To study the dependence of bleomycin acute injury on WBCs, rabbits (n = 4) were given 10 units/kg of bleomycin after depletion of granulated WBCs by nitrogen mustard. A marked reduction in injury was seen (day 8 PaO₂, 25.8 ± 1.8 mm Hg for bleomycin vs 53.5 ± 6.8 mm Hg for bleomycin with WBC depletion).

DISCUSSION

Previous investigators have shown that long-term exposure to oxygen after administration of bleomycin can enhance early mortality and subsequent fibrosis. This study now demonstrates that as little as 2 minutes of oxygen exposure enhances bleomycin lung injury and that oxygen pulses can be used to titrate injury. That such very short exposures have an effect supports the probable role of free radicals in producing the initial effects of bleomycin on the lung.

A second way to influence the severity of early bleomycin injury is variation in dose size. Finally, polymorphonucleated WBC depletion suppresses falls in PaO₂. This suggests that granulocytes may play a role in the development of acute bleomycin lung injury and provides a third modality for changing the spectrum of lung injury. Granulocytes are stimulated to migrate into the lung following intratracheal bleomycin (see Haslett article, session 1), and depletion by nitrogen mustard might protect the lung by blocking their release of free radicals, proteolytic enzymes, neutral proteases, etc.

Therefore, acute bleomycin lung injury can be titrated by the use of short oxygen pulses, the size of bleomycin dose given, and the depletion of granulated WBCs by nitrogen mustards. Current studies are directed at determining if these manipulations modulate subsequent chronic lung inflammation and fibrosis. If so, the relative importance of the many factors thought to regulate the transition from acute lung injury to chronic inflammation and fibrosis can be studied and evaluated.

REFERENCES


Reduction in Bleomycin-induced Lung Hydroxyproline Content by an Iron Chelating Agent*

John I. Kennedy, M.D.; David B. Chandler, Ph.D.; Robert M. Jackson, M.D., F.C.C.P., and Jack D. Fulmer, M.D., F.C.C.P.

Bleomycin, an antineoplastic compound, is used therapeutically against a variety of squamous cell carcinoma and lymphomas. A dose- and time-dependent pulmonary toxicity develops that progresses to pulmonary fibrosis in approximately 1% of patients. Intratracheal bleomycin is widely used to produce an animal model for the study of pulmonary fibrosis. The cytotoxicity of bleomycin has been suggested to be related to a ferrous iron-molecular oxygen mechanism that induces intracellular DNA chain breakage. Furthermore, studies have shown that bleomycin produces superoxide and hydroxyl radicals and initiates lipid peroxidation in the presence of iron and oxygen. Although bleomycin-induced pulmonary fibrosis has been investigated biochemically and morphologically, its mechanism has not been established. It is believed that lipid peroxidation might be related to the pulmonary fibrosis, but involvement of the inflammatory and immune systems has also been suggested. Therefore, iron-catalyzed oxygen radical formation and lipid peroxidation might participate in bleomycin-induced pulmonary fibrosis following deferoxamine treatment.

MATERIALS AND METHODS

Adult male golden Syrian hamsters were used. Each group was pretreated with either saline or deferoxamine IM for 5 days before intratracheal treatment. Intramuscular treatment was continued after intratracheal administration of saline solution or bleomycin until sacrifice. Four groups of animals were compared in this study: (A) a saline-saline (SAL-SAL) group in which animals received 0.33 ml of sterile saline intratracheally and 0.05 ml of sterile saline IM twice daily; (B) a saline-deferoxamine (SAL-DF) group in which animals received 0.33 ml of sterile saline intratracheally and 5 mg of deferoxamine (CIBA Pharmaceutical Co) in 0.05 ml of sterile saline IM twice daily; (C) a bleomycin-saline group (BLEO-SAL) in which animals received 1 unit of bleomycin intratracheally and 5 mg of deferoxamine (CIBA Pharmaceutical Co) in 0.05 ml of sterile saline IM twice daily; (D) a bleomycin-deferoxamine (BLEO-DF) group in which animals received 1 unit of bleomycin intratracheally in 0.33 ml of sterile saline and 0.05 ml of sterile saline IM twice daily; (E) a bleomycin-saline group (BLEO-SAL) in which animals received 1 unit of bleomycin intratracheally in 0.33 ml of sterile saline and 0.05 ml of sterile saline IM twice daily; (F) a bleomycin-deferoxamine (BLEO-DF) group in which animals received 1 unit of bleomycin intratracheally in 0.33 ml of sterile saline and 0.05 ml of sterile saline IM twice daily. Intratracheal injections were performed using pentobarbital anesthesia (60-70 mg, IP) as described. Twenty-one days after intratracheal treatment, hamsters were weighed and sacrificed by exsanguination, and the lungs were weighed and evaluated.

*From the Division of Pulmonary and Critical Care Medicine, Department of Medicine, The University of Alabama at Birmingham and the Veterans Administration Medical Center, Birmingham, supported by NIH training grant HL07553-01, the Research Services of the Veterans Administration, and a grant from the American Lung Association.

Reprint requests: Dr. Fulmer, Department of Medicine, University of Alabama in Birmingham, Birmingham 35294
harvested. Right lung was dissected free of all major structures and used for collagen determination. Left lung was then filled with 10% Millonig's buffered formalin via the trachea at a constant pressure of 25 cm H$_2$O for 24 hours. The tissue was embedded in paraffin and 5μm coronal sections cut, mounted on glass slides and stained with hematoxylin and eosin (H & E). The severity of lesions in these sections was graded using a modification of the criteria described by Szapiel et al. 

Hydroxyproline determination was performed on the right lung using the colorimetric assay as described previously.

RESULTS

The effect of intratracheal bleomycin with and without deferoxamine on body weight are shown in Table 1. The SAL-SAL group and the SAL-DF group attained 120.8% and 120.6% of their initial body weight, respectively, in 21 days. The BLEO-DF group exhibited a weight gain to 104.5% of their initial body weight. There were no significant differences between these groups. In contrast, the BLEO-SAL group showed a significant weight loss to 95.0% of its initial body weight compared with either the SAL-SAL or SAL-DF group.

The 21-day cumulative mortality for the hamsters in various treatment groups are shown in Table 1. The mortality rate in the bleomycin groups was 60%; there appears to be no direct or synergistic effect of deferoxamine on mortality.

The effects of intratracheal bleomycin with and without deferoxamine on hydroxyproline (OH-PRO) content are summarized in Figure 1. Significant increases in lung OH-PRO in the BLEO-SAL group were found relative to SAL-SAL and SAL-DF controls. Furthermore, the lung OH-PRO content of the BLEO-SAL group was significantly elevated when compared with the BLEO-DF treatment group (p<0.05). No differences in the lung OH-PRO content among the BLEO-DF, SAL-DF, and SAL-SAL groups were found.

The lungs of hamsters in the 2 control groups, SAL-SAL and SAL-DF, were essentially normal. The histopathologic changes observed in the lungs of hamsters treated with bleomycin were similar to those previously reported. The grade of lesion severity (Table 1) was significantly different among groups (p<0.001). The most severe lesion score (+3.1) was in the BLEO-SAL group, which was significantly higher than that of all other groups (p<0.03). The BLEO-DF group also exhibited a significant elevation in lesion grade (+1.2), but only when compared with the SAL-SAL treatment group.

DISCUSSION

In this study, we examined the effect of deferoxamine, a ferric iron chelator, on bleomycin-induced pulmonary fibrosis. The ability of iron to serve as a redox reagent in the metabolism of oxygen and the mechanism of iron-catalyzed hydroxy radical formation by reaction of ferric and superoxide ions has been well established. Hydroxyl radicals are known to be associated with the peroxidation of membrane lipids, which might lead to loss of membrane function and cellular death. Current data indicate that deferoxamine is able to prevent the formation of iron-catalyzed hydroxyl radicals from superoxide anion-radical and associated lipid peroxidation, wherein ferric iron is believed to be required as an initiation factor.

These findings are interesting in view of recent data on the mechanism of action of bleomycin. Bleomycin has been shown to produce reactive oxygen species such as superoxide.
and hydroxyl radicals. In addition, bleomycin was found to increase lipid peroxidation in lung and liver microsomal fractions. What roles superoxide or hydroxyl radicals and lipid peroxidation play in the pathophysiology of bleomycin-induced pulmonary fibrosis is not known.

There are, however, several routes aside from the prevention of lipid peroxidation, where hydroxyl radical formation may have additional effects. Hydroxyl radical scavengers have recently been shown to inhibit lymphocyte mitogenesis. The prevention of lymphocyte mitogenesis might be important, since lymphocyte function can modulate collagen synthesis and secretion by fibroblasts as well as the proliferation of fibroblasts. Support for this possible mechanism comes from the present study wherein the lesion of the BLEO-DF group was more diffuse and appeared to have fewer mononuclear cells compared with the BLEO-SAL treatment group.

Deferoxamine also has been reported to modulate directly fibroblast proliferation and collagen synthesis. In 24-day cultured lung fibroblasts, deferoxamine was shown to decrease proliferation and prolyl hydroxylase activity. However, in an earlier study, prolyl hydroxylase activity was stimulated by deferoxamine. It appears that the effect of deferoxamine on prolyl hydroxylase activity is controversial.

Bleomycin-induced pulmonary fibrosis is reduced by parental defereroxamine as determined by lung hydroxyproline content and lesion severity. The mechanism by which deferoxamine is acting to reduce lung injury in unknown. However, deferoxamine’s effect on hydroxyl radical formation and lipid peroxidation, as well as possible secondary effects on lymphocyte proliferation, might contribute to the decrease in bleomycin-induced pulmonary fibrosis.

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