

Effect of Platelet Activating Factor Antagonist (TCV-309) on Lung Injury in Dogs with Experimentally Endotoxin-Induced Shock

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ABSTRACT. The effect of TCV-309, a newly developed platelet activating factor (PAF) antagonist, on the wet/dry weight ratio of the lung (index of pulmonary edema) and the pulmonary surface activity (index of pulmonary compliance) was evaluated in comparison with that of CV-3988 (PAF-antagonist). Administration of TCV-309 (1 mg/kg) or CV-3988 (10 mg/kg) significantly reduced the wet/dry weight ratio which was increased by endotoxin administration (3 mg/kg). It also augmented the pulmonary surface activity. Administration of either TCV-309 or CV-3988 alleviated the histologic lesions caused by endotoxic shock. These results suggest that lung injury during endotoxic shock can be controlled by TCV-309 as by CV-3988.—**KEY WORDS:** endotoxic shock, lung injury, platelet activating factor antagonist.

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Lung injury occurring after endotoxic shock tends to induce acute respiratory failure and to develop into a condition called “adult respiratory distress syndrome (ARDS)” [1]. Active oxygen, lysosomal enzyme and chemical mediators such as platelet activating factor (PAF) are considered to be involved in the pathogenesis of endotoxic shock-induced lung injury [2, 7]. Our previous study has shown the rapid progression of lung injury during endotoxic shock [16] and the remission of injury following the administration of proteolytic enzyme inhibitors [14].

Of all factors involved, PAF in particular has attracted much attention. It is known that lung injury is induced in experimental animals by administration of PAF and is alleviated by treatment with PAF-antagonist, CV-3988 [3], during endotoxic shock [15]. TCV-309, a newly developed PAF-antagonist, has been estimated to have several hundred times as much antagonistic action against PAF as CV-3988 [17]. Its effect on the relief of endotoxic shock has been reported [17], but the therapeutic effect of TCV-309 on lung injury during endotoxic shock has not been studied.

In this study, we evaluated the effect of TCV-309 on lung injury in dogs with endotoxic shock in comparison with that of CV-3988, using the wet/dry weight ratio of the lung, pulmonary surface activity and histopathological evaluation as indices.

The experiment was performed basically by the method previously described [14]. Seventeen clinically healthy beagle dogs (ranging in age from 1 to 5 years and in weight from 8 to 14 kg) without filarial infection were used. The experiment was carried out under general anesthesia induced with sodium pentobarbital and under artificial ventilation. Endotoxin (3 mg/kg) (lipopolysaccharide; *Escherichia coli* 055: B5 Difco, Detroit, U.S.A.) was intravenously injected over a 5 min period. In the treatment groups, TCV-309 (n=5, 1 mg/kg) or CV-3988 (n=5, 10 mg/kg) was intravenously injected 10 min after the administration of endotoxin. In the control group

(n=7), physiological saline was similarly injected. TCV-309 and CV-3988 were supplied by Takeda Chemical Industries Ltd. (Osaka, Japan). The experimental dogs were sacrificed by euthanasia with a sufficient dose of sodium pentobarbital at 360 min after the administration of endotoxin. The wet/dry weight ratio and pulmonary surface activity were determined as early as possible after resection of the lobe.

The wet weight of the lung was measured after removing as much blood as possible from the resected lobe. The lung was then dried at 80°C for more than 48 hr in a hot-air oven to measure its dry weight. From the values obtained, the wet/dry weight ratio was calculated.

The lungs were removed and the right middle lobe of the lung was inflated with air to 40 cm H₂O. The pressure-volume relation in deflation was recorded. The pulmonary surface activities obtained from the deflation curve were expressed as Gruenward's stability index (SI) [8], Clements' expansion index (EI) [4] and percent lung volume (V_{p15cm}) at 15 cm H₂O (which closely agrees with the γ -minimum according to Johnson [10]).

The data were expressed as the mean \pm standard deviation. Statistical analysis of the data was carried out by Mann-Whitney test and $p < 0.05$ was defined as statistically significant.

The wet/dry weight ratio in the control group was 5.0 ± 0.2 . In contrast, the ratios in the TCV-309 and CV-3988 groups were 3.7 ± 0.7 and 3.5 ± 0.8 , respectively. The values in the TCV-309 and CV-3988 groups were significantly low ($p < 0.01$), compared with the control group (Fig. 1).

The pulmonary surface activity at V_{p15cm} was significantly ($p < 0.01$) higher in the TCV-309 and CV-3988 groups than in the control group (Fig. 2).

The histopathological findings in the control group consisted of extensive infiltration of blood in parenchymal tissue and enlargement of pulmonary alveoli. In the control group, pulmonary alveoli were filled with hydropic fluid due to the development of pulmonary edema. In the TCV-309 and CV-3988 groups, a few animals developed only mild interstitial edema.

Chemical mediators such as thromboxane A₂, leukot-

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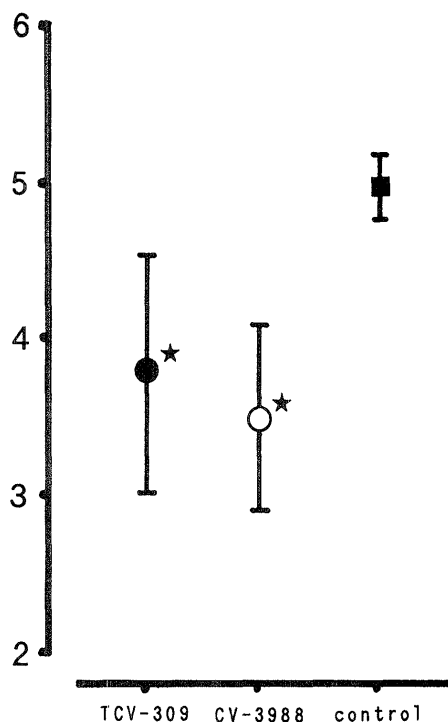


Fig. 1. Effects of TCV-309 and CV-3988 on wet/dry weight ratio of lung in dogs with experimentally-induced endotoxic shock. Data are the mean \pm standard deviation. \star ; $p < 0.01$ versus control.

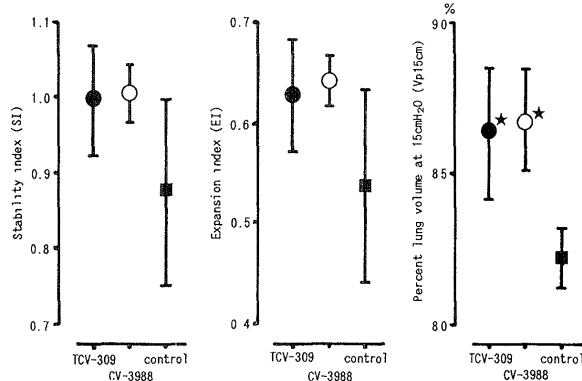


Fig. 2. Effects of TCV-309 and CV-3988 on pulmonary surface activity in dogs with experimentally-induced endotoxic shock. Data are the mean \pm standard deviation. \star ; $p < 0.01$ versus control.

riene and PAF, in addition to active oxygen and elastase released from neutrophils, are considered to be involved in the mechanism of lung injury occurring after endotoxic shock [2, 7]. PAF activates neutrophils and induces the release of lysosomal enzyme and production of active oxygen [18]. This results in damage to vascular endothelial cells. Furthermore, PAF activates platelets and induces the release of arachidonic acid, thromboxane A_2 and 12-hydroxy-eicosatetraenoic acid [9]. The released substances activate neutrophils and augment the permeability

of blood vessels [5]. With these major actions, PAF causes pulmonary edema by damaging vascular endothelial cells and augmenting the permeability of pulmonary capillaries.

The wet/dry weight ratio of the lung was increased in the control group. This is attributable to the augmented pulmonary vascular permeability [6] as a result of the release of PAF caused by the administration of endotoxin [3]. In contrast, the ratios in the treatment groups were significantly lower than those in the control group. The decreased wet/dry weight ratios seem to have resulted from alleviation of pulmonary edema, because lung injury caused by the active oxygen-producing, neutrophil-activating actions [18] and arachidonic acid-producing [9] of PAF was inhibited by TCV-309 and CV-3988. In fact, we have found a decreased thromboxane A_2 concentration in blood after the administration of CV-3988 during endotoxic shock [13].

The stability index, EI and V_{p15cm} for the removed lungs, were examined to assess the influence of TCV-309 and CV-3988 on pulmonary surface activity in endotoxic shock. Lipoprotein lipase seems to be involved in the depression of pulmonary surface activity [12]. In conjunction with the activity of lipoprotein lipase, lecithinase in blood has been found to increase its activity during shock [11]. PAF seemed to have augmented vascular permeability [6] and thereby increased the inflow into pulmonary alveoli of blood lecithinase which was increased during shock to result in the depression of pulmonary surface activity. On the other hand, TCV-309 and CV-3988 seemed to have prevented the depression of pulmonary surface activity by inhibiting the outflow of lecithinase into pulmonary alveoli, because the drugs suppressed the damage to vascular endothelial cells and augmentation of vascular permeability.

TCV-309 and CV-3988, the PAF-antagonists used in this experiment, were found to be more effective in reducing lung injury during endotoxic shock than the proteolytic enzyme inhibitors used in the previous experiment [14]. This suggests that PAF plays a more important role than the lysosomal enzyme in the pathogenesis of lung injury induced by endotoxic shock.

The results of this experiment suggested the possibility of alleviating lung injury during endotoxic shock by treatment with TCV-309, as treatment with CV-3988 and the important role of PAF in lung injury induced by endotoxic shock was re-recognized.

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