Effect of Intratracheal Dexamethasone on Oleic Acid-induced Lung Injury in the Rat*

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The therapeutic efficacy of single-dose intratracheal dexamethasone (2.5 mg/kg) in oleic acid-induced acute lung injury in the rat was established by showing significant improvement in bronchoalveolar lavage parameters and lung compliance at 24 h after oleic acid as compared with animals not treated with oleic acid. An equivalent dose of intraperitoneal dexamethasone demonstrated no therapeutic benefit at 24 h after oleic acid. The therapeutic effect of this single-dose intratracheal dexamethasone regimen was accompanied by recovery to normal bronchoalveolar parameters and lung compliance at 7 days, in contrast to previous observations made with high-dose sustained intraperitoneal dexamethasone (4 mg/kg for 7 days). This improved benefit-toxicity ratio of intratracheal dexamethasone compared with systemic dexamethasone may be due to enhanced topical anti-inflammatory potency relative to systemic potency and toxicity. (Chest 1994; 106:388-397)

ARDS=adult respiratory distress syndrome; BSA=bovine serum albumin; BAL=bronchoalveolar lavage; DX=dexamethasone; IP=intraperitoneal; IT=intratracheal; MP=methylprednisolone; PMN=neutrophil; OA=oleic acid; UIP=usual interstitial pneumonitis

The adult respiratory distress syndrome (ARDS) can be conceptualized as a dynamic lung injury response to a variety of stimuli, which begins as patchy diffuse alveolar damage, and then follows one of two paths: either resolution to a normal anatomic state or transformation into a diffuse alveolar-interstitial and microvascular fibrosis. This description is also recognized as the pathologic spectrum of usual interstitial pneumonitis (UIP) as first described by Liebow and Carrington, although the time course from initial injury to diffuse fibrotic change is accelerated in ARDS. Failure of the lung repair process to restore normal lung architecture has been implicated in the mechanisms proposed to explain fibroproliferative transformation from acute lung injury in ARDS and UIP. Unfortunately, clinical prognosis after acute lung injury is largely dependent on this unpredictable and poorly controllable fibroproliferative response. Thus, the ideal therapeutic goal aims at effectively impeding progressive inflammatory lung injury and yet not impeding those inflammatory mechanisms needed in the lung repair process. At present, the role of corticosteroids, our most widely used and potent anti-inflammatory agents, remains unclear with respect to the management of ARDS and UIP.

Several recent large-scale clinical trials on ARDS of various causes suggest that high-dose intravenous (IV) methylprednisolone (MP) therapy (60 to 120 mg/kg/day for 1 to 2 days) offers no improvement in lung mechanics or gas exchange and may increase both incidence of infection and mortality. However, other investigators have shown that sustained high-dose corticosteroid therapy may improve the clinical course and survival in ARDS of various causes. Van der Merwe and coworkers did a controlled study on 92 trauma patients and found that when 60 mg/kg of IV MP was given within 24 h of trauma onset, a significant fourfold reduction in ARDS incidence was observed.

Several studies using animal models of ARDS have shown that systemic MP or dexamethasone (DX), in the ranges of 20 mg/kg/day for MP and 4 mg/kg/day for DX, improved pulmonary inflammation and mechanics during the acute lung injury stage. The effect of IV administration of oleic acid (OA) on the lungs of several animal species is well established as a model of acute diffuse lung injury resembling the initial phase of ARDS. Theoretically, the OA model is most closely related to the subset of ARDS caused by fat embolism. In the rat model of OA-induced lung injury, we have shown previously that bronchoalveolar lavage (BAL) inflammatory cell presence and protein content, histopathologic changes of pulmonary hemorrhage and edema, and reduced lung compliance are invariably most severe from 4 h to 3 days after OA, but return to normal by 3 to 7 days without therapeutic intervention. The consistent recovery phase observed in the OA model can be exploited in the laboratory by examining how anti-inflammatory interventions, instituted at the onset of acute lung injury, may affect not only the injurious response, but also recovery and repair.

The advent of direct delivery systems of corticosteroids into the airway has revolutionized the...

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therapeutic approach in many patients with chronic airways disease. The design and selection of the ideal corticosteroids for this application have been based on the concept of the maximization of topical potency relative to systemic potency and toxicity. To our knowledge, there are no published studies that have investigated the effect of direct airway delivery of corticosteroids in ARDS or ARDS animal models. We have chosen to examine the effects of intratracheal (IT) corticosteroids on OA-induced lung injury and recovery in the rat using DX at a reduced dose relative to previously examined systemic corticosteroid doses for ARDS treatment.

METHODS

Animals

Adult male pathogen-free Fischer 344 rats, weighing 175 to 200 g, were obtained (Charles River Laboratories, Worthington, Mass). The animals were free from respiratory disease and housed in isolation from all other laboratory animals.

Reagents

Bovine serum albumin (BSA) and OA were purchased (Sigma Chemical Company, St. Louis, Mo). Dexamethasone sodium phosphate at 4 mg/ml was purchased from one company (American Reagent Laboratories, Shirley, NY), and ketamine hydrochloride was purchased from another one (Parke Davis Company, Detroit).

Administration of OA

Rats were anesthetized via an intraperitoneal (IP) injection of ketamine hydrochloride (75 mg/kg). Each animal then received an IV injection of 300 µl of an OA suspension immediately after varying (30 µl of pure OA suspended in 270 µl of 0.1 percent BSA). Control animals for the OA-treated groups received volume equivalent IV 0.1 percent BSA.

Corticosteroid Therapy

All corticosteroid-treated animals received a single dose of 2.5 mg/kg of DX. Animals that received both OA (IV) and DX (IT) were administered both agents simultaneously via their respective routes. Animals receiving IT DX were anesthetized by the method described above for OA administration. Intratracheal DX administration was accomplished by performing a tracheal cutdown and injecting DX, via a 1-ml insulin syringe, into the midline anterior trachea with the animal mounted in a supine position and head up at 45°. Animals that received IP DX and no OA did not require anesthesia. Control animals for the DX-treated groups received volume equivalent IT or IP sterile physiologic saline solution.

Assessment of Lung Injury

Analysis of BAL Fluid: Animals were killed at times representative of peak injury and recovery in the OA model (4 h, 24 h, and 7 days after OA). Control animals that received DX or saline solution alone were killed at similar times. Lungs were lavaged in situ by infusion of 50 ml (10-ml aliquots) sterile saline solution via a cannula ligated in the trachea. Approximately 40 ml of fluid was obtained from BAL in each animal. Total nucleated cell counts were made using a standard American Optical (AO) hemocytometer and cell viability was determined by 0.1 percent trypan blue dye exclusion. Differential cell counts were done from cytacentrifuge smears stained with Wright’s stain. The protein concentration in BAL fluid was calculated according to the method described by Lowry and associates, using BSA as a standard.

Lung Compliance Measurement: Air-filled lung compliance measurements were performed in situ on anesthetized animals as previously described. Briefly, a plastic cannula was inserted into the trachea and ligated in place. The animal was then put in a constant-volume body plethysmograph and a water-filled esophageal catheter was inserted to monitor pleural pressure. The changes of pressure and volume were recorded on a recorder (XY, Houston Instrument). Lungs were inflated slowly to a pleural pressure of 25 cm H2O and a quasistatic deflation curve was recorded for 10 to 12 s down to functional residual capacity (FRC). The compliance was measured as the slope of a straight line fitted to the steepest segment of the deflation curve above FRC. Measurements were repeated three times and the mean value was recorded as a percent of the control mean value.

Histologic Evaluation of Lungs: Two animals in each group were killed for histologic examination to evaluate overall lung injury. These animals were not lavaged so as to preserve the natural state as much as possible. Briefly, uninfused lungs were removed after death and fixed in 10 percent buffered formaldehyde for 24 h. Tissue sections were processed in the usual manner for light microscopic examination with hematoxylin-eosin stain. Only qualitative histologic evaluations were made.

Statistical Analysis for Animals

Student’s unpaired t test and Bonferroni’s adjustment for multiple comparisons were used for data analysis.

RESULTS

Intratracheal Dexamethasone Study

Effects of IT DX on Normal Animals: There were no significant differences in BAL total inflammatory cell count, protein level, or lung compliance in IT DX-treated normal animals compared with untreated control animals at 4 and 24 h after IT DX injection (Table 1). Intratracheal DX was noted to depress peripheral blood lymphocyte counts to 20 to 25 per-

Table 1—Effect of IT DX Therapy on BAL Inflammatory Cell Presence, BAL Protein Content, and Lung Compliance in Normal Animals at Various Times After DX*

<table>
<thead>
<tr>
<th>Time After DX, h</th>
<th>Group†</th>
<th>Nucleated Cells, X10⁶</th>
<th>PMN, X10⁶</th>
<th>Protein, mg/ml</th>
<th>Compliance, % Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (4)</td>
<td>0.9 ± 0.03</td>
<td>0.01 ± 0.003</td>
<td>0.2 ± 0.01</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>DX (5)</td>
<td>0.8 ± 0.1</td>
<td>0.01 ± 0.003</td>
<td>0.1 ± 0.03</td>
<td>127 ± 18</td>
</tr>
<tr>
<td>24</td>
<td>Control (4)</td>
<td>0.9 ± 0.03</td>
<td>0.01 ± 0.003</td>
<td>0.2 ± 0.01</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>DX (5)</td>
<td>1.2 ± 0.6</td>
<td>0.01 ± 0.002</td>
<td>0.1 ± 0.01</td>
<td>109 ± 3</td>
</tr>
</tbody>
</table>

*Data represent the mean ± SEM. Numbers in parentheses indicate number of animals in each group.
†Control = animals received IT saline solution; DX = animals received IT DX.

Effect of Dexamethasone on Lung Injury in the Rat (Volpe, Lin, Thrall)
cent of normal levels at 24 h after IT DX injection, indicating that systemic absorption of the corticosteroid from the lung did occur. However, protein levels were significantly lower in the DX-treated OA-treated animals compared with OA-treated animals at 7 days. The percentages of PMN found in BAL were increased in OA-treated animals at 4 (88 percent) and 24 (49 percent) h as compared with control (1 percent) and 7-day OA-treated (0.4 percent) animals. The OA-DX-treated animals had similar percentages of PMN at 4 h and 7 days but the percentage was reduced at 24 h to 38 percent as compared with the 49 percent in animals that received OA alone.

The lung compliance value for untreated control animals was 1.1 ± 0.05 ml/cm H2O (n=8). Lung compliance was significantly (p<0.05) decreased in OA-treated animals by approximately 50 percent when compared with control animals at 4 and 24 h (Fig 1). The effect of IT DX therapy on lung compliance in OA-treated animals parallels the effect of IT DX on BAL inflammatory cell count and protein in OA-treated animals. At 4 h, there was no significant difference between IT DX-treated and
untreated OA animal groups. However, at 24 h, lung compliance in the IT DX-treated OA group improved significantly compared with untreated animals that received OA. This improved compliance value was greater than 80 percent of the control mean value. The 24-h lung compliance in the IT DX-treated OA group was also significantly greater than observed in the 4-h IT DX-treated OA group. At 7 days, reductions in lung compliance were no longer present in both the DX-treated and untreated animals that received OA.

At both 4 and 24 h after OA, histologic examination of the animal lungs that were exposed to OA alone showed patchy diffuse alveolo-interstitial regions of edema, PMN, and mononuclear influx, vascular congestion, and hemorrhage. Similar histologic changes were observed in the IT DX-treated OA-treated animals at 4 and 24 h, except that there was noticeably less PMN and mononuclear influx compared with the untreated animals that received OA.

Comparison of IT and IP DX Therapy on OA-Induced Lung Injury: Animals that received an equivalent dose (2.5 mg/kg) of IP DX, unlike their IT DX-treated counterparts, showed no significant alteration of BAL total inflammatory cell count, BAL protein level, or lung compliance observed in the animals receiving OA alone at 24 h after OA (Table 3). Peripheral blood lymphocyte counts were depressed in IP DX-treated animals and there was no significant difference between the IP DX and IT DX-depressed lymphocyte counts.

**DISCUSSION**

Treatment of OA-treated animals with IT DX significantly reduced BAL inflammatory cell presence and BAL protein content, and improved lung compliance at 24 h after injury. This effect was accompanied by normal BAL inflammatory cell presence and protein content as well as normal lung compliance at 7 days after OA. The single dose of IT DX used (2.5 mg/kg) was considerably less than previously examined multiple-day dose ranges of DX (4 to 6 mg/kg/d) or MP (20 to 120 mg/kg/d) in both clinical trials5-11 and animal studies12-14 of ARDS. When 2.5 mg/kg of DX was given by the IP route to OA-injured animals, there was no significant improvement in these BAL parameters or lung compliance at 24 h.

A beneficial therapeutic effect of single-dose IT DX (2.5 mg/kg) in the OA-induced acute lung injury model has been established. This effect concurs with the responses observed by Shiue and Thrall12 in their 7-day treatment courses with IP DX (4 mg/kg/d) and MP (20 mg/kg/d) during OA-induced injury. Both of their systemic corticosteroid regimens proved efficacious in reducing pulmonary inflammation, minimizing protein leakage, and improving lung compliance during the acute injury phase, but prevented the return to normal ranges in these parameters that are typically observed in the untreated OA model by 3 to 7 days. Therefore, the IT modality suggests two important therapeutic advantages over the systemic treatment courses used by Shiue and Thrall. First, the beneficial effect on OA-induced injury was feasible with IT DX at a reduced dose. Second, IT DX did not impair the recovery phase of OA-induced injury. One possible explanation for the reduced therapeutic dose requirement of the IT delivery route is that direct contact of unmetabolized DX at the air/alveolar epithelial interface may have a topical anti-inflammatory potency analogous to that observed with topical dermatologic corticosteroids25 and aerosolized inhaled corticosteroids for chronic airways disease.21

The normal recovery phase associated with the IT DX modality is likely due to the fact that a substantial 20-fold reduction in total dose of DX was given to each animal over the 7-day injury-recovery period. Thus, potential adverse side effects due to high sustained levels of circulating DX are greatly diminished. Shiue and Thrall12 hypothesized in their study that the indiscriminate anti-inflammatory effects of high-dose systemic MP and DX, while showing therapeutic benefit during the OA-induced acute injury phase, may have impaired the lung repair process following injury. Interestingly, their study also showed that significantly greater recovery of normal lung compliance was observed in DX-treated OA-treated animals as compared with MP-treated OA-treated animals. Although our present study suggests that the systemic lymphopenia observed in IT DX-treated animals at 24 h indicates a potential systemic immunologic alteration may have occurred, this finding was not accompanied by any evidence.

**Table 3—Effect of IP DX Therapy on BAL Inflammatory Cell Presence, BAL Protein Content, and Lung Compliance at 24 h After OA***

<table>
<thead>
<tr>
<th>Group†</th>
<th>Nucleated Cells, 10⁶</th>
<th>PMN, 10⁶</th>
<th>Protein, mg/ml</th>
<th>Compliance, % Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>OA (4)</td>
<td>6.9±1.3</td>
<td>5.4±0.9</td>
<td>2.7±0.5</td>
<td>55±2</td>
</tr>
<tr>
<td>OA-DX (5)</td>
<td>5.8±0.6</td>
<td>3.6±0.4</td>
<td>1.9±0.1</td>
<td>55±1</td>
</tr>
</tbody>
</table>

*Data represent the mean±SEM. Numbers in parentheses indicate number of animals in each group.
†OA=animals received IV OA and IP saline solution; OA-DX=animals received both IV OA and IP DX.

586 Effect of Dexamethasone on Lung Injury in the Rat (Volpe, Lin, Thrall)
suggesting a detrimental effect on the lung repair process.

Dexamethasone was selected for this study for several reasons: (1) previously established ability of DX to reduce OA injury;\(^2\)\(^\text{12}\) (2) evidence that DX stimulates pulmonary surfactant production in the rat,\(^\text{27,29}\) while other corticosteroids, such as MP, may have a detrimental effect on pulmonary surfactant metabolism;\(^\text{20}\) and (3) good animal tolerance of DX administered via the IT route. The unique property of DX pulmonary surfactant stimulation may explain the observation of Shiue and Thrall\(^\text{12}\) that recovery of lung compliance after OA injury is significantly greater for DX than for MP.

This OA animal model of fat embolism-induced ARDS represents one of the various diseases that make up the syndrome of ARDS. Future considerations for the application of IT corticosteroid therapy in ARDS and/or UIP should include both investigations of other animal models of ARDS, such as sepsis, and the use of other corticosteroid agents by the IT route. Methylprednisolone would be of interest to examine since this is one of the more commonly employed systemic corticosteroids used in ARDS clinical trials.\(^5\)\(^\text{11}\) Aerosolized inhaled corticosteroid preparations or their liquid-based forms, such as beclomethasone dipropionate and triamcinolone acetonide, would be ideal agents to study for IT application in ARDS due to their much greater topical potency: systemic potency ratios compared with DX aerosol preparations.\(^2\)\(^\text{11}\) An analysis of the effects of single-dose IT DX on pulmonary surfactant would also be of interest in an attempt to elucidate possible mechanisms responsible for the therapeutic benefit of IT DX in this lung injury model.

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