

Involvement of Oxidative Stress in Increases in the Serum Levels of Various Enzymes and Components in Rats with Water-Immersion Restraint Stress

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Summary The plasma or serum levels of various enzymes and components are known to increase in rats with water-immersion restraint stress (WIRS). We examined whether oxidative stress is involved in increases in the serum levels of various enzymes and components in rats with WIRS. Rats were exposed to WIRS for 6 h after oral administration of vitamin E (VE) (50 or 250 mg/kg). Rats with WIRS had increased serum alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, creatine kinase, urea nitrogen, creatinine, glucose, corticosterone, adrenocorticotrophic hormone and lipid peroxide (LPO) levels, increased kidney and heart VE levels, decreased skeletal muscle VE level, and increased LPO levels in all tissues studied. Pre-administered VE (50 or 250 mg/kg) attenuated the increased serum alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, creatine kinase, urea nitrogen, creatinine, and LPO levels, the decreased skeletal muscle VE level, and the increased LPO levels in all tissues studied more effectively at its higher dose than at its lower dose. However, either dose of the pre-administered VE did not affect the increased serum glucose, corticosterone, and adrenocorticotrophic hormone levels. These results suggest that oxidative stress is involved in increases in the serum levels of various enzymes and components in rats with WIRS.

Key Words: water-immersion restraint stress (rat), serum enzymes and components, vitamin E, lipid peroxide, oxidative stress

Introduction

Acute emotional stress (immobilization or restraint, alone or in combination with cold exposure) in rats and mice causes increases in creatine kinase (CK), alanine amino-

transferase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) activities in the plasma or serum [1–4]. The increases in the activities of these enzymes in the plasma or serum of rats and mice by acute emotional stress have been suggested to result from damage of various tissues [1–4].

Water-immersion restraint stress (WIRS), one form of emotional stress, has been widely used as a technique for making an animal model of gastric mucosal lesions. The technique involves restraining an animal in a small fitted

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cage and immersing the animal in 23°C water to the xyhoid process [5]. Arakawa *et al.* [6] have shown in rats exposed to WIRS for 6 h that this stress causes increases in plasma CK, ALT, AST, and LDH activities and urea nitrogen, creatinine, and glucose levels. The authors have suggested that excessive peripheral sympathetic activity possibly plays an important role in the WIRS-induced increases in the plasma enzyme activities primarily via β -adrenoceptors, while α -adrenoceptors and the cholinergic nerves might be involved in the stress-induced increases in plasma urea nitrogen and glucose [6]. Starec *et al.* [7] have shown in rats exposed to WIRS for 4 h that pre-administration of argoclavine, a natural alkaloid with D₁ dopamine and α -adrenoceptor agonistic properties, stimulates increases in the activities of serum creatine kinase-MB (CK-MB), an isoenzyme of CK, which is specific for cardiac tissues, and ALT. Iwai *et al.* [8] have reported that the plasma activities of CK, AST, LDH, and amylase and the gastric, hepatic, and plasma levels of lipid peroxide (LPO), which is generated via reactive oxygen species, are increased in rats exposed to WIRS for 6 h. Furthermore, the authors have reported that pre-administration of gamazumi fruit juice or (-)-epigallocatechin gallate, each of which has antioxidant activity, for 2 weeks attenuates the increased plasma CK, LDH, and amylase activities and gastric, hepatic, and plasma LPO levels found in rats with 6 h of WIRS [8]. Our previous report has shown that when rats are exposed to WIRS for 1.5, 3 or 6 h, serum ALT and AST activities increase at 1.5 h and further increase time-dependently, while serum and hepatic LPO concentrations increase at 3 h and further increase at 6 h [9]. These findings may allow us to think that exposure of rats to WIRS causes increases in the plasma or serum levels of various enzymes and components not only by stimulating the activity of the autonomic nervous system but also by inducing oxidative stress in various tissues. However, it is still unclear whether exposure of rats to WIRS causes increases in the plasma or serum levels of various enzymes and components by inducing oxidative stress in various tissues of the animals.

The purpose of the present study was to clarify whether exposure of rats to WIRS causes increases in the serum levels of various enzymes and components by inducing oxidative stress in the various tissues of the animals. Namely, we examined the effect of pre-administered vitamin E (VE), a well-known lipid soluble antioxidant, on CK, ALT, AST, and LDH activities and urea nitrogen, creatinine, glucose, VE, and LPO concentrations in the serum of fasted rats exposed to WIRS for 6 h. We also examined the effect of pre-administered VE on LPO and VE concentrations in the liver, kidney, heart, and skeletal muscle of the stressed rats. It has been reported that rats with 6 h of WIRS have increases in the plasma levels of adrenocorticotrophic hormone (ACTH) and corticosterone which occur during

stress response via activation of the hypothalamic-pituitary-adrenal (HPA) axis [10]. It has been reported that chronic VE administration reduces an increase in plasma corticosterone level in rats with cold stress or immobilization stress [11, 12]. Therefore, the effect of pre-administered VE on increases in serum ACTH and corticosterone levels was further examined in rats exposed to WIRS for 6 h in order to verify whether the pre-administered vitamin affects stress response via activation of the HPA axis directly.

Materials and Methods

Chemicals

RRR- α -tocopherol was purchased from Sigma-Aldrich (St. Louis, MO). Tween-80, ethylenediaminetetra acetic acid (EDTA), thiobarbituric acid (TBA), and other reagents were obtained from Wako Pure Ind. Ltd. (Osaka, Japan).

Animals

Male Wistar rats were purchased from Nippon SLC (Hamamatsu, Japan) at the age of 6 weeks and kept in our laboratories for 1 week before the experiment under a 12:12 h light/dark cycle with light on at 7:00 AM. The rats were deprived of food for 24 h, but permitted water *ad libitum* before use. All animals received humane care in compliance with the Guidelines of the Management of Laboratory Animals in Fujita Health University. This animal experiment was approved by the Institutional Animal Care and Use Committee and its approved protocol number was M14-02.

WIRS induction and VE administration

Fasted rats were restrained in wire cages and immersed up to the depth of the xiphoid process in a water bath (23°C) for 6 h to induce WIRS, as described by Takagi and Okabe [5]. VE, dissolved in 5% Tween 80, at a dose of 50 or 250 mg/kg body weight (BW) was orally administered to rats with and without WIRS just before the onset of WIRS and this administration was conducted at a constant dosing volume of 5 ml/kg BW with a stomach tube. Stressed and unstressed rats without vitamin E administration were orally given the same volume of 5% Tween 80 at the same time point.

Sample preparation

Rats were sacrificed under ether anesthesia at which time blood was collected from the inferior vena cava. Serum was obtained from the collected blood by centrifugation. Immediately after sacrifice, perfusion was conducted through the portal vein with ice-cold 0.9% NaCl to remove blood remaining in tissues and then livers, kidneys, hearts, and skeletal muscles were removed. The obtained serum and tissues were stored at -80°C until use.

Assays of serum enzymes and components and tissue LPO and VE

Serum transaminases (ALT and AST), LDH and CK were assayed using commercial kits of Transaminase C II-Test Wako, LDH-Test Wako, and CK-Test Wako (Wako Pure Ind. Ltd. Co.), respectively. These enzyme activities are expressed as an international unit (IU/l). Serum urea nitrogen and creatinine were assayed using commercial kits of UN B-Test Wako and creatinine-Test Wako (Wako Pure Ind. Ltd. Co.), respectively. Serum LPO was fluorometrically assayed by the TBA method of Yagi [13]. Serum corticosterone was fluorometrically assayed by the method of Guillemin [14]. Serum ACTH was assayed using a commercial kit, ACTH EIA kit (Phoenix Pharmaceutical Inc., Burlingame, CA). Tissue LPO was spectrophotometrically assayed by the TBA method of Ohkawa *et al.* [15] except that 1mM EDTA was added to the reaction medium. Liver, kidney, heart, and skeletal muscle tissues were disrupted using a micro-homogenizer in 9 volumes of ice-cold 0.05 M Tris-HCl buffer (pH 7.4) containing 1 mM EDTA and the prepared homogenate was sonicated on ice for 60 s using a Handy Sonic model UR-20P (Tomy Seiko Co. Ltd., Tokyo, Japan). The concentration of LPO in serum and tissues is expressed as that of malondialdehyde (MDA) equivalents. VE in serum and tissues was assayed by the high-performance liquid chromatographic method with electrochemical detection as described in our previous report [16]. The concentration of VE in serum and tissues is expressed as that of α -tocopherol (α -Toc).

Statistical analysis

All results obtained are expressed as the mean \pm SD. The statistical analyses of the results were performed using a computerized statistical package (Stat View). Each mean value was compared by one-way analysis of variance (ANOVA) and Fisher's protected least significance (PLSD) for multiple comparisons as the post-hoc test. The significance level was set at $p < 0.05$.

Results

Effect of pre-administered VE on serum corticosterone and ACTH concentrations

Serum corticosterone and ACTH concentrations in rats exposed to WIRS for 6 h were significantly higher than those in control rats without the stress (Fig. 1). VE administered at a dose of 50 or 250 mg/kg BW just before the onset of WIRS did not affect the increases in serum corticosterone and ACTH concentrations in the stressed rats (Fig. 1). The same doses of VE given to rats without WIRS in the same manner did not affect the serum corticosterone and ACTH concentrations (Fig. 1).

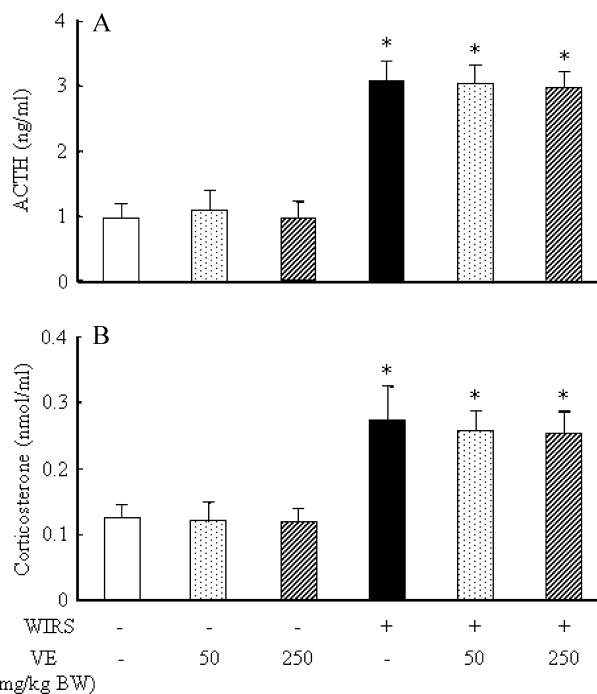


Fig. 1. Effect of pre-administered VE on serum ACTH (A) and corticosterone (B) concentrations in rats with 6 h of WIRS. Each value is the mean \pm SD ($n = 5$ for unstressed groups; $n = 8$ for stressed groups). *Significantly different from control rats without WIRS.

Effect of pre-administered VE on serum glucose, creatinine and urea nitrogen concentrations

Serum glucose, creatinine, and urea nitrogen concentrations in rats exposed to WIRS for 6 h were significantly higher than those in control unstressed rats (Fig. 2). Pre-administered VE (50 or 250 mg/kg BW) did not affect the increase in serum glucose concentration in the stressed rats but reduced the increase in serum urea nitrogen concentration in the stressed rats partially but significantly and this reducing effect of VE was larger at its higher dose than at its lower dose (Fig. 2). VE pre-administered at a dose of 50 mg/kg BW reduced the increase in serum creatinine concentration in the stressed rats partially but significantly and its dose of 250 mg/kg caused an almost complete reduction of the increased serum creatinine concentration (Fig. 2B). The same doses of VE given to rats without WIRS in the same manner did not affect the serum glucose, creatinine, and urea nitrogen concentrations (Fig. 2).

Effect of pre-administered VE on serum ALT, AST, LDH, and CK activities

Serum ALT, AST, LDH, and CK activities in rats exposed to WIRS for 6 h were significantly higher than those in control unstressed rats (Fig. 3). Pre-administered VE at a dose of 50 mg/kg BW reduced the increases in serum ALT,

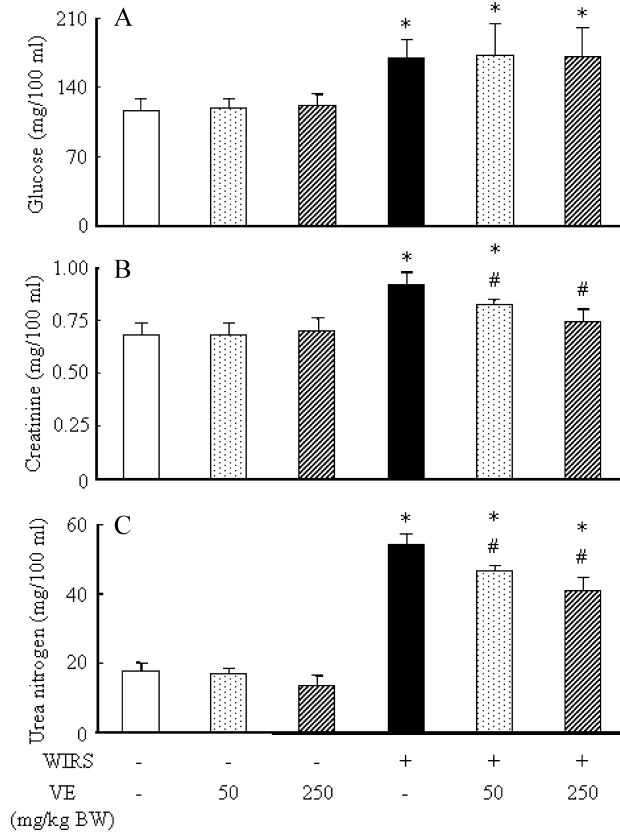


Fig. 2. Effect of pre-administered VE on serum glucose (A), creatinine (B), and urea nitrogen (C) concentrations in rats with 6 h of WIRS. Each value is the mean \pm SD ($n = 5$ for unstressed groups; $n = 8$ for stressed groups). *Significantly different from control rats without WIRS, $p < 0.05$; #Significantly different from rats with WIRS alone, $p < 0.05$.

AST, and LDH activities in rats with WIRS significantly and its dose of 250 mg/kg BW reduced the increases in serum CK activity significantly with further reduction of the increases in serum ALT, AST, and LDH activities in the stressed rats (Fig. 3). However, the preventive effects of pre-administered VE on the WIRS-induced increases in serum ALT, AST, LDH, and CK activities were partial (Fig. 3). The same doses of VE given to rats without WIRS in the same manner had no effect on the serum ALT, AST, LDH, and CK activities (Fig. 3).

Effect of pre-administered VE on serum LPO and VE concentrations

Serum LPO concentration in rats exposed to WIRS for 6 h was significantly higher than that in control unstressed rats (Fig. 4A). Pre-administered VE (50 or 250 mg/kg BW) reduced the increase in serum LPO concentration in the stressed rats significantly, but not completely, and the pre-administered VE exerted this reducing effect more effi-

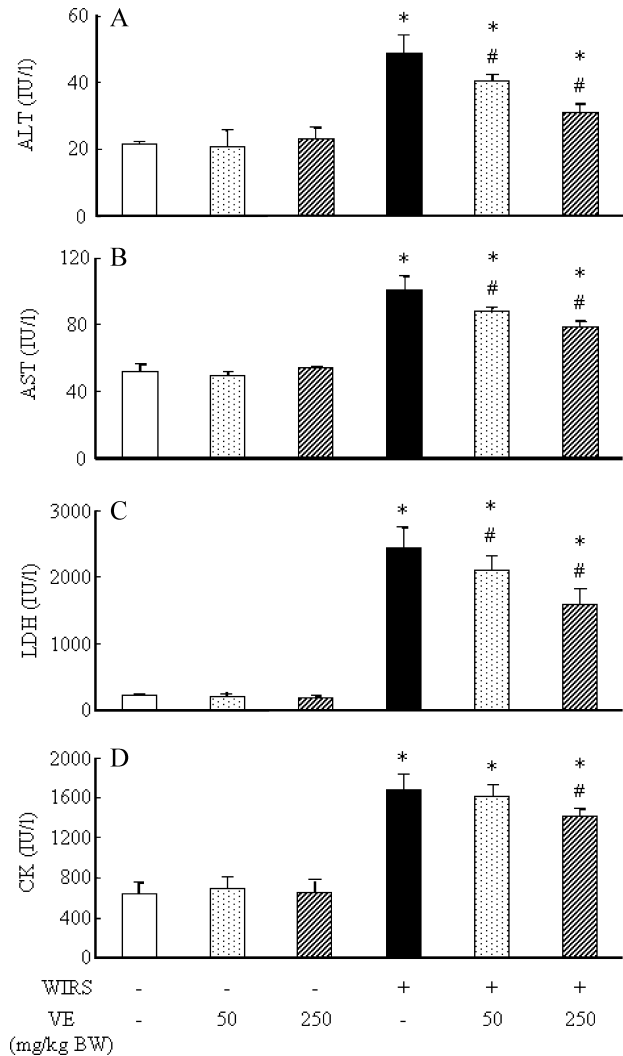


Fig. 3. Effect of pre-administered VE on serum ALT (A), AST (B), LDH (C), and CK (D) activities in rats with 6 h of WIRS. Each value is the mean \pm SD ($n = 5$ for unstressed groups; $n = 8$ for stressed groups). *Significantly different from control rats without WIRS, $p < 0.05$; #Significantly different from rats with WIRS alone, $p < 0.05$.

ciently at its higher dose than at its lower dose (Fig. 4A). There was no difference in serum VE concentration between the stressed and unstressed rats (Fig. 4B). Pre-administered VE (50 or 250 mg/kg BW) caused a significant increase in serum VE concentration in the stressed rats and the increase in serum VE concentration tended to be higher at its higher dose than at its lower dose (Fig. 4B). VE (50 or 250 mg/kg BW) given to rats without WIRS in the same manner did not affect the serum LPO concentration but increased the serum VE concentration significantly, although the increased serum VE concentration was significantly higher at its higher dose than at its lower dose ($p < 0.05$) (Fig. 4).

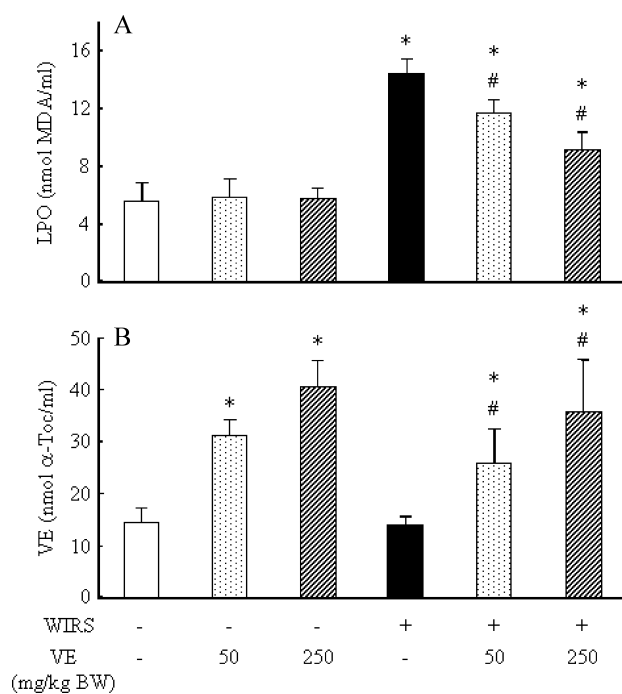


Fig. 4. Effect of pre-administered VE on serum LPO (A) and VE (B) concentrations in rats with 6 h of WIRS. Each value is the mean \pm SD ($n = 5$ for unstressed groups; $n = 8$ for stressed groups). *Significantly different from control rats without WIRS, $p < 0.05$; #Significantly different from rats with WIRS alone, $p < 0.05$.

Effect of pre-administered VE on tissue LPO and VE contents

LPO contents in the liver, kidney, heart, and skeletal muscle tissues of rats exposed to WIRS for 6 h were significantly higher than those in control unstressed rats (Fig. 5). Pre-administered VE (50 mg/kg BW) reduced the increases in liver, kidney, and skeletal muscle LPO contents, but not the increase in heart LPO content, in the stressed rats significantly (Fig. 5). Pre-administered VE (250 mg/kg BW) reduced the increase in heart LPO content in the stressed rats significantly and further reduced the increases in liver, kidney, and skeletal muscle LPO contents in the stressed rats (Fig. 5). However, the preventive effects of pre-administered VE on the WIRS-induced increases in liver, kidney, heart, and skeletal muscle LPO contents were partial (Fig. 5). VE (50 or 250 mg/kg BW) given to rats without WIRS in the same manner had no effect on the kidney, heart, and skeletal muscle LPO contents but its higher dose reduced the liver LPO content significantly (Fig. 5).

VE contents in the kidney and heart tissues of rats exposed to WIRS for 6 h were significantly higher than those in control unstressed rats but there were no significant differences in liver and skeletal muscle VE contents between the stressed and unstressed groups (Fig. 6). Pre-administered

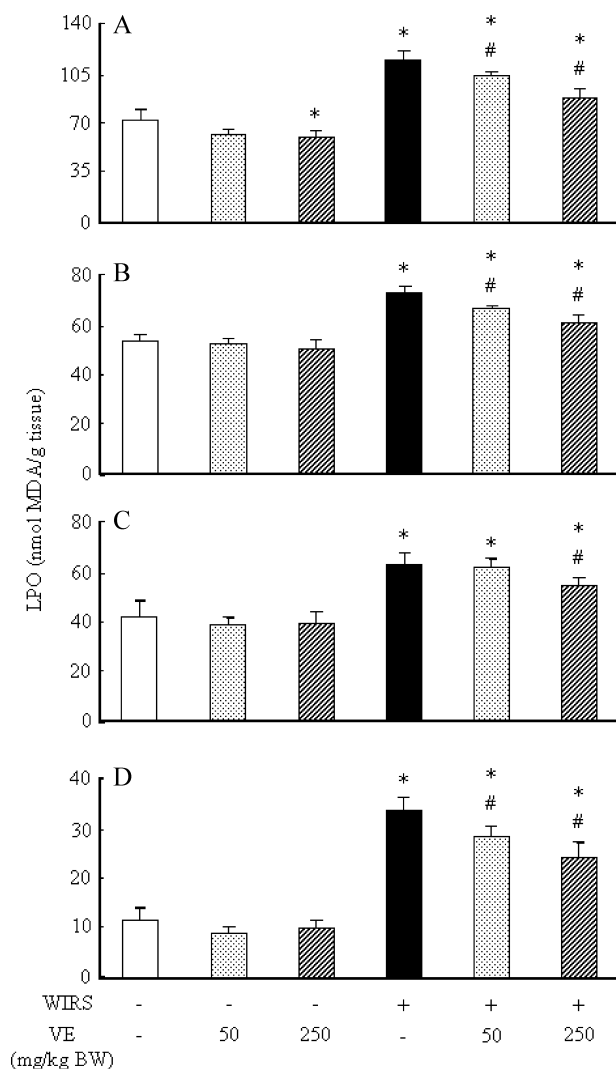


Fig. 5. Effect of pre-administered VE on LPO contents in the liver (A), kidney (B), heart (C), and skeletal muscle (D) of rats with 6 h of WIRS. Each value is the mean \pm SD ($n = 5$ for unstressed groups; $n = 8$ for stressed groups). *Significantly different from control rats without WIRS, $p < 0.05$; #Significantly different from rats with WIRS alone, $p < 0.05$.

VE (50 or 250 mg/kg BW) increased the hepatic VE content in the stressed rats significantly and the pre-administered VE exerted the increasing effect more efficiently at its higher dose than at its lower dose (Fig. 6A). Pre-administered VE at a dose of 250 mg/kg BW, but not 50 mg/kg BW, further increased the kidney and heart VE contents in the stressed rats significantly (Fig. 6B and C). Pre-administered VE (50 or 250 mg/kg BW) attenuated the decreased skeletal muscle VE content in the stressed rats partially but significantly and the pre-administered VE exerted this attenuating effect more efficiently at its higher dose than at its lower dose (Fig. 6D). Pre-administered VE (250 mg/kg BW) further

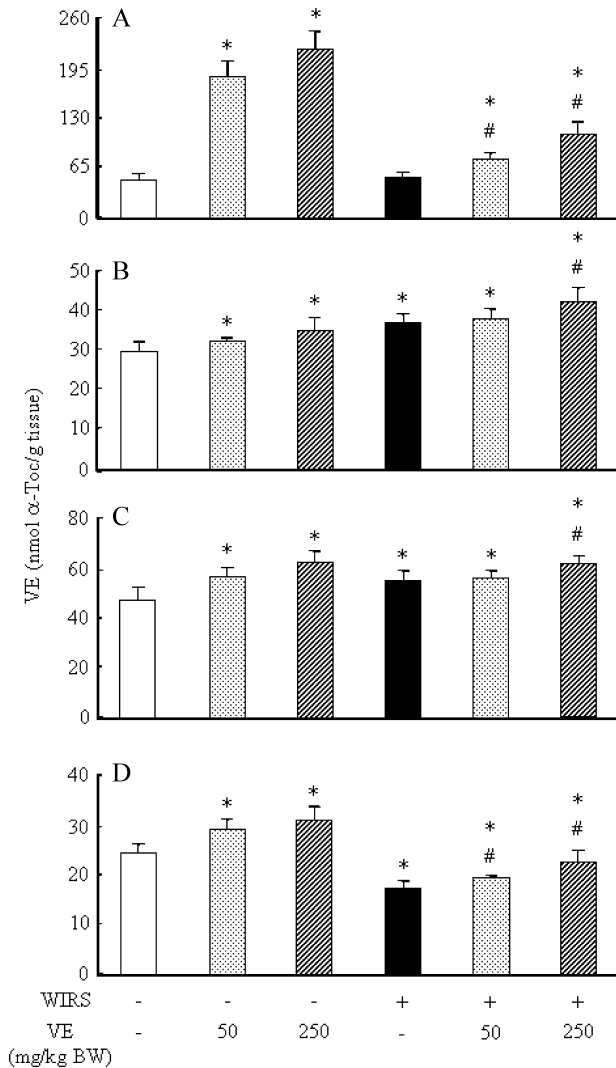


Fig. 6. Effect of pre-administered VE on VE contents in the liver (A), kidney (B), heart (C), and skeletal muscle (D) of rats with 6 h of WIRS. Each value is the mean \pm SD ($n = 5$ for unstressed groups; $n = 8$ for stressed groups). *Significantly different from control rats without WIRS, $p < 0.05$; #Significantly different from rats with WIRS alone, $p < 0.05$.

increased the increase in kidney and heart VE contents in the stressed rats significantly (Fig. 6A, B, and C). VE (50 or 250 mg/kg BW) given to rats without WIRS in the same manner increased the liver, kidney, heart, and skeletal muscle VE contents significantly and these increasing effects of VE were larger at its higher dose than at its lower dose (Fig. 6).

Discussion

In the present study, fasted rats exposed to WIRS for 6 h showed increases in serum ACTH and corticosterone

concentrations, as reported previously [10]. Thus, stress response via activation of the HPA axis occurred in the stressed rats. Oral administration of VE, a well known lipid soluble antioxidant, at a dose of 50 or 250 mg/kg BW before the onset of WIRS did not affect the increases in serum ACTH and corticosterone concentrations. The same doses of VE given to rats without WIRS had no effect on the serum ACTH and corticosterone concentrations. These results indicate that pre-administered VE has no effect on stress response via activation of the HPA axis in rats with WIRS. Fasted rats with 6 h of WIRS had increased serum glucose concentration, as reported previously [6]. The increase in serum glucose concentration in the stressed rats was not reduced by pre-administered VE. Arakawa *et al.* [6] have shown that plasma epinephrine and norepinephrine levels are increased in fasted rats with 6 h of WIRS, and have suggested that hyperglycemia occurring in the stressed rats may be mediated by α -adrenoceptors and the cholinergic nerves. Yamada *et al.* [17] have shown that hyperglycemia in fasted rats with immobilization stress is mediated by the elevation of epinephrine in the adrenal medulla, and have suggested that the main source of increase in plasma glucose is epinephrine-induced hepatic gluconeogenesis in immobilization stress-induced hyperglycemia. These findings suggest that VE pre-administered to fasted rats with 6 h of WIRS has no effect on epinephrine-mediated hyperglycemia via α -adrenoceptors and the cholinergic nerves. Accordingly, it can be thought that pre-administered VE does not affect stress responses via activation of the HPA axis and the autonomic nerve system in rats with 6 h of WIRS.

In the present study, increases in plasma ALT, AST, LDH, and CK activities and creatinine and urea nitrogen concentrations were found in rats exposed to WIRS for 6 h, as reported previously [6, 8, 9]. It has been suggested that increases in plasma ALT, AST, LDH, and CK activities and creatinine and urea nitrogen concentrations in rats exposed to WIRS for 6 h are caused by activation of the autonomic nervous system [6]. However, it has been shown that increases in AST, LDH, and CK activities in the plasma of rats exposed to WIRS for 6 h are reduced with attenuation of increased plasma LPO concentration by pre-administration of gamazumi fruit juice or (-)-epigallocatechin gallate, each of which has antioxidant activity, for 2 weeks [8]. In the present study, VE pre-administered once to rats exposed to WIRS for 6 h at a dose of 50 mg/kg BW reduced the increases in serum ALT, AST, and LDH activities and creatinine and urea nitrogen concentrations partially. VE pre-administered at a dose of 250 mg/kg BW attenuated the increased CK activity in the stressed rats partially, further attenuated the increased serum ALT and AST activities and urea nitrogen concentration in the stressed rats although partially, and the increased serum creatinine concentration almost completely. The same doses of VE given to rats

without WIRS had no effect on serum ALT, AST, LDH, and CK activities and creatinine and urea nitrogen concentrations. An increase in serum LPO concentration was found in rats with 6 h of WIRS, as reported previously [8, 9]. VE (50 or 250 mg/kg BW) pre-administered to the stressed rats attenuated the increased serum LPO concentration more effectively at its higher dose than at its lower dose. However, this attenuating effect of VE was partial. Both doses of pre-administered VE increased serum VE concentrations in rats with and without WIRS more effectively at its higher dose than at its lower dose, although there was no difference in serum VE concentration between the stressed and unstressed groups without pre-administration of VE. These findings suggest that not only stimulation of the activity of the autonomic nervous system but also oxidative stress could be involved in increases in serum ALT, AST, LDH, and CK activities and urea nitrogen concentration in rats exposed to WIRS for 6 h and that an increase in serum creatinine concentration in the stressed rats could be mainly due to oxidative damage.

The plasma or serum levels of various enzymes and components are increased when tissues are damaged: Creatinine level increases in plasma or serum when kidney tissue and/or skeletal muscle tissue are damaged. Urea nitrogen level increases in plasma or serum when kidney tissue is damaged. An increase in ALT activity in plasma or serum is mainly due to the release of the enzyme from damaged liver tissue into bloodstream. Increases in AST and LDH activities in plasma or serum are mainly due to the release of the two enzymes from damaged liver, heart, and skeletal muscle tissues into bloodstream. An increase in CK activity in plasma or serum is mainly due to the release of the enzyme from damaged heart and skeletal muscle tissues into bloodstream. It has been reported that oxidative stress occurs in the liver of rats with WIRS [8, 9]. Therefore, we examined the effect of pre-administered VE on LPO and VE contents in the liver, kidney, heart, and skeletal muscle tissues of rats exposed to WIRS for 6 h. LPO content was increased in the liver, kidney, heart, and skeletal muscle tissues of rats with 6 h of WIRS. Pre-administered VE (50 or 250 mg/kg) attenuated the increased LPO contents found in the liver, kidney, heart, and skeletal muscle tissues of the stressed rats partially, although these attenuating effects of VE were larger at its higher dose than at its lower dose. The same doses of VE given to unstressed rats had no effect on the kidney, heart, and skeletal muscle LPO contents, although the higher dose reduced the liver LPO content. There was no difference in hepatic VE content between rats with and without 6 h of WIRS, but the kidney and heart tissues of the stressed rats had higher VE contents than those of the unstressed rats, while the skeletal muscle tissue of the stressed rats had lower VE content than that of the unstressed rats. Pre-administered VE (50 or 250 mg/kg) attenuated the

decreased VE content in the skeletal muscle tissue of the stressed rats partially and increased the VE content in the liver tissue of the stressed rats more effectively at its higher dose than at its lower dose. In addition, pre-administered VE at a dose of 250 mg/kg further increased the kidney and heart VE contents in the stressed rats. Both doses of VE given to rats without WIRS increased the liver, kidney, heart, and skeletal muscle VE contents more effectively at its higher dose than at its lower dose. These results indicate that oxidative stress occurs in the liver, kidney, heart, and skeletal muscle tissues of rats exposed to WIRS for 6 h and that pre-administered VE attenuates increased ALT, AST, LDH, and CK activities and creatinine and urea nitrogen concentrations in rats with 6 h of WIRS by preventing oxidative stress occurring in the tissues such as liver, kidney, heart, and skeletal muscle. Therefore, it can be thought that the increases in serum ALT, AST, CK, and LDH activities and creatinine and urea nitrogen concentrations in rats exposed to WIRS for 6 h are due to the release of these enzymes and components from specific tissues damaged by oxidative stress into the bloodstream. We have shown in rats with WIRS that increases in serum ALT and AST activities and cell damage occur before the appearance of oxidative stress in the liver tissue [9]. Erin *et al.* [18] have reported that systemic denervation of afferent fibers with capsaicin inhibits cell damage in the liver of rats with cold-restraint stress without affecting LPO and reduced glutathione levels in the tissue. Accordingly, it is suggested that increases in the activities of ALT, AST, LDH, and CK and the concentration of urea nitrogen, but not creatinine, in the plasma or serum of rats exposed to WIRS are caused by activation of the autonomic nervous system and subsequently these increases are enhanced by oxidative damage occurring in the specific tissues.

In conclusion, the results of the present study indicate that orally pre-administered VE attenuates increases in serum ALT, AST, LDH, and CK activity and creatinine and urea nitrogen concentrations in rats with 6 h of WIRS by preventing oxidative stress occurring in the specific tissues such as liver, kidney, heart, and skeletal muscle without affecting stress responses per se. These results also suggest that oxidative stress is, at least in part, involved in increases in the serum levels of various enzymes and components in rats with WIRS.

References

- [1] Highman, B. and Altland, P.D.: Serum enzyme and histopathologic changes in rats after cold exposure. *Proc. Soc. Exp. Biol. Med.*, **109**, 523–526, 1962.
- [2] Pearl, W., Balazs, T., and Buyske, D.A.: The effect of stress on serum transaminase activity in the rat. *Life Sci.*, **5**, 67–74, 1966.

- [3] Meltzer, H.Y.: Plasma creatine phosphokinase activity, hypothermia, and stress. *Am. J Physiol.*, **221**, 896–901, 1971.
- [4] Sánchez, O., Arnau, A., Pareja, M., Poch, E., Ramirez, I., and Soley, M.: Acute stress-induced tissue injury in mice: differences between emotional and social stress. *Cell Stress Chaperones*, **7**, 36–46, 2002.
- [5] Takagi, K. and Okabe, S.: The effects of drugs on the stress ulcer in rat. *Jpn. J. Pharmacol.*, **18**, 9–18, 1968.
- [6] Arakawa, H., Kodama, H., Matsuoka, N., and Yamaguchi, I.: Stress increases plasma enzyme activity in rats: differential effects of adrenergic and cholinergic blockers. *J. Pharmacol. Exp. Ther.*, **280**, 1296–1303, 1997.
- [7] Starec, M., Fišerová, A., Rosina, J., Málek, J., and Kršiak, M.: Effect of agroclavine on NK activity *in vivo* under normal and stress conditions in rats. *Physiol. Res.*, **50**, 513–519, 2001.
- [8] Iwai, K., Onodera, A., and Matsue, H.: Antioxidant activity and inhibitory effect of Gamazumi (*Viburnum dilantaum* THUNB) on oxidative damage induced water immersion restraint stress in rats. *Int. J. Food Sci. Nutr.*, **52**, 443–451, 2001.
- [9] Ohta, Y., Chiba, S., Tada, M., Imai, Y., and Kitagawa, A.: Development of oxidative stress and cell damage in the liver of rats with water-immersion restraint stress. *Redox Rep.*, **12**, 139–147, 2007.
- [10] Lou, L.-X., Geng, B., Du, J.-B., and Tang, X.-C.: Hydrogen sulfide-induced hypothermia attenuates stress-induced ulceration in rats. *J. Exp. Pharmacol. Physiol.*, **35**, 223–228, 2008.
- [11] Yargıçođlu, P., Yaraş, N., Ađar, A., Gümüşlü, S., Bilmen, S., and Özkaya, G.: The effect of vitamin E on stress-induced changes in visual evoked potentials (VEPs) in rats exposed to different experimental stress models. *Acta Ophthalmol. Scand.*, **81**, 181–187, 2003.
- [12] Azlina, M.F.N. and Nafeeza, M.I.: Tocotrienol and α -tocopherol reduce corticosterone and noradrenalin levels in rats exposed to restraint stress. *Pharmazie*, **63**, 890–892, 2008.
- [13] Yagi, K.: A simple fluorometric assay for lipoperoxides in blood sample. *Biochem. Med.*, **15**, 212–216, 1975.
- [14] Guillemain, R., Clayton, G.W., Lipscomb, H.S., and Smith, J.D.: Fluorometric measurement of rat plasma and adrenal corticosterone concentration. A note on technical details. *J. Lab. Clin. Med.*, **53**, 830–832, 1959.
- [15] Ohkawa, H., Ohishi, N., and Yagi, K.: Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.*, **95**, 351–358, 1979.
- [16] Kamiya, Y., Ohta, Y., Imai, Y., Arisawa, T., and Nakano, H.: A critical role of gastric ascorbic acid in the progression of acute gastric mucosal lesions induced by compound 48/80 in rats. *World J. Gastroenterol.*, **11**, 1324–1332, 2005.
- [17] Yamada, F., Inoue, S., Saitoh, T., and Tanaka, K.: Glucoregulatory hormones in the immobilization stress-induced increase of plasma glucose in fasted and fed rats. *Endocrinology*, **132**, 2199–2205, 1993.
- [18] Erin, N., Ercan, F., Yegen, B.Ç., Arbak, S., Okar, I., and Oktay, S.: Role of capsaicin-sensitive nerves in gastric and hepatic injury induced by cold-restraint stress. *Dig. Dis. Sci.*, **45**, 1889–1899, 2000.