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Effect of Misoprostol Treatment on Oleic Acid-Induced ARDS in Rats.

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ABSTRACT

Adult respiratory distress syndrome (ARDS) is a clinical condition associated with high mortality. Prostaglandins are involved in the pathophysiology of ARDS but the results of earlier studies are not conclusive. Therefore, this study was aimed to determine the role of misoprostol (Prostaglandin E1 agonist) in oleic acid (OA)-induced ARDS in rats. Anaesthetized animals were divided in to three groups. In group I (control group), rats were treated with saline. In group II (OA only group), OA was injected to induce ARDS. In group III (OA + M group), misoprostol was administered after OA injection and the dose was repeated after every 20 min. In all groups respiratory frequency (RF), pulmonary water content, mean arterial pressure (MAP), heart rate (HR), P/F ratio, and survival time were determined. OA produced ARDS as indicated by ventilatory changes, decreased P/F ratio, pulmonary edema, and death within 90 min. Severe changes in heart rate and mean arterial pressure were also observed. Misoprostol post-treatment prevented pulmonary edema and initial tachypnea and also improved P/F ratio in OA-induced ARDS but overall survival time was not altered. The MAP changes were reversed initially but not the heart rate changes. The results indicate that misoprostol post treatment ameliorates the OA-induced ARDS in the initial phase but can not prevent the lethality.

Keywords: ARDS, Hypoxemia, Pulmonary edema, Oleic acid, Misoprostol

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INTRODUCTION

Adult respiratory distress syndrome (ARDS) is a major cause of morbidity and mortality. The mortality rate is still very high (27-48%) in ARDS despite better understanding of the pathophysiology [1, 2]. Although many treatment strategies have been proposed no single therapy or maneuver has improved the outcome or mortality in ARDS. ARDS is an acute condition characterized by pulmonary edema, hypoxemia and decreased compliance of the lungs leading to respiratory failure. According to the American Thoracic Society, the features of ARDS in experimental animal models include acute onset, histological evidence of lung injury, increased permeability of the alveolar capillary barrier, pulmonary edema, inflammatory response and hypoxemia [3]. Earlier, we have reported OA model of ARDS in rats which exhibited all these characteristic features of ARDS as per ATS guidelines [4]. Earlier studies indicate that prostaglandins play an important role in the pathphysiology of ARDS [5]. Prostaglandins (PGs) are important mediators of inflammation in various diseases. In animal models of acute lung injury, intravenous PGE1 has demonstrated an anti inflammatory response. In addition, PGE1 is also reported to decrease pulmonary capillary leak and increase oxygen extraction capabilities [6, 7]. So, we hypothesized that PGE1 may have a beneficial role in reversing ARDS. Therefore, in the present study we investigated the role of prostaglandin E1 analogue (misoprostol) in OA- induced ARDS in rats.

MATERIALS AND METHODS

Animals, anesthesia and recording procedure

Approval from the ethical clearance committee of the Institute of Medical Sciences, Banaras Hindu University, Varanasi, India, was obtained before the commencement of animal experiments. Adult male albino rats of Charles-Foster strain weighing 175-225 g were selected for experiments. Urethane (Sigma Aldrich Inc; St Louis; USA; 1.5 g/kg body weight i.p) was used to anaesthetize the animal. Additional bolus dose of urethane (0.1-0.15 g/kg i.p.) was injected if required. Tracheal cannulation was done to keep the respiratory tract patent; jugular venous cannulation was done for drug administration; and carotid artery cannulation was done for recording blood pressure via pressure transducer. The blood pressure was computed to determine the mean arterial pressure (MAP). The skin over the xiphisternum was secured with the help of a thread and was attached to a force displacement transducer. The respiratory movements were recorded on a chart recorder via a bridge amplifier and respiratory frequency (RF) was computed from these recordings. The electrocardiographic potentials were recorded by needle electrodes using limb lead II configuration. Heart rate (HR) was calculated from R-R interval.

Experimental Protocol

The animals were divided in to 3 groups. In all groups, the animals were allowed to stabilize for 30 min after dissection and cannulation. In group I (saline group, n= 4), after stabilization of animals, initial recordings of respiratory movements, ECG and arterial blood pressure were taken and then saline (75 µL i.v.) was injected. Subsequent recordings were taken continuously for 5 min and then at the interval of 15 min till the end of observation period (120). This group served as time-matched control group. In group II (OA only group, n= 5), after initial recordings, each animal received OA (75 µL i.v.) and Subsequent recordings were taken continuously for 5 min and then at the interval of 15 min till the end of observation period (120) or death of the animals. In group III (OA+M group, n= 4), after initial recordings oleic acid (OA 75 µl) was injected i.v. followed by immediate injection of misoprostol (6µg/kg i.v.) and subsequent recordings were taken as mentioned in group II. The dose of misoprostol was repeated at every 20 min interval as the half life of misoprostol is 20-40 min [8].

Determination of Pulmonary water content

The pulmonary water content was determined by physical method as described earlier [9]. In brief, at the end of each experiment the lungs were excised, weighed and dried to a constant weight in an electric oven (at 90º C for 48 h). The difference between wet weight and dry weight was calculated to determine the pulmonary water content.

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Determination of P/F ratio

Blood sample from Carotid artery was collected in a heparin-rinsed syringe 15 min after the injection of saline in group I and OA in group II and III. This blood sample was subjected to Roche OMNI C blood gas analyzer to determine P/F ratio as reported earlier [4].

Drugs and solutions

Urethane was obtained from Sigma Aldrich Inc, St Louis, USA and was injected i.p. in the dose of 1.5 g/kg body weight. OA was obtained from Hi Media Laboratories Pvt. Limited Mumbai, India and was injected i.v. (75 µL bolus). Misoprostol was obtained from Cipla Ltd., Sikkim and was used i.v. in the dose of 6 µg/kg.

Analysis of Data

The changes in RF, HR and MAP were expressed as % of initial. The data were pooled and 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 and P/F ratio in different groups. $P<0.05$ was considered significant.

RESULTS

Effect of saline on cardiopulmonary parameters

The basal RF, HR and MAP values in this group (n = 4) of animals were 70 ± 6 breaths/ min, 276 ± 3 beats/min and 78 ± 6 mm Hg, respectively (Fig 1, 2, 3). These values did not vary significantly throughout the observation period of 120 min (Fig 1, 2, 3). The P/F ratio in this group of animals was 464 ± 8.1mm Hg and pulmonary water content was 78 ± 0.2% (Table 1, Fig 4). All the animals in this group survived throughout the period of observation (120 min; Table 1). This group served as time-matched control group.

Effect of OA on cardiopulmonary parameters

The basal RF in this group of animals was similar to control group (78 ± 5 breaths/min). After OA administration the RF increased and by 60 min it was about 2 times that of initial value followed by decrease leading to respiratory arrest and death of the animals around 90 min (Fig 1).

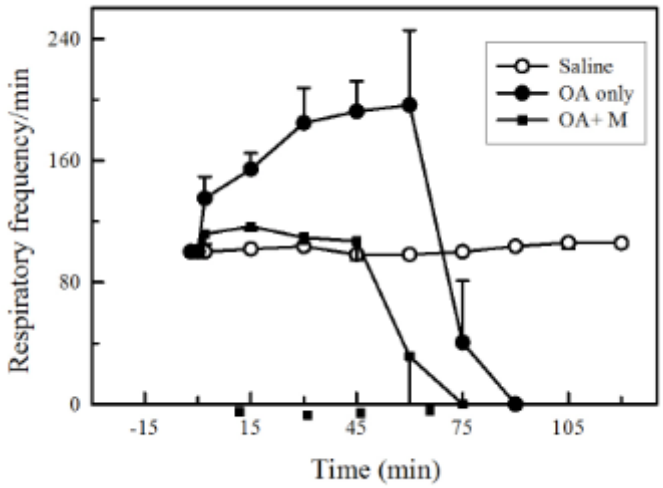

Fig 1: Effect of OA (75 µL) on respiratory frequency (RF) in rats with or without misoprostol treatment. Each point depicts mean ± SEM values obtained from 4-5 experiments in each group. Saline/ OA was injected at '0' time. The changes seen in RF in M + OA group are significantly different from OA only group ($P<0.05$; Two way Anova). Saline = saline control group; OA only = oleic acid treated group; M + OA = misoprostol treatment after oleic acid injection. Time interval of repeat dose of misoprostol has been marked with (■).

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The basal HR and MAP in this group of animals was 261 ± 25 beats/ min and 76 ± 9 mm Hg respectively. Injection of OA (75 µl) produced an immediate fall in HR and MAP followed by some recovery and then a progressive fall and by 90 min the heart stopped (Fig 2, 3).

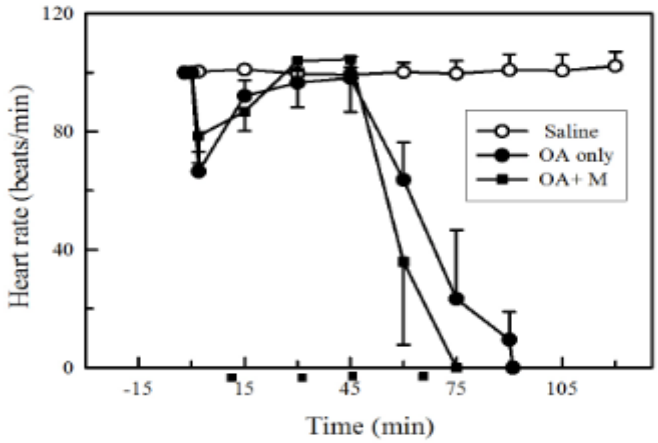

Fig 2: Effect of OA (75 µL) on heart rate (HR) in rats with or without misoprostol treatment. Each point depicts mean ± SEM values obtained from 4-5 experiments in each group. Saline/ OA was injected at '0' time. The changes seen in HR in M + OA group are not significantly different from OA only group (P> 0.05; Two way Anova). Saline = saline control group; OA only = oleic acid treated group; M + OA = misoprostol treatment after oleic acid injection. Time interval of repeat dose of misoprostol has been marked with (■).

The P/F ratio in this group of animals was 186 ± 3 mm Hg (Table 1). This value was significantly different from the control group (464 ± 8 mm Hg, Table 1). It was found that the pulmonary water content in this group was 85 ± 0.3% indicating significant increase as compared to control group (78 ± 0.2%, Table 1, Fig 4)). The mean ± SEM of survival time in this group of animals was 70 ± 8 min (Table 1)

Effect of misoprostol on cardiopulmonary parameters in OA-induced ARDS

The basal RF in this group of animals was similar to control group (76 ± 3 breaths/min). Initial profound tachypnea as observed in OA only group was not seen after misoprostol treatment in animals injected with OA. The RF remained at the basal value (76 ± 3 breaths/min) and was maintained at that level up to 45 min. After 45 min there was progressive fall in the RF and it became '0' by around 75 min (Fig 1).

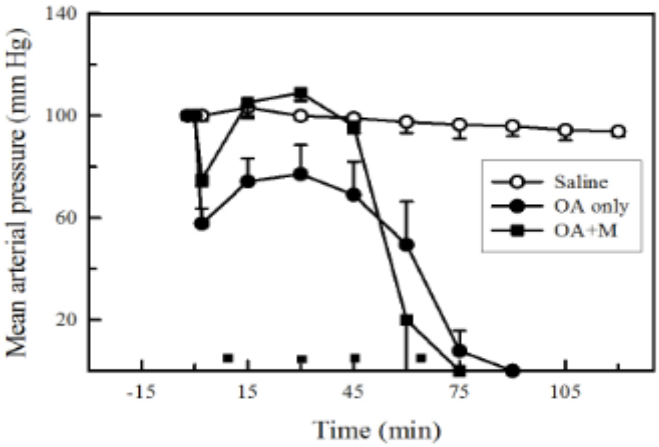

Fig 3: Effect of OA (75 µL) on mean arterial pressure (MAP) in rats with or without misoprostol treatment. Each point depicts mean ± SEM values obtained from 4-5 experiments in each group. Saline/ OA was injected at '0' time. The changes seen in MAP in M + OA group are significantly different from OA only group in the initial phase (P <0.05; Two way Anova) but not in the later phase. Saline = saline control group; OA only = oleic acid treated group; M + OA = misoprostol treatment after oleic acid injection. Time interval of repeat dose of misoprostol has been marked (■).

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The basal HR and MAP in this group of animals was 292 ± 28 beats/ min and 79 ± 4 mm Hg respectively. After immediate fall (within 2 min), the HR returned back to basal level within 15 min and was maintained at that level till 45 min. Following this there was progressive fall in HR and by 75 min, HR became '0' (Fig 2). MAP followed the pattern similar to HR (fig 3).

The P/F ratio in this group of animals was 249 ± 4.2 mm Hg. This value was significantly greater than P/ F value of OA only group but lesser than OA only group (Table 1).

Table 1: The mean ± SEM values of survival time, P/F ratio and pulmonary water content in various groups.

Group	Survival time (min)	P/F ratio	Pulmonary water conten
I (Control; n=4)	>120	464 ± 8.1	78 ± 0.2
II (OA only; n=5)	70 ± 8	186 ± 3	85 ± 0.3
III (OA+M; n=4)	61 ± 8	249 ± 4.2	82 ± 0.3

Survival time in group II (OA only) and group III (OA+M) are significantly different as compared to group I (control). P/F ratio in group II (OA only) is significantly decreased as compared to group I (control). In group III, P/F ratio is increased as compared to group II (OA only) but decreased as compared to group I (control). Pulmonary water content is increased in group II (OA only) as compared to group I (control). In group III, pulmonary water content is decreased as compared to group II (OA only) but increased as compared to group I (control). $P < 0.05$ is considered significant (Student's *t* test for unpaired observations).

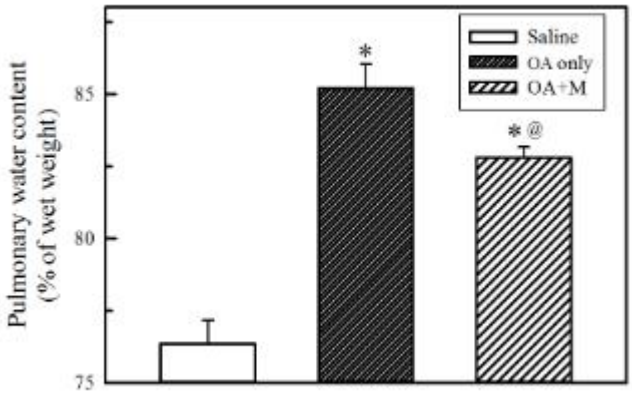

Fig 4: Effect of OA (75 µL) on pulmonary water content in rats with or without misoprostol treatment. Each bar depicts the mean ± SEM values obtained from 4-5 experiments in each group. The (*) indicates significant difference of OA only group and M + OA group from saline group (P< 0.05; Student's t test for unpaired observations). The (∞) indicates significant difference of M + OA group from OA only group. Saline = saline control group; OA only = oleic acid treated group; M + OA = misoprostol treatement after oleic acid.

Pulmonary water content in this group of animals was 82 ± 0.3%. This value was significantly greater than control group (78 ± 0.2) but was significantly less than OA only group (85 ± 0.3%, Table 1, Fig 4). The mean survival time for this group was about 61 ± 8 min (Table 1).

DISCUSSION

Results of the present study show that OA produced ARDS in rats as reported earlier [4]. Further misoprostol post-treatment prevented early deterioration of cardio respiratory parameters in OA-induced ARDS but could not alter the survival time.

ARDS is an inflammatory condition characterized by pulmonary edema, atelectasis of lungs, hypoxemia and infiltration of lungs by inflammatory cells [4, 10]. In this study also we observed the development of pulmonary edema and hypoxemia as indicated by tachypnea followed by bradypnea and finally respiratory failure leading to death of the animals within 90 min (Fig 1) as reported earlier [4]. In

addition P/F ratio was also significantly decreased as compared with control group (P< 0.05, Students t test for unpaired observation; Table 1).

ARDS is associated with inflammation and various inflammatory mediators are implicated in the pathophysiology of ARDS. Prostaglandin is one of the inflammatory mediator but its role in ARDS is still not clear [5]. In some studies it has been reported that infusion of prostaglandin E1 (PGE1) reduced lung water, pulmonary micro-vascular permeability and sequestration of neutrophils within the pulmonary microvasculature in lung injury associated with pancreatitis [11]. Further pulmonary hypertension is reduced by PGE1 at the cost of deterioration of pulmonary gas exchange [12]. PGE1 is also reported to increase oxygen extraction capabilities in the setting of reduced oxygen delivery [7]. In our study also misoprostol (PGE1 agonist) post-treatment decreased the pulmonary edema and improved the oxygenation as indicated by increased P/F ratio and absence of tachypnic response in the initial phase in OA-induced ARDS (fig 4, Table 1). These findings favor the beneficial effects of misoprostol in ARDS possibly operating through the above mentioned mechanisms. Along with the improvement in the respiratory parameters, cardiovascular parameters (HR and MAP) were also maintained in the intial phase.

Prostaglandin one of the mediators in many inflammatory diseases has anti inflammatory role as well [13]. It can modulate neutrophil function and inhibit the release of chemical mediators also [14]. This may explain its efficacy in preventing early deterioration of cardiopulmonary parameters in OA-induced ARDS in this study. However, we found deterioration in various cardiopulmonary parameters in the later phase resulting in lethality indicating that prostaglandins are not effective in the later phase of ARDS. It suggests involvement of factors other than prostaglandin which dominate over the beneficial effect of misoprostol causing deterioration in the later phase.

CONCLUSION

Prostaglandin may be beneficial in early phase of ARDS. Other factors are also involved in the pathophysiology of ARDS in addition to prostaglandins and may deteriorate the condition of animals in later phase. Further research is required to identify these factors.

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