Effects of Calcium Dobesilate on Adrenomedullin, Nitric Oxide and Superoxide Dismutase Levels in Experimental Stress Ulcer Formation

Deneysel Stres Ülseri Oluşumunda Kalsiyum Dobesilatın Adrenomedüllin, Nitrik Oksit ve Süperoksit Dismutaz Düzeyleri Üzerine Etkileri

Yeşim TEMİZ, MD,[a]
Hakan EKMEKÇİ, MD,[b]
Ezel USLU, MD,[a]
Özlem BALCI EKMEKÇİ, MD,[b]
Tuncay ALTUĞ, MD,[c]
Koray GÜMÜŞTAŞ, MD[a]

[a]Department of Biochemistry, İstanbul University, Cerrahpaşa Faculty of Medicine,
[b]Department of Pediatric Hematology/Oncology, Bone Marrow Transplantation Unit, İstanbul University, İstanbul Faculty of Medicine,
[c]Center for Reproduction and Research of Experimental Animals, İstanbul University, Cerrahpaşa Faculty of Medicine, İstanbul

Geliş Tarihi/*Received:* 17.06.2008
Kabul Tarihi/*Accepted:* 01.04.2009

This study was funded by the İstanbul University Scientific Research Projects Unit. (Project No:T-401/08032004)

The study was presented as a poster at the "2nd World Conference of Stress, Budapest, Hungary" August 23-26, 2007.

Yazışma Adresi/*Correspondence:*
Hakan EKMEKÇİ
İstanbul University, İstanbul Faculty of Medicine, Department of Pediatric Hematology/Oncology, Bone Marrow Transplantation Unit, İstanbul, TÜRKİYE/TURKEY
hakekmekci@yahoo.com

ABSTRACT Objective: In the present study, we investigated the adrenomedullin (AM) and total nitric oxide (NOx) [nitrite plus nitrate] levels in experimentally induced stress ulcer model in rats. To examine the relation with oxidative stress, we measured the levels of thiobarbituric acid-reactive substances (TBARS) as an index of lipid peroxidation, and superoxide dismutase (SOD) activity together with the effect of calcium dobesilate (Ca-D) as well. **Material and Methods:** In this study, 33 female Wistar-Albino rats weighing about 230 g (200-250) aged 7-8 months were used. The rats were divided into 3 groups each containing 11 rats. The Ca-D-treated stress group received daily single oral dose of calcium dobesilate for 10 days (group 1). The saline-treated stress group received daily single oral dose of saline (same volume with calcium dobesilate) for 10 days (group 2). The non-stressed control group received daily single oral dose of saline (same volume with calcium dobesilate) for 10 days (group 3). In all groups of rats, plasma AM, NOx and TBARS levels as well as gastric mucosa NOx, TBARS levels, and SOD activity were determined. **Results:** In group 2 plasma AM ($p< 0.001$), plasma NOx ($p< 0.001$), gastric mucosa NOx ($p< 0,001$), and gastric mucosa TBARS levels ($p< 0.01$) were significantly higher than the levels in the control group. In group 1, elevated plasma NOx, gastric mucosa NOx, and plasma TBARS levels were found compared to the control group. On the other hand, the average plasma adrenomedullin and gastric mucosa TBARS levels in group 2 were significantly higher ($p< 0.001$) and plasma NOx levels were lower ($p< 0.01$) compared to group 1. In addition, in group 1 and 2, decreased SOD activity ($p< 0.001$) was found in gastric mucosa compared to the control group. **Conclusion:** Elevated levels of AM and NOx may be generated to protect the organism as a response to increased oxidative stress. Calcium dobesilate may partially protect the organism against oxidative stress in stress ulcer pathogenesis, probably in relation to NO. However, further studies will be required to evaluate the role of calcium dobesilate in stress ulcer pathogenesis because of low levels of plasma AM and high levels of plasma TBARS in Ca-D-treated stress group.

Key Words: Nitric oxide; superoxide dismutase; ulcer; calcium dobesilate; adrenomedullin

ÖZET Amaç: Bu çalışmada, deneysel olarak stres ülseri oluşturulan sıçanlarda adrenomedullin (AM) ve total nitrik oksit (NOx) [nitrit + nitrat] düzeyleri araştırıldı. Süperoksit dismutaz aktivitesi (SOD) ve lipid peroksidasyonunun bir göstergesi olarak "Thiobarbituric asit reactive substance (TBARS)" düzeylerinin oksidatif stresle ilişkisine ek olarak kalsiyum dobesilat (Ca-D)'ın etkileri incelendi. **Gereç ve Yöntemler:** Bu çalışmada yaklaşık 230 g (200-250 g) ağırlığında 7-8 aylık 33 dişi Wistar Albino sıçan kullanıldı. Bu sıçanlar, her biri 11 adet sıçan içeren 3 gruba bölündü. Kalsiyum dobesilat verilen stres grubundaki (grup 1) hayvanlara, 10 gün boyunca ağızdan tek doz Ca-D verildi. Serum fizyolojik verilen stres grubundaki (grup 2) hayvanlara, 10 gün boyunca serum fizyolojik (Ca-D ile aynı miktarda) verildi. Stres oluşturulmayan kontrol grubundaki (grup 3) hayvanlara, 10 gün boyunca serum fizyolojik (Ca-D ile aynı miktarda) tek doz olarak ağızdan verildi. Plazma AM, NOx, TBARS düzeyleri tüm gruplarda tayin edildi. Ayrıca bu grupların mide mukozasında NOx, TBARS düzeyleri ve SOD aktivitesi de belirlendi. **Bulgular:** Grup 2'de, plazma AM ($p< 0.001$), plazma NOx ($p< 0.001$), gastrik mukoza NOx ($p< 0.001$) ve gastrik mukoza TBARS düzeyleri ($p< 0.01$) kontrol grubuna göre anlamlı derecede yüksek bulundu. Grup 1'de, plazma NOx, gastrik mukoza NOx ve plazma TBARS düzeyleri kontrol grubuna göre daha yüksekti. Diğer taraftan, grup 2'deki plazma NOx düzeyi grup 1 ile kıyaslandığında anlamlı ölçüde daha düşük ($p< 0.01$), adrenomedullin ve gastrik mukoza TBARS düzeyleri ise grup 1 ile kıyaslandığında grup 2'de anlamlı ölçüde daha yüksek bulundu ($p< 0.001$). Ek olarak gastrik mukoza SOD aktivitesi, kontrol grubuyla kıyaslandığında grup 1 ve grup 2'de istatistiksel olarak anlamlı düzeyde azalmış bulundu ($p< 0.001$). **Sonuç:** Adrenomedullin ve NOx düzeyleri, artmış oksidatif strese yanıt olarak organizmayı korumaya yönelik artış göstermiş olabilir. Kalsiyum dobesilat, muhtemelen NO ile ilişkili olarak, stres ülseri patogenezinde oksidatif strese karşı organizmanın korunmasında kısmen etkili olabilir. Ancak Ca-D uygulanan stres grubunda artmış plazma TBARS ve azalmış plazma AM düzeylerinden dolayı stres ülseri patogenezinde kalsiyum dobesilatın rolünün araştırılacağı yeni çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: Nitrik oksit; süperoksit dismutaz; ülser; kalsiyum dobesilat; adrenomedullin

Turkiye Klinikleri J Med Sci 2009;29(6):1450-6

Stress ulcer is a common cause of gastrointestinal bleeding and its etiology is multifactorial. A reduction in gastric mucosal blood flow and oxygen derived free oxygen radicals plays an important role in the stress ulcer pathogenesis.[1-6]

Reactive oxygen species (ROS) such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH°) have emerged as highly toxic agents responsible for a wide variety of tissue damages.[7,8] Their involvement in gastric ulceration was realized when they were shown to mediate gastric mucosal lesions in experimental animals.[9-11] A growing body of experimental and clinical evidence suggest that gastric mucosal damage by ethanol, non-steroidal antiinflammatory drugs, and by *Helicobacter pylori* is mediated through the ROS.[12-18] ROS are also involved in gastric ulceration induced by several kinds of stress.[18,19]

Adrenomedullin (AM), a potent vasodilator peptide originally isolated from human pheochromocytoma, has recently been shown to be abundantly expressed by and to be secreted from vascular endothelial cells and functions in an autocrine/paracrine fashion.[20] Immunoreactive AM has been detected by radioimmuno- assay in plasma and various tissues including kidney, pancreas and intestine.[21]

Some studies have shown that AM prevents gastric mucosal damage, reduces gastric acid secretion and decreases the vasoconstriction to 5-hydroxytryptamine in gastric arteries, suggesting that its gastro-protective effects may partially be related to an increase in blood flow in the gastric mucosa, by a mechanism involving nitric oxide (NO).[21,22] Moreover, Shimosawa et al have recently shown that AM-deficient mice generated by gene targeting is associated with perivascular inflammation in coronary artery and increases in systemic and local oxidative stress and reversal of increased urinary isoprostane excretion by exogenous AM supplementation.[23] These data are consistent with the assumption that AM may play a protective role against oxidative stress as an endogenous antioxidant in vivo.

Calcium dobesilate (Ca-D) possesses angioprotective properties in chronic venous insufficiency and diabetic retinopathy .[24] Among other effects, Ca-D has been shown to enhance NO synthase activity in vascular endothelial cells.[25] Furthermore, it has been recently shown that Ca-D possesses antioxidant properties in vitro[26] and protects capillary permeability by reactive oxygen species in the rat periteonal cavity.[27] In addition, radical scavengers such as superoxide dismutase (SOD) have been shown to be effective in reducing the adverse effects of free radicals on gastric mucosa.[9,28,29]

In our study, we investigated the AM and total nitric oxide (NOx) [nitrite plus nitrate] levels in experimentally induced stress model in rats. To examine the relation with oxidative stress, we measured the levels of thiobarbituric acid reactive substances (TBARS) as an index of lipid peroxidation, and SOD activity together with the effect of Ca-D as well.

MATERIAL AND METHODS

STUDY DESIGN

Interventions concerning animals were performed according to the Guide for the Care and Use of the Laboratory Animals.[30] In this study, 33 female Wistar-Albino rats weighing about 230 g (200-250) aged 7-8 months were used. The rats were divided into 3 groups each containing 11 rats. The Ca-D-treated stress group received daily single oral dose of Ca-D for 10 days (group 1). Ca-D (Doxium©) was diluted in 1 mL saline and was administered orally by gavage through esophageal catheter. The saline-treated stress group received daily single oral dose of saline, same volume with Ca-D, for 10 days (group 2). Stress ulcer was induced in these two groups of rats (group 1 and 2). The non-stressed control group that received daily single oral dose of saline at the same volume with Ca-D formed the control group (Group 3).

Experimental Model

All groups of rats were starved 12 hours before the experiment with free access to water. Then the rats (group 1 and 2) were anesthetized lightly with ether and were restrained on a rodent immobilization device space 15 cm apart from each other for 6 hours at 16°C.[31] At the end of the restraint period

blood was collected from all rats in plain tubes and tubes containing EDTA and aprotinin (Roche, Germany). After the blood collection process, rats were sacrificed by prolonged ether anesthesia for laparotomy; the stomach was placed on an ice-bearing surface and was opened along the grater curvature. The mucosal surface was gently rinsed by cold saline solution. Stomach was stretched over the ice. Mucosal lesions were inspected and rated for gross pathology according to the scale described by Dekansky et al[32] as follows: 0= no damage, 1= blood at the lumen, 2= pin point erosions, 3= 1-5 small erosions < 2 mm, 4= > 5 small erosions < 2 mm, 5= 1-3 large erosions > 2 mm, 6= >3 large erosions > 2 mm.

Biochemical Assay

Collected blood was centrifuged at 1500 g for 10 min at 4°C. Plasma and gastric mucosa specimens were frozen at -80°C in aliquots until assayed. Tissue samples were homogenized in phosphate buffer (Ph: 7.4) with a mechanic homogenizer with a Teflon-coated piston (RZR 2021; Heildolph, Schwabach, Germany) forming a 10% (w/v) homogenate. The homogenate was sonicated (MSE, 11-73/ PG597, England) and centrifuged at 4.000 g for 10 min at 4°C for TBARS analysis or at 15.000 g for 15 min at 4°C for NOx and SOD analysis (Heraeus, Germany). The resultant supernatant was used for the measurement of TBARS, NOx, and SOD activity.

Plasma AM concentrations were determined by enzyme-linked immunosorbent assay (ELISA) (Phoenix Pharmaceuticals; Cat no: EK-010-08). Plasma and tissue NOx levels were determined by colorimetric method (Roche; Cat no: 1746-081, Germany). Plasma and tissue TBARS and tissue SOD activity levels were measured according to the method of Buege and Aust,[33] and Sun et al,[34] respectively. Tissue TBARS, NOx and SOD activity results were expressed as μmol/g wet tissue, μmol/g wet tissue and U/g wet tissue, respectively.

Statistical Analysis

Conventional methods were used for the calculation of means and standard deviation (SD). The data were expressed as the mean ± SD. Data were analyzed with a one way ANOVA followed by multiple comparisons by Bonferroni and Dunnett t-test, as appropriate. For correlation analysis Pearson's correlation was used. p values equal to or less than 0.05 were considered significant.

RESULTS

In group 1 and 2, gross gastric lesions were visualized in the fundus and corpus of the stomach. There was no significant difference between the two groups regarding gross pathology ($p > 0.05$).

In group 2, plasma AM ($p < 0.001$), plasma NOx ($p < 0.001$), gastric mucosa NOx ($p < 0.001$), and gastric mucosa TBARS levels ($p < 0.01$) were significantly higher than in the control group. In group I, elevated plasma NOx, gastric mucosa NOx, and plasma TBARS levels were found compared to the control group. On the other hand, the average plasma AM and gastric mucosa TBARS levels in group 2 were significantly higher ($p < 0.001$) and plasma NOx levels were lower ($p < 0.01$) compared with group 1. In addition, in group 1 and 2 SOD activity ($p < 0.001$) in gastric mucosa was decreased compared to the control group (Table 1).

According to Pearson's correlation test, there was a positive correlation between plasma adrenomedullin and gastric mucosa TBARS levels ($r = 0.616$; $p < 0.01$). We also found positive correlations between plasma TBARS and plasma NOx/gastric mucosa NOx levels [($r = 0.440$; $p < 0.05$) / ($r = 0.544$; $p < 0.01$) respectively]. Moreover, a positive correlation was detected between plasma NOx and gastric mucosa NOx levels ($r = 0.803$; $p < 0.001$). In contrast, we found negative correlations between gastric mucosa SOD activity and plasma AM ($r = -0.376$; $p < 0.05$), plasma TBARS ($r = -0.523$; $p < 0.01$), plasma NOx ($r = -0.510$; $p < 0.01$), and gastric mucosa NOx ($r = -0.698$; $p < 0.01$) levels.

DISCUSSION

The etiology of gastroduodenal ulcers is influenced by various aggressive and defensive factors such as acid-pepsin secretion, parietal cell, mucosal barrier, mucus secretion, blood flow, cellular regeneration, and endogenous protective agents.[35]

TABLE 1: Results of gastric mucosa TBARS, NOx levels and SOD activity, and plasma TBARS, NOx and AM levels in experimental groups (mean ± SD).

Variables	Group 1 (n= 11)	Group 2 (n= 11)	Group 3 (n= 11)
p AM (ng/mL)	3.52 ± 0.88	4.81 ± 0.50*,**	3.43 ± 0.26
p TBARS (µmol/L)	4.52 ± 0.52+	4.08 ± 0.62	3.67 ± 0.24
m TBARS (µmol/g wet tissue)	47.74 ± 4.71	55.62 ± 4.17**,***	49.88 ± 2.58
p NOx (µmol/L)	3.09 ± 0.29++,+++	2.73 ± 0.17*	2.25 ± 0.09
m NOx (µmol/g wet tissue)	10.90 ± 0.83++	10.26 ± 0.50*	7.16 ± 0.44
m SOD (U/g wet tissue)	107.231 ± 5.26	107.789 ± 3.48	116.248 ± 4.69++,*

AM: Adrenomedullin, TBARS: Thiobarbituric acid reactive substances, NOx: Nitric oxide, SOD: Superoxide dismutase.
p= plasma, m= gastric mucosa)
Group I; Ca-D-treated stress group,
Group II; Saline-treated stress group,
Group III; Non-stressed control group,
*p< 0.001; (group 2 - group 3), **p< 0.001; (group 2 - group 1), ***p< 0.01; (group 2 - group 3), +p< 0.01; (group 1 - group 3),
++ p< 0.001;(group 1 - group 3), +++ p< 0.01; (group 1 - group 2).

The ROS generated by the metabolism of arachidonic acid, platelets, macrophages, and smooth muscle cells may contribute to gastric mucosal damage. Therefore, by scavenging free radicals, the reactive oxygen metabolites might be useful by protecting the gastric mucosa from oxidative damage or accelerating healing of gastric ulcers.[35]

Recently, it has been implicated that NO is important in the regulation of acid and alkaline secretion, gastric mucosal blood flow, and gastric mucus secretion. It is also known that NO is involved in the modulation of gastric mucosal integrity. Three genetically distinct NOS isoforms have been identified. These include a constitutive, low output endothelial isoform (eNOS) that modulates vascular tone, a neuronal isoform (nNOS) that modulates synaptic plasticity and neurotransmission, and an inducible, high output immune/inflammatory isoform (iNOS) that functions as an effector component of the cell mediated immune response. nNOS and eNOS are dependent on Ca^{++}/calmodulin complex for NO production, whereas iNOS is Ca^{++} independent.[36,37] cNOS and iNOS have been detected in gastric mucosal cells isolated from rats.[38] It has been widely accepted that in the digestive system, NO produced by cNOS is cytoprotective, while excessive NO produced by iNOS is cytotoxic, although there is a report suggesting that NO produced by iNOS may have protective actions on gastrointestinal mucosa.[39] While low levels of NO is essential in preventing the lipid peroxidation, excessive amounts of NO react with ROS and produce peroxynitrite, singlet oxygen, and hydroxyl radical which are highly toxic.[40-42] Thus, the role of NO in pathological states is complex, and balance between ROS and NO may be important.

AM is an aminoacid peptide with a potent hypotensive/vasodilatory action. Levels of AM are significantly elevated in patients with hypertension, and reports have suggested that stressor-related increases may serve as a regulatory or protective function. Acute restraint stress is known to stimulate sympathetic activity as well as hypothalamic-pituitary-adrenal (HPA) axis, producing significant increase in AM levels in pituitary gland, plasma and adrenal glands all of which are key components of the HPA axis, suggesting a regulatory and/or protective role for AM in countering HPA activation following a variety of physiological and psychological stressors.[43]

AM has vascular, renal, and endocrine effects. The vasodilator effects of AM were suggested to be mediated via calcitonin-gene-related peptide (CGRP) receptors coupled to the accumulation of intracellular cAMP, NO release and increased intracellular Ca^{+2} concentrations or activated phospholipase C.[44] In addition, AM has been described to increase interleukin-(IL)1-induced NO production. NO not only inhibits vascular smooth muscle cell (VSMC) proliferation and DNA synthesis but also

exerts antioxidant effects, probably through NAD(P)H oxidase inhibition. Therefore, AM deficiency can increase ROS generation, possibly through activation of nicotinamide adenine dinucleotide phosphate, reduced form (NAD(P)H) oxidase.[45,46] In our study, plasma AM and gastric mucosa TBARS levels in group 2 were significantly higher than in group 1 and the control group. Elevated plasma adrenomedullin levels in group 2 may be a result of compensatory mechanisms. Moreover, this may be the cause of higher plasma and gastric mucosa NOx levels in group 2 than in the control group.

Ca-D has been recently reported to express antioxidant properties in vitro. Ca-D appears to act as a scavenger for hydroxyl radicals and as an inhibitor of membrane lipid peroxidation generated by oxygen free radicals in human erytrocytes and polymorphonuclear cells.[47] Moreover, this study suggested that Ca-D acted predominantly in the extracellular compartment, as expected from its hydrophilic properties. In addition, previous in vitro studies showed that Ca-D potently scavenges superoxide anion.[25,27] On the other hand, Ruiz et al[25] showed that Ca-D increased NOS activity in vascular endothelial cells. Thus, Ca-D may contribute to the increase in gastric mucosa blood flow due to the increased NO production.

In the present study, we also found lower plasma AM levels in group 1 than in group 2. In addition, plasma NOx levels were found to be significantly higher in group 1 than in group 2 and the Control group. Consequently, Ca-D administration may partially contribute to high plasma NOx, low plasma AM and gastric mucosa TBARS levels in group 1 when compared to group 2. In addition, possible effects of Ca-D are not reflected to plasma TBARS levels in group 1.

On the other hand, in this study, gastric mucosa SOD activity was significantly lower in group 1 and group 2 than in the control group. We also found negative correlations between gastric mucosa SOD activity and other study parameters. Preventive antioxidants, such as SOD, catalase and glutathione peroxidase (GPx) are the first line of defense against reactive oxygen species. The activity of SOD and GPx were initially increased during fasting but prolonged fasting as well as ulceration significantly reduced their activity.[46] Exogenous administration of SOD was found to reduce ulcer formation and to prevent the decrease of SOD activity in the gastric mucosa following indomethacin-induced ulceration.[48] A decrease in the SOD activity following ulceration induced by intraperitoneal injection of 0.6 M HCl has also been demostrated by Tanaka and Yuda.[49] Similar to our results, Kwiecien et al[50] showed decreased SOD activity and increased TBARS levels in experimental mucosal damage.

CONCLUSION

Our results suggest that;

1. Elevated levels of AM and NOx may be generated to protect the organism as a response to increased oxidative stress and decreased gastric mucosal blood flow.

2. The pathogenesis of experimental mucosal damage in rat stomach includes the generation of ROS that seems to play an important role, namely due to generation of lipid peroxides, accompanied by impairment of antioxidative enzyme activity of cells.

3. Ca-D may partially protect the organism against oxidative stress in stress ulcer pathogenesis, probably related to NO but further studies will be required to evaluate the role of Ca-D in stress ulcer pathogenesis because of low levels of plasma AM and high levels of plasma TBARS in Ca-D-treated stress group.

REFERENCES

1. Repetto MG, Llesuy SF. Antioxidant properties of natural compounds used in popular medicine for gastric ulcers. Braz J Med Biol Res 2002;35(5):523-34.
2. Rastogi L, Patnaik GK, Dikshit M. Free radicals and antioxidant status following pylorus ligation induced gastric mucosal injury in rats. Pharmacol Res 1998;38(2):125-32.
3. Uslu E, Aydin S, Carkman S, Uzun H, Altinli E, Apaydin BB, et al. Effects of gender on stress ulcer formation in rats. Tohoku J Exp Med 2002;197(1):17-26.
4. Das D, Bandyopadhyay D, Bhattacharjee M, Banerjee RK. Hydroxyl radical is the major causative factor in stress-induced gastric ulceration. Free Radic Biol Med 1997;23(1):8-18.
5. Hiramoto JS, Terdiman JP, Norton JA. Evidence-based analysis: postoperative gastric bleeding: etiology and prevention. Surg Oncol 2003;12(1):9-19.
6. Ito M, Suzuki Y, Ishihara M, Suzuki Y. Anti-ulcer effects of antioxidants: effect of probucol. Eur J Pharmacol 1998;354(2-3):189-96.
7. Halliwell B, Gutteridge JM. Oxygen free radicals and iron in relation to biology and medicine: some problems and concepts. Arch Biochem Biophys 1986;246(2):501-14.
8. Halliwell B, Gutteridge JM. Role of free radicals and catalytic metal ions in human disease: an overview. Methods Enzymol 1990;186: 1-85.
9. Itoh M, Guth PH. Role of oxygen-derived free radicals in hemorrhagic shock-induced gastric lesions in the rat. Gastroenterology 1985;88(5 Pt 1):1162-7.
10. Perry MA, Wadhwa S, Parks DA, Pickard W, Granger DN. Role of oxygen radicals in ischemia-induced lesions in the cat stomach. Gastroenterology 1986;90(2):362-7.
11. Yoshikawa T, Ueda S, Naito Y, Takahashi S, Oyamada H, Morita Y, et al. Role of oxygen-derived free radicals in gastric mucosal injury induced by ischemia or ischemia-reperfusion in rats. Free Radic Res Commun 1989;7(3-6):285-91.
12. Mizui T, Doteuchi M. Lipid peroxidation: a possible role in gastric damage induced by ethanol in rats. Life Sci 1986;38(23):2163-7.
13. Szelenyi I, Brune K. Possible role of oxygen free radicals in ethanol-induced gastric mucosal damage in rats. Dig Dis Sci 1988; 33(7):865-71.
14. Pihan G, Regillo C, Szabo S. Free radicals and lipid peroxidation in ethanol- or aspirin-induced gastric mucosal injury. Dig Dis Sci 1987;32(12):1395-401.
15. Vaananen PM, Meddings JB, Wallace JL. Role of oxygen-derived free radicals in indomethacin-induced gastric injury. Am J Physiol 1991;261(3 Pt 1):G470-5.
16. Yoshikawa T, Naito Y, Kishi A, Tomii T, Kaneko T, Iinuma S, et al. Role of active oxygen, lipid peroxidation, and antioxidants in the pathogenesis of gastric mucosal injury induced by indomethacin in rats. Gut 1993;34(6):732-7.
17. Davies GR, Simmonds NJ, Stevens TR, Sheaff MT, Banatvala N, Laurenson IF, et al. Helicobacter pylori stimulates antral mucosal reactive oxygen metabolite production in vivo. Gut 1994;35(2):179-85.
18. Phull PS, Green CJ, Jacyna MR. A radical view of the stomach: the role of oxygen-derived free radicals and anti-oxidants in gastroduodenal disease. Eur J Gastroenterol Hepatol 1995;7(3):265-74.
19. Das D, Banerjee RK. Effect of stress on the antioxidant enzymes and gastric ulceration. Mol Cell Biochem 1993;125(2):115-25.
20. Yoshimoto T, Fukai N, Sato R, Sugiyama T, Ozawa N, Shichiri M, et al. Antioxidant effect of adrenomedullin on angiotensin II-induced reactive oxygen species generation in vascular smooth muscle cells. Endocrinology 2004;145(7):3331-7.
21. Salomone S, Caruso A, Cutuli VM, Mangano NG, Prato A, Amico-Roxas M, et al. Effects of adrenomedullin on the contraction of gastric arteries during reserpine-induced gastric ulcer. Peptides 2003;24(1):117-22.
22. Clementi G, Caruso A, Cutuli VM, Mangano NG, Salomone S, Lempereur L, et al. Gastroprotective effect of adrenomedullin administered subcutaneously in the rat. Peptides 2002;23(6):1149-53.
23. Shimosawa T, Shibagaki Y, Ishibashi K, Kitamura K, Kangawa K, Kato S, et al. Adrenomedullin, an endogenous peptide, counteracts cardiovascular damage. Circulation 2002;105(1):106-11.
24. Gepdiremen A, Süleyman H. Intraperitoneal administration of salicylate dose-dependently prevents stress-induced ulcer formation in rats. Pol J Pharmacol 2003;55(2):209-12.
25. Ruiz E, Lorente R, Tejerina T. Effects of calcium dobesilate on the synthesis of endothelium-dependent relaxing factors in rabbit isolated aorta. Br J Pharmacol 1997; 121(4):711-6.
26. Brunet J, Farine JC, Garay RP, Hannaert P. In vitro antioxidant properties of calcium dobesilate. Fundam Clin Pharmacol 1998; 12(2):205-12.
27. Brunet J, Farine JC, Garay RP, Hannaert P. Angioprotective action of calcium dobesilate against reactive oxygen species-induced capillary permeability in the rat. Eur J Pharmacol 1998;358(3):213-20.
28. Salim AS. Removing oxygen-derived free radicals stimulates healing of ethanol-induced erosive gastritis in the rat. Digestion 1990;47(1):24-8.
29. Ohara T, Yuki T, Hamada S, Asaki S, Toyota T. [A cytoprotective role of extracellular superoxide dismutase in experimental stress ulcer models]. [Article in Japanese] Nippon Shokakibyo Gakkai Zasshi 1991;88(3):659-63.
30. Guide for the Care and Use of Laboratory Animals. National Academy Press Washington DC: National Academy Press; 1996.
31. Brodie DA, Hanson HM. A study of the factors involved in the production of gastric ulcers by the restraint technique. Gastroenterology 1960;38:353-60.
32. Dekanski JB, Macdonald A, Sacra P. Effects of fasting, stress and drugs on gastric glycoprotein synthesis in the rat. Br J Pharmacol 1975;55(3):387-92.
33. Buege JA, Aust SD. Microsomal lipid peroxidation. Methods Enzymol 1978;52:302-10.
34. Sun Y, Oberley LW, Li Y. A simple method for clinical assay of superoxide dismutase. Clin Chem 1988;34(3):497-500.
35. Repetto MG, Llesuy SF. Antioxidant properties of natural compounds used in popular medicine for gastric ulcers. Braz J Med Biol Res 2002;35(5):523-34.
36. Xing G, Chavko M, Zhang LX, Yang S, Post RM. Decreased calcium-dependent constitutive nitric oxide synthase (cNOS) activity in prefrontal cortex in schizophrenia and depression. Schizophr Res 2002;58(1):21-30.
37. Cekmen MB, Turgut M, Turkoz Y, Aygun D, Gozukara EM. Nitric oxide (NO) and nitric oxide synthase (NOS): physiologic and pathologic characteristics. Turkiye Klinikleri J Pediatr 2001;10(4):226-36.
38. Vento P, Kiviluoto T, Järvinen HJ, Soinila S. Changes in distribution of three isoforms of nitric oxide synthase in ulcerative colitis. Scand J Gastroenterol 2001;36(2):180-9.
39. Nishida K, Ohta Y, Ishiguro I. Role of gastric mucosal constitutive and inducible nitric oxide synthases in the development of stress-induced gastric mucosal lesions in rats. Biochem Biophys Res Commun 1997;236(2):275-9.
40. Gewaltig MT, Kojda G. Vasoprotection by nitric oxide: mechanisms and therapeutic potential. Cardiovasc Res 2002;55(2):250-60.
41. Hummel SG, Fischer AJ, Martin SM, Schafer FQ, Buettner GR. Nitric oxide as a cellular antioxidant: a little goes a long way. Free Radic Biol Med 2006;40(3):501-6.

42 Ishii M, Shimizu S, Nawata S, Kiuchi Y, Yamamoto T. Involvement of reactive oxygen species and nitric oxide in gastric ischemia-reperfusion injury in rats: protective effect of tetrahydrobiopterin. Dig Dis Sci 2000;45(1):93-8.

43. Yüksel S, Akbay A, Yürekli M. Contribution of adrenomedullin to homeostatic response to cold stress in rat model. Pathophysiology 2002;8(4):243-7.

44. Oliver KR, Kane SA, Salvatore CA, Mallee JJ, Kinsey AM, Koblan KS, et al. Cloning, characterization and central nervous system distribution of receptor activity modifying proteins in the rat. Eur J Neurosci 2001;14(4):618-28.

45. Ando K, Shimosawa T, Fujita T. Adrenomedullin in vascular diseases. Curr Hypertens Rep 2004;6(1):55-9.

46. Shimosawa T, Matsui H, Xing G, Itakura K, Ando K, Fujita T. Organ-protective effects of adrenomedullin. Hypertens Res. 2003;26 Suppl:S109-12.

47. Brunet J, Farine JC, Garay RP, Hannaert P. In vitro antioxidant properties of calcium dobesilate. Fundam Clin Pharmacol 1998;12(2): 205-12.

48. Rastogi L, Patnaik GK, Dikshit M. Free radicals and antioxidant status following pylorus ligation induced gastric mucosal injury in rats. Pharmacol Res 1998;38(2):125-32.

49. Tanaka J, Yuda Y. Role of lipid peroxidation in gastric mucosal lesions induced by ischemia-reperfusion in the pylorus-ligated rat. Biol Pharm Bull 1993;16(1):29-32.

50. Kwiecień S, Brzozowski T, Konturek SJ. Effects of reactive oxygen species action on gastric mucosa in various models of mucosal injury. J Physiol Pharmacol 2002;53(1):39-50.