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Sleep deprivation increase the expression of inducible heat shock protein 70 in rat gastric mucosa

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Abstract

AIM To investigate if sleep deprivation is able to increase the expression of inducible heat shock protein 70 in gastric mucosa and its possible role in mucosal defense.

METHODS Rats for sleep disruption were placed inside a computerized rotating drum, gastric mucosa was taken from rats with 1, 3 and 7 days sleep deprivation. RT-PCR, immunohistochemistry and Western blotting were used to determine the expression of heat shock protein 70. Ethanol (500mL·L⁻¹, i.g.) was used to induce gastric mucosa damage.

RESULTS RT-PCR, Western blotting and immunostaining confirmed that the sleep deprivation as a stress resulted in significantly greater expression of inducible heat shock protein 70 in gastric mucosa of rats. After the 500mL·L⁻¹ ethanol challenge, the ulcer area found in the rats with 7 days sleep deprivation (19.15±4.2 mm²) was significantly lower (P<0.01) than the corresponding control (53.7±8.1 mm²).

CONCLUSION Sleep deprivation as a stress, in addition to lowering the gastric mucosal barrier, is able to stimulate the expression of inducible heat shock protein 70 in gastric mucosa of rats, the heat shock protein 70 may play an important role in gastric mucosal protection.

Subject headings sleep deprivation; heat shock proteins 70/biosynthesis; gastric mucosa; rats

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INTRODUCTION

Stress has been shown to induce gastric mucosal lesions and lower the effectiveness of the mucosa as a barrier⁴⁴-⁴⁸. In rats, gastric ulcers can be produced by cold-restraint stress⁴⁷⁹ and it is frequently employed as a model for the study of the mechanisms of stress on ulcer formation. Cold-restraint stress, however, is not normally encountered in human subjects while sleep deprivation is a common experience among city dwellers, shift workers and medical professionals. It imposes stress on the body, and produces a variety of health problems⁴⁰-⁴⁴. Sleep deprivation is associated with poor cognitive ability, and shortening of longevity⁴⁵. Studies have found that the cognitive function of doctors after a night shift was considerably decreased. Sleep deprivation is also a major problem in the intensive care units and it has been suggested to affect the healing process of patients thus contributing to an increase in morbidity and mortality⁴⁶. Sleep deprivation has been shown to induce typical dermatitis in experimental animals. Severe ulcerative and hyperkeratotic skin lesions localized to the paws and tails developed in rats deprived of sleep⁴⁷. This effect of sleep deprivation may affect the epithelial linings of the gastrointestinal tract, because stress has been demonstrated to produce gastric mucosal lesions in rats⁴⁸. Our previous works showed that the food and water consumption in sleep disturbed rats were not affected but they had a smaller percentage gain in body weight. The locomotion activities of sleep disturbed rats were similar to the controls, however, their adrenal weights were increased⁴⁹. The mucosal epithelial cell proliferation rate was also suppressed by sleep disturbance. Although various factors have been proposed to account for this process, the precise mechanism of how sleep deprivation affects the gastric mucosa barrier, especially at the molecular level, still remains unclear. Previously, we used cDNA expression arrays to identify genes that abnormal expressed in gastric mucosa of sleep deprivation rats⁵⁰. In this project, inducible heat shock protein 70, one candidate gene emerging from cDNA array for its potential significance was further analyzed.

MATERIALS AND METHODS

Rats and reagents

Male Sprague Dawley rats weighing 180g-200g were used in the experiments. They were housed in a temperature 22°C ± 1°C and humidity 65% - 70% controlled room with a day night cycle of 12h. The rats were given standard laboratory diet (Ralston Purina Co., Chicago, IL) and tap water ad libitum. Rats were starved for 24h and water withdrawn 1 hour prior to any oral or intragastric administration of agents in order to obtain a uniform distribution of those agents onto the gastric mucosa. All chemicals used in this study were purchased from Sigma Chemical Co. (St. Louis, USA) unless specified otherwise. The present study has been examined and approved by the Committee on the Use of Live Rats for Teaching and Research of the University of Hong Kong.

Sleep disturbance

Rats for sleep disruption were placed inside a computerized rotating drum while the control animals were left undisturbed in a stationary drum. The drum was rotated 180° in 30s at 5 minutes intervals and was programed to switch off for 1h...
every day at 13:00 to allow for an hour of undisturbed sleep. Sleep disturbance was continued for 1 wk before the animals were killed.

Collection of gastric mucosa

Rats were killed by ether anesthesia followed by cutting off the abdominal aortic artery. The stomachs were removed rapidly, opened along the greater curvature, and rinsed with cooled normal saline thoroughly. A longitudinal section of gastric tissue was taken from the anterior part of the stomach and then fixed in 100mL·L⁻¹ buffered formalin for 24h. It was cut into sections of 5μm and then used in immunostaining. Gastric mucosa was taken from the remaining part of the stomach by scraping with a glass slide on a glass dish on ice. They were wrapped by a piece of aluminum foil, immediately froze in liquid nitrogen and stored at -70°C until assayed.

Detection of inducible heat shock protein 70 mRNA expression by RT-PCR²³

Total RNA was extracted from gastric mucosa of rats by using Trizol reagent (Gibco BRL, Gaithersburg, MD). First-strand complementary DNAs were synthesized from 5μg RNA by using oligo dt primer and Thermoscript RT-PCR system (Gibco BRL, Gaithersburg, MD). The PCR cycle was performed for inducible heat shock protein 70 and β-actin from the same complementary DNA sample using a PCR Thermal Cycler (Gene Amp PCR System 9700, The Perkin-Elmer Corporation, Norwalk, CT). The sequence of the oligonucleotide primers are as follows: sense inducible heat shock protein 70, 5'-TCGTCGATCTCCAGGC-3'²⁴ and sense β-actin, 5'-GTGCGCCGCCAGGCACCA-3'; antisense inducible heat shock protein 70, 5'-AGAGTCGA TCTCCAGGC-3'²⁴ and sense inducible heat shock protein 70, 5'-TGCTGACCAAGA TGAAG-3'; antisense β-actin, 5'-CTCCTTAATGTCAGGCAGATTC-3'²⁵. After-denaturation for 10 min at 95°C, 30 cycles of amplification were carried out followed by final extension of 10 min at 72°C, each step for 1 min. After amplification, 10μL of PCR products were electrophoresed in a 1% agarose gels containing 0.5μg/mL ethidium bromide.

Immunohistochemical detection of inducible heat shock protein 70 in gastric mucosa²⁶⁻³⁰

Fixed tissue sections (5μm) were mounted on Vectabond Reagent-coated slides, deparaffinized and rehydrated through xyline, graded ethanol to distilled water. After blocking endogenous peroxidase with 3mL·L⁻¹ hydrogen peroxide in methanol for 30 min, sections were treated with 0.05mol·L⁻¹ phosphate-buffered saline containing 30mL·L⁻¹ normal horse serum and 5g·L⁻¹ Trition X-100 for 30min, then the sections were rinsed with PBS, incubated with mouse anti-rat monoclonal antibody (StressGen Biotechnologies Corp. Victoria, Canada, Cat # SPA-810) at dilution of 1:200 overnight at 4°C in a humidity chamber. The secondary, biotinylated antibody (LSAB kit, Dako, Denmark) was then applied for 30 min followed by rinsing with PBS. Staining was performed by the addition of streptavidin (LSAB kit, Dako, Denmark) for 30 min, rinsed in PBS and developed in 3, 3'-diaminobenzidine tetrahydrochloride for about 3 min. Sections were counterstained with Mayer’s hematoxylin, dehydrated, cleared and mounted.

Detection of inducible heat shock protein 70 proteins expression by Western blotting

The gastric mucosa were homogenized at 4°C in RIPA buffer (50mmol·L⁻¹ Tris-HCl, pH 7.5, 150mmol·L⁻¹ NaCl, 1g·L⁻¹ sodium dodecyl sulfate, 5g·L⁻¹ a-cholate, 2mmol·L⁻¹ EDTA, 10 g·L⁻¹ Triton X-100, 100mL·L⁻¹ glycerol), containing 1 mmol·L⁻¹ PMSF and 10 mg·L⁻¹ apro tinin. After centrifuged at 10000×g at 4°C for 20 min, the supernatant (50μg of total protein) were denatured and separated on 75g·L⁻¹ sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and then transferred to nitrocellulose membrane (Bio-Rad, Hercules, CA)³¹,³². The membranes were blocked with blocking buffer (50mL·L⁻¹) nonfat milk in wash buffer (20mmol·L⁻¹ PBS, pH 7.5 containing 100mmol·L⁻¹ NaCl and 1g·L⁻¹ Tween 20) for 1h at room temperature, and subsequently incubated at 4°C with mouse monoclonal antibody against rat inducible heat shock protein 70 (StressGen Biotechnologies Gorp. Victoria, Canada, Cat # SPA-810) diluted in blocking buffer (1:1000). Membranes were washed 6 times and incubated with a rabbit-anti-mouse immunoglobulin G conjugated with the horseradish peroxidase (1:4000) (Bio-Rad Laboratories) for 1h . After six times additional washes, membranes were developed by a commercial chemiluminescence system (Amersham, Arlington Heights, IL) and exposed to X-ray film. Protein determinations were made with Bio-Rad protein assay kit with bovine serum albumin as a standard. Prestained molecular-weight standards (Bio-Rad) were used as markers.

Ethanol-induced gastric mucosal damage

Rats were starved for 24h before 1mL of 500mL·L⁻¹ ethanol was administered orally to induce acute gastric mucosal damage³³. Rats were killed 2h later by a sharp blow on the heads followed by cervical dislocation. The stomach was removed and opened along the greater curvature. The gastric lesion area (mm²) was traced onto a glass plate and subsequently measured on a graph paper with 1mm² division³⁴. The lesion index was calculated by dividing the total lesion area with the number of rats in each group.

Statistics

The data were statistically analyzed with the unpaired two-tailed Student’s t test.

RESULTS

Sleep deprivation increase the expression of inducible heat shock protein 70

Total RNA and protein were isolated from rats’ gastric mucosa at various times of sleep deprivation. As shown in Figure 1, RT-PCR results indicated that the expression of inducible heat shock protein 70 mRNA was low in normal gastric mucosa. Following the sleep deprivation, inducible heat shock protein 70 mRNA expression was elevated. The pattern of inducible heat shock protein 70 protein accumulation showed a similar trend to mRNA expression (Figure 2).

Inducible heat shock protein 70 immunohistochemistry in gastric mucosa

To further prove that inducible heat shock protein 70 is increased in gastric mucosa of sleep deprived rats, confirm their expression at protein level, and determine their cellular sources, immunostaining for iHsp 70 was performed on gastric mucosa of sleep deprived rats and normal rats. The results showed that inducible heat shock protein 70 staining was nearly absent in normal mucosa but in mucosa of sleep deprived rats was detected in epithelium (Figure 3).
Sleep deprivation decrease ethanol induced gastric ulceration

After the 500mL·L⁻¹ ethanol challenge, the ulcer area found in the rats with 7d sleep deprivation (19.2±4.2) mm² was significantly lower ($P<0.01$) than the corresponding control (53.7±8.1) mm², as shown in Figure 4.
to speculate that the heat shock protein 70 may play an important role in gastric mucosal protection.

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