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Morphine as a drug for stress ulcer prevention and healing in the stomach

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Abstract

Morphine pretreatment protects against stress-induced gastric ulceration, however, the exact mechanism is still undefined. Interestingly, the effect of morphine on ulcer healing has not been investigated. In this report, we would like to study these effects in a defined stress ulcer model and to delineate a new implication for morphine to promote stress ulcer healing in rats. Our study showed that cold-restraint stress for 3 h induced hemorrhagic lesions and increased myeloperoxidase activity in the gastric mucosa. Stress also reduced the dimension of layer of periodic acid-Schiff reagent-stained cells in the gastric mucosa by about 50%. Morphine pretreatment (2 or 8 mg/kg, given intraperitoneally) at the time of stress dose-dependently reversed stress-induced gastric ulceration, increase of myeloperoxidase activity and reduction of thickness of mucus-stained cells in the gastric mucosa. Morphine treatment after stress (given at the end of a 3-h stress and also at 3 h thereafter) increased ulcer healing by reducing the ulcer size measured 24 h later. Such action was blocked by naloxone (8 mg/kg) given intraperitoneally 15 min before morphine treatment. Morphine also increased the number of cell proliferation and dimension of layer of cells stained for mucus but not the number of microvessels in the gastric mucosa. Moreover, the number of apoptotic cells was less evidenced in the morphine-treated rats. This study reports for the first time that morphine not only prevents stress ulceration but also promotes healing of stress ulcer through a defined mechanism.

Keywords: Cell proliferation; Gastric ulcer; Morphine; Mucus; Stress; Myeloperoxidase

1. Introduction

It has been suggested that endogenous opioids released during stress may attenuate the pathological effects of stress. Indeed, morphine treatment prior to cold-restraint stress reduced ulcer severity (Glavin, 1985). It was postulated to be due to the activation of kappa opioid receptor (Ray et al., 1993) and induction of prostaglandin production in the gastric mucosa (Ferri et al., 1983). However, blockade of the opioid receptor gave controversial effects. It was reported that opioid receptor antagonists could worsen gastric ulceration (Glavin, 1985; Arrigo-Reina and Ferri, 1980). Other report showed that these blockers protected against the formation of stress ulcer in the stomach (Morley et al., 1982; Dai and Chan, 1983). How exactly opioid receptor involves in stress ulceration and the mechanisms underlying these actions are yet to be defined. Moreover, there is no report concerning the action of morphine on ulcer healing after ulcer is formed. This action could be equally important as far as the clinical implication for morphine is concerned because patients, especially after surgery, are under different kinds of stress conditions in which complications such as gastric injury and bleeding would likely occur (McGuigan, 1991). Such therapeutic indication is interesting and requires further study. We aim to study whether morphine is effective to prevent ulceration and promote ulcer healing in the stomach in stress conditions. These actions, if any, could likely be due to the strengthening of the defensive mechanisms and increasing the repair system, respectively, in the gastric mucosa.

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2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats, weighing 200–230 g, were obtained from the Laboratory Animal Unit of the University of Hong Kong. The animals were housed in cages with wide mesh wire bottoms to prevent coprophagy and fed a standard laboratory diet and given free access to tap water. The cages were kept in a room with controlled temperature (22 ± 1 °C), relative humidity (65–70%) and day/night cycle (12:12 light/dark). The protocols of the following experiments were approved by the Committee on the Use of Live Animals in the University of Hong Kong.

2.2. Induction of stress ulceration

The method of restraint stress coupled with cold stress was adopted in the present experiment. This method was originally devised by Senay and Levine (1967) and modified in our laboratory (Wong et al., 2002). Rats were starved for 24 h and restrained inside individual close-fitting tubular wire mesh cages and exposed to a room temperature of 4 ± 0.3 °C for 3 h. Rats were either killed immediately after the 3-h stress for the morphine pretreatment study or sacrificed 24 h later after stress for the morphine posttreatment experiment. At the end of the experiment, the animals were anesthetized and the stomach was opened along the greater curvature. Lesion size (in mm) was determined by measuring each lesion along its greater diameter. In the case of petechiae, five such lesions were taken as the equivalent of a 1-mm lesion. The total lesion lengths in each group of rats were averaged and expressed as the lesion index. After measurement of mucosal lesions, part of the tissue was fixed in 10% buffered formalin solution. The tissue was then sectioned and stained for mucus-secreting layer, cell proliferation and apoptosis.

2.3. Administration of morphine and naloxone

In the pretreatment experiments, rats were pretreated with morphine sulphate (Macfarlan Smith) in the doses of 2 or 8 mg/kg or saline solution (0.9% NaCl), injected intraperitoneally in the volume of 1 ml/kg at the time just before cold-restraint stress. These animals were killed immediately after stress. In the posttreatment study, rats were stressed for 3 h and then returned back to normal room temperature without restraint. At this time, morphine sulphate or its vehicle (saline solution) was injected with the same doses. Drug treatment was repeated 3 h later. Rats were killed at 24 h after completion of stress. In examining the effect of blockade of the opioids receptor on ulcer healing, naloxone (8 mg/kg) was given intraperitoneally 15 min before morphine administration. Again, animals were killed 24 h later.

2.4. Measurement of myeloperoxidase activity in the gastric mucosa

Myeloperoxidase activities were determined by the method described by Bradley et al. (1982) with modifications (Chow et al., 1997). Gastric mucosa was homogenized in 0.5% hexadecyltrimethylammonium bromide (Sigma) in a potassium phosphate buffer (British Drug House). The supernatant was assayed for myeloperoxidase activity. The samples were mixed with hydrogen peroxide (Sigma) and *o*-dianisidine (Sigma) prepared in potassium phosphate buffer solution. The end point absorbance of the mixture was measured at 460 nm using as a spectrophotometer (Beckman DU 650; Beckman Instruments) with horseradish peroxidase (Sigma) as standard. Protein assay was conducted using the method described by Lowry et al. (1951). The final values were expressed as mU/mg protein.

2.5. Measurement of thickness of the layer of periodic acid-Schiff reagent (PAS)-stained positive cells

After fixation in buffered formalin for 24 h, 5-μm sections were made and stained with periodic acid followed by Schiff's reagent (PAS). Finally, they were counterstained with Harris hematoxylin and mounted in Permount. The dimension of the layer of cytoplasmic PAS-stained positive cells and the thickness of the whole glandular mucosa were measured using an image analyzer (Q500IW, Leica Image Systems) throughout the section along the length of each longitudinal strip. The ratio between the thickness of the mucus-secreting layer and the total mucosal thickness (measured from the surface of the epithelium of the glandular mucosa to the point just above the submucosa) was taken (Ma et al., 2000).

2.6. Determination of angiogenesis in the gastric mucosa

The number of microvessels in the gastric mucosa was identified by immunohistochemical staining with von Willebrand factor antibody (DAKO) following the method by Augustin et al. (1995). The number of microvessels was randomly counted under the microscope (200 ×) for 10 fields in each section of tissue. They were averaged and expressed as the number of microvessels per mm^2.

2.7. Immunohistochemical staining with proliferating cell nuclear antigen (PCNA)

Mucosal cell proliferation was assessed by immunohistostaining as described by Tarnawski et al. (1992) with modifications. The sections were digested with trypsin for 15 min at room temperature and incubated with a blocking agent (LSAB kit, DAKO) for 1 h. After blocking, they were incubated with a monoclonal primary antibody (1:200) (sc-56) (Santa Cruz Biotechnology) against mouse PCNA overnight at 4 °C. After washing with Tris buffer saline (TBS),

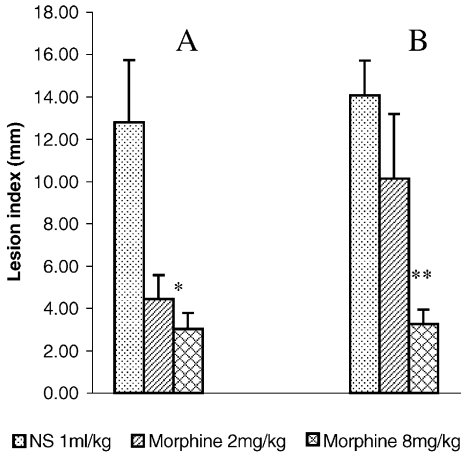

Fig. 1. Effects of morphine pretreatment (A, given intraperitoneally just before stress, rats killed immediately after the 3-h stress) or posttreatment (B, given immediately after stress and also 3 h later, rats killed 24 h after the 3-h stress) on cold-restraint stress (restrained at 4 °C for 3 h)-induced gastric mucosal injury. *$P<0.01$, **$P<0.001$ when compared with the saline control (NS).

sections were incubated with Link reagent (DAKO) for 1 h at room temperature. Another washing with TBS, they were incubated with streptavidin (DAKO) for 1 h. The sections were further incubated with H_2O_2-diaminobenzidine to visualize the PCNA-positive cells. Finally, the sections were counterstained with Mayer's hematoxylin for 1–2 min, and cleared by graded ethanol and xylene solutions and mounted. PCNA-positive cells were stained brown and counted under an image analyzer with a magnification of 200 ×.

2.8. Measurement of apoptotic cells in the gastric mucosa

DNA breaks were detected in situ by terminal deoxytransferase (TdT)-mediated dUTP-biotin nick end labeling (TUNEL) according to the method of Gavrieli et al. (1992). Briefly, tissue sections were digested with proteinase K to permeate the nucleus. Tissues were treated with H_2O_2 solution and washed with distilled water. Subsequently, the sections were covered with TdT buffer containing TdT and biotinylated dUTP. The reaction was stopped by washing sections with terminated buffer at room temperature. After blocking nonspecific binding with normal diluted serum, sections were incubated with peroxidase-labeled streptavidin and stained with diaminobenzidine-H_2O_2. Finally, the sections were counterstained with Mayer's hematoxylin. Sections treated with DNase I in buffer solution were served as positive control. Negative control was prepared with omission of TdT from the buffer solution. The TUNEL-positive cells were dark-brown in colour and they were counted at a magnification of 400, under an image analyzer (Q500IW, Leica). They were defined as the mean of data from eight 25-gland of each mucosal section.

2.9. Statistical analysis

The results were expressed as means ± S.E.M. The number of animals was ranging from four to eight in each group. Differences between the means were analyzed with Student's unpaired *t*-tests and also by the one-way analysis of variance (ANOVA) when appropriate. *P* values of <0.05 were considered statistically significant. In order to avoid subjective bias on the assessment of histological parameters measured in this study, samples were blinded from the observers when they were determined.

3. Results

3.1. Effects of morphine pre- and posttreatment on stress-induced gastric mucosal damage and its influence by naloxone pretreatment

Cold-restraint stress for 3 h markedly induced gastric mucosal damage together with severe hemorrhage in the stomach. Pretreatment with morphine at doses of 2 or 8 mg/kg dose-dependently and significantly reduced the severity of mucosal damage (Fig. 1A). Stress significantly also increased the mucosal myeloperoxidase activity (Table 1), and morphine pretreatment partially reversed such action (Table 1). The lesion size was similar at 24 h later after a 3-h stress (immediately after a 3-h stress: 12.8 ± 3.0 mm versus 24 h later after a 3-h stress: 14.1 ± 1.6 mm). Morphine posttreatment given after stress ulceration also reduced the lesion severity measured at 24 h after a 3-h stress in a dose-dependent manner (Fig. 1B). However, the mucosal myeloperoxidase activity at this time was significantly less

Table 1
Effects of stress (3-h cold restraint) and morphine treatment on the thickness of PAS-stained cells layer, myeloperoxidase activity and number of apoptotic cells in the gastric mucosa

Treatment (i.p.)		Thickness of mucus-stained layer (mm)	Myeloperoxidase (mU/mg)	Apoptosis (apoptotic cell/25 glands)
(A) Nonstressed control				
Saline	1 ml/kg	0.39 ± 0.02	15.2 ± 2.1	5.2 ± 0.6
(B) Morphine given before the 3-h stress				
Saline	1 ml/kg	0.14 ± 0.02[a]	25.4 ± 2.9[b]	10.8 ± 1.0[a]
Morphine	2 mg/kg	0.18 ± 0.01		
	8 mg/kg	0.22 ± 0.01[c]	19.9 ± 2.3	13.9 ± 2.3
(C) Morphine given after the 3-h stress				
Saline		0.19 ± 0.01	16.5 ± 2.2	8.6 ± 1.1
Morphine	2 mg/kg	0.21 ± 0.01		
	8 mg/kg	0.25 ± 0.01[c]	16.9 ± 1.9	3.1 ± 0.5[c]

Each value represents mean ± S.E.M.
[a] $P<0.01$ when compared with the nonstressed saline control.
[b] $P<0.05$ when compared with the nonstressed saline control.
[c] $P<0.05$ when compared with the respective saline-treated control.

($P<0.05$) than that of the mucosa immediately after a 3-h stress (Table 1). Morphine treatment did not affect the myeloperoxidase activity in this group of animals (Table 1). In the morphine posttreatment experiment, naloxone pretreatment partially abrogated the lesion healing effect of morphine after stress ulceration (morphine alone: 1.05 ± 0.51 versus morphine plus naloxone: 5.64 ± 2.05, $P<0.05$).

3.2. Effects of stress and morphine on mucus-stained layer, angiogenesis, cell proliferation and apoptosis in gastric mucosa

The thickness of the mucus-stained cells layer was profoundly depleted by cold-restraint stress. It was dose-dependently prevented by morphine pretreatment when compared to the saline control (Table 1). Stress for 3 h did not significantly affect the number of microvessels in the gastric mucosa (nonstress group: 4.13 ± 0.19 versus stress group: 3.70 ± 0.59 number of vessels/mm^2). However, 24 h after stress, the number of blood vessels was significantly increased (from 3.70 ± 0.59 to 5.19 ± 0.18, $P<0.05$) suggesting that the increase of angiogenesis was evidenced at this time point. Morphine treatment neither affected angiogenesis immediately after the 3-h stress (morphine 2 mg/kg: 3.91 ± 0.28; morphine 8 mg/kg: 4.16 ± 0.32), nor 24 h later (morphine 2 mg/kg: 4.58 ± 0.13; morphine 8 mg/kg: 4.59 ± 0.28). The number of PCNA-stained cells was slightly increased in the morphine-treated group immediately after the 3-h stress (Fig. 2). At 24 h after a 3-h stress, the mucus-stained layer remained at a lower level. Morphine posttreatment increased the thickness of the PAS-stained cells layer in the gastric mucosa (Table 1). The number of PCNA-positive cells in the gastric mucosa was also significantly elevated in the morphine-treated group (Fig. 2). The number of apoptotic cells in the gastric mucosa was significantly increased ($P<0.05$) after a 3-h stress (Table 1). Morphine pretreatment (8 mg/kg) slightly further increased the number of apoptotic cells. The same dose of morphine given after stress, however, markedly decreased apoptosis in the gastric mucosa (Table 1).

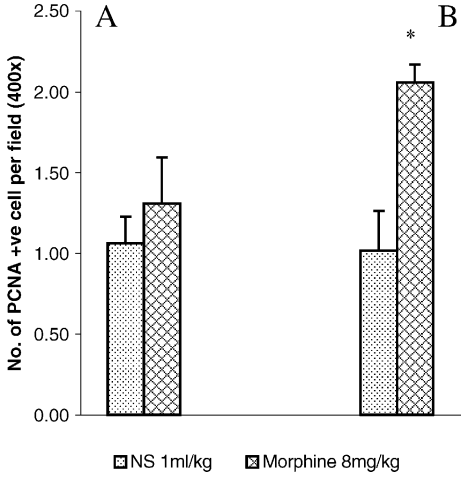

Fig. 2. Effects of morphine pretreatment (A, given intraperitoneally just before stress, rats killed immediately after the 3-h stress) or posttreatment (B, given immediately after stress and also 3 h later, rats killed 24 h after the 3-h stress) on number of positive (+ve) cells with proliferating cell nuclear antigen (PCNA)-staining in the gastric mucosa of cold-restraint rats. *$P<0.02$ when compared with the saline control (NS).

4. Discussion

In this study, the antiulcer effect of morphine pretreatment was confirmed and it was found to be in a dose-dependent manner. Cold-restraint stress induced severe hemorrhagic lesions together with a mark increase in myeloperoxidase activity. This finding may indicate that stress ulceration is a neutrophil-dependent ulcerogenic process, because myeloperoxidase is a marker for neutrophil infiltration during inflammation in the stomach (Chow et al., 1997; Tepperman et al., 1991). The increase of myeloperoxidase activity declined at 24 h after ulceration, suggesting that mucosal inflammation was peaked at the end of the 3-h stress and subsided thereafter in the animals. The severity of ulceration immediately after stress was not different from that of 24 h later. Unlike other acute gastric damage, this finding also indicates that stress ulcers in the gastric glandular mucosa could sustain for more than 24 h. This animal setting would provide a good model to assess acute ulcer healing in the stomach. In this connection, it is anticipated that any agent, which can promote the healing process through the decrease of apoptosis and increase of cell proliferation and angiogenesis together with the attenuation of inflammation at the ulcer crater, is useful to ameliorate the complications of stress in the stomach.

Indeed, morphine pretreatment reduced the elevation of myeloperoxidase activity during the active stage of ulceration, implicating that the drug could have some degree of anti-inflammatory action. In fact, morphine has been reported to exert protection against certain inflammatory models, such as carrageenan edema and ethanol-induced gastric damage (Gyires, 1992, 1994; Bhounsule et al., 1994), through the mμ receptors and stimulation of the endogenous nitric oxide production (Bhounsule et al., 1994; Gyires, 1994). Furthermore, it is known that mucus plays a significant role in the defensive mechanism in the gastric mucosa (Allen and Carrol, 1985; Allen et al., 1986) and morphine could partially reverse the depletion of mucus-stained cell layer induced by stress. All these actions could explain in part why morphine can inhibit the formation of gastric lesions under stress conditions. However, it is unlikely that the antiulcer action of morphine is mediated through the suppression of gastric acid secretion, as the current doses of morphine have been reported not to affect gastric acid secretion in conscious rats (Ho et al., 1984).

Interestingly, morphine treatment, when given after ulceration, markedly increased the healing process in stressed animals. The lesion size was significantly reduced by more than 80%. This was partially blocked by naloxone, indicating that the action of morphine was mediated through the opioid receptor. Such effect could have significant implication in clinical setting. Since opioids are given usually after surgical operation for their analgesic effect, morphine given in current study not only reduces pain caused by gastric ulceration but also promotes lesion repair by stimulating cell proliferation in the gastric tissue (Fig. 2). Morphine also increased the amount of secreting mucus in the gastric mucosa that would strengthen the healing process in the stomach by providing a physical barrier and a stable unstirred layer between the apical surfaces of the epithelial cells and the lumen. All these can reduce the aggressive action of acid on the epithelium and at the ulcer site to provide a better environment for wound repair (Allen and Carrol, 1985; Allen et al., 1986). As nitric oxide has been shown to be responsible for the antiulcer action of morphine (Gyires, 1994), it is also involved in the mucus secretion (Brown et al., 1992; Li et al., 2000) and cell proliferation (Li and Cho, 1999; Li et al., 2000; Ma et al., 1999a) during ulcer healing in the stomach. It is envisaged that morphine could act through these mechanisms and thereby promote ulcer healing in stressed animals. In addition, stress for 3 h significantly increased the number of apoptotic cells in the gastric mucosa and partially recovered 24 h later. Morphine treatment after stress significantly enhanced such recovery. This action can promote tissue regeneration and restitution in the gastric mucosa (Ma et al., 1999b). Therefore, the increase of ulcer healing by morphine could in part be due to the decrease of apoptosis in the gastric mucosa.

The increase of blood vessel number in the gastric mucosa 24 h after stress could be important for ulcer healing in the stomach (Ma et al., 1999a). Morphine treatment, however, did not affect the number of blood vessels in the gastric mucosa immediately and also 24 h after the 3-h stress, suggesting that angiogenesis was not a factor responsible for the ulcer healing action of morphine in the current stress ulcer model. It is unlikely that the action of morphine is mediated through the improvement of blood flow in the stomach.

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References

Allen, A., Carrol, N.J.H., 1985. Adherent and soluble mucus in the stomach and duodenum. Dig. Dis. Sci. 30, 55S–62S (Suppl).

Allen, A., Hutton, D.A., Leonard, A.J., Pearson, J.P., Sellers, L.A., 1986. The role of mucus in the protection of the gastroduodenal mucosa. Scand. J. Gastroenterol. 21 (Suppl. 25), 71–77.

Arrigo-Reina, R., Ferri, S., 1980. Evidence of an involvement of the opioid peptidergic system in the reaction to stressful conditions. Eur. J. Pharmacol. 30, 85–88.

Augustin, H.G., Braun, K., Telmenakis, I., Modlich, U., Kuhn, W., 1995. Ovarian angiogenesis, phenotypic characterization of endothelial cells in a physiological model of blood vessel growth and regression. Am. J. Pathol. 147, 339–351.

Bhounsule, S.A., D'Souza, R.S., Dhume, V.G., 1994. Gastric cytoprotective effect of morphine is probably not mediated by mμ-receptors. Arch. Int. Pharmacodyn. Ther. 328, 99–105.

Bradley, P.P., Priebat, D.A., Christensen, R.D., Rothstein, G., 1982. Measurement of cutaneous inflammation: estimation of neutrophil content with an enzyme marker. J. Invest. Dermatol. 78, 206–209.

Brown, J.F., Hanson, P.J., Whittle, B.J., 1992. Nitric oxide donors increase mucus gel thickness in rat stomach. Eur. J. Pharmacol. 223, 103–104.

Chow, J.C.Y., Ma, L., Zhu, M., Cho, C.H., 1997. The potentiating actions of cigarette smoking on ethanol-induced gastric mucosal damage in rats. Gastroenterology 113, 1188–1197.

Dai, S., Chan, M.Y., 1983. Effects of naloxone on serum corticosterone and gastric lesions in stressed rats. Pharmacology 27, 180–184.

Ferri, S., Arrigo-Reina, R., Candeletti, S., Costa, G., Murari, G., Speroni, E., Scoto, G., 1983. Central and peripheral sites of action for the protective effect of opioids of the rat stomach. Pharmacol. Res. Commun. 15, 409–418.

Gavrieli, Y., Sherman, Y., Ben Sasson, S.A., 1992. Identification of programmed cell death in situ via specific labeling of nuclear DNA fragmentation. J. Cell Biol. 119, 493–501.

Glavin, G.B., 1985. Effects of morphine and naloxone on restraint-stress ulcers in rats. Pharmacology 31, 57–60.

Gyires, K., 1992. Are all "cytoprotective" drugs gastroprotective? Acta Physiol. Hung. 80, 247–255.

Gyires, K., 1994. The role of endogenous nitric oxide in the gastroprotective action of morphine. Eur. J. Pharmacol. 255, 33–37.

Ho, M.M., Dai, S., Ogle, C.W., 1984. Decreased acid secretion and gastric lesion production by morphine in rats. Eur. J. Pharmacol. 102, 117–121.

Li, Y., Cho, C.H., 1999. The ulcer healing effect of protamine sulphate in rat stomach. Aliment. Pharmacol. Ther. 13, 1351–1362.

Li, Y., Wang, W.P., Wang, H.Y., Cho, C.H., 2000. Intragastric administration of heparin enhances gastric ulcer healing through a nitric oxide-dependent mechanism in rats. Eur. J. Pharmacol. 399, 205–214.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.L., 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193, 255–257.

Ma, L., Chow, J.Y.C., Cho, C.H., 1999a. Cigarette smoking delays ulcer healing: role of constitutive nitric oxide synthase in rat stomach. Am. J. Physiol. 39, G238–G248.

Ma, L., Wang, H.Y., Chow, Y.Y.C., Cho, C.H., 1999b. Cigarette smoke increases apoptosis in the gastric mucosa: role of epidermal growth factor. Digestion 60, 461–468.

Ma, L., Wang, W.P., Chow, J.Y.C., Lam, S.K., Cho, C.H., 2000. The role of polyamines in gastric mucus synthesis inhibited by cigarette smoke or its extract. Gut 47, 170–177.

McGuigan, J.E., 1991. Peptic ulcer and gastritis. In: Wilson, J.D., Braunwald, E., Isselbacher, K.J., Petersdorf, R.G., Martin, J.B., Fauci, A.S., Root, R.K. (Eds.), Harrison's Principles of Internal Medicine. McGraw-Hill, NY, pp. 1229–1244.

Morley, J.E., Levine, A.S., Silvis, S.E., 1982. Endogenous opiates and stress ulceration. Life Sci. 16, 693–699.

Ray, A., Gulatim, K., Puri, S., Sen, P., 1993. Role of κappa opioid receptors during stress responsiveness in rats. Indian J. Exp. Biol. 31, 116–119.

Senay, E.C., Levine, R.J., 1967. Synergism between cold and restraint for rapid production of stress ulcer in rats. Proc. Soc. Exp. Biol. Med. 124, 1221–1223.

Tarnawski, A., Lu, S.Y., Stachura, J., Sarfeh, I.J., 1992. Adaptation of gastric mucosa to chronic alcohol administration is associated with increased mucosal expression of growth factors and their receptor. Scand. J. Gastroenterol., Suppl. 193, 59–63.

Tepperman, B.L., Besco, J.M., Kiernan, J.A., Soper, B.D., 1991. Relationship between myeloperoxidase activity and the ontogenic response of rat gastric mucosa to ethanol. Can. J. Physiol. Pharm. 69, 1882–1888.

Wong, D., Koo, M.W.L., Shin, V.Y., Liu, E.S.L., Cho, C.H., 2002. Pathogenesis of nicotine treatment and its withdrawal on stress-induced gastric ulceration in rats. Eur. J. Pharmacol. 434, 81–86.