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# Mechanisms in pulmonary toxicology

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Pulmonary toxicology can be divided in two broad categories based on the route of exposure to the offending agent. The two routes that lead to the lungs are the airway (inhalation) and the blood stream. For the most part, inhalation implies nontherapeutic agents, such as cigarette smoke, sniffed drugs, and other environmental and occupational pollutants; however, therapeutic agents also can be administered through the respiratory tract. This includes the administration of oxygen, anesthetic gases, and various respiratory, as well as non respiratory, medications (eg, bronchodilators, insulin). Ocular medications may also reach the airways. The blood stream gives another route to the lungs and can be tainted with toxic and therapeutic agents. Illicit drugs, and environmental or dietary substances, such as pesticides, solvents, soil, and water or food contaminants are toxic agents that can be found in the blood stream. Despite the use of the airway tract as a mean of administrating therapeutic drugs, the blood stream remains the most relevant route for dispensing medications. Thus, the lung circulation can be spoiled with medications that can threaten the homeostasis of the respiratory tract. Several single-case reports presented examples of adverse respiratory tract reactions following the use of certain medications [1,2]. The true mechanism of drug-induced lung injury has yet to be elucidated. Most of what we know about the mechanism of this type of lung injury comes from studies of agents that cause hepatotoxicity or of pneumotoxic compounds. This article discusses the general issue of drug-induced lung injury and attempt to answer to these three questions. How do drugs cause (lung) cell injury? Why do drugs cause cell injury in the lung? Why are some individuals more sensitive to drug-induced lung injury?

## How do drugs cause lung cell injury?

### *Bioactivation*

Most chemicals do not cause cell toxicity directly, except in the case of membrane irritants or receptor agonists/antagonists. An example of adverse respiratory effects that are caused by pharmacologic interactions with specific receptors includes induced bronchospastic reactions (mainly in asthmatics) by β-adrenergic antagonists or by anticholinesterase agents. Nevertheless, direct respiratory mucosal irritation is almost solely limited to cases of inhaled pollutants.

Usually, some form of biotransformation (“bioactivation”) is required for chemical agents to cause cell injury. The process of biotransformation of foreign chemicals (or xenobiotics) is generally described as a succession of phase I reactions followed by phase II reactions (Fig. 1). Phase I reactions consist primarily of oxidative reactions that are catalyzed by a variety of enzymes, such as the cytochrome P450-dependent mono-oxygenases, flavin-containing mono-oxygenases, or prostaglandin synthase. The cytochrome P450 (CYP) superfamily of enzymes is the main system that is involved in the initial biotransformation of xenobiotics. This system is characterized by several enzymes that have different substrate specificities and variable degrees of genetic polymorphisms. Phase II reactions consist of conjugate reactions whereby the metabolites are coupled to yield more water-soluble

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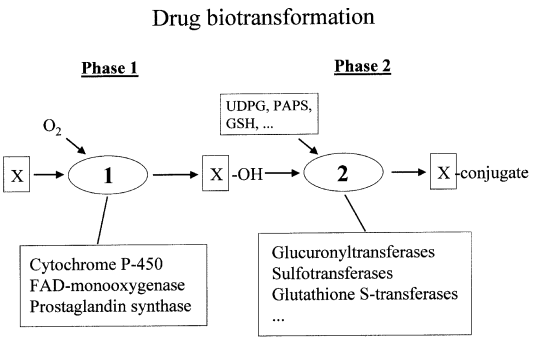


Fig. 1. General principle of the biotransformation of drugs. Oxidative reactions in phase I. Conjugate reactions in phase II. X, drug concerned; FAD, flavin-containing mono-oxygenase; PAPS, phosphosulfate; UDPG, uridin diphospho glucuronic acid.

conjugates that can be excreted. The coupling of the metabolites is governed by the action of glucuronyl transferases; sulfotransferases; glutathione-S-transferases; and other transferases with endogenous molecules; such as glucuronic acid, sulfate, glutathione, and so forth. Thus, biotransformation is essentially a detoxification process that accelerates the excretion of lipid-soluble molecules by rendering them more water-soluble. Conversely, this process may increase the toxicity of chemicals by producing reactive metabolites. Most of the time, these reactive metabolites result from phase I reactions. If these reactive metabolites are not readily removed by enzymatic (phase II reactions) or nonenzymatic reactions, they may cause cell injury and death by covalently binding to macromolecules, such as functionally or structurally important proteins, and nucleic acids. When this occurs, biotransformation becomes a toxic activation or "bioactivation" process.

*Bioactivation and oxidative stress*

Such metabolic activation leads to the production of reactive electrophilic metabolites or activated oxygen species (see Fig. 1; Fig. 2). The potential covalent binding of reactive electrophilic metabolites to essential macromolecules (eg, enzymes, nucleic acids) leads to cell dysfunctions or mutations. In addition to producing electrophilic metabolites, drug bioactivation may also lead to the production of toxic oxygen species (TOS) (see Fig. 2), such as superoxide anion ($O_2^{-\bullet}$), hydrogen peroxide ($H_2O_2$), and the hydroxyl radical ($OH^\bullet$). The TOS depletes the reducing equivalents and oxidative stress, which eventually results—when the available antioxidant defense systems are overwhelmed—in cell dysfunction and lipid peroxidation. These oxygen radicals generate singlet electron transfers that can disrupt critical cellular functions provided that they overcome the powerful and complex antioxidative defense systems. Amid those radicals, oxidant scavenging molecules such as glutathione (GSH), vitamin E [3], ascorbic acid, and uric acid and the enzymes superoxide dismutase (SOD), catalase, GSH peroxidase, GSH synthetase, glutathione disufilde (GSSG) reductase, GSH tranferase, and semidehydroascorbate reductase are included [4]. Three different types of SOD are found: (1) CuZnSOD

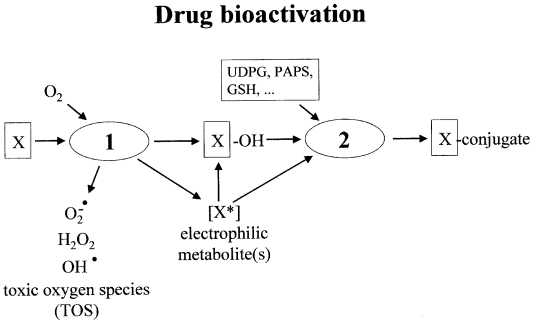


Fig. 2. Bioactivation of a drug to produce toxic oxygen species or reactive electrophilic metabolites. *, electrophilic; •, electron.

that is constitutively expressed in bronchial epithelium, (2) MnSOD that is induced by oxidants, and cytokines in alveolar macrophages and epithelium, and (3) extracellular SOD in alveolar macrophages and extracellular fluids [5]. Catalase is constitutively expressed in pneumocytes, neutrophils, macrophages, and bronchial epithelium. High levels of glutathione are found in lung epithelial lining fluid and type II pneumocytes [6]. Bilirubin is another powerful antioxidant. Hyperbilirubinemia increases the excretion of oxidative metabolites in the urine of bleomycin-injured rats and simultaneously decreases lung inflammation and pulmonary fibrosis [7]. Some individuals may be deficient in cellular antioxidant GSH, a protective agent against oxygen radicals that makes these individuals more prone to developing drug-induced lung injury. Toxicants also can upset the oxidant-antioxidant balance of cells. They do by inhibiting the enzymes, reacting with oxidant scavengers, or forming free radical intermediates that initiate uncontrolled tissue reactions with molecular oxygen.

The biomembranes are composed primarily of phospholipids and proteins. The membrane phospholipids are rich in polyunsaturated fatty acids that are highly susceptible to oxidative damage [4]. When lipid peroxidation of the membrane is induced, oxygen free radicals are produced endogenously. This endogenous production of oxygen free radicals can be assessed by quantifying the primary and secondary peroxidation end-products. Amid those peroxidation end-products, the prostaglandins [8,9] and the $F^{2-}$ isoprostanes, prostaglandin-like compounds that do not require the cyclooxygenase enzyme for their formation, seem to be reliable markers of oxidant stress in various animal models [10,11]. Cell injury in the form of cell membrane peroxidation is followed by inflammation and repair (Fig. 3), which, in some circumstances, may be excessive or uncontrolled [10].

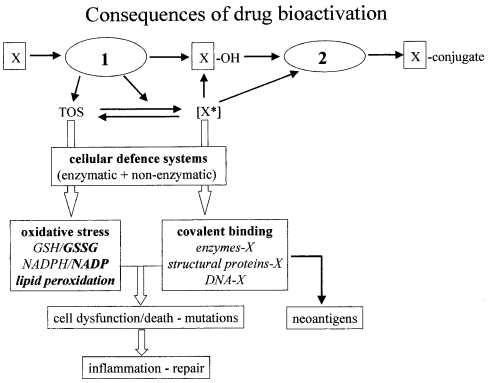


Fig. 3. Consequences of cell bioactivation.

*Inflammation and repair*

Blood circulating leukocytes reach and infiltrate the lung tissue through the capillary endothelial wall (recruitment by chemotactic cytokine wave, such as chemokines from the CXC, CC,C and $CX^3C$ families). Once in the lung tissue, the leukocytes become activated and the phagocytic leukocytes (macrophages) can settle in the lung tissue. This process of lung tissue infiltration is clinically illustrated by the perpetuation of neutrophilic alveolitis. Neutrophilic alveolitis is correlated with an increased level of interleukin (IL)-8 (from CXC chemokine family) whose cellular sources seem to be alveolar macrophages and pulmonary fibroblasts [12]. Furthermore, with neutrophilic alveolitis the presence of CC chemokines, such as monocyte chemoattractant protein-1 and macrophage inflammatory protein-1$\alpha$, all coming from macrophages, eosinophils, and epithelial cells underscores the link between neutrophilic alveolitis and the phagocytic leukocytes.

Depletion of these chemokines results in the reduction of the infiltrating cells and in the total lung collagen as determined by lung hydroxyproline content [12–14]. The resolution of that inflammation can either be of no consequence (predominance of type-1 [Th1] cytokines, including interferon-$\gamma$ and IL-2) or result in fibrosis. The fibrosis arises from fibroblast stimulation by way of the elaboration of type-2 (Th2) cytokines (IL-4, IL-5, IL-10, IL-13) [12]. Therefore, the progression—leading to fibrosis—or the resolution of the inflammatory process depends on the ratio of Th2:Th1 cytokines.

Lung injury that is induced by pneumotoxic agents or by oxidant gases gives rise to alveolitis and edema. In animal models, alveolitis and edema can be detected by means of bronchoalveolar lavage (BAL) fluid analysis. The bronchoalveolar lavage fluid is analyzed for the presence of neutrophils, protein, and lactate dehydrogenase (LDH), a cytoplasmic enzyme whose extracellular levels increase if cells are necrotic [15], and angiotensin-converting enzyme (ACE) that accompanies the endothelial cell injury [16,17]. Oxygen-driven type II cells' toxicity of paraquat and its enhancement by increasing partial pressure of oxygen were assessed through the same means and targeted on the release of LDH in culture medium [18]. This type of tissue inflammation is usually followed by fibrosis. The fibrosis is best evidenced by treating animals with bleomycin. Following bleomycin treatment the animals' BAL fluids were analyzed and showed a fibroblast-stimulating activity and an increased amount of fibronectin (a fibroblast chemoattractant and a fibroblast growth factor). The

analysis pointed to the alveolar macrophages as the origin of the fibrosis [15].

*Bioactivation in the lung*

The processes of drug biotransformation and bioactivation have been extensively studied in the liver, which is the main drug-metabolizing organ of the body. Drug biotransformation and bioactivation, however, occur in other organs, including the lungs [8,19,20]. The lungs contain the necessary enzymatic systems to metabolize foreign compounds [21,22]; however, the contribution of the lungs to the overall metabolism of chemicals is much less than that of the liver. The cytochrome P450 system exists in the lung at 10% to 20% of the hepatic levels. Although the overall cytochrome P450 lung concentration is lower than in the liver, in specific lung cells the levels may be as high as in hepatocytes.

Moreover, the types of cytochrome P450 "isozymes" and their relative distribution in these lung cells are not necessarily the same as in hepatocytes. With the advent of the reverse transcriptase-polymerase chain reaction technology, it is possible to detect minute amounts of mRNA in tissue samples. Expression of various CYP enzymes has been shown at mRNA and protein level in the human lung [23]. Thus, CYP1A1 has been detected only in the lungs of smokers [24,25], where it is located mainly in terminal cuboidal epithelial cells and in type II alveolar cells [26]. Both cell types are involved in the development of peripheral lung cancers. Other subtypes (CYP2B6/7, 2 E1, 2F1, CYP3A4, 3A5) have a widespread, but variable, distribution in bronchi, bronchioli, macrophages, alveolar epithelium, and endothelium [27]. CYP2B7 has been detected in human Clara cells [28], whereas CYP2C and likely CYP4B1 have been found in serous cells of bronchial glands [29]. Various CYP450 forms can be induced in the lung, either by smoking (1A1 & 1B1) [25] or by glucocorticoids [30]. The expression of CYP enzymes also seems to be regulated by genetic polymorphism. Furthermore, constitutional variability could contribute to the individual sensitivity to environmental chemicals [31], drug activity, and toxicity [9,22,32].

Certain lung cells are capable of biotransforming some foreign chemicals; however, this was demonstrated mainly in animal models and only for model compounds rather than for well-established pneumotoxic drugs. Recent in vitro studies of alveolar macrophages and type II pneumocytes that were isolated from rat lungs suggest that pulmonary CYP450 and likely other enzyme systems (prostaglandin H synthetase) can bioactivate the analgesic and antipyretic paracetamol (acetaminophen) to a cytotoxic metabolite [33]. According to Dimova et al (unpublished data), these observations that were made on isolated rat lung cells are applicable to human lung cells. If this pertains to in vivo situations, theoretically it could promote inflammation and subsequent atopy [34].

The production of oxidative stress in the lung has been best described for a nontherapeutic agent, the herbicide, paraquat [35]. Paraquat is reduced to the paraquat radical by a one-electron reduction reaction catalyzed by a nicotinamide-adenine-dinucleotide-phosphate (NADPH)–dependent reductase that is associated with cytochrome P450 (Fig. 4). The paraquat radical is unstable and is reoxidized immediately by molecular oxygen; the end result is the production of a superoxide anion. The cyclic reduction-oxidation of paraquat leads on one hand to the nonstoichiometric production of superoxide anions, which, in turn, produces further reactive oxygen species, and, on the other hand, to the progressive depletion of NADPH and of the antioxidant defense systems. Several studies suggested that these events are also applicable to the human lung [18].

This oxidative stress also is encountered upon exposure of the lung to high concentrations of oxygen and in the pulmonary toxicity that is caused by nitrofurantoin and bleomycin [8]. The mechanism of nitrofurantoin toxicity bears a strong resemblance to that of paraquat. Under aerobic conditions and in the presence of NADPH, microsomes catalyze a one-electron reduction of the nitro group of nitrofurantoin to yield a nitrofree radical. The nitrofree radical spontaneously reacts with oxygen to regenerate the parent compound and the superoxide anion radical ($O^{2-\bullet}$). In the case of bleomycin, the cellular toxicity seems to be due to the existence of a bleomycin-iron complex that generates oxygen-derived species within the lung (see Fig. 4). The role of metals (particularly of iron, the concentration of which is increased in smokers) in pulmonary

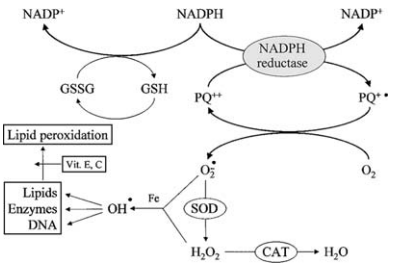


Fig. 4. Oxidative stress caused by paraquat (PQ). Reduction of PQ by NADPH reductase. PQ reoxidation produces superoxide anion. CAT, catalase; NADPH, nicotinamide-adenine-dinucleotide-phosphate; •, electron.

toxicity is put forward by the observation that depletion of iron, by dietary means or by deferoxamine, an iron chelator, reduces the risk of bleomycin-induced pulmonary toxicity [8]. The concept that some drugs may cause oxidative stress and that their toxicity may be enhanced by the (therapeutic) administration of oxygen is of paramount importance in clinical settings. In animal and human experiments on paraquat poisoning, nitrofurantoin and bleomycin, such synergy between drug-induced injury and oxygen was well-demonstrated [8].

*Direct cytotoxicity in the lung*

Lung injury that is associated with chemotherapeutic drugs (eg, bleomycin) also may be due to a direct cytotoxic reaction. Drug accumulation in the cells leads to DNA fragmentation and atypical cells generation. Contrary to other epithelial cells, lung and skin epithelial cells contain lower levels of a specific enzyme that inactivates bleomycin. Therefore, bleomycin can accumulate within the cell and induce DNA fragmentation [36].

Bleomycin toxicity seems to be related to dosage and age. A large total dose can overwhelm the bleamycin hydrolase enzyme, especially in enzyme-deficient individuals. Individuals who are older than 70 years of age are deficient in the enzyme. Therefore, the dosage of bleomycin at which toxicity occurs is, in fact, closely related to the individual levels of inactivating enzyme. The injury starts on type I alveolar epithelial cells.

Dead cells are replaced by an alveolar type II repairing proliferation, which differentiate into type I cells to restore the normal air–blood barrier. The vulnerability of the type II cell to bleomycin depends on the state of its cell cycle. If the type II cell is in the resting G0 phase, the cell seems to be resistant to injury, whereas if the cell is proliferating or differentiating, atypical exaggerated metaplasia occurs [37]. Type I cells are particularly vulnerable to injury by cytotoxic drugs, whereas in the steady state the type II cell is generally considered to be injury resistant. By dosing the drug repeatedly, it increases the likelihood that type II cells will be exposed while they are vulnerable during their proliferative or differentiating periods. It is not known if chemotherapeutic drugs directly stimulate the formation of oxygen radicals; however, when injury has taken place there is a loss of the normal cellular antioxidant substances (GSH, superoxide dismutase), which leads to oxygen toxicity. The histologic findings of cytotoxicity are characteristic of a chemotherapeutic drug effect by virtue of the marked atypia that is produced in type II pneumocytes. Fewer type I cells are present; there are more type II cells with bizarre nonmalignant changes, the nuclear to cytoplasmic ratio remains normal, and there is not an increased number of mitotic abnormalities [37,38]. A marked inflammatory reaction takes place in the interstitium. First, there is a neutrophil adhesion to vascular endothelial cells, then their migration; activation in lung parenchyma follows. In recent studies of bleomycin-challenged mice, the neutrophil recruitment parallels, with a slight delay, the mRNA induction of adhesion molecules, E-selectin, P-selectin, intercellular adhesion molecule 1 (ICAM-1), and vascular adhesion molecule 1 (VCAM-1) [39,40].

One of these studies showed that during the early phase of injury 14-membered ring macrolides inhibited the expression of VCAM-1 mRNA, and, perhaps, ICAM-1. This inhibition produced an attenuated inflammatory cell migration and subsequent fibrosis, which suggests a potential therapeutic role for the 14-membered ring macrolides [40].

*Cytotoxicity, inflammation, and fibrosis*

As a consequence of the net loss of type I alveolar epithelial cells, a fibroblastic reparative process is initiated. Fibroblasts then are directly stimulated to increase collagen synthesis. Next, there is deposition of fibrin and collagen in the septal wall [38].

After lung injury, apoptosis, an active form of cell death that requires the activation of specific enzymes and other components of signaling pathways, seems to play a role in lung tissue remodeling. The involvement of apoptosis in lung injury is at least two fold: after hyperplastic repair for the clearance of excess epithelial stem cells and in the resolution of fibrotic lesions for the removal of mesenchymal cells in excess [41].

Another potential involvement of apoptosis is in the pathogenesis of lung fibrosis. In lung presenting fibrosis, and especially in bronchiolar and alveolar epithelial cells, heavy labeling of fragmented DNA (apoptosis marker) colocalizes with regions of heaviest myofibroblast activity and collagen accumulation. A human fibroblast-derived factor that was responsible for the killing of alveolar cells was isolated and identified as angiotensin II. Apoptosis of epithelial cells, rather than inflammation, could lead to a fibrogenic response; intratracheal instillation of bleomycin in mice is associated with the upregulation of Fas (the ''death receptor'' of apoptosis in bronchial and alveolar cells) and the concomitant induction of epithelial apoptosis as a prelude to fibrogenesis [42]. Knockout mice who were deficient in Fas were resistant to this profibrotic effect [43]. An inhibitor of apoptosis

should prevent the subsequent fibrosis. This was confirmed with the ACE inhibitor, captopril [41].

Another element in cellular injury that is due to bleomycin is the release of 5-cystineyl-leukotrienes. High levels of 5-cystineyl-leukotrienes can be found in BAL of bleomycin-injured wild type mice. The high levels of 5-cystineyl-leukotrienes are concomitant with collagen synthesis, high hydroxyproline levels, and inflammatory cell recruitment. Lower levels of collagen, hydroxyproline, and inflammatory cells are found in bleomycin-injured, 5-cystineyl-leukotriene gene knock-out mice; this underscores the role played by leukotrienes in lung inflammation when the inflammation is related to cytotoxic drugs. Moreover, higher levels of $\gamma$-interferon and prostaglandin E2 are found in these knock-out mice [44].

## Why do drugs cause cell injury in the lung?

We have discussed that xenobiotic metabolism and bioactivation can take place in the lungs, but why certain drugs cause toxicity specifically in the lungs and not in the liver or other organs that are also capable of drug bioactivation, remains to be explained. Various reasons may be put forward to explain such organ specificity: (1) some substances reach higher cell or tissue concentrations in the lung rather than in other organs, (2) a specific pattern or extent of bioactivation occurs in the lung, and (3) the consequences of bioactivation are lung specific.

Some foreign compounds may accumulate preferentially in lung tissue. Various mechanisms may underlie such pulmonary accumulation: (1) the drug may be sequestered in the lungs as a result of its chemical properties, (2) the specific anatomical situation of the lungs may be critical, and (3) a specific pulmonary uptake system may exist.

Passive sequestration in the lungs has been well described for amphiphilic drugs: chlorphentermine, imipramine, quinine, chlorcyclizine, propanolol, and, especially, amiodarone. This cellular sequestration in lung macrophages and alveolar type II cells induces phospholipidosis; presumably the presence of the drugs in lysosomes interferes with the normal catabolism of surfactant phospholipids [8].

In individuals who react adversely to amiodarone, there is a marked accumulation of abnormal alveolar macrophages that contain lamellar inclusions with a variety of phospholipids. It is not known whether the accumulation is a secondary effect or if the interstitial pneumonitis primarily results from phospholipid accumulation with infiltration by neutrophilic polymorphonuclear cells and lymphocytes [45]. The cellular profile of BAL is highly variable (neutrophilic, mixed, lymphocytic patterns); a cellular pattern is neither predictive of a detrimental outcome (fibrosis) nor related to a daily or a total dose [8,46]. More than 20 cationic amphiphilic drugs are known to induce phospholipid storage disorder in cells. The process is reversible when discontinuing the drugs, but it may induce fibrosis or bronchiolitis obliterans with organizing pneumonia (BOOP). The process may also trigger an acute respiratory distress syndrome (ARDS) if an additional oxidative aggression occurs as a result of oxygen therapy [47,48].

Selective drug toxicity in the lungs also may result from the release of toxic metabolites by the liver. The first capillary bed that is reached by such metabolites consists of the pulmonary endothelium. This mechanism has been invoked to explain the pronounced pulmonary toxicity of the pyrrolizidine alkaloid, monocrotaline. Monocrotaline is a toxic compound for producing and investigating pulmonary hypertension in animal models. In rats, this compound is mainly, if not exclusively,metabolized in the liver. From the liver, the metabolites that have a high toxicity for the pulmonary endothelium are released. It is not known whether this drug-induced pulmonary hypertension has any direct relevance to human disease.

Another argument for the existence of an active uptake system to explain the pulmonary toxicity of a drug is the mechanism of paraquat toxicity. Paraquat (1,1′-dimethyl-4,4′-bipyridylium chloride) is a contact herbicide with high systemic toxicity, especially for the lung where paraquat concentrations are higher than in the blood. These high pulmonary levels are due to an active uptake that occurs there and not in other major organs [49]. The paraquat uptake system has been demonstrated in the lungs of all mammalian species that have been examined [50], including humans [51]. The endogenous substrates for the uptake system consist of the diamine 1,4-diaminobutane (putrescine) and other oligoamines, such as spermidine and spermine. The specific cellular sites of the polyamine uptake system were shown to be mainly the alveolar epithelial cells [16,52–54]. The physiologic reason for the existence of a particularly active polyamine uptake system in these cells is not known and apart from paraquat, no other drugs seem to be implicated in this process.

Besides the possibility that higher concentrations of a drug or its metabolites accumulate in the lungs, specific pneumotoxicity may result from a particular type of biotransformation in the lung. This could involve a higher degree of bioactivation to a toxic

metabolite in the lung than elsewhere, a lesser degree of detoxication of active metabolites, or a combination. Such processes have been documented mainly with experimental pneumotoxic agents, such as butylated hydroxytoluene, 4-ipomeanol, O,S,S-trimethylphosphorodithioate, naphthalene, and 3-methylindole.

*Intrapulmonary vasoactive substances could induce lung toxicity by a direct activity on the pulmonary vessels*

In theory, pulmonary vascular damage can be revealed by increases in serum ACE levels, because this enzyme is localized on the plasma membrane of the pulmonary endothelial cells and can be released into the circulation. In animals, serum ACE was elevated transiently after administration of thiourea, paraquat, bleomycin, and bis-chloro-nitrosurea (BCNU) [55]. With BCNU, however, decreases of serum ACE also are found. Careful attention must be given to the time course of changes in serum ACE levels in drug-induced damage; its use to assess microvascular injury cannot be considered conclusive [16].

Recent evidence indicates that the induction of apoptosis of alveolar epithelial cells requires the de novo synthesis of angiotensin II (ANG) by the epithelial cell, and can be prevented by ACE inhibitors, ANG receptor antagonists, and other agents that are capable of blocking ANG synthesis or function [41]. The antifibrotic effect of these agents could at least partly be attributed to their ability to prevent the apoptotic death of the epithelial layer. Investigations of epithelial cells apoptosis in response to amiodarone found that apoptosis could be completely abrogated by ACE inhibitors or ANGII receptor antagonists [41]. ANG inhibition prevents fibrosis and inversely β2-agonists induced edema [56]. Based on normal capillary pressures during edema and the discovery of near plasmatic protein levels in bronchial suctions, an increased permeability of the lung capillaries was incriminated [57]. This increased permeability must be major because cyclic adenosine monophosphate (cAMP) agonists (ie, β-adrenergic agonists) were shown to enhance alveolar fluid clearance in multiple models of lung injury, including hyperoxic lung injury [58]. Amitriptyline and likely other tricyclic antidepressants induce ARDS, probably by increasing endothelial permeability due to impaired tight junction function mediated by way of intracellular calcium changes [8]. In addition to synthetic drugs, biomolecules can induce infiltrative lung disease. Infiltrative lung disease may result from damage to pulmonary vessels [59] during the transit, aggregation, and sequestration of activated blood cells or progenitors within the pulmonary circulation including immunoglobulins and antithymocyte globulin [60,61]; substances that modulate the growth, release, or maturation of blood cells or progenitors (granulocyte [G]- or granulocyte-monocyte [GM]–colony stimulating factor [CSF]) [62,63]; blood transfusions; blood products that contain antileukocyte or anti-HLA of donor origin [64]; and pulmonary cytolytic thrombi from stem cells transplantation [65]. In a bone marrow transplant model in mice, intense monocytic cellular infiltrate of activated macrophages that was preceded by an acute increase in monocyte chemotactic protein-1 and macrophage inflammatory protein-1α caused substantial oxidative stress that was manifested by increases in lung lipid peroxidation and oxidized glutathione [66]. These mechanisms could explain the ''idiopathic pneumonia syndrome'' that can occur after autologous transplantation [67].

## Why do drugs cause cell injury in the lungs of only some individuals?

Although the aforementioned biologic processes are all plausible mechanisms to explain the pneumotoxic properties of some chemicals, adverse effects of drugs are only seen in a minority of treated patients. In most instances, the individual susceptibility factors that determine why a person experiences serious adverse effects from a particular drug, whereas most other patients remain unaffected, are not clear. Nevertheless, several scenarios can be proposed to explain why drugs cause lung injury in only some individuals.

The delivery of a drug through the lung depends on its route of administration (1) inhalation for aerosolized substances or gases or (2) blood flow for infused or ingested drugs. The pattern of inhaled particle deposition in the lung is influenced by the anatomic characteristics of the subject (increased if small size) [68], physiologic conditions (enhanced during exercise) [69], or diseases (greater deposition in smokers and in chronic obstructive pulmonary disease, with formation of ''hot spots'' and reduced particle clearance) [70].

Drug toxicity also may be predictable when the drug is administered in excessive amounts [71], either intentionally, as in suicide by overdose [72], or unintentionally through therapeutic misadventure. Therapeutic misadventure may happen because the effects of a drug are influenced by the previous or simultaneous administration of another drug (drug-drug interactions) or by dietary or environmental factors (drug-environment interactions).

For some drug-induced reactions (eg, amiodarone, bleomycin), the risk of lung disease is related to the amount of material that the individual ingested. Nevertheless, even for these dose-related conditions, some subjects show high susceptibility, whereas others seem to be resistant to the development of significant pulmonary disease. Then, cumulative exposure is less critical and host susceptibility plays a more prominent role.

The following scenarios may be possible reasons for increased susceptibility that are not based on idiosyncrasy: (1) the underlying disease for which the drug is being given (rheumatoid arthritis and methotrexate) [73], (2) occupational factors potentiate the noxious effects of the drug (asbestos and bromocriptine) [74], (3) hazardous associations (radiotherapy, chemotherapy and high inspired concentrations of oxygen) [75], (4) concomitant renal failure (bleomycin) [76], (5) rate of infusion (bleomycin) [77], and (6) the impact of drugs that are taken concomitantly on cytochrome P450 systems, on detoxication pathways, or by way of altered pharmacokinetics of the offending drugs [78]. Alternatively, drug toxicity also may be unpredictable and even can occur following the administration of small amounts of the agent [79,80]. This may be due to (metabolic) idiosyncrasy, which implies a genetically determined intolerance to the agent whereby the person experiences toxicity because, for example, there is an innate inability to biotransform the agent or to cope with its metabolites, or the patient has developed an immunologically-acquired intolerance to the drug. Unlike metabolic idiosyncrasy, drug hypersensitivity (or allergy) implies a previous contact with that, or a similar, drug.

*Metabolic idiosyncrasy*

Drug activation can be enhanced through induction of activating CYP450 enzymes, selective inhibition of the detoxification pathways, or competition for these detoxification pathways [81,82].

The variability of drugs' local persistence and toxicity depends on the biotransformation which is determined by species or genetic polymorphism (leading to so called "idiosyncratic" reactions) or environmental factors (interactions with other drugs, pollutants, dietary factors). For instance, the toxicity of coumarin on the Clara cells in mice depends on the presence of CYP2B enzymes [83]. Another example is the toxicity of bleomycin, which, in mice, is due to lower levels of conjugating and detoxifying bleomycin hydrolase enzyme [84].

Defense can be decreased against toxic metabolites by decreasing GSH levels or antioxidant protective mechanisms (Vitamin E [3], selenium). Alterations in biotransformation and defense systems may be acquired and affected by dietary factors (chronic alcohol abuse [85]), drugs, including oxygen, and environmental agents, including smoking [33,86]. Thus, CYP1A1 and CYP 1B1, which are known to be inducible by polycyclic aromatic hydrocarbons, are induced in the lungs by smoking [25], whereas CYP3A5 is induced by glucocorticoids [30]. Incubation of alveolar macrophages and type II pneumocytes with paracetamol at concentrations around normal therapeutic levels was shown recently to lead to a significant decrease in intracellular GSH [33]. The pulmonary consequences of such effect are not known, but it is conceivable that oxidant-mediated drug toxicity could be potentiated by a common nonprescription drug, such as paracetamol. Recent epidemiologic studies suggest that frequent use of paracetamol can be associated with an increased risk of wheezing in the offspring [34].

Genetic polymorphism was shown to occur for many enzyme systems that are involved in the biotransformation of xenobiotics: N-acetyltransferase, debrisoquine polymorphism related to CYP2D6 gene with extensive (rapid) versus poor (slow) metabolizers, and Ah locus polymorphism (Ah receptor) with high versus low susceptibility to induction of CYP1A1 and CYP1A2 by TCDD or PAH, gluthatione-s-transferase. Genetic susceptibility to oxidative stress was shown in a variety of mice strains (eg, mice with the resistant phenotype linked to chromosome 11 [87]; mice genetically deficient in Clara cell protein). The latter mice strain indicates a protective role of the Clara cell protein in the defense against oxidative stress [88]. These polymorphisms can be responsible for 10 to 200 fold differences in the individual response to some chemicals, either in terms of their effectiveness or with respect to their toxicity or carcinogenic effects. The expression of CYP1A1 is regulated by genetic polymorphism. It is likely that this variability contributes to the individual susceptibility to environmental chemicals [31] and to interindividual variations in drug activity and toxicity [9,22,32]. Among phase II enzymes, glutathione S-transferase M1 shows a considerable polymorphism in human lungs and may be associated with differential susceptibility to lung cancer [89,90]. Although this susceptibility has been well-studied regarding the susceptibility to smoking, there are no examples, to our knowledge, where this has been documented for pneumotoxic drugs.

Genetic deficiencies in enzymes that are involved in oxidant defense have been invoked in at least two cases of drug-induced pulmonary toxicity. Acker-

man et al [91] described the case of a 26-month-old child who had partial monosomy 21 and blood Cu,Zn-superoxide dismutase at 45% of normal values and developed pulmonary edema following a short (4.5 hour) normally nonhazardous exposure to 100% $O_2$ during anesthesia [91]. Drent [92] attributed the occurrence and recurrence of diffuse interstitial pneumonia in a 64-year-old nonsmoking man to the antimalarial drug, mefloquine. It was theorized that because he was hemizygote for a glucose-6-phosphate dehydrogenase deficiency it rendered him more sensitive to oxidative stress [92].

*Genetic predisposition*

Large interindividual differences exist in cytokine levels (tumor necrosis factor [TNF]-α, IL-6) that are released during an inflammatory process. This variability may be due to inborn or acquired factors (smoking, viral or *Mycoplasma pneumoniae* infections). The genetic variability was observed in beryllium lung disease (HLA-DPB1Glu69) [93] and in extrinsic allergic alveolitis (high-producing TNF-α2 genotype) [94]. The level of exposure to these incriminated substances also is an important determinant of disease incidence (dose-effect relationship), but genetic predisposition plays the major role and has an additive/supramultiplicative effect upon exposure intensity [95]. Smoking seems to be protective and infections to be adjuvants in hypersensitivity pneumonitis [81].

Unexpected drug-related pulmonary reactions are often attributed to immunologic ‘‘hypersensitivity’’ or ‘‘allergy.’’ Evidence for a specific immune-mediated sensitization to the drug is often circumstantial and based only, for example, on the presence of eosinophils in the lungs or the fact that a previous administration of the drug was well-tolerated. Specific antibodies or other cell-mediated reactions are rarely, if ever, documented. This does not mean that such mechanisms could not operate in some forms of drug-induced lung disease, because they were shown to occur for drug-induced liver disease. Moreover, covalent binding of a drug’s reactive metabolite can take place with cellular enzymes or structural proteins (Fig. 3); this can generate neoantigens. Formation of neoantigens was shown with the anesthetic, halothane ($CF_3CHClBr$), the oxidation of which generates $CF_3CO-$ adducts with various proteins (microsomal carboxylesterase, cytochrome P450(2B1), calreticulin) that are no longer recognized as self-proteins [96]. In the serum of some patients who suffer from halothane hepatitis, antibodies against trifluoroacetyl-polypeptides that are derived from hepatic microsomes from rats who were treated in vivo with halothane or from rat liver microsomes that were incubated in vitro with halothane, were detected. This formation of neoantigens could also happen in the lung. Like liver cells, various lung cells have the capability to activate chemicals to reactive metabolites that can bind covalently to cellular proteins [81]. No evidence of such mechanisms has been shown with pneumotoxic agents.

A diffuse, infiltrating, immunity-induced pulmonary reaction could start with the process of recognition and fixation of the drug to an HLA receptor of dendritic Langerhans cells or a macrophage that leads to the activation of T lymphocytes, cytokine production (IL-1, TNF), and increased expression of adhesion molecules for leukocyte trafficking that results in cytotoxic activity on alveolar type I epithelial cells and endothelial cells and inflammation. This inflammatory process can induce cellular infiltration that progresses toward resolution or pulmonary fibrosis.

*Susceptibility to healing of inflammation or fibrogenesis*

The local tissue outcome from an inflammatory attack can evolve either to ad integrum healing with minimal residual damage or to scarring or fibrosis with respiratory insufficiency.

Genetic and environmental factors lead to healing or fibrosis: the cytokine phenotype that concern TNF-α, transforming growth factor (TGF), and IL-6 seem to play an important role in initiation and progression of fibrosis. The capacity of the host to mount a Th2 immune response increases this susceptibility [81]. A stimulation of the Th1 lymphocytes (eg, in mycobacterial infection) increases the release of interferon-γ, IL-2, IL-12, and IL-18 which leads to cell-mediated immunity and tissue restoration [12] in animal models of bleomycin-induced lung interstitial fibrosis. Stimulation of the Th2 lymphocytes on the other side increases the release of cytokines IL-4, IL-5, IL-10, and IL-13 and leads to antibody-mediated immunity and an inflammatory process that involves TNF-α and repair/growth factor, TGF-β. This stimulates fibroblast activation and collagen matrix deposition [12]. TGF-β exists in three closely homologous to dimeric forms (TGF-β1, TGF-β2, TGF-β3) that are differentially expressed during bleomycin-induced lung fibrosis; expression of TGF-β1 predominates and is produced primarily by the macrophages [97,98]. ANGII is well-documented as an inducer of the TGF-β1 expression [41]. Increased expression of TGF-β1 was shown in the lung of rats who were fed ethanol and had increased expression and deposition of fibronectin. Fibronectin leads to fibrotic remodel-

ing (model of dietary susceptibility to fibrosis) [85]. Angiogenesis is a fundamental component of inflammation and wound repair. During bleomycin-induced fibrosis, macrophage inflammatory protein (MIP)-2 (murine functional homolog of IL-8) and angiostatic interferon-inducible CXC chemokine inflammatory protein (IP)-10 are found in lung tissue; this correlates with fibrosis [99–101]. This pathologic process seems to be blocked by interferon-γ that has since been suggested for the treatment of usual interstitial pneumonitis. A compromised function of P53 tumor suppression protein, which mediates cellular response to DNA damage and induces apoptosis, impaired recovery of the lungs in mice who were exposed to bleomycin [102]. Although progression toward fibrosis can be found following bleomycin or amiodarone treatment or radiation therapy, individuals have different risks of developing pulmonary fibrosis after an apparently similar pulmonary insult [103]. For instance, only a fraction of patients who have definite amiodarone pneumonitis develop irreversible pulmonary fibrosis [46,48,59].

### *Specific models of pleuropulmonary drug-induced immunologic injury*

A drug that acts as an adjuvant or immunostimulant may induce a monoclonal expansion of cell-reacting lymphocytes that are ordinarily held in check by helper and suppressor influences that balance each other. The ensuing monoclonal expansion secretes homogenous antibodies against nuclear proteins (histones H2A-H2B dimer in procainamide, H3 and H4 in hydralazine-induced lupus [104]), in contrast to idiopathic systemic lupus erythematosus where the antinuclear antibodies are heterogeneous (for example, antibodies to native DNA, histone, and nonhistone ribonuclear proteins) as well as antibodies formed against extranuclear host components, such as clotting factors [104,105]. Nevertheless, the same nuclear immunofluorescence with anti-immunoglobulin G (IgG), anti-IgM, and anti-C3 is found in pleural biopsies of patients with lupus and with procaine-induced patients who have lupus [106]. The antinuclear factor can be found, either in a large percentage of treatments with the drug but of no to few pathologic consequences, or conversely in few treatments but all subjects suffering from lupus disease [37]. Early studies suggested an association of HLA DR4 with hydralazine-induced lupus, but this was not confirmed [104]. A genetic predisposition comes from study of the patient acetylator status; slow acetylators are homozygous for this gene and have low levels of N-acetyl transferase in the liver. Procainamide and hydralazine are metabolized by this pathway; slow acetylators have a higher risk of developing drug-induced lupus following exposure to these drugs [104].

Procainamide and hydralazine are able to bind polynucleotides in vitro and render DNA or histones antigenic. Drugs could also interfere with the normal process of DNA methylation. Following DNA replication, cytosine residues are methylated at the position 5 by the enzyme methyltransferase. Failure of methylation of regulatory sequences is associated with gene expression, whereas methylation is associated with suppression of gene transcription. DNA methylation, is, therefore, a mechanism that regulates gene expression [104,107]. Human T cells that are treated by both drugs have a reduction in total genomic deoxymethylcytosine that is compatible with hypomethylation and autoreactivity. These autoreactive cells induce B cells to differentiate into IgG secreting cells without any antigen or mitogen; this coincides with the polyclonal B cells' activation that is seen in induced lupus [104]. The drug-induced systemic lupus erythematosus syndrome [108] may result from exposure to a wide array of drugs including, penicillamine, nonsteroidal anti-inflammatory drugs, hydralazine, hydantoin, and β-blockers.

Drug-induced hypersensitivity syndromes with involvement of the liver, brain, heart, digestive system, bone marrow, lymph nodes, or any combination of these may follow exposure to antiviral nevirapine [109] or anticonvulsants [110]; alveolar hemorrhage with concomitant renal failure (mimicking a Goodpasture syndrome that is known to be due to a immune-complexes pathology) may follow penicillamine [1,71]. Antineutrophil cytoplasmic antibody-positive angiitis, with or without capillaritis and alveolar hemorrhage, was related recently to the use of the antithyroid drug propylthiouracil, and the drugs minocycline, levamisole, sulfasalazine, and allopurinol. Drug-induced Churg-Strauss syndrome (asthma plus systemic eosinophilic vasculitis) was described after aspirin and leukotriene antagonists.

## References

[1] Limper AH, Rosenow EC. Drug-induced interstitial lung disease. Cur Opin Pulm Med 1996;2:396–404.
[2] Foucher P, Biour M, Blayac JP, Godard P, Sgro C, Kuhn M, et al. Drugs that may injure the respiratory system. Eur Respir J 1997;10:265–79.
[3] Rocksen D, Ekstrand-Hammastron B, Johansson L, Bucht A. Vitamin E reduces transendothelial migration

of neutrophils and prevents lung injury in endotoxin-induced airway inflammation. Am J Respir Cell Mol Biol 2003;28:199–207.
[4] Massaro EJ, Grose EC, Hatch JE, Slade R. Antioxidant defense mechanisms of the lung. In: Gardner DE, Crapo JD, Massaro EJ, editors. Toxicology of the lung. New York: Raven Press; 1988. p. 201–18.
[5] Tsan MF. Superoxide dismutase and pulmonary oxygen toxicity. Proc Soc Exp Biol Med 1997;214: 107–13.
[6] Vanklaveren RJ, Demedts M, Nemery B. Cellular glutathione turnover in vitro, with emphasis on type II pneumocytes. Eur Respir J 1997;10:1392–400.
[7] Wang HD, Yamaya M, Okinaga S, Jia YX, Kamanaka M, Takahashi H, et al. Bilirubin ameliorates bleomycin-induced pulmonary fibrosis in rats. Am J Respir Crit Care Med 2002;165:406–11.
[8] Ryrfeldt Å. Drug-induced inflammatory responses to the lung. Toxicol Lett 2000;112–113:171–6.
[9] Valero FC, Pardo JM. Pulmonary toxicity of pharmaceutical drugs. [Toxicidad pulmonar por farmacos]. Arch Bronconomeul 1999;35:550–9.
[10] Wood LG, Gibson PG, Garg ML. Biomarkers of lipid peroxidation, airway inflammation and asthma. Eur Respir J 2000;21:177–86.
[11] Morrow JD, Roberts LJ. The isoprostanes. Their role as an index of oxidant stress status in human pulmonary disease. Am J Respir Crit Care Med 2002;166: S25–30.
[12] Strieter RM, Keane MP. Cytokine biology and the pathogenesis of interstitial lung disease. In: King TE, editor. New approaches to managing idiopathic pulmonary fibrosis. ATS monography. New York: American Thoracic Society; 2000. p. 27–35.
[13] Zhang K, Gharaee-Kermani M, Jones ML, Warren JS, Phan SH. Lung monocyte chemoattractant protein-1 gene expression in bleomycin-induced pulmonary fibrosis. J Immunol 1994;153:4733–41.
[14] Smith RE, Strieter RM, Phan SH, Kunkel SL. CC chemokines: novel mediators in the profibrotic inflammatory response to bleomycin challenge. Am J Respir Cell Mol Biol 1996;15:693–702.
[15] Henderson RF. Use of bronchoalveolar lavage to detect lung damage. In: Gardner DE, Crapo JD, Massaro EJ, editors. Toxicology of the lung. New York: Raven Press; 1988. p. 239–68.
[16] Nemery B, Dinsdale D, Verschoyle RD. Detecting and evaluating chemical-induced lung damage in experimental animals. Bull Eur Physiopathol Resp 1987; 23:501–28.
[17] Shasby DM, Shasby SS, Bowman CM. Angiotensin-converting enzyme concentrations in the lung lavage of normal rabbits and rabbits treated with nitrogen mustard exposed to hyperoxia. Am Rev Respir Dis 1981;124:202–3.
[18] Hoet PHM, Demedts M, Nemery B. Effects of oxygen pressure and medium volume on the toxicity of paraquat in rat and human type II pneumocytes. Hum Exp Toxicol 1997;16:305–10.
[19] Vanklaveren RJ, Roelant C, Boogaerts M, Demedts M, Nemery B. Involvement of an NAD(P)H oxidase-like enzyme in superoxide anion and hydrogen peroxide generation by rat type II cells. Thorax 1997;52: 465–71.
[20] Forman HJ, Torres M. Reactive oxygen species and cell signaling respiratory burst in macrophage signaling. Am J Respir Crit Care Med 2002;166:S4–8.
[21] Hoet PH, Demedts M, Nemery B. In vitro modulation of the P450 activities of hamster and human lung slices. Cell Biol Toxicol 1997;13:185–92.
[22] Weinshilboum R. Inheritance and drug response. N Engl J Med 2003;348:529–37.
[23] Hukkanen J, Pelkonen O, Raunio H. Expression of xenobiotic-metabolizing enzymes in human pulmonary tissue: possible role in susceptibility for ILD. Eur Respir J 2001;18(Suppl.32):122S–6S.
[24] Shou M, Korzekwa KR, Crespi CL, Gonzalez FJ, Gelboin HV. The role of 12cDNA-expressed human, rodent and rabbit cytochrome P450 in the metabolism of benzo(a)pyrene trans 7–8-diol. Mol Carcinog 1994; 10:159–68.
[25] Antilla S, Hietanen E, Vainio H. Smoking and peripheral type of cancer are related to high levels of pulmonary cytochrome P450IA in lung cancer patients. Int J Cancer 1991;47:681–5.
[26] Saarikoski ST, Husgafvel-Pursiainen K, Hirvonen A, Vainio H, Gonzalez FJ, Antilla S. Localization of CYP1A1 mRNA in human lung by in situ hybridization: comparison with immunohistochemical findings. Int J Cancer 1998;77:33–9.
[27] Anttila S, Hukkanen J, Hakkola J, Stjernwall T, Beaune P, Edwards R, et al. Expression and localization of CYP3A4 and CYP 3A5 in human lung. Am J Respir Cell Mol Biol 1997;16:242–9.
[28] Mori M, Tezuka F, Chiba R. Atypical adenomatous hyperplasia and adenocarcinoma of the human lung. Their heterology in form and analogy in immunohistochemical characteristics. Cancer 1996;77:665–74.
[29] Yokose T, Yokose T, Doy M, Tanigushi T. Immunohistochemichal study of cytochrome P450 2 C and 3 A in human nonneoplastic and neoplastic tissues. Virchows Arch 1999;434:401–11.
[30] Hukkanen J, Lassila A, Paivarinta K. Induction and regulation of xenobiotic-metabolizing cytochrome P450s in the human A549 lung adenocarcinoma cell line. Am J Respir Cell Mol Biol 2000;22:360–6.
[31] Hirvonen A. Polymorphisms of xenobiotic-metabolizing enzymes and susceptibility to cancer. Environ Health Perspect 1999;107:37–47.
[32] Evans WE, McLeod HL. Pharmacogenomics-drug deposition, drug targets and side effects. N Engl J Med 2003;348:538–49.
[33] Dimova S, Hoet P, Nemery B. Paracetamol (acetaminophen) cytotoxicity in rat type II pneumocytes and alveolar macrophages in vitro. Biochem Pharmacol 2000;59:1467–75.
[34] Shaheen SO, Newson RB, Sherriff A, Henderson AJ, Heron JE, Burneu PGJ, et al. Paracetamol use in preg-

nancy and wheezing in early childhood. Thorax 2002; 57:958–63.
[35] Lewis CPL, Nemery B. Pathophysiology and biochemical mechanisms of the pulmonary toxicity of paraquat. In: Bismuth C, Hall AH, editors. Paraquat poisoning. Mechanisms, prevention, treatment. New York: Marcel Dekker Inc.; 1995. p. 107–40.
[36] Adamson IY. Drugs induced pulmonary fibrosis. Environ Health Perspect 1984;55:25–36.
[37] Limper AH, Rosenow EC. Drug-induced pulmonary disease. In: Murray J, Nadel J, Mason R, Boushey A, editors. Textbook of respiratory medicine. Philadelphia: Saunders; 2000. p. 1971–94.
[38] Fisher GL, Placke ME. Cells and organ cultures models of respiratory toxicity. In: Gardner DE, Crapo JD, Massaro EJ, editors. Toxicology of the lung. New York: Raven Press; 1988. p. 285–314.
[39] Serrano-Mollar A, Closa D, Cortijo J, Morcillo EJ, Prats N, Gironella M, et al. P-selectin upregulation in bleomycin induced lung injury in rats: effects of N-acetyl-l-cysteine. Thorax 2002;57:629–34.
[40] Li Y, Azuma A, Takahashi S, Usuki J, Matsuda K, Aoyama A, et al. Fourteen-membered ring macrolides inhibit vascular cell adhesion molecule 1 messenger RNA induction and leukocyte migration. Role of preventing lung injury and fibrosis in bleomycin-challenged mice. Chest 2002;122:2317–45.
[41] Uhal BD. Apoptosis in lung fibrosis and repair. Chest 2002;122:293S–8S.
[42] Hagimoto N, Kuwano K, Nomoto Y. Apoptosis and expression of FAS/FAS ligand mRNA in bleomycin-induced pulmonary fibrosis in mice. Am J Respir Cell Mol Biol 1997;16:91–101.
[43] Kuwano K, Hagimoto N, Kawasaki M. Essential roles of the Fas/Fas ligand pathway in the development of pulmonary fibrosis. J Clin Invest 1999;104:13–9.
[44] Peters-Golden M, Bailie M, Marshall T, Wilke C, Phan SH, Toews GB, et al. Protection of pulmonary fibrosis in leukotrien-deficient mice. Am J Respir Crit Care Med 2002;165:229–35.
[45] Martin WJ, Rosenow EC. Amiodarone pulmonary toxicity recognition and pathogenesis (part2). Chest 1988;93:1242–8.
[46] Coudert B, Bailly F, Lombard JN, Andre F, Camus P. Amiodarone pneumonitis. Bronchoalveolar lavage findings in 15 patients and review of the literature. Chest 1992;102:1005–12.
[47] Camus P. Respiratory disease induced by drug. Eur Respir J 1997;10:260–4.
[48] Ott MC, Khoor A, Leventhal JP, Paterick TE, Burger CD. Pulmonary toxicity in patients receiving low-dose amiodarone. Chest 2003;123:646–51.
[49] Rose MS, Smith LL, Wyatt I. Evidence for energy-dependent accumulation of paraquat into rat lung. Nature 1974;252:314–5.
[50] Smith LL, Wyatt I. The accumulation of putrescine into slices of rat lung and brain and its relationship to the accumulation of paraquat. Biochem Pharmacol 1981;30:1053–8.
[51] Hoet PHM, Dinsdale D, Lewis CPL, Verbeken EK, Lauwerijs JM, Nemery B. Kinetics and cellular localization of putrescine in human lung. Thorax 1993;48: 1235–41.
[52] Nemery B, Smith LL, Aldridge WN. Putrescine and 5-hydroxytryptamine accumulation in rat lung slices: cellular localization and responses to cell-specific lung injury. Toxicol Appl Pharmacol 1987;91:107–20.
[53] Hoet PH, Lewis CP, Demedts M, Nemery B. Putrescine and paraquat uptake in human lung slices and isolated type II pneumocytes. Biochem Pharmacol 1994;48:517–24.
[54] Hoet PH, Lewis CP, Dinsdale D, Demedts M, Nemery B. Putrescine uptake in hamster lung slices and primary cultures of type II pneumocytes. Am J Physiol 1995;269:L681–9.
[55] Smith AC, Boyd MR. Effects of bis-chloronitrosourea (BCNU) on pulmonary and serum angiotensin converting enzyme activity in rats. Biochem Pharmacol 1983;32:3719–22.
[56] Hamel H, Bonniaud P, Baudouin N, Spagnolo G, Sagot P, Camus P. Pulmonary edema and tocolysis with beta-agonists. Rev Mal Respir 2002;19:241–4.
[57] Mabie WC, Pernoll ML, Witty JB. Pulmonary edema induced by betamimetic drugs. South Med J 1983;16: 1354–60.
[58] Berthiaume Y, Folkesson HG, Matthay MA. Lung edema clearance: 20 years of progress. Invited review: alveolar edema fluid clearance in the injured lung. J Appl Physiol 2002;93:2207–13.
[59] Camus PH, Foucher P, Bonniaud PH, Ask K. Drug-induced infiltrative lung disease. Eur Respir J 2001; 18(Suppl 32):93S–100S.
[60] Dooren MC, Ouwehand WH, Verhoeven AJ, Vondemborne AE, Kuijpers RW. Adult respiratory distress syndrome after experimental intra-venous gamma-globulin concentrate and monocyte-reactive IgG antibodies. Lancet 1998;352:1601–2.
[61] Maillard N, Foucher P, Caillot D, Durand C, Sgro C, Camus P. Transient pulmonary infiltrates during treatment with anti-thymocyte globulin. Respiration (Herrlisheim) 1999;66:279–82.
[62] Couderc LJ, Stellianides S, Frachon I. Pulmonary toxicity of chemotherapy and G/GM CSF: a report of 5 cases. Respir Med 1999;93:65–8.
[63] Ruiz-Arguelles GJ, Arizpe-Bravo D, Sanchez-Soza S, Rojas-Ortega S, Moreno-Ford V, Ruiz-Arguelles A. Fatal-G-CSF-induced pulmonary toxicity. Am J Hematol 1999;60:82–3.
[64] Kopko PM, Holland PV. Transfusion related lung injury. Br J Haematol 1999;105:322–9.
[65] Woodard JP, Gulbache E, Shreve M. Pulmonary cytolytic thrombi: a newly recognized complication of stem cell transplantation. Bone Marrow Transplant 2000;25:293–300.
[66] Bhalla KS, Folz RJ. Idiopathic pneumonia syndrome after syngeneic bone marrow transplant in mice. Am J Respir Crit Care Med 2002;166:1579–89.
[67] Haddad IY. Idiopathic pneumonia after marrow trans-

plantation. When are antioxidants effective? Am J Respir Crit Care Med 2002;166:1532–3.
[68] Beclacke MR, Toyota B, Stewart M, Hanson R, Hanley J. Lung structure as a risk factor in adverse pulmonary responses to asbestos exposure. A case reference study in Quebec chrysotile miners and millers. Am Rev Respir Dis 1983;128:385–8.
[69] Bennett WD, Zeman KL, Kim C, Mascarella J. Enhanced deposition of fine particles in COPD patients spontaneously breathing at rest. Inhal Toxicol 1997;9: 1–14.
[70] Svartengren K, Ericsson CH, Svartengren M, Mossberg B, Philipson K, Camner P. Deposition and clearance in large and small airways in chronic bronchitis. Exp Lung Res 1996;22:555–76.
[71] Touraine R, Bernard JP, Cordier JF. Les maladies respiratoires induites par les médicaments [Respiratory ailments induced by medicine]. Rev Prat 1984; 34:1235–50 [in French].
[72] Repetto MR, Repetto M. Therapeutic, toxic and lethal concentrations of 73 drugs affecting respiratory system in human fluids. J Toxicol Clin Toxicol 1998;36: 287–93.
[73] Salaffi F, Manganelli P, Carotti M, Subiaco S, Lammana G, Cervini C. Methotrexate –induced pneumonitis in patients with rheumatoid arthritis and psoriatric arthritis: report of 5 cases and review of the litterature. Clin Rheumatol 1997;16:296–304.
[74] Hillerdal G, Lee J, Blomkvist A. Pleural disease during treatment with bromocriptine in patients previously exposed to asbestos. Eur respir J 1997;10: 2711–5.
[75] Gilson AJ, Sahn SA. Reactivation of bleomycin lung toxicity following oxygen administration. A second response to corticosteroids. Chest 1985;88:304–6.
[76] Sleijfer S, Vandermark TW, Schraffordt Koops H, Mulder N. Enhanced effects of bleomycin on pulmonary function disturbances in patients with decreased renal function due to cisplatin. Eur J Cancer 1996; 32A:550–2.
[77] Gerson R, Tellz-Bermal E, Lazaro-Leon M. Low toxicity with continuous infusion of high-dose bleomycin in poor prognostic testicular cancer. Am J Clin Oncol 1993;16:323–6.
[78] Lin JH, Lu AYH. Inhibition and induction of cytochrome P450 and the clinical implications. Clin Pharmacokinet 1998;35:361–90.
[79] Monfort L, Bosly A, Dehasque N, Delaunois L. Acute pulmonary fibrosis due to bleomycin. [Fibrose pulmonaire aiguë à la bléomycine]. Louv Med 1980; 99:103–10.
[80] Real E, Roca MJ, Vinuales A, Pastor E, Grau E. Life threatening lung toxicity induced by low doses of bleomycin in a patient with Hodgkin's disease. Haematologica 1999;84:667–8.
[81] Nemery B, Bast A, Behr J, Borm PJ, Bourke SJ, Camus PH, et al. Interstitial lung disease induced by exogenous agents: factors governing susceptibility. Eur Respir J 2001;18(Suppl 32):30S–42S.
[82] Hukkanen J, Pelkonen O, Raunio H. Expression of xenobiotic-metabolizing enzymes in human pulmonary tissue: possible role in susceptibility for ILD. Eur Respir J 2001;18(Suppl 32):122S–6S.
[83] Born SL, Fix AS, Caudill D, Lehman-McKeeman LD. Selective Clara cell injury in mouse lung following acute administration of coumarin. Toxicol Appl Pharmacol 1998;151:45–56.
[84] Lazo JS, Hoyt DG, Sebti SM, Pitt BR. Bleomycin: a pharmacologic tool in the study of the pathogenesis of interstitial pulmonary fibrosis. Pharmacol Ther 1990; 47:347–58.
[85] Guidot DM, Roman J. Chronic ethanol ingestion increases susceptibility to acute lung injury. Role of oxidative stress and tissue remodeling. Chest 2002; 122:309S–14S.
[86] Dimova S, Hoet PH, Nemery B. Xenobiotic-metabolizing enzyme activities in primary cultures of rat type II pneumocytes and alveolar macrophages. Drug Metab Dispos 2001;29:1349–54.
[87] Prows DR, Daly MJ, Shertzer HG, Leikauf GD. Ozone induced acute lung injury: genetic analysis of F(2) mice generated from A/J and C57BL/6J strains. Am J Physiol 1999;277:L372–80.
[88] Mango GW, Johnston CJ, Reynolds SD, Finkelstein JL, Poppler CG, Stripp BR. Clara cell secretory protein deficiency increases oxidant stress response in conducting airways. Am J Physiol 1998;275: L348–56.
[89] Antilla S, Hirvonen A, Vainio H, Husgavesel-Pursiainen K, Hayes JD, Ketterer B. Immunohistochemical localization of glutathion S-transferase in human lung. Cancer Res 1993;53:5643–8.
[90] Antilla S, Hirvonen A, Husgavsel-Pursiainen K, Karjalainen A, Nurminen T, Vainio H. Combined effect of CYP1A1 inducibility and GSTM1 polymorphism on histological type of lung cancer. Carcinogenesis 1994;15:1133–5.
[91] Ackerman AD, Fackler JC, Tuck-Muller CM, Tarpey MM, Freeman BA, Rogers MC. Partial monosomy 21,diminished activity in superoxide dismutase and pulmonary oxygen toxicity. N Engl J Med 1988;318: 1666–9.
[92] Drent M. Drug induced pneumonia associated with hemizygote glucose-6-phosphate deshydrogenase deficiency. Eur J Haematol 1998;61:218–20.
[93] Richeldi L, Sorrentino R, Saltini C. HLA-DPB1 glutamate 69: a genetic marker of beryllium disease. Science 1993;262:242–4.
[94] Zabel P, Schlaak M. Tumor necrosis factor alpha in lung disease. [Tumor nekrose faktor alpha bei lungenerkrankungen]. Pneumologie 1993;47:49–52.
[95] Richeldi L, Kreiss K, Mroz MM, Zhen B, Tartoni P, Saltini C. Interaction of genetic and exposure factors in the prevalence of berylliosis. Am J Ind Med 1997; 32:337–40.
[96] Bourdi M, Chen W, Peter RM. Human cytochrome P450 2 E1 is a major utoantigen associated with halothane hepatitis. Chem Res Toxicol 1996;9:1159–66.

[97] Branton MH, Kopp JB. TGF-β and fibrosis. Microbes Infect 1999;1:1349–65.
[98] Coker RK, Laurent JS, Shahzeidi S. Transforming growth factors-beta 1,-beta 2 and -beta 3stimulate fibroblast procollagen production in vitro but are differentially expressed during bleomycin-induced lung fibrosis. Am J Pathol 1997;150:981–91.
[99] Keane MP, Arenberg DA, Lynch JP. The CXC chemokines IL-8 and IP-10 regulate angiogenic activity in idiopathic pulmonary fibrosis. J Immunol 1997;159: 1437–43.
[100] Kean MP, Belperio JA, Moore TA. Neutralization of the CXC chemokine, macrophage inflammatory protein-2 attenuates bleomycin induced pulmonary fibrosis. J Immunol 1999;162:5511–8.
[101] Keane MP, Belperio JA, Arenberg DA. IFN-gamma-induced pulmonary fibrosis via inhibition of angiogenesis. J Immunol 1999;163:5686–92.
[102] Ghosh S, Mendoza T, Ortiz LA, Hoyle GW, Fermin CD, Brody AR, et al. Bleomycin sensitivity in mice expressing dominant-negative p53 in the lung epithelium. Am J Respir Crit Care Med 2002;166: 890–7.
[103] Avilla JJ, Lympani PA, Pantedilidis P, Welsch KI, Back CM, Dubois RM. Fibronectin gene polymorphisms associated with fibrosing alveolitis in systemic sclerosis. Am J Respir Cell Mol Biol 1999; 20:106–12.
[104] D'Cruz D. Autoimmune diseases associated with drugs, chemicals and environmental factors. Toxicol Lett 2000;112–113:421–32.
[105] Schoen RT, Trentham DE. Drug induced lupus: an adjuvant disease. Am J Med 1981;71:5–8.
[106] Wang DY. Diagnosis and management of lupus pleuritis. Curr Opin Pulm Med 2002;8:312–6.
[107] Yung RL, Quddus J, Crisp CE, Johnson KJ, Richardson BC. Mechanisms of induced drug lupus. I. Cloned Th2 cells modified with DNA methylation inhibitors in vitro cause autoimmunity in vivo. J Immunol 1995;154:3025–35.
[108] Skaer TL. Medication-induced systemic lupus erythematosus. Clin Therap 1992;14:496–506.
[109] Bourezane Y, Salar D, Hoen B, Vandel S, Drobacheff C, Laurent R. Dress (drug rash with eosinophilia and systemic symptoms)syndrome associated with nevirapine therapy. Clin Infect Dis 1998;27:1321–2.
[110] Marik P. Anticonvulsant hypersensitivity syndrom occuring as sepsis with multiorgan dysfunction. Pharmacotherapy 1999;19:346–8.