Attenuation of Oxidant-induced Lung Injury by 21-Aminosteroids (Lazaroids): Correlation with the mRNA Expression for E-selectin, P-selectin, ICAM-1, and VCAM-1

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We compared the effects of treatment with methylprednisolone or the 21-aminosteroids, U-74389 and U-74006F (Tirilizad mesylate), on hyperoxic lung injury and the associated expression of mRNA for several adhesion molecules in rats. Inhalation of >95% oxygen for up to 72 hr in Sprague-Dawley rats produced a marked increase in lung weight and an accumulation of fluid in the thorax when compared with air-breathing controls. Hyperoxia also induced a marked neutrophil-rich influx of inflammatory cells into the bronchial lumen as measured by bronchoalveolar lavage. Neutrophil numbers in bronchoalveolar lavage fluid peaked after 60 hr of exposure to >95% oxygen; this was associated with a marked upregulation of mRNA for the adhesion molecules P-selectin and E-selectin but not VCAM-1. mRNA for ICAM-1 was constitutively expressed at high levels in both air-breathing controls and in the lungs of rats exposed to high concentrations of oxygen. Pretreatment with the 21-aminosteroids reduced hyperoxic lung damage and improved survival times in animals exposed to >95% oxygen. However, treatment with methylprednisolone significantly decreased survival times. Treatment with U-74389 did not significantly (p > 0.05) inhibit the BAL neutrophilia and did not significantly (p > 0.05) reduce hyperoxia-induced increases in mRNA expression for P-selectin and E-selectin. The inhibition of hyperoxic lung damage coupled with improved survival seen in treated animals suggests that 21-aminosteroids may provide valuable treatments for pulmonary disorders in which oxidant damage has been implicated. — Environ Health Perspect 102(Suppl 10):193–200 (1994)

Key words: hyperoxic lung injury, hyperoxia, P-selectin, E-selectin, ICAM-1, VCAM-1, 21-aminosteroids

Introduction

Although oxygen therapy has proved invaluable in infants, children, and adults with respiratory insufficiency, prolonged exposure to oxygen can lead to lung cell damage, lung fibrosis, bronchopulmonary dysplasia, organ dysfunction, and death from pulmonary edema (1). Presently, there is no commonly accepted pharmacologic treatment for patients suffering from the harmful effects of hyperoxia. In addition to hyperoxic lung injury, a number of clinical and experimental pulmonary disorders have been associated with increased oxidant stress, where the local antioxidant defenses in the lungs are overwhelmed by the oxidant burden arising from diverse sources. These include ischemia followed by tissue reoxygenation, the administration of toxic chemicals that augment intracellular oxidant formation, and the accumulation of activated leukocytes in the lung tissues. Clinical pulmonary disorders in which oxidant damage has been implicated include adult respiratory distress syndrome (ARDS) (2–4), idiopathic pulmonary fibrosis (5,6), cystic fibrosis (7–9), emphyma (10,11), and asthma (12,13).

Modification of the basic structure of methylprednisolone led to the introduction of a new class of compounds, 21-aminosteroids or "first-generation" Lazaroids, originally designed as nonglucocorticoids, iron-dependent inhibitors of lipid peroxidation (14,15). Members of this class of compound have greater potency than methylprednisolone in animal models of central nervous system trauma and neuronal membrane damage (16,17). Close structural analogs also inhibit antigen-induced lung eosinophilia in animals (18) and inhibit oxidant-mediated injury in isolated rat lungs after ischemia/reperfusion or after tert-butylhydroperoxide challenge (19). It would be of obvious interest to explore the potential of these compounds in lung diseases in which oxidant damage has been implicated. In this article, we describe the attenuation of hyperoxic lung injury in rats following treatment with the 21-aminosteroids, U-74389* (21-[4-(2,6-di-1-pyrrolidinyl-4-pyrimidinyl)-1-sulfonate (Figure 1) or U-74006F (21-[4-(2,6-di-1-pyrrolidinyl-4-pyrimidinyl)-1-piperazinyl]-16α-methylpregna-1,4,9(11)-triene-3,20-dione monomethane sulfonate (Figure 1), but not following treatment with methylprednisolone. We also compared the effects of treatment with methylprednisolone or the 21-aminosteroids on survival times of rats held in a hyperoxic environment for up to 96 hr.

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*U-74389 was tested as either the methane sulfonic acid (U-74389F) or maleic acid (U-74389G) salts.
The role of the neutrophil in contributing to hyperoxic lung injury is controversial, although it is well established that the neutrophil burden in the lungs is markedly increased after exposure to oxygen for 36 hr or more (20). The recruitment of neutrophils into inflamed lung tissue involves multiple adhesion molecules and several discrete steps, including rolling of the cells on the activated endothelial cells, firm adhesion to and migration through the endothelial cells of the blood vessel wall into the surrounding tissues, and in some cases a further migration of the cells through the airway epithelium into the bronchial lumen (21–28).

In the present experiments we attempted to correlate the time course of neutrophil infiltration into rat lungs during exposure to >95% oxygen, with the expression in lung tissues of mRNA, by Northern blot analysis, for the adhesion molecules, P-selectin, vascular cell adhesion molecule-1 (VCAM-1), endothelial leukocyte adhesion molecule-1 (ELAM-1; E-selectin), and intercellular adhesion molecule-1 (ICAM-1). We also investigated the effects of treating rats with the 21-aminosteroid U-74389F on the hyperoxia-induced lung neutrophilia and on the expression of mRNA for the above adhesion molecules during hyperoxic lung injury.

Methods

Induction and Time Course of Hyperoxic Lung Injury in Rats

Groups of 6 to 12 male Sprague–Dawley rats, 225 to 250 g, were housed in clear 40-l plexiglass exposure chambers for the duration of the period of oxygen exposure. The animals were supported on a stainless steel mesh grid and were allowed food and water ad libitum. The chambers were cleaned twice daily and were equipped with separate air-tight openings to allow feeding, cleaning, and monitoring oxygen concentrations. One hundred percent oxygen was delivered and exhausted through ports at approximately five to seven volume changes per hour to maintain a concentration of >95% oxygen in the chambers. After exposure to >95% oxygen or air for 24, 48, 60, or 72 hr, the animals were killed by an overdose of urethane, ip, the lungs were removed, weighed, dried overnight in an oven, and reweighed. The volume of fluid accumulating in the thorax was also measured.

Animals were dosed, ip, 1 hr prior to oxygen exposure and then twice daily throughout the period of oxygen exposure with vehicle (CS4), U-74389F, U-74006F, or methylprednisolone. At various time points after oxygen exposure, the rats were killed with an overdose of urethane.

Bronchoalveolar Lavage

At 24, 48, 60, and 72 hr after exposure to >95% oxygen, each rat was anesthetized with 1.5 g/kg urethane, ip, the trachea was cannulated and 5.0 ml of PBS was instilled into the lungs. The tracheal cannula was clamped and the thorax was massaged for 30 sec before recovering the bronchoalveolar lavage (BAL) fluid. A further 5 ml PBS was then instilled into the lungs, and the procedure was repeated. Ten milliliters of Hank’s balanced salt solution (HBSS) containing 5% fetal calf serum was then added to the pooled and recovered lavage fluid and vortexed. After centrifugation (150g, 10 min, 4°C) the supernatant was discarded and the cells were resuspended in 5.0 ml HBSS containing 5% fetal calf serum. For cytologic examination, cytospin preparations were made using a Shandon cytocentrifuge (150g, 10 min, room temperature) (Shandon Southern Instruments, Sewickley, PA). The cells were fixed and stained using Diff QuickR (American Scientific Products, Mcgraw Park, IL). Differential cell counts on at least 100 cells were made using standard morphologic criteria to classify the cells into eosinophils, neutrophils, and mononuclear cells.

Preparation of cDNA Probes

cDNA clones encoding rat P-selectin and E-selectin were isolated by homology cloning (Manning et al., manuscript in preparation). A cDNA clone encoding rat ICAM-1 (29) was kindly provided by Tadashi Horiuchi (Daiichi Pharmaceutical Co., Tokyo, Japan). A rat VCAM-1 cDNA fragment was isolated by polymerase chain reaction (PCR) from first-strand cDNA synthesized from total RNA isolated from the heart tissue of rats 3 hr after administration of bacterial endotoxin. Gene-specific oligonucleotides were designed based on the available cDNA sequence for rat VCAM-1 (30). The resulting PCR product was subcloned and its authenticity confirmed by DNA sequence analysis.

For use in hybridization protocols, DNA fragments were prepared from these cDNA clones by the PCR. Gene-specific oligonucleotides were designed to generate fragments of approximately 500 bp in length from the P-selectin, VCAM-1, and ICAM-1 cDNA clones, and 1.6 Kb in length from the rat E-selectin cDNA clone. The following oligonucleotide pairs were used (5’oligo/3’oligo; each sequence as 5’ to 3’): P-selectin: CGACCTTGAATGTCAACCTCA/ACAAGTGAGATACACAG; VCAM-1: TATATATCGACC/AAACGCCATATCAACCTCA/ACAAGTGAGATACACAG; ICAM-1: CACCTCA/ACAAGTGAGATACACAG. cDNA fragments were amplified with the appropriate primers using the polymerase chain reaction (PCR). The amplified DNA fragments were ligated into Bluescript plasmid and sequenced using the dideoxynucleotide chain-terminating method. The sequences obtained were compared with the sequences of the available cDNA clones. The amplified DNA fragments were ligated into Bluescript plasmid and sequenced using the dideoxynucleotide chain-terminating method. The sequences obtained were compared with the sequences of the available cDNA clones. The amplified DNA fragments were ligated into Bluescript plasmid and sequenced using the dideoxynucleotide chain-terminating method. The sequences obtained were compared with the sequences of the available cDNA clones.
3 untranslated region; and VCAM-1 CCA-AGCTATGCATTCCAGACT/CTGAAA-GTCAACCCAGTGAC encompassing the 3 untranslated region.

RNA Extraction and Northern Blot Analysis

Total rat lung RNA was isolated using the RNAzol (Cinna/Biotex Laboratories International, Inc., Friendswood, TX) method, which is a modification of the Chomczynski and Sacchi (31) single step procedure. Equivalent amounts of RNA (10 μg/lane) were denatured with glyoxal and DMSO and applied to 1.5% agarose gels. The RNA was transferred to Nylon 66 Plus membranes (Hoeffer Scientific, San Francisco, CA) by vacuum blotting and UV cross-linked. The blots were prehybridized in hybridization buffer (1% BSA, 1 mM EDTA, and 7% SDS in 0.5 M NaHPO4, pH 7.2) for 60 min and hybridized overnight at 65°C. The blots were then washed twice with 0.5% BSA, 1 mM EDTA, and 5% SDS in 40 mM NaHPO4, pH 7.2, and four times with 1 mM EDTA and 1% SDS in 40 mM NaHPO4, pH 7.2, all for 20 min at 65°C. For probing with β-actin, blots were prehybridized in Hybrisol 1 (Oncor, Gaithersberg, MD) for 60 min and hybridized overnight at 52°C. Blots were washed at 52°C in 0.1x SSC and 0.1% SDS. After a brief rinse in 1x SSC, the blots were exposed to Kodak XAR-2 X-ray film for 1 to 3 days. Quantitation was performed using a PhosphorImager 425 (Molecular Dynamics, Sunnyvale, CA).

The probe for the metabolic enzyme GAPDH was a 1.2-kb PstI insert fragment from a plasmid containing the rat GAPDH cDNA. The β-actin cDNA probe was purchased from Oncor (Gaithersburg, MD). All probes were labeled with [γ-32P]dATP (6000 Ci/mmole; Dupont NEN, Boston, MA) using the Prime-It Random Priming Kit (Strategene, La Jolla, CA) to a specific activity of 106 dpm/μg.

Statistical Analysis

Student’s t-test for independent values was used to test the significance of the differences for mean values of lung weights or pleural fluid volumes between vehicle-treated and methylprednisolone or 21-aminosteroid-treated rats. An extension of Fisher’s exact test was used at each time point to test for significant differences in numbers of animals surviving among the four groups (vehicle-, methylprednisolone-, U74389G-, or U-74006F-treated) taken collectively. If this test was significant (p < 0.05) at a given time point, pairwise Fisher’s exact tests were carried out to determine significant differences between the groups. Petro and Petro’s log rank test (32) also was applied across time points to test for differences in the distributions of survival times among the four groups. Differences that produced p-values less than 0.05 were accepted as significant.

Care and Use of Animals

All procedures in this study are in compliance with the Animal Welfare Act Regulations, 9CFR Parts 1, 2, and 3 and with the Guide for the Care and Use of Laboratory Animals, DHEW Publication (NIH) 85-23, 1985.

Results

Time Course of Hyperoxic Lung Injury, Neutrophil Influx, and Expression of P-selectin, E-selectin, VCAM-1, and ICAM-1 mRNA in Hyperoxic Lungs

Exposure of rats to >95% oxygen for up to 72 hr induced a progressive decrease in body weight and increasing lung damage, demonstrated by a marked and significant (p < 0.05) increase in wet lung weight (Figure 2A), but no significant (p > 0.05) change in dry lung weight. Consequently, hyperoxia also induced a significant increase in the lung wet weight to dry weight ratio (Figure 2B). Hyperoxia also produced a marked and significant (p < 0.05) accumulation of fluid in the thorax at 60 hr after the initial exposure to >95% oxygen (Figure 2C). These changes were associated with a neutrophil-rich accumulation of inflammatory cells in the lungs (Figure 3A,B). Forty-eight hours after exposure to >95% oxygen there was a small but significant (p < 0.05) increase in the numbers of neutrophils recovered from BAL fluid. Sixty hours after exposure there was a marked infiltration of the bronchial lumen with inflammatory cells, neutrophils comprising approximately 30% of the total.
cells in the BAL fluid (Figure 3B). The time course of neutrophil infiltration correlated with a marked upregulation of mRNAs for P-selectin and E-selectin, and a small upregulation of mRNA for VCAM-1 (Figure 4). ICAM-1 was constitutively expressed at a high level in both air-breathing and hyperoxic rats, and the levels did not change significantly during the progression of hyperoxic lung injury (Figure 5).

**Effect of Methylprednisolone, U-74389F, and U-74006F on Hyperoxic Lung Injury**

Because some animals died after exposure to >95% oxygen for periods longer than 60 hr, we decided to evaluate compound activity in this model in animals exposed to >95% oxygen for 60 hr. At this time point all animals survived but there were marked and significant (p < 0.05) increases in lung weight and fluid accumulation in the thorax when compared with air-breathing controls. Pretreatment with 1, 3, and 10 mg/kg methylprednisolone, ip, 1 hr before oxygen exposure and then twice daily throughout the period of oxygen inhalation produced no significant (p > 0.05) effect on the hyperoxia-induced increases in lung wet weight (Figure 6) or pleural fluid accumulation (Figure 7). U-74006F, at all the dose levels tested and using the same dosing regimen as that for methylpred-

**Figure 4.** Northern blot analysis of mRNA for P-selectin, VCAM-1, and E-selectin before (0 hr) and 48, 54, 60, and 66 hr exposure to >95% oxygen. Lung tissues were taken from rats treated with U-74389G (30.0 mg/kg, ip) bid (+) or vehicle (−) throughout the period of oxygen exposure.

**Figure 5.** Northern blot analysis of mRNA for ICAM-1, before (0 hr) and 48, 54, 60, and 66 hr exposure to 95% oxygen. Blots were probed for GAPDH and β-actin.

methylprednisolone, produced a marked and statistically significant (p < 0.05) inhibition of the oxygen-induced increases in pleural fluid, but not on the changes in lung weights. U-74389F also produced a significant (p < 0.05) inhibition of the oxygen-induced increases in pleural fluid at all doses and significantly inhibited (p < 0.05) the increase in lung weight at the 10 mg/kg, ip, dose level (Figures 6, 7).

After demonstrating inhibition of hyperoxic lung injury with the 21-aminosteroids, but not with methylprednisolone, we wished to determine if these agents would influence the survival rates of rats exposed to >95% oxygen. When survival times were compared in groups of 12 animals exposed to >95% oxygen for up to 96 hr, we found that methylprednisolone 10 mg/kg, bid, treatment significantly (p < 0.05) decreased survival time, while both of the 21-aminosteroids (U-74389G and U-74006F) 10 mg/kg bid significantly (p < 0.05) increased survival times (via the logrank test as well as the Fisher’s exact tests shown in Table 1). Under the experimental conditions described here, we found that exposure to 95% oxygen for 72 hr resulted in the death of approximately 50% of untreated rats or rats treated with methylprednisolone.
vehicle alone. All rats treated with methylprednisolone died by 72 hr, whereas approximately 50% of animals treated with the 21-aminosteroids survived to 96 hr.

We also explored the effects of treatment with one of the 21-aminosteroids, U-74389, (30.0 mg/kg, bid, ip) on the accumulation of neutrophils in the lung, and the expression of mRNA for P-selectin, E-selectin, ICAM-1, and VCAM-1 during the development of hyperoxic lung injury. We found that U-74389 did not significantly affect the numbers of neutrophils appearing in BAL fluid, nor did it significantly affect the levels of expression of mRNA for P-selectin, VCAM-1, ICAM-1, or E-selectin (Table 2).

**Discussion**

It is now well established that prolonged oxygen therapy causes a progressive pulmonary edema and lung damage in all species studied so far, including humans (1,33,34). To date, there is no commonly accepted pharmacologic intervention for the treatment of this disorder and the mechanisms by which hyperoxia induces cell damage have not been fully elucidated. However, there is strong evidence that oxygen-derived free radicals (superoxide anion and hydroxyl radical), hydrogen peroxide, and peroxidation products of lipids play a significant role and therefore lead to an increase in capillary permeability and lung edema. Thus, studies in animal models of hyperoxic lung injury show that interventions which increase antioxidant enzymes correlate positively with increased survival (35–39), while those that compromise antioxidant defenses decrease survival (40–44). There are numerous other examples. Preexposure of rats to 85% oxygen induces tolerance to the effects of subsequent exposure to 100% oxygen and this is associated with an increase of lung levels of superoxide dismutase, an enzyme which converts $O_2^-$ to $H_2O_2$ (35). In accordance with these findings, treatments with superoxide dismutase or catalase have been shown to be protective against oxygen toxicity (45,46). In addition, hyperoxia also has been shown to increase oxygen radical production in rat lung homogenates (47), while oxygen metabolite scavengers inhibit hyperoxic lung injury (48) and protect alveolar macrophages from hyperoxic injury in vitro (49). Lipid peroxides also have been shown to be directly toxic to alveolar cells (50). The above findings support the view that hyperoxic lung injury occurs as a result of the natural antioxidant defenses being overwhelmed by the increased oxidant stress resulting from breathing high oxygen concentrations. Therapeutic interventions that might attenuate the oxidant stress would be of obvious value in this disorder.

Previously, we demonstrated an inhibition of hyperoxic lung injury after treatment with the nonglucocorticoid 21-aminosteroid U-74389F (51). 21-Aminosteroids were originally designed as inhibitors of iron-dependent lipid peroxidation (15) and are effective in animal models of CNS trauma and neuronal membrane damage (16,17). In the present study, we showed that the inhibitory properties of U-74389F on hyperoxic lung injury, as measured by the inhibition of the hyperoxia-induced accumulation of pleural fluid, was shared by a close structural analog, U-74006F (Tirilizad mesylate), a compound that is currently being evaluated for the treatment of head and spinal cord injury in humans. In contrast, methylprednisolone, when tested at the same dose levels and under the same dosing regimen, failed to demonstrate any protection of hyperoxic lung injury. Furthermore, when we compared survival times of rats exposed to >95% oxygen, methylprednisolone treatment reduced survival times, while the nonglucocorticoid 21-aminosteroids U-74389G and U-74006F significantly increased survival times. Although we have not explored the mechanisms by which methylprednisolone exacerbated oxygen toxicity, a similar decrease in survival times has been described by other investigators after glucocorticoid pretreatment of sheep (52), mice (53), and rats (54). Interestingly, the latter investigators showed that, although dexamethasone exacerbated lung damage and diminished survival when given early during exposure to hyperoxia, they were able to demonstrate an improved survival if given when the hyperoxia was soon to be terminated. In sheep, glucocorticoid treatment potentiated oxygen toxicity irrespective of the time of treatment (52). The abundance of experimental data, including our own, that suggests glucocorticoid treatment is deleterious to animals breathing high concentrations of oxygen may indicate that these agents should be avoided in clinical settings that require inhalation of high fractions of inspired oxygen. However, at the present time, high-dose steroids provide the only available pharmacologic treatment for patients with severe spinal cord injuries, and these patients often require ventilation with oxygen.

The effects of U-74389 and U-74006F in preventing hyperoxic lung injury and prolonging survival times in rats in our experiments are consistent with a recent report by Frank and McLaughlin (55) that showed treatment of neonatal rats with U-74389F protected against the oxygen-induced inhibition of normal lung development. These investigators showed that the normal separation of the large air sacs at birth to form smaller diameter alveoli, with a much increased surface area, is markedly inhibited by hyperoxia. The model showed a very similar lung pathology to that which occurs in infants who had died with bronchopulmonary dysplasia. Treatment of 4-day-old rat pups for 10 days with U-74389F markedly reduced the oxygen-induced inhibition of normal lung development. Similarly, the oxygen-induced inhibition of elastin deposition, which is intimately involved in the sepsis process, was ameliorated by U-74389F treatment.

In addition to hyperoxic lung injury, 21-aminosteroids have also been shown to be efficacious in other models of pulmonary oxygen damage. Thus U-74500A, a close structural analog of the compounds tested here, has been shown to prevent oxidant-mediated injury in isolated rat lungs after ischemia/reperfusion or after $\beta$-butylhydroperoxide challenge (19). U-74389F also offers some protection against bleomycin-induced pulmonary fibrosis in rats (56). U-74500A and U-74389F also protect pulmonary endothelial cells against oxidant-dependent injury induced by neutrophils (57).

The mechanisms by which 21-aminosteroids inhibit hyperoxic lung injury are yet to be elucidated. However, these compounds are potent inhibitors of lipid peroxidation (15,58) and are highly lipophilic, distributing preferentially to the lipid bilayer of cell membranes where they have been shown to exert potent stabilizing effects (59,60). Consequently, it has been proposed (61) that these compounds exert their antilipid peroxidation activity through cooperative mechanisms, a radical scavenging action, and a physicochemical interaction with cell membranes that decreases membrane fluidity. This proposition is consistent with the compounds' effects on hyperoxic lung injury in vivo, where stabilization of endothelial cell membranes would reduce hyperoxia-induced increases in capillary permeability and pulmonary edema.

In our experiments, hyperoxic lung injury in rats was associated with a progressive increase in the numbers of neutrophils that could be washed out of the bronchial...
lumen by lavage. This was consistent with findings from other laboratories in which increases in the numbers of radiolabeled neutrophils were detected during hyperoxic lung injury (20). To determine if there was a temporal association between the appearance of neutrophils in the bronchoalveolar lavage fluid and the expression of mRNA for the adhesion molecules, ICAM-1, E-selectin, VCAM-1, or P-selectin, we exposed rats to an atmosphere of >95% oxygen for up to 72 hr. Quantitation of mRNA for E-selectin and P-selectin in hyperoxic lung tissue demonstrated marked increases in both selectin molecules after prolonged exposure to oxygen, at 60 and 66 hr. This was temporally associated with the influx of neutrophils into the lungs and is consistent with the view that P-selectin expression on activated endothelial cells first promotes rolling of these cells (22,25) via interaction with neutrophil carbohydrate ligands (e.g., sialyl Lewis) presented by L-selectin, which is expressed at high levels on circulating resting neutrophils, and is constitutively functional (21). Following rolling of neutrophils, firm adhesion and transmigration through endothelial cells may then be mediated through the integrins LFA-1 and MAC-1 on the neutrophil surface with ICAM-1 on the endothelial cells. The increase in mRNA for E-selectin in hyperoxic lung tissue, and the associated neutrophilia seen in our experiments in rats, also is consistent with the observation that adhesion of neutrophils to endothelial cells can be mediated via L-selectin on the neutrophil surface with upregulated E-selectin on endothelial cells (22). This is in contrast to studies in mice (62) in which no upregulation of E-selectin mRNA was observed. Messenger RNA for ICAM-1 was constitutively expressed at high levels in air-breathing and hyperoxic rats, and no perceptible change was observed during the development of hyperoxic lung damage. This is in contrast to hyperoxic lung injury in murine lung tissue in which an increase in mRNA for ICAM-1 has been reported (63) and antiICAM-1 monoclonal antibody treatment partially inhibits hyperoxic lung injury (64). Signals for VCAM-1 were weak in rat lung tissues, but nonetheless, a slight increase in expression occurred at >54 hr of hyperoxia. The precise contribution made by each of the different adhesion molecules to leukocyte trafficking in hyperoxic lung injury may be best examined using deficient or mutant mice that lack the expression of P-selectin, CD18, and ICAM-1 (65–67).

When rats were pretreated with the 21-aminosteroid U-75389, we saw no significant inhibition of the neutrophil influx into bronchoalveolar lavage fluid and no significant inhibition of the bronchoalveolar neutrophilia or the expression of mRNA for the different adhesion molecules. We conclude from this that the effects of U-74389 cannot be ascribed to the inhibition of neutrophil trafficking into the lungs. The ability of 21-aminosteroids to prevent lung damage and promote survival of hyperoxic rats also adds further support to the view that neutrophil products do not contribute to the early (<60 hr) lung damage that occurs during exposure to very high concentrations of oxygen. Ibuprofen has been shown to inhibit neutrophil influx during hyperoxia in rabbits without preventing lung damage (66), and neutrophil depletion with cytoxic drugs does not prevent acute hyperoxic injury (69,70). However, it is likely that reactive oxygen metabolites from neutrophils serve to exacerbate lung damage during long-term exposure to high oxygen concentrations. It would be interesting to explore the effects of 21-aminosteroids in such a model.

The inhibition of hyperoxic lung damage by 21-aminosteroids, coupled with the improved survival seen in treated animals, suggests that 21-aminosteroids and their derivatives may be of potential clinical use in treating hyperoxic lung injury and other pulmonary conditions, such as ARDS, in which oxidant injury may contribute to the pathophysiology.

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