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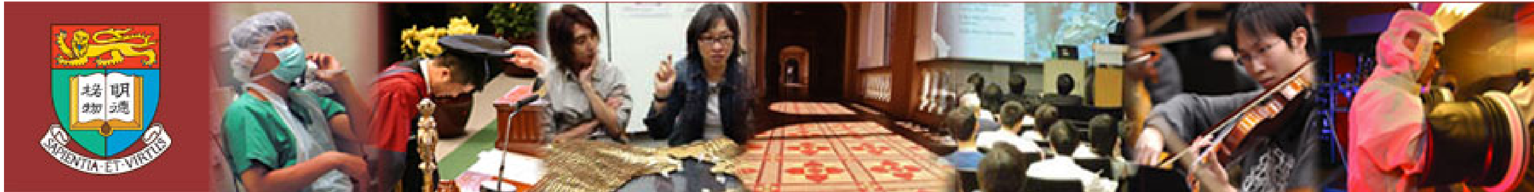

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Gene expression profiles in gastric mucosa of sleep deprivation rats

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Subject headings gene expression; mucosa, gastric; stress ulcer; GI tract; sleep deprivation; cDNA; ethanol

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INTRODUCTION

Stress has been shown to induce gastric mucosal lesions and lower the effectiveness of the mucosa as a barrier[1-6]. In rats, gastric ulcers can be produced by cold-restraint stress[7-9] 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, night shift workers and medical professionals. It imposes stress on the body, and produces a variety of health problems[10-14]. Sleep deprivation may affect the epithelium linings of the gastrointestinal tract, because stress been demonstrated to produce gastric mucosal lesions in rats[15,16]. Although various factors has been proposed to account for this process, the precise mechanism of how sleep deprivation to affect the gastric mucosa barrier, especially at the molecular level, still remains unclear. In this project we observed the effect of sleep deprivation on the defensive factors of gastric mucosa, and used cDNA expression arrays to identify genes expressed abnormally in gastric mucosa of sleep deprivation rats.

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℃ + 1℃) and humidity (65% - 70%) controlled room with a day night cycle of 12 hours. The rats were given standard laboratory diet (Ralston Purina Co. Chicago, IL) and tap water *ad libitum*. Rats were starved for 24 hours and water withdrawn 1 hours prior to any oral or intragastric administration of agents in order to obtain an uniform distribution of those agents onto the gastric mucosa.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 5min intervals and was programmed to switch off for 1h every day at 1:00 p.m. to allow for an hour of undisturbed sleep. Sleep disturbance was continued for 1wk before the animals were killed and the organ weight was determined. Daily water and food consumption as well as the body weight was recorded throughout the whole experimental period.

Ethanol-induced gastric mucosal damage

Rats were starved for 24h before 1mL of 500mL/L ethanol was administered orally to induce acute gastric mucosal damage[17]. 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 (mm^2) was traced onto a glass plate and subsequently measured on a graph paper with 1mm^2 divisions. The total lesion lengths divided by the number of rats in each group was expressed as the mean ulcer index[18].

Cold-restraint-induced gastric mucosal damage

Rats were put inside the close-fitting tubular wire-mesh cages and restrained inside a cold room for 2h. At the end of the experimental period they were killed and stomachs prepared for ulcer measurement as described previously[18].

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

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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 buffered formalin for 24h. It was cut into sections of 5μm and then used in histological examination. 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℃ until assayed.

cDNA expression arrays[19-24]

Total RNA for cDNA expression arrays was isolated from the gastric mucosa of rat s by using the Atlas™ Pure Total RNA Isolation Kit (CLONTECH Laboratories, Inc. CA. Cat.#: K1038-1). cDNA was synthesized and radioactive labeled using 5μg total RNA from sleep deprivation rat and normal control rat according to standardized protocols (CLONTECH Laboratories, Inc. CA. PT3140-1). Atlas™ cDNA Expression Arrays (CLONTECH Laboratories, Inc. CA.) was used for differential expression screening. Each Atlas Array includes 588 of cDNA spotted in duplicate on a positively charged nylon membrane. Plasmid and bacteriophage DNAs are included as negative controls to confirm hybridization specificity, along with several house keeping cDNA as positive controls for normalizing mRNA abundance. After a high-stringency wash, the membranes were exposed to X-ray film (Kodak BioMax MS film Cat.# 118 8077) at -70℃ with an intensifying screen for 3d. The gene expression pattern of 588 genes in gastric mucosa of normal and sleep deprivation rats was analyzed and compared on a computerized densitometer. Signals that genes were absent or present on one of the two membranes were identified visually.

Statistics

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

RESULTS

Effect of sleep deprivation on body weight

Sleep disturbed rats had the same water and food consumption when compared with the control however there was a slower increase in the percentage (%) of body weight among the sleep disturbed animals (Table 1). The decrease in % body weight gain was observed as early as 2d after sleep disturbance, and after that they gain in weight; although slower; but in a parallel fashion to that of the control (Table 1, all values at P<0.05). Sleep disturbance ind uced a significant increase in adrenal weight (240μg/g ± 8μg/g body weight) when compared with the control (215μg/g ± 6μg/g body weight). There was no difference in weights for the thymus and spleen between the two groups.

Effect of sleep deprivation on cold-restraint stress induced gastric ulceration

After rats were restrained in 4℃ for 2h, the ulcer index in gastric mucosa of sleep deprivation rats (41.7mm^2 ± 8.3mm^2) was significantly higher (P<0.01) then it in control rats (Figure 1). The results indicated that the sleep disturbance aggravated cold-restraint stress induced gastric ulceration.

Effect of sleep deprivation on ethanol induced gastric ulceration

After the 500mL/L ethanol challenge, the ulcer area found in the rats with 7d sleep deprivation (19.15mm^2 ± 4.2mm^2) was significantly lower (P<0.01) then the corresponding control (53.7mm^2 ± 8.13mm^2), as shown in Figure 2.

cDNA expression array

Figure 3 shows the result obtained by hybridizing two cDNA array membranes with radioactive-labeled cDNA from gastric mucosa of control normal rat and from gastric mucosa of 7d sleep deprivation rat. More than 10 differentially expressed genes were found in total 588 genes, most of them were digestive enzyme related genes, one of the overexpression gene in the gastric mucosa of sleep deprivation rat was identical to that of inducible heat shock protein 70.

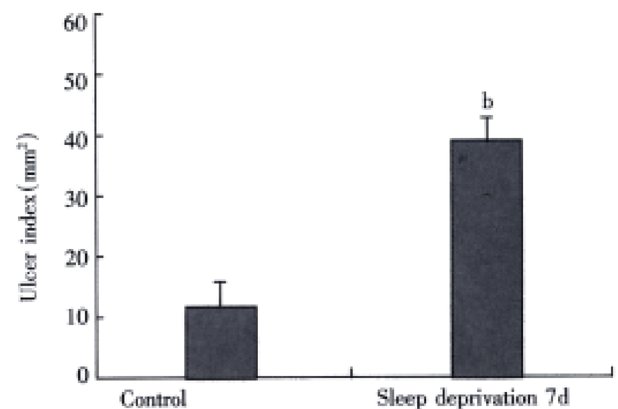

Figure 1 Effect of sleep disturbance on cold-restraint stress induced (4℃ for 2h) gastric ulceration in rats. Values are means ±SEM of 12 rats in each group; bP<0.01, *vs* control group.

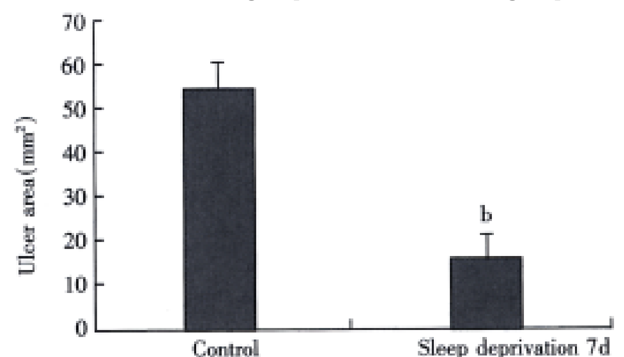

Figure 2 Effect of sleep deprivation on ethanol in duced (500mL/L ethanol 1mL p.o. for 2h) gastric ulceration in rats. Error bars represent SEM, n = 10 for each group. bP<0.01, *vs* control group.

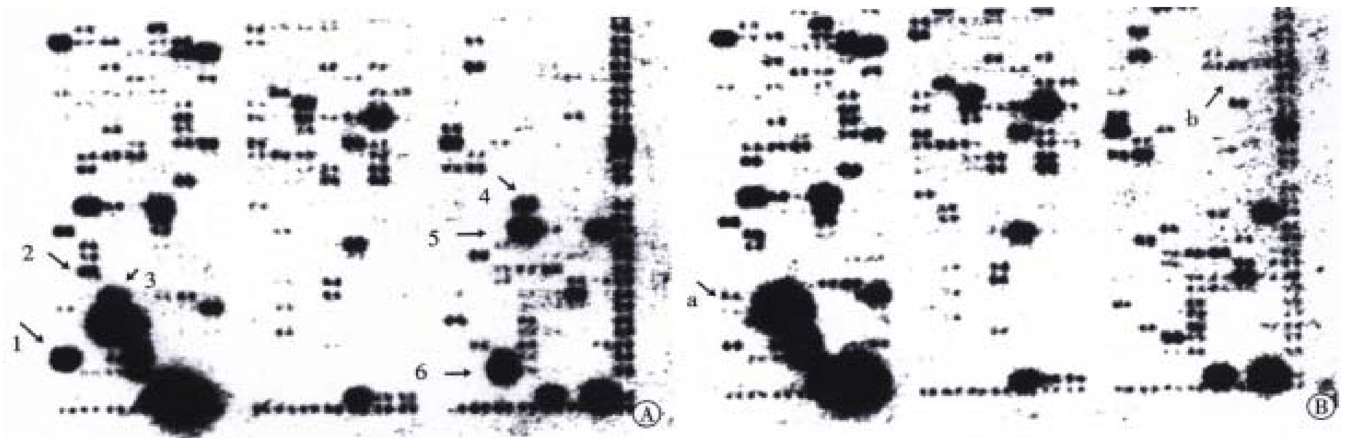

Figure 3 cDNA array: Differential gene expression in gastric mucosa of normal control rat (A) and sleep deprivation rat (B) . Sleep deprivation may decrease the following genes expression (Arrow 1-6) 1: bile-salt-stimulated lipase; 2: pancreatic lipase related protein 2 precu rsor; 3: triacylglycerol lipase precursor; 4: elastase 2 precursor; 5: trypsinogen II; 6: chymotrypsinogen B precursor, and increase the following genes expression (Arrow a-c) a: low-density lipoprotein receptor precursor; b: glucose transporter type 1; c: transferrin receptor protein. The dot for over expressed inducible heat shock protein 70 gene is circled.

Table 1 Effect of sleep disturbance on the percentage gain in body weight

Groups	Percentage gain in body weight						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Control	7.2±2.6	14.4±2.2	18.3±2.4	23.9±2.2	28.5±3.1	33.1±3.6	37.7±3.2
Sleep disturbed	4.8±2.5	7.7±2.3[a]	10.5±2.3[a]	16.2±2.5[a]	19.9±2.4[a]	24.6±2.7[a]	27.2±3.1[a]

Values are means±SEM of 12 rats in each group; [a]$P<0.05$, *vs* control group.

DISCUSSION

In this experiment a rotating drum was used to produce sleep disturbance in rats and it was found to be an effective model for stress induction. Sleep disturbed rats had a smaller percentage gain in body weight and this was observed 2d after sleep disturbance. This suppressive effect was not intensified after d2 and the sleep deprived rats gained in weight in a fashion paralleled to that of the controls. The statistical differences between all values of percent age gain in body weight were maintained at $P<0.05$ level (Table 1). The slowing of weight gain was not due to a reduction in food intake as there was no difference in food and water consumption between the two groups. This may be the result of an increase in catabolic process that was generally observed in sleep deprived animals[25-27].

It has been found that sleep disturbance aggravated cold-restraint stress induced gastric ulceration (Figure 1). The aggravation may be the result of lowered effectiveness of the mucosal barrier. Cold-restraint stress has been shown to produce blood stagnation in the gastric mucosa[25], and sleep disturbance imposed psychological stress on the rats as was demonstrated by an increase in the adrenal weight. The increase in adrenal weight could be due to the activation of the hypothalamus-pituitary- adrenal axis producing an overstimulation on the adrenal glands[28-31]. All these factors may lead to the decrease in basal gastric mucosal blood flow and affect the defensive function of gastric mucosa. Results of this experiment indicated that the sleep deprivation did not aggravate the 500mL/L ethanol induced gastric ulceration as expected, on the contrary, it did show some protective effect for ethanol challenge.

The human genome project's large-scale sequencing efforts have generated partial sequence data for thousands of genes. Although many of these genes have been assigned to functional classes, the roles they play in various biological processes have yet to be elucidated. An important step toward understanding these roles is defining gene expression profiles, i.e. comparing patterns of expression in different tissues and developmental stages, in normal and disease states, or in distinct *in vitro* cell conditions. This can be accomplished using RT-PCR, RNase protection assays, Northern blot analysis, *in situ* hybridization, immunohisto-chemistry or Western blotting[32-44], but these methods focus on only a few genes at a time. A more promising approach for analyzing multiple genes simultaneously is the hybridization of entire cDNA population to nucleic acid arrays. This technology has a wide range of application, including investigating normal biological and disease processes, profiling differential gene expression, and discovering potential therapeutic and diagnostic drug targets[19-24]. In this experiment, for further

investigation the reason why sleep deprivation protect gastric mucosa from ethanol insult, cDNA arrays were used to search for genes that were differentially expressed in gastric mucosa of sleep deprivation rats compared to gastric mucosa of control rats. More than 10 differentially expressed genes were found in total 588 genes (Figure 3), most of these were digestive enzyme related genes, one of the overexpression gene was inducible heat shock protein 70 gene. A variety of chemicals, viruses, and noxious stimuli such as trauma, hypoxia, or ischemia trigger the heat shock response and the subsequent synthesis of heat shock proteins[45-47]. Substantial evidence showed that heat shock is capable of protecting cells, tissues, organs, and animals from a subsequent, normally lethal heating, as well as from other types of noxious condition[48]. The protective effect of heat shock is likely mediated by overexpressed heat shock protein 70, because there is a lag between heat shock and the development of protection correlated with the production of heat shock protein 70, and protection is affected when heat shock protein 70 production is inhibited by treatment with inhibitors[49-51]. The overexpression of heat shock protein 70 was reported to protect guine a pig gastric mucosal cells from ethanol damage[52]. In conclusion, sleep disturbance imposed psychological stress on the rats as was demonstrated by decreasing body weight gain and increasing in the adrenal weight. Defensive function of the gastric mucosa was weakened by sleep deprivation thus predisposing it to ulcer formation induced by cold-restraint stress. On other hand, sleep deprivation decreased ethanol induced mucosa damage. This protective effect may be mediated by over expression of inducible heat shock protein 70 in gastric mucosa. Our experiment also showed that the cDNA arrays are the powerful approach to rapidly identify the gene expression profiles.

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