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Effects of Mild Chronic Heat Exposure on the Concentrations of Thiobarbituric Acid Reactive Substances, Glutathione, and Selenium, and Glutathione Peroxidase Activity in the Mouse Liver

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KASANUMA, Y., WATANABE, C., KIM, C.-Y., YIN, K. and SATOH, H. *Effects of Mild Chronic Heat Exposure on the Concentrations of Thiobarbituric Acid Reactive Substances, Glutathione, and Selenium, and Glutathione Peroxidase Activity in the Mouse Liver.* Tohoku J. Exp. Med., 1998, **185** (2), 79-87 —— To determine whether mild and chronic heat stress leads to oxidative stress and to differenciate such effects of different exposure periods, we kept male ICR-mice at an ambient temperature of either 35°C or 25°C for 6 hours, 3 days, or 7 days and measured the concentrations of thiobarbituric acid reactive substances (TBARS), glutathione (GSH), selenium (Se), and glutathione peroxidase (GSH-Px) activities in the liver. Since the food consumption of the heat-exposed group was only half that of the control, we prepared pair-fed groups, which were kept at 25°C and whose food consumption were limited to those of the heat-exposed group for the 3-day and the 7-day exposure.

TBARS concentrations of the liver was significantly higher in the heat group than the control after the 3-day exposure, while there was no significant difference among the groups after the 7-day exposure. There was no significant difference in GSH concentrations between the heat-exposed group and the control after the 7-day exposure, when the GSH concentration of the pair-fed group was significantly lower than that of the control.

Hepatic cytosolic Se GSH-Px activity in the heat group was significantly less than that in the control group after the 6-hour exposure and it tended to be lower in the heat group than that of the control group after the 7-day exposure, while there was no difference in the total GSH-Px activity among the three groups. Our results showed that mild and chronic heat exposure may cause oxidative damage to organisms and that GSH-related anti-oxidative systems would play an important role to defensive reaction. ——— heat exposure; oxidative damage; glutathione; selenium; liver

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Global warming is presently and will continue to be one of the most important problems in the world. From the viewpoint of environmental health, the harmful effects of hyperthermia on organisms has become a matter of concern. Several studies have suggested that heat exposure could result in oxidative stress, which in turn can lead to cytotoxicity (Mitchell and Russo 1983; Mitchell et al. 1983; Shrieve et al. 1986; Skibba et al. 1986, 1987). For example, Skibba et al. (1986) have shown that when human liver was perfused at 42–43°C, increases of malondialdehyde occur in the perfusate. They (Skibba et al. 1987) also showed that an elevated ratio of xanthine dehydrogenase to superoxide-generating xanthine oxidase occurs with increasing temperature in vitro. Moreover, it was shown that glutathione (GSH)-depleted cells were more susceptible to cytotoxic effects of heat exposure (Mitchell and Russo 1983; Mitchell et al. 1983; Shrieve et al. 1986).

However, all of these studies are in vitro experiments with cells or organs being exposed to heat at 42°C or higher. Only one study employed chronic and mild heat exposure to whole organisms to investigate the effects of oxidative stress under conditions simulating global warming (Ando et al. 1994). Ando et al. (1994) reported that an environmental temperature of 35°C increased thiobarbituric acid reacting substances (TBARS) in the liver of rats and guinea pigs and increased selenium-dependent glutathione peroxidase (Se GSH-Px) activity in rat liver, even though there was no change in Se-independent GSH-Px.

If heat exposure increases Se GSH-Px activity and has no effect on Se-independent GSH-Px, as was shown by Ando et al. (1994), Se would play an important role in protecting organisms from oxidative stress caused by heat exposure. Therefore, it is necessary to investigate effects of heat exposure in vivo by measuring not only GSH-Px activity but also selenium concentration and GSH concentration.

In the present study, we examine the effect of mild heat exposure on oxidative damage, and compare the effects that depend on the exposure period. We investigate the changes in (i) the concentration of TBARS, which is an indicator of oxidative stress, (ii) the concentration of GSH, which is the one of the important antioxidants, (iii) activity of the anti-oxidative enzyme GSH-Px, and (iv) the concentration of Se, which is a constituent element of GSH-Px, in the liver.

Materials and Methods

Animal treatment

This study was carried out after permission from the Committee of Animal Experimentation, Tohoku University School of Medicine.

Seven-week-old male ICR-mice (purchased from Clea Japan Inc., Tokyo) were acclimated in cages placed in an incubator at 25 ± 1°C for 5 days, and then the heat exposure periods were started. The exposures were done 3 series; 6-hour

exposure for acute period effects, 3-day for relatively chronic period effects, and 7-day for chronic period effects.

For 6-hour heat exposure, the mice were divided into 2 treatment groups (7 animals/group). One group was exposed to heat (the heat group), and the other was used for a control. For the mice receiving the 3-day and 7-day exposure, it was expected that their food intake would decrease due to the high ambient temperature and the decreases could weaken the systems that protect against oxidative stress. Therefore, two other groups were prepared as pair-fed groups for the 3-day (6 animals/group) and the 7-day experiment (8 animals/group).

The cages of the heat group were placed in an incubator in which the temperature was kept at $35 \pm 1^{\circ}C$, and the control group cages were kept in another incubator at $25 \pm 1^{\circ}C$. In each incubator, the lighting condition was maintained at 12 hours light and 12 hours dark. The mice of these groups were able to get food and water ad libitum. The third group, designated the pair-fed group, was kept in an incubator at $25 \pm 1^{\circ}C$, and was fed with the amount of food consumed by the heat group during the previous day. The rectal temperature of each mouse and the food consumption of each cage were measured daily between 1400 and 1600. After being kept in incubators for pre-determined periods (6 hours, 3 days or 7 days), mice were sacrificed by severing the cervical artery under ether anesthesia. The tissues were perfused with ice-cold saline, removed, and immediately stored at $-80^{\circ}C$ until analysis.

Chemicals

Reduced GSH, NADPH, 2-thiobarbituric acid (TBA), and *o*-phthalaldehyde (OPT) were purchased from Wako Pure Chemical Co. (Osaka). Glutathione reductase (EC 1.6.4.2) and xanthine oxidase (EC 1.1.3.22) were purchased from Boehringer Mannheim GmbH (Mannheim, Germany). All other chemicals were of the highest purity commercially available.

Analytical methods

TBARS were determined by a method slightly modified from the original method of Ohkawa et al. (1979). Briefly, to 200 μl of 2.5% homogenate in 1.15% KCl, 200 μl of 0.8% sodium dodecyl sulfate, 1.5 ml of 20% acetate solution (pH 3.5), 1.5 ml of 0.5% TBA, and 10 μl of 10 mM butylated hydroxytoluene were added, and the mixture was heated at $95^{\circ}C$ for 60 minutes. Then the TBARS were measured spectrometrically at 532 nm and the values were converted as equivalent to malondialdehyde (MDA). The concentration of GSH was determined by the method of Cohn and Lyle (1966) using OPT. Briefly, liver was homogenized in 10 mM EDTA containing 5% trichloroacetic acid (TCA), and the homogenate was centrifuged at $800 \times g$ for 10 minutes to remove proteins. OPT of 0.35 mM in 0.4 M Tris-HCl buffer (pH 8.9) containing 10 mM EDTA was added to the supernatant, and then the GSH content was determined fluorometrically.

For the measurement of GSH-Px activity, liver was homogenized with 9 volumes of 0.25 M sucrose in a Potter-Elvehjem glass-Teflon homogenizer and the homogenates were centrifuged at 105 000×g for 60 minutes at 4°C. Cytosolic GSH-Px activities were measured by the method of Paglia and Valantine (1967), using cumen-OOH as a substrate for measuring total GSH-Px and H_2O_2 as a substrate for determination of Se GSH-Px. Se concentrations in the cytosol and whole tissue were determined fluorometrically by the method of Watkinson (1966). Protein was determined by the procedure of Lowry et al. (1951).

Statistical analyses

Statistical analyses were performed by t-test for 2 group comparison and ANOVA with Tukey's multiple comparison test for 3 group comparison. Differences were considered statistically significant when $p<0.05$.

Results

Rectal temperatures of the mice of the heat group were maintained significantly higher than those of the control group after the exposure started (Fig. 1). As expected, the mean individual food intake of the heat group was only about half that of the control (Fig. 2).

TBARS concentrations (Fig. 3) of the liver were not significantly different between the heat group and the control group after the 6-hour exposure. But after the 3-day exposure, TBARS concentration was significantly higher in the heat group than in the other groups ($p<0.01$). After the 7-day exposure, there

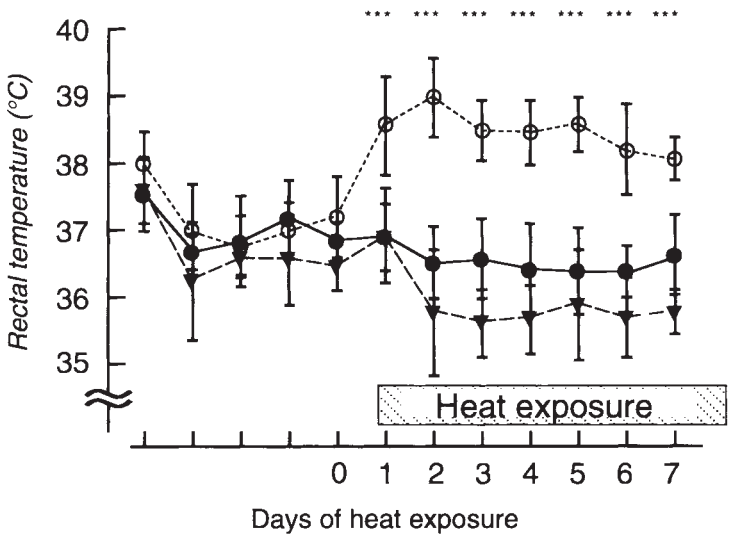

Fig. 1. Effect of environmental temperature on rectal temperature of mice. Temperatures were measured between 1200 and 1400 daily. Immediately after the measurement on day 0, the heat exposure was started. ●, control group (25°C); ○, heat group (35°C); ▼, pair-fed group (25°C and dietary restriction). Data are means±standard deviation for 8 animals. ***Significantly different from control value at 25°C ($p<0.001$).

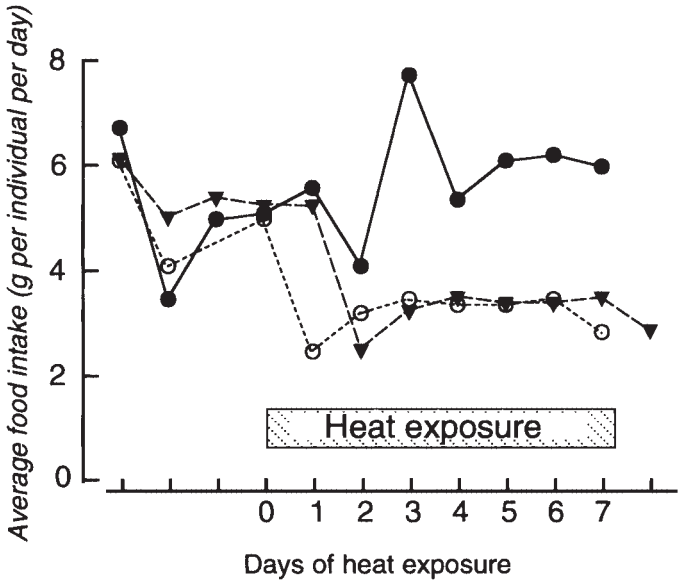

Fig. 2. Effect of environmental temperature on the food intake of mice.
Average food intakes of each treatment are shown. Food intakes were measured between 1200 and 1400 daily. Immediately after the measurement on day 0, the heat exposure was started. ●, control group (25°C); ○, heat group (35°C); ▼, pair-fed group (25°C and dietary restriction). Tic marks on x-axis correspond to 1200–1400 hour.

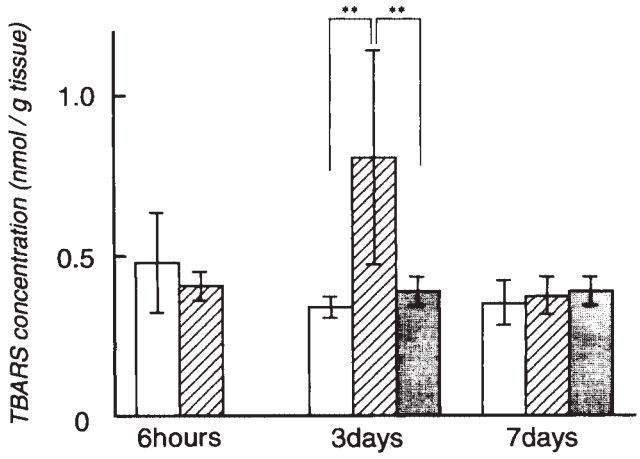

Fig. 3. Effect of environmental temperature on TBARS production (equivalent to malondialdehyde: MDA) in homogenate of mouse liver.
□, control group (25°C); ▨, heat group (35°C); ▩, pair-fed group (25°C and dietary restriction). Data are means±standard deviation. **Significantly different ($p<0.01$).

was no significant difference in TBARS concentrations among the groups.

GSH concentrations of the liver (Fig. 4) were not significantly different among the groups after the 6-hour or 3-day exposures. After the 7-day exposure, although there was no significant difference in GSH concentrations between the heat and control groups, the GSH concentration of the pair-fed group was significantly lower than that of the control ($p<0.05$). In addition, the GSH

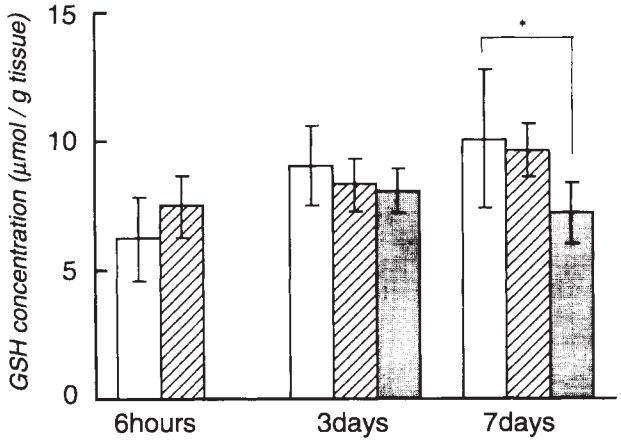

Fig. 4. Effect of environmental temperature on GSH concentration in mouse liver. □, control group (25°C); ▨, heat group (35°C); ▩, pair-fed group (25°C and dietary restriction). Data are means±standard deviation. *Significantly different ($p<0.05$).

concentration of the heat group tended to be higher than that of the pair-fed group, though the difference was not statistically significant ($p=0.056$).

As shown in Fig. 5, hepatic cytosolic Se GSH-Px activity in the heat group was significantly less than that in the control group after the 6-hour exposure ($p<0.05$). There were no significant differences in Se GSH-Px activity among the groups after the 3-day and 7-day exposures, though the Se GSH-Px activity tended to be lower in the heat group than in the control group after the 7-day exposure ($p=0.081$). There was no differences in the total GSH-Px activity among the three groups (Fig. 6).

After the 6-hour exposure, there was no difference in the liver Se concentrations (Fig. 7) between the heat group and the control. After the 3-day exposure, the Se concentration in the heat group was significantly lower than it was in the

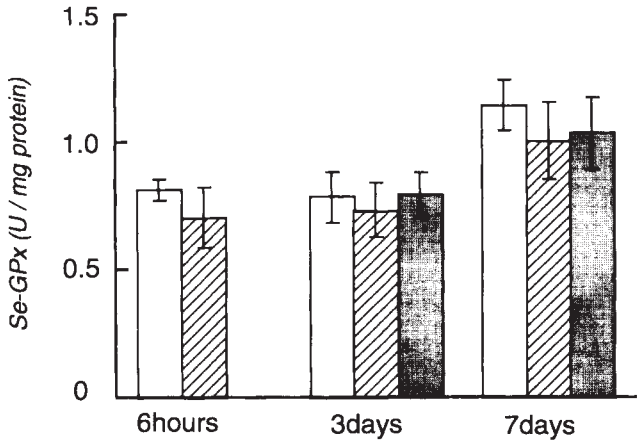

Fig. 5. Effect of environmental temperature on hepatic cytosolic Se GSH-Px in mice.
□, control group (25°C); ▨, heat group (35°C); ▩, pair-fed group (25°C and dietary restriction). Data are means±standard deviation.

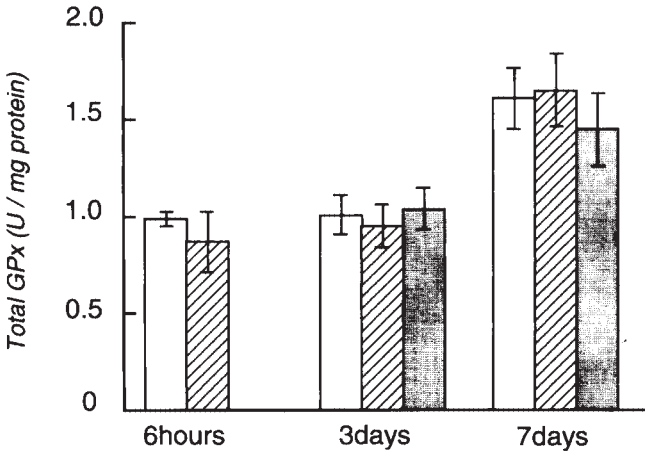

Fig. 6. Effect of environmental temperature on hepatic cytosolic total GSH-Px in mice.
□, control group (25°C); ▨, heat group (35°C); ▩, pair-fed group (25°C and dietary restriction). Data are means±standard deviation.

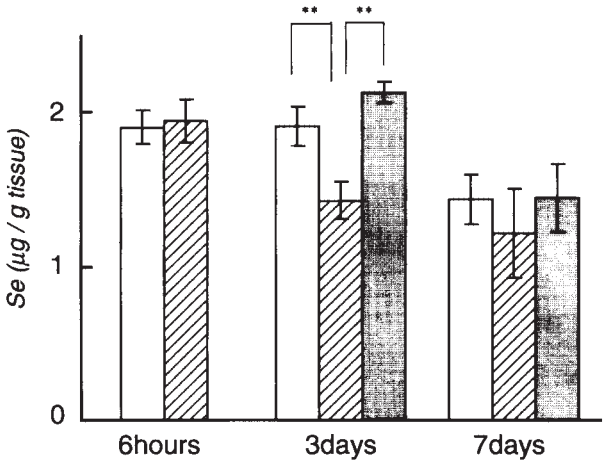

Fig. 7. Effect of environmental temperature on Se concentration in mouse liver.
□, control group (25°C); ▨, heat group (35°C); ▩, pair-fed group (25°C and dietary restriction). Data are means±standard deviation. **Significantly different ($p<0.01$).

other groups ($p<0.01$). There were no significant differences in the Se concentrations among the groups after the 7-day exposure, though the Se concentration tended to be lower in the heat group than in either the pair-fed group ($p=0.079$) or the control group ($p=0.062$).

Discussion

The comparison of TBARS concentrations between the heat group and the control at each exposure period is of interest: The concentration was not different between these group after the 6-hour exposure, and was significantly higher in the heat group than in the control after the 3-day exposure, and then was not different between these groups after the 7-day exposure again (Fig. 3). This change of

TBARS with exposure period shows that lipid peroxidation of the liver increases by prolonged heat exposure and decreases to the same degree of that of the control with a much longer period of exposure. It is presumed that some defense mechanisms against oxidative stress may take part in the change of TBARS.

In the present experiment, although there was no significant difference in GSH concentrations between the heat group and the control after any of the exposure periods, the GSH concentration of the heat group tended to be higher than that of the pair-fed group after the 7-day exposure. In the 7 day experiment, the GSH concentration of the pair-fed group was significantly lower than that of the control, indicating that the 7-day food restriction caused a lower GSH concentration, although the amount of food given to the pair-fed group was adjusted to the heat group food intake of the previous day. How did the heat group avoid lowering the liver GSH concentration in spite of a low food intake ? It is suspected that maintaining the GSH concentration had some relation to the defensive reaction against oxidative damage caused by heat exposure, because more GSH would be needed to scavenge peroxidates.

After the 7-day exposure, Se GSH-Px activity in the heat group tended to be lower than that in the control. The concentration of Se, which is needed for the synthesis of Se GSH-Px, also tended to be lower in the heat group than in the control. Toyoda et al. (1990) showed that Se restriction causes a lowering of amount of Se GSH-Px protein. It is suggested that the lower Se GSH-Px activity of the heat group of our experiment may also be related to the lower Se concentration.

On the other hand, the Se concentration of the heat group was significantly lower than the pair-fed group after the 3-day exposure or tended to be lower than the pair-fed group after the 7-day exposure. Since the pair-fed group was given the same amount of food as the heat group, lowering the Se concentration of the heat group was not caused by the restriction of food. Watanabe et al. (1990), who injected mice with toxic doses of Se and then exposed them to different temperatures for 4.5 hour, reported that the Se concentration of the liver was lower in mice that were exposed to 33°C than in the mice exposed to 22 or 8°C. Their report, like the present results, suggests that heat exposure may reduce the hepatic Se concentration, although there are differences in the dose, the temperature, and the period of heat exposure. However, the mechanisms of the suppressive effects of heat exposure on the liver Se concentration are unknown. It is speculated that heat exposure may suppress the accumulation of Se in the liver or prompt the secretion of Se from the liver. To clarify these mechanisms, changes in Se distribution in the body and the biochemical changes related to Se metabolism in the liver, blood, and other organs and secretions will need to be investigated.

A lower Se GSH-Px activity may be unfavorable for defending organisms against oxidative damage. However, even after the 7-day exposure, there were no differences in total GSH-Px activity among the groups. It is thought that

Se-independent GSH-Px increased to compensate for the lowering of Se GSH-Px activity. This may one of the adaptations of mice to high ambient temperature.

In this experiment, we showed that mild heat exposure may cause oxidative damage to organisms and that GSH-related anti-oxidative systems appear to play an important role to defensive reaction. Further investigation into the relationship between heat exposure and defense mechanisms against oxidative stress will be needed to avoid damage to organisms from global warming.

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