Appl Biochem Biotechnol (2009) 152:449–459
DOI 10.1007/s12010-008-8384-z

Gastroprotective Effect of Nicorandil in Indomethacin and Alcohol-Induced Acute Ulcers

M. A. El-Moselhy · N. M. Abdel-Hamid · S. R. Abdel-Raheim

Received: 11 June 2008 / Accepted: 23 September 2008 /
Published online: 19 August 2008

Abstract Despite the fact that dietary habits and lifestyles are incredibly advancing, gastric ulceration is still a terrible complaint. Extensive use of non-steroidal anti-inflammatory drugs (NSAIDs) and alcohol, in addition to stress, are all predisposing factors for ulcers. Most medical treatments are always time consuming and not efficient or satisfactory to the patients. Cardiovascular patients always need NSAIDs, or mostly cannot quit alcohols, while using many cardiovascular drugs. We aim to study a possible benefit of a common nitrogen oxide donor, anti-anginal drug, nicorandil [*N*-(2-hydroxyethyl) nicotinamide nitrate ester], in managing acute gastric ulcers through studying its effect on some relevant intermediates to ulcerogenesis as lipid peroxidation, tumor necrosis factor-alpha (TNF-α), and nitric oxide (NO). In addition, gastric mucosal histology was studied to pursue the drug effects on tissue level. Our study revealed that both indomethacin and alcohol induced gastric ulcer mainly through up-regulation of gastric mucosal lipid peroxidation, local tissue inflammation, leukocytic infiltration, and necrosis. Both ulcerogens significantly elevated TNF-α and decreased NO, initiating ulcer formation. Nicorandil pretreatment depicted a higher preventive index in indomethacin- (89.8%) and alcohol-induced (77.7%) acute ulceration. On the tissue level, it also protected the gastric mucosa combating leukocyte infiltration and tissue congestion. Nicorandil protected tissue necrosis through decreasing oxidative stress, elevating NO levels, and down-regulating the ulcerogen-induced TNF-α elevation and improved sub-mucosal blood supply. We conclude that nicorandil may be a suitable bimodal treatment for cardiovascular patients who are at high risk of gastric ulcers

M. A. El-Moselhy
Department of Pharmacology, Minia University, Minia, Egypt

N. M. Abdel-Hamid (✉)
Department of Biochemistry, Faculty of Pharmacy, Minia University, Minia, Egypt
e-mail: nabilmohie@yahoo.com

N. M. Abdel-Hamid
College of Pharmacy, Minia University, Minia, Egypt

S. R. Abdel-Raheim
College of Medicine, Minia University, Minia, Egypt

by using variable analgesics to alleviate possible cardiac pain episodes, and probably frequent doses will offer a more established and long-lasting protection.

Keywords Gastric ulcer · Indomethacin · Alcohol · Nicorandil · Lipoperoxidation · Nitric oxide · TNF-α

Introduction

Gastric ulcers are known as erosions of the gastric mucosa that may penetrate the muscle layer and perforate the stomach wall [1]. It is induced by predominance of aggressive factors to defensive factors, as the balance between both factors is necessary for maintaining intact and healthy gastric mucosa [2]. Of these aggressive factors are lipid peroxides and inflammatory mediators. The most common lipid peroxide product is malondialdehyde (MDA), which is well known to cause cross-linking and polymerization of membrane components. Being diffusible, it also reacts with nitrogenous bases of DNA leading to more complicated sequelae [3]. The mucosal content of MDA (known alternatively as thiobarbituric acid reactive species, TBARS) was observed to increase with the development of gastric ulcers [4, 5].

Mostly, lipid peroxidation is one of several factors that act to produce observable gastric lesions. It initiates a series of complementary events disturbing the balance between the aggressive and defensive factors leading ultimately to gastric ulceration [6]. Another aggressive factor in ulcerogenesis is tumor necrosis factor-alpha (TNF-α), which is a potent stimulator of neutrophil infiltration into gastric mucosa [7]. Overproduction of TNF-α increases the risk of gastric ulcer and cancer [8]. The inhibition of TNF-α and neutrophil infiltration will ultimately inhibit tissue destruction by reactive oxygen species [9]. TNF-α is a pro-inflammatory cytokine secreted by macrophages increasingly during ulcerative stress [10] and it is a potent stimulator of inducible nitric oxide (NO) expression [11]. NO (commonly known as endothelium-derived relaxing factor) is about nine times more lipid than water soluble, so it is highly diffusible. Because it is very difficult to measure NO in biological fluids, it is usually measured after oxidative inactivation into nitrite (NO_2^-) or (NO_3^-) [12]. Mostly, both NO and TNF-α are changed dually in inflammatory gastric lesions [13].

The use of non-steroidal anti-inflammatory drugs (NSAIDs) is frequently associated with gastrointestinal toxicity [14]. One of these NSAIDs is indomethacin; it was suggested to break the mucosal barrier, leading to back diffusion of hydrogen ions and extensive mucosal damage [15]. Indomethacin was also reported to up-regulate the synthesis of pro-inflammatory molecules like TNF-α, contributing to mucosal injury [16]. It also decreases gastric mucosal blood flow, epithelial cell turnover, and bicarbonate and mucous secretion [17].

Ethanol is another common ulcerogen known to induce gastric ulcer in a dose-dependent fashion. It causes mucosal cellular injury through activation of matrix metalloproteinase enzymes, TNF-α release, lipid peroxidation, and protein carbonyl formation [18]. Thus, ethanol was frequently used as an experimental inducer of acute gastric ulcers [19].

Nicorandil [*N*-(2-hydroxyethyl) nicotinamide nitrate ester] is a potent NO donor, generally accepted as an effective therapy for the treatment of ischemic heart diseases. It also acts as a potassium channel opener [20]. It is effective in the treatment of several diseases as bronchial asthma, urinary incontinence, erectile dysfunction, and neurodegenerative diseases [21, 22]. It was reported by Patel and co-workers [23] that nicorandil depicted a gastroprotective potential in many drug-induced ulcers.

Our study was conducted to elucidate the different mechanisms underlying a possible gastroprotective action of nicorandil due to its complex nature as a NO donor, estimating some specific parameters known to mediate gastric ulcers as TBARS, NO, and TNF-α, challenged by two common ulcerogens indomethacin and ethanol. Histological changes of gastric mucosa will be examined in all groups in comparison to non-treated controls.

Materials and Methods

Materials

Forty male Wistar rats weighing 100–110 g (8–10 weeks old), were randomly classified into five groups (eight rats per each). Animals were held in polyethylene cages, given free access to tap water, and fed with standard commercial rat chow. Animals were left to accommodate for 1 week before dosing and kept on 12 h light/12 h dark regular cycle in partially humid and well-aerated room. The animals of the first group were not given any medication and served as control. The second group was given a single indomethacin (Nile Co., Egypt) dose (40 mg/kg), dissolved in sterile water, intraperitoneally [24]. Animals of the third group were given a single nicorandil (Torrent Pharmaceuticals, India) dose (10 mg/kg), intraperitoneally [25], 30 min before a single indomethacin dose as in group two. The fourth group animals were given a single oral ethanol 100% (Prolabo) dose as 1 ml/100 g [19]. The fifth group animals were given a single nicorandil dose, 30 min before a single ethanol dose.

All animals given ethanol were killed 1 h after the end of dosing, while animals given indomethacin were killed 3 h after the end of dosing. Blood samples were taken, centrifuged, sera were kept frozen (at −80 °C) and stomach tissues were excised, washed with ice-cold saline, opened along the greater curvature, re-washed by cold saline, and scored for macroscopical mucosal lesions. Then, the tissue was divided into two parts; one was kept frozen for biochemical assays of TBARS and NO in the scraped mucosa and the other portion was kept in 10% formalin saline for histological examinations.

Methods

Both mucosal lesions, counted as ulcer index (UI) and the severity factor, were determined according to the method of Peskar et al. [26]. The severity factor was measured by the gross lesion size. It was considered 0 if no lesions, 1 when lesion size is smaller than 2 mm, 2 when lesion size is 2–4 mm, or 3 if the lesion exceeds 4 mm. The mean lesion score for each group is the UI of the group. The preventive index of nicorandil was calculated from the equation of Hano et al. [27], as follows:

$$\frac{(\text{UI of control group} - \text{UI of drug treated group}) \times 100}{\text{UI of control group}}$$

TBARS-mucosal content was spectrophotometrically estimated as malondialdehyde in 10% tissue homogenate in saline [28]. Serum TNF-α was determined by ELISA (R & D, USA), according to the method of de Kossodo et al. [29]. Mucosal content of NO was then spectrophotometrically determined in the tissue homogenates utilizing copper–cadmium alloy as a reducing agent. This method basically relied on the reduction of nitrate into stable nitrite (Gries reaction) [30]. Formalin-preserved tissues were stained by hematoxylin–eosin and examined microscopically for tissue changes per drug challenges.

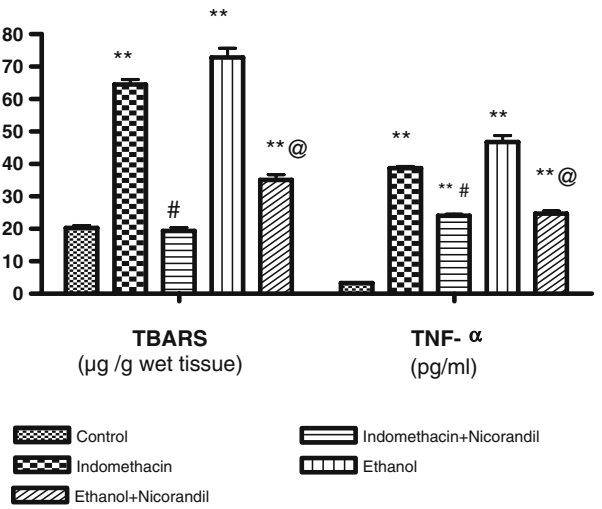

Fig. 1 Stomach mucosal TBARS content and serum TNF-α level in rats given a single dose of nicorandil (IP) before indomethacin (IP) and ethanol (per oral) single doses (mean±SE), $N=8$. **Significantly different from control at $p<0.01$. #Significantly different from indomethacin group at $p<0.001$. @Significantly different from alcohol group at $p<0.001$

Statistical Analysis

Data were analyzed using one-way analysis of variance. Graph Pad Prism (version 3.02 for Windows) software, San Diego, CA, USA, was used. A p value less than 0.05 was considered significant.

Results

Administration of indomethacin significantly up-regulated stomach mucosal lipid peroxidation, serum TNF-α, but down-regulated mucosal NO levels with a significant elevation of ulcer index. Pretreatment with nicorandil significantly decreased TBARS and TNF-α but increased NO values. It significantly decreased ulcer index and severity factor presenting a highly significant ulcer prevention (89.8%). Ethanol administration significantly elevated both TBARS and TNF-α with non-significant decrease of NO values. It also significantly elevated both ulcer index and severity factor. Nicorandil pretreatment significantly decreased TBARS and TNF-α with significant increase of NO values, decreasing both

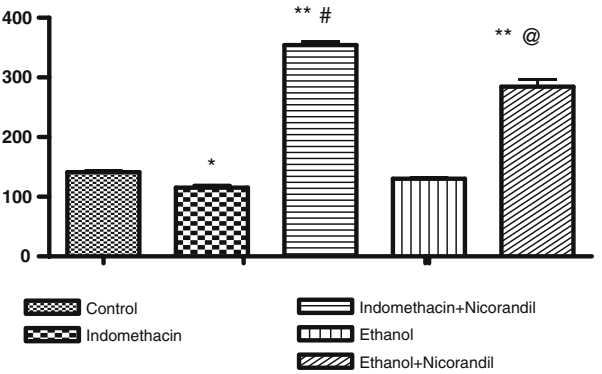

Fig. 2 Stomach mucosal NO (nmol/g wet tissue) in rats given a single dose of nicorandil (IP) before indomethacin (IP) and ethanol (per oral) single doses (mean±SE), $N=8$. *Significantly different from control at $p<0.05$. **Significantly different from control at $p<0.01$. #Significantly different from indomethacin group at $p<0.001$. @Significantly different from alcohol group at $p<0.001$

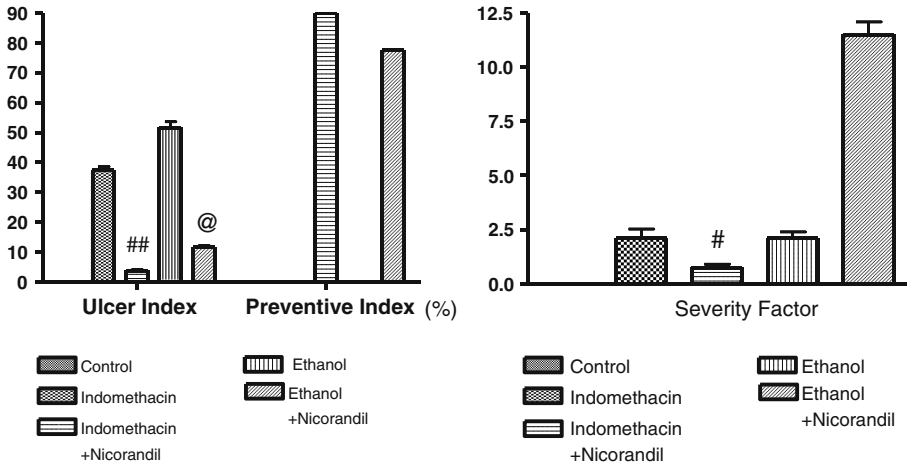

Fig. 3 **a** Ulcer indices, preventive indices (percentile), and **b** severity factors in rats given a single dose of nicorandil (IP) before indomethacin (IP) and ethanol (per oral) single doses (mean±SE), $N=8$. $^{\#}$Significantly different from indomethacin group at $p<0.05$. $^{\#\#}$Significantly different from ethanol group at $p<0.001$. $^{@}$Significantly different from alcohol group at $p<0.001$

ulcer index and severity factor with a significant preventive index (77.7%) (Figs. 1, 2, and 3a,b).

Histological examination of the stomach revealed the following: Control group (Fig. 4): normal microscopic structures of examined stomach were seen without any abnormalities. The parietal cells located in the upper half of gastric glands were spherical to pyramidal in shape with eccentric nuclei and pale eosinophilic vacuoles in the cytoplasm. Chief cells, which reside primarily in the lower half of the gastric glands, were filled with zymogenic granules. In indomethacin-treated group (Fig. 5), the gastric mucosa was focally necrotic, ulcerated, and infiltrated with intense leukocytes (mostly lymphocytes and few eosinophils) beside extensive hemorrhages. The epithelial lining of gastric mucosa adjacent to the ulcers showed over-distended cytoplasm with mucus. The sub-mucosal blood vessels were congested and the muscular coat was hypertrophied and partially hyalinized. Nicorandil-pretreated indomethacin group showed rarely vacuolated surface epithelium of gastric mucosa. The mucosa, sub-mucosa, and lamina propria were slightly infiltrated with eosinophils, lymphocytes, and macrophages. Congested capillaries, extravasated erythrocytes, and edema were also seen at the mucosa and the lamina propria (Figs. 6 and 7). In

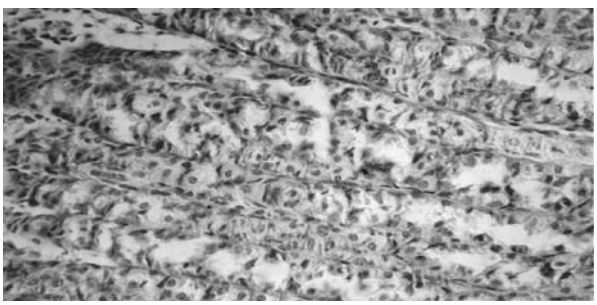

Fig. 4 Control group (H & E, ×300)

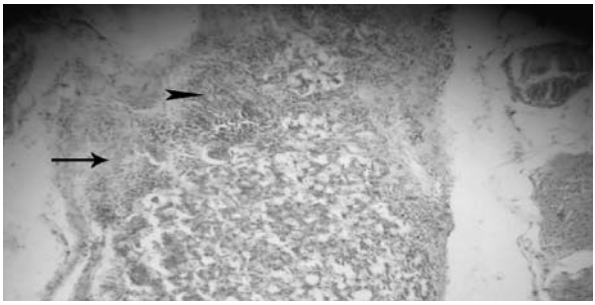

Fig. 5 Indomethacin-treated group (H & E, ×100)

ethanol-treated group, the stomach showed severe desquamation, erosions, and ulcerations with golden yellow pigment of hemosiderosis. Moderate lymphocytic infiltrations were observed among the gastric glands and invaded to include the eroded and ulcerated areas. Edema, congestion of the gastric blood vessel, and few leukocytes were detected in the sub-mucosa and lamina propria (Fig. 8). In nicorandil-pretreated ethanol group, the stomach showed mild mucosal congestion, slight desquamation of the lining epithelium, and few lymphocyte infiltrations (Fig. 9).

Histological Results

In Fig. 5, stomach of untreated indomethacin group shows gastric ulcer (arrow) with necrosis of the mucosa, hemorrhage, and intense leukocyte infiltration (arrowhead). In Fig. 6, stomach of nicorandil plus indomethacin-treated group shows congested capillaries in the sub-mucosa (arrows). In Fig. 7, stomach of nicorandil plus indomethacin shows focal hemorrhage (arrowhead), mucosal edema, and leukocyte infiltration (arrow). In Fig. 8, stomach of untreated ethanol group shows ulcer (arrow) with golden yellow pigment on the mucosal surface. In Fig. 9, stomach of nicorandil-treated ethanol shows mucosal congestion (arrow) and few leukocyte infiltration.

Discussion

The diversity of etiological factors underlying gastric ulcers and the complex nature of pathways participating in healing always make peptic ulcer treatment a complicated

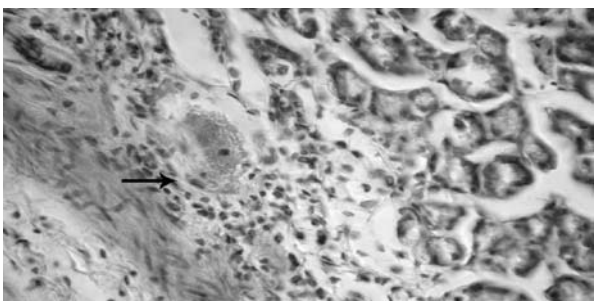

Fig. 6 Nicorandil-pretreated indomethacin group (H & E, ×300)

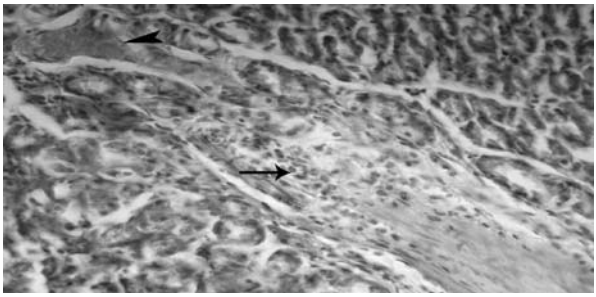

Fig. 7 Nicorandil-pretreated indomethacin group (H & E, ×300)

challenge. Maintaining equilibrium between aggressive and defensive factors is always a critical objective in peptic ulcer management [31]. Our finding revealed that indomethacin administration induced a significant and severe mucosal ulceration, associated with significant lipid peroxidation. This was manifested as mucosal necrosis, hemorrhage, and leukocytic infiltration. This effect on mucosal oxidative stress and histological derangement is in accordance with the report of Valecheva-Kuzmanova et al. [32]. Meanwhile, it significantly up-regulated serum TNF-α. Indomethacin-induced serum TNF-α and mucosal TBARS up-regulation at the ulcer site seems mostly to be responsible for ulcerogenesis [33].

Polymorphonuclear migration is an early and critical event in the pathogenesis of gastric mucosal injury caused by indomethacin. TNF-α was previously reported to be a pro-inflammatory cytokine that causes polymorphnuclear neutrophil migration, through up-regulating the expression of adhesion molecules in both neutrophil and endothelial cells [34]. Probably, prostaglandins inhibition by NSAIDs is responsible for the TNF-α rise, as these class of drugs markedly reduce prostaglandin synthesis, which were known to be potent inhibitors of TNF-α release from both macrophages [35] and mast cells [36].

On the other hand, the up-regulatory action of indomethacin to serum TNF-α is possibly responsible for the decrease in mucosal NO. This action depicted an agreement with reported results of Bauer et al. who registered that TNF-α is a potent inhibitor to constitutive NO [37]. NO mediated a protective effect in the stomach, mostly through modulation of cytokine production. One of the suggested pathways through which TNF-α contributes to mucosal injury induced by NSAIDs is through activation of caspases triggering apoptosis. NO can inhibit activation of these caspases through *S*-nitrosylation; this had been speculated to be a major reason why NO donors could protect against mucosal damage [38–40]. Histological studies on the gastric mucosa although showed that

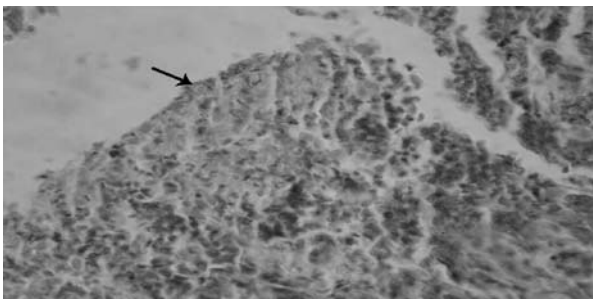

Fig. 8 Ethanol-treated group (H & E, ×500)

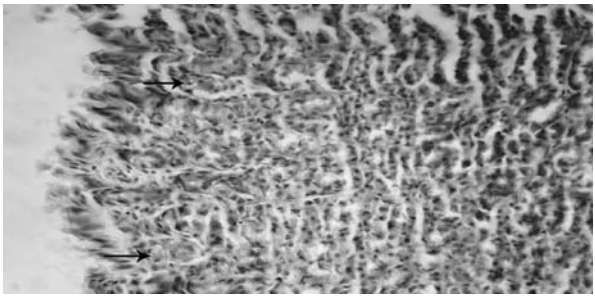

Fig. 9 Nicorandil-pretreated ethanol group (H & E, ×300)

nicorandil had corrective action against indomethacin-induced inflammatory infiltration and congestion at the ulcer site through increased blood supply. This nicorandil gastroprotective action was previously depicted by Ismail and co-workers [41], whose results coincide with the present results, except the effect of nicorandil on NO, as they noticed that it was consequently decreased. This contradiction is mostly referred to the ulcerogen in our study, taking into account that nicorandil is a classical NO donor.

This significant up-regulation of TNF-α, induced by alcohol treatment, is in accordance with the results of Kiefer and co-workers [42] in a study on group of alcohol abusers. Additionally, the highly significant alcohol-induced mucosal lipoperoxidation coincided with the findings of Khalil et al. [43], who tried different natural product extracts for protection against alcohol-induced gastric oxidative stress. The effect of alcohol on the gastric mucosa initiating ulcers is mediated also by its depressive action on mucosal NO content as seen in our results and in other recent publication [44].

Collectively, alcohol is considered a more potent ulcerogenic model if compared to indomethacin through their effects on mucosal TBARS and NO levels and inflammatory cytokine activation. These actions were mirrored on the mucosal tissue histology as polymorphonuclear infiltration and stomach lining epithelial derangements.

In addition, the association between the severity of gastric ulceration, up-regulated tissue lipoperoxidation, and TNF-α values was previously registered by Singh et al. [45], who found that antioxidant treatment could alleviate alcohol-induced gastric ulceration. Up-regulation of TNF-α was mostly depicted to be responsible for recurrence of healed ulcers [46].

Pretreatment with nicorandil decreased leukocyte infiltration probably through elevated NO levels at the ulcer sites, with a concomitant significant reduction of TBARS and TNF-α, when compared to indomethacin- and alcohol-treated animals. The lower preventive index of nicorandil in ethanol, in comparison to indomethacin-induced ulcers, may be attributed to the lower ability of the drug to reduce the rise in TBARS contents at the ulcer sites [47].

In conclusion, alcohol is a more powerful inducer of gastric ulcer than indomethacin. Gastric damaging action is mediated by elevated mucosal TBARS with a reduction of NO content, with a consequent elevation of serum TNF-α, which all participate in gastric necrosis, inflammation, congestion, and ulcer development. Nicorandil represents a more suitable cardiovascular therapy for patients who are at risk of gastric ulcers, through decreasing both drug-induced lipoperoxidation and TNF-α, with a parallel increase in NO availability. It is recommended as a more effective and convenient gastroprotective drug for cardiovascular patients using NSAIDs and/or alcohol; this may be a useful drug for possible acute ulcer seizures.

Acknowledgement Histological examination was kindly executed by Dr. Mohamed Hamed Mohamed, Histology Department, College of Veterinary Medicine, Zagazig University, Egypt.

References

1. Anderson, L. E. (1994). Mosby's medical, nursing and allied health dictionary. In K. N. Anderson, L. E. Anderson, & W. D. Glaze (p. 656). St Louis: Mosby.
2. Bose, M., Motghare, V. M., Dakhale, G. N., & Turankar, A. V. (2003). Antiulcer activity of levcromakalim and nicorandil in albino rats: a comparative study. *Polish Journal of Pharmacology*, *55*, 91–95.
3. Giust, A. M., Raimondi, M., Ravagnan, G., Sapora, O., & Parasassi, T. (1998). Human cell membrane oxidative damage induced by single and fractional doses of ionizing radiation: a fluorescence spectroscopy study. *International Journal of Radiation Biology*, *74*, 595–605. doi:10.1080/095530098141177.
4. Kwiecien, S., Brzozowski, T., Konturek, P. C., Pawlik, M. W., Pawlik, W. W., Kwiecien, N., et al. (2004). Gastroprotection by pentoxyifilline against stress-induced gastric damage. Role of lipid peroxidation, antioxidizing enzymes and proinflammatory cytokines. *Journal of Physiology and Pharmacology*, *55*, 337–355.
5. Jiang, P., Chang, L., Pan, C. Z., Qi, Y. F., & Tang, C. S. (2005). Protective role of metallothionein in stress induced gastric ulcer in rats. *World Journal of Gastroenterology*, *11*, 2739–2743.
6. Shian, W. M., Sasaki, I., Kamiyama, Y., Naito, H., Matsuno, S., & Miyazawa, T. (2000). The role of lipid peroxidation in gastric mucosal lesions induced by water water-immersion-restraint stress in rats. *Surgery Today*, *30*, 40–53. doi:10.1007/PL00010046.
7. Wei, X. M., Heywood, G. J., Di Girolamo, N., & Thomas, P. S. (2003). Nicorandil inhibits the release of TNF alpha from a lymphocytes. *International Immunopharmacology*, *3*, 1581–1588. doi:10.1016/S1567–5769(03)00176–0.
8. Mitsushige, S., Takahisa, F., Naohito, S., Akiko, N., Fang, X., Masayoshi, K., et al. (2007). Different effects of polymorphisms of tumor necrosis factor-alpha and interleukin-1 beta on development of peptic ulcer and gastric cancer. *Gastroenterologia y Hepatologia*, *22*(1), 51–59. doi:10.1111/j.1440–1746.2006.04442.x.
9. Kwiecien, S., Brzozowski, T., & Konturek, S. J. (2002). Effects of reactive oxygen species on gastric mucosa in various models of mucosal injury. *Journal of Physiology and Pharmacology*, *53*, 39–50.
10. Hamaguchi, M., Watanabe, T., Higuchi, K., Tominaga, K., Fujiwara, Y., & Arkawa, T. (2001). Mechanisms and roles of neutrophil infiltration in stress-induced gastric injury in rats. *Digestive Diseases and Sciences*, *46*, 2708–2715. doi:10.1023/A:1012779530004.
11. Calatayud, S., Barrachina, D., & Esplugues, J. V. (2001). Nitric oxide: relation to integrity, injury and healing of the gastric mucosa. *Microscopy Research and Technique*, *53*, 325–335. doi:10.1002/jemt.1100.
12. Wallace, J. L., & Miller, M. J. (2000). Nitric oxide mucosal defense: a little goes a long way. *Gastroenterol*, *119*, 512–520. doi:10.1053/gast.2000.9304.
13. Fox, J. G., Back, P., Dangler, C. A., Whary, M. T., Wang, T. C., Shi, H. N., et al. (2000). Concurrent enteric helminth infection modulates inflammation and gastric immune responses and reduce *Helicobacter*-induced gastric atrophy. *Nature Medicine*, *6*, 536–542. doi:10.1038/75015.
14. Griffin, M. R., & Scheiman, J. M. (2001). Prospects for changing the burden of non-steroidal anti-inflammatory drug toxicity. *The American Journal of Medicine*, *110*, 33S–37S. doi:10.1016/S0002–9343(00)00634–3.
15. Clinch, D., Banerjee, A. K., Levy, D. W., Ostick, G., & Faragher, E. B. (1987). Non-steroidal anti-inflammatory drugs and peptic ulceration. *Journal of the Royal College of Physicians of London*, *21*(3), 183–187.
16. Appleyard, C. B., McCafferty, D. M., Tigley, A. W., Swain, M. G., & Wallace, J. L. (1996). Tumor necrosis factor mediation of NSAID-induced gastric damage: role of leukocyte adherence. *American Journal of Physiology. Gastrointestinal and Liver Physiology*, *270*, G42–G48.
17. Bech, P. L., Xavier, R., Lu, N., Nanda, N. N., Dinauer, M., Podolsky, D. K., et al. (2000). Mechanism of NSAID-induced gastrointestinal injury defined using mutant mice. *Gastroenterol*, *119*, 699–705. doi:10.1053/gast.2000.16497.
18. Swarnakar, S., Mishra, A., Ganguly, K., & Sharma, A. V. (2007). Matrix metalloproteinase-9 activity and expression is reduced by melatonin during prevention of ethanol-induced gastric ulcer in mice. *Journal of Pineal Research*, *43*(1), 56–64. doi:10.1111/j.1600–079X.2007.00443.x.

19. Khosla, P., Karan, R. S., & Bhargava, V. K. (2004). Effect of garlic oil on ethanol-induced gastric ulcers in rats. *Phytotherapy Research*, *18*(1), 87–91. doi:10.1002/ptr.1349.
20. Sakai, K., Akima, M., Saito, K., Saitoh, M., & Matsubara, S. (2000). Nicorandil metabolism in rat myocardial mitochondria. *Journal of Cardiovascular Pharmacology*, *35*, 723–728. doi:10.1097/00005344–200005000–00007.
21. Hedlund, P., Holmquist, F., Hedlund, H., & Andersson, K. E. (1994). Effects of nicorandil on human isolated corpus cavernosum and cavernous artery. *The Journal of Urology*, *151*, 1107–1113.
22. Zhou, Q., Satake, N., & Shibata, S. (1995). The inhibitory mechanisms of nicorandil in isolated rat urinary bladder and femoral artery. *European Journal of Pharmacology*, *273*, 153–159. doi:10.1016/0014–2999(94)00685-Z.
23. Patel, H. M., Santani, D. D., & Goswami, S. G. (2001). Evaluation of the effects of nicorandil on experimentally induced gastric ulcers. *Pharmacol*, *63*, 154–159. doi:10.1159/000056127.
24. Toroudi, H. P., Rahgozar, M., Bakhtarian, A., & Djahanguiri, B. (1999). Potassium channel modulators and indomethacin-induced gastric ulceration in rats. *Scandinavian Journal of Gastroenterology*, *34*, 962–966. doi:10.1080/003655299750025048.
25. Sakai, K., Akima, M., & Katsuyama, I. (1999). Effects of nicorandil on experimentally induced gastric ulcers in rats: a possible role of K(ATP) channels. *Japanese Journal of Pharmacology*, *79*, 51–57. doi:10.1254/jjp.79.51.
26. Peskar, B. M., Ehrlich, K., & Peskar, B. A. (2002). Role of ATP-sensitive channels in prostaglandin-mediated gastroprotection in the rat. *The Journal of Pharmacology and Experimental Therapeutics*, *301* (3), 969–974. doi:10.1124/jpet.301.3.969.
27. Hano, J., Bugajski, J., & Danek, L. (1976). Effect of adrenergic blockade on gastric secretion altered by catecholamines in rats. *Archivum Immunologiae et Therapiae Experimentalis*, *24*(4), 507–524.
28. Mihara, M., & Uchyama, M. (1978). Determination of malondialdehyde precursor in tissues by thiobarbituric acid test. *Analytical Biochemistry*, *86*(1), 271–278. doi:10.1016/0003–2697(78)90342–1.
29. de Kossodo, S., Houba, V., & Grau, G. E. (1995). Assaying tumor necrosis factor concentrations in human serum. A WHO International Collaborative study. *Journal of Immunological Methods*, *182*(1), 107–114. doi:10.1016/0022–1759(95)00028–9.
30. Sastry, K. V. H., Moudgal, R. P., Mohan, J., Tyagi, J. S., & Rao, G. S. (2002). Spectrophotometric determination of serum nitrite and nitrate by copper-cadmium alloy. *Analytical Biochemistry*, *306*(1), 79–82. doi:10.1006/abio.2002.5676.
31. Glavin, G. B., & Szabo, S. (1992). Experimental gastric mucosal injury: laboratory models reveal mechanisms of pathogenesis and new therapeutic strategies. *The FASEB Journal*, *6*, 825–831.
32. Valcheva-Kuzmanova, S., Krasnaliev, I., Galunska, B., & Belcheva, A. (2007). Influence of DL-alpha-tocopherol acetate on indomethacin-induced gastric mucosal injury in rats. *Autonomic & Autacoid Pharmacology*, *27*(3), 131–136. doi:10.1111/j.1474–8673.2007.00402.x.
33. Swarnakar, S., Ganguly, K., Kundu, P., Banerjee, A., Maity, P., & Sharma, A. V. (2005). Curcumin regulates expression and activity of matrix metalloproteinase 9 and 2 during prevention and healing of indomethacin-induced gastric ulcer. *The Journal of Biological Chemistry*, *280*(10), 9409–9415. doi:10.1074/jbc.M413398200.
34. Santucci, L., Fiorucci, S., Di Matteo, F. M., & Morelli, A. (1995). Role of tumor necrosis factor alpha release and leukocyte margination in indomethacin-induced gastric injury in rats. *Gastroenterology*, *108* (2), 393–401. doi:10.1016/0016–5085(95)90065–9.
35. Kunkel, S. L., Wiggins, R. C., Chensue, S. W., & Larrick, J. (1986). Regulation of macrophage tumor necrosis factor production by prostaglandin E2. *Biochemical and Biophysical Research Communications*, *137*, 404–410. doi:10.1016/0006–291X(86)91224–6.
36. Hogaboam, C. M., Bissonnete, E. Y., Chin, B. C., Befus, A. D., & Wallace, J. L. (1993). Prostaglandins inhibit inflammatory mediator release from rat mast cells. *Gastroenterol*, *104*, 122–129.
37. Bauer, H., Jung, T., Tsikas, D., Stichtenoth, D. O., Frolich, J. C., & Neumann, C. (1997). Nitric oxide inhibits the secretion of T-helper 1- and T-helper 2-associated cytokines in activated human T cells. *Immunology*, *90*, 205–211. doi:10.1046/j.1365–2567.1997.00161.x.
38. Wallace, J. L., McKnight, W., Del Soldato, P., Baydoun, A. R., & Cirino, G. (1995). Antithrombotic effects of a nitric oxide-releasing, gastric sparing aspirin derivative. *The Journal of Clinical Investigation*, *96*, 2711–2718. doi:10.1172/JCI118338.
39. Fiorucci, S., Antonelii, E., Santucci, L., Morelli, O., Miglietti, M., Federici, B., et al. (1999). Gastrointestinal safety of nitric oxide-derived aspirin is related to inhibition of ICE-like cysteine proteases in rats. *Gastroenterol*, *116*(5), 1089–1106. doi:10.1016/S0016–5085(99)70012–0.
40. Fiorucci, S., Santucci, L., Gresele, P., Faccino, R. M., del Soldato, P., & Morelli, A. (2003). Gastrointestinal safety of NO-aspirin (NCX-4016) in healthy human volunteers: a proof of concept endoscopic study. *Gastroenterol*, *124*, 600–607. doi:10.1053/gast.2003.50096.

41. Ismail, H. A. F., Khalifa, M. M. A., Hassan, M. K., & Ashour, O. M. (2007). Investigation of the mechanisms underlying the gastroprotective effect of nicorandil. *Pharmacology*, *79*, 76–85. doi:10.1159/000097817.
42. Kiefer, F., Jahn, H., Schick, M., & Wiedemann, K. (2002). Alcohol intake, tumor necrosis factor-α, leptin and craving: factors of a possibly vicious circle. *Alcohol and Alcoholism (Oxford, Oxfordshire)*, *37* (4), 401–404. doi:10.1093/alcalc/37.4.401.
43. Khalil, H., Ismail, H., Taye, A., & Kamel, M. (2007). Gastroprotective effect of *Lippia nodiflora* L. extracts in ethanol-induced gastric lesions. *Pharmacognosy Magazine*, *3*(12), 259–262.
44. Duffin, R., Shaw, C. A., & Rossi, A. G. (2008). Sildenafil reduces alcohol-induced gastric damage: just say 'NO'. *British Journal of Pharmacology*, *153*(4), 623–624. doi:10.1038/sj.bjp.0707642.
45. Singh, L. P., Kundu, P., Ganguly, K., Mishra, A., & Swarnakar, S. (2007). Novel role of famotidine in down-regulation of matrix metalloproteinase 9 during protection of ethanol-induced acute gastric ulcer. *Free Radical Biology & Medicine*, *43*(2), 289–299. doi:10.1016/j.freeradbiomed.2007.04.027.
46. Bhattacharya, S., Chatterjee, S., Bauri, A., Bandivdeker, A. H., Chattopadhyay, S., Bandyopadhyay, S. K., et al. (2007). Immunopharmacological basis of the healing of indomethacin-induced gastric mucosal damage in rats by the constituents of *Phyllanthus emblica*. *Current Science*, *93*(1), 47–53.
47. Sivalingam, N., Hanumantharaya, K., Faith, M., Basivireddy, J., & Balasubramanian, K. A. (2007). Curcumin reduces indomethacin-induced damage in the rat small intestine. *Journal of Applied Toxicology*, *27*(6), 551–560. doi:10.1002/jat.1235.