

## Cardiovascular and Blood Gas Responses to Ketanserin in Canine Pulmonary Edema Induced by Oleic Acid

S. Koyama, M.D.,\* S. Kiyono, M.D.,† K. Kayaba, M.D.,‡ M. Kimura, M.D.,§ M. Nishizawa, M.D.¶

This study was performed to determine the cardiovascular and respiratory effects of ketanserin, a specific 5-HT<sub>2</sub> antagonist, following oleic acid lung injury in anesthetized dogs. Following intravenous administration of oleic acid (0.1 ml/kg) to a control group (N = 7), systemic blood pressure decreased significantly. This lowered level of systemic blood pressure was maintained throughout the experiment. Cardiac output gradually decreased following oleic acid administration, while total peripheral resistance, pulmonary vascular resistance, and pulmonary arterial pressure were increased significantly. In a group treated with intravenous ketanserin (0.16 mg/kg, N = 7) 60 min after the injection of oleic acid, no decrease in cardiac output was seen. The increased total peripheral resistance, pulmonary vascular resistance, and pulmonary arterial pressure following injection of oleic acid also were returned toward preoleic acid levels. However systemic blood pressure showed no significant improvement after treatment with ketanserin nor did ketanserin protect against progressive hypoxemia following pulmonary injury with oleic acid. A progressive increase in hemoglobin concentration was seen after oleic acid in the control group. This recovered toward the preoleic acid level following treatment with ketanserin. The postmortem lung wet-dry weight ratio was significantly lower in the treated group compared with the control group. In conclusion, these data suggest that serotonin may have a role in including cardiopulmonary hemodynamic disturbances and in producing increases in extravascular lung water when pulmonary edema is induced by oleic acid injection in anesthetized dogs. (Key words: Antagonists, miscellaneous: ketanserin-serotonin. Hypoxia. Lung: pulmonary edema.)

PULMONARY EDEMA in humans commonly is associated with acute circulatory failure. It has been suggested that pulmonary edema may develop either because of an increase in vascular hydrostatic pressure in the nonobstructed portion of the lung or because of an increase

in pulmonary vascular permeability<sup>1-3</sup> or both mechanisms. Increased pulmonary vascular permeability following pulmonary microembolization may result from release of humoral factors such as histamine and serotonin.<sup>1,4</sup> It has been reported that serotonin increases pulmonary vascular resistance by causing small pulmonary veins to constrict leading to pulmonary edema.<sup>5-7</sup> If serotonin is involved in the circulatory disturbances associated with pulmonary edema, a selective antagonist for serotonin should improve hemodynamic perturbations accompanying the pulmonary edema. However, the lack of specific antagonists has hampered the precise delineation of the role of serotonin (5-HT) in the circulatory disturbances. Recently, ketanserin (R41 468) was introduced as a selective 5-HT<sub>2</sub> antagonist at receptors in vascular and bronchial smooth muscle.<sup>8-10</sup> To determine if serotonin is involved in the development of acute circulatory failure following pulmonary edema, we studied cardiovascular and blood gas responses to ketanserin in canine lung injury induced by oleic acid.

### Methods

Fourteen adult mongrel dogs, 7-12 kg, were anesthetized with sodium pentobarbital (25 mg/kg) intravenously. Artificial respiration was carried out with room air using a Harvard respirator. Ventilation rate (12-18 cycles/min) and tidal volume (20-35 ml/kg) were adjusted to maintain arterial blood pH and PaO<sub>2</sub> within physiologic ranges. The animals were paralyzed with 0.2 mg/kg of pancuronium bromide. Lactated Ringer's solution was infused at a rate of 5 ml·kg<sup>-1</sup>·h<sup>-1</sup> by means of a Harvard infusion pump for maintaining hydration. Body temperature was measured by a rectal probe and maintained at 37 ± 1° C by use of a heating lamp. A polyethylene tube was placed in the lower abdominal aorta through a femoral artery for measurement of systemic blood pressure. Heart rate was measured by a cardiometer triggered by lead II of the electrocardiogram. Cardiac output (CO) was measured by thermal dilution from a flow-directed #7 F Swan-Ganz® catheter, with the thermister tip in the main pulmonary artery, with the use of a cardiac output computer (Nihonkoden, Japan). A volume of 3 ml of physiologic saline at room temperature was injected into

\* Associate Professor, Department of Physiology, Kagawa Medical College.

† Professor and Chairman, Department of Anesthesiology, Shinshu University School of Medicine.

‡ Associate Professor, Department of Anesthesiology, Shinshu University School of Medicine.

§ Instructor, Department of Anesthesiology, Shinshu University School of Medicine.

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Address reprint requests to Dr. Kiyono: Department of Anesthesiology, Shinshu University School of Medicine, 3-1-1 Asahi-machi, Matsumoto, Nagano 390, Japan.

TABLE 1. Baseline Values for Each Cardiovascular Variable before and 60 min after Oleic Acid Administration

	Before Oleic Acid		60 Min after Oleic Acid	
	Group 1	Group 2	Group 1	Group 2
MBP (mmHg)	139 ± 11	137 ± 12	109 ± 11*	104 ± 12*
HR (beats/min)	176 ± 9	182 ± 8	162 ± 7	168 ± 9
CO (l/min)	1.95 ± 0.23	1.98 ± 0.24	1.45 ± 0.25*	1.36 ± 0.22*
TPR (dyn · s · cm <sup>-5</sup> )	4,584 ± 644	4,627 ± 1,577	9,368 ± 1,720*	8,727 ± 1,470*
PAP (mmHg)	14.2 ± 1.2	14.8 ± 0.7	17.4 ± 1.5*	17.4 ± 1.0*
PVR (dyn · s · cm <sup>-5</sup> )	306 ± 83	390 ± 126	552 ± 109*	704 ± 129*
PCWP (mmHg)	8.6 ± 1.0	8.5 ± 0.5	8.4 ± 0.9	8.9 ± 1.2

Values are mean ± SE. Group 1 = control group; Group 2 = treated group. MBP (mean blood pressure); HR (heart rate); CO (cardiac output); TPR (total peripheral resistance); PAP (pulmonary arterial

pressure); PVR (pulmonary vascular resistance); and PCWP (pulmonary capillary wedge pressure). The asterisk (\*) indicates statistical significance ( $P < 0.05$ ) as compared with each preoleic acid level.

the right atrium. CO was measured in duplicate and the averaged used. Thermodilution curves were checked periodically to confirm exponential form. Pulmonary arterial pressure (PAP) and central venous pressure (CVP) were measured via the Swan-Ganz® catheter. Pulmonary capillary wedge pressure (PCWP) was measured after inflation of the balloon on the catheter tip. Total peripheral resistance (TPR) and pulmonary vascular resistance (PVR) were calculated using the following formulae:

$$\text{TPR (dyn} \cdot \text{s} \cdot \text{cm}^{-5}) = (\text{MBP} - \text{CVP}) \times 79.9 / \text{CO}$$

$$\text{PVR (dyn} \cdot \text{s} \cdot \text{cm}^{-5}) = (\text{PAP} - \text{PCWP}) \times 79.9 / \text{CO}$$

Arterial blood samples were drawn before and at 30-min intervals after the injection of oleic acid and analyzed (ABL-II) for PaO<sub>2</sub>, PaCO<sub>2</sub>, and pH at 37° C. The metabolic component was calculated automatically by ABL-II as base excess (BE) from blood pH and PaCO<sub>2</sub> with correction for hemoglobin concentration and O<sub>2</sub> saturation. All animals were given 0.1 ml/kg of oleic acid (Wako-Junyaku, Japan) by bolus injection into the right atrium. Sixty minutes after the injection of oleic acid, the treated group (seven dogs) received 0.16 mg/kg of ketanserin intravenously. Control animals (seven dogs) received 2 ml of physiologic saline as placebo. Postmortem examination was performed immediately after the experiment. Sections of both right and left lungs were fixed in 10% formalin solution and processed for routine light microscopy using hematoxylin and eosin staining. The major pulmonary vessels were drained of blood. Excised samples of the lung were weighed wet and then dried in an oven at a temperature of 80° C for determining lung wet-dry weight ratio as an index of extravascular lung water. All values are reported as the mean ± standard error. Statistical comparisons between control values before and values after treatment or placebo were made using Student's *t* test for paired data. When multiple comparisons were made

within groups, a one-way analysis of variance was performed. Results from the time course studies were compared using two-way analysis of variance for repeated measurements. A *P* value of 0.05 or less was considered statistically significant.

## Results

Table 1 shows values for each cardiovascular variable before and 60 min after oleic acid in both groups. Values before oleic acid did not differ significantly between groups. Following the oleic acid, mean blood pressure (MBP) and cardiac output (CO) decreased significantly, while pulmonary arterial pressure (PAP), total peripheral resistance (TPR), and pulmonary vascular resistance (PVR) were increased significantly. There were no significant differences at 60 min after oleic acid between groups.

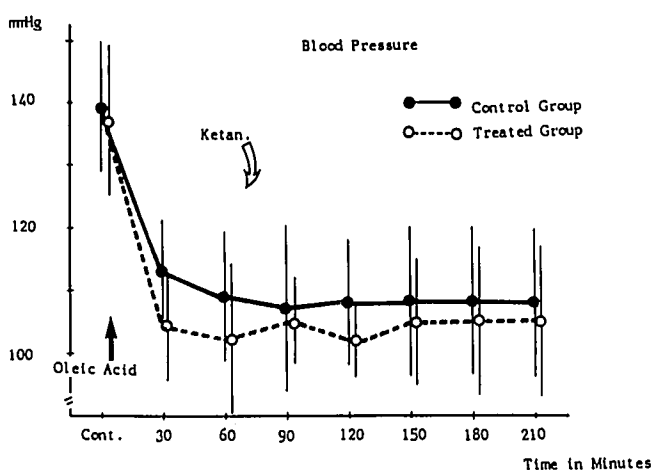


FIG. 1. Time course of changes in mean blood pressure following oleic acid and ketanserin. Each value is the mean ± standard error. Control group is shown in solid circle connected with solid line, and treated group is open circle with a dashed line. The arrow (a) indicates the injection point of oleic acid and the arrow (b) that of ketanserin.

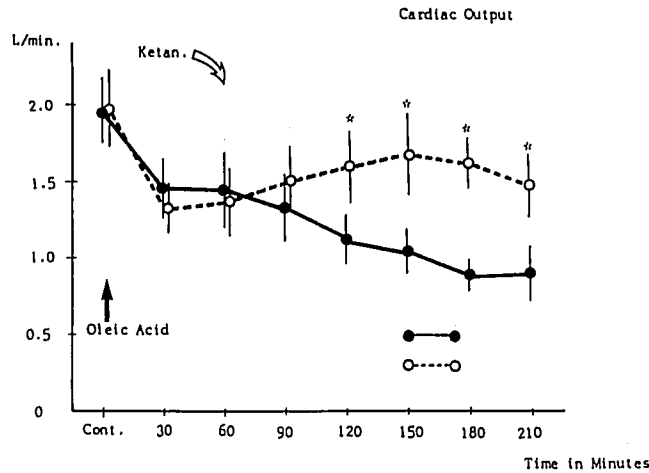


FIG. 2. Time course of changes in cardiac output following oleic acid and ketanserin. For further explanations of the figure see legend of figure 1.

The time course of changes in MBP after ketanserin and placebo are demonstrated in figure 1. Following placebo injection, 60 min postoleic acid, hypotension was maintained for the duration of the experiment without any significant differences from the MBP at 60 min after injection of oleic acid. There was no significant difference between the time course of changes in MBP in both groups. In the control group given the placebo, CO continued to decrease, reaching  $0.90 \pm 0.15$  l/min at 210 min postoleic acid (fig. 2). Ketanserin given 60 min postoleic acid reversed this trend. Following ketanserin, CO recovered toward the preoleic acid level, so that the level of CO at 120 min postoleic acid in the treated group was significantly greater than that in the control group. This effect was maintained throughout the experiment. Following oleic acid, TPR in the control

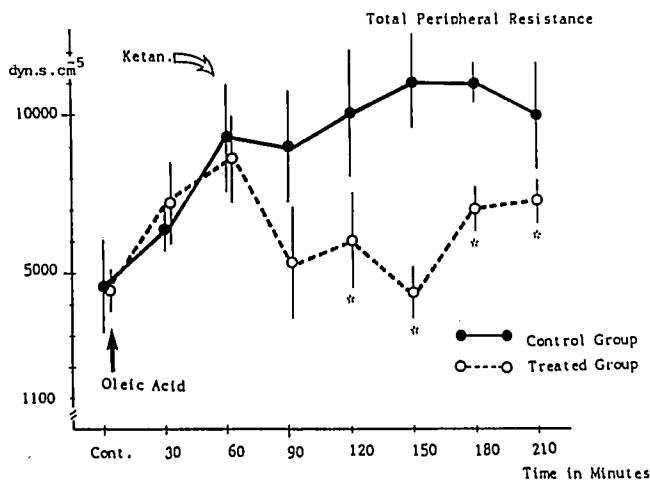


FIG. 3. Time course of changes in total peripheral resistance following oleic acid and ketanserin. For further explanations of the figure see legend of figure 1.

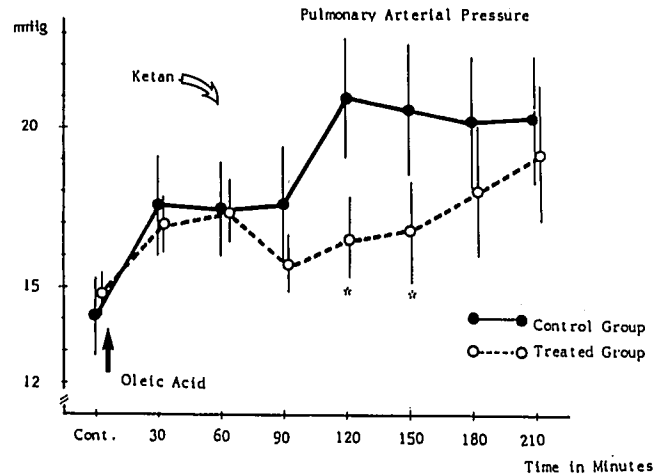


FIG. 4. Time course of changes in pulmonary arterial pressure following oleic acid and ketanserin. For further explanations of the figure see legend of figure 1.

group increased significantly, reaching  $10,800 \pm 1,780$  dyn  $\cdot$  s  $\cdot$  cm<sup>-5</sup> at 210 min postoleic acid. Ketanserin given at 60 min postoleic acid significantly reduced the TPR in parallel with the increase in CO (fig. 3), so that 210 min after the injection of oleic acid TPR was  $7,233 \pm 725$  dyn  $\cdot$  s  $\cdot$  cm<sup>-5</sup>. As shown in figures 4 and 5, PAP and PVR in the control group gradually increased following oleic acid so that 120 min postoleic acid PAP and PVR were  $21 \pm 2$  mmHg and  $913 \pm 190$  dyn  $\cdot$  s  $\cdot$  cm<sup>-5</sup>, respectively. These levels of PAP and PVR were significantly greater than the preoleic acid levels and were maintained until the end of experiment (210 min postoleic acid). In the treated group, ketanserin prevented the progressive increase in PAP induced by oleic acid, so that PAP in the treated group were significantly less than in the control group (fig. 4). Following ketan-

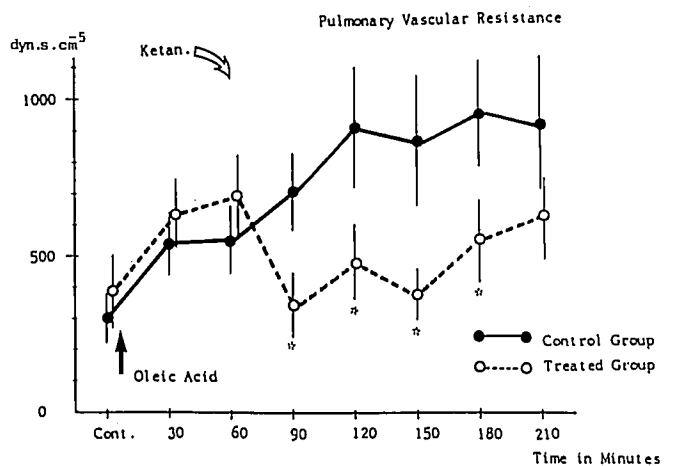


FIG. 5. Time course of changes in pulmonary vascular resistance following oleic acid and ketanserin. For further explanations of the figure see legend of figure 1.

serin, PVR significantly decreased, reaching  $331 \pm 101$  dyn  $\cdot$  s  $\cdot$  cm<sup>-5</sup> 90 min postoleic acid (30 min after ketanserin). There followed a gradual return toward the preoleic acid levels of PVR. However, there were significant differences between the levels of PVR in both groups at each time interval from 90 min to 180 min postoleic acid (from 30 min to 120 min after ketanserin) (fig. 5). Heart rate was not altered significantly even when ketanserin was given after the oleic acid. There also were no significant alterations in central venous pressure (CVP) and pulmonary capillary wedge pressure (PCWP) throughout the experiment in either group.

Table 2 shows changes in arterial gas tension before and after oleic acid in the control group and in the treated group. Following oleic acid, arterial pH, PaCO<sub>2</sub>, and BE decreased progressively. There were no significant differences between the time course of changes in each variable between groups. Arterial PaCO<sub>2</sub> in the control group tended to increase after oleic acid but did not differ significantly from the preoleic acid value nor from the treated group. Hemoglobin (Hb) concentration gradually increased after oleic acid, from  $12.2 \pm 1.0$  g/dl to  $19.1 \pm 1.4$  g/dl 210 min postoleic acid in the control group. However, in the treated group, ketanserin prevented this increase in Hb concentration.

Gross appearance of the lung after the completion of the experiment showed that the trachea and main bronchi usually contained large amounts of bloody serosanguinous fluid in the control group and moderate amounts in the treated group. No gross thrombus was found in the pulmonary arteries in either group. Microscopically, arterioles generally were filled with erythrocytes. Occasionally microscopic thrombi were found in smaller vessels. Interstitial edema and congestion were common features. Intraalveolar hemorrhage and exudates were found in varying degrees. The lung wet-dry weight ratios at the postmortem examination were significantly different between the groups (control group ( $6.28 \pm 0.12$ ) and treated group ( $5.41 \pm 0.11$ )).

### Discussion

This study demonstrated that ketanserin, a specific 5-HT<sub>2</sub> receptor-blocking agent, is effective in causing improvement of cardiovascular disturbances following pulmonary edema due to oleic acid. Ketanserin (0.16 mg/kg) prevented progression or reversed the reduction of cardiac output and the increases in TPR, PVR, and PAP seen following oleic acid in control animals. The postmortem lung wet-dry weight ratio was decreased significantly in the treated group. However, ketanserin did not protect against progressive deterioration of arterial gas tensions and arterial pH.

Pulmonary injury due to oleic acid increased PVR

TABLE 2. Blood-Gas Responses to Oleic Acid in Control and Treated Group

Group	Before	After the Injection of Oleic Acid (h)							
		0.5	1.0	1.5	2.0	2.5	3.0	3.5	
pH	1	7.30 ± 0.16	7.23 ± 0.10	7.18 ± 0.13*	7.15 ± 0.10*	7.07 ± 0.07*	6.61 ± 0.05*	7.02 ± 0.06*	
	2	7.24 ± 0.06	7.24 ± 0.07	7.14 ± 0.05*	7.09 ± 0.08*	7.08 ± 0.10*	7.05 ± 0.06*	7.05 ± 0.06*	
PaO <sub>2</sub> (mmHg)	1	83 ± 15	80 ± 10*	71 ± 12*	66 ± 8*	58 ± 7*	54 ± 8*	51 ± 5*	
	2	75 ± 6*	68 ± 5*	63 ± 5*	56 ± 5*	53 ± 5*	54 ± 7*	50 ± 4*	
PaCO <sub>2</sub> (mmHg)	1	24 ± 8	29 ± 7	31 ± 10	33 ± 11	37 ± 14	39 ± 13	38 ± 14	
	2	26 ± 5	31 ± 2	33 ± 6	35 ± 9	37 ± 10	39 ± 12	40 ± 16	
BE (mEq/l)	1	-7.6 ± 0.7*	-10.5 ± 0.9*	-12.9 ± 0.8*	-13.3 ± 1.1*	-15.1 ± 0.6*	-14.9 ± 0.7*	-15.3 ± 1.9*	
	2	-8.4 ± 0.9*	-8.4 ± 0.9*	-12.1 ± 0.6*	-14.2 ± 0.8*	-14.9 ± 1.0*	-15.9 ± 1.3*	-15.3 ± 1.9*	
Hb (g/dl)	1	14.5 ± 0.8	14.9 ± 0.7*	15.9 ± 0.3*	16.5 ± 0.2*	18.0 ± 1.2*	18.3 ± 1.1*	19.1 ± 1.4*	
	2	14.8 ± 1.6	15.6 ± 1.6*	15.2 ± 1.5*	16.0 ± 1.3*	15.4 ± 1.4**†	14.5 ± 1.4**†	14.1 ± 0.8**†	

Values are mean ± SE. Group 1 = control group; Group 2 = treated group. The asterisk (\*) indicates statistical significance from control values within group ( $P \leq 0.05$ ); †from Group 1c.

and PAP, leading to an increase in net transvascular fluid filtration by the lung.<sup>11-14</sup> Increased pulmonary vascular permeability following pulmonary injury has been postulated to result from physical injury of the vascular wall or from release of humoral factors, such as serotonin, by oleic acid<sup>14-16</sup> or from both mechanisms. Experimental data from anesthetized dogs and isolated, perfused canine lungs in agreement with the present study, suggest that 5-HT (serotonin) causes an increase in PVR and leads to pulmonary edema.<sup>5-7,16</sup> Serotonin not only acts as a direct vasoconstrictor but also amplifies the vasoconstrictor responses to agents such as noradrenaline.<sup>10,16,17</sup> Serotonin is released by aggregating platelets.<sup>18-20</sup> A recent report indicated that ketanserin antagonizes not only the direct vasoconstrictor effect of serotonin but also amplifies the effects of other vasoactive substances.<sup>9</sup> Therefore, the results of this study support at least in part the view that serotonin may be involved in cardiovascular hemodynamic alterations associated with pulmonary edema induced by oleic acid. Another interesting finding of this study was that ketanserin prevented the progressive increase in hemoglobin concentration and the increase in extravascular lung water following oleic acid. This effect may result from a decrease in net transvascular permeability due to the improvement of cardiopulmonary hemodynamic disturbances following pulmonary edema. However, the exact mechanism remains obscure.

Recently it was reported that ketanserin reversed changes in pulmonary perfusion pressure occurring in patients with the adult respiratory distress syndrome.<sup>7</sup> Furthermore, a favorable response to ketanserin has been reported for congestive heart failure.<sup>21</sup> Ketanserin produced a significant decrease in PAP, PCWP, and MBP; TPR and PVR also were decreased.<sup>21</sup> Cardiac output increased without any significant changes in heart rate. These cardiovascular effects of ketanserin are in agreement with the present data. However, the use of ketanserin in the present study did not increase the lowered blood pressure following pulmonary edema nor improve impaired gas exchange. The failure of improvement of arterial gas tension in the present study may be dependent on physical injury of pulmonary endothelium,<sup>13,14</sup> leading to disturbances in gas exchange. Further studies are required to determine whether this difference might be dependent on systemic cardiovascular conditions before treatment with ketanserin.

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