Gastric Mucosal Phosphatidylcholine Hydroperoxide Increases during Cold Water-Immersion Restraint Stress in Rats

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SHIAN, W.-M., SASAKI, I., KAMIYAMA, Y., NAITO, H., FUNAYAMA, Y., MATSUNO, S. and MIYAZAWA, T. *Gastric Mucosal Phosphatidylcholine Hydroperoxide Increases during Cold Water-Immersion Restraint Stress in Rats.* Tohoku J. Exp. Med., 1995, 176 (2), 127-130 — To know the role of lipid peroxidation in the pathogenesis of stress-induced gastric mucosal lesions, we performed the present study. Both gastric mucosal lesions and gastric mucosal phosphatidylcholine hydroperoxides (PCOOH) levels during cold water-immersion restraint (CWIR) stress were measured by the chemiluminescence-HPLC method in Sprague-Dawley rats. Ulcer indices increased time-dependently during the stress. Gastric mucosal PCOOH levels also significantly increased and reached a peak level (250% of untreated level) after 2 hr of stress. These results suggest that lipid peroxidation may play an important role in the pathogenesis of gastric mucosal lesions induced CWIR stress. ——— phosphatidylcholine hydroperoxides (PCOOH); cold water-immersion restraint stress; lipid peroxidation; gastric mucosal lesions; CL-HPLC method

Oxygen-derived free radicals (oxyradicals) have been implicated in the pathogenesis of several experimental models of gastric ulceration. Cold water immersion plus restraint (CWIR) stress develops gastric corpus erosions in the rat. This effect depends upon the presence of intraluminal acid and high-amplitude, prolonged-duration contractions associated with diminished mucosal blood flow (Garrick et al. 1986a, b; Livingston et al. 1988) which might result in cycles of ischemia and reperfusion. Although numerous studies have been undertaken to determine the relationship between gastric mucosal lesions and lipid peroxidation in CWIR stress, direct evidence for such a relationship is still lacking. In previous studies (Yoshikawa et al. 1986; Tarnasky et al. 1990), the link between gastric mucosal lesions and tissue lipid peroxidation could not be adequately assessed because the thiobarbituric acid (TBA) test commonly employed in such experiments is not specific for lipid peroxides. Miyazawa and colleagues (1993, 1994) have recently developed a new,

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sensitive and selective assay for phospholipid hydroperoxides using high performance liquid chromatography (HPLC) combined with chemiluminescence (CL) detection. Using this CL-HPLC method, phospholipid hydroperoxides in human plasma and serum lipoproteins and in liver and brain of rodents were determined (Miyazawa et al. 1993).

In the present study, we investigated the changes in gastric mucosal phosphatidylcholine hydroperoxides (PCOOH) levels during CWIR stress in rats by the CL-HPLC method.

MATERIALS AND METHODS

Seventy two male Sprague-Dawley rats, weighing around 200 g, were used. After over 18-hr food deprivation, CWIR stress was performed according to Takagi and Okabe's method (1968). Each animal was tightly restrained inside a screen wire mesh, which was secured with staples. Animals were then immersed to the level of the xyphoid in water maintained at 22°C. Before the stress and 30 min, 1, 2, 3 and 4 hr after the stress, animals were removed and sacrificed under ether anesthesia. The stomachs in 32 rats were harvested and opened along the greater curvature for assessment of lesions. In the remainder of the animals, the stomach was obtained immediately after laparotomy under ether anesthesia and gastric mucosa was scraped. Specimens were stored at -80°C until the measurement of the PCOOH content.

Ulcer index was expressed by measuring the total length (mm) of all lesions in the stomach induced during the stress. Gastric mucosal levels of PCOOH were measured by Miyazawa's method (1987).

Results are presented as the mean $\pm$ s.e. Data were analyzed by the PLSD method of Stat-View (Abacus Concepts Inc., Berkeley, CA, USA). Differences at $p < 0.05$ were defined as significant.

RESULTS

Ulcer index

The ulcer indices are presented in Fig. 1. No macroscopic lesions were observed in untreated rats. Ulcer indices increased time-dependently during CWIR stress.

Mucosal PCOOH levels

In untreated rats, mucosal PCOOH level was 663.8 ± 329.5 pmol/100 mg protein. It gradually increased during stress and reached a value of 1903.3 ± 408.3 pmol/100 mg protein at 2-hr (Fig. 2). After the stress treatment, mucosal PCOOH levels were significantly elevated compared with untreated levels at all time points.

Fig. 1. Ulcer index induced by cold water-immersion restraint (CWIR) stress in rats.

() number of rats, values are expressed as mean $\pm$ s.e.

*significant difference ($p < 0.05$; vs. untreated rats)

Fig. 2. Gastric mucosal PCOOH levels in cold water-immersion restraint (CWIR) stress in rats.

() number of rats, values are expressed as mean $\pm$ S.E.

*significant difference ($p < 0.05$; vs. untreated rats)

DISCUSSION

The nature of the gastric mucosal barrier to chemical insult is not completely understood. One possible explanation for the efficient barrier function has been provided by Hills et al. (1983) and Schmitz and Renooij (1990) who proposed that the surface of the gastric mucosa is relatively hydrophobic, and thus may repel the aqueous gastric acid. The hydrophobic nature of the gastric mucosa is well documented; Wassef et al. (1979) reported that phosphatidylcholine constituted about half of the total phospholipids. Lichtenberger et al. (1983) reported that the gastric mucosal concentration of both phosphatidylcholine and phosphatidylethanolamine was increased around six times by administration of 16-16-dimethyl prostaglandin E_2 . They suggested that local accumulation of these phospholipids might be an integral component of the cytoprotective mechanism activated by prostaglandin treatment. Although there has been no report that phosphatidylcholine is more highly susceptible to peroxidation than other phospholipids in the gastric mucosa, one of possible means of damage to the gastric mucosa may be via oxidative degradation of mucosal barrier lipids.

It has been proposed that free radical-mediated lipid peroxidation may play an important role in the generation of acute gastric mucosal lesions in CWIR stress in rats. Yoshikawa et al. (1986) reported that TBA-reactive substances, an indirect index of lipid peroxidation, were significantly increased in gastric mucosa after CWIR stress in rats. They suggested that lipid peroxidation may play a role in the pathogenesis of gastric mucosal lesions. However, because measurement of TBA-reactive substance is not specific for lipid peroxidation, more exact method is necessary to verify their hypothesis. In the present study, we used a sensitive and selective assay method which measures phospholipid hydroxides. The present results provide unequivocal evidence that the early phase of the CWIR stress is associated with a marked increase in PCOOH in gastric mucosa. These data support, but do not prove, the hypothesis that lipid peroxidative damage plays an important role in the development of CWIR stress-induced gastric mucosal lesions. Other pieces of evidence implicating oxidative damage after CWIR stress includes a decrease in reduced glutathion (a free radical scavenger) content in both liver (Hirota et al. 1989) and gastric mucosa (Shian et al. 1994) after CWIR stress.

The precise role of lipid peroxidative damage in mediating the development of gastric mucosal lesions after CWIR stress is not clear. Although the present results and those of others (discussed above) demonstrate an association between lesion formation and the presence of indicators of lipid peroxidation, whether the relationship is a causal one is controversial. For example, Tarnasky et al. (1990) reported that inhibition of oxygen radical formation by chronic feeding of a sodium tungstate diet, oral administration

allopurinopl, or intraperitoneal administration of dimethylsulfoxide did not significantly attenuated gastric lesions produced by CWIR stress in rats. The present study, for the first time, clearly indicated that CWIR stress produced peroxidative damage to gastric mucosal lipids. However, more studies will be required to define the precise role of lipid peroxidation in the pathogenesis of stress-induced gastric mucosal lesions.

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