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Worsening effect of partial sleep deprivation on indomethacin-induced gastric mucosal damage

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Abstract

The present study was to investigate the roles of cyclooxygenase-1 and -2 (COX-1 and COX-2) and prostaglandin (PG) on gastric mucosal integrity of partially sleep deprived (PSD) rats. A slowly moving drum was used to induce PSD. The PG levels in the gastric mucosa of PSD rats, with or without indomethacin or rofecoxib treatment, were determined. Exogenous prostaglandin E (PGE) analog, misoprostol, was administered to PSD rats to investigate the modulating effect of PG in indomethacin-induced gastric damage. It was observed that COX-1 mRNA and protein were up-regulated in the gastric mucosa of PSD rats. Selective COX-2 inhibition by rofecoxib failed to decrease mucosal PGE_2 levels nor to affect mucosal integrity in both PSD and sleep undisturbed rats. However, indomethacin, a COX-1 preferential non-selective COX inhibitor, significantly reduced mucosal PGE_2 content and produced more severe mucosal damage in PSD rats than in the controls. The deleterious effect of indomethacin on gastric mucosal integrity of PSD rats was significantly attenuated with the administration of misoprostol. These results suggest that PSD enhances COX-1 biosynthesis of gastroprotective PGE_2 as an adaptive response of the stomach to stress. The administration of non-selective COX inhibitors to subjects with chronic sleep deprivation may induce more gastric damages.

Keywords: Partial sleep deprivation; Stress; Cyclooxygenase; Indomethacin; Prostaglandin

1. Introduction

The stomach is a stress vulnerable organ that can be damaged by a variety of stresses such as sepsis, burn injury, trauma and multiple organ failure. Stress has been considered as a major risk factor for peptic ulcer diseases regardless of *Helicobacter pylori* infection or the use of non-steroidal anti-inflammatory drugs (Levenstein, 1998). Several mechanisms have been implicated in the pathogenesis of stress-induced gastric lesions, including alterations of gastric luminal factors, mucosal blood flow, gastric motility, superoxide generation, epithelial cytoprotectants, cell proliferation, and neuroendocrine mediators. The pathogenesis of stress-induced gastropathy is complex and may vary with different forms of stress.

Sleep is an essential biological behaviour of animals and humans. Sustained sleep loss imposes stress on the body and may have significant contribution to a variety of health problems, including gastrointestinal disorders (Naitoh et al., 1990; Orton and Gruzelier, 1989; Rechtschaffen and Bergmann, 2002). Although epidemiological studies have demonstrated that sleep deprivation is a risk factor for peptic ulcers (Levenstein, 2000), and can induce gastric lesions in sleep deprived animals (Murison et al., 1982; Guo et al., 2005), the effects and mechanisms of sleep deprivation on gastric mucosal integrity have not yet been fully elucidated.

It is well known that endogenous PG plays a crucial role in the maintenance of gastric mucosal integrity. It is involved in mucus and bicarbonate secretions, gastric microcirculation, epithelial cell turnover, and function of mucosal immunocytes (Miller, 1983). Supplementation of PG analogs have been shown to reduce gastric damages induced by different kinds of stress (Brzozowski et al., 1993; Ranta-Knuuttila et al., 1989; Victor et al., 1989; Yoshimura et al., 1989). There are two isoforms of cyclooxygenase in catalyzing the first committed step in the biosynthesis of PG, i.e., the conversion of arachidonic acid to prostaglandin H_2. It is generally accepted that the constitutive isoform of cyclooxygenase, cyclooxygen-

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ase-1 (COX-1), is highly expressed in normal gastric tissues, and produces cytoprotective PG. The ulcerogenic property of conventional non-steroidal anti-inflammatory drugs (non-selective COX inhibitors), such as aspirin and indomethacin, is mainly related to their inhibition of COX-1 in the gastric and duodenal mucosae. Another isoform of cyclooxygenase, cyclooxygenase-2 (COX-2), which is encoded by a different gene and can be induced by various stimuli, appears to contribute to the inflammatory reaction and wound healing in the stomach. Its expression is normally low or undetectable in healthy gastric tissues (Kargman et al., 1996). However, when rats were exposed to water-immersion restraint stress or long-term endotoxin administration, both COX-1 and COX-2 mRNA were found to be up-regulated in the gastric mucosa (Ferraz et al., 1997; Kato et al., 2002; Konturek et al., 1998), indicating a possible role of both COX isoforms and their derived PG in the homeostatic protective response of the gastric mucosa to various kinds of stress (Brzozowski et al., 2000).

The roles of the endogenous PG/COX system in the response of the gastric mucosa to psychosomatic stress induced by partial sleep deprivation (PSD) are unknown. Our previous studies have demonstrated that PSD compromised gastric mucosal integrity in rats (Guo et al., 2004, 2005). Increased gastric acid secretion and reduced gastric mucosal blood flow were suggested to be involved in the PSD-induced gastric mucosal damage. All of these could be related to changes in PG production by the COX enzymes. Since down-regulation of the PG/COX system may weaken the gastric mucosal defense in PSD rats, while up-regulation as a homeostasis response of gastric mucosa to stress may confer resistance of the gastric mucosa to damage. The objective of the present study was therefore to investigate the involvement of COX isoforms and PG in the maintenance of mucosal integrity in PSD rats. The changes in mRNA and protein expressions of COX-1 and COX-2 in the gastric mucosa of PSD rats were determined. The gastric levels of PGE_2 in rats treated with indomethacin or rofecoxib were also examined.

2. Materials and methods

2.1. Induction of partial sleep deprivation

The protocol for PSD was approved by the Committee on the Use of Live Animals for Teaching and Research of The University of Hong Kong. Male Sprague Dawley rats weighing 160±20 g were housed in a temperature (22±1 °C) and humidity (65–70%) controlled room. They were fed with a standard laboratory chow (Ralston Purina Co., Chicago, IL, USA) and tap water ad libitum. Procedures for partial sleep deprivation were described previously (Guo et al., 2004, 2005; Shen et al., 2000). Briefly, rats for PSD were placed inside specially constructed slow revolving cylindrical drums (Fig. 1). An opening on the cage allows free handling of the rats and bottle of water for drinking can be inserted through an inlet hole on the side wall of the drum. The drum was belt driven with a slow moving motor, which was set to one rotation per 2 min. It was programmed to switch off at 3:00 p.m. to 4:00 p.m. daily, permitting the animals to have an hour of undisturbed sleep. Partial sleep deprivation was conducted continuously for 14 days. The control rats were left undisturbed inside stationary drums for the same period of the experiment.

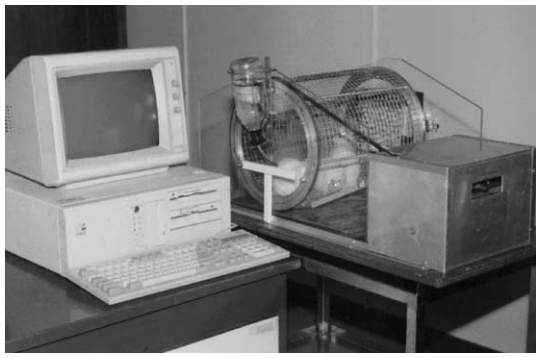

Fig. 1. Photograph of the sleep deprivation drum.

2.2. Collection of gastric samples

Six each of the PSD (PSD group) and sleep undisturbed control (S group) animals were killed at day 14 by cervical dislocation. Their stomachs were excised and opened along the greater curvature followed by rinsing with cold saline. Gastric mucosal samples were obtained by gently scraping the inner wall of the stomach with a glass slide in ice-cold condition. The samples were then frozen in liquid nitrogen and stored at −70 °C until assayed.

2.3. RT-PCR for COX mRNA expression

Total RNA was extracted from the gastric mucosa with Trizol reagent (Gibco BRL, Gaitherburg, MD, USA). First-strand complementary DNA was synthesized from 5 μg RNA using oligo dT_{20} primer and Thermoscript RT-PCR system (Gibco BRL). PCR cycles were performed for COX-1, COX-2 and β-actin from the same complementary DNA sample in a PCR Thermal Cycler (Gene Amp PCR System 9700, The Perkin-Elmer Corporation, Norwalk, CT, USA). The oligonucleotide primers were designed according to previously published sequences. The primers for COX-1 were sense 5′-TGCTGCTGAGAAGGGAGTTCATTC-3′ and antisense 5′-CAAGTCACACACACGGTTATGCTC-3′ (Genbank accession no. S67721, nt 548–571 and 928–951, with 404 bp amplicon). For COX-2, the primers used were sense 5′-ACACTCTATCACTGGCATCC-3′ and antisense 5′-GAAGGGACACCCTTTCACAT-3′ (S67722, nt 1229–1249 and 1794–1813, with 585 bp amplicon). β-actin was employed as an internal control, and the primers for β-actin were sense 5′-GTGGGGCCGCCCTAGGCACCA-3′ and antisense 5′-CTCCTTAATGTCACGCACGATTTC-3′ (BC063166, nt 184–203 and nt 700–723, with 539 bp amplicon). The PCR programme for COX-1 and β-actin, which was prepared in a 25 μl mixture containing 2 mM dNTP, 1× PCR buffer, 15 mM $MgCl_2$ and 0.025 U of Taq DNA–polymerase (Gibco-PRL, Gaithersburg, MD), consisted of 30 cycles at 94, 55, and 72 °C

each for 1 min, followed by a final extension at 72 °C for 10 min. For COX-2 the optimized PCR reaction was carried out for 36 cycles at 94, 54, and 72 °C each for 1 min. After amplification, 10 μl of PCR products were electrophoresed in a 1% agarose (Gibco BRL) gels containing 0.5 μg/ml ethidium bromide. Localization of the predicted products was performed using ϕX 174 RF DNA/Hae III fragments (Gibco BRL) as standard size markers. The intensity of bands was quantified with a computerized densitometer. The amplification signal of COX cDNA fragment was standardized against the β-actin signal for each sample and the result was expressed as COX/β-actin mRNA ratio.

2.4. Western blot analysis for COX protein

Gastric mucosal samples were homogenized at 4 °C in homogenizing buffer solution (50 mM Tris/HCl, pH 7.5, 150 mM NaCl, 0.1% sodium dodecyl sulfate, 0.5% α-cholate, 2 mM EDTA, 1% Triton X-100, 10% glycerol) containing 1 mM phenylmethylsulfonyl fluoride (Sigma Chemical Co, St Louis, MO, USA) and 10 μg/ml aprotinin. After centrifugation at 10,000 *g* at 4 °C for 20 min, the supernatant was collected and the protein content was determined with the dye-binding method (Bio-Rad, Hercules, CA, USA). The same amount of total protein (40 μg) of each sample were loaded onto a SDS–polyacrylamide gel and blotted onto Hybond-C membranes (Amersham Life Science, Little Chalfont, Buckinghamshire, England) by electrophoresis. Pre-stained rainbow recombinant protein molecular weight markers (Amersham International plc, Little Chalfont, Buckinghamshire, England) were used for molecular weight determination. Membranes were blocked with a buffer containing 5% non-fat milk powder, 10 mM Tris/HCl (pH 7.5), 100 mM NaCl and 0.1% Tween 20 for 1 h at room temperature. The blots were incubated overnight at 4 °C with 1:500 dilutions of polyclonal antibodies against COX-1, COX-2, and β-actin (Santa Cruz Biotechnology INC, Santa Cruz, California, USA). They were then washed 6 times and incubated with rabbit–anti-mouse immunoglobulin G conjugated (1:5000) with horseradish peroxidase (Bio-Rad) for 1 h. After six changes of additional washing, the blots were developed by an ECL Western blotting system (Amersham, Arlington Heights, IL, USA) in accordance to the manufacturer's instructions for chemiluminescence of proteins and then exposed to photographic films (Fuji Photo Film Co, Tokyo, Japan). The bands of COX-1, COX-2 and β-actin proteins were quantified with densitometry. The signals of COX protein bands were normalized with their corresponding β-actin signal and the results were expressed as COX protein/β-actin ratio.

2.5. Administration of COX inhibitors

Rats were randomly assigned into 6 groups of 12 rats each. PSD was conducted for 14 days in 3 groups of rats before the administration of indomethacin (PSDI group), rofecoxib (PSDR group), or methylcellulose vehicle (PSD group). Rats in the remaining three groups were similarly treated with indomethacin (SI group), rofecoxib (SR group), or methylcellulose (S group) but were allowed to sleep undisturbed. At day 14, after an overnight fast, rats in groups PSDI and SI were orally administered indomethacin, suspended in 0.5% methylcellulose (Sigma), in a dose of 20 mg/kg 6 h before gastric samples collection. Groups SR and PSDR were similarly treated with rofecoxib (Merck and Company, Inc., Rahway, NJ) in a dose of 10 mg/kg. The doses of indomethacin and rofecoxib used in the present study have previously been shown to inhibit gastric PG production, induce mucosal damage (Gretzer et al., 2001; Ferraz et al., 1997), and delay ulcer healing (Guo et al., 2002). Groups PSD and S were treated with the vehicle, i.e., 0.5% methylcellulose. All animals were sacrificed 6 h after treatments and their stomachs were removed for gastric damage assessment. Gastric mucosal samples were obtained by scraping with an ice-cold glass slide and stored at −70 °C until used for PGE_2 determination.

2.6. Measurement of PGE_2 level in gastric mucosal tissues

Gastric tissues were homogenized in ice-cold Tris/HCl buffer containing 50 mM Tris/HCl (pH 7.4), 100 mM NaCl, 1 mM $CaCl_2$, 1 mg/ml D-Glucose, and 28 μM indomethacin. Protein level in the homogenate was determined with the dye-binding method (Bio-Rad). The tissue homogenate was boiled and centrifuged at 12,000 *g* for 30 min before the PGE_2 level in the supernatant was determined with a PGE_2 immunoassay kits (R&D Systems, Inc. Minneapolis, MN, USA). The results were expressed as nanograms PGE_2 per milligram protein.

2.7. Administration of prostaglandin analog

The hypothesis that indomethacin-induced gastric mucosal damage in PSD and sleep undisturbed rats involves inhibition of PGs was evaluated with the use of misoprostol, a prostaglandin analog. Rats were randomly divided into 4 groups of 12 rats each. PSD was conducted for 14 days in 2 groups (PSDIM group and PSDI group), while the remaining two groups (SIM group and SI group) acted as sleep undisturbed controls. Indomethacin was administered to all of the PSD and sleep undisturbed rats at day 14 with similar treatment protocol as previously described. Misoprostol (300 μg/kg suspended in 0.5% methylcellulose) was orally given to rats in the PSDIM and SIM groups 5 min after indomethacin administration. Gastric mucosal damages were assessed in all rats after 6 h of drug administration.

2.8. Measurement of gastric damage

Gastric mucosal damage was examined by an experienced investigator blinded to the treatments given. Lesion size as observed under an illuminated magnifying lens (3×) was determined by summing up each lesion along its greatest length. In case of petechiae, five such lesions were taken as the equivalent to 1 mm lesion.

The lesions length (mm) of gastric mucosa in PSD or sleep undisturbed rats with or without the administration of COX inhibitors were scored with an arbitrary 0–4 scale as described

by Stroff et al. (1996) and Peskar et al. (2002), with modification. Briefly, the lesions were scored as 0=no lesion, 1=lesions <4 mm, 2=lesions 4–8 mm, 3=lesions 8–12 mm, and 4=lesions >12 mm. The sum of the lesion length (mm) in each group was divided by the number of rats in that group and expressed as the mean lesion index.

2.9. Statistical analysis

Wilcoxon rank test was performed in the statistical analysis of nonparametric data on gastric lesion scores. For other data, statistical differences between groups were determined with one-way ANOVA followed by post hoc Bonferroni test, or unpaired, two-tailed, Student's *t*-test where appropriate (SPSS statistical package, version 11.0, SPSS Inc., Chicago, IL). All data are expressed as means±S.E.M. Values of $P<0.05$ were considered statistically significant.

3. Results

3.1. Increased expression of COX-1 mRNA and protein in the gastric mucosa of PSD rats

As was shown in Fig. 2A, the expression of β-actin mRNA was well preserved in the mucosal samples taken from PSD and sleep undisturbed rats. COX-1 and COX-2 mRNA were detectable in normal gastric mucosa and their expressions were markedly increased in rats exposed to PSD (Fig. 2A). The ratio of COX mRNA over β-actin mRNA confirmed that the expressions of COX-1 and COX-2 were increased in rats of PSD group when compared with the S group (Fig. 2B).

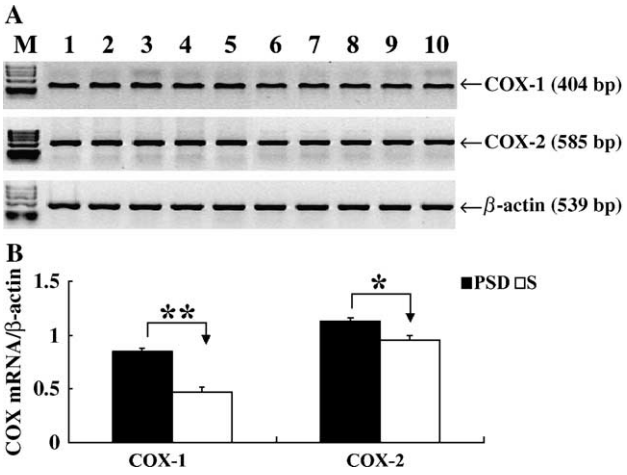

Fig. 2. (A) mRNA expression for cyclooxygenase-1 (COX-1), cyclooxygenase-2 (COX-2), and β-actin in the gastric mucosa of partially sleep deprived (PSD) (lane 1–5) and sleep undisturbed (S) (lane 6–10) rats. The mRNA expressions of COX-1 and COX-2 were all detectable in the gastric mucosa of sleep undisturbed rats and their expression was markedly increased in PSD rats. ϕX 174 RF DNA/Hae III fragments (M) were used as standard size markers to confirm the locations of the predicted products. (B) The ratios of COX-1 and COX-2 mRNA signals over their corresponding β-actin mRNA signal confirmed that their expressions were increased in the gastric mucosa of PSD rats (PSD) when compared with the sleep undisturbed rats (S). *$P<0.05$, **$P<0.01$ when compared with the S group.

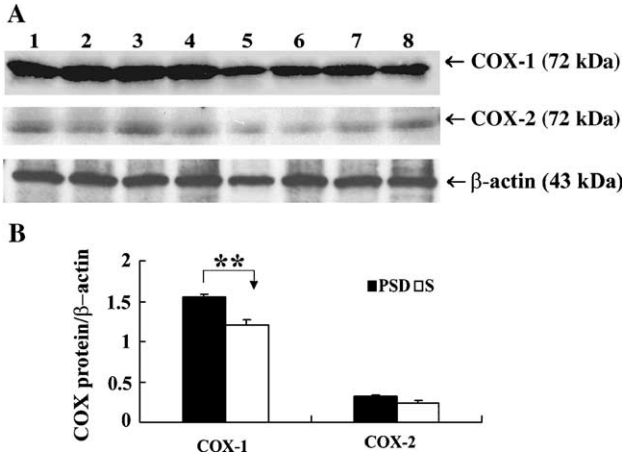

Fig. 3. (A) Western blot analysis of cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) proteins in the gastric mucosa of partially sleep deprived (PSD) (lane 1–4) and sleep undisturbed (S) (lane 5–8) rats. A basal level of COX-1 protein and a trace amount of COX-2 protein were detectable in the gastric mucosa of sleep undisturbed rats. (B) The ratios of COX-1 and COX-2 protein signals over their corresponding β-actin signal confirmed that the expression of COX-1, but not COX-2, was significantly increased in the gastric mucosa of PSD rats (PSD) when compared with the sleep undisturbed rats (S). **$P<0.01$ when compared with the S group.

A basal level of COX-1 protein and a trace amount of COX-2 protein were detectable in the gastric mucosa of sleep undisturbed rats (Fig. 3A). The ratio of COX protein over β-actin protein indicated that the protein level of COX-1 but not COX-2 in the gastric mucosa was significantly increased in the rats exposed to PSD (Fig. 3B).

3.2. Indomethacin but not rofecoxib induced substantial gastric mucosal damage in PSD rats

Mild lesion was found in the corpus and/or pylori of stomach in about 30% of the PSD rats. No lesion was found in the stomachs of sleep undisturbed rats (Fig. 4). The lesion score of PSD rats is higher than sleep undisturbed rats with or without rofecoxib treatment (Fig. 4). Indomethacin administration induced substantial gastric mucosal damage in PSD rats

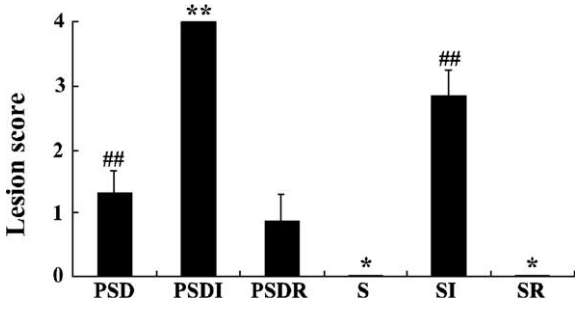

Fig. 4. Lesion score of the gastric mucosal damage. No lesion was found in the stomachs of sleep undisturbed rats with (SR) or without (S) rofecoxib treatment. The administration of indomethacin produced substantial gastric mucosal damage in PSD rats (PSDI) and the damage was much more severe when compared with the sleep undisturbed rats (SI). There was no difference between the lesion scores of PSD rats with (PSDR) and without (PSD) rofecoxib treatment. *$P<0.05$, **$P<0.01$ when compared with the corresponding PSD groups; ##$P<0.01$ when compared with the PSDI group.

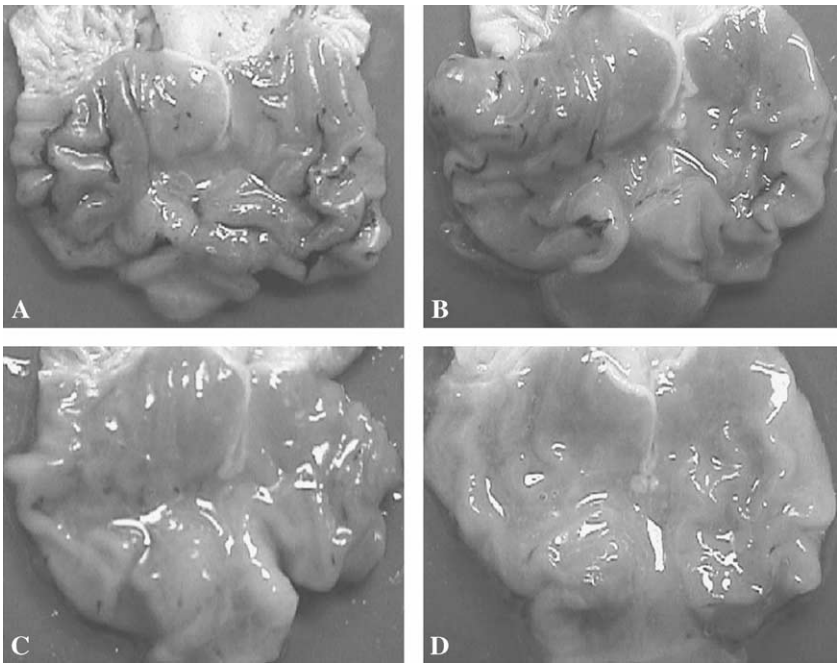

Fig. 5. Gross appearance of indomethacin-induced gastric mucosal damage. Indomethacin administration produced substantial gastric mucosal damage in PSD rats (A) and the damages were much more severe than the sleep undisturbed rats (C). Oral administration of 300 μg/kg misoprostol significantly reduced the indomethacin-induced gastric mucosal damage in PSD rats (B) as well as in sleep undisturbed rats (D).

and the damage was much more severe when compared with the sleep undisturbed rats (Figs. 5A,C and 6). On the contrary, the lesion scores of PSD rats treated with rofecoxib were not different from their vehicle-treated PSD controls (Fig. 4). No gastric lesion was observed in the sleep undisturbed rats treated with rofecoxib.

3.3. Differential effect of indomethacin and rofecoxib on PGE_2 synthesis in gastric mucosa of PSD rats

The PGE_2 level in the gastric mucosa of PSD rats was significantly increased when compared with the sleep undisturbed rats (Fig. 7). Indomethacin significantly decreased mucosal PGE_2 level in both the PSD and sleep undisturbed rats, while rofecoxib treatment did not change the mucosal PGE_2 levels in both groups (Fig. 7).

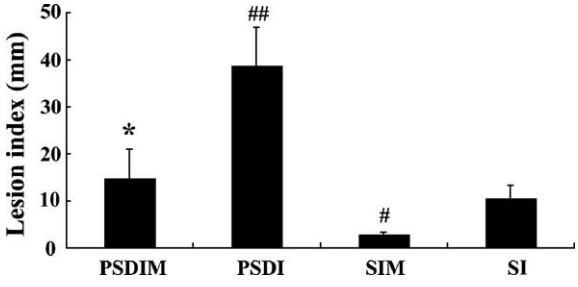

Fig. 6. Lesion index of indomethacin-induced gastric damage. The lesion index of indomethacin-induced gastric mucosal damage in partial sleep deprived rats (PSDI) was significantly higher than the sleep undisturbed rats (SI). Oral administration of 300 μg/kg misoprostol significantly reduced the lesion index of indomethacin-induced gastric mucosal damage in PSD rats (PSDIM) as well as in sleep undisturbed rats (SIM). *$P<0.05$ when compared with PSDI group; $^{\#}P<0.05$, $^{\#\#}P<0.01$ when compared with SI group.

3.4. Protective effect of misoprostol on indomethacin-induced gastric damage

Oral administration of 300 μg/kg misoprostol significantly protected the PSD rats as well as sleep undisturbed rats against indomethacin-induced gastric mucosal damage (Figs. 5B,D and 6).

4. Discussion

The results of this study showed that partial sleep deprivation for 14 days up-regulated the expression and activity of COX in the gastric mucosa of rats. Moreover, PSD increased the susceptibility of the gastric mucosa to indomethacin-induced injury in rats. Exogenous supplementa-

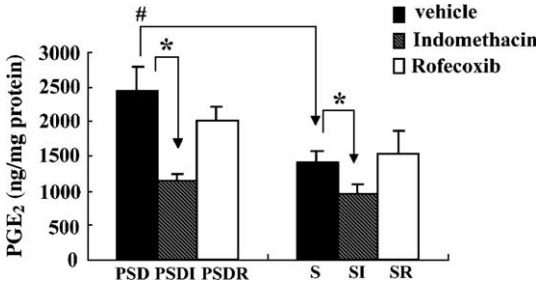

Fig. 7. Effects of indomethacin and rofecoxib on PGE_2 synthesis in the gastric mucosa. The PGE_2 level in the gastric mucosa of PSD rats (PSD) was significantly higher than the sleep undisturbed rats (S). Indomethacin administration decreased mucosal PGE_2 level in both PSD (PSDI) and sleep undisturbed rats (SI). While rofecoxib treatment did not change the mucosal PGE_2 level significantly in the PSD (PSDR) and sleep undisturbed rats (SR). *$P<0.01$ when compared with the indomethacin treated groups; $^{\#}P<0.05$ when compared with the S group.

tion of misoprostol to PSD and sleep undisturbed rats reversed the indomethacin-induced mucosal damage. These results suggested that the increased expression of COX as well as PG production in the gastric mucosa of PSD rats might be a protective homeostatic response of the stomach to stress produced by PSD.

PGE_2 level was found to be elevated in the stomach of PSD rats, although the mucosal integrity was compromised. This result indicated that suppression of COX activity or PG production was not involved in the mechanisms of PSD-induced mucosal damage. Our previous findings have shown that PSD increased gastric acidity, plasma levels of noradrenaline, gastrin, and histamine but reduced gastric mucosal blood flow (Guo et al., 2005). The up-regulation of COX activity and PG production in the gastric mucosa of PSD rats, as was demonstrated in the present study, may therefore be a protective homeostatic response of the stomach to these stressful changes, thus minimizing gastric injury. This was supported by the finding that further inhibition of COX activity and PG production by indomethacin, a non-selective COX inhibitor, aggravated gastric lesions in PSD rats. It is possible that the increased prostaglandin production observed after partial sleep deprivation is not sufficient to counteract the processes that produce damage, as it has already been found that a consecutive administration of misoprostol for 5 days could decrease PSD-induced gastric mucosa damage (unpublished data). It is well known that exposure of the stomach to mild irritants can increase its resistance to necrotic injury (Robert et al., 1983; Takeuchi et al., 2002). This type of gastric protection, termed as adaptive cytoprotection, is largely mediated by endogenous PG. This response was also observed in long-term administration of endotoxin and aspirin (Ferraz et al., 1997). Similar phenomenon was reported in PSD rats as it was found that PSD reduced HCl-induced gastric damage (Guo et al., 2004). These results suggest that PSD imposes stress on the gastric mucosa and induces a protective increase of PG production in the stomach. Besides PG produced by COX pathway, leukotrienes (LT) produced by lipoxygenase pathway of arachidonic acid metabolism has also been documented to be associated with enhanced injury susceptibility of the rat stomach to cold-restraint stress. Whether there is a role of LT in the enhanced susceptibility of gastric mucosa of PSD rats to injury, warrant further investigation.

The results of the present study also demonstrated that the increased production of PG in PSD rats was most likely due to the activity of COX-1 rather than COX-2. This was supported by the finding that the expression of COX-1 protein, but not COX-2, was increased in the gastric mucosa of PSD rats. In addition, indomethacin, a COX-1 preferential non-selective COX inhibitor, significantly inhibited the mucosal PG synthetic capacity of PSD rats; whereas rofecoxib, a highly selective COX-2 inhibitor, when used in a dose sufficient to suppress PG production in gastric ulcer tissues (Guo et al., 2002), did not affect mucosal PG synthesis in PSD rats. Similar findings were observed in the sleep undisturbed rats. These results provided strong evidence for the importance of endogenous PG in the maintenance of mucosal integrity, and that COX-1 rather than COX-2, is important for the protection of the stomach against mild and persistent stress, such as PSD. This is in line with previous findings that COX-1 plays an important role in minimizing mucosal damage by noxious agent (Gretzer et al., 2001). However, the present data can not rule out the requirement for inhibiting both COX-1 and COX-2 in the worsening effect of PSD on indomethacin-induced gastric mucosal damage. Further study with the use of a highly selective COX-1 inhibitor is needed to delineate this issue.

The regulations of COX-1 and COX-2 expression have been studied in many in vitro and in vivo systems. In most instances, COX-2 was found to be regulated by a variety of stimuli, whereas COX-1 behaved as a house keeping gene, which is constitutively expressed in most cell types. COX-2 can be up-regulated 20-fold in macrophages, monocytes, synoviocytes, chondrocytes, fibroblasts and endothelial cells by various inflammatory or mitogenic stimuli. In contrast, COX-1 activity is unaffected or only marginally (2–4 fold) increased (Brooks et al., 1999). It has been shown that both COX-1 and COX-2 mRNA are constitutively expressed in normal gastric mucosa with COX-1 protein and activity predominant. The expression of COX-2 in normal gastric mucosa has previously been reported (Tomomasa et al., 2002; To et al., 2001), and this was also observed in the present study. The increase in COX-2 mRNA but not its protein level in the gastric mucosa of PSD rats suggested that there may be a posttranscriptional down-regulation of COX-2 protein expression, thus contributing little to the PG production in PSD rats. There was also no excessive infiltration of inflammatory cells, which are capable of generating PG from COX-2, in the gastric mucosa of rats subjected to PSD (Guo et al., 2005). Thus COX-1 expression and its moderate increase in protein activity could have contributed to the enhanced PG production in these PSD animals. The exact mechanism of this adaptive change of COX-1 expression in the gastric mucosa of PSD rats is currently unknown, and whether this involves changes in neuroendocrine mediators, gastric hyperacidity or hypoanemia warrants further investigations.

Conventional non-steroidal anti-inflammatory agents are well recognized to produce gastric mucosal damage due to their capacity to inhibit COX-1 and the synthesis of gastric protective PG. The newly developed highly selective COX-2 inhibitors significantly reduce the risk of gastrointestinal damage by sparing COX-1 activity (Bombardier et al., 2000; Cannon and Breedveld, 2001; Weaver, 2001). However, COX-2 inhibitors still exacerbate inflammation-associated injury in the stomach and block angiogenesis that is essential for the healing of gastric ulcer, thus raising doubt of their use in patients with this gastric condition (Guo et al., 2002). In the present study, indomethacin, but not rofecoxib, was found to exacerbate PSD-induced gastric mucosal damage in rats, suggesting that it may not be safe to use non-selective NSAIDs in subjects with sleep deprivation. Further evidences from clinical and epidemiological studies are needed to substantiate this potential adverse effect of NSAIDs in PSD subjects.

In conclusion, the present study demonstrates that PSD imposes stress on the gastric mucosa and activates an adaptive response of the stomach by increasing biosynthesis of gastroprotective prostaglandins. COX-1 derived PG plays an important role in gastric protection and care should be taken in PSD subjects using non-selective COX inhibitors.

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