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Propofol attenuates oxidant-induced acute lung injury in an isolated perfused rabbit-lung model

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Abstract
Purpose. Reactive oxygen species have been strongly implicated in the pathogenesis of acute lung injury (ALI). Some animal studies suggest that free radical scavengers inhibit the onset of oxidant-induced ALI. Propofol (2,6-diisopropylphenol) is chemically similar to phenol-based free radical scavengers such as the endogenous antioxidant vitamin E. Both in vivo and in vitro studies have suggested that propofol has antioxidant potential. We hypothesized that propofol may attenuate ALI by acting as a free-radical scavenger.
Methods. We investigated the effects of propofol on oxidant-induced ALI induced by purine and xanthine oxidase (XO), in isolated perfused rabbit lung, in two series of experiments. In series 1, we examined the relationship between the severity of ALI and the presence of hydrogen peroxide (H_2O_2). In series 2, we evaluated the effects of propofol on attenuating ALI and the dose dependence of these effects. The lungs were perfused for 90 min, and we evaluated the effects on the severity of ALI by monitoring the pulmonary capillary filtration coefficient (Kfc), pulmonary arterial pressure (Ppa), and the pulmonary capillary hydrostatic pressure (Ppc).
Results. In series 1, treatment with catalase (an H_2O_2 scavenger) prior to the addition of purine and XO resulted in complete prevention of ALI, suggesting that H_2O_2 may be involved closely in the pathogenesis of ALI. In series 2, pretreatment with propofol at concentrations in excess of 0.5 mM significantly inhibited the increases in the Kfc values, and that in excess of 0.75 mM significantly inhibited the increase in the Ppa values.
Conclusion. Propofol attenuates oxidant-induced ALI in an isolated perfused rabbit lung model, probably due to its antioxidant action.

Key words Propofol · Free-radical scavenger · ALI · Rabbit

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Introduction

Reactive oxygen species (ROS) are constantly formed under normal metabolic conditions in aerobic organisms. In addition, activated phagocytes produce ROS during the process of capturing and ingesting microorganisms and other pathogens (i.e., foreign proteins) invading our bodies. Many recent studies have indicated that ROS may contribute significantly to the pathogenesis of adult respiratory distress syndrome (ARDS) and acute lung injury (ALI) [1,2]. Propofol, as a free-radical scavenger, has been reported to exert antioxidant effects [3,4]. Recently, in vitro, in vivo, and clinical studies have revealed that propofol inhibits lipid peroxidation and possesses antioxidant potential [5–7].

Propofol, a useful drug in the field of anesthesia because of its excellent sedative and hypnotic properties, is now also used widely as a major sedative agent in intensive care units (ICUs). Because ALI and ARDS are treated in many ICUs, we may expect beneficial effects of propofol in the management of patients with ALI and ARDS. We therefore investigated the effects of propofol on an oxidant-induced ALI, induced by the addition of purine and xanthine oxidase (XO) to the blood-free perfusate of isolated perfused rabbit lung (IPRL) [8,9].

Materials and methods

IPRL preparation

The protocol conformed with the guidelines for the conduct of animal experiments issued by the Nagoya City University Graduate School of Medical Science and by the Japanese government (Law No. 105, notification No. 6), and was approved by the Ethics Committee on Animal Experiments of our institution. A modified version of a previously described method of preparation of IPRL was used [8].

Male Japanese white rabbits that weighed 2.5 to 3.5 kg were anesthetized by intramuscular injection of ketamine (85 mg·kg^{-1}) and intravenous injection of pentobarbital sodium (15–25 mg·kg^{-1}), and anticoagulated with intravenous heparin (300 units·kg^{-1}). Repeated bolus injections of pentobarbital (5 mg·kg^{-1}) were administered as required. Pancuronium bromide was administered (1 mg intravenously) after first confirming the depth of anesthesia by the absence of response to skin clamping, and a sternotomy was performed. Then, the lungs were mechanically ventilated, using a Mid-Range Animal Ventilator (Model 122-D; Nemi, Medway, MA, USA) with 100% O_2 at the rate of 20 breaths·min^{-1}, a tidal volume of 4 ml·kg^{-1}, and a positive end-expiratory pressure (PEEP) of 2 cm H_2O. The carotid artery was cannulated for arterial pressure measurements, with additional pentobarbital administered for any elevation greater than 10% from the baseline of the mean arterial pressure; the ribs were then retracted to expose the heart and lungs. The main pulmonary artery (PA) and the left atrium (LA) were cannulated via right and left ventriculostomy incisions, and the rabbits were killed by rapid bleeding. The lung was then ventilated with 45% O_2-5% CO_2-50% N_2 under the same ventilator settings as mentioned above, and the pulmonary circulation was perfused with Krebs-Henseleit solution containing 3% bovine serum albumin, 0.373 g $CaCl_2$ (to obtain a calcium ion concentration of about 1.2 mM), and 2.1 g $NaHCO_3$ at 37°C (pH 7.4), using a peristaltic pump (Minipuls 3; Gilson, Villiers-le-Bel, France). The pulmonary circulation was initially washed with 500 ml of perfusate at the rate of 20–40 ml·min^{-1} to reduce the red cell, neutrophil, and platelet counts in the subsequent recirculating perfusate to less than 3% of the normal counts in rabbit blood [9]. The total circuit volume of 500 ml was maintained at 37°C via a heated water bath (EL-15F Coolnit Bath; Taitec, Saitama, Japan). Perfusion was then interrupted for 5 min while the lungs and heart were dissected free and suspended by the trachea from a force displacement transducer (isometric transducer TB-652T; Nihon Kohden, Tokyo, Japan) that allowed continuous measurement of the lung weight gain (LWG). Residual atelectasis was reversed by brief hyperinflation, and ventilation was continued as mentioned before. The lungs were then perfused in a recirculating manner at a flow rate of 40 ml·kg^{-1}·min^{-1}. The pulmonary arterial pressure (Ppa) and left atrial pressure (Pla) were monitored continuously through side holes in the cannula, using pressure transducers (pressure monitoring system MK12030UW; Baxter, Irvine, CA, USA), and recorded on a polygraph (thermal array recorder WS-681G; Nihon Kohden). All pressures were referenced to the level in the LA. The height of the venous reservoir was adjusted to maintain the Pla at 2 mmHg. After about 10 min of stable perfusion, the pulmonary capillary hydrostatic pressure (Ppc), isogravimetric pressure (Piso), pulmonary capillary filtration coefficient (Kfc), and the static lung compliance (SLC) were measured.

Measurement of Ppc

Ppc was estimated by the double vascular occlusion method [8,10]. This method is based on the finding that pulmonary vascular compliance is located for the most part in the pulmonary capillary bed. For measurement of the Ppc, ventilation was discontinued, the PA and LA cannulae were occluded simultaneously, and the Ppc was measured as the mean of the PAP and LAP measured 3 s after the double occlusion. Using the value of Ppc, the pulmonary vascular resistance (Rp), divided into the precapillary (arterial, Ra) and postcapillary (venous, Rv) components, was calculated, so that Rp = Ra + Rv, Ra = (Ppa − Ppc)/Q, and Rv = (Ppc − Pla)/Q, where Q is the pulmonary flow.

Measurement of Piso and Kfc

Piso is the pulmonary capillary pressure at which the lung neither gains nor loses weight, where the Starling forces are balanced [11–13]. Piso was determined by discontinuing perfusion and opening a shunt between the two cannulae, so that the Ppa and Pla were equal. Pla was then altered in 1-mmHg increments by elevating the venous reservoir, and the effect on lung weight (LW) was examined. Piso was defined as the highest Pla over a 3-min period during which the lung did not gain weight.

Kfc was then calculated by a modified version of the method of Wakerlin et al. [8] and Drake et al. [11]. When the pulmonary capillary pressure is equal to Piso, the Starling forces are balanced, so that there is no net transfer of fluid across the pulmonary capillary membrane. After the determination of Piso, the Pla was increased to 7 mmHg over the Piso; at this time, other Starling forces were kept unchanged, so that edema occurred at the rate of 7 × Kfc. However the initial LWG is due to both an increase of the intravascular volume and edema formation. This rapid increase in LW is essentially completed within 3 min. Therefore, the rate of LWG was recorded on a semilog plot every min from 3 to 10 min, and extrapolated back to time 0 by linear regression.

Measurement of SLC

The SLC was determined after reversal of visible residual atelectasis by the application of PEEP. A fixed tidal volume (12 mg·kg^{-1}) was then injected, and

the plateau airway pressure was used to calculate the SLC.

Induction of ALI with purine and XO

After measurements of the Ppc, Piso, Kfc, and SLC, the lungs were perfused for 15 min to establish a stable baseline LW and Ppa. To establish oxidant-induced ALI, 0.5 mM purine (Sigma Chemical, St Louis, MO, USA) was added to the perfusate. About 5 min later, XO (2 mU·ml^{-1}; XO grade IV from milk; Sigma Chemical) was added to the perfusate of the specimens of the study groups [9,12,13]. The lung preparations were perfused for 90 min after the addition of XO to the perfusate. The Ppa, Pla, airway pressure, and LW were continuously monitored for 90 min after the addition of XO to the perfusate. The final Ppc, Piso, and Kfc were measured after perfusion had taken place for 90 min. If ALI was absent, physiological saline was administered at the same time as purine and XO as described above, and the same measurements were performed.

Measurement of wet/dry lung weight ratio (wet lung weight – dry lung weight)/dry lung weight) (W/D)

The left lung was excised after the end of the experiment, and the W/D was determined after sequential weighings demonstrated maximal dehydration of the lung specimens placed in a drying oven.

Definition of ΔKfc ratio and ΔPpa

We defined the value of (final Kfc – baseline Kfc)/baseline Kfc as the ΔKfc ratio, according to a previous report [13]. We defined the value of (final Ppa – baseline Ppa) as ΔPpa.

Series 1

To examine the relationship between the severity of ALI and the presence of H_2O_2 (hydrogen peroxide), the animals were divided at random into three groups: the control group (perfused for 90 min without administration of purine or XO; $n = 5$), the radical group (perfused for 90 min after treatment with purine and XO; $n = 5$), and the catalase group (given purine and XO after pretreatment with catalase [100 μg·ml^{-1}; from bovine liver, Wako Pure Chemical Industries, Tokyo, Japan], and subsequently perfused for 90 min; $n = 5$).

Series 2

To evaluate the effects of propofol on attenuating ALI and the dose dependence of these effects, the animals were divided into six groups: the lung-injury-free intralipid group (given no purine or XO and perfused with fluid containing 2.5 ml of 10% fat emulsion ([FE; Intralipid; Pharmacia AB, Stockholm, Sweden] $n = 8$), and five lung-injury groups, i.e., the P (0) group (given purine and XO and then perfused with fluid containing 2.5 ml of 10% FE; $n = 8$), the P (0.25) group (given purine and XO and perfused with fluid to which 2.5 ml of 10% FE containing 0.25 mM propofol had been added; $n = 8$), the P (0.5) group (given purine and XO and then perfused with fluid to which 2.5 ml of 10% FE containing 0.5 mM propofol had been added; $n = 8$), the P (0.75) group (given purine and XO and then perfused with fluid to which 2.5 ml of 10% FE containing 0.75 mM propofol had been added; $n = 8$), and the P (1) group (given purine and XO and then perfused with fluid to which 2.5 ml of 10% FE containing 1 mM propofol had been added; $n = 8$).

Statistical analysis

Values were expressed as means ± SD. Differences between two groups were examined by Mann-Whitney's *U*-test. Differences between the baseline and the final data were examined by Wilcoxon's signed-rank test. Kruskal-Wallis one-way analysis of variance (ANOVA) was used to compare multiple groups, and Scheffe's test was employed for the post-hoc test (Stat View 4.02; Abacus Concepts, Berkeley, CA, USA). A probability value of less than 0.05 was considered as denoting significance.

Results

Series 1

There were no significant differences in the rabbits' body weights or in the baseline Kfc, Ppa, and Rp among the three groups. The LWG, W/D, and the final Kfc, Ppa, and Rp values were significantly increased in the radical group as compared to the values measured in the control group; furthermore, the increases in the values of all these five parameters were significantly attenuated in the catalase group (Table 1).

The ΔKfc ratio was significantly increased in the radical group as compared to that in the control group, and this increase was significantly attenuated in the catalase group (Fig. 1A).

The ΔPpa increased by 2 mmHg, on average, in the radical group, and this increase was significantly attenuated in the catalase group (Fig. 1B).

Series 2

There were no significant differences in the rabbits' body weights, or in the baseline Kfc, peak inspiratory

Table 1. Variations in parameters of lung injury in each group (series 1)

	Control group ($n = 5$)	Radical group ($n = 5$)	Catalase group ($n = 5$)
Body weight (kg)	3.4 ± 0.2	3.3 ± 0.4	3.5 ± 0.5
Lung weight gain (g)	0.3 ± 0.6	19.3 ± 4.3*	0.9 ± 1.3**
Wet/dry lung weight ratio	5.0 ± 1.5	15.7 ± 5.7*	5.4 ± 1.4**
Kfc ($ml \cdot min^{-1} \cdot mmHg^{-1} \cdot 100\,g^{-1}$)			
Baseline	0.27 ± 0.13	0.29 ± 0.13	0.32 ± 0.22
Final	0.27 ± 0.32	1.33 ± 0.70*	0.35 ± 0.22**
Ppa (mmHg)			
Baseline	6.4 ± 1.9	6.5 ± 2.4	5.9 ± 2.2
Final	6.6 ± 1.3	8.5 ± 2.1*	6.0 ± 3.2**
Rp ($mmHg \cdot min \cdot l^{-1}$)			
Baseline	34.9 ± 11.4	35.8 ± 19.0	37.2 ± 23.6
Final	34.1 ± 11.3	55.5 ± 16.9*	36.9 ± 18.7**

* $P < 0.05$ vs control group; ** $P < 0.05$ vs radical group
Data values are presented as means ± SD
Kfc, pulmonary capillary filtration coefficient; Ppa, pulmonary arterial pressure; Rp, pulmonary vascular resistance

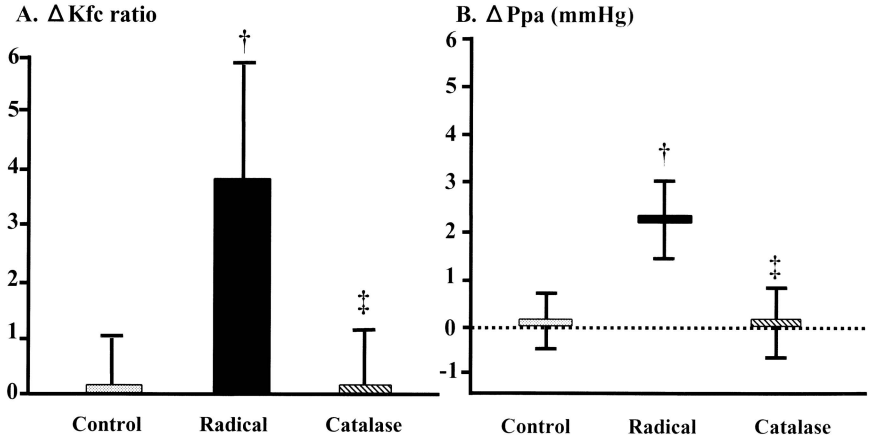

Fig. 1. Effects of catalase on oxidant-induced lung injury (series 1). **A** Δ Pulmonary capillary filration coefficient (*Kfc*) ratio; **B** Δ pulmonary arterial pressure (*Ppa*). Data values are presented as means ± SD. †$P < 0.05$ vs control group; ‡$P < 0.05$ vs radical group; *ΔKfc ratio*, (final Kfc – baseline Kfc)/baseline Kfc; *ΔPpa*, final Ppa – baseline Ppa

pressure (PIP), and SLC values among the groups (Table 2). The LWG and the W/D were increased significantly in all the lung-injury groups as compared to the values measured in the intralipid group. However, this increase in the LWG and the W/D was significantly suppressed in the P (0.5), P (0.75), and P (1) groups as compared to that in the P (0) group. The final Kfc was significantly increased in the P (0), P (0.25), P (0.5), and P (0.75) groups as compared to the value in the intralipid group, but there was no significant difference in the final Kfc between the Intralipid group and the P (1) group. The final Kfc values in the P (0.5), P (0.75), and P (1) groups were significantly lower than that in the P (0) group. The final PIP was significantly increased in the P (0), P (0.25), and P (0.5) groups as compared to the value in the intralipid group. The final PIP value in the P (1) group was significantly lower than that in the P (0) group. The final SLC was significantly decreased in the all lung-injury groups. The final SLC values in the P (0.5), P (0.75), and P (1) groups were significantly higher than that in the P (0) group.

There were no significant differences in any of the baseline pulmonary vascular parameters among the groups (Table 3). The final Ppa and Rp were significantly higher in the P (0) than in the intralipid group, and this increase in the values of the two parameters was significantly suppressed in the P (0.5), P (0.75), and P (1) groups as compared to that in the P (0) group. The final Ppa values were significantly increased as compared to the respective baseline values in the P (0), P (0.25), and P (0.5) groups. The final Rp values were significantly increased as compared to the respective baseline values in the P (0) and P (0.25) groups. There were no significant differences in the Ppc values between the baseline and the final measurements in any of the groups.

The values of the ΔKfc ratio were significantly increased in the P (0), P (0.25), P (0.5), and P (0.75) groups as compared to that in the intralipid group. These increases were suppressed partially but significantly in the P (0.5) and P (0.75) groups, as compared to that in the P (0) group. There was no significant differ-

Table 2. Variations in parameters of lung injury in each group (series 2)

	Intralipid ($n = 8$)	P (0) ($n = 8$)	P (0.25) ($n = 8$)	P (0.5) ($n = 8$)	P (0.75) ($n = 8$)	P (1) ($n = 8$)
Body weight (kg)	3.2 ± 0.5	3.1 ± 0.7	3.1 ± 0.2	3.5 ± 0.7	3.3 ± 0.4	3.2 ± 0.4
Lung weight gain (g)	0.7 ± 1.1	16.9 ± 4.8*	14.3 ± 3.4*	10.5 ± 1.8*;**	5.3 ± 2.9*;**	5.6 ± 2.9*;**
Wet/dry lung weight ratio	4.8 ± 1.7	14.5 ± 4.1*	12.6 ± 4.1*	9.4 ± 3.3*;**	5.9 ± 2.8*;**	7.5 ± 1.9*;**
Kfc ($ml \cdot min^{-1} \cdot mmHg^{-1} \cdot 100\,g^{-1}$)						
Baseline	0.25 ± 0.10	0.27 ± 0.14	0.24 ± 0.11	0.21 ± 0.06	0.29 ± 0.12	0.26 ± 0.09
Final	0.27 ± 0.22	1.21 ± 0.57*	1.21 ± 0.41*	0.68 ± 0.26*;**	0.45 ± 0.10*;**	0.38 ± 0.09**
Peak inspiratory pressure (mmHg)						
Baseline	4.3 ± 1.2	4.0 ± 0.7	4.4 ± 0.8	5.1 ± 1.1	4.0 ± 1.0	4.8 ± 1.4
Final	4.5 ± 1.3	6.3 ± 2.2*	6.4 ± 1.5*	6.5 ± 1.7*	5.0 ± 1.0	4.5 ± 1.1**
Static lung compliance ($ml^{-1} \cdot cmH_2O$)						
Baseline	5.9 ± 1.9	6.0 ± 2.1	5.8 ± 2.2	6.3 ± 1.9	6.1 ± 1.6	5.6 ± 1.3
Final	5.8 ± 0.9	2.5 ± 0.9*	2.4 ± 0.6*	4.0 ± 1.5*;**	4.0 ± 1.1*;**	4.5 ± 1.3*;**

* $P < 0.05$ vs intralipid group; ** $P < 0.05$ vs P (0) group
Data values are presented as means ± SD
Intralipid, group without radicals; P (0), group with no added propofol; P (0.25), P (0.5), P (0.75), P (1), groups with added 0.25, 0.5, 0.75, and 1 mM propofol, respectively
Kfc, pulmonary capillary filtration coefficient

Table 3. Variations in pulmonary vascular parameters in each group (series 2)

	Intralipid ($n = 8$)	P (0) ($n = 8$)	P (0.25) ($n = 8$)	P (0.5) ($n = 8$)	P (0.75) ($n = 8$)	P (1) ($n = 8$)
Ppa (mmHg)						
Baseline	6.3 ± 2.0	6.6 ± 2.4	5.9 ± 1.2	6.0 ± 1.1	5.2 ± 1.0	5.8 ± 1.1
Final	6.4 ± 1.5	8.2 ± 2.1*;***	7.4 ± 1.8***	6.6 ± 1.2**;***	5.1 ± 1.2**	5.8 ± 1.1**
Ppc (mmHg)						
Baseline	4.4 ± 1.7	4.4 ± 2.1	3.0 ± 0.5	3.5 ± 0.7	3.2 ± 0.6	4.1 ± 0.8
Final	4.0 ± 1.2	4.4 ± 1.6	3.3 ± 1.1	3.7 ± 0.6	3.2 ± 0.8	3.9 ± 0.9
Rp ($mmHg \cdot min \cdot l^{-1}$)						
Baseline	37.2 ± 10.3	38.1 ± 19.8	31.8 ± 10.3	33.0 ± 6.3	25.7 ± 8.1	31.3 ± 10.6
Final	38.2 ± 9.3	51.0 ± 17.0*;***	42.7 ± 14.1***	37.1 ± 9.2**	24.3 ± 11.0**	29.3 ± 8.7**
Ra ($mmHg \cdot min \cdot l^{-1}$)						
Baseline	16.8 ± 10.3	18.6 ± 8.9	23.5 ± 10.6	19.6 ± 10.8	15.9 ± 4.2	12.9 ± 6.4
Final	19.5 ± 10.2	30.2 ± 8.8*;***	31.1 ± 15.6***	24.5 ± 11.2	15.3 ± 7.6**	15.0 ± 6.7**
Rv ($mmHg \cdot min \cdot l^{-1}$)						
Baseline	20.4 ± 13.8	19.6 ± 18.0	11.5 ± 8.2	13.4 ± 8.5	9.8 ± 4.9	18.4 ± 8.9
Final	18.7 ± 8.1	19.5 ± 15.1	15.4 ± 11.5	12.7 ± 5.3	9.1 ± 6.6	14.3 ± 7.0

* $P < 0.05$ vs intralipid group; ** $P < 0.05$ vs P (0) group; *** $P < 0.05$ vs baseline value within a group
Data values are presented as means ± SD
Intralipid, group without radicals; P (0), group with no added propofol; P (0.25), P (0.5), P (0.75), P (1), groups with added 0.25, 0.5, 0.75, and 1 mM propofol, respectively
Ppa, pulmonary arterial pressure; Ppc, pulmonary capillary hydrostatic pressure; Rp, pulmonary vascular resistance; Ra, pulmonary precapillary (arterial) resistance; Rv, pulmonary postcapillary (venous) resistance

ence in the ΔKfc ratio between the intralipid group and the P (1) group (Fig. 2).

The values of ΔPpa increased by about 1.5 mmHg, on average, in the P (0) and P (0.25) groups, and by about 1 mmHg in the P (0.5) group, as compared to that in the intralipid group. The values of ΔPpa in the P (0.75) and P (1) groups were significantly lower than that in the P (0) group, and that in the P (1) group was significantly lower than that in the P (0.25) group (Fig. 3).

Discussion

XO activity is induced by stresses such as ischemia, hypoxia, and inflammation, and ROS are produced, which induce cellular injury leading to the formation, in particular, of vascular endothelial lesions [14]. Furthermore, numerous data indicate that XO may play an important role in the pathogenesis of ALI or ARDS [15,16]. Therefore, our isolated perfused ALI model,

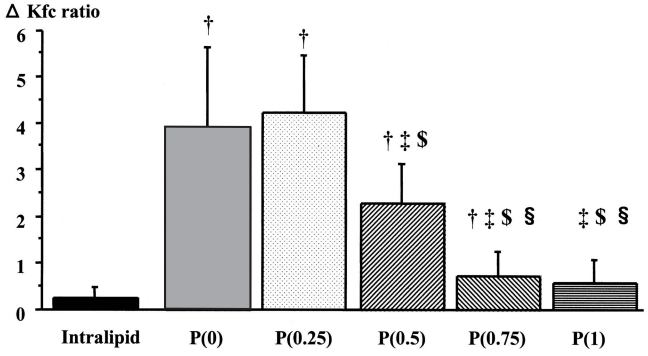

Fig. 2. ΔKfc ratios compared among the six groups (series 2). Data values are presented as means ± SD. *Intralipid*, group without radicals; *P (0)*, group with no added propofol; *P (0.25)*, *P (0.5)*, *P (0.75)*, *P (1)*, groups with added 0.25, 0.5, 0.75, and 1 mM propofol, respectively; $^{\dagger}P < 0.05$ vs intralipid group; $^{\ddagger}P < 0.05$ vs P (0) group; $^{\$}P < 0.05$ vs P (0.25) group; $^{\S}P < 0.05$ vs P (0.5) group. *ΔKfc* ratio, (final Kfc – baseline Kfc)/baseline Kfc

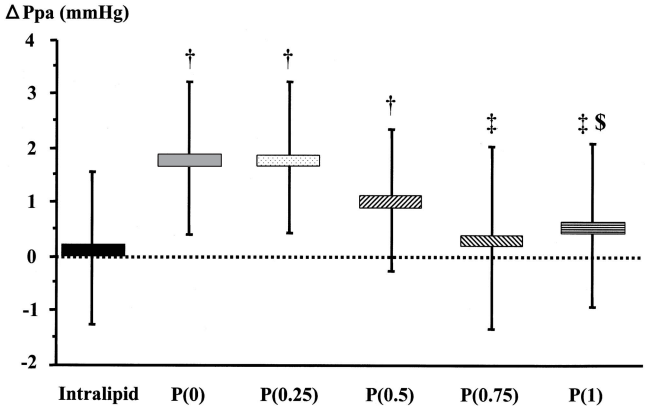

Fig. 3. ΔPpa values compared among the six groups (series 2). Data values are presented as means ± SD. *Intralipid*, group without radicals; *P (0)*, group with no added propofol; *P (0.25)*, *P (0.5)*, *P (0.75)*, *P (1)*, groups with added 0.25, 0.5, 0.75, and 1 mM propofol, respectively; $^{\dagger}P < 0.05$ vs intralipid group; $^{\ddagger}P < 0.05$ vs P (0) group; $^{\$}P < 0.05$ vs P (0.25) group. *ΔPpa*, final Ppa – baseline Ppa

established by the addition of purine and XO to the lung perfusate, may be representative of the pathogenesis of ALI in humans.

In our series 1 experiment, the ΔKfc ratio showed an approximately fourfold increase, and ΔPpa was increased by about 2 mmHg or more in the radical group compared with the control group. These results indicate that considerably severe ALI was induced during the 90-min observation period. Our oxidant-induced ALI model has been well established in many studies [9,12,13]. We also tried to carry out a histological examination. However, because the perfusate did not contain any blood cells, it was impossible to confirm pulmonary edema from the microscopic findings.

The increases in the the ΔKfc ratio and ΔPpa in the radical group were significantly attenuated in the catalase group. The ROS formed following treatment with purine and XO primarily assume the form of O_2^-, but H_2O_2 is also formed. In the absence of a substrate, the O_2^- is converted by dismutation to yield H_2O_2 and O_2. In neutral aqueous solutions, such as the perfusate used in this study, the O_2^- species disappear very soon. ALI was found to be completely prevented by treatment with catalase, which suggested that H_2O_2, rather than O_2^-, may be primarily involved in the development of ALI. H_2O_2 is a stable substance which does not directly exert any cytotoxic effects. In the presence of transition metals, OH^- is formed from H_2O_2. OH^- is a highly reactive radical and has potent cytotoxic actions. However, unlike H_2O_2, which can easily pass through the cell membrane, O_2^- and OH^- ions cannot pass through the cell membrane. Furthermore, the lifespan of OH^- in aqueous solutions is very short, and this substance shows little diffusion. Therefore, it seems likely that the H_2O_2 formed within the perfusate enters the cells where OH^- is formed, with resultant cell injury. Because catalase does not usually enter cells, the degradation of H_2O_2 present in the perfusate into H_2O and O_2 by its catalytic action probably prevented the development of ALI in this study.

In our series 2 experiment, pretreatment with high-dose propofol inhibited the increase in the ΔKfc ratio and the ΔPpa. Thus, it was clarified that propofol inhibited not only the increase in pulmonary capillary permeability, but also attenuated the increase of pulmonary vascular resistance. These results suggested that two possible factors; namely, enhanced pulmonary capillary permeability and an increase in the pulmonary vascular resistance, may have been responsible for the induction of ALI. However, we inferred that increased pulmonary vascular resistance was probably not involved, as there was no significant increase in the Ppc. In our experiment, pretreatment with a propofol concentration in excess of 0.5 mM significantly inhibited the increase in the ΔKfc ratio. However the increase in the ΔPpa was prevented significantly only with propofol concentrations in excess of 0.75 mM. From these results, we may speculate that pretreatment with 0.5 mM propofol inhibited pulmonary edema mainly by the suppression of pulmonary capillary permeability in our ALI model. Tate et al. [9] also reported that enhanced pulmonary capillary permeability, induced by purine and XO, caused ALI, even when an increase in the Ppa was inhibited by papaverine, a vasodilator. These findings suggest that enhanced pulmonary capillary permeability was probably responsible for the oxidant–induced ALI.

Propofol has been shown to act as a free-radical scavenger. Like DL-α-tocopherol (vitamin E), an endogenous antioxidant, propofol is considered to exert its radical scavenger activity by the release of a hydrogen atom from its hydroxyl group, which is then transferred to a free radical [3–7,17,18]. Many studies have shown that propofol and α-tocopherol could eliminate OH^-, but not O_2^- [4,19]. As stated earlier, ALI in our model seemed to be primarily attributable to intracellular OH^-. It would therefore be reasonable to assume that propofol, which serves as an OH^- scavenger, can alleviate ALI.

In our series 2 study, fat emulsion (FE) was added to the perfusate, because a high dose of propofol with its hydrophobic properties necessitates the use of a solvent. The propofol formulation actually includes a 10% FE as the solvent. The FE has been reported to mediate the production of prostanoids, which causes the elevation of pulmonary vascular resistance and is therefore associated with worsening of the lung injury [20,21]. But, because no changes in the Ppa, Ppc, or Rp were observed in the intralipid group, the FE was considered to have scarcely affected the pulmonary vascular resistance. Also, no changes were seen in the LW, W/D, Kfc, PIP, or SLC in the intralipid group, further validating the assumption that the FE did not worsen the lung injury.

The effective concentration of propofol in the perfusate was 0.5 mM. The dose of 0.5 mM is 20 times higher than the minimal effective blood concentration of propofol (25 μM) required to achieve adequate sedation in humans. However, we usually use about 2–3 $mg \cdot kg^{-1}$ of propofol for induction of anesthesia. The blood concentration of propofol may reach 20 times the maintenance concentration (namely, about 0.5 mM) temporarily during induction. The propofol concentration used in our study may therefore not be far from that used in clinical practice.

Blood-free perfusate containing 3% bovine serum albumin was used in our study. There were two reasons why we did not add blood to the perfusate. First, we wanted to observe the effects of propofol itself, ruling out the effects of blood cells. The second reason was that if we had used blood-containing perfusate, we may have needed huge quantities of purine and XO for the induction of oxidant-induced ALI. Erythrocytes contain high concentrations of superoxide dismutase, catalase, glutathione, and other enzymes and agents, which can efficiently degrade extracellular ROS. Therefore, in the clinical setting, where we infuse propofol into the bloodstream, even a clinical dose of propofol may be effective to prevent oxidant-induced lung injury in the presence of erythrocytes.

In fact, Tsuchiya et al. [22] reported that even a clinically used concentration of propofol showed antioxidant activities in the presence of erythrocytes. They also showed that the decrease in red blood cell count caused by oxidative injury after surgery was smaller in patients who received propofol anesthesia as compared with those given sevoflurane. Sayin et al. [23] reported that the clinical dose of propofol strongly attenuated lipid peroxidation, as assessed by the measurement of thiobarbituric acid reactive substances in atrial tissue samples, during coronary artery bypass grafting surgery.

We may conclude that propofol attenuates oxidant-induced ALI in an isolated perfused rabbit-lung model, probably due to its antioxidant action. Further study on the clinical significance of the antioxidant action of propofol is warranted.

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