Pheniramine maleate attenuates oleic acid-induced acute respiratory distress syndrome in rats

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Received 08 May 2015; revised 20 February 2016

Acute respiratory distress syndrome (ARDS) is a common clinical syndrome of acute lung injury with considerable mortality rate of 26-58%. Oleic acid (OA)-induced lung injury that reproduces the early exudative phase of ARDS is an established experimental model of acute lung injury in animals. In this study, we examined the role of antihistaminic drug pheniramine maleate in reversing the oleic acid-induced acute respiratory distress syndrome compared to methylprednisolone, generally used in the treatment of ARDS. Trachea, jugular vein and carotid artery were cannulated in anesthetized rats. Lethal dose of OA (75 µL) was injected i.v. and respiratory frequency (RF), heart rate (HR) and mean arterial pressure (MAP) were determined. At the end of experiment, the lungs were excised for estimation of pulmonary water content and the histological examination. OA produced typical manifestations of ARDS as indicated by profound increase in RF, injury to alveolar-capillary barrier, flooding of alveolar spaces with fluid, influx of inflammatory cells and lethality within 60 min. Along with these changes there was progressive decrease in HR and MAP. In pheniramine maleate pretreated animals, OA did not produce immediate increase in RF and after 60 min there was progressive decrease. There was no pulmonary edema and the histology revealed nearly normal lung parenchyma, less exudation and infiltration. HR and MAP were maintained till 75 min followed by decrease. Survival time was prolonged and 50 % of the animals survived up to 120 min. In another group pretreated with methylprednisolone, OA failed to produce severe changes in RF up to 90 min. Pulmonary water content was significantly less in this group and the histological features exhibited less lung injury as compared to OA treated group. The HR and MAP were maintained till 75 min followed by decrease. Mean survival time of these animals was significantly greater (105 min) than only OA treated animals. Present observations reveal that both pheniramine maleate and methylprednisolone ameliorated OA-induced ARDS in rats.

Keywords: Acute lung injury, Antihistamine, ARDS, Methylprednisolone, Pulmonary edema

Acute respiratory distress syndrome (ARDS) is a common devastating clinical syndrome of acute lung injury with high mortality rate, 26 to 58% ¹,². Oleic acid (OA)-induced lung injury is an established experimental model of acute lung injury in animals and is considered to reproduce the early exudative phase of ARDS³,⁴. The earliest abnormalities seen in ARDS is loss of alveolar-capillary barrier integrity. Histological examination exhibits vascular leak with flooding of alveolar space with protein rich edema fluid⁴,⁵. This can occur as a result of direct injury to the lungs or in response to systemic inflammation and cytokine production. It is reflected by infiltration of inflammatory cells and the presence of cells in bronchoalveolar lavage fluid⁶,⁷. In addition to inflammation, congestion of the capillaries and increased capillary permeability resulting in the development of pulmonary edema and respiratory failure are also reported in ARDS⁴. Also, the alveolar epithelial injury decreases the surfactant synthesis which further enhances pulmonary edema production and hypoxemia⁸.

Increased capillary permeability can be produced by a number of mediators including histamine. Histamine also causes bronchoconstriction leading to hypoxia. Hypoxia in turn produces pulmonary hypertension and development of pulmonary edema. Thus, histamine plays an important role in ARDS. However, the role of histamine in ARDS is yet not clear. In the present study, we observed the effect of an antihistaminic drug, pheniramine maleate, on OA-induced ARDS and compared it with methyl-prednisolone, an anti-inflammatory drug used in the treatment of ARDS.
Materials and Methods

Animals, anesthesia, dissection and recording

All the experiments were performed according to the guidelines of the ethical committee of Institute of Medical Sciences, Banaras Hindu University, Varanasi, India for conducting animal experimentation. Adult male albino rats belonging to Charles Foster strain were kept in a temperature (25±0.5°C), humidity (50% RH) and light (12:12 light/dark cycle) controlled room. They were provided ad libitum food and water. The animals were anesthetized with an i.p. injection of urethane (1.5 g/kg body wt. i.p.).

Under the anesthetic effect trachea, jugular vein and carotid artery were cannulated. Trachea was cannulated to keep the respiratory tract patent; jugular venous cannulation for saline/drug administration; and carotid artery cannulation for recording blood pressure via pressure transducer. ECG recordings were made by needle electrodes connected in limb lead II configuration. The respiratory movements were recorded by connecting the force-displacement transducer with a thread secured to the skin over the xiphisternum. All the recordings were made on a computerized chart recorder.

Experimental protocol

The animals were stabilized for 30 min before the experimental procedure. The animals were divided into four groups. In group I (saline control group, n=6), 75 µL of saline was administered through the jugular vein and the recording of respiratory rate (RF), heart rate (HR), and mean arterial pressure (MAP) were made continuously for initial 5 min and then intermittently at 15 min intervals for 120 min. This group served as time-matched control group. In group II (OA only group, n=4), after initial recordings, OA (75 µL) was injected and the recordings were taken as mentioned for group I. In group III (Phe+OA, n=4), pheniramine maleate, an antagonist of histamine receptor (3 mg/kg) was injected after initial recordings and 15 min after this OA (75 µL) was administered. The recordings were taken at each step and at 15 min interval for 120 min. In group IV (MP+OA group, n=4), methylprednisolone (60 mg/kg) was injected after initial recordings and after 15 min, OA (75 µL) was administered. The responses were recorded as mentioned in group III. In all the groups, the lungs were taken out at the end of experimentation for determination of pulmonary water content and for the histological examination.

Determination of pulmonary water content

The pulmonary water content was determined by physical method as described earlier. At the end of each experiment, the lungs were excised. One lung was preserved in formal saline for histological examination and the other was weighed and dried in an electric oven (at 90°C for 48-72 h) to a constant weight. The difference between wet weight and dry weight was calculated to determine the water content.

Histology of lungs

The lungs preserved in formal saline was subjected to standard histological protocol and stained with haematoxylin and eosin (H & E) for microscopic examination.

Drugs and solutions

Urethane was obtained by Sigma Aldrich Inc, St Louis, USA, oleic acid from Hi-Media Laboratories Pvt Limited Mumbai, India and was used in bolus dose of 75 µL; methylprednisolone from Neon Laboratories Limited Mumbai (India) and was used in the dose of 60 mg/kg; pheniramine maleate was from Unimark remedies Ltd, Bangalore (India) and was used in the dose of 3 mg/kg. The dose of methylprednisolone and pheniramine were used as reported in earlier studies.

Statistical analysis

All the data were presented as Mean ± SEM. The time-response relation of oleic acid only group was compared with “methylprednisolone + oleic acid” or “pheniramine + oleic acid” group by using two-way ANOVA. Student’s t test for unpaired observation was also used for comparing pulmonary water content with different groups against saline treated group. A P value <0.05 was considered significant. Kaplan Meier’s survival analysis was done for determining the survival time.

Results

Pulmonary and cardiac parameters in saline treated group

The initial RF, HR and MAP in control group was 76±6.8 breaths/min, 262±22.5 beats/min and 67±6.5 mm Hg, respectively. No significant change was observed in RF in the control group for the entire period of observation (Fig. 1). The pulmonary water content in this group was 77.67 ± 0.28 % (Fig. 2). Histological examination of lung showed the well aerated alveoli with no infiltration or exudates (Fig. 3). There were no significant alterations in the HR and MAP (Fig. 4 A and B). All the animals in this group survived throughout the period of observation (120 min, Fig. 5).
Oleic acid produced ARDS

The initial RF in oleic acid treated group, was 74 ± 6.9 breaths/min and was similar to the control group. RF increased by about 44% immediately after the injection of oleic acid. Subsequently, there was a progressive fall in RF (Fig. 1, Table 1) and all the animals died by 60 min (Fig. 5, Table 1). The pulmonary water content was significantly increased in this group as compared with the saline (control) group (Fig. 2, Table 1). Histological examination of

Table 1—Qualitative assessment of various parameters in Phe + OA group and MP + OA group as compared to oleic acid group

<table>
<thead>
<tr>
<th>Parameters</th>
<th>OA group</th>
<th>Phe+OA group</th>
<th>MP+OA group</th>
</tr>
</thead>
<tbody>
<tr>
<td>PO as compared to saline control</td>
<td>+++</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Histopathological alteration as compared to saline control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alveolar damage</td>
<td>+++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Infiltration of inflammatory cells and RBC</td>
<td>+++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Exudation</td>
<td>++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Respiratory frequency</td>
<td>Stopped within 60 min</td>
<td>Continued for &gt;120 min</td>
<td>Stopped by 120 min</td>
</tr>
<tr>
<td>Survival time</td>
<td>&lt;60 min</td>
<td>&gt;120 min</td>
<td>&gt;90 min</td>
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[The ‘+’, ‘++’ and ‘+++’ or ‘-’ sign depicts comparative increase or decrease in the parameters, respectively. RF, respiratory frequency, and PO, pulmonary edema]

Fig. 1—Effect of OA (75 µL) on respiratory frequency (RF) in rats without or with pheniramine (A)/methylprednisolone (B) pre-treatment. [Each point depicts the mean ± SEM values obtained from 4 experiments in each group. Saline/OA was injected at 0 time. The changes in RF in OA group are significantly different from pheniramine maleate/methylprednisolone pre-treated group (P <0.05 is considered significant, Two-way ANOVA). Saline, saline control group; OA only, oleic acid treated group; Phe + OA group, pheniramine maleate + oleic acid group; and MP + OA, methylprednisolone + oleic acid group]

Fig. 2—Effect of OA (75 µL) on pulmonary water content in rats without or with pheniramine/methylprednisolone pretreatment are compared with saline control group. [Each bar depicts the mean ± SEM values obtained from 4 experiments in each group. The (*) indicates significant difference from the saline group and @ indicates significant difference from OA only group (P <0.05; Student’s t test for unpaired observations). Saline = saline control group; OA only, oleic acid treated group; Phe + OA group, pheniramine maleate + oleic acid group; and MP + OA, methylprednisolone + oleic acid group]

Fig. 3—Photomicrograph of rat lung in (A) saline control; (B) Oleic acid treated; (C) pheniramine maleate + Oleic acid; and (D) methylprednisolone + oleic acid groups are shown. [Magnification = 400X. “<” depicts alveolar damage, “-” depicts infiltration of inflammatory cells and “*” depicts exudates]
lungs showed massive destruction of lung parenchyma with alveolar collapse, interstitial edema, and infiltration by inflammatory cells (Fig. 3, Table 1). Oleic acid produced progressive decrease in the HR and MAP till death (Fig. 4 A and B). All the animals (in this group) died by 60 min (Fig. 5, Table 1). Effect of oleic acid in animals pretreated with pheniramine maleate

The RF before administration of pheniramine maleate was 76 ± 4 breaths/min (Fig. 1). Pheniramine maleate per se did not produce significant change in RF. No immediate tachypneic response was observed in this group and RF was maintained at initial level till 60 min. Subsequently, RF decreased and it was 22 % of initial by 120 min (Fig. 1, Table 1). The pulmonary water content in this group was similar to the saline treated group (Fig. 2, Table 1). Thus, there was blockade of OA-induced pulmonary edema. The histological examination of the lungs in this group showed less lung injury as compared with the rats treated with OA only. Alveolar architecture of lungs exhibited nearly normal pattern and there was lesser parenchymal injury, exudation and infiltration of inflammatory cells (Fig. 3, Table 1).

In pheniramine maleate pretreated group (Phe + OA), the initial value of HR was 295 ± 31.8 beats/min; and after pheniramine maleate treatment, HR decreased slightly. Oleic acid administration in these animals did not produce any significant change in HR up to 75 min. However, the HR decreased progressively thereafter and at 120 min, it was about 33% of the initial value (Fig. 4A).

Initial MAP in pheniramine maleate pretreated group was 74 ± 6 mm Hg. Administration of pheniramine maleate increased the MAP by about 21%. The MAP was not significantly altered after oleic acid injection in this group and was maintained up to 75 min. After this MAP decreased and at 120 min it was 21% of the initial value (Fig. 4B). Mean survival time of animals in this group was >120 min and 50% of the animals survived even after 120 min (Fig. 5, Table 1). Effect of oleic acid in animals pretreated with methylprednisolone

The RF before administration of methylprednisolone was 80 ± 8.6 breaths/min. Injection of methylprednisolone increased the RF by about 26%. Oleic acid administration in this group did not alter the RF significantly up to 90 min. After this MAP decreased and at 120 min it was 21% of the initial value (Fig. 4B). Mean survival time of animals in this group was >120 min and 50% of the animals survived even after 120 min (Fig. 5, Table 1).
damage and alveolar collapse as compared to OA only group. Thickening of the interalveolar septum due to fluid accumulation and infiltration by inflammatory cells was observed in this group but was lesser than OA only group (Fig. 3, Table 1).

The initial HR in this group was 276 ± 13 beats/min. Methylprednisolone administration did not alter the HR significantly. Oleic acid injection in these animals failed to produce significant change in HR in the initial phase and was maintained till 75 min. Subsequently, HR decreased till death (Fig. 4A). The MAP before methylprednisolone administration was 79.7 ± 7.6 mm Hg. Methylprednisolone increased the MAP by about 32%. Oleic acid administration in these animals did not alter the MAP up to 75 min. After this, MAP decreased and all the animals died by 120 min (Fig. 4B). The mean survival time of animals in this group was more than OA only group (Fig. 5, Table 1).

Discussion

In the present study, we demonstrated the antihistaminic drug pheniramine maleate protecting the rats against acute respiratory distress syndrome (ARDS) and increased the survival time of rats. Methylprednisolone, a drug indicated in the treatment of ARDS, also attenuated the oleic acid (OA)-induced toxicity in rats but failed to prevent the lethality in all the animals. These observations indicate that histamine plays an important role in the pathophysiology of OA-induced ARDS.

ARDS in animals can be produced by administration of OA. OA has been shown to produce mild to severe form of ARDS in rats. Further, OA-induced ARDS is characterized by pulmonary damage, alteration of alveolar-capillary barrier permeability, hypoxemia and inflammation. In the present study, the dose of OA (75 µL) that produced severe form of ARDS in rats was used. Similar to earlier report, OA produced typical features of ARDS as indicated by severe changes in RF, development of pulmonary edema, histopathological evidences of lung injury and infiltration of inflammatory cells leading to death of the animals within 60 min in the present study also.

ARDS is associated with loss of alveolar capillary barrier and flooding of air spaces with protein rich edema fluid. Our results also indicate damage to the alveolar capillary membrane and development of pulmonary edema. It is shown that OA induces lung injury (demonstrated by electron microscopy) as early as 10 min after administration. Thus, the observation period of 120 min selected in the present study is sufficient to manifest with all the characteristics of ARDS.

Histological studies of lung specimen obtained after oleic acid injection showed marked infiltration by neutrophils and macrophages as reported earlier indicating inflammation. The protective effect of methylprednisolone is attributed to its anti-inflammatory action. Further, glucocorticoid is known to cause inhibition of IgE-dependent release of histamine. Inhibition of histamine release may be another mechanism of protection against ARDS by steroids (methylprednisolone). Therefore, we hypothesized that histamine may be involved in the pathophysiology of lung injury in ARDS. Our results confirm the above hypothesis as pheniramine maleate (an antihistaminic agent) pretreated animals survived after OA administration for a longer period. Pulmonary pathology was attenuated significantly in these animals and there was no development of pulmonary edema.

Thus, histamine seems to be a key factor in the development of pulmonary edema and has been shown in isolated perfused lung following paraquat-induced vascular injury. It is known that lungs are rich in histamine which is present mostly within the storage granules of mast cells. Histamine release produces marked dilatation of smaller blood vessels and causes increased capillary permeability. Thus, histamine released by lung injury increases the permeability of alveolar capillary membrane leading to development of pulmonary edema. Further, histamine is also known to cause bronchoconstriction which further enhances the hypoxia and produces pulmonary hypertension and pulmonary edema. Similar to any form of inflammation, ARDS represent a complex process in which multiple pathways are involved in producing lung injury. Involvement of multiple pathways may be responsible for the ineffectiveness of pheniramine maleate or methylprednisolone pretreatment in the later phase. This is indicated by decrease in RF after OA in the later phase of experiment even in pheniramine/methylprednisolone pretreated animals. The decreased respiratory activity may be due to the failure of medullary centers. This is supported by the parallel decrease in RF, HR and MAP after OA even in pheniramine/methylprednisolone pretreated animals (Fig. 1, Fig. 4 A and B).

Methylprednisolone is used for treating hypersensitivity or inflammatory conditions like ARDS but it can suppress the necessary protective responses.
against infection and also inhibits essential repair and healing processes due to decreased fibroblast function. On the other hand, pheniramine maleate by opposing vasodilatation and decreasing alveolar-capillary membrane permeability tends to prevent pulmonary edema.

In conclusion, our data suggest the protective role of antihistaminic drug in ARDS. Considering the inhibition of tissue repair/healing properties and other side effects of methylprednisolone, pheniramine could be a better choice for the management of ARDS.

Conflict of interest
The authors declare no conflict of interest.

References