide dismutase activity of about 20 U/mg protein compared to whole lung which was 15–16 U/mg protein. These studies suggest that superoxide dismutase is not homogeneously distributed throughout the lung. The consequences of these observations with respect to pulmonary toxicity of xenobiotics are unknown.

Catalase is responsible for the reduction of hydrogen peroxide to nontoxic molecular oxygen and water. In most tissues, catalase is associated with specific microperoxisomes that can be identified histochemically. Peroxisomes have been found in the lungs of mouse and rat (93), pig (94), rabbit, and monkey (95). Bucher & Roberts (96) have shown that rat pulmonary catalase increases from approximately 400 IU/lung at birth to nearly 600 IU/lung at day 12. Exposure of neonatal rats to oxygen concen-

trations greater than 40% produced a significant increase in the pulmonary catalase activity, which indicates that, like superoxide dismutase, catalase is inducible by high oxygen tensions (91).

In pig lung, Goldenberg and coworkers (94) histologically demonstrated that "catalase-positive particles" were localized within the Type II pneumocytes and bronchiolar Clara cells. In the type II cells, the catalase peroxisomes often appeared to be associated with the smooth endoplasmic reticulum cisterns. No such relationship was obvious within the Clara cells. Isolated rat macrophages have also been shown to contain significant catalase activity (97). Histochemically, macrophagal catalase was localized within a membrane-lined granule associated with the smooth endoplasmic reticulum.

Peroxides can be formed in vivo and produce significant tissue damage if the cellular concentration is not maintained at low levels. Glutathione peroxidase is able to reduce peroxides via a reaction involving the transfer of electrons from reduced glutathione to the peroxide (Figure 1*B*). Unlike superoxide dismutase and catalase, glutathione peroxidase does not appear to be induced by exposure of the lungs to high oxygen tensions (10, 25).

Peroxidase activity in guinea pig pulmonary macrophages was primarily found in the microsomal and cytosolic fractions of sonicated cells (70). Exposure of the macrophages to 85% O_2 produced a time-dependent decrease in enzyme activity. Forman & Fisher (10) found that type II cells isolated from rat lungs contain substantially higher glutathione peroxidase activity than whole lung. The localization of catalase within the type II pneumocyte has been suggested to indicate a possible role of this enzyme in cellular functions other than simply protection from hydrogen peroxide (94). Likewise, glutathione peroxidase may have specific functions within the cell apart from peroxide detoxification.

CONCLUSION

The lung is a complex organ with a great deal of histological and biochemical heterogeneity. The distribution of systems responsible for the metabolic activation of lung toxins and/or their detoxification can vary substantially from one cell-type to another. This suggests that localized damage by xenobiotics requiring metabolic activation can, at least in part, be attributed to the localization of such systems. Recent advances in the isolation of different lung cell-types in reasonably pure fractions have allowed for investigation of the metabolic capabilities of some individual cell-types.

A more complete understanding of the mechanisms of pulmonary metabolic activation and deactivation of toxic chemicals and drugs may contribute to our knowledge of the underlying causes of various pathological

processes such as cellular necrosis, fibrosis, emphysema, and cancer, any of which may arise from the interaction of chemical toxins with lung tissue. We hope that this review has drawn attention to some of the newer and less understood aspects of pulmonary activation/deactivation of toxic drugs and chemicals.

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