INTRODUCTION

Alcohol, which is a potent immunosuppressive drug, is widely recognized as an important risk factor in the development of infections. Indeed, several investigators have identified alcohol abuse as a risk and prognostic factor for infections (Fernandez-Sola et al., 1995; Cook, 1998). In contrast, the acute and low-dose intake of alcohol has anti-inflammatory effects (Mandrekar et al., 2006; Karavitis et al., 2008).

Severe infection, which causes endotoxia, induces the continuous release of cytokines involved in the pathogenesis of endotoxin-induced shock, metabolic acidosis, and multiple organ dysfunctions (Tracey et al., 1986). Chronic alcohol intake is associated with increased proinflammatory cytokine activation (Cook, 1998). On the other hand, the acute and low-dose intake of alcohol can inhibit the proinflammatory cell activation that is pivotal for innate immune activation (Karavitis et al., 2008; Crews et al., 2006). However, there are no reports on therapeutic effects of low-dose alcohol. Therefore, we hypothesized that acute and low-dose ethanol administration would be useful therapy for endotoxin-induced shock to inhibit the development of cardiovascular dysfunction and metabolic acidosis by the attenuation of cytokine responses to endotoxia. In order to test this hypothesis, we studied the effects of ethanol on hemodynamics, acid-base statuses, and the plasma concentrations of cytokines in endotoxin-exposed rats.

MATERIALS AND METHODS

All methods, which were approved by the Animal Care Committee of Kanazawa University School of Medicine, were performed in accordance with the National Institutes of Health guidelines for animal use.
A total of 20 male Wistar rats, weighing 350 ± 20 g (mean ± S.D.), were anesthetized with an intraperitoneal injection of 30 mg/kg of pentobarbital sodium and connected to a volume-controlled ventilator (tidal volume, 10 ml/kg × respiratory rate, 60 breaths/min) (SN-480-7, Shinano manufacturing Co., Ltd. Tokyo, Japan), delivering 100% oxygen through a tracheotomy. Lactated Ringer’s solution was infused continuously at a rate of 10 ml·kg⁻¹·hr⁻¹, and pancuronium bromide and pentobarbital sodium were injected at doses of 0.1 mg/kg and 2.5 mg/kg, respectively, every 30 min. The femoral artery was cannulated in order to monitor blood pressure and to draw blood samples. The heart rate was recorded from lead II of the electrocardiographic device (Dynascope DS-5300, Fukuda Denshi Co., Ltd, Tokyo, Japan). Rectal body temperature was maintained between 36 and 38°C with a heating pad.

The animals were allowed to rest for at least 15 min until the blood gases and hemodynamic variables had stabilized. After baseline measurements were taken of systolic arterial pressure and heart rate, the animals were randomly allocated to one of the following four groups: the control group, the ethanol group, the endotoxemia group, or the endotoxemia-ethanol group. The control group (n = 5) was not exposed to endotoxin. The animals in this group received a bolus injection of 0.9% saline (1.0 ml/kg) through the femoral vein catheter. The ethanol group (n = 5) was not exposed to endotoxin, too. However, anhydrous ethanol for injection (FUSO Pharmaceutical Industries, Tokyo, Japan) was administered intravenously at a rate of 100 mg·kg⁻¹·hr⁻¹ for 2 hr. The endotoxemia group (n = 5) was exposed to endotoxin. Escherichia coli lipopolysaccharide (LPS) that was derived from E. coli 0111:B4 (Difco Laboratories, Inc., Detroit, MI, USA) was injected intravenously at a dose of 15 mg/kg over a period of 2 min. The endotoxemia-ethanol group (n = 5) was exposed to endotoxin. Endotoxemia was induced as for the endotoxemia group. However, ethanol was administered intravenously at a rate of 100 mg·kg⁻¹·hr⁻¹ for 2 hr immediately after the injection of endotoxin.

An arterial blood sample (1.5 ml) was drawn for the measurement of pH, PaCO₂, PaO₂, and plasma cytokine (tumor necrosis factor (TNF)-α, interleukin (IL)-6, and IL-10) concentrations were measured using ELISA kits (Biosource, Camarillo, CA, USA). Each sample was assayed in triplicate, and the mean value was used. The lower limits of detection for TNF-α, IL-6, and IL-10 were 4, 8, and 5 pg/ml, respectively.

Results were compared using one-way ANOVA and Tukey HSD post-hoc test. Statistical significance was defined at α = 0.05.

RESULTS

Haemodynamics

There were no significant differences in systolic arterial pressure between the groups before endotoxin or saline injections (Fig. 1). After the injection of endotoxin, the systolic arterial pressure was decreased in endotoxemia group and endotoxemia-ethanol group. Furthermore, the systolic arterial pressure in endotoxemia-ethanol group was significantly greater than that in endotoxemia group from 0.5 to 1.0 hr after the injection (135 ± 17.3 mmHg vs. 97 ± 14.3 mmHg to 122 ± 2.7 mmHg vs. 97 ± 2.7 mmHg).

Blood gases

Carbon dioxide and oxygen pressure did not differ significantly between the 4 groups at any point during the experimental period. After the injection of endotoxin, the base excess was decreased in endotoxemia group and endotoxemia-ethanol group. Furthermore, the base excess in endotoxemia-ethanol group was significantly greater than that in endotoxemia group from 3 to 4.5 hr after the injection (-1.4 ± 2.7 mEq/l vs. -4.8 ± 1.1 mEq/l to -1.7 ± 1.0 mEq/l vs. -3.3 ± 0.8 mEq/l; Fig. 2).

Plasma cytokine concentrations

All baseline cytokine concentrations were similar in the four groups, and endotoxin injections significantly increased the TNF-α (Fig. 3), IL-6 (Fig. 4), and IL-10 (Fig. 5) concentrations in endotoxemia group and endotoxemia-ethanol group.

After the injection of endotoxin, the plasma TNF-α concentrations were increased in the groups that received endotoxin. Furthermore, the plasma TNF-α concentration of endotoxemia-ethanol group was significantly lower than that in endotoxemia group at 3 hr after the injection (1737 ± 643.0 pg/ml vs. 2419 ± 255 pg/ml).

The plasma IL-6 concentrations were increased in the groups that received endotoxin, and there was no significant difference between endotoxemia group and endotoxemia-ethanol group.

The plasma IL-10 concentrations were increased in the
Fig. 1. Systolic arterial pressure at baseline and after endotoxin or saline administration. After the injection of endotoxin, the systolic arterial pressure was decreased in endotoxemia group and endotoxemia-ethanol group. Furthermore, the systolic arterial pressure in endotoxemia-ethanol group was significantly greater than that in endotoxemia group from 0.5 to 1.0 hr after the injection. Values represent the mean ± S.D. for 5 rats. ***: lower than other groups, **: lower than control group and ethanol group.

Fig. 2. Base excess of the arterial blood at baseline and after endotoxin or saline administration. After the injection of endotoxin, the base excess was decreased in endotoxemia group and endotoxemia-ethanol group. Furthermore, the base excess in endotoxemia-ethanol group was significantly greater than that in endotoxemia group from 3 to 4.5 hr after the injection. Values represent the mean ± S.D. for 5 rats. ***: lower than other groups, **: lower than control group and ethanol group.
Fig. 3. Changes in plasma tumor necrosis factor (TNF)-α concentration. After the injection of endotoxin, plasma TNF-α concentrations were increased in the groups that received endotoxin. Furthermore, the plasma TNF-α concentration of endotoxemia-ethanol group was significantly lower than that in endotoxemia group at 3 hr after the injection. Values represent the mean ± S.D. for 5 rats. ***: higher than other groups, **: higher than control group and ethanol group.

Fig. 4. Changes in plasma interleukin (IL)-6 concentration. After the injection of endotoxin, the plasma IL-6 concentrations were increased in the groups that received endotoxin, and there was no significant difference between endotoxemia group and endotoxemia-ethanol group. Values represent the mean ± S.D. for 5 rats. **: higher than control group and ethanol group.
groups that received endotoxin. Furthermore, the plasma IL-10 concentration in endotoxemia-ethanol group was significantly higher than that in endotoxemia group from 3 to 4.5 h after the injection (1073 ± 571.1 pg/ml vs. 535 ± 133.8 pg/ml to 471 ± 168.6 pg/ml vs. 223 ± 45.2 pg/ml).

**DISCUSSION**

In the present study, the injection of the endotoxin LPS produced hypotension and metabolic acidosis. Low-dose ethanol administration suppressed these responses.

The ratio of inflammatory to anti-inflammatory cytokines is one of the prognostic factors in patients with endotoxin-induced shock (Taniguchi et al., 1999). LPS is a component of gram-negative bacteria, and it activates leukocytes through Toll-like receptor 4 (TLR4). The leukocytes produce inflammatory cytokines, such as TNF-α and IL-6, as a result of the activation of the nuclear regulatory factor κB (NF-κB) signaling pathway (Takeda and Akira, 2005). The release of these cytokines produces endotoxin shock, which may lead to multiple organ dysfunctions (Moscovitz et al., 1994). IL-10, which is an anti-inflammatory cytokine, is induced by TNF-α and IL-6, and it diminishes the cytokine response (Fiorentino et al., 1991). An increase in the ratio of inflammatory to anti-inflammatory cytokines is thought to indicate that the inflammatory response is stronger than the anti-inflammatory response. Thus, it is necessary to decrease the ratio in the treatment of endotoxin-induced shock. It has been reported that ethanol affects immune responses, including these cytokines, and the effects appear to depend on the amount and duration of its consumption. In vitro, acute and moderate ethanol administration inhibited LPS-induced NF-κB activation by TLR4 and interfered with the production of inflammatory cytokines (Szabo et al., 1995). In addition, the treatment increased IL-10 production by leukocytes (Mandrekar et al., 1996). The present study of TNF-α and IL-6 as inflammatory cytokines, and IL-10 as an anti-inflammatory cytokine, showed that ethanol administration at a rate of 100 mg·kg⁻¹·hr⁻¹ for 2 hr decreased TNF-α and increased IL-10 in rats with endotoxin-induced shock.

In addition to its anti-inflammatory effects, acute, low-dose ethanol treatment for sepsis seems to have other effects. First, this treatment has been reported to enhance the functional recovery of damaged organs by improving hemodynamics through ATP channels of the myocardium (Gross et al., 2001). The improvement of hemodynam-
ics and metabolic acidosis in the early stages when there were no differences in cytokine levels in the present study may be a result of this effect. Next, critically ill patients with sepsis suffer a high degree of stress due to pain, anxiety, and organ-specific responses to sepsis and require adequate sedation (Tonner et al., 2003). Ethanol administration at a rate of 100 mg•kg⁻¹•hr⁻¹ for 2 hr was compatible with a blood alcohol level of 0.03%, and this was thought to lead to mild euphoria (Ellenhorn and Barcelou, 1988). Therefore, acute, low-dose ethanol treatment may be useful for sedation.

In summary, the results of the present study showed that acute, low-dose ethanol treatment improved hypotension and metabolic acidosis that is produced by endotoxins, and the anti-inflammatory action of ethanol contributed to this effect.

REFERENCES

Takeda, K. and Akira, S. (2005): Toll-like receptors in innate immu-