SELECTIVITY AND MOLECULAR MECHANISMS OF TOXICITY

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Preface

A symposium was held in September 1985 at the University of Kent to mark the retirement of Dr W. Norman Aldridge, the Deputy Director of the Toxicology Unit of the Medical Research Council and the first Chairman of the British Toxicology Society. The title of the Symposium was *Selectivity and Molecular Mechanisms of Toxicity* and it was intended to highlight the major guiding principles in Norman's distinguished scientific career which made him a recognised pioneer of toxicological research both in the UK and worldwide. Most of the contributors had been closely associated with Norman, either as students or as collaborators, and we were very happy to organise the scientific programme with the help of Martin Johnson, which we gratefully acknowledge.

This book is aimed at providing a more permanent tribute to Norman's influential achievements. It is a collection of short reviews which have been written by the contributors to the symposium, all specialists in various fields of human and experimental toxicology, and which illustrate some of the most interesting and topical problems in present-day toxicological research. Examples are given of the molecular basis for tissue-selective toxicity in the lung, peripheral nerves, kidney and immune system; and the mechanisms of toxicity of toxic lectins, organophosphate inhibitors, genotoxic chlorinated hydrocarbons and suicide substrates of cytochrome P-450 are then considered. Finally, human and experimental studies on the toxicity of three classes of compounds (MPTP, hexane and impurities of commercial parathion) are reviewed: these have received a great deal of interest in recent years for their involvement in human toxicity, being associated with the development of previously undescribed toxic syndromes.

Our thanks go to the British Toxicology Society who supported the symposium and encouraged the publication of this book.

Carshalton and Alderley Park, 1987

F. De M.
E. A. L.

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Tissue-selective Toxicity

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Cellular Specific Toxicity in the Lung

Lewis L. Smith and Benoit Nemery

INTRODUCTION

The architectural structure of the lung is designed to provide and protect a vast surface area within the chest cavity which allows the effective exchange of respired gases with the bloodstream. This means that the lung has numerous cell types with specific functions and when the cell types in the blood are taken into consideration, over forty individual cell types have been identified (Sorokin, 1970). Since the total cardiac output passes through the lung, the lung can be exposed to toxic xenobiotic compounds and their metabolites present in the blood. The lung is also exposed to gases, vapours and particles (if small enough) present in the inspired air. Even toxins present at very low concentrations in the atmosphere may present a risk to the lung, especially when one considers that the adult human lung respires approximately three tons of air per year (Mustafa and Tierney, 1978).

The selective vulnerability of lung cells will depend on several factors. These will include:

1. The route of exposure (i.e. inhalation or via the bloodstream).
2. The mean aerodynamic diameter (particulates).
3. Solubility of inhaled gases (e.g. sulphur dioxide compared with ozone).
4. Selective uptake mechanism (e.g. paraquat).
5. Selective metabolic activation (e.g. 4-ipomeanol).
6. Susceptibility of individual cell types.
7. Species susceptibility (e.g. butylated hydroxytoluene, BHT; trialkylphosphorothioates; α-naphthylthiourea, ANTU; 4-ipomeanol; paraquat).

It is the purpose of this review to consider in some detail the mechanism of toxicity of some chemicals which damage the lung. In doing so we shall attempt to highlight the cellular specific toxicity which these chemicals provoke. In particular we shall

discuss the pulmonary toxicity of hyperoxia, ANTU, BHT, 4-ipomeanol, and paraquat.

OXYGEN TOXICITY

Prolonged exposure to elevated oxygen tensions is toxic to most animal species including man (Clark and Lambertsen, 1971). The effects of hyperoxia on various aspects of lung structure, function and biochemistry and its mechanism of toxicity have been the subject of numerous experimental studies. Indeed the investigation of pulmonary oxygen toxicity is of direct relevance to man, not only because humans are often exposed to high levels of oxygen (usually for therapeutic purposes), but also because hyperoxia can be used as a model of the pathogenesis of adult respiratory distress syndrome, pulmonary fibrosis and for processes such as inflammation, ageing and carcinogenesis (Autor, 1982; Parke, 1982; Pryor, 1982).

Pathology

Several ultrastructural studies (Kistler *et al.*, 1967; Kapanci *et al.*, 1969; Meyrick *et al.*, 1972; Crapo *et al.*, 1978, 1980) have shown that breathing high concentrations of oxygen leads to the initial destruction of the alveolar capillary endothelial cells with few signs of damage to the alveolar type I epithelial cells. This process provokes interstitial and alveolar oedema and haemorrhage, some infiltration by monocytes and polymorphonuclear leucocytes and eventually fibroblast proliferation. If the animal survives this early destructive phase, a proliferative phase follows characterised by hypertrophy and proliferation of type II epithelial cells and a large increase in the number of interstitial fibroblasts.

There is considerable species variability in susceptibility to pulmonary oxygen poisoning. The time-course of tissue changes and the survival times vary with species (rats being more susceptible than monkeys), age (immature animals being often more resistant than mature animals, Frank *et al.*, 1978) and other factors such as nutritional levels of antioxidants (Deneke *et al.*, 1983) or the induction of oxygen tolerance as a result of prior exposures to oxygen or other pretreatments which increase the levels of antioxidant systems (Frank, 1983).

Mechanism of toxicity

The primary mechanism of oxygen toxicity is thought to result from an imbalance between the increased generation of toxic oxygen radical species during hyperoxia and the available cellular defence mechanisms against these species (Frank, 1983; Fisher *et al.*, 1984). Toxic oxygen species result from the reduction of molecular oxygen to superoxide anion (O_2^-) and this radical may in turn dismutate, either spontaneously or catalysed by superoxide dismutase, to form hydrogen peroxide (H_2O_2). Superoxide anion is also able to reduce transition metals such as Fe^{3+} to

Fe^{2+} and the ferrous iron is capable of reacting with H_2O_2 to form the hydroxyl radical ($OH^{\cdot}$) by processes which have been well described by Freeman and Crapo (1982) and Halliwell and Gutteridge (1984). The $OH^{\cdot}$ is thought to react with, and modify structural, metabolic and genetic material. In this way it may lead to perturbations of cell function and cause cell death, if the protective mechanisms (low-molecular weight scavengers and enzymatic systems) are overwhelmed.

Although it is generally accepted that the primary mechanism of oxygen toxicity is the increased generation of free radicals of oxygen, the cellular source of these radicals, during hyperoxic exposure, is not known. There are at least two hypotheses which have been advanced: one is that high concentrations of oxygen lead to an excessive production of oxygen radicals *within the alveolar cells themselves*; and the other is that oxygen radicals are generated, mainly by *activated polymorphonuclear leucocytes* which accumulate in oxygen-damaged lungs.

This latter hypothesis would explain the apparent paradox that the endothelial cells appear to be damaged by hyperoxia before the more directly exposed epithelial cells. Neutrophils are well equipped to inflict tissue damage, since they can release potent oxidants (including reactive oxygen-radical species) as well as proteolytic enzymes, metabolites of arachidonic acid and other amplifiers of the inflammatory response.

Circumstantial evidence incriminating the neutrophil comes from the observation that circulating leucocytes are sequestered by the lungs of patients developing adult respiratory distress syndrome, thus resulting in high numbers of neutrophils or their products in broncho-alveolar lavage fluid and in the lungs at autopsy (Anon., 1984; Tate and Repine, 1983). In rats exposed to greater than 95% oxygen a close temporal relationship was observed between the chemotactic activity of the lavage fluid, the influx of neutrophils to the lavaged lung compartment and the subsequent mortality (Fox *et al.*, 1981). In sheep, the chemotactic activity for granulocytes in lung lymph increased eight-fold within 72 to 96 h of oxygen exposure (Newman *et al.*, 1983). This neutrophil recruitment to the lungs has recently been attributed to an increased elaboration of chemotactic factors by macrophages as a result of hyperoxic exposure (Christman *et al.*, 1985) although the respiratory burst of alveolar macrophages is in fact decreased by oxygen exposure *in vitro* (Suttorp and Simon, 1983).

The importance of the infiltration of inflammatory cells into the lung has been further supported by the observation that neutropenia results in a decreased severity of lung injury from hyperoxia and that the degree of lung injury correlates with the number of remaining circulating polymorphs (Shasby *et al.*, 1982). Lung vascular permeability changes induced by other treatments have also been shown to be attenuated in leukopenic animals (Brigham, 1982; Eiermann *et al.*, 1983).

It is not generally accepted that lung injury is mediated by neutrophils in oxygen toxicity (Fisher *et al.*, 1984; Glauser and Fairman, 1985). Indeed ultrastructural evidence of endothelial cell injury seems to precede the accumulation of inflammatory cells, and the correlative studies of Fox *et al.* (1981) indicate that neutrophil infiltration is associated with onset of mortality rather than with the initiation of lung injury. Also, much larger numbers of neutrophils may be seen in lobar

pneumonia and inflammatory conditions other than adult respiratory distress syndrome. Moreover, pulmonary oxygen toxicity and the adult respiratory distress syndrome have been shown to occur despite neutropenia in animals (Raj *et al.*, 1985) and patients (Braude *et al.*, 1985; Rinaldo and Borovetz, 1985). Oxygen toxicity can also clearly occur *in vitro* without the participation of inflammatory cells (Martin *et al.*, 1981; Block and Stalcup, 1981; Housset *et al.*, 1983; Block *et al.*, 1985), and signs of oxygen toxicity may occur *in vivo* without evidence of pulmonary inflammation, as shown by analysis of bronchoalveolar lavage from human volunteers exposed for 17 h to $> 95\%$ O_2 (Davis *et al.*, 1983).

In conclusion, it seems likely that blood leucocytes are not essential for the initiation of acute lung injury but they may enhance pulmonary dysfunction by the self-propagating effects of the released tissue-damaging proteases and oxygen radicals. The two concepts, i.e. direct injury by the intracellular generation of oxygen radicals or inflammatory cell mediated injury are not necessarily mutually exclusive, since hyperoxia may cause the initial damage which in turn causes an influx of inflammatory cells. This combination of injurious processes is strongly supported by the *in vitro* experiments of Suttorp and Simon (1982), Bowman *et al.* (1983) and Krieger *et al.* (1985). The latter authors showed a synergistic interaction between hyperoxia and granulocytes in producing acute lung injury, with hyperoxia priming the lung for further injury by granulocytes.

α-NAPHTHYLTHIOUREA

α-Naphthylthiourea (ANTU) was developed as a selective rodenticide following the observation that phenylthiourea killed rats in low doses while being virtually non-toxic to man (Murphy, 1980; Gosselin *et al.* 1984). Rats and dogs are the most sensitive species tested but even in rats there is considerable variation in the LD_{50} of ANTU, depending on the strain, age and diet (Roszkowski, 1967). This factor and the rapid induction of tachyphylaxis (Van den Brenk *et al.*, 1976) have detracted from the use of thioureas as rodenticides. However, ANTU has been widely used as a model toxin for the study of the pathophysiology of pulmonary oedema since it produces pulmonary toxicity by causing massive, non-haemorrhagic pulmonary oedema with fibrin-rich pleural effusion.

Pathology

The most characteristic feature of ANTU toxicity is the rapid onset and resolution of pulmonary oedema. In rats, pulmonary oedema and pleural effusion begin 2 h after injection and are maximal by 4 h; death generally occurring between 8 and 10 h. In survivors the exudate is reabsorbed by 24 h (Cunningham and Hurley, 1972; Van den Brenk *et al.*, 1976). The same pattern of toxicity has been described in mice (Mais and Bosin, 1984).

Cunningham and Hurley (1972) and Meyrick *et al.* (1972) have described the ultrastructural characteristics of rat lungs at various time-points after ingestion of

lethal or sub-lethal doses of ANTU. The earliest changes (2 h) consist of perivascular and interstitial oedema with localised sub-endothelial blebbing (i.e. accumulation of fluid between the endothelium and its basement membrane). Later, interstitial oedema and endothelial blebbing become more marked. Ultimately, intra-alveolar oedema containing fibrin becomes apparent. This classical (Staub *et al.*, 1967; Staub, 1974) sequence of events in pulmonary oedema is interpreted as follows: interstitial fluid is normally drained away from the gas exchange region to juxta-alveolar sumps formed by lymphatic capillaries in the perivascular, peribronchiolar and septal connective tissue beds, possibly as a consequence of the interstitial pressure gradient created by the distribution and interaction of mechanical forces in the alveolus-capillary region (Weibel and Bachofen, 1979). If altered haemodynamics or altered permeability cause the rate of fluid accumulation in the interstitium to exceed the rate of lymphatic drainage, then the peribronchial and perivascular spaces will become dilated, forming 'cuffs'. Fluid will then accumulate in the alveolar walls and eventually it will enter the air spaces causing alveolar flooding and severe impairment of gaseous exchange. Thus, pulmonary oedema is best defined in functional terms as a condition of altered fluid dynamics in the lung leading to excessive accumulation of fluid within one or more lung compartments. Using a dog lung lymphatic preparation, Rutili *et al.* (1982) have recently confirmed that the oedema caused by ANTU is produced by increased vascular permeability.

Despite the evident fluid leakage, definite signs of endothelial cell injury (swelling, loss of organelles, vacuolation, breaks in endothelium, increased electron density) are either uncommon (Cunningham and Hurley, 1972; Machado *et al.*, 1977) or occur only at a late stage (Meyrick *et al.*, 1972). The pulmonary oedema caused by ANTU is remarkably short-lived. By 24–48 h after an oedemagenic dose the exudate and pleural effusion are reabsorbed and minimal signs of injury are present (Cunningham and Hurley, 1972). No long-term sequelae seem to result from a single dose of ANTU (Van den Brenk *et al.*, 1976), but a recent study has demonstrated right ventricular hypertrophy after four weekly doses of ANTU (Hill *et al.*, 1984).

Mechanisms of toxicity

The mechanism by which ANTU causes endothelial dysfunction is still unknown. Boyd (1980a) and Neal and Halpert (1982) have reviewed the experimental evidence indicating that the lung toxicity of ANTU is brought about by its metabolism and in particular that its desulphuration results in the formation of reactive intermediates.

Resistance to ANTU toxicity has been induced by pretreatments with small doses of ANTU itself, with other thiourea derivatives, sulphydryl compounds including glutathione, 2-aminoethylisothiouronium bromide, possibly cysteine, 3-methylcholanthrene, or piperonyl butoxide (Boyd and Neal, 1976; Van den Brenk *et al.*, 1976). On the other hand, depletion of glutathione by diethylmaleate potentiated the toxicity of ANTU (Boyd and Neal, 1976). Administration of

(carbonyl-^{14}C) and (^{35}S)-ANTU led to covalent binding of radioactivity to macromolecules in the rat lung and liver, with relatively more binding taking place in the lung. In contrast, little radioactivity became bound after the administration of the non-toxic (carbonyl-^{14}C) labelled α-naphthylurea (Boyd and Neal, 1976). The latter observation provides compelling evidence that the thiono-sulphur group is required for both covalent binding and toxicity.

Incubation of (^{14}C) or (^{35}S)-ANTU with rat liver or lung microsomes also led to covalent binding of radioactivity to protein (Boyd and Neal, 1976). Most of this binding was NADPH dependent. The proportion of bound sulphur was considerably higher than that of bound carbon and, as with other thiono-sulphur compounds (Neal and Halpert, 1982), incubation of microsomes with ANTU resulted in a decreased activity of mixed function oxidase. The latter phenomenon also occurred in the lungs following *in vivo* treatment of rats with ANTU (Boyd and Neal, 1976).

Taken together the results from the *in vivo* and *in vitro* studies with labelled ANTU suggest that its lung toxicity results from the biotransformation of the thiocarbonyl moiety giving rise to reactive products that can covalently bind to macromolecules in the lung. It is clear that the lungs are capable of metabolising ANTU products that bind to macromolecules and it is therefore reasonable to assume that it is metabolism *in situ* which is responsible for lung damage (Boyd, 1980a). However, as pointed out by Boyd (1980a) the possibility that toxic metabolites released by the liver reach the pulmonary vascular endothelium has not been conclusively ruled out. Moreover, even if ANTU is metabolised to toxic products in the lung, the cellular sites of the biotransformation of ANTU within the lung and the reasons for the peculiar susceptibility of endothelial cells are still unknown. Both the cytochrome P450-dependent and the FAD-containing monoxygenase systems have been implicated in the activation of ANTU (Poulsen *et al.*, 1974, 1979; Lee *et al.*, 1980).

Two reactive metabolites have been proposed: (1) atomic sulphur released in the desulphuration of ANTU and (2) another metabolite containing the carbonyl carbon (Lee *et al.*, 1980). Approximately half the atomic sulphur bound to liver and lung microsomes appears to have reacted with cysteine of the microsomal proteins to form hydrodisulphides (Lee *et al.*, 1980). However, by virtue of its high reactivity, atomic sulphur possibly binds exclusively at the site of its production thereby only inactivating cytochrome P450 and suggesting that it will be the less reactive metabolites that are more likely to contribute to toxicity (Neal and Halpert, 1982). Fox *et al.* (1983) have recently proposed that thiourea-induced lung injury results from $OH^{\cdot}$ mediated oxidation of thiourea to formamidine disulphide which rapidly decomposes to yield thiourea, atomic sulphur and cyanamide. Fox and co-workers (1983) based their suggestion on the fact that $OH^{\cdot}$ scavengers prevent the induction of oedema by thiourea and they speculated that $OH^{\cdot}$ radicals were generated during microsomal electron transfer. They further hypothesised that cyanamide was the ultimate toxin on the basis that iv injection of high doses of cyanamide also result in lung oedema. This hypothesis seemed to be supported by the fact that singly N-substituted thioureas such as ANTU can be oxidised to cyano-containing compounds whilst the non-oedemagenic N,N′-disubstituted thioureas (Dieke *et al.*, 1947) cannot (Fox *et al.* 1983).

Secondary mechanisms may also be critically involved in ANTU induced oedema. Polymorphonuclear leukocytes do not seem to be crucially involved in this model of pulmonary oedema (Fantone *et al.*, 1984; Cunningham and Hurley, 1972; Meyrick and Reid, 1979) thus possibly explaining its rapid reversibility and the lack of long-term response. Platelet thrombi are a constant if not prominent feature of ANTU lung injury (Cunningham and Hurley, 1972; Meyrick *et al.*, 1972). The possible role of platelets is not easy to determine. They may be involved both in the causation and limitation of pulmonary oedema (Mais and Bosin, 1984; Fantone *et al.*, 1984). It seems possible that ANTU metabolites cause some alteration in the membrane of vascular endothelial cells. This may trigger off a 'rescue operation' by platelets which attempt to seal off gaps. The release of 5HT by aggregating platelets would cause increased vascular permeability and further recruitment of platelets. However, this phenomenon would be self limiting because of the effective sealing off of gaps by the platelets themselves. These considerations illustrate the possible sequence of events which lead to increased lung permeability via complex pathways that include direct interaction of toxins with 'permeability receptors' (possibly involving sulphydryl bonds) and the triggering off of 'second messengers' and the release of mediators (Brigham, 1978).

4-IPOMEANOL

The furan derivative, 4-ipomeanol, has been isolated from spoiled sweet potatoes infected with the common fungus *Fusarium solani* (Boyd *et al.*, 1974a). It has been reported that cattle develop pulmonary injury characterised by oedema, congestion and haemorrhage after consuming sweet potatoes contaminated with this fungus (Wilson *et al.*, 1970; Boyd and Wilson, 1972). 4-Ipomeanol is probably the most studied derivative of a number of furans which have been shown to cause acute lung injury in experimental animals (Boyd, 1980a).

Pathology

After the oral, iv or ip administration of 4-ipomeanol there can be extensive damage to the terminal airways and the development of pulmonary effusions and oedema. The pulmonary effusion is often more extensive in the mouse compared with the rat in which inter-alveolar or perivascular oedema occurs (Boyd, 1980b). If the effusion or oedema is severe enough it will cause the death of the animal. However, after dosing with minimally toxic doses of 4-ipomeanol, the lung lesion is restricted to the smaller bronchioles, with selective necrosis of the non-ciliated bronchiolar epithelial (Clara) cells (Boyd, 1977). The damage which results from the administration of higher doses of 4-ipomeanol may involve other cell types such as the alveolar type II epithelial cell or the bronchiolar ciliated cells (Newton *et al.*, 1985). In mice, the earliest damage after large doses of 4-ipomeanol is found in capillary endothelial cells (Durham *et al.*, 1985). When Clara cells, or indeed other epithelial