National Journal of Physiology, Pharmacy and Pharmacology

RESEARCH ARTICLE

Protective role of prostaglandin E1 analog in indomethacin-induced deterioration in acute respiratory distress syndrome in rats

Parul Sharma, Ratna Pandey, Shripad B Deshpande

Department of Physiology, Institute of Medical Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

Correspondence to: Ratna Pandey, E-mail: ratna93@yahoo.co.in

Received: March 06, 2017; **Accepted:** March 24, 2017

ABSTRACT

Background: Acute respiratory distress syndrome (ARDS) is a severe inflammatory condition. Our earlier studies have characterized oleic acid (OA)-induced rat model of ARDS which was exacerbated by indomethacin (prostaglandin [PG] synthesis inhibitor). **Aim and Objectives:** The role of PGs in ARDS is ill defined as the results of earlier studies are conflicting. This study was undertaken to determine the effect of PGE1 analog (misoprostol) in indomethacin-induced exacerbation of ARDS in rats. **Materials and Methods:** The rats were anesthetized with urethane. Tracheal and jugular vein cannulation was done to keep the respiratory tract patent and deliver drugs, respectively. Respiratory excursions were recorded with the help of force displacement transducer. Cannulated carotid artery was connected to pressure transducer for recording of blood pressure. Electrocardiographic potentials were recorded by needle electrodes. Animals were divided into four groups. In Group I, OA (75 µl) was used to induce ARDS in rats. In Group II, OA was injected in indomethacin-pretreated rats. In Group III (control group), animals were treated with ethanol. In Group IV, OA was administered after indomethacin + misoprostol pretreatment. Misoprostol treatment was repeated after OA injection at 20 min interval. Cardiorespiratory parameters (respiratory frequency, heart rate, mean arterial pressure, and pulmonary water content) were determined, and histological examination of the lung was done in all groups. **Results:** Indomethacin pretreatment drastically advanced the OA-induced ARDS. Misoprostol protected against the deterioration as indicated by improvement in all the parameters and increase in survival time. **Conclusion:** Results of this study indicate that PGs have protective role in ARDS.

KEY WORDS: Acute Respiratory Distress Syndrome; Prostaglandins; Oleic Acid; Prostaglandin E1 Analog

INTRODUCTION

Acute respiratory distress syndrome (ARDS) is an inflammatory condition characterized by pulmonary edema, hypoxia, and massive influx of inflammatory cells as per the American Thoracic Society (ATS) guidelines.[1] Despite the advancement in the treatment strategies and good supportive care, the mortality rate is very high (27-48%) even in developed countries like the USA.[2,3] Of the various animal models of ARDS, oleic acid (OA)-induced ARDS is considered to mimic the ARDS condition observed in humans.[1,3-6] The pathogenic mechanisms include damage to the alveolar-capillary membrane barrier due to epithelial or endothelial injury or by both and increased permeability causing massive influx of inflammatory cells in the lungs.[7,8] The injured cells and the inflammatory cells release prostaglandins (PGs) which are implicated as important mediators of inflammation in ARDS.[9] Liposomal PGE1 caused statistically significant improvement in the oxygenation, lung compliance, and ventilator dependency in ARDS patients. Unfortunately, these

Access this article online	
Website: www.njppp.com	Quick Response code
DOI: 10.5455/njppp.2017.7.0305924032017	

encouraging results were not confirmed in later studies.[10] In animal models of acute lung injury, intravenous PGE1 has demonstrated anti-inflammatory response.[10] In our earlier study, we have reported an acute model of ARDS in rats using OA which exhibited all the characteristic features of ARDS in experimental animal models as defined by the ATS.[7] Further, it was observed that OA-induced ARDS was worsened when the animals were pretreated with a PG synthesis inhibitor (indomethacin).[9] The results of earlier study indicate the involvement of PGs in the pathophysiology of ARDS. Therefore, the present study was undertaken to identify the role of PGE1 analog in the indomethacin-induced exacerbation in ARDS and to explore the possible mechanism.

MATERIALS AND METHODS

Animals, Anesthesia, and Recording Procedure

All the animal experiments were performed after approval from the Ethical Clearance Committee of the Institute of Medical Sciences, Banaras Hindu University, Varanasi, India. Adult albino rats of Charles-Foster strain weighing (175-225 g) were selected for experiments. Animals were kept in a temperature, and light (12 h: 12 h, light dark period) controlled room. Food and water was given *ad libitum*. Urethane (Sigma Aldrich Inc.; St. Louis; USA; 1.5 g/kg body weight i.p.) was given to anesthetize the animals. Additional bolus dose of urethane (0.1-0.15 g/kg i.p.) was injected if required. Tracheal, jugular vein, and carotid artery cannulation was done to keep the respiratory tract patent, drug administration, and recording blood pressure, respectively. The skin over the xiphisternum was secured with the help of a thread and attached to a force displacement transducer. The respiratory excursions were recorded on a chart recorder through a bridge amplifier, and respiratory frequency (RF) was computed from these recordings. The electrocardiographic (ECG) potentials (for heart rate [HR]) were recorded by needle electrodes connected to bioamplifier (ADI) using limb lead II configuration. HR was calculated manually from R to R interval. The recorded blood pressure was computed to determine the mean arterial pressure (MAP). At the end of each experiment, the lungs were excised. Lungs of one side were kept for the estimation of pulmonary water content and other side was sent for histological examination.

Experimental Protocol

The animals were stabilized for 30 min after dissection and cannulation. The animals were divided into four groups (N = 4-6).

Group I (E + OA)

After initial recordings of respiratory excursions, ECG, and blood pressure, 75 μL of ethanol was injected, and after 30 min, respiratory excursions, ECG, and blood pressure were recorded. OA (75 μL) was now injected, and the respiratory excursions, blood pressure, and ECG were recorded for the initial 5 min continuously and then at the interval of 15 min up to 120 min.

Group II (I + OA)

After initial recordings, indomethacin (10 mg/kg) was injected, and respiratory excursions, ECG, and blood pressure were recorded. 30 min after indomethacin treatment, OA (75 μL) was injected, and the respiratory excursions, ECG, and blood pressure were recorded for the initial 5 min continuously and then at interval of 15 min up to 120 min.

Group III (ethanol/control)

After initial recordings, 75 μL of ethanol was injected. The respiratory excursions, ECG, and blood pressure were recorded for the initial 5 min continuously and then at the interval of 15 min up to 120 min. As indomethacin was dissolved in ethanol, this group served as time-matched control group.

Group IV (I + M + OA group)

After initial recordings, indomethacin (10 mg/kg) was injected, and blood pressure, ECG, and respiratory excursions were recorded. 25 min after indomethacin treatment, misoprostol was injected (6 μg/kg). 5 min later, OA (75 μL) was injected. The respiratory excursions, ECG, and blood pressure were recorded for the initial 5 min continuously and then at the interval of 15 min up to 120 min. The dose of misoprostol was repeated after every 20 min.

In all groups, RF, pulmonary water content, MAP, HR, and survival time (ST) were determined, and histological examination of the lung was done.

Drugs and Solutions

OA was obtained from HiMedia Laboratories Pvt. Limited Mumbai. Indomethacin (PG synthesis inhibitor) was obtained from Sigma Chemical Company, St. Louis, MO, USA. Indomethacin was prepared by dissolving in 100% ethanol. Misoprostol was obtained from Cipla Ltd. Sikkim, India and was prepared in normal saline.

Determination of Pulmonary Water Content

The pulmonary water content was determined by physical method as described earlier.[11] In brief, 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 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 preserved in formal saline was subjected to standard histological protocol and stained with hematoxylin and eosin for microscopic examination.

Analysis of Data

The changes in RF, HR, and MAP were expressed as % of initial. The data were pooled and mean ± standard error mean (SEM) was calculated. The data were compared using two-way ANOVA. Student's *t*-test for unpaired observations was used for comparing pulmonary water content among different groups.

RESULTS

OA Produced ARDS in Rats

OA injection in ethanol-pretreated rats (ethanol + OA group) increased the RF (twice the initial) by 30 min followed by progressive decrease. RF was maintained at reasonably good level till 60 min. By 105 min, respiration stopped and all the animals died (Figure 1). RF in this group was significantly different from ethanol-treated (control) group. Mean ± SEM of ST in this group was 83.25 ± 7.87 min and pulmonary water content was 85.36 ± 0.35% (Figure 2). Histological examination of lungs shows multifocal and heterogeneous injury. The entire lung architecture of the lung parenchyma was destroyed in certain areas (Figure 3b). OA administration produced immediate decrease in HR (24%) and MAP (32%) followed by recovery and then progressive decrease till death of the animals (Figures 4 and 5).

Indomethacin Advanced the OA-induced Injury

OA administration in indomethacin-pretreated rats (I + OA group) produced immediate and progressive decrease in RF and by 15 min all the animals died (Figure 1). The RF in this group was significantly different from E + OA and I + M + OA group. Mean ± SEM of ST in this group was 7.42 ± 0.98 and the pulmonary water content was 80.81 ± 0.5% (Figure 2). On histological examination of lungs, extensive damage to the lung parenchyma was found in multifocal areas. Destruction of the alveolar septa and hemorrhagic infiltrate forming a homogenous solid area was exhibited. Thickening of alveolar septa with an infiltration of leukocytes was also observed (Figure 3c). HR and MAP followed the pattern similar to RF (Figures 4 and 5).

No Significant Change in Control Group

Ethanol-treated animals (ethanol group) were taken as control group (since indomethacin was dissolved in ethanol) to see the *per se* effect of ethanol on various parameters. Ethanol treatment produced immediate increase in RF which recovered to normal value within 15 min and was maintained throughout the observation period. All the animals in this group survived for the entire observation period of 120 min, and the pulmonary water content in this group was 76.28 ± 1.25 (Figure 2). Histological examination of lung treated with ethanol exhibited the well-aerated alveoli which form the parenchyma of the lung and give it a lace-like appearance. Alveoli were lined by a simple squamous epithelium. Adjacent alveoli shared a common interalveolar

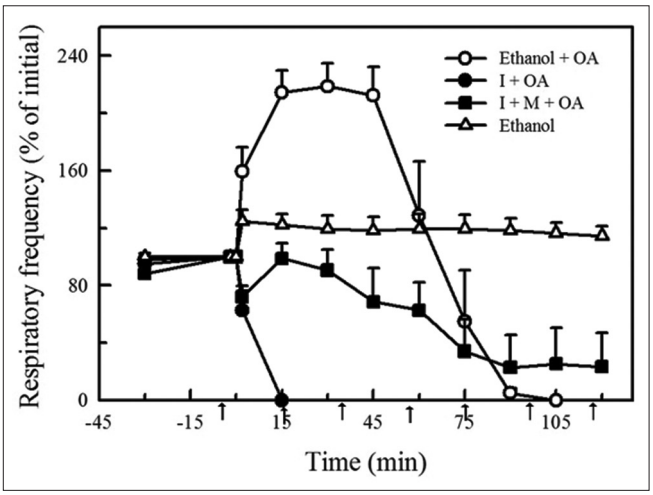

Figure 1: Effect of oleic acid (OA) (75 µL) on respiratory frequency in different groups in rats as compared to ethanol (control) group. Each point depicts the mean ± standard error mean values obtained from 4 to 6 experiments in each group. Ethanol/OA was injected at 0 time. ($P < 0.05$ is considered significant, two-way ANOVA). Ethanol + OA = Ethanol + OA-treated group; I + OA = Indomethacin + OA group; I + M + OA = Indomethacin + Misoprostol + OA group; Ethanol = Ethanol-treated (control) group

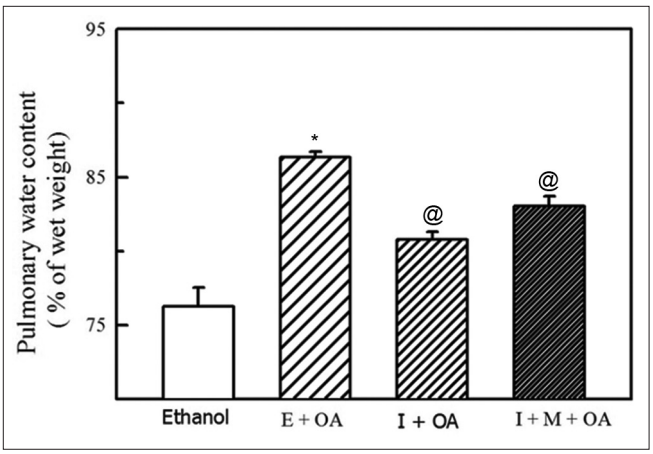

Figure 2: Effect of oleic acid (OA) (75 µL) on pulmonary water content in different groups in rats as compared with ethanol (control) group. Each bar depicts the mean ± standard error mean values obtained from 4 to 6 experiments in each group. The * indicates a significant difference from the control group ($P < 0.05$) and @ indicates significant difference from E + OA group" before ($P < 0.05$); Student's *t*-test for unpaired observations). Ethanol + OA = Ethanol + OA-treated group; I + OA = Indomethacin + OA group; I + M + OA= Indomethacin + Misoprostol + OA group; Ethanol = Ethanol-treated (control) group

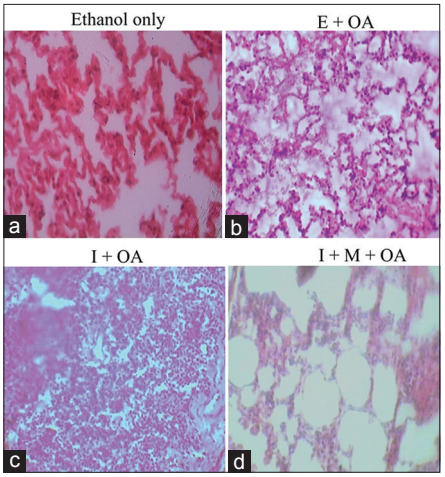

Figure 3: Photomicrograph of rat lung in different groups. Ethanol only (a) = Ethanol-treated (control) group; Ethanol + oleic acid (OA) (b) = Ethanol + OA-treated group; I + OA (c) = Indomethacin + OA group; I + M + OA (d) = Indomethacin + Misoprostol + OA group

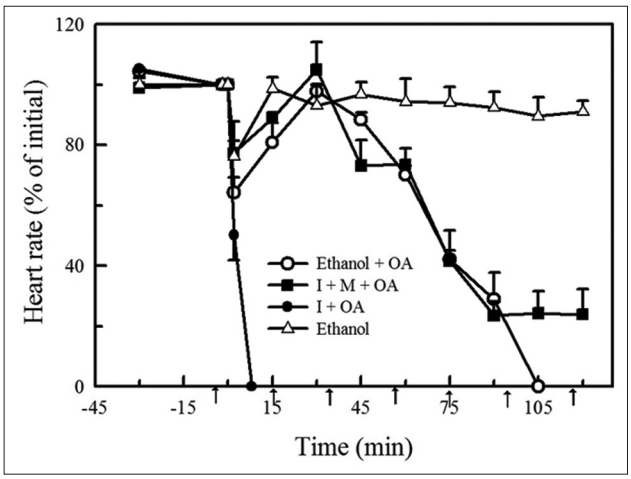

Figure 4: Effect of oleic acid (OA) (75 µL) on heart rate in different groups in rats as compared to ethanol (control group). Each point depicts the mean ± standard error mean values obtained from 4 to 6 experiments in each group. Ethanol/OA was injected at 0 time ($P < 0.05$ is considered significant, two-way ANOVA). Ethanol + OA = Ethanol + OA-treated group; I + OA = Indomethacin + OA group; I + M + OA= Indomethacin + Misoprostol + OA group; Ethanol = Ethanol-treated (control) group

septum. (Figure 3a). HR and MAP followed the pattern similar to RF (Figures 4 and 5).

PGE1 Analog (Misoprostol) Protected the Animals against Indomethacin-induced Deterioration in ARDS

After OA injection in animals pretreated with both indomethacin and misoprostol (I + M + OA group), the immediate progressive decrease in RF as observed in only indomethacin-treated rats was not found. Instead, after initial decrease (about 28%) in RF, recovery occurred and RF was maintained at reasonably good level up to 60 min. In 3 out of 4 animals, it became "0" by 90 min, and in one animal, the respiration was maintained till 120 min although at low level (23%, Figure 1). The mean ± SEM of ST in this group was 79.75 ± 15.41. Pulmonary water content in this group was 83.31 ± 0.79% (Figure 2). Histological examination of lungs in this group does not show the severe injury as observed in only indomethacin-pretreated animals. Airspace was not as much reduced as in indomethacin-pretreated group (Figure 3d). HR and MAP followed the pattern similar to RF in this group (Figures 4 and 5).

Pulmonary water content in all groups was increased as compared to control group (Figure 2). Pulmonary water content in I + OA and I + M + OA group was significantly reduced as compared to E + OA group (Figure 2).

DISCUSSION

Results of the present study show that PGE1 analog, misoprostol, prevented the indomethacin-induced deterioration in ARDS. ARDS is an acute condition associated with inflammatory changes in the lungs, pulmonary edema, and hypoxemia. In this study also, administration of OA produced pulmonary edema as indicated by increase in the pulmonary water content and widening of interalveolar septum in histological examination of the lungs. Profound (two times) increase in the RF after OA administration reflects hypoxemia. Further, loss of integrity of alveolar-capillary membrane and infiltration of inflammatory cells in the lungs suggest inflammation. Our earlier report shows that indomethacin, an anti-inflammatory drug, failed to protect the animals against ARDS, rather than it worsened the condition and all the animals died within a very short time (15 min) as compared to OA treated group (105 min). It was intriguing that indomethacin, in contrary to expected improvement, proved devastating in OA-induced ARDS animal model.[9]

Various inflammatory mediators are proposed to be involved in the pathophysiology of ARDS. PG is one of them but its role in ARDS is not clear.[12-15] In the present study also, inhibition of PG synthesis by indomethacin pretreatment killed the animals within 15 min of OA administration as reported earlier indicating a beneficial role of PG in ARDS. Further, improvement in the RF, HR, MAP, and lung injury when PGE1 analog (misoprostol) was given as pretreatment along with indomethacin, indicates that PGs play a protective role in ARDS.

PGs are possibly not effective in the later phase of ARDS as the ST of I + M + OA group was not significantly different from E + OA group. It suggests the involvement of factors other than PG which dominate over the beneficial effect of misoprostol causing deterioration in the later phase.

Less pulmonary water content in I + OA group than E + OA group can be attributed to decrease ST which did not allow a significant pulmonary edema to develop.

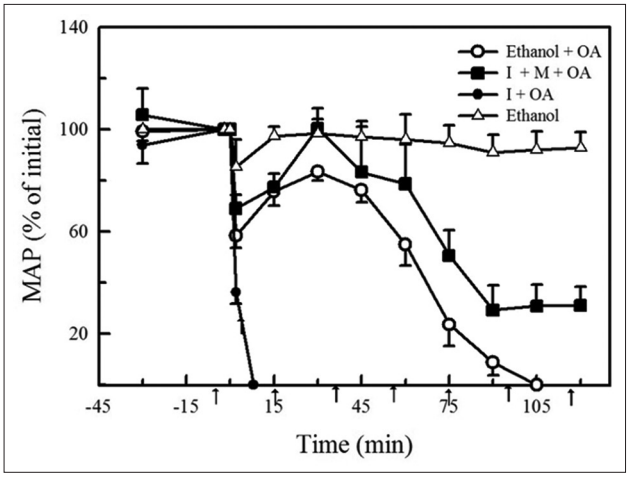

Figure 5: Effect of oleic acid (OA) (75 µL) on mean arterial pressure in different groups in rats as compared to ethanol (control group). Each point depicts the mean ± standard error mean values obtained from 4 to 6 experiments in each group. Ethanol/OA was injected at 0 time ($P < 0.05$ is considered significant, two-way ANOVA). Ethanol + OA = Ethanol + OA-treated group; I + OA = Indomethacin + OA group; I + M + OA= Indomethacin + Misoprostol + OA group; Ethanol = Ethanol-treated (control) group

CONCLUSION

PGE1 analog (misoprostol) protects the animals against indomethacin-induced deterioration in ARDS. Further, the PGs may play a protective role in the early phase of OA-induced ARDS in rats.

REFERENCES

1. Matute-Bello G, Downey G, Moore BB Groshong SD, Malthay MA, Slutsky AS, et al. Acute lung injury in animal study group. An official Americal thoracic society workshop report: Feature and measurements of experimental acute lung injury in animals. Am J Respir Cell Mol Bio. 2011;44:725-38.
2. ARDS Definition Task Force, Ranieri VM, Rubenfeld GD, Thompson BT, Ferguson ND, Caldwell E, et al. Acute respiratory distress syndrome: The Berlin definition. JAMA. 2012;307(23):2526-33.
3. Zambon M, Vincent JL. Mortality rates for patients with acute lung injury/ARDS have decreased over time. Chest. 2008;133(5):1120-7.
4. Ware LB. Pathophysiology of acute lung injury and the acute respiratory distress syndrome. Semin Respir Crit Care Med. 2006;27(4):337-49.
5. Easley RB, Fuld MK, Fernandez-Bustamante A, Hoffman EA, Simon BA. Mechanism of hypoxemia in acute lung injury evaluated by multidetector-row CT. Acad Radiol. 2006;13(7):916-21.
6. Matute-Bello G, Frevert CW, Martin TR. Animal models of acute lung injury. Am J Physiol Lung Cell Mol Physiol. 2008;295(3):L379-99.
7. Akella A, Sharma P, Pandey R, Deshpande SB. Characterization of oleic acid-induced acute respiratory distress syndrome model in rat. Indian J Exp Biol. 2014;52(7):712-9.
8. Husain AN. The Lung. In: Kumar V, Abbas AK, Aster JC, editors. Robbins and Cartan Pathologic Basis of Disease. 9th ed. Philadelphia, PA: Elsevier; 2014. p. 674.
9. Sharma P, Pandey R, Deshpande SB. Indomethacin exacerbates oleic acid induced acute respiratory distress syndrome in adult rats. IJPP. 2016;60(1):82-9.
10. Cepkova M, Matthay MA. Pharmacotherapy of acute lung injury and the acute respiratory distress syndrome. J Intensive Care Med. 2006;21(3):119-43.
11. Deshpande SB, Bagchi S, Rai OP, Aryya NC. Pulmonary oedema produced by scorpion venom augments a phenyldiguanide-induced reflex response in anaesthetized rats. J Physiol. 1999;521:537-44.
12. Fuller BM, Mohr NM, Skrupky L, Fowler S, Kollef MH, Carpenter CR. The use of inhaled prostaglandins in patients with ARDS: A systematic review and meta-analysis. Chest. 2015;147(6):1510-22.
13. Zhang H, Benlabed M, Spapen H, Nguyen DN, Vincent JL. Prostaglandin E1 increases oxygen extraction capabilities in experimental sepsis. J Surg Res. 1994;57(4):470-9.
14. Yamanaka K, Saluja AK, Brown GE, Yamaguchi Y, Hofbauer B, Steer ML. Protective effects of prostaglandin E1 on acute lung injury of caerulein-induced acute pancreatitis in rats. Am J Physiol. 1997;272:G23-30.
15. Melot C, Lejeune P, Leeman M, Moraine JJ, Naeije R. Prostaglandin E1 in adult respiratory distress syndrome. Benefit for pulmonary hypertension and cost for pulmonary gas exchange. Am Rev Respir Dis. 1989;139(1):106-10.

How to cite this article: Sharma P, Pandey R, Deshpande SB. Protective role of prostaglandin E1 analog in indomethacin-induced deterioration in acute respiratory distress syndrome in rats. Natl J Physiol Pharm Pharmacol 2017;7(7):749-753.

Source of Support: Nil, **Conflict of Interest:** None declared.