Melatonin Attenuates Noise Stress-induced Gastrointestinal Motility Disorder and Gastric Stress Ulcer: Role of Gastrointestinal Hormones and Oxidative Stress in Rats

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Background/Aims

There are increasing evidences for gastrointestinal motility disorder (GIMD) and gastric stress ulcer induced by noise stress. The present study was to investigate the reversed effect of melatonin on GIMD and gastric stress ulcer induced by noise stress and potential mechanism.

Methods

Noise stress was induced on rats, and melatonin (15 mg/kg) was administered to rats by intraperitoneal injection. Differences were assessed in gastric residual rate (GRR), small intestine propulsion rate (SPR), Guth injury score, cortisol, gastrointestinal hormones (calcitonin-gene-related peptide and motilin) and oxidative stress markers (superoxide dismutase and malondialde

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hyde) in blood plasma as well as gastric mucosa homogenate with or without melatonin. The pathological examination of gastric mucosa was also performed.

Results
The GRR and SPR were improved by noise stress compared with control ($P < 0.05$). The pathological examination and Guth injury score revealed gastric stress ulcer. Moreover, the levels of cortisol, motilin and malondialdehyde in blood plasma and malondialdehyde in gastric mucosa homogenate were increased by noise stress ($P < 0.05$). CGRP and superoxide dismutase activity in both of blood plasma and gastric mucosa homogenate were significantly decreased ($P < 0.05$). Furthermore, melatonin reversed changes in GRR, SPR, pathological examination, Guth injury score, cortisol, motilin, CGRP, superoxide dismutase activity and malondialdehyde ($P < 0.05$).

Conclusions
Melatonin is effective in reversing the GIMD and gastric stress ulcer induced by noise stress. The underlying mechanism may be involved in oxidative stress and gastrointestinal hormones.
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Key Words
Gastrointestinal hormones; Gastrointestinal motility; Melatonin, Oxidative stress

Introduction

Stress is a state of threatened homeostasis provoked by psychological, physiological or environmental stressors.[1] It activates the hypothalamic pituitary adrenal (HPA) axis and the sympathetic nervous system resulting in a physiological change.[2] Noise stress has been linked to a variety of health issues including cardiovascular diseases,[3] mental illness,[4] sleep disorder[5] and hypertension.[6] Furthermore, gastrointestinal motility disorder (GIMD), gastritis and peptic ulcers[7,8] have been noticed in populations who are exposed to high levels of noise. However, little is known about prevention and treatment of noise on GIMD and gastric stress ulcer.

Stress would induce activation of HPA axis that leads to the excessive production of free radicals. Generation of free radicals is an integral feature of normal cellular functions, on the other hand, excessive generation and/or inadequate removal of free radical results in destructive and irreversible damage to cells.[9] Corticotrophin-releasing hormone is released during stress and stimulates the release of adrenocorticotropic hormone.[10] It is well established that activation of HPA axis disorganizes gastrointestinal (GI) hormones and up regulates oxidative stress.[11] GI hormones and oxidative stress may play a critical role in the process of GIMD and gastric stress ulcer induced by noise stress.

Various evidences based on studies conducted in rodents that melatonin (N-acetyl-5-methoxytryptamine, MT), a pineal gland neurohormone, is a neuroendocrine transducer of environmental information with a key role in the circadian organization of vertebrates.[12] MT is best known for its antioxidant activities and free radical scavenging ability.[13,14] The anti-oxidative effect of MT is attributed to both its free radical scavenging activity and the reducing activation of enzymes on the production of reactive oxygen species.[15,16] Moreover, treatment of pharmacologically effective does of MT during the day to rodents, reported to induce sedation, hypothermia and suppression of HPA axis.[17]

Enterochromaffin cells of the GI tract are the main source of extra pineal MT and substantially contribute to the peripheral blood concentration of MT.[18,19] While MT acts mostly as an endocrine substance, extra pineal-derived MT functions not only as endocrine, but also as autocrine or paracrine substance and regulates many GI functions such as water and ion transport, proliferation of epithelium, secretion of acid, immune system and motility.[19-21] Thus, small does of MT intraperitoneal injecting accelerates intestinal transit and gastric emptying in rats besides high does delays the latter action.[12] But the mechanism underlying MT on GIMD and gastric stress ulcer have not been elucidated.

Considering these data, the aims of the present study were to investigate the effect of MT on noise stress-induced GIMD and gastric stress ulcer in rats. The possible mechanisms were focused on the HPA axis, GI hormones and oxidative stress.

Materials and Methods

Animal Preparation

Animal care was in accordance with the Principles of Medical Laboratory Animal Care issued by the National Ministry of Health. All experimental procedures were performed under the guidelines of the National Ordinances on Experimental animals for the ethical use of animals. The present study was approved from IRB of the Fourth Military Medical University. Adult male SD rats (n = 40, 220 ± 20 g) obtained from the Laboratory Animal Center of the Fourth Military Medical University were used in this study. They were housed 4 rats to a plexiglas cage in a climate-controlled room (20-22°C) on a 12/12 hour light/dark schedule (lights on at 7 AM) with a free access to food and water. All rats were allowed to acclimate for 1 week before the experiment.

Drugs Used

MT (Sigma-Aldrich, St. Louis, MO, USA), superoxide dismutase activity and malondialdehyde kit (Nanjing Jian-Cheng Biotechnology Research, Nanjing, China), calcitonin gene-related peptide (CGRP) and motilin kit (Military 301 Hospital Immunity Technique Research, Beijing, China).

Study Protocol

Forty rats were divided into 5 groups including 1 control group, 2 stressed groups (1 and 2) and 2 experimental groups (1 and 2) with 8 rats per group. Rats in stressed groups and experimental groups were given to noise stress (120 dB) 8 hours everyday: stressed group 1 and experimental group 1 for 1 day; while stressed group 2 and experimental group 2 for 3 days. The rats were fast for 12 hours before the last noise stress. MT was dissolved in a small amount of ethanol and diluted with saline to give a final concentration of ethanol less than 5%. Rats in experimental groups 1 and 2 were given to intraperitoneal injection of MT at a dose of 15 mg·kg^{-1}·day^{-1} (body weight) 30 minutes before the noise stress. The MT does used in this study was chosen on the basis of our previously published experiment.[16] The other groups of rats received the same volume of saline intraperitoneal injection with coordinate content of ethanol at the same time. All experiments were carried out between 8 AM and 5 PM.

Establishment of the Noise-stress Model

The noise-stress model used in the study was modified from that of Mu et al.[11] The rats in stressed groups and experimental groups were confined in an isolated room. Firing noise of submachine guns was recorded on a tape and introduced as an inducing factor, which was played to the rats through a loudspeaker at a distance of 20-30 cm. The intensity of firing noise was measured by a precision pulse counter and a frequency-spectrum analyzer as 120 dB (A) with a frequency of 0.25-4.00 kHz.

Measurement of Gastric Residual Rate and Small Intestine Peristalsis Rate

After the last noise stress, rats in each group were given to 2.5 mL carbon powder suspension which was made of 5% carbon powder and 10% arabic gum in distilled water[22] by intragastric administration. And 20 minutes later, all rats were narcotized by intraperitoneal injecting napental. The abdomen was opened and the stomach was rapidly clamped above the lower esophageal sphincter and beneath the pylorus in order to prevent further passage of carbon powder suspension. The protocol from anaesthesia to abdominal anatomy was taken within one minute by experienced hands. The GI tract from proventriculus to terminal ileum were separated and gently stretched. The stomach was wiped dry and weighted (weight 1, G1), and then it was cut open along the arcus major ventriculi. After the gastric contents were washed out, the stomach was weighted again (weight2, G2). The gastric residual rate (GRR) was calculated from the equation: GRR = [(G1-G2)/weight of 2.5 mL carbon powder suspension] × 100%.

The distances from sphincter pylori to the end of carbon powder suspension (distance 1, D1) and to ileocecal junction (distance 2, D2) were measured respectively. The small bowel peristalsis rate (SPR) was calculated from the equation[23]:

SPR = (D1/D2) × 100%.

Measurements of Gastric Mucosa Damage

The gastric mucosa damage was scored according to the Guth method[24]: the length (L) of a gastric mucosa damage area <1 mm was scored as 1 point; 1 mm ≤ L < 2 mm, 2 points; 2 mm ≤ L < 3 mm, 3 points; 3 mm ≤ L < 4 mm, 4 points; and L ≥ 4 mm, scored segmentally. The total score summed over the whole stomach was used as the ulcer index.

Pathological Examination of Gastric mucosa

Small pieces of ulcerative gastric mucous membrane were taken and fixed in formalin and kept in 4°C for subsequent pathological examination.

Evaluation of Cortical, Oxidative Stress and Gastrointestinal Hormone in Blood Plasma

Two 2 mL blood samples were taken by thoracotomy from heart: one was mixed well by centrifugation at 3,000 rpm/min for 10 minutes and kept at 4°C, which was ready for detecting superoxide dismutase activity and malondialdehyde level; the other was added with EDTA and retardant peptidase for separation of plasma and kept at −20°C in a refrigerator, which was ready for detecting cortisol, CGRP and motiln.

Detection of Oxidative Stress in Gastric Mucosa

Gastric mucosa was homogenated to the concentration of 100 g/L in ice-cold physiological saline by a homogenizer, and then well mixed with hydroextractor at 3,000 rpm/min for 10 minutes. The supernatant fluid was harvested and stored in −4°C for the detection of superoxide dismutase activity and motilin level.

Statistical Methods

All data were calculated as mean ± SD, with n equal to the number of involved rats. Differences among means of 5 groups were tested with one-way ANOVA. For post hoc testing, Fisher's least significant difference was used in multiple comparisons. A P-value < 0.05 was considered statistically significant. All analyses were performed with SPSS (version 17.0 for Windows, SPSS Inc., Chicago, IL, USA).

Results

Cortisol of stressed group rats was obviously higher than that of control group or experimental group rats ($P < 0.01$, Fig. 1) and it was higher in experimental group rats than in control group rats ($P < 0.01$, Fig. 1).

As shown in Figure 2 noise stress significantly enhanced GRR (Fig. 2A) and SPR (Fig. 2B) in stressed group rats. The noise stress effect on the stressed group 2 rats was more apparent
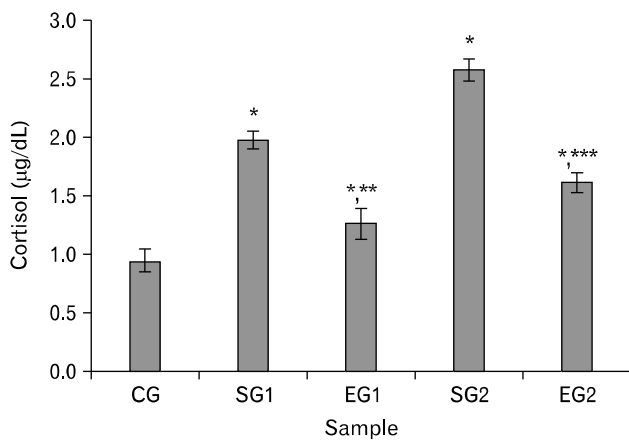

Figure 1. Effect of noise stress on cortisol of blood plasma in rats and the reverse effect of melatonin. Results are shown as mean ± SEM. For n = 8, *$P < 0.05$, significantly different from control group (CG) rats; **$P < 0.05$, significantly different from stressed group 1 (SG1) rats; and ***$P < 0.05$, significantly different from SG2 rats. One-way ANOVA and Fisher's least significant difference tests were used.
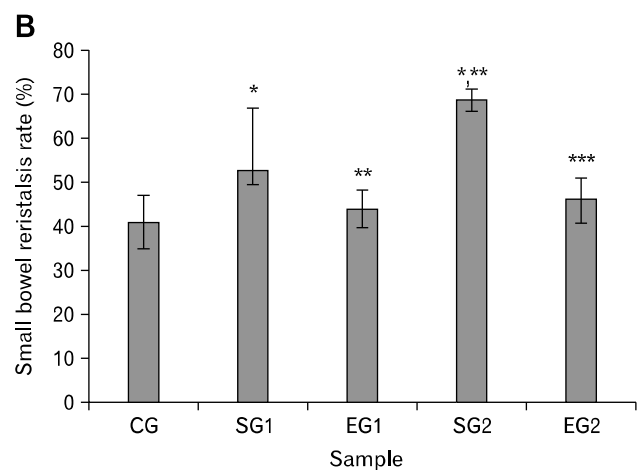

Figure 2. Effect of noise stress on gastric residual rate (A) and small bowel peristalsis rate (B) in rats and the reverse effect of melatonin. Results are shown as mean ± SEM. For n = 8, *$P < 0.05$, significantly different from control group (CG) rats; **$P < 0.05$, significantly different from stressed group 1 (SG1) rats; and ***$P < 0.05$, significantly different from SG2 rats. One-way ANOVA and Fisher's least significant difference tests were used.
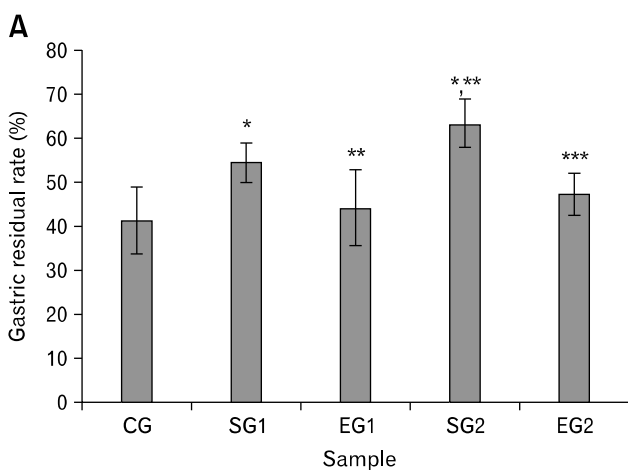
than that on the stressed group ones. However, the influence of noise stress on GRR and SPR induced by noise stress were reversed by MT (Fig. 2).

The results of Guth injury sore showed that it was significantly higher in stressed groups than in control groups and experimental groups ($P < 0.01$, Fig. 3). Under a microscope, our pathological observation showed that there was gastric mucosa damage in stressed groups. Meanwhile, it is observed that gastric mucosa discontinuation, gland disorganization and remote hemorrhage on the surface as well as interstitial edema with inflammatory cell infiltration (Fig. 4). Due to the MT treatment, the damage score of experimental group rats was apparently reduced ($P < 0.05$, Fig. 3), and the pathological damages of mucosa were obviously palliated (Fig. 4).
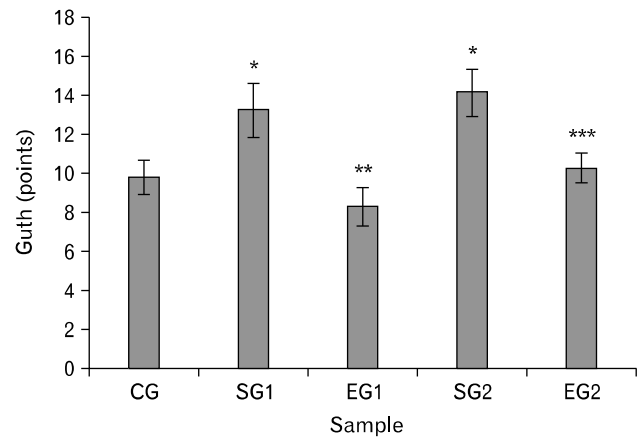
Figure 3. Effect of noise stress on Guth injury sore in rats and the reverse effect of melatonin. Results are shown as mean ± SEM. For n = 8, *$P < 0.05$, significantly different from control group (CG) rats; **$P < 0.05$, significantly different from stressed group 1 (SG1) rats; and ***$P < 0.05$, significantly different from SG2 rats. One-way ANOVA and Fisher's least significant difference tests were used.
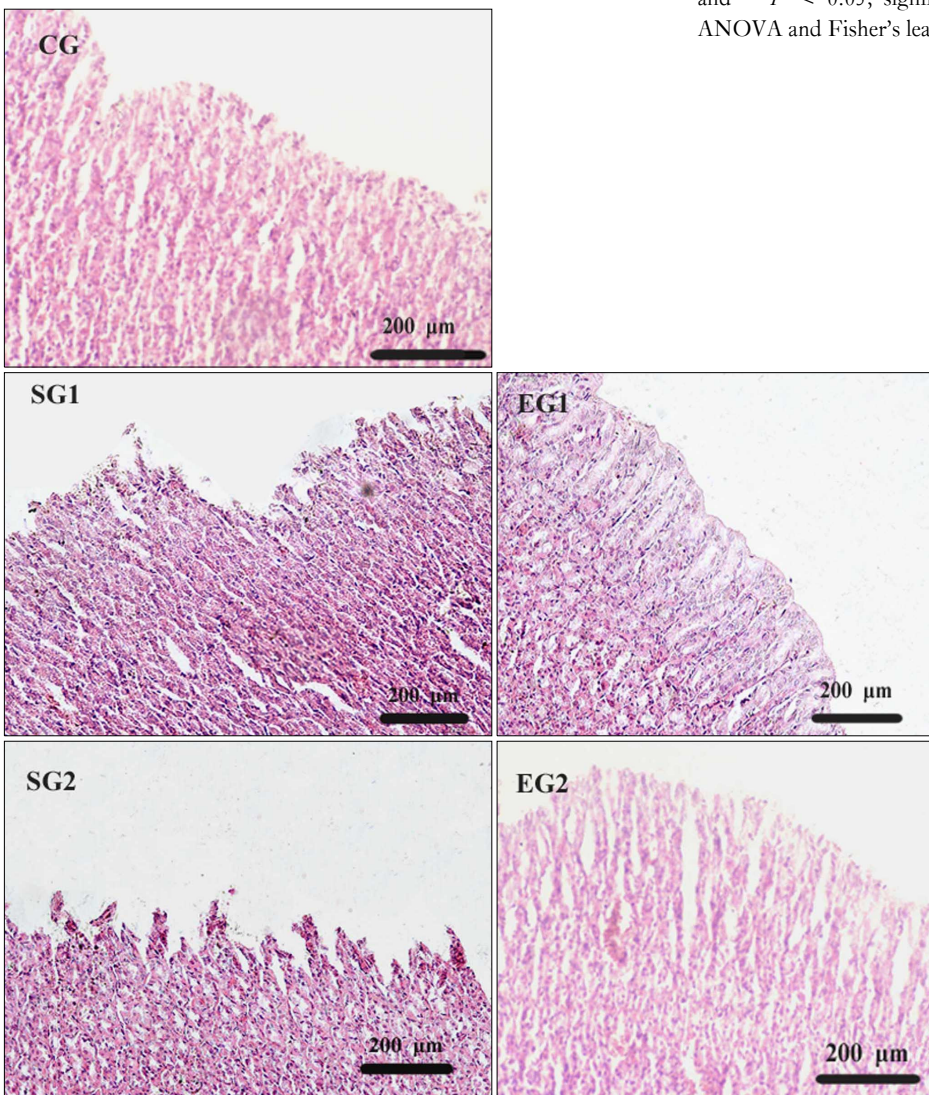
Figure 4. Pathological examination of gastric mucosa in rats (H&E stain, ×10).
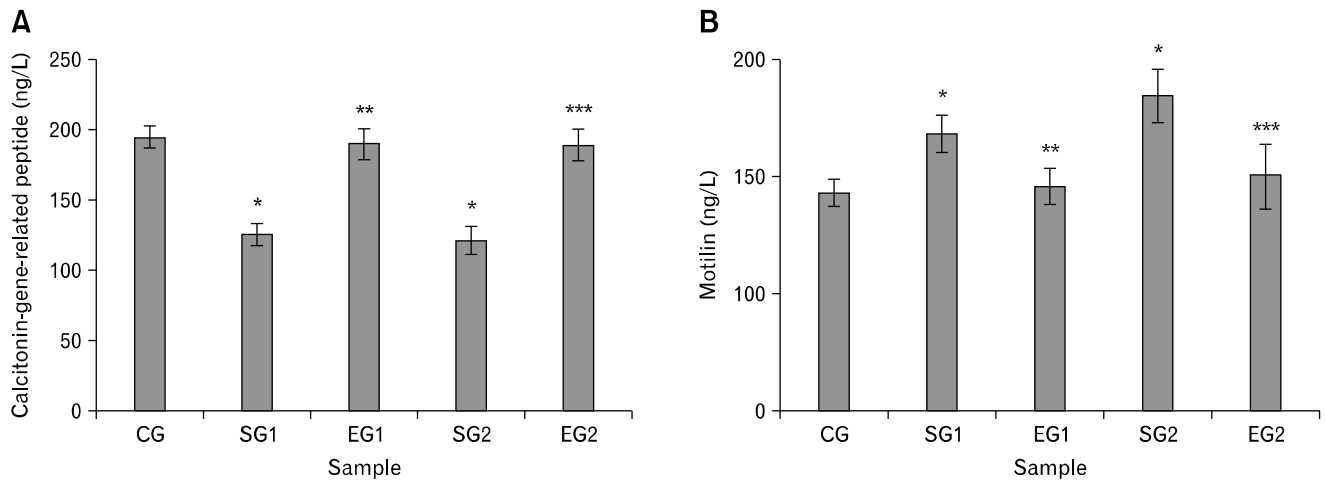
Figure 5. Effect of noise stress on calcitonin-gene-related peptide (A) and motilin (B) of blood plasma in rats and the reverse effect of melatonin. Results are shown as mean ± SEM. For n = 8, *$P < 0.05$, significantly different from control group (CG) rats; **$P < 0.05$, significantly different from stressed group 1 (SG1) rats; and ***$P < 0.05$, significantly different from SG2 rats. One-way ANOVA and Fisher's least significant difference tests were used.

Figure 6. Effect of noise stress on superoxide dismutase activity (A and C) and malonaldehyde (B and D) of blood plasma (A and B) and gastric mucosa homogenate (C and D) in rats and the reverse effect of melatonin. Results are shown as mean ± SEM. For n = 8, *$P < 0.05$, significantly different from control group (CG) rats; **$P < 0.05$, significantly different from stressed group 1 (SG1) rats; and ***$P < 0.05$, significantly different from SG2 rats. One-way ANOVA and Fisher's least significant difference tests were used.

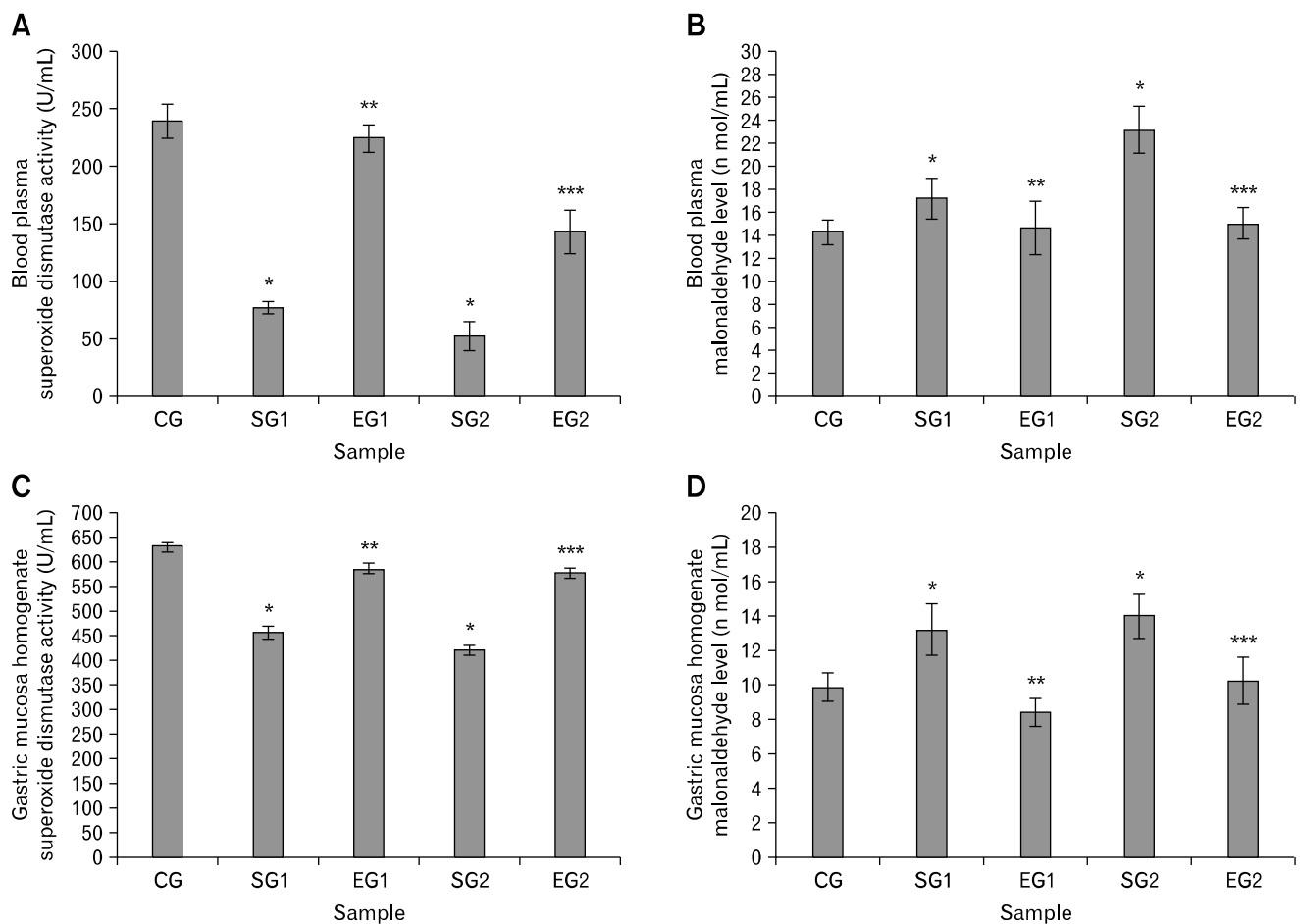The levels of GI hormones in plasma are shown in Figure 5. To the rats exposed to noise stress, the CGRP level was decreased ($P < 0.05$, Fig. 5A) whereas the motilin level was increased ($P < 0.05$, Fig. 5B). Moreover, there were no differences between experimental group and control group rats in terms of motilin and CGRP levels ($P > 0.05$, Fig. 5).

Quantity of lipid peroxidation was used to represent oxidative stress, which was measured by the thiobarbituric acid colorimetric assay of malondialdehyde level and superoxide dismutase activity. For both blood plasma and gastric mucous homogenate, the superoxide dismutase activity was lower in stressed groups than that in control and experimental groups ($P < 0.05$, Fig. 6A and 6C); whereas the malondialdehyde level was higher in stressed groups than that in control and experimental groups ($P < 0.05$, Fig. 6B and 6D).

Discussion

In the present study, noise stress is confirmed to induce GIMD and gastric stress ulcer due to oxidative stress and activation of HPA axis which changes gut hormones. The results of present work show that MT plays a significant protective role in attenuating noise stress-induced GIMD and gastric stress ulcer. In addition, MT suppresses HPA axis activation and reverses gut hormones (motilin and CGRP) as well as oxidative stress markers (superoxide dismutase activity and malondialdehyde level).

Noise is one of important factors which induce human body stress and many non-auditory effects on GI tract.[25,26] In general terms, these non-auditory responses tend to increase the level of alertness and activate HPA axis. These reflexes include a focused posture directed to the source of noise, a shunting blood away from vegetative regions like the stomach, a rise in adrenalin, noradrenalin and 17-hydroxycorticosteroids, and a release of glucose into the blood stream.[27,28] Corticotropin-releasing factor (CRF) is a major signaling molecule released by hypothalamus into the portal circulation. Both of CRF and glucocorticoid receptor are found to localize in the cochlea. In addition, CRF expression has been described in the cochlea, indicating that not only the start point (CRF) but also the end point (glucocorticoid receptors) of the systemic HPA axis signaling are expressed in the cochlea.[29] It is believed that cochlea is an independent neuroendocrine organ. Besides other extra-hypothalamic that impacts within the brain, these hormones increase the activity of the HPA-axis and the release of corticosterone (eg, cortisol) from the adrenal cortex.[30] Therefore, noise may affect the HPA-axis through the auditory

system and result in the increased cortisol,[31] which has been also confirmed in our study. Our results show that the level of cortisol in the plasma of stressed groups is higher than that of control group under noise stress. That maybe due to activate HPA axis and stimulate cochlea.

The secretion of MT with circadian rhythm in the blood of mammals is functionally linked to the adjustment of 24-hour cycles and to the circannual rhythm regulation.[32] And the circadian change of MT is opposite to that of HPA-related hormones, suggesting maybe a connection between these two factors. It is reported that MT could reverse the HPA-axis activation induced by different stress which is similar to our results.[33] However, our results also indicate that cortisol of blood plasma in experimental groups is different from that in control group, indicating the limitation of MT effect.

A growing number of studies have shown that stress could induce GIMD and gastric stress ulcer. For experimental animals, accumulating evidences have demonstrated that the most consistent patterns of GI motility alternations induced by various stresses (eg, restraint stress, foot shock stress, cold stress, and immersion stress) including delayed gastric emptying[32,34,35] and accelerated colon transit.[36,37] In the present study, noise stress inhibits gastric emptying of rats, which is supported by previous research.[32,34,35] However, there are some controverts about small intestine motility under stress in previous studies. Some reports show that stress restrains small intestine motility,[38,39] whereas the others suggest an accelerating effect.[40,41] This may be attributed to the differences in the type of motility tested, complexity of the tasks, manner of stress induction, and the experimental protocols. We find that the noise stress accelerates food march in small intestine. Based on our results, the Guth injury score and pathology observation show that noise stress could produce gastric stress ulcer. In support of our findings, numerous reports indicate gastric stress ulcer is closely associated with stress including psychological stress[42] and water immersion stress.[43]

The underlying mechanism of GIMD and gastric stress ulcer may be associated with abnormal excrete of gut hormones as a result of HPA axis activation. The present study reveals that noise stress increases motilin of but decreased CGRP of blood plasma. CGRP and motilin are known as key factors of gastric and duodenum ulcer. Besides regulating blood current and GI motility, CGRP maintains the homeostasis of intracellular Ca^{2+} and decreases the permeability of cell membrane to Ca^{2+}.[44] Therefore, it can affect Ca^{2+} concentrations in and outside the cell, a key factor in the relaxation-contraction activities. In the

previous study, gastric CGRP levels are found to decrease rapidly under water immersion restraint stress in both male and female mice.[45] It is also suggested that both of mucosal expression of the CGRP gene and its serum levels are decreased with gastric damage in rat water immersion restraint stress model.[46,47] It indicates that stress could down-regulate the level of CGRP, in both of serum and gastric mucosa which is similar to our results. The CGRP level in blood is a crucial regulating factor for gastric acid and ulcer development, and the exogenous CGRP can prevent lesion and accelerate lesion healing.[48,49] Motilin can facilitate the intra-cellular calcium library to release Ca^{2+}, which results in the contraction of smooth muscle, the acceleration of GI motility, the elevation of lower esophageal sphincter pressure and the contract cholecyst.[50] Moreover, motilin participates in initiating the migrating motor complex (MMC) in the stomach, stimulates GI motility and accelerates gastric emptying.[51] A previous study continuously monitored of fecal occult blood and GI motility and analyzes the plasma motilin concentration, GI endoscopy and gastric emptying in dogs. The results show that motilin is connected with ulcer and GI motility induced by nonsteroidal anti-inflammatory drugs.[52] The motilin level in rats is also related to ulcerated antral mucosa tissue.[53]

Enterochromaffin cells in GI tract could secrete MT and motilin.[54] The finding that the concentration of MT in the GI tissues surpasses that in the blood by 10-100 times and in pineal gland by nearly 400 times suggests MT may also play an important role in digestive system.[55] It has been shown that central nervous stimuli can increase duodenal bicarbonate secretion by release of mucosa MT.[56] MT is reported to inhibit irregular spiking activity and reinforces the cyclic MMC pattern.[57] These results suggest that MT affects GI motility in rats, which might be mediated by MT receptors in the GI tract, although central receptors for the hormone is possibly involved.[58] Results of lots of articles suggest that CGRP plays an important role in the pathogenesis of gastric ulcer[45,47,59] and GI motility disorder.[60] Exogenous CGRP shows protective effect to reflux esophagitis[61] and gastric mucosa ulcer.[48,49] However, it is reported that GIMD is observed in patients with ulcers and those with non-ulcer dyspepsia compared with asymptomatic controls.[62] That may indicate that gastric ulcer may influent GI motility itself. With the MT treatment, CGRP of blood plasma in experimental group rats was significantly different from that in stressed group rats. CGRP is involved in regulation of GIMD due to stress, no matter by affecting GI motility directly or through preventing gastric ulcer. These observations indicate that MT can reverse GIMD

and gastric stress ulcer maybe through adjusting gut hormone secretion derangement induced by noise stress.

An important form of management for gastric ulcer diseases is to administer agents that strengthen the gastric mucosal barrier by reducing oxidative stress. Recent studies show that oxidative stress is involved in stress ulcer and GIMD in many animal stress models and peptic ulcer diseases of human beings. MT, the primary secretory product of the pineal gland, is a potent antioxidant and has been shown to be highly effective in reducing oxidative stress. In addition, MT or L-tryptophane accelerates ulcer healing with omeprazole treatment and this likely depends mainly upon the significant increments in plasma MT.[63] Lipid peroxidation resulted from unsaturated fatty acids could induce the injury in biological membrane by oxygen free radicals. Malondialdehyde[64] level and superoxide dismutase activity are used to estimate the oxidative stress in degrading and eliminating oxygen free radicals caused by lipid peroxidation[65] that is also performed in the present study. It is well known that oxygen free radicals play an important role in gastric stress ulcer[66] induced by different stress. In our study, noise stress leads to an increased lipid peroxidation of blood and gastric mucosa, as indicated by an increased malondialdehyde level of and a decreased superoxide dismutase activity.

MT is considered to be of a series of properties that would characterize an ideal free radical scavenger. It is believed that MT is present in all cells[67] and concentration is in adequate amounts to protect tissues. Furthermore, MT is a broad-spectrum antioxidant by scavenging hydroxyl radical, alkoxyl radical, peroxyl radical, nitric oxide, and singlet oxygen. As an antioxidant, MT may be regenerated after radical quenching through different processes.[68] It can also assist in stimulating antioxidant enzymes including superoxide dismutase, glutathione, peroxidase, and so on.[69] MT is also an indirect antioxidant[70,71]; metabolites of MT including N1-acetyl-N2-formyl-5-methoxykynuramine and N1-acetyl-5-methoxykynuramine possess free radical-scavenging activities.[72] After reaction between MT and a free radical, it yields an oxidized form of itself; its reactivity is so low that it is not toxic to cells.[73] Many reports have shown that acute and chronic toxicity of MT is extremely low.[74] Doses even up to one g daily by human volunteers revealed no negative side effects.[75] There is widespread agreement that MT has minimal toxicity over a very wide dose range. Taken together, MT has most of characteristics of an ideal free radical scavenger.

However, molecular biology methods are not used in the present study that may provide further evidence for related mo-

lecular mechanism. It is suggested that MT protects the gastric damage under diabetes through regulation of both matrix metalloproteinase-1 and -13 which are mediated by activator protein-1 activation via extracellular signal-regulated kinase 1/2.[76] We have focused on the MT as a free radical scavenger but the effect on the matrix metalloproteinase would also be of great interest for future studies.

In conclusion, the main finding of this study is that MT is a protective agent that prevents GIMD and gastric stress ulcer associated with noise stress. The possible mechanism may be attributed to its ability to reduce oxidative stress and attenuate the activation of HPA-axis as well as gut hormone disorder. Since MT has not been found to be of toxic activities in the body and its lethal dose 50 cannot be established in many animals, it may be relatively safe for clinical application.

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