Characterization of oleic acid-induced acute respiratory distress syndrome model in rat

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Animal studies using oleic acid (OA) model to produce acute respiratory distress syndrome (ARDS) have been inconsistent. Therefore, the present study was undertaken to establish an acute model of ARDS in rats using OA and to characterize its effect on cardio-respiratory parameters and lethality. The trachea, jugular vein and femoral artery of anesthetized adult rats were cannulated. A dose of OA (30-90 µL; iv) was injected in each animal and changes in respiratory frequency (RF), heart rate (HR) and mean arterial pressure (MAP) were recorded. Minute ventilation and PaO₂/FiO₂ (P/F) ratio were also determined. At the end, lungs were excised for determination of pulmonary water content and histological examination. At all doses of OA, there was immediate decrease followed by increase in RF, however at 75 and 90 µL of OA, RF decreased abruptly and the animals died by 63 ± 8.2 min and 19 ± 6.3 min; respectively. In all the groups, HR and MAP changes followed the respiratory changes. The minute ventilation increased in a dose-dependent manner while the values of P/F ratio decreased correspondingly. Pulmonary edema was induced at all doses. Histological examination of the lung showed alveolar damage, microvascular congestion, microvascular injury, infiltration of inflammatory cells, pulmonary edema and necrosis in a dose-dependent manner. With these results, OA can be used to induce different grades of ARDS in rats and OA doses of 50, 60 and 75 µL resemble mild, moderate and severe forms of ARDS respectively. Hence, OA model serves as a useful tool to study the pathophysiology of ARDS.

Keywords: Acute lung injury (ALI), Minute ventilation, P/F ratio, Pulmonary edema, Surfactant

Acute respiratory distress syndrome (ARDS) is a fulminant disease characterized by pulmonary edema, hypoxemia and decreased pulmonary compliance leading to respiratory failure. The pathological changes seen in ARDS include infiltration of neutrophils into the alveoli, alterations in the alveolar permeability barrier, deposition of hyaline membrane in the inter-alveolar septa and also formation of microthrombi¹⁻⁴. High mortality rate (27-48%) has been observed in patients with ARDS even in developed countries like USA with good treatment strategies and supportive care.⁵,⁶ The exact cause of mortality remains unclear. Though ARDS is primarily a respiratory disorder, it produces multi-system failure and death. It is associated with the development of pulmonary edema, surfactant insufficiency, inflammation and tissue injury leading to insufficient oxygenation of blood (hypoxemia)⁴,⁷. All these mechanisms operate synergistically culminating in multi-organ failure and death. Hence, the changes produced in respiratory system, cardio-vascular system, hypoxemic status, pulmonary pathology and pulmonary water content have to be considered for understanding the pathophysiology of ARDS.

Presently the following animal models of ARDS are in use: lipopolysaccharide-induced injury (LPS, intra tracheal or intra venous), ventilator-induced lung injury (VILI), surfactant lavage-induced injury, reperfusion injury, oleic acid (OA)-induced injury etc.³,⁸ Amongst these models, OA model is considered to mimic the ARDS condition as seen in humans.³ However, the animal studies using OA model for ARDS are inconsistent with respect to the dose of OA, administration route, experimentation time, parameters under study, ventilator usage and others.⁹ In addition, most of the studies using OA model of ARDS have been done for wide range of durations (from 0.5 to 72 h)⁹⁻¹⁵. Since ARDS is an acute condition, the present study has been undertaken to determine whether OA can induce ARDS acutely.

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within 2 h, and to assess whether the alterations in respiratory parameters, hypoxemic status, cardiovascular parameters, pulmonary pathology, pulmonary water content and survival time can be used to grade the OA-induced changes.

The results of the present study will provide an acute model of ARDS which will be helpful in understanding the pathophysiology of ARDS and for designing novel management strategies.

**Materials and Methods**

*Animals, anesthesia and recording procedure*—The animal experiments were performed after obtaining approval from the Ethical Clearance Committee (Approval No. Dean/12-13/CAEC/333 dated 2/7/2012) of the Institute of Medical Sciences, Banaras Hindu University, Varanasi, India. Adult albino rats (either sex) of Charles-Foster strain (175-225 g) were used. The animals were obtained from the Central Animal House, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India and were housed in a temperature, humidity (50% RH) and light (12 h: 12 h light dark period) controlled room with *ad libitum* food and water. The animals were anaesthetized with urethane (Sigma Aldrich Inc, St Louis, USA; 1.5 g/kg body weight, ip). Additional bolus dose of urethane (0.1-0.15 g/kg, ip) was injected if required.

Trachea, jugular vein and femoral artery were cannulated as reported earlier. Tracheal cannulation was used to keep the respiratory tract patent; jugular venous cannulation for drug administration; and femoral artery cannulation for recording blood pressure via pressure transducer. Electrocadiographic (ECG) potentials were recorded by connecting the needle electrodes in standard limb lead-II configuration. Respiratory movements were recorded by securing a thread to the skin over xiphisternum to a force-displacement transducer. All the recordings were made on a computerized chart recorder.

*Experimental protocol*—The animals were stabilized for 30 min before subjecting to experimental procedure. The animals were divided in to two groups.

In group I (n=6), 100 µL saline was injected and the changes in respiratory frequency (RF), heart rate (HR) and mean arterial pressure (MAP) were recorded at every 15 min up to 120 min. These animals served as time-matched control group.

Group II (n=28) was divided in to 5 sub-groups (n=3-8 as mentioned in Table 1), depending upon the dose. Each animal received a given dose of OA (30, 50, 60, 75, or 90 µL; Hi-Media Laboratories Pvt Limited Mumbai, India). The changes in RF, HR and MAP were recorded at every 15 min for 120 min or till the death of the animal, whichever was earlier.

*Determination of P/F ratio*—The femoral arterial blood sample was collected into a heparin-rinsed syringe after 30 min of OA injection in all the groups except in 90 µL group, where blood was drawn 20 min after OA (as the animals were dying by 30 min in this group). The blood sample was subjected to arterial blood gas analysis using Roche OMNI C blood gas analyzer to determine the P/F ratio, where P stands for partial pressure of oxygen in arterial blood (PaO$_2$) and F for fraction of oxygen in the inspired air (FiO$_2$; considered as 0.21).

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

*Histology of lungs*—The lung tissue was fixed in formal saline and was subjected to standard histological protocol and stained with Haematoxyline and Eosin (H&E) for microscopic examination.

*Analysis of data*—The changes in RF, HR and MAP were expressed as % of initial. The data were pooled and mean ± SE was calculated. The data were compared using two-way ANOVA followed by Berfonni’s test. Student’s *t* test for unpaired observations was used for comparing pulmonary water content, minute ventilation and P/F ratio in OA treated group with saline group. Kaplan Meier’s survival analysis was done for determining the survival time.

Since respiratory wave is triangular, the area under each respiratory wave was calculated. It was assumed that the area of a triangle represents the respiratory activity per breath (representing time and amplitude). The area under one respiratory wave was multiplied by the respiratory frequency per min to obtain the minute ventilation. The minute ventilation was hence expressed as arbitrary units (AU).
Results

Control group—Saline treated (control) animals showed stable cardio-respiratory parameters for the entire observation period of 120 min. The basal RF, HR and MAP values in saline control group were 60 ± 3.5 breaths per min, 291 ± 23.3 beats per min and 92.6 ± 7.6 mmHg, respectively (Table 1). There was no significant change in these values throughout the observation period of 120 min (Fig. 1).

Effect of OA on survival time—Animals injected with 30, 50 and 60 µL OA survived for 120 min (Table 2). However, the mean survival time for the animals injected with 75 µL OA was 63 ± 8.2 min and the maximum survival time was 95 min (Table 2; Fig. 2). 90 µL OA killed the animals within 30 min and the mean survival time in this group was 19 ± 6.3 min (Table 2; Fig. 2).

Effect of OA on RF—The initial RF values in OA treated groups were not different from control group (Table 1). RF increased (by 10-50%) immediately after injection of OA in all the groups (Fig. 1B). With 30 and 50 µL of OA, the increase in RF was 100% by 30 min and the increase remained at that level up to 120 min. With 60 µL OA, there was a time-dependent increase in RF up to 50% by 30 min and remained at the same level thereafter (Fig. 1B). With 75 µL OA, the time-dependent increase in RF was observed

<table>
<thead>
<tr>
<th>OA (µL)</th>
<th>n</th>
<th>RF (breaths/min)</th>
<th>HR (beats/min)</th>
<th>MAP (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline (0)</td>
<td>6</td>
<td>60 ± 3.5</td>
<td>291 ± 23.3</td>
<td>92.6 ± 7.6</td>
</tr>
<tr>
<td>30</td>
<td>3</td>
<td>56 ± 1.7</td>
<td>243 ± 6.4</td>
<td>71 ± 11.2</td>
</tr>
<tr>
<td>50</td>
<td>8</td>
<td>69 ± 10.2</td>
<td>273 ± 30.9</td>
<td>100 ± 7.7</td>
</tr>
<tr>
<td>60</td>
<td>5</td>
<td>76 ± 9.9</td>
<td>265 ± 11.3</td>
<td>100 ± 11.2</td>
</tr>
<tr>
<td>75</td>
<td>8</td>
<td>77 ± 4.6</td>
<td>253 ± 16.8</td>
<td>95 ± 7.2</td>
</tr>
<tr>
<td>90</td>
<td>4</td>
<td>74 ± 13.3</td>
<td>333 ± 11.0</td>
<td>107 ± 3.4</td>
</tr>
</tbody>
</table>

*P < 0.05, Student’s t test for unpaired observations as compared to saline group.

Table 2—Survival time, P/F ratio and minute ventilation in various experiments after injection of different doses of oleic acid.

<table>
<thead>
<tr>
<th>OA (in µL)</th>
<th>n</th>
<th>Survival time (min)</th>
<th>P/F ratio (mm Hg)</th>
<th>Minute ventilation (AU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline (0)</td>
<td>6</td>
<td>120</td>
<td>448 ± 10</td>
<td>11.1 ± 0.2</td>
</tr>
<tr>
<td>30</td>
<td>3</td>
<td>120</td>
<td>354 ± 10</td>
<td>20.1 ± 3.3</td>
</tr>
<tr>
<td>50</td>
<td>8</td>
<td>120</td>
<td>297 ± 8.5</td>
<td>17.2 ± 2.5</td>
</tr>
<tr>
<td>60</td>
<td>5</td>
<td>120</td>
<td>256 ± 6.6</td>
<td>22.5 ± 5.5</td>
</tr>
<tr>
<td>75</td>
<td>8</td>
<td>63 ± 8.2*</td>
<td>188 ± 7.2*</td>
<td>28.5 ± 4.5*</td>
</tr>
<tr>
<td>90</td>
<td>4</td>
<td>19 ± 6.3*</td>
<td>123 ± 6.4*</td>
<td>29.2 ± 5.7*</td>
</tr>
</tbody>
</table>

Table 1—Basal respiratory frequency (RF), heart rate (HR) and mean arterial pressure (MAP) in different groups

Fig. 1—Dose-response of oleic acid (OA)-induced cardio-respiratory changes in rats. A: Original tracings of an experiment showing respiration (Resp), blood pressure (BP) and electrocardiographic (ECG) recordings before OA, 0, 30 and 60 min after OA (75 µL) administration. Vertical dashed line indicates the point of OA administration. Horizontal line (time scale) = 5 sec for all. B, C and D: OA (30-90 µL)-induced changes in respiratory frequency (RF), heart rate (HR) and mean arterial pressure (MAP), respectively. OA was injected at 0 time. Each point depicts the mean ± SE values obtained from 3-8 experiments in each group. The changes produced by oleic acid at all the doses were significantly different as compared to the control group (P < 0.05; Two-way ANOVA).
up to 45 min subsequently the RF showed an abrupt fall leading to the death of the animals by 60 min (Fig. 1A and B). With 90 µL OA, the immediate increase in RF was followed by time-dependent decrease leading to the death of animals by 20 min. The RF values after injection of different doses of OA were significantly different from control values ($P < 0.05$, Two-way-ANOVA).

**Effect of OA on minute ventilation and P/F ratio**—The minute ventilation in control group was $11.1 \pm 0.2$ AU and P/F ratio was $448 \pm 10$ mmHg. After OA treatment, the minute ventilation increased and P/F ratio decreased in a dose-dependent manner (Fig. 3). The mean values of minute ventilation (AU) and P/F ratio (mmHg) are given in Table 2.

**Effect of OA on pulmonary water content**—The pulmonary water content in saline control group was $76.3 \pm 0.08\%$. It was significantly increased in all the OA treated groups after injection of OA as compared to saline control group (Fig. 4; $P < 0.05$ Student’s $t$ test for unpaired observations).

**Histopathological evidences**—In comparison to saline control group, marked histopathological changes in lung tissues were observed with different doses of OA (Figs 5 and 6). With 30 µL of OA, the alveoli were enlarged or bloated as compared to saline control. With 50 µL of OA, there was alveolar shrinkage at some places and coalescing of smaller alveoli into larger air sacs was seen in some areas (Fig. 5). With higher doses of OA (60-90 µL), large air spaces were seen implicating the extent of alveolar damage (Fig. 6).

Along with gross changes in the alveolar morphology, the lungs treated with 30 µL of OA, showed capillary congestion and alterations in the capillary wall (Fig. 5). At 50 µL OA, capillary congestion, and increased number of inflammatory cells, alveolar damage and alveolar shrinkage were seen (Fig. 5). At 60 µL OA, damage to pulmonary parenchyma, intra alveolar edema, infiltration of inflammatory cells in the alveoli and capillary breakage were seen (Fig. 6). With 75 µL OA, the damage to the pulmonary parenchyma was extensive along with increased interstitial edema, infiltration of inflammatory cells and intra alveolar RBCs (Fig. 6). At 90 µL OA, the normal morphology of lung was destroyed, masses of necrosed tissue and whorling of capillaries were seen (Fig. 6).

**Effect of OA on cardio-vascular parameters**—The basal HR and MAP values in OA treated groups were not different from control group (Table 1). HR and
MAP decreased (by 20-50%) immediately after injection of OA in all the groups.

With 30, 50 and 60 µL of OA, the decrease in HR and MAP was followed by recovery in case of HR and partial recovery in case of MAP. They were maintained till the end of the observation period. With 75 µL of OA, HR changes manifested as immediate fall, followed by increase (20% of initial at 15-45 min) and subsequent fall (30% of initial by 60 min; Fig. 1A and C). MAP in this group showed an immediate fall (40%) and remained at that level up to 30 min followed by time-dependent fall till the death of the animal (up to 60 min; Fig. 1A and D). With 90 µL OA, HR and MAP decreased in a time-dependent manner and the animal died within 20 min (Fig. 1C and D). The HR and MAP values after injection of different doses of OA were significantly different from control ($P < 0.05$; Two-way ANOVA).

**Discussion**

ARDS is a clinical syndrome marked by hypoxemia and pulmonary pathology. The pathology of the disease is characterized by the alteration in the pulmonary permeability barrier, inflammation, cellular infiltration, exudation and necrosis resulting in ventilation-perfusion failure$^{1-4}$. Based on the degree of hypoxemia, clinically ARDS has been categorized into mild ($P/F=200-300$ mmHg), moderate ($P/F=100-200$ mmHg) and severe ($P/F<100$ mmHg) forms, respectively$^5$.

Earlier, oleic acid (OA) has been used to induce lung injury, using bovine serum albumin (BSA) or...
ethanol as solvent\textsuperscript{3,10-13}. These vehicles have inherent effects on the physiological system for example, ethanol is known to alter the membrane properties while BSA being a foreign protein, possesses antigenic properties and can interfere with the pulmonary water clearance\textsuperscript{19,20}. Considering these points, bolus injection of OA without any solvents was used in the present study.

Most of the studies using bolus injection of OA (low doses) examined the responses for longer durations\textsuperscript{14,15}. Since, ARDS is an acute and fulminant disorder, it was thought to produce the acute effects of OA within 120 min. ARDS-like situation using OA was induced in such a short time in the present study. The present OA model exhibited important features of ARDS in rats as proposed by the American Thoracic Society report which includes histological evidences of tissue injury, alteration of the alveolar capillary barrier, inflammatory response and physiological dysfunction\textsuperscript{8}. Utilizing these parameters, OA-induced ARDS was categorized into different grades viz. mild, moderate and severe, as reported clinically\textsuperscript{5}.

Animals injected with OA up to 60 \( \mu \)L doses survived the entire period of observation (120 min) however, based on the degree of hypoxemia (P/F~300 mmHg), pulmonary pathology and cardio-vascular parameters 30 and 50 \( \mu \)L OA groups were categorized as mild form of ARDS. Although the important features of ARDS were comparable in both 30 and 50 \( \mu \)L of OA, the increase in minute ventilation, degree of hypoxemia and features of histological alterations were considerably greater in 50 \( \mu \)L of OA as compared to 30 \( \mu \)L (Table 2; Fig. 5).
Therefore, 30 µL OA group was further categorized into very mild form of ARDS. Even though the animals injected with 60 µL of OA survived for 120 min, there was a greater degree of hypoxemia (P/F<300 mmHg) and pulmonary pathology than the previous dose (50 µL of OA). Therefore, 60 µL was categorized as moderate form of ARDS. The animals injected with 75 µL of OA died around 63 min (Table 2). The cardio-vascular alterations appear to be reflexly driven by the respiratory changes (arrest) leading to lethality in these animals (Fig. 1). Considering the lethality, severity of pulmonary pathology and hypoxemia (P/F<200 mmHg), 75 µL of OA was categorized to produce severe form of ARDS. Animals injected with 90 µL OA, died within 20 min (Table 2) associated with simultaneous decrease in cardio-respiratory parameters (Fig. 1). As the animals started dying within 20 min, hence the P/F ratio could not be determined in all the animals. However, the P/F ratio was determined in 2 animals that survived for more than 20 min and it was <125 mmHg (Table 2). Considering the acute lethality, severe degree of pulmonary pathology and hypoxemia, this dose was categorized as very severe form of ARDS.

The present results indicate that, OA induced ARDS can be classified into very mild (30 µL), mild (50 µL), moderate (60 µL), severe (75 µL) and very severe (90 µL) forms based on survival time, respiratory, cardio-vascular and histopathological data. The P/F ratios in the mild, moderate and severe forms were ~300, < 300 and < 200 mmHg, respectively. These values did not coincide with the P/F values given for different phases of ARDS clinically. The difference may be due to the fact that, in the present study the assessment of P/F ratio was done at very early time phase (30 min/20 min after OA administration) of the disease.

In the present study, the assessment of pulmonary water content by physical method demonstrated that even with 30 µL of OA, the pulmonary edema was maximal (Fig. 4). However at this dose, the lungs did not show any signs of edema histologically except for some degree of microvascular congestion (Fig. 5). On the other hand, higher doses (60 and 75 µL) of OA showed physical pulmonary edema along with the presence of colloidal substances in the inter- and intra-alveolar regions histologically (Fig 4 and 6). Further, the histological changes can be positively correlated with hypoxic status of the animals. Therefore, pulmonary edema can be considered as an initial feature in the development of ARDS. It may begin as a transudate and end-up with severe inflammatory reaction and exudation.

**Conclusion**

The results of the present study thus demonstrate that OA model exhibits all the characteristic features of ARDS. Further, the study differentiates the different degrees of ARDS (very mild, mild, moderate, severe and very severe). In addition, the study provides evidences for the cardio-vascular alterations associated with OA-induced ARDS. The severe form of ARDS with 75 µL of OA can be considered as the representative of the critical human ARDS cases encountered. This is supported by the increased OA levels were increased in the broncho-alveolar lavage from patients suffering from ARDS. OA model can be used to study different phases of ARDS and design suitable therapeutic strategies to prevent lethality in ARDS.

**Conflict of interest**

The authors declare no conflict of interest.

**Acknowledgement**

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