NORMOBARIC PULMONARY OXYGEN TOXICITY

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the protective role of endotoxin
and the role of surfactant

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NORMOBARE PULMONALE ZUURSTOF TOXICITEIT

experimentele studies naar het mechanisme van de beschermende werking van endotoxine en de rol van surfactant

PROEFSCHRIFT

ter verkrijging van de graad van doctor
aan de Erasmus Universiteit Rotterdam
op gezag van de rector magnificus
Prof. Dr. C.J. Rijnvos
en volgens besluit van het college van dekanen.
De openbare verdediging zal plaats vinden op
woensdag 13 maart 1991 om 15.15 uur

door

Jan Klein

geboren te Schiedam

1991
Offsetdrukkerij Haveka B.V.,
Alblasserdam

PROMOTIECOMMISSIE:

Promotor	: Prof. Dr. W. Erdmann
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De uitgave van dit proefschrift werd mede mogelijk gemaakt dankzij financiële steun van:

de Stichting Dr. A.A. van Puyvelde Fonds

nv W.A. Hoek's Machine- en Zuurstoffabriek

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PREFACE

Administration of above-ambient oxygen tensions, necessary for treatment of severe hypoxemia caused by respiratory failure or acute lung injury, is potentially toxic for the lungs. This thesis is based on six articles dealing with this topic: one review article and five articles describing original experimental work.

The literature review (Chapter 1) aims to put the five original studies in a broader perspective. Chapters 2, 3 and 4 are based on studies to analyze endotoxin's mechanism of action: endotoxin is reported, in rats, to be the most effective pharmacologic agent for increasing oxygen tolerance.

Working under the supervision of Professor Dr. B. Lachmann, one of the leading experts on the pulmonary surfactant system, Chapters 5 and 6 investigate the role of surfactant in pulmonary oxygen toxicity.

Although the five original studies have their own Discussion and Conclusion sections, some aspects are elaborated upon in the literature review (Chapter 1) and, consequently, there is no separate Discussion chapter in this thesis.

ABBREVIATIONS

atm	=	Atmosphere
BAL	=	Broncho-alveolar lavage
FOR	=	Free oxygen radicals
5-HETE	=	5-hydroxy-6,8,11,14-eicosa tetraenoic acid
12-HETE	=	12-hydroxy-5,8,10,14-eicosa tetraenoic acid
15-HETE	=	15-hydroxy-5,8,11,13-eicosa tetraenoic acid
HHT	=	12(S)-hydroxy-5,8,10-heptadeca trienoic acid
HPLC	=	High performance liquid chromatography
IP	=	Intraperitoneal
$6kPGF_{1\alpha}$	=	6-keto-prostaglandin $F_{1\alpha}$
L-ASA	=	Lysine salt of acetylsalicylic acid
LTB_4	=	Leukotriene B_4
NS	=	Natural surfactant
PGDH	=	Prostaglandin dehydrogenase
PGE_2	=	Prostaglandin E_2
PGI_2	=	Prostacyclin
PMN	=	Polymorphonuclear leukocytes
P-V	=	Pressure-volume
RDS	=	Respiratory distress syndrome
SH	=	Sulfhydryl
SOD	=	Superoxide dismutase
TxA_2	=	Thromboxane A_2
TxB_2	=	Thromboxane B_2
VC	=	Vital capacity
XO	=	Xanthine oxidase

CHAPTER 1

NORMOBARIC PULMONARY OXYGEN TOXICITY

This review article has been published before in: Anesth Analgesia 1990; 70: 195-207

NORMOBARIC PULMONARY OXYGEN TOXICITY

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INTRODUCTION

At the time of the discovery of oxygen (O_2) Joseph Priestley speculated that it might prove of benefit in the treatment of some disease. At the same time he warned that the effects of O_2 might not be uniformly beneficial: "*From the greater strength and vivacity of the flame of a candle, in this pure air, it may be conjectured, that it might be peculiarly salutary to the lungs in certain morbid cases, when the common air would not be sufficient to carry off the phlogistic putrid effluvium fast enough. But, perhaps, we may also infer from these experiments, that though pure dephlogisted air might be very useful as a medicine, it might not be so proper for us in the usual healthy state of the body: for, as a candle burns out much faster in dephlogisticated than in common air, so we might, as may be said, live out too fast, and the animal powers be too soon exhausted in this pure kind of air. A moralist, at least, may say, that the air which nature has provided for us is as good as we deserve.*" [1]

The classic nineteenth-century experiments of Paul Bert and Lorraine Smith proved that O_2 in high concentration was indeed toxic to healthy mammalian lungs [2,3]. Bert demonstrated that it was the increase in partial pressure rather than concentration of O_2 in the inspired atmosphere that was responsible for these deleterious effects. In recent years, a biochemical mechanism involving cellular production of partially reduced metabolites of O_2 has been proposed as a basis for O_2 toxicity. The importance of enzymatic and other intracellular antioxidant defenses against pulmonary O_2 toxicity is now appreciated. Experimental animal models of increased O_2 tolerance have been extensively investigated but, as yet, there is no clinically useful means of reducing or preventing O_2-induced lung injury in humans.

Although this review article presents information on the clinical manifestations, pathology, mechanism and prevention of pulmonary O_2 toxicity, recent developments concerning the mechanisms and prevention of hyperoxic damage in animal models will be emphasized because an increased understanding of these mechanisms may lead

to a more rational basis for the clinical use of O_2 and the development of therapeutic measures effective in preventing or decreasing the effects of O_2 toxicity. Data presented here on mechanisms of hyperoxic injury and protection are obtained from experiments done at normobaric conditions and elevated partial pressures of O_2. The reader is also referred to other reviews [4,5,6,7,8,9] where certain aspects of the subject may be covered in more detail.

CLINICAL MANIFESTATIONS

With exposure to hyperoxia at 1 atmosphere (atm) of pressure, the lung is the organ most severely damaged because pulmonary tissue PO_2 is the highest in the body. As pulmonary tissue PO_2 is directly determined by the alveolar PO_2 [10], arterial hypoxemia does not delay the development of pulmonary O_2 toxicity at 1 atm of pressure [11]. Exposure to O_2 at a partial pressure in excess of 2 atm of pressure also damages the central nervous system and may result in convulsions (the Paul Bert effect) due to sharply increased brain tissue pO_2 [10]. The rate at which O_2 toxicity develops is directly related to the partial pressure of inspired O_2. Until the Apollo fire of 1967 American astronauts breathed 100% O_2 at a pressure of one-third of an atmosphere without showing any sign of pulmonary O_2 toxicity. Hence, a high concentration of O_2 may be less damaging at high altitude where the atmospheric pressure is reduced than is the same concentration at normobaric pressure [10].

The precise concentration of O_2 that is toxic to humans has, for obvious reasons, been difficult to establish. Most data regarding the tolerable limits of O_2 breathing have been obtained from normal, healthy, young subjects. Thus, the effects of underlying disease and other factors such as age, nutritional status, endocrinologic status and the history of previous exposures to oxidants or other substances that may alter protective mechanisms against O_2 toxicity are largely unknown.

The onset of O_2 toxicity may occur after an asymptomatic period, during which no physiologic changes are detectable. In nine young men who breathed 100% O_2 for 6-12 h, no abnormality could be detected in the alveolar-arterial O_2 gradient, pulmonary-artery pressure, total pulmonary resistance, cardiac output or pulmonary extravascular water volume; in addition, there were no symptoms and no x-ray changes [12].

In conscious subjects, the earliest manifestations of O_2 toxicity are symptoms of tracheobronchial irritation like cough and substernal discomfort. The onset of symptoms of tracheobronchial irritation, roughly 4-22 h after the start of O_2 breathing [13], parallels the occurrence of tracheitis and decreased tracheal clearance of mucus [14]. The symptoms of this O_2-induced tracheobronchitis precede changes in pulmonary function tests, but the rate of development of these symptoms is so variable as to be a poor index of O_2 tolerance.

The most widely applied index of O_2 toxicity in humans has been the vital capacity (VC), as early respiratory physiologists reported that subjects who breathed 90%-100% O_2 for 25-30 h had a decreased VC [13,15]. In 1970 it was suggested that decreases in VC could be used to predict the onset, rate of development, and degree of severity of the toxic process in the lung caused by O_2 exposure [16]. Subsequently, a mathematical description was developed [17] and named the "unit pulmonary toxicity dose". The unit pulmonary toxicity dose is still used as a guideline for O_2 exposures by the U.S. Navy [18] and others [19,20]. Recently, however, the available data set was updated and a quantitative statistical analysis was performed to evaluate VC as an index of pulmonary O_2 toxicity [21]. As previously noted by others [4,22], it showed that a decrease in VC is not an ideal index of O_2 toxicity development. The VC measurement requires a trained subject, is effort-dependent and does not take into account the recovery periods as during intermittent exposure; moreover the response varies between individuals. As the index is based on the response of an individual of median susceptibility, more susceptible individuals would be at increased risk.

In four healthy subjects, decreases in vital capacity were followed by small decreases in both static compliance and carbon monoxide diffusing capacity after breathing 0.98 atm of O_2 for 48 h [15].

Pulmonary physiologic changes observed and reproduced in normal subjects exposed to O_2 under experimental conditions may be obscured in the clinical setting. For example, patients exposed to 100% O_2 for 21-44 h, compared with a control group exposed to less than 42% O_2, had no detectable physiologic alterations following cardiac surgery [23]. Similarly, there was no evidence of pulmonary O_2 toxicity, judged by respiratory function, in 41 patients having high-frequency jet ventilation of the lungs with at least 80% O_2 for up to 12 days [24]. On the other hand, increased ratios of dead space to tidal volume and increased arteriovenous shunting have been reported

in patients with irreversible brain damage after ventilation with 100% O_2 for 40 h [25]. However, the patients in this study received steroid therapy which may have affected the outcome.

Although physiologic changes attributable to O_2 breathing include decreases in VC, pulmonary compliance, and diffusing capacity, together with increases in arteriovenous shunting and ratio of dead space to tidal volume, early detection of O_2 toxicity requires more sensitive and specific tests.

Using a bronchoalveolar lavage (BAL) technique in volunteers exposed to more than 95% O_2 for 17 h, a significant alveolar-capillary leak expressed by the presence of increased plasma albumin and transferrin in lavage fluid was detected [26]. Similarly, increases of albumin in BAL fluid occurred in a dose-dependent manner in subjects inhaling 30-50% O_2 for a mean of 45 h [27]. In the same study, clearance of inhaled technetium-labeled diethylenetriamine pentaacetate, a measure to assess lung epithelial permeability, was increased in subjects inhaling 50% O_2. Quantitation of hydrocarbons such as pentane or ethane in expired alveolar gas is another direction in the development of possible indices of early oxidant damage. Because ethane and pentane are volatile hydrocarbons formed during free-radical induced lipid peroxidation, the presence of these gases indicates ongoing free radical formation in lung tissue [28]. Although in humans pentane production increases within 30-120 minutes of breathing 100% O_2 [29], a dose relationship between inspired O_2 concentration and ethane or pentane excretion is not yet established. In the future, it may be possible to develop a metabolic index for detection of hyperoxic pulmonary damage. The fact that the lungs' ability to metabolize biogenic amines, polypeptides, and prostaglandins [30,31,32] decreases soon after hyperoxic exposure might also be used to develop an "early warning" test of O_2 toxicity. To date, use of the tests described above to detect pulmonary O_2 toxicity in the clinical setting in an early phase has been limited for reasons including lack of specificity, problems with reproducibility and the need for sophisticated equipment.

The above studies, however, do indicate that although early (reversible) physiologic, anatomic and biochemical changes can be detected after short exposure to hyperoxia using sensitive tests, humans can tolerate 100% O_2 at sea level for 24-48 h without serious pulmonary injury. Pulmonary damage results only with longer periods of exposure in normal subjects (but pulmonary tolerance to hyperoxia may be

altered by the underlying disease and other physiologic factors). Inspired O_2 in concentrations of 50-100% over long periods of time carries a risk of lung damage, and the duration of exposure required to produce damage seems to be proportional to the concentration of inspired O_2. The "safe" level of inspired O_2 is not established, but we know that less than 50% O_2 can be tolerated for extended periods of time without serious deleterious effects [4].

PATHOLOGY OF PULMONARY OXYGEN TOXICITY

Most studies of pulmonary O_2 toxicity, including ultrastructural morphometry, have been conducted in experimental animals. Few studies have been performed in human subjects; those that have been done have typically been done at autopsy after severe illnesses requiring high concentrations of O_2 (usually delivered by mechanical ventilation). Consequently, although the details and time- course of pulmonary O_2 toxicity are well documented in experimental animals, only the end stages of O_2 toxicity have been studied in humans. However, the sequence of morphologic changes that occurs in the lungs in response to pulmonary O_2 toxicity seems to be quite similar in different animal species [4,33,34,35,36] and humans [37], but the duration and relative severity of each phase of the process show species variability.

In most species, exposure to 100% O_2 at 1 atm for 24-72 h is associated with an initial phase of injury during which no significant evidence of morphologic injury is apparent. This phase is characterized by augmentation in the production rates of partially reduced O_2 species [38] due to increased intracellular metabolism of O_2 [39]. These free radicals are associated with alterations in cell metabolism that are not initially associated with changes in lung structure or ultrastructure.

The earliest morphologic changes seen in the inflammatory phase involve subtle changes in endothelial cell structure, which result in pericapillary accumulation of fluid [33,37,40]. This stage of lung injury is associated with, or rapidly followed by, accumulation of thrombocytes, macrophages and neutrophils in the lung and the release of soluble mediators of inflammation [40,41,42,43].

After exposure of rats to O_2 for 36 h neutrophils are rapidly recruited to the lung [43], and after 48 h, the volume of platelets retained in the pulmonary capillary bed almost doubles [41]. The appearance of neutrophils in the lung is associated with a rapid increase in the extent of morphologic lung injury [33,40]. Neutrophils probably

initiate the final stage of lethal pulmonary O_2 toxicity by releasing further mediators of inflammation and, once activated, by producing toxic O_2 species via oxidases on their plasma membranes [7,44]. Recently, it was suggested that one of these mediators, leukotoxin [9,10-epoxy-12-octadecenoate), plays an important role in the genesis of acute edematous lung damage in pulmonary O_2 toxicity [45]. However, the exact role of the neutrophil as a primary mediator of hyperoxic lung injury is under debate. Depletion of neutrophils decreases the toxic effects of hyperoxia [46], but neutropenia induced in rabbits by the administration of nitrogen mustard does not prevent development of lung microvascular injury and pulmonary edema caused by exposure to hyperoxia [47]. Moreover, the presence of pre-existing lung damage with accumulation of neutrophils in the lung is generally associated with decreased rather than increased sensitivity to O_2 toxicity. Examples include increased O_2 tolerance in animals following preexposure to sublethal doses of O_2 [33] or pretreatment with Bacille de Calmetter et Guétin (BCG) [48], endotoxin [49], oleic acid [50], phosgene [51] or α-naphthylthiourea [52]. These observations suggest that the neutrophil may contribute to but is not essential for the development of pulmonary O_2 toxicity.

The contribution of alveolar macrophages to pathologic effects in the lung is not clear. Oxygen in vivo appears to increase the number of macrophages in sections of rat lung but may not increase the number of cells obtainable with standard methods of lung lavage [53]. It is suggested that the macrophage is responsible for the influx of neutrophils into the lung by the release of chemotactic factors under hyperoxia [54]. Bacterial clearance in animal lungs in vivo decreases after exposure of animals to 100% O_2 [55]. Although bactericidal dysfunction of alveolar macrophages of neonatal rabbits exposed to hyperoxia has been reported [56,57], phagocytotic ability of pulmonary macrophages isolated from adult rats exposed to hyperoxia is normal [58], and the impaired bacterial clearance seen may be due to impaired mucociliary clearance [14]. Production of factors such as O_2 radicals and eicosanoids by alveolar macrophages probably contributes to the pathology of lung damage in hyperoxia [59,60], but such an effect has not yet been shown directly.

In the final phase, overt destruction of the capillary endothelium takes place. In rats exposed to lethal hyperoxia approximately 50% of capillary endothelial cells are destroyed in the few hours preceding the death of the animal [33]. However, this destruction of endothelial cells does not result in overt lung edema; a pleural effusion,

nearly equal in volume to the total lung capacity, and associated plasma volume depletion leading to respiratory or perhaps cardiovascular failure probably constitute the immediate cause of death in O_2-poisoned rats [61,62,63,64]. Although the mechanism of this effusion deserves further study, the pleural effusion is the hallmark of O_2 toxicity in the rat.

At time of death in rats exposed to a lethal dose high concentration of O_2 there is no significant change in the number of type 1 or type 2 alveolar epithelial cells, even though some ultrastructural changes occur, including ruffling of the membranes of alveolar type 1 cells and blunting of the microvilli on alveolar type 2 cells. A significant epithelial cell proliferative response or frank epithelial cell destruction has not been documented [33].

In primates, including humans, there appears to be proportionally greater injury to the alveolar epithelium during the destructive phase of O_2 toxicity. In monkeys, the alveolar type 1 epithelium is almost completely destroyed after 4 days in 100% O_2. Hyperplasia of type 2 alveolar epithelial cells leads to almost total replacement of the alveolar epithelial lining with type 2 cells by the seventh day of exposure [35,40].

With discontinuation of exposure to hyperoxia, or during chronic exposure to sublethal hyperoxia, at least three events may develop as a result of the exposure. The first is the proliferation of type 2 alveolar cells that appears to constitute a restructuring of the alveoli of the lung. The second is a fibroblastic proliferation that may lead to an interstitial fibrosis, one that does not seem to have any utility in recovery and may simply be a manifestation of the aberrant proliferation of a cell type (the fibroblast) that is relatively insensitive to hyperoxia [33,36]. The third event is the development of pulmonary hypertension with major restructuring of the walls of large and small pulmonary arteries. Obliterative and restrictive rather than constrictive changes of the precapillary alveolar unit due to fibrosis and extension of the muscle in the microcirculation appear to be the basis for pulmonary hypertension induced by hyperoxia [65,66].

IN VITRO MODEL SYSTEMS

In cell cultures individual cells exposed to hyperoxia can be damaged without interaction with other cells. This is most extensively demonstrated in endothelial cells. Aortic endothelial cells show evidence of impaired uptake of serotonin after 24 h of

exposure to 95% O_2 [67], possibly due to decreased fluidity of the plasma membranes of these cells [68]. Although endothelial cells show morphologic changes within 24 h of exposure to 80% O_2 [69], increased albumin permeability of cultured endothelial monolayers becomes detectable only after exposure to 95% O_2 for 3 days [70].

In addition to cell cultures, organ cultures and lung explants have been used to evaluate the effects of O_2 on the lung. Ciliary activity ceases in organ cultures of tracheal epithelium after 2-6 days of exposure to 60-80% O_2 [71]. The exposure of tissue slices of rat lungs to hyperoxia results in a degradation of collagen [72]. Damage due to hyperoxia has also been reported in explants of parenchymal tissues from rat and rabbit lung [71,73]. Release of chromium 51 indicator from labeled lung tissue in culture showed that significant cell damage occurs within 18 h of exposure to 95% O_2.

A third in vitro model, the perfused lung has proven to be a sensitive model for detection of early functional damage to the pulmonary alveolar endothelium. Hyperoxia impairs the ability of pulmonary capillary endothelium in the perfused lung to remove various compounds, including serotonin and prostaglandins, from the pulmonary circulation [30,74,75].

MECHANISMS OF PULMONARY OXYGEN TOXICITY

BIOCHEMISTRY OF OXYGEN TOXICITY

The mechanism of O_2 toxicity at the molecular level is now generally attributed to O_2 free-radical reactions with cellular components. Oxygen free radicals are highly reactive O_2 metabolites which have an unpaired orbital electron. The so-called "free radical theory of O_2 toxicity" attributes the damaging effects of hyperoxia to these highly reactive metabolites of molecular O_2. These O_2 free radicals are products of normal cellular oxidation-reduction processes. Under conditions of hyperoxia, their production increases markedly. The sources of O_2 free radicals in hyperoxia are unknown but may be the accelerated oxidative processes in pulmonary parenchymal cells and phagocytes [38]. The enzyme xanthine oxidase, present in endothelial cells, has also been implicated as a source of toxic O_2 metabolites during hyperoxia [76,77].

The O_2 molecule is normally susceptible to univalent reduction reactions in the cell to form a superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) [39,78]. Although

it is likely that both the superoxide anion and hydrogen peroxide have direct toxic effects, they interact to produce even more dangerous species [79]. Figure 1 shows the Fenton reaction, which is catalyzed by metals, particularly ferrous iron, and which results in the formation of the harmless hydroxyl ion together with two extremely reactive species, the hydroxyl free radical ($OH^{\bullet}$) and singlet O_2 [$^1O_2$]. Although all O_2 radicals are capable of various toxic activities, including lipid peroxidation, depolymerisation of mucopolysaccarides, protein sulfhydryl oxidation, cross linking that can lead to enzyme inactivation, and nucleic acid damage, it seems likely that the hydroxyl free radical and singlet O_2 are mainly responsible for the toxic effects of O_2 [80].

Much evidence has recently appeared concerning endogenous defense systems evolved by organisms to protect their biologic integrity from destruction by free radicals. As O_2 free radicals are products of normal cellular oxidative processes, a multilayered biochemical defense system exists that protects organisms against excessive free radical damage (Fig. 1).

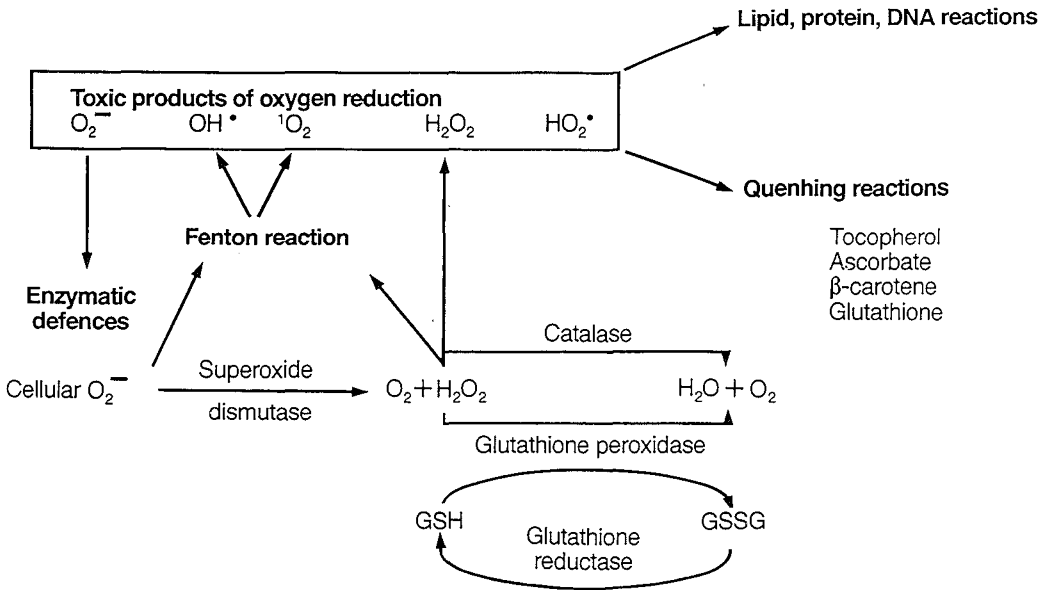

Fig. 1: Scheme of free radical reactions and defense systems.

These biochemical defenses, which probably began evolving as soon as the first photosynthetic organisms began discharging O_2 into the atmosphere, an event that can be dated to about 2 billion years ago [81], include both complex enzyme systems and low-molecular-weight free radical scavengers and are a prerequisite for aerobic life. Prototypes of these antioxidant enzymes are the metalloproteins, termed superoxide dysmutases (SODs), which neutralize superoxide by conversion to hydrogen peroxide [82]. Two enzymes subsequently guard against damage from hydrogen peroxide: catalase and glutathione peroxidase, both of which are capable of degradating intracellular hydrogen peroxide to water; glutathione peroxidase has a more general action and catalyzes the reduction of many hydroperoxides.

The cytoplasmatic enzyme glutathione reductase participates in antioxidant defense by reforming reduced glutathione to glutathione. Glutathione, a preferential substrate for many oxidizing agents, is of primary importance sparing protein sulfhydryl (SH) groups from oxidation.

The low-molecular-weight free radical scavengers include α-tocopherol, ascorbate and ß-carotene, a variety of molecules that preferentially partition into membranes and function by reducing lipophilic free-radical species to less toxic forms. Any molecule that reacts with a free radical can be termed "scavenger"; thus cell components such as sugars, unsaturated amino acids, sulfur-containing amino acids, and unsaturated fatty acids can also scavenge free radicals.

ROLE OF ARACHIDONIC ACID METABOLITES

Arachidonic acid metabolites have biologic properties that can mimic the pulmonary changes produced by hyperoxic exposure. They have potent vasoactive, bronchoactive, and chemoattractant properties, and can increase vascular permeability; all of these are features of hyperoxic lung injury.

Mounting evidence suggests that reactive O_2 metabolites can initiate the release and metabolism of arachidonic acid [60,83,84,85]. Increases in levels of cyclooxygenase as well as lipoxygenase pathway products in BAL fluid have been associated with hyperoxic lung injury [49,86,87,88], but the administration of a cyclooxygenase inhibitor to block the synthesis of prostaglandins does not result in a decrease but rather an increase in hyperoxic lung injury [87]. The early increases in prostaglandin levels in BAL fluid that have been documented [49,86,87], therefore, may

rather reflect an overall increase in arachidonic acid metabolism, with the increase in lipoxygenase pathway products being at least as important or, perhaps, having a more primary role in mediating the hyperoxic lung injury. Recent reports show reduced mortality, inhibition of neutrophil influx and a reduction in the increase of BAL leukotriene B_4 levels in a rat hyperoxia model after treatment with the lipoxygenase inhibitor AA861 [89], and attenuation of rat and rabbit lung injury induced by hydrogen peroxide or an oxidant lipid peroxide using various leukotriene antagonists and inhibitors [83,90]. A primary etiologic role for lipoxygenase pathway products would provide an explanation for the seemingly contradictory results of studies in which the use of a cyclooxygenase inhibitor resulted in exacerbation of prostaglandin-associated lung injury [87,91]. Blockade of just the cyclooxygenase pathway probably results in shunting of arachidonic acid metabolism to the lipoxygenase pathway [92] (Fig. 2). This shunting would result in increased production of lipoxygenase products and, as a consequence, increased lung injury [83,93,94]. A complete understanding of this seemingly paradoxical effect of cyclooxygenase inhibitors and the role of lipoxygenase pathway products as mediators of hyperoxic lung injury awaits studies in which measurements of both prostaglandins and leukotrienes in BAL fluid can be performed and the effects of selective inhibitors can be determined.

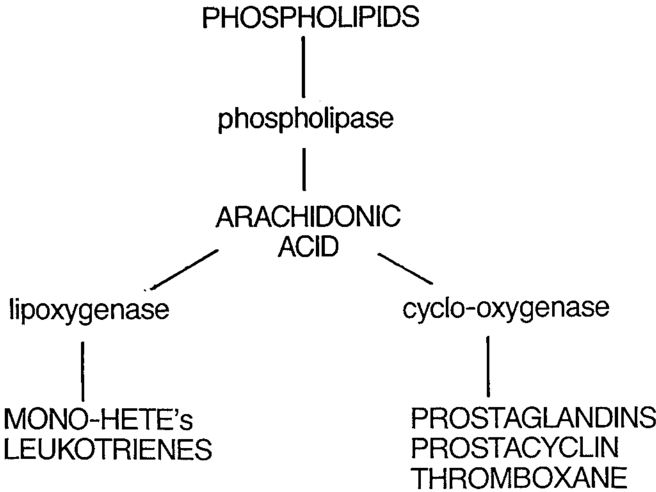

Fig. 2: Arachidonate pathway.

ROLE OF PULMONARY SURFACTANT

The change in lung compliance in animals exposed to high concentrations of O_2 suggests involvement of the surfactant system. Although most investigators report that surface activity of the material lining the alveoli is reduced by exposure to O_2 at increased partial pressures [61,95,96,97], others found normal or increased surface activity, even in the presence of severe pulmonary O_2 intoxication [98].

Increases as well as decreases in surfactant-associated protein synthesis and decreased rates of incorporation of radiolabeled precursors into surfactant phospholipid have been reported [96,99]. There are many possible reasons for such inconsistency. One is the large species and age differences in susceptibility to O_2 toxicity; another is the intensity of hyperoxia used and the stage at which animals were studied. Nevertheless, it appears to be reasonably well established that the surface activity of the alveolar lining material is significantly decreased in the lungs of animals exposed to hyperoxia until death from pulmonary O_2 intoxication. Whether the reduction of pulmonary surfactant function occurs as a direct toxic effect of O_2 or as a consequence of other adverse effects of pulmonary O_2 poisoning (for instance, inactivation of surfactant by intra-alveolar edema [100], remains to be determined. In either case, reduced surfactant function contributes to the pathophysiological changes found during the terminal stages of O_2 intoxication (Fig. 3).

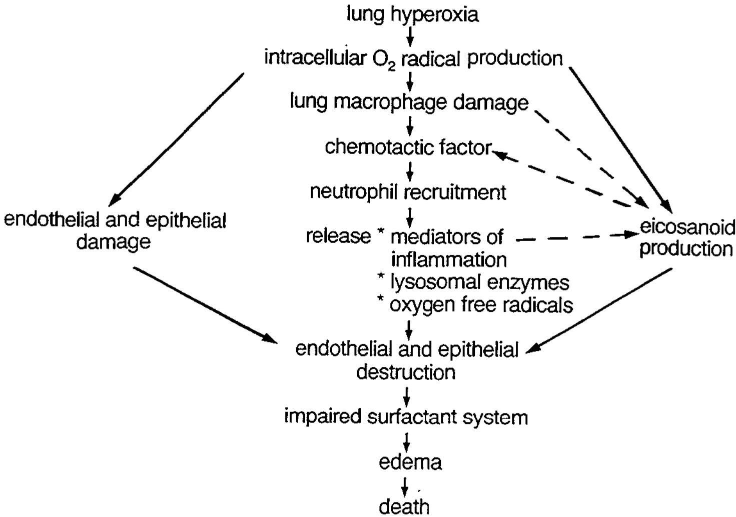

Fig. 3: Summary of events leading to hyperoxic death.

TOLERANCE AND FACTORS INFLUENCING TOLERANCE TO PULMONARY OXYGEN TOXICITY

Susceptibility of animals to O_2-induced lung injury varies widely among species [4], which may in part be based on differences in metabolic rate, including the degree of cytochrome P-450 inducibility [101]. The response to O_2 is also age-dependent: immature animals are less sensitive to O_2 toxicity than adult animals [53]. The increased threshold of young animals to O_2 toxicity appears to be correlated with their ability to increase concentrations of protective enzymes described above in response to exposure to O_2 [102].

Several constitutional and environmental factors may also influence tolerance to hyperoxia. Among factors best explored experimentally are metabolic alterations, diet, administered medications and chemicals, and prior exposure to hyperoxia or hypoxia.

Because hyperoxic damage is dependent on the rate of free-radical production by intracellular metabolic processes, factors that increase cell metabolism, such as epinephrine [103], hyperthermia [104], testosterone [105] and thyroid hormones [106], exacerbate O_2 toxicity in experimental animals and may have similar effects in humans. Dexamethasone treatment of rats exposed to hyperoxia also increases O_2-induced injury and decreases survival, but this effect seems to be dependent on the time of dexamethasone administration; if given when pulmonary inflammation due to hyperoxia is marked, dexamethasone improves survival and decreases lung damage [107].

Deficiencies of vitamins or trace metals in diets increase the susceptibility of the experimental animal to hyperoxia. The adverse effects of vitamin E and A deficiency in hyperoxic exposure have been especially well documented [52,108,109]; while selenium- or copper-deficient diet also lead to increased mortality of rats under hyperoxic conditions [110,111].

Dietary deficiency of protein in rats potentiates toxicity to exposure to hyperoxia due to a lack of sulfur-containing amino acids, which are critical for glutathione synthesis [112]. A negative nitrogen balance and deprivation of protein may make patients in the intensive care unit more susceptible to O_2 toxicity. Administration of sulfur-containing amino acids may protect against this possible potentiation of lung injury.

Many compounds used therapeutically are metabolized with the production of free-radical intermediates (these may be O_2-derived) and may worsen O_2-induced lung injury. A prominent example is the glycoprotein antibiotic bleomycin, which is used clinically in treatment of squamous-cell and germ-cell carcinomas. In animal models, the toxic effects of hyperoxia and bleomycin are synergystic, resulting in more extensive lung injury and fibrosis [113]. Exacerbations of recognized or occult pulmonary fibrosis may also occur as a result of the therapeutic use of other antineoplastic agents (including busulfan, methotrexate, cyclophosphamide) and fortified inspired O_2 concentrations during, for example, anesthesia and the immediate postoperative period [114,115]. The mechanism of bleomycin-induced lung injury involves the formation of a DNA-bleomycin-Fe^{+2} complex that has oxidase-like activity, producing a superoxide anion after binding to nuclear DNA [116,117]. Disulfiram and nitrofurantoin are similarly metabolized with intermediate production of superoxide or hydroxyl radicals and O_2 expectedly increases its cytotoxicity [44,118]. Paraquat, a herbicide which occasionally causes human poisoning, is also more toxic to lungs under hyperoxic conditions [119]. Herbicides initiate plant death in a variety of ways, but in many instances they do so by overtaxing or destroying the protective mechanisms which control toxic O_2 species and free radicals [120].

To date there are no studies available on the effects of general anesthesia on pulmonary O_2 toxicity apart from studies in which patients have been described who showed an increased susceptibility to O_2-induced lung injury after the use of fortified O_2 concentrations during general anesthesia due to drug treatment or herbicide intoxication. This could be due to the fact that, generally, anesthesia procedures are too brief to induce pulmonary O_2 toxicity.

The rat is capable of responding to 80-85% O_2 by increasing concentrations of SOD and the glutathione-related protective enzymes within 3-5 days of exposure [121]. Animals pre-exposed to a sublethal concentration of O_2 are able to tolerate prolonged exposures to 100% O_2. Pre-adaptation of adult rats to hypoxia (10% O_2 for 7 days) also results in tolerance to O_2-induced lung injury and is associated with an increase in SOD concentration [122]. On the other hand, pre-exposure of rats to 40-60% O_2 does not increase protective- enzyme concentrations and decreases tolerance to subsequent exposure to 100% O_2 [123]. These findings suggest that almost lethal levels of superoxide radical production or cell damage are required to increase

protective-enzyme concentrations by hyperoxia or hypoxia.

THERAPEUTIC APPROACHES TO PROTECTION FROM PULMONARY OXYGEN TOXICITY

Many animal models of increased O_2 tolerance have been investigated, but to date no clinically useful means of reducing O_2-induced lung injury in humans exists. Some experimental models will be discussed here because therapeutic measures effective in preventing or decreasing the effects of O_2 toxicity, based on the results of these animal studies, may be introduced to clinical practice in the near future.

To this point, the most effective pharmacologic agent described for increasing O_2 tolerance in rats is bacterial endotoxin. The protection provided by endotoxin is species-specific (rats and lambs develop O_2 tolerance, but mice and hamsters do not; primates have not been tested) [109,124,125]. The mechanism of endotoxin protection against hyperoxic injury is not known. The improved tolerance has been associated with increases in lung SOD and other antioxidant enzymes during hyperoxic exposure [42,123,126]. However, the protective effect of endotoxin is blocked by acetylsalicylic acid [91] which interferes with prostaglandin metabolism. Although production of lipoxygenase metabolites by BAL lavage cells is not inhibited by endotoxin [127], inhibition of in vivo free-radical release by lung neutrophils has been proposed as the mechanism by which endotoxin protects rats from O_2 toxicity [128]. Endotoxin treatment stimulates the production of at least three potent cytokines: tumor necrosis factor/cachectin, interleukin 1, and interferon. All three factors have been implicated as playing an important role in endotoxin's protective action; pretreatment of rats with either tumor necrosis factor/cachectin and interleukin 1 [129], interferon inducers [130], or simply serum of endotoxin-protected rats [131] decreases lung injury and mortality in hyperoxia. Endotoxin also protects against hyperoxic injury to porcine endothelial cells [132]. It has been suggested that endotoxin protects these cultured endothelial cells by prevention of the hyperoxia-induced decrease in plasma membrane fluidity [133]. Currently, there is great interest in the attempt to modify the endotoxin molecule to produce protective substances which have low inherent toxic action, so-called endotoxoids [134].

Difficulties arise with the therapeutic use of SOD or catalase because they are intracellular enzymes with very short half-lives in plasma. There is, therefore, little scope

for their use by direct intravenous injection. However, it is possible for these enzymes to enter cells if they are administered in liposomes and their plasma half-life may also be extended by conjugation with polyethylene glycol. Experimental use of SOD and catalase in these forms results in a substantial protection [135,136,137,138,139]. Polyethylene glycol conjugation with antioxidants may be more effective than liposome encapsulation [140]. Delivery of antioxidants conjugated to polyethylene glycol is improved and their half-lives prolonged compared with liposome encapsulation.

Instillation of exogenous surfactant in rabbits exposed to 100% O_2 for 64 h prevents the development of abnormal lung mechanics and alveolar collapse and mitigates the degree of lung edema, once animals are returned to room air; at least part of this beneficial effect appears to be related to the action of exogenous surfactant in preventing an increase in alveolar surface tension [141].

The leukotriene synthesis blocker AA861 significantly reduces mortality of rats caused by O_2 toxicity when administered intraperitoneally [89]. AA861 inhibits leukotriene B_4 production, a chemotactic agent for neutrophils and, thus, AA861 would reduce the accumulation of neutrophils in the lung.

Intact erythrocytes placed in the tracheobronchial tree of hyperoxic rats dramatically improves their chances for survival [142]. Lungs from erythrocyte-protected rats show almost none of the morphologic damage suffered by untreated animals. These protective effects of insufflated erythrocytes would be based on their recyclable glutathione. An ironic connotation of these experiments is that small amounts of spontaneous alveolar hemorrhage, a common feature in respiratory distress situations, may actually be beneficial in patients ventilated with O_2 at high inspired tensions.

Continuous infusion of the sulfhydryl compounds cysteamine or N-acetylcysteine in rats exposed to 100% O_2 results in a reduced mortality and lung edema caused by hyperoxia [143]. As sulfhydryl compounds are among the most important endogenous antioxidant agents, administration of these cell-permeable sulfhydryl compounds probably prevents the oxidation of lung nonprotein sulfhydryls such as glutathione [144].

Desferioxamine is an iron-chelating agent that may prove to have a therapeutic role, as ferrous iron is both a potent source of electrons for conversion to the superoxide anion and a catalyst in the Fenton reaction. In rats, the administration of

desferioxamine provides partial protection against hyperoxic lung damage [145].

Pretreatment of rats by inducers of pulmonary cytochrome P-450 results in a marked protection against pulmonary O_2 toxicity. This protection is associated with a substantial increase in the components of the pulmonary cytochrome P-450 system, its peroxidase activity and an increased response to hyperoxia by lung antioxidant enzyme activities [146].

Although dietary supplementation of vitamins, proteins and trace metals provides only partial protection in animals with a deficiency of these factors, dietary supplementation of polyunsaturated fatty acids in newborn rats results in increased lung polyunsaturated fatty acid content and improved hyperoxic survival [147]. How lung lipid composition works to influence tolerance to pulmonary O_2 toxicity is not yet known.

CONCLUSION

The last 25 years have seen major progress in our understanding of the mechanisms and pathophysiology of pulmonary O_2 toxicity and, in particular, the elucidation of the role of free radicals. Important problems that remain include methods for early detection of hyperoxic damage and the means to augment antioxidant defenses. With solution of these problems it should be possible to expand significantly the value and indications for the therapeutic use of O_2.

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CHAPTER 2

ENDOTOXIN PROTECTION AGAINST OXYGEN TOXICITY AND ITS REVERSAL BY ACETYLSALICYLIC ACID

This article has been published before in: Crit Care Med 1986; 14: 32-33

ENDOTOXIN PROTECTION AGAINST OXYGEN TOXICITY AND ITS REVERSAL BY ACETYLSALICYLIC ACID

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ABSTRACT

This study investigated the involvement of substances derived from arachidonic acid in the mechanism of endotoxin's protective action against pulmonary oxygen toxicity. Eighty-three percent of rats treated with a small dose of endotoxin (1 mg/kg) survived exposure to over 95% oxygen for 7 days. In contrast, all control rats exposed to the same oxygen concentration died within 3 days. When the endotoxin-treated rats were also treated with the soluble lysine salt of acetylsalicylic acid (100 mg/kg), 7-day survival decreased to 25%. This suggests that prostaglandin metabolism may play an important role in the protective action of endotoxin during hyperoxia.

INTRODUCTION

Despite the potential dangers of hyperoxia on the lung, administration of above-ambient oxygen tensions is necessary for treatment of severe hypoxemia caused by respiratory failure or acute lung injury.

Small doses of bacterial endotoxin markedly increase the survival rate of adult rats exposed to 98% oxygen for periods that are normally lethal (60-72 h) [1,2]. Details concerning the mechanism and even the cellular site(s) of endotoxin's action are not yet known, but there is evidence that the pulmonary responses after bacterial endotoxin administration are due to release of substances derived from arachidonic acid [3] and that these responses can be prevented by concurrent treatment with various cyclooxygenase inhibitors [4]. We investigated the involvement of arachidonic acid derivatives in the mechanism of endotoxin's protective action against pulmonary toxicity, by combined treatment with endotoxin and the soluble lysine salt of acetylsalicylic acid (L-ASA).

METHODS

Forty-eight male Sprague-Dawley rats (TNO, Rijswijk, NL) weighing 250-300 g and maintained on a standard laboratory diet were assigned at random to four different treatment groups: an endotoxin group, a control group, an endotoxin with L-ASA group, and an L-ASA group. Each group had 12 rats.

Endotoxin-treated rats were given a single intraperitoneal (ip) injection of 1 mg/kg endotoxin (Salmonella typhimurium lipopolysaccharide, phenol water extraction: Sigma Chemical Co, London, UK) dissolved in normal saline. Control rats received an equal volume of ip normal saline. L-ASA treated rats received 100 mg/kg L-ASA subcutaneously (sc), 30 min before the ip endotoxin or saline administration, and every 24 h thereafter.

Directly after endotoxin or saline administration, half of the rats in each group (chosen at random) were exposed to compressed air at 1 atm of pressure, while the other half were exposed to 100% oxygen at 1 atm of pressure. Exposures lasted a maximum of 7 days and were performed in special airtight cages with an overflow hole; each cage contained 12 rats. Oxygen concentration was continuously measured (oxygen monitor, Instrumentation Laboratories, Lexington, MA) and was constantly higher than 95% in the oxygen-perfused cages. The CO_2 concentration was held constant at a level similar to that of room air (0.033%) by means of a high oxygen flow (7-8 complete gas changes per hour) and by placing containers of soda lime chips in the cage. The cage temperature was held constant between 23° and 26°C. Water and food were provided ad libitum. The cages were opened once a day for a 10-15 min period to facilitate injection, replenishment of food and water, and waste removal. Survival was monitored on a daily basis. Changes in duration of survival were reflected by a shift of the survival curve to the right or left. We used the Wilcoxon test to determine whether such shifts were statistically significant at a level of 0.05.

RESULTS

Although the saline-treated rats and the L-ASA-treated rats exposed to 100% oxygen all died within 3 days, 83% of the endotoxin-treated rats survived for 7 days (Fig. 1). Of the rats treated with endotoxin plus L-ASA, 25% survived 7 days of oxygen exposure. By contrast, there were no deaths in identically treated groups exposed to compressed air. The survival curve for rats pretreated with endotoxin was significantly

(p < 0.01) to the right of the survival curve for saline-treated rats. This prolonged survival was partly reversed by concurrent treatment with L-ASA (p < 0.05).

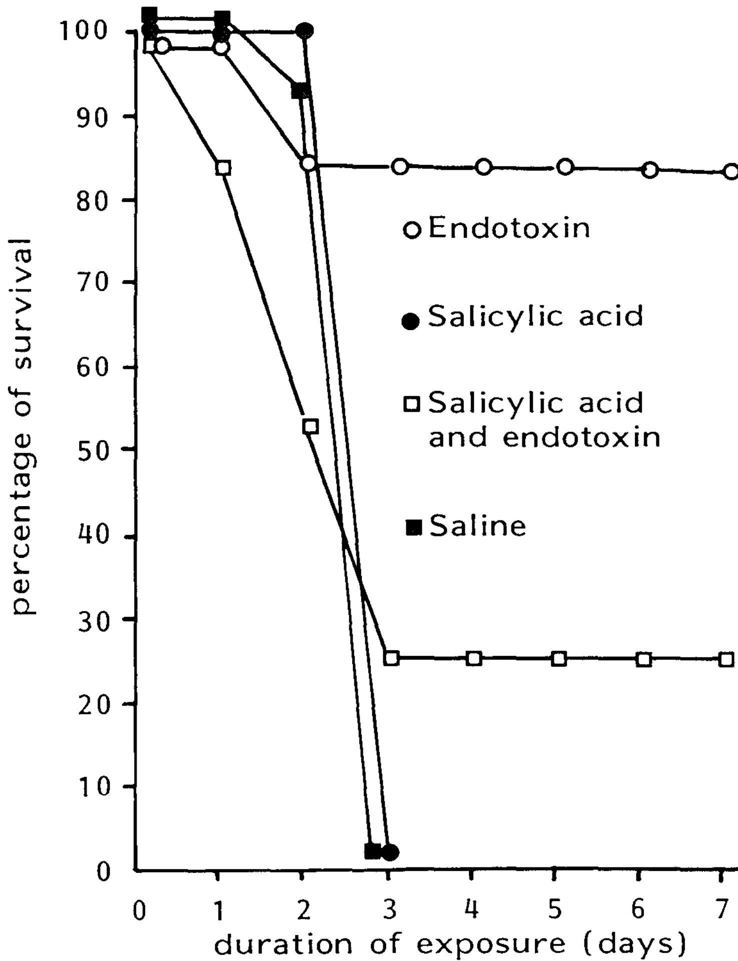

Fig. 1: Survival of rats of exposed to over 95% oxygen for 7 days. The survival curve for rats pretreated with endotoxin is significantly (p < 0.01) to the right of the survival curve for saline-treated rats. This prolonged survival was partly reversed by concurrent treatment with L-ASA (p < 0.05).

DISCUSSION

According to the free-radical theory of oxygen toxicity, exposure to hyperoxia elevates the intracellular oxygen concentration and increases the production of highly reactive oxygen species such as superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) [5]. The possible relationship between free-radical reactions and the microcirculation stems from work revealing the dependency of a patent microcirculation on the continuing balanced production of prostaglandin I_2 (PGI_2) [6]. PGI_2, which is produced by blood vessel walls, is essential to keep the endothelial surface free of platelets and other adhering blood elements. Platelets and lung parenchyma are constantly producing thromboxane A_2 (TxA_2), a potent vasoconstrictor which has marked pro-aggregating and pro-adhering effects on platelets. The continuing synthesis of PGI_2 by blood vessel walls constantly opposes the effects of TxA_2; this synthesis is inhibited by lipid hydroperoxide products of free-radical reactions, but can be stimulated by a single dose of endotoxin [7]. Thus, it seems likely that endotoxin protects the pulmonary microcirculation from hyperoxic injury [8].

We found that repeated administration of L-ASA partly reversed endotoxin-induced protection. Frank and Roberts [1] also administered a cyclooxygenase inhibitor (ip indomethacin) to endotoxin-treated rats exposed to over 95% oxygen concentrations, but they did not find any detrimental effect on endotoxin-induced protection expressed as survival. Although this discrepancy is difficult to interpret, it is possible that the single 3-mg/kg dose of indomethacin was not sufficient to block the cyclooxygenase enzyme for the total period of endotoxin-induced activation, whereas repeated doses of 100 mg/kg L-ASA inhibited cyclooxygenase throughout the exposure to high oxygen concentrations [9].

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CHAPTER 3

CELLULAR AND EICOSANOID COMPOSITION OF BRONCHOALVEOLAR LAVAGE FLUID IN ENDOTOXIN PROTECTION AGAINST PULMONARY OXYGEN TOXICITY

This article has been published before in: Crit Care Med 1989; 17: 247-250

CELLULAR AND EICOSANOID COMPOSITION OF BRONCHOALVEOLAR LAVAGE FLUID IN ENDOTOXIN PROTECTION AGAINST PULMONARY OXYGEN TOXICITY

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ABSTRACT

Endotoxin protects against pulmonary oxygen toxicity in rats, and both prostaglandins and polymorphonuclear leukocytes (PMN) are implicated as playing an important role in this protective action. In this study, a bronchoalveolar lavage (BAL) technique was used to analyze cellular and eicosanoid composition of the lavage fluid of endotoxin-protected oxygen-exposed rats. The BAL fluid of the endotoxin-protected oxygen-exposed rats contained the highest number of PMN, while the BAL fluid of the nonprotected oxygen-exposed rats contained the highest number of macrophages. Thus, morbidity due to pulmonary oxygen toxicity was correlated with the number of macrophages but not with the number of PMN present in the BAL fluid. Leukotriene B_4, thromboxane B_2, and prostaglandin E_2 levels were significantly higher in the lavage fluid of nonprotected oxygen-exposed rats compared to the levels in the lavage fluid of air-exposed rats. Eicosanoid levels in the BAL fluid of endotoxin-protected oxygen-exposed rats did not differ significantly from the levels found in air-exposed control rats. These findings suggest that endotoxin protects against hyperoxia-induced changes in eicosanoid metabolism.

INTRODUCTION

Prolonged exposure to hyperoxia can cause extensive lung injury in many mammalian species. Small doses of endotoxin protect rats from the lung damage and edema of oxygen toxicity [1]. We reversed the protective action of endotoxin by concomitant administration of the lysine salt of acetyl salicylic acid [2]. This could indicate that prostaglandins play an important role in endotoxin protective action.

Polymorphonuclear leukocytes (PMN) have been implicated as playing an important role in acute lung edema resulting from hyperoxic exposure [3]. Several arachidonic acid metabolites (eicosanoids) are thought to have an effect on PMN adherence [4] and ability to produce superoxide radicals [5]. More recently, it has been suggested that leukotriene B_4 (LTB_4) mediates the recruitment of PMN in the lung and that PMN recruitment contributes to lung injury during hyperoxia [6]. In addition to their effect on PMN adherence, chemotaxin, and function, eicosanoids may also contribute to the increased vascular permeability found in pulmonary oxygen toxicity [7]. In this randomized comparative study, a bronchoalveolar lavage (BAL) was performed to determine the number and type of cells and eicosanoid levels in the lavage fluid of endotoxin-protected oxygen-exposed rats and control rats, respectively.

MATERIALS AND METHODS

Experimental Design

Male Sprague Dawley rats weighing 200 to 250 g and maintained on a standard laboratory diet were treated in randomized blocks according to three different treatment regimes: oxygen exposure after endotoxin treatment, oxygen exposure after saline pretreatment, and air exposure after saline pretreatment. Each group consisted of nine rats. Endotoxin-treated rats were given a single intraperitoneal (ip) injection of 1 mg/kg endotoxin (Salmonella typhimurium lipopolysaccharide, phenol water extraction Sigma Chemical, London, UK) dissolved in normal saline. Saline-treated rats received an equal volume of ip normal saline. Directly after endotoxin or saline administration, the rats were exposed to air or 100% oxygen at 1 atm of pressure. Exposures lasted 48 h and were performed in special airtight cages with an overflow hole; each cage contained a maximum of six rats housed in separate compartments. Oxygen concentration was measured continuously with an oxygen monitor (Instrumentation Laboratories, Lexington, MA) and was constantly >95% in the oxygen-perfused cages. The CO_2 concentration was held constant at a level similar to that of room air (0.033%) by means of a high oxygen flow (seven to eight complete gas changes per hour) and by placement of soda lime chip containers in the cage. The cage temperature was held constant between 23° and 26°C. Water and food were provided ad libitum.

Blood Sampling

After exposure for 48 h, the rats were anesthetized with sodium pentobarbital (60 mg/kg ip). The abdomen was opened and the animals were exsanguinated via a puncture of the abdominal aorta. Heparinized blood was collected for estimation of Hct and plasma protein content (Biuret method [8]).

Bronchoalveolar Lavage

A thoracotomy was performed and the volume of pleural fluid was measured by careful suction with a syringe. Total protein content of the pleural effusion fluid was measured (Biuret method [8]). After an anterior neck incision, the trachea was cannulated with a polyethylene catheter. BAL was performed by infusion of 6-ml aliquots of phosphate-buffered saline. The procedure was performed five times so that a total volume of 30 ml was installed and >80% was recovered. After lavage, all aliquots were centrifuged at 450 rpm for 10 min. The supernatant fraction was decanted for analysis of the eicosanoid and albumin content. The lowest detectable albumin concentration was 0.2 mg/ml (Biorad method [9]).

Cell Counts

Amounts of cells present in the lavage fluid were counted by a standard hemacytometer. The number of PMN, macrophages and lymphocytes were determined from Wright-Giemsa-stained differential counts of centrifuged preparations.

Assay of Eicosanoids

The lavage fluid was applied to a column consisting of a Sep-pak C 18 and a silica cartridge (Waters Assoc., Etten-Leur, The Netherlands) which had previously been washed with 10 ml of absolute ethanol and 10 ml of distilled water. The column was rinsed with 2 ml of distilled water and the eicosanoids eluted with 2 ml of absolute ethanol. Radioimmunoassay was used to detect LTB_4, 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$), thromboxane B_2 (TxB_2) and prostaglandin E_2 (PGE_2) levels.

Anti-LTB_4 and standard were obtained from Wellcome Research Laboratories (Dartford, UK), anti-6-keto-$PGF_{1\alpha}$ from Seragen (Boston, MA), antisera to TxB_2 and PGE_2 from l'Institut Pasteur (Paris, France). Prostaglandin standards were obtained from Sigma Chemical. Tritiated antigens were purchased from the Radiochemical

Centre of Amersham (Amersham, UK). Cross reactivities for relevant compounds were <2% at 50% bound/bound$_o$ [10].

Statistical Analysis

Analysis of variance was used to establish proper error variances and to test the statistical significance of differences between group means. Student's two-sample t-test and Spearman rank correlation test were applied in appropriate situations. Level of significance was set at $p < 0.05$.

RESULTS

Volume and Protein Content Pleural Effusion Fluid

All oxygen-exposed saline-treated rats showed pleural effusion with a mean volume of 3.8 ± 0.7 (SD) ml. The total protein content of the pleural effusion fluid was 43.3 ± 0.5 mg/ml, which was almost equal to the protein content of plasma (48.0 ± 1.39 mg/ml). In the oxygen-exposed endotoxin-treated group, only one rat showed pleural effusion with a volume of 5.5 ml and a total protein content of 41.7 mg/ml. None of the air-exposed rats showed pleural effusion.

Hematocrits

The Hct values in the saline-treated oxygen-exposed group were significantly higher than those in the endotoxin-treated and in the air-exposed groups (0.48 ± 0.01% vs 0.40 ± 0.01% and 0.40 ± 0.01%, respectively). There was a significant correlation between the height of the Hct and the volume of pleural effusion fluid.

Albumin Content Lavage Fluid

The albumin content of the lavage fluid of the oxygen-exposed saline-treated rats was 0.48 ± 0.07 mg/ml. This was significantly higher than the albumin content in the other two groups (median concentration <0.2 mg/ml). The albumin content of the lavage fluid showed significant correlation with the volume of pleural effusion fluid.

Cells Counts and Differential

Considerable differences were found in the total number of cells present in the lung lavage fluid (Table 1). The lavage fluid of the oxygen-exposed groups contained the

TABLE 1. Difference in cellular composition of lavage fluid (mean ± SE)

Treatment	Mean (% of lavaged cells)	O_2 Control	Endotoxin + O_2
Number of Cells			
Air control	431	1125 ± 161[a]	1303 ± 586[a]
O_2 control	1556	—	177 ± 503
Endotoxin + O_2	1733	—	—
Number of PMNs			
Air control	1 (0.2)	248 ± 90[a]	913 ± 443[a]
O_2 control	249 (17.5)	—	664 ± 442
Endotoxin + O_2	913 (63.4)	—	—
Number of Macrophages			
Air control	427 (98.8)	715 ± 148[a]	93 ± 195
O_2 control	1142 (80.5)	—	622 ± 225[a]
Endotoxin + O_2	519 (36.0)	—	—
Number of Lymphocytes			
Air control	4 (0.9)	23 ± 7[a]	3 ± 4
O_2 control	27 (1.9)	—	20 ± 7[a]
Endotoxin + O_2	7 (4.9)	—	—

$\alpha = p < 0.05$. Total number of cells per lavage: number.10^4

TABLE 2. Difference in eicosanoid composition of lavage fluid (mean ± SE)

Treatment	Mean	O_2 Control	Endotoxin + O_2
LTB_4 Level			
Air control	2573	2716 ± 807[a]	1142 ± 807
O_2 control	5289	—	1574 ± 807
Endotoxin + O_2	3714	—	—
PGE_2 Level			
Air control	57	30 ± 9[a]	12 ± 9
O_2 control	88	—	19 ± 9[a]
Endotoxin + O_2	69	—	—
6-keto-$PGF_{1\alpha}$ Level			
Air control	2428	465 ± 748	743 ± 748
O_2 control	1963	—	278 ± 748
Endotoxin + O_2	1685	—	—
TxB_2 Level			
Air control	274	167 ± 66[a]	9 ± 66
O_2 control	441	—	176 ± 66[a]
Endotoxin + O_2	265	—	—

$\alpha = p < 0.05$. All eicosanoid levels are shown pg/lavage fluid.

highest number of cells. The lavage fluid of the air-exposed rats contained mainly macrophages, while there was an influx of neutrophils in the saline-treated oxygen-exposed group which increased in the endotoxin-treated oxygen-exposed group. In absolute numbers, the lavage fluid of the endotoxin-treated oxygen-exposed group contained mostly neutrophils, while the lavage fluid of the saline-treated oxygen-exposed rats contained mostly macrophages.

Eicosanoid Content Lavage Fluid

The eicosanoid levels in the lavage fluid of the endotoxin-protected oxygen-exposed rats did not differ significantly from the levels found in the lavage fluid of air-exposed controls (Table 2). The lavage fluid of the saline-treated, oxygen-exposed rats contained significantly more LTB_4, TxB_2, and PGE_2 than the lavage fluid of air-exposed rats. There was a clear correlation between the level of 6-keto-$PGF_{1\alpha}$ and TxB_2 in all three groups.

Relationship between LTB_4 and Number of PMN in Lavage Fluid

Although there was a significant correlation between the level of LTB_4 and the number of PMN present in the lavage fluids of the air-exposed group, no correlation could be found in the other two groups.

DISCUSSION

Although hyperoxic lung injury is well recognized pathologically, its pathogenesis has not been fully elucidated. It has been postulated that the source of oxygen-derived radicals associated with oxygen toxicity may be the PMN [3]. A close temporal association between the appearance of PMN in the lung lavage fluid and death of animals exposed to hyperoxia has been reported [11]. Endotoxin protects against pulmonary damage due to oxygen toxicity [12]. The albumin content of the lavage fluid and the amount of pleural effusion are good indications of the extent of pulmonary damage [13]. Although the numbers of neutrophils present in the lavage fluid of the endotoxin-protected rats were at least as high as the numbers found in the lavage fluid of the nonprotected oxygen-exposed group, eight of nine animals in the endotoxin group showed signs of protection (no pleural effusion and a low albumin content of the

lavage fluid). This could be due to a decreased production of free radicals by PMN in the endotoxin-treated rats, as suggested by Berg and Smith [14].

The numbers of macrophages in the lavage fluid of the oxygen-exposed saline-treated group were significantly higher than the numbers found in the endotoxin-protected, oxygen-exposed group and the air-exposed group. These findings resemble the observations of Glass et al. [15], who found that in rats the lethality of oxygen exposure correlated with the number of mononuclear cells, but not with the number of PMN present in BAL fluid.

Recently, Taniguchi et al. [6] were able to improve survival of oxygen-exposed rats using a leukotriene synthesis blocker. They suggested that LTB_4 is responsible for the accumulation of PMN in the lung and that this PMN recruitment contributes to hyperoxic lung injury. In the air-exposed group, there was a significant correlation between the number of neutrophils (although very small) and the concentration of LTB_4 on the lavage fluid. In the other two oxygen-exposed groups, no correlation could be found. This could be due to the fact that neutrophils themselves, present in abundancy in these two groups, break down LTB to degradation products which could be detected in this study, or it could indicate that in these two groups LTB_4 is not responsible for the attraction of PMN to the lungs.

Thromboxane A_2 (TxA_2) stimulates PMN adherence while prostacyclin (PGI_2) opposes this effect [4]. We found a clear correlation between the level of the hydration product of PGI_2 (6-keto-$PGF_{1\alpha}$) and the level of the hydration product of TxA_2 (TxB_2) in each group. The fact that TxB_2 levels were significantly higher in the oxygen-exposed group could indicate that the leukoadherent effect of TxA_2 dominates in the oxygen-exposed, saline-treated group. We found the highest concentration of PGE_2 in the oxygen-exposed, saline-treated group. This could be due to a decreased activity of the enzyme prostaglandin dehydrogenase (PGDH) which is responsible for the degradation of PGE_2. The PGDH activity in lung homogenates from guinea pigs exposed in vivo to 100% oxygen was found to be decreased [16]. In perfused rat lungs, a decrease in PGE_2 inactivation has been correlated with the duration of exposure to high oxygen concentrations [17]. PGE_2 is reported to inhibit the ability of PMN to release LTB_4 and oxygen-free radicals [6]. Toxic oxygen metabolites are thought to play an important role in pulmonary oxygen toxicity. Reactive oxygen

derivatives, including hydrogen peroxide (H_2O_2) and superoxide (O_2^-) may themselves influence arachidonic acid metabolism. H_2O_2, the predominant dismutation product of O_2^-, has been shown to stimulate both PGE and prostaglandin F production after its degradation to an oxygen-centered radical [18]. Organic hydroperoxides, products of oxygen-induced lipid peroxidation of cell membranes, stimulate prostaglandin synthesis by accelerating the release of arachidonate from lipid stores [19].

We found no significant differences in eicosanoid levels between the air-exposed and oxygen-exposed endotoxin-treated groups. This could indicate that endotoxin protects against hyperoxia-induced changes in eicosanoid metabolism and may be explained by either a prevention of the hyperoxia-induced increase in eicosanoid production or a protection against hyperoxia-induced inhibition of eicosanoid degradation [16]. Similar endotoxin action has been described concerning serotonin and norepinephrine degradation [20]. Eicosanoid metabolism may play an important role in the protective action of endotoxin during hyperoxia [2]. Further studies are needed to elucidate the role of eicosanoids in endotoxin-induced protection against pulmonary oxygen toxicity.

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CHAPTER 4

ENDOTOXIN PROTECTION AGAINST PULMONARY OXYGEN TOXICITY AND ITS REVERSAL BY ACETYL SALICYLIC ACID: ROLE OF EICOSANOID PRODUCTION BY BRONCHO-ALVEOLAR LAVAGE CELLS

This article has been published before in: Agents and Actions 1989; 26: 246-248

ENDOTOXIN PROTECTION AGAINST PULMONARY OXYGEN TOXICITY AND ITS REVERSAL BY ACETYL SALICYLIC ACID: ROLE OF EICOSANOID PRODUCTION BY BRONCHO-ALVEOLAR LAVAGE CELLS

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ABSTRACT

Small doses of endotoxin markedly increase the survival rate of adult rats exposed to 98% oxygen for periods that are normally lethal. The lysine salt of acetyl salicylic acid (L-ASA) partially reverses this protective effect of endotoxin. In this pilot study we investigated the level of eicosanoid production by broncho-alveolar lavage (BAL) cells and found that BAL cells of endotoxin protected rats, present in abundancy, have an equal or increased capacity of HHT, 15-HETE, 12-HETE, LTB_4 and 5-HETE production. These data suggest that production of the lipoxygenase products by BAL cells does not seem to play an important role in the pathogenesis of pulmonary oxygen toxicity. We did not find any indication for the occurrence of shunting of arachidonic acid metabolism to the lipoxygenase pathway as an explanation for the reversal of endotoxin's protective action by L-ASA.

INTRODUCTION

Endotoxin protects against the development of pulmonary oxygen toxicity in the rat. The lysine salt of acetyl salicylic acid (L-ASA) reverses this effect [1]. In a former study [2], we found that inhibition of prostaglandin synthesis does not seem to be responsible for this reversal. We also found that the broncho-alveolar lavage (BAL) fluid of the endotoxin-protected oxygen-exposed rats contained more cells when compared with the BAL fluid of the saline-treated oxygen-exposed rats and the saline-treated oxygen-exposed rats (17.3×10^6 vs 15.6×10^6 and 4.3×10^6 per lavage, respectively).

In this pilot study we measured eicosanoid production by BAL cells after calcium ionophore stimulation:

1. to test the capacity of eicosanoid production by BAL cells obtained from endotoxin-protected oxygen-exposed rats.
2. to test the hypothesis that the reversal of survival by L-ASA was based on shunting of arachidonic acid metabolism to the lipoxygenase pathway.

METHODS

Male Sprague Dawley rats weighing 200-250 g and maintained on a standard laboratory diet were treated in randomised blocks according to five different treatment regimes: air exposure after saline treatment, oxygen exposure after saline treatment, oxygen exposure after endotoxin treatment, oxygen exposure after L-ASA treatment and oxygen exposure after endotoxin + L-ASA treatment. Each group consisted of nine rats. Endotoxin-treated rats were given a single intraperitoneal (i.p.) injection of 1 $mg.kg^{-1}$ endotoxin (Salmonella typhimurium lipopolysaccharide, phenol water extraction: Sigma Chemical Co. London, UK) dissolved in normal saline. Saline-treated rats received an equal volume i.p. of normal saline. L-ASA treated rats received 100 $mg.kg^{-1}$ L-ASA subcutaneously, 30 min before saline administration, and every 24 h thereafter. Directly after endotoxin or saline administration, the rats were exposed to air, or 100% oxygen at 1 atm of pressure. Exposures were performed in special airtight cages. Food and water were provided ad libitum.

After 48 h of exposure, BAL was performed by infusion of 6 ml aliquots of normal saline. The procedure was performed 5 times so that a total volume of 30 ml was installed and more than 80% recovered. After lavage, all aliquots were centrifuged at 450 x g for 10 min. Numbers of cells were counted by a standard hemacytometer. Eicosanoid production by BAL cells was measured after Ca^{2+} ionophore A23187 stimulation (final concent. 2 μM) and a 10 min incubation with 14C-arachidonic acid (1 μCi, 58 mCi/mmol) and glutathione (final concent. 2 μM), using a HPLC method.

RESULTS

Eicosanoid production by BAL cells is shown in Fig. 1. Other eicosanoids were not present in detectable levels. BAL cells of endotoxin-protected oxygen-exposed rats produced equal amounts of HHT, 15-HETE and 12-HETE but more ($p < 0.05$, unpaired Student's t-test) LTB_4 and 5-HETE when compared with BAL cells of saline-treated oxygen-exposed rats.

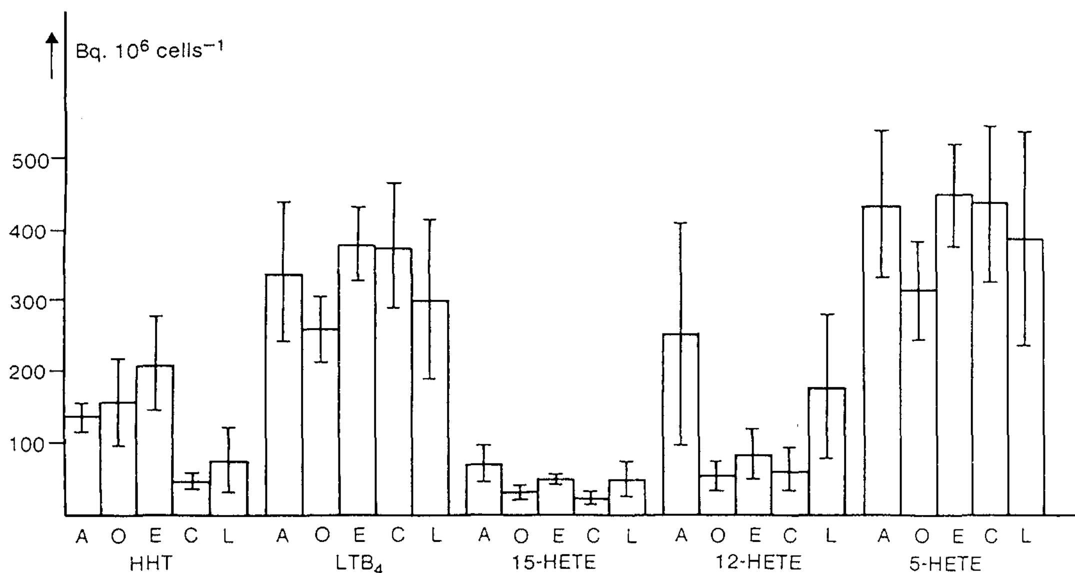

Fig. 1: Eicosanoid production by BAL cells (mean ± SD).

A = Air exposure for 48 h

O = Oxygen exposure for 48 h

E = Oxygen exposure for 48 h + endotoxin 1 mg.kg^{-1} i.p.

C = Oxygen exposure for 48 h + endotoxin 1 mg.kg^{-1} i.p. + L-ASA 100 mg.kg^{-1} 24 h^{-1} s.c.

L = Oxygen exposure for 48 h + L-ASA 100 mg.kg^{-1} 24 h^{-1} s.c.

DISCUSSION

Although hyperoxic lung injury is well recognised pathologically, its pathogenesis has not been fully elucidated. The most effective pharmacologic agent yet described for increasing oxygen tolerance in rats is bacterial endotoxin. Lipoxygenase products may play a role in the pathogenesis of pulmonary oxygen toxicity, although the source of these lipoxygenase products is not yet known [3]. Pulmonary macrophages and polymorphonuclear leukocytes (PMN) may also play a role in the pathogenesis of hyperoxic damage [4]. A close temporal association between the appearance of PMN in the lung lavages and death of animals exposed to hyperoxia has been reported. In a former study, we found that PMN were present in abundancy in the lavage fluid of endotoxin-protected oxygen-exposed rats [2]. We have measured the capacity of eicosanoid production of BAL cells from these endotoxin-protected rats after Ca^{2+} ionophore A23187 stimulation. The results seem to indicate that these BAL cells have an equal or increased capacity of 15-HETE, 12-HETE, LTB_4 and 5-HETE production. Assuming that eicosanoid production measured in vitro after Ca^{2+} ionophore A23187 stimulation reflects the level of eicosanoid production by BAL cells prior to lavage, the data suggest that production of these lipoxygenase products by BAL cells does not seem to play an important role in the pathogenesis of pulmonary oxygen toxicity.

The protective effect of endotoxin is blocked by acetyl salicylic acid. In an earlier study we found that inhibition of prostaglandin synthesis does not seem to be responsible for this effect [2]. It is possible that blockade of the cyclooxygenase pathway results in shunting of arachidonic metabolism to the lipoxygenase pathway [5]. Having measured the eicosanoid production by BAL cells after Ca^{2+} ionophore stimulation in vitro, we did not find any indication for the occurrence of shunting of arachidonic acid metabolism to the lipoxygenase pathway. However, since there is little information on the source of leukotrienes which may play a role in pulmonary oxygen toxicity, there is still a possibility that shunting to the lipoxygenase pathway does take place in lung tissue. Future studies will concentrate on this aspect.

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CHAPTER 5

SURFACTANT IN PULMONARY OXYGEN TOXICITY

This article (with minor editorial revisions) has been published before in:
Adv Exp Med Biol 1987; 215: 345-349.

SURFACTANT IN PULMONARY OXYGEN TOXICITY

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INTRODUCTION

Prolonged continuous exposure to high concentrations of oxygen can lead to respiratory failure and death. However, the exact pathogenesis of respiratory failure induced by oxygen toxicity has not yet been fully established. This study was designed to investigate the role of pulmonary surfactant in the development of respiratory failure induced by high concentrations of oxygen.

METHODS

Thirty-two Sprague Dawley rats weighing 250-300 g and maintained on a standard laboratory diet were used. Twenty-two rats were exposed to 100% oxygen at 1 atm of pressure in a specially designed polystyrene chamber. Oxygen concentration was continuously monitored (Oxygen Monitor, Instrumentation Labs. Lexington, USA) and kept at a level above 95% throughout the study. Carbon dioxide concentration was held constant at a level similar to room air (0.33%) by means of a high oxygen flow and placement of soda lime chip containers in the chamber; temperature in the chamber was maintained between 23-26°C. Survival of animals was checked on an hourly basis; all oxygen-exposed rats died within 72 h of exposure.

Directly after death a thoracotomy was performed and the volume of pleural effusion was measured. Lungs were dissected, weighed and the trachea was cannulated. Pressure-volume (P-V) diagrams were recorded with a maximum insufflation pressure of 40 cm H_2O. Following this procedure, broncho-alveolar lavage was performed by infusion and gentle aspiration of 9 ml physiological saline; the surface tension activity of the lavage fluid was measured with a Wilhelmy balance (Biegler, Mauerbach, Austria).

In a control group of four air-exposed rats, in vivo broncho-alveolar lavage was performed according to the method of Lachmann et al [1980], to produce surfactant-deficient lungs. Following this procedure, the lungs were ventilated for 15

min; the animals then received a lethal intraperitoneal injection of barbiturate. Another untreated control group of six air-exposed rats also received a lethal injection of barbiturate. Lung weight and surface tension in both control groups were measured and P-V diagrams recorded, following the same procedure as for oxygen-exposed animals.

RESULTS

Pressure-volume diagrams

The lungs of all oxygen-exposed rats were completely atelectatic. The P-V curves in Fig. 1 show that an opening pressure of more than 10 cm H_2O was needed to open the lung. At an insufflation pressure of 40 cm H_2O, the inflated volume was less than 50% of that in normal lungs (air-exposed, untreated group; Fig. 2).

The characteristic deflation limb of the P-V curves recorded in oxygen-exposed lungs indicates that these lungs are over-stabilized compared with lungs of the air-exposed group. This was confirmed by the fact that oxygen-exposed lungs did not collapse after inflation; they remained completely aerated.

Lungs in which surfactant deficiency was induced had the same retractive forces as oxygen-exposed lungs, with similar opening pressure, but the deflation curve was almost linear, an obvious sign of stiffness of the lungs (Fig. 3).

Surface tension measurements

No significant difference was found between surface tension activity of lavage fluid from oxygen-exposed lungs and air-exposed lungs (Figs. 1, 2 and Table 1). In surfactant-deficient, air-exposed lungs, however, surface tension activity was very low (Fig. 3 and Table 1).

Table 1. Surface tension measurements (mean ± SD)

	Gamma max. mN/m	Gamma min. mN/m
Air-exposed lungs	51.3 ± 3.2	20.7 ± 1.2
Oxygen-exposed lungs	51.5 ± 1.6	22.9 ± 3.5
Surfactant-deficient lungs	71.6 ± 1.4	43.4 ± 5.2

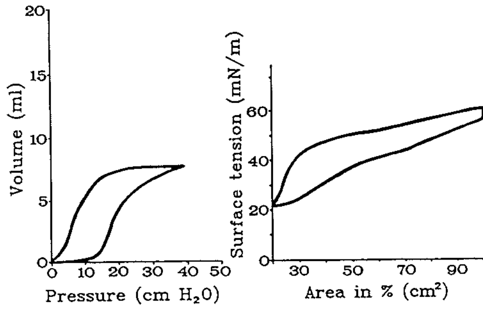

Fig. 1. Left: Pressure-volume diagram. Right: Surface tension/surface area diagram. Both diagrams show mean values from 22 rats exposed to 100% oxygen at 1 atm of pressure.

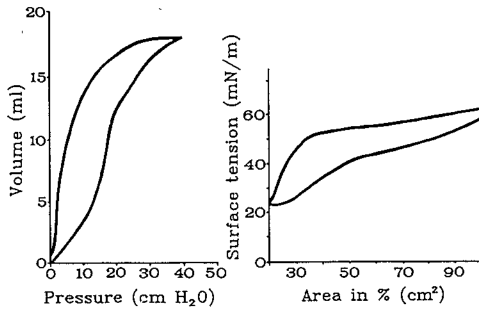

Fig. 2. Left: Pressure-volume diagram. Right: Surface tension/surface area diagram. Both diagrams show mean values from 6 air-exposed control rats.

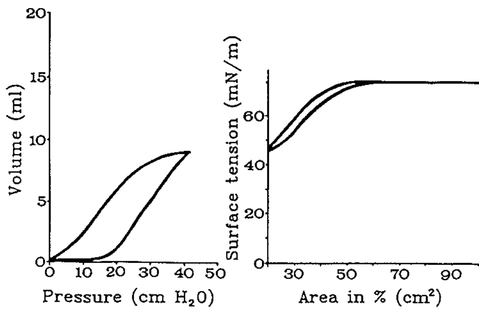

Fig. 3. Left: Pressure-volume diagram. Right: Surface tension/surface area diagram. Both diagrams show mean values from 4 rats following lung lavage to produce surfactant-deficient lungs.

Wet lung measurements

The wet weight (x ± SD) of oxygen-exposed lungs was slightly higher than that of air-exposed lungs: 3.05 ± 0.15 g vs 1.9 ± 0.18 g, respectively. The wet weight of surfactant-deficient, air-exposed lungs was 5.8 ± 1.3 g.

Pleural effusion volume measurements

In the oxygen-exposed group pleural effusion values (x ± SD) were 12.8 ± 2.6 ml with a serum protein content of 4.1 ± 0.2 g%.

DISCUSSION

Prolonged exposure to high concentrations of oxygen can lead to death from respiratory failure. Interestingly, this study revealed that lung failure induced by oxygen exposure is not the primary cause of death. This is substantiated by the following findings:

1. the lavage fluid of oxygen-exposed lungs showed normal surface tension activity;
2. the average wet weight of oxygen-exposed lungs was only slightly higher than that of air-exposed lungs;
3. although normal surface tension activity was measured in oxygen-exposed lungs, the P-V curves showed high retractive forces. This might be due to changes in anatomical structure, but this idea is not supported by pathological examination of oxygen-exposed lungs in other studies [Crapo et al, 1980]. Another explanation could be that high retractive forces are induced by dysfunction of the pulmonary surfactant system, probably due to an inhibitor which is not yet detectable by surface tension measurements in vitro. Stabilization of the deflation limb in these lungs can be explained by the presence of fluid with high surface tension in the small airways which leads to air trapping [Lachmann, 1988];
4. the P-V curves of the oxygen-exposed group, compared to controls, clearly showed changes in lung mechanics. Nevertheless, we do not assume that these changes in lung mechanics are responsible for the respiratory failure and ultimate death. Previous studies have demonstrated that guinea pigs with similar P-V curves could survive [Lachmann et al, 1980].

Thus, in accordance with Smith et al [1981] we opine that the large amount of pleural effusion found in oxygen-exposed rats, resulting in severe compression of the lung, is the only possible explanation remaining as the primary cause of death of rats exposed to 100% O_2.

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CHAPTER 6

ACUTE RESPIRATORY FAILURE INDUCED BY TRACHEAL INSTILLATION OF XANTHINE OXIDASE, ITS PREVENTION AND THERAPY BY EXOGENOUS SURFACTANT INSTILLATION

This article (with minor editorial revisions) has been published before in:
Adv Exp Med Biol 1987; 215: 351-4.

ACUTE RESPIRATORY FAILURE INDUCED BY TRACHEAL INSTILLATION OF XANTHINE OXIDASE, ITS PREVENTION AND THERAPY BY EXOGENOUS SURFACTANT INSTILLATION

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INTRODUCTION

Free oxygen radicals (FOR) play an important role in a variety of diseases. The hypoxanthine-xanthine oxidase system, which generates the superoxide radical, exerts a damaging effect on several organs, including the lung [review: Saugstad, 1985]. It has been shown that intravenous hypoxanthine in rats breathing 100% oxygen can cause lung damage, in contrast to hypoxanthine alone or oxygen alone [Saugstad et al, 1984a]. Further, it has been shown [Johnson et al, 1981] that the hypoxanthine-xanthine oxidase system acutely induces increased capillary permeability in the rat lung. We have demonstrated that xanthine oxidase (XO) applied to the trachea of guinea-pigs induces dramatic changes in lung-thorax compliance, in the course of a few minutes, by destroying the functional integrity of the bronchial and alveolar surfactant system, probably by formation of FOR. This effect could be partly prevented by superoxide dismutase (SOD) which is a superoxide radical scavenger [Saugstad et al, 1984b]. The purpose of this study was to investigate whether it is possible to influence the functional changes induced by FOR by tracheal instillation of natural surfactant (NS).

MATERIAL AND METHODS

Preparation and in vivo characterization of surfactant

The surfactant used in this study was a natural surfactant isolated from adult rabbit lungs in basically the same manner as previously described [Metcalfe et al, 1980] but with some modifications. In brief, immediately after the animals received a lethal dose of pentobarbital sodium, the lungs were dissected, minced and the tissue washed with saline (100 ml/20-25 g tissue) for 30 min, filtered and the filtrates centrifuged at 500 g at room temperature to remove cell debris. The supernatant was then centrifuged for

one hour at 8000 g at 2-4°C. The surfactant phospholipids were extracted from the resulting white pellet with chloroform, methanol, ethanol and acetone. The latter procedure was repeated at least twice.

The resulting surface active material was resuspended in saline so that a concentration of 60 mg total phospholipids/ml resulted. The activity of this preparation was tested in vivo [Lachmann, 1986] in immature rabbit fetuses from day 27 of gestation. In these premature rabbits the instilled surfactant increased lung-thorax compliance more than eleven-fold.

Experimental procedure

The study was performed using young guinea-pigs, weight range 330-390 g. Following anesthesia with pentobarbital sodium (60 mg/kg body weight) the animals were tracheotomized. After relaxation with pancuronium (2 mg/kg body weight) animals' lungs were ventilated in parallel (6 animals simultaneously) with a Servo ventilator 900 B (Siemens Elema, Solna, Sweden) according to Lachmann [1985]. The respiratory rate was 20 per min, peak pressure 15 cm H_2O, inspiratory time 50% and inspiratory oxygen concentration 100%.

One unit XO (Sigma Chemical Co. St. Louis, USA) dissolved in 1 ml saline was instilled into the trachea of the animals after a ventilation period of 10 min. Simultaneous to the application of the fluid, the peak pressure of the ventilator was raised to 35 cm H_2O and positive end-expiratory pressure of 4 cm H_2O; this was necessary to keep the animals alive. This working pressure was maintained in all animals throughout the study period, except for the few minutes when pressure-volume diagrams were recorded. Pressure-volume diagrams were recorded in each animal by placing them alternately into a specially constructed pressure-constant body plethysmograph connected to a Servo ventilator 900 B. Volume changes were recorded with a Fleisch tube connected to the body plethysmograph via a differential transducer (Siemens Elema EMT 34) and amplifier (EMT 31), and an integrator unit (Siemens Elema Mingograph 81).

Five groups of animals were studied:

1) animals receiving 1 unit XO plus, 35-40 min later, 1.5 ml surfactant (n=7)

2) animals receiving 1 unit XO plus, 35-40 min later, 1.5 ml saline (n=8)
3) animals receiving nothing, only ventilated (n=4)
4) animals receiving 1 unit XO followed 1-2 min later by 1.0 ml surfactant plus, 35-40 min later, additional 1.0 ml surfactant (n=7)
5) animals receiving 1 unit XO followed 1-2 min later by 1.0 ml saline plus, 35-40 min later, 1.0 ml surfactant (n=5).

RESULTS AND DISCUSSION

Table 1 shows that the decreased lung-thorax compliance after XO instillation could be almost completely restored by tracheal instillation of surfactant (Group 1).

Table 1. Lung-thorax compliance from the deflation curve of the P-V diagrams.

	Initial values	30 min after XO	15 min after surf. or saline
Group 1 (n=7) XO+1.5 ml surf. 35-40 min later	100 ± 26%	31 ± 11%	84 ± 19%
Group 2 (n=8) XO+1.5 ml saline 35-40 min later	100 ± 16%	33 ± 13%	25 ± 8%
(X±SD)			

This effect must be related to the surfactant lipids and not to fluid administration since the lung-thorax compliance deteriorated further in those animals receiving saline (Group 2) instead of surfactant. In earlier investigations we demonstrated that the combination of fluid with artificial ventilation itself leads to damage, mainly of the bronchial surfactant system, which results in an increased opening pressure without significant restrictive lung volume changes, at a pressure of 30 cm [Lachmann, 1985]. Similarly in this study, one factor of functional disturbance can be attributed to the fluid

administration. However, we observed both an increased opening pressure and a significant decrease in volume at a pressure of 30-35 cm H_2O after XO instillation. In those healthy animals which were artificially ventilated only (Group 3, n=4) there was no effect on lung-thorax compliance (initial values, 1.11 ± 0.23; after 30 min ventilation, 1.23 ± 0.22; after 45 min ventilation, 1.21 ± 0.14).

Tracheal instillation of surfactant 1-2 min after XO instillation partly prevents the effect of XO instillation (Table 2). One possible explanation for the latter result could be that lipid peroxidation by FOR takes place with exogenous surfactant lipids, thus preventing peroxidation of the lipids of the surfactant system, which line the alveoli and airways. While surfactant replacement partly prevented and restored the functional changes after tracheal XO instillation, it can be concluded from the results that FOR, generated by the xanthine oxidase system, also destroy the functional integrity of the bronchial and alveolar surfactant system.

Table 2. Lung-thorax compliance from the deflation curve of the P-V diagrams.

	Initial values	30 min after 1st instill. (surf. or sal.)	15 min after 2nd instill. (surf.)
Group 4 (n=7) XO, 2 min later 1 ml surf; 35-40' later 1 ml surf.	100 ± 16%	77 ± 21%	92 ± 12%
Group 5 (n=5) XO, 2 min later 1 ml sal; 35-40' later 1 ml surf. (X±SD)	100 ± 13%	35 ± 17%	84 ± 14%

CONCLUSION

The general application of radical scavengers in patients who are at risk of developing acute respiratory distress syndrome (RDS) might be one important therapeutic approach in the future. However, in all cases where one of the primary causes of respiratory failure are free oxygen radicals, additional "local" surfactant replacement might be an important step for a successful treatment of RDS.

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SUMMARY AND CONCLUSIONS

Prolonged exposure to hyperoxia can cause extensive lung injury in many mammalian species (Chapter 1).

It has been shown that small doses of endotoxin protect rats from the lung damage and edema caused by oxygen toxicity. The protective action of endotoxin was reversed by concomitant administration of the lysine salt of acetylsalicylic acid (L-ASA) (Chapter 2). This could indicate that prostaglandins play an important role in endotoxin's protective action.

A subsequent study (Chapter 3) on the cellular and eicosanoid composition of broncho-alveolar lavage (BAL) fluid in endotoxin protection against pulmonary oxygen toxicity, however, indicated not only that in rats morbidity due to pulmonary oxygen toxicity is correlated with the number of macrophages and not with the number of leukocytes present in BAL fluid, but also that endotoxin protects against hyperoxia-induced increases in eicosanoid levels in BAL fluid. This makes it unlikely that inhibition of prostaglandin synthesis by L-ASA is responsible for the reversal of the protective action of endotoxin.

Blockade of only the cyclooxygenase pathway of arachidonic acid metabolism by L-ASA probably results in shunting to the lipoxygenase pathway. Lipoxygenase products have potent properties which all are features of hyperoxic injury. Having measured eicosanoid production by BAL cells after Ca^{2+} ionophore stimulation in vitro (Chapter 4) no indication for the occurrence of shunting of arachidonic acid metabolism to the lipoxygenase pathway was found. Since the formation of lipoxygenase products after Ca^{2+} stimulation by BAL cells of endotoxin protected rats was equal (15-HETE, 12-HETE) or increased (LTB_4 and 5-HETE) when compared with the formation of lipoxygenase products by BAL cells of non-protected oxygen-exposed rats, the formation of lipoxygenase products by BAL cells does not seem to play an important role in the pathogenesis of pulmonary oxygen toxicity. However, there is little information on the source of lipoxygenase products which may play a role in pulmonary oxygen toxicity, thus the possibility remains that L-ASA induced shunting to the lipoxygenase pathway does take place in lung tissue.

Investigating the role of surfactant in the development of respiratory failure due to oxygen exposure in rats (Chapter 5), we found that in rats, lung failure (including

failure of the surfactant system) is not the primary cause of death.

In guinea-pigs, however, induction of free oxygen radical mediated lung damage by tracheal instillation of xanthine oxidase and ventilation with 100% O_2 resulted in decreased lung-thorax compliances which could almost completely be prevented, as well as restored, by instillation of exogenous surfactant (Chapter 6). This could indicate that surfactant replacement might be effective in the prevention and treatment of pulmonary oxygen toxicity.

SAMENVATTING EN CONCLUSIES

Langdurige blootstelling aan hoge concentraties zuurstof kan uitgebreide long schade veroorzaken bij de meeste zoogdieren (hoofdstuk 1).

Lage doseringen endotoxinen beschermen de rat tegen longbeschadiging en longoedeem veroorzaakt door zuurstof toxiciteit. Deze beschermende werking van endotoxine kan teniet gedaan worden door de gelijktijdige toediening van het lysine zout van acetyl-salicylzuur (L-ASA) (hoofdstuk 2). Dit zou kunnen betekenen dat prostaglandines een belangrijke rol spelen bij de beschermende werking van endotoxine.

Een volgend onderzoek echter, (hoofdstuk 3) naar de cellulaire en eicosanoide samenstelling van broncho-alveolaire lavage (BAL) vloeistof bij endotoxine bescherming tegen pulmonale zuurstof toxiciteit, toonde niet alleen aan dat de morbiditeit van ratten ten gevolge van pulmonale zuurstof toxiciteit gecorreleerd is met het aantal macrofagen en niet met het aantal leukocyten aanwezig in BAL vloeistof, maar ook dat endotoxine beschermt tegen de door hyperoxie veroorzaakte **verhoging** in eicosanoid spiegels in BAL vloeistof. Dit maakt het onwaarschijnlijk dat remming van de prostaglandine synthese door L-ASA verantwoordelijk is voor het teniet doen van de beschermende werking van endotoxine.

Remming van alleen de cylcooxygenase route van het arachidonzuur metabolisme door L-ASA resulteert mogelijk in shunting naar de lipoxygenase route. Lipoxygenase producten hebben eigenschappen welke een rol zouden kunnen spelen bij de pathogenese van pulmonale zuurstof toxiciteit. Bij bepaling van de eicosanoid productie door BAL cellen na Ca^{2+} ionophoor stimulatie echter, werd geen aanwijzing gevonden voor het optreden van een shunt van het arachidonzuur metabolisme naar de lipoxygenase route. Omdat de vorming van lipoxygenase producten door BAL cellen van de door endotoxine beschermde ratten gelijk (15-HETE, 12-HETE) of groter (LTB_4 en 5-HETE) was vergeleken met de vorming van lipoxygenase producten door BAL cellen van niet beschermde aan zuurstof blootgestelde ratten, lijkt de vorming van lipoxygenase produkten door BAL cellen geen belangrijke rol te spelen in de pathogenese van pulmonale zuurstof toxiciteit. Daar er weinig bekend is over de oorsprong van de lipoxygenase producten welke mogelijk een rol spelen bij pulmonale zuurstof toxiciteit, blijft de mogelijkheid bestaan dat door L-ASA veroorzaakte shunting

naar de lipoxygenase route plaats vindt in long weefsel.

Bij onderzoek naar de rol van surfactant bij het ontstaan van respiratoire problemen bij ratten blootgesteld aan hoge zuurstof concentraties (hoofdstuk 5), vonden wij dat pathofysiologische afwijkingen van de long (waar onder falen van het surfactant systeem) niet de primaire oorzaak zijn van de mortaliteit.

In cavia's daarentegen, resulteerde de inductie van long schade met zuurstof radicalen door middel van de intratracheale inspuiting van xanthine oxidase en beademing met 100% O_2 in een verminderde long-thorax compliance welke zowel voorkomen als teniet gedaan kon worden door de intratracheale inspuiting van exogeen surfactant (hoofdstuk 6). Dit zou kunnen betekenen dat de intratracheale toediening van surfactant effectief is bij zowel de preventie als de behandeling van pulmonale zuurstof toxiciteit.

ACKNOWLEDGEMENTS

The experimental work described in this thesis was performed in the research laboratories of the Departments of Anesthesiology of the University of Cambridge, UK and Erasmus University Rotterdam, The Netherlands.

I am indebted to:

- Professor Dr. W. Erdmann
  His encouragement and willingness to provide facilities were essential for the realisation of these studies.

- Professor Dr. B. Lachmann
  For introducing me to the field of surfactant and for his efforts as a member of the manuscript committee.

- Dr. P.J. Salt
  For the exciting stay in Cambridge, UK.

- Professor Dr. J.L. Bonta, Dr. F.J. Zijlstra, Dr. J.E. Vincent and Corné Tak of the Department of Pharmacology, EUR.
  Their collaboration was the backbone of the endotoxin studies.

- Professor R. van Strik
  For valued advice concerning medical statistical analysis.

- Co-authors, the expertise contributed by each of them is greatly appreciated.

- Professor Dr. C. Hilvering and Professor Dr. H.A. Bruining for their efforts as members of the manuscript committee.

- Laraine Visser-Isles for extensive editorial work.

- Arie Kok for techical assistance and non-technical discussions.

- The experimental animals......

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