The effect of simvastatin pretreatment on stress-induced gastric ulcer in rats

Sıçanda stres ile uyarılan mide ülserinde simvastatin'in etkisi

Elif BAHADIR, Muhammet BULUT, Beyza KABAYUKA, Ece Söylem ŞENKAL, Abdullah AVLAÇ, Meral YÜKSEL, Feriha ERCAN, İnci ALİCAN

ABSTRACT

Objectives: This study aimed to elucidate the possible protective effect of simvastatin (SIM) pretreatment on stress-induced gastric ulcer in rats.

Materials and Methods: Gastric ulcer was produced in Sprague-Dawley rats (250-300 g) by cold-restraint stress. SIM (10 mg/kg/day; per oral) or saline was administered for 21 days prior to stress. On day-21, a group of animals was treated with the non-selective nitric oxide synthase (NOS) inhibitor L-NAME (50 mg/kg; intraperitoneally) or the non-selective cyclooxygenase (COX) inhibitor indomethacin (Indo; 5 mg/kg; subcutaneously) prior to SIM. The stomachs were examined macroscopically and microscopically and stored for biochemical analyses.

Results: The severity of the lesions of the stress group was decreased by SIM, but this was not altered significanty by L-NAME or Indo. Stress increased gastric myeloperoxidase activity comparised to control level ($p<0.01$); however, SIM did not cause a significant change on this parameter. Stress increased gastric chemiluminescence levels ($p<0.001$) which were reversed by SIM ($p<0.001$) and this effect continued in L-NAME- or Indo-treated animals.

Conclusion: SIM pretreatment of rats with cold-restraint stress provided protection against gastric lesion formation via supression of oxidants derived from sources other than neutrophils without the involvement of NOS and COX systems.

Keywords: Ulcer, Nitric oxide synthase, Cyclooxygenase, Rat, Stress

ÖZ

Amaç: Bu çalışmada sıçanlarda stres ile uyarılan mide ülserinde simvastatin'in (SİM) olası koruyucu etkisini değerlendirmek amaçlanmıştır.

Gereç ve Yöntem: Sprague-Dawley sıçanlarda (250-300 gr) soğuk-kısıtlama stres uygulaması ile mide ülseri oluşturuldu. Stres öncesi 21 gün süreyle SİM (10 mg/kg/day; oral yolla) veya fizyolojik tuzlu su (1 ml; oral yolla) uygulandı. Bir grup sıçan 21. günde SİM öncesi selektif olmayan nitrik oksit sentaz (NOS) inhibitörü L-NAME (50 mg/kg; intraperitoneal) veya selektif olmayan siklooksijenaz (COX) inhibitörü indometazin (İndo; 5 mg/kg; subkutan) verildi. Mideler makroskopik ve mikroskopik olarak incelendi ve biyokimyasal analizler için saklandı.

Bulgular: Stres grubunda lezyonların şiddeti SİM ile azaldı ancak L-NAME veya İndo ile anlamlı bir değişiklik göstermedi. Stres mide miyeloperoksidaz aktivitesini kontrol düzeyine kıyasla artırdı ($p<0.01$); ancak, SİM bu parametre üzerinde anlamlı bir değişikliğe neden olmadı. Stres mide kemiluminisans düzeylerini artırdı ($p<0.01$); bu etki SİM ile geri döndürüldü ($p<0.001$); L-NAME veya İndo ile tedavi edilen hayvanlarda ise devam etti.

Sonuç: Soğuk-kısıtlama stresine maruz bırakılan sıçanlara SİM öntedavisi mide lezyon oluşumunda NOS ve COX sistemlerinden ayrı mekanizmalarla nötrofil dışı hücrelerden kaynaklanan oksidanları baskılayarak koruma sağlamaktadır.

Anahtar kelimeler: Ülser, Nitrik oksit sentaz, Siklooksijenaz, Sıçan, Stres

Elif Bahadır, İnci Alican (✉)
Department of Physiology, School of Medicine, Marmara University, Istanbul, Turkey
e-mail. incialican@yahoo.com

Muhammet Bulut, Beyza Kabayuka, Ece Söylem Şenkal, Abdullah Yalvaç,
Student, School of Medicine, Marmara University, Istanbul, Turkey

Meral Yüksel
Vocational School of Health Related Professions, Marmara University, Istanbul, Turkey

Feriha Ercan
Department of Histology and Embriology, School of Medicine, Marmara Univiversity, Istanbul, Turkey

Introduction

Statins are widely used clinically for lowering hypercholesterolemia because of their inhibitory effect on 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the enzyme that catalyzes the rate-limiting step of the cholesterol synthesis in the liver and other tissues [1]. Clinical studies have shown that statins reduce cardiovascular related morbidity and mortality in patients with or without coronary artery disease with or without high cholesterol

levels [2-4]. Besides the therapeutic use in hyperlipidemia, the antioxidant, anti-inflammatory and immunomodulatory benefits of statins have been reported in many studies [5,6]. They promote endogenous nitric oxide (NO) production [7-9], decrease platelet aggregation and inhibit thromboxane formation [10]. Simvastatin (SIM) is a commonly prescribed statin with anti-inflammatory [11,12] and antioxidant effects [13]. Studies on experimental animals revealed that SIM protects against ischemia-reperfusion injury of the lung [14], kidney [15], heart [16] and brain [17]. A study by Tariq et al. [18] demonstrated the gastric antisecretory and antiulcer effects of SIM in pylorus-ligated rats. SIM was also found to protect gastric mucosa from indomethacin-induced ulceration via promoting gastric mucosal NO and prostaglandin E2 levels, stimulating gastric mucin release in addition to its direct antioxidant activity [19].

Stress ulcer characterized by diffuse lesions of the gastric mucosa and duodenum frequently occurs as a result of various stressful events, including extensive burns, shock, sepsis, major surgery, and severe trauma. The pathological basis for the development of stress-induced gastric lesions has been postulated to be multifactorial. The contribution of gastric neutrophil accumulation, inflammatory cytokine production, free radical production, decreased antioxidants and decreased mucosal blood flow have been reported to be involved in the pathogenesis of stress-induced gastric lesions [20].

The present study was conducted to elucidate the possible gastroprotective effect of SIM pretreatment on stress-induced gastric ulcer in rats and the mechanisms underlying this protection.

Materials and Methods

Animals

Adult Sprague-Dawley rats of either sex (250-300 g) were housed in a temperature-controlled room (22 ± 1 $^\circ$C) with a 12 h light/dark cycle and free access to food and water. The study protocol was approved by Marmara University, Animal Care and Use Committee.

Stress-induced gastric ulceration model

Gastric ulcer was produced in rats by cold-restraint stress, as described previously [21]. Briefly, the animals were immobilized in metallic restraining devices for 3 h at 4 $^\circ$C following a starvation period of 24 h in mesh-bottomed cages to minimize coprophagia. The control group was kept at room temperature without any stress.

Experimental design

Rats were randomized into five groups: Control group (n=7), stress group (n=7), SIM+stress group (n=7), L-NAME+SIM+stress group (n=7) and indomethacin (Indo)+SIM+stress group (n=7). In treatment groups, the animals were given SIM (10 mg/kg/day) or saline daily for 21 days by a gastric tube. On day-21, among SIM-treated rats, a group of animals was treated with the non-selective nitric oxide synthase (NOS) inhibitor L-NAME (50 mg/kg; intraperitoneally) or the non-selective cyclooxygenase (COX) inhibitor Indo (5 mg/kg; subcutaneously) 60 min prior to SIM administration. Then, gastric ulceration was induced by cold-restraint administration. The effect of L-NAME and Indo alone was also assessed in animals exposed top stress (n=5 per group). Immediately after the induction of cold-restraint stress, all rats were decapitated. The stomach was removed, opened along the greater curvature and washed with ice-cold saline. Gastric lesions were examined macroscopically to measure the length of ulcers. The length (mm) of each lesion was summed per stomach and used as lesion index. Tissue samples were taken for histologic evaluation of the lesions by light microscopy or stored at -70 $^\circ$C for subsequent measurement of malondialdehyde (MDA) and glutathione (GSH) levels, and myeloperoxidase (MPO) activity. Formation of reactive oxygen species in gastric samples was monitored by using chemiluminescence (CL) method.

Histological evaluation

For light microscopic investigation, tissue samples from the fundic region of the stomach were placed in 10% formaldehyde, dehydrated in ascending alcohol series (70%, 90%, 96% and 100%), and embedded in paraffin. For each animal, four randomly taken tissue sections (5 μm) were stained with hematoxylin and eosin (H&E) and examined under an Olympus BH-2 photomicroscope. The gastric injury based on epithelial desquamation, mucosal hemorrhage, glandular damage and inflammatory cell infiltration was scored using a scale ranging from 0 to 3 (0: none, 1: mild, 2: moderate, and 3: severe) for each criterion. The total score was 12 [22]. All tissue sections were examined by an experienced histologist (F.E.) who was unaware of the treatment groups.

Measurement of MDA and GSH levels

The gastric samples were homogenized in 10 volumes of ice-cold 10% trichloracetic acid and centrifuged at 700 g for 15 min at 4 $^\circ$C. Supernatant was removed and recentrifuged at 10,000 g at 4 $^\circ$C for 8 min. GSH was determined by a spectrophotometric method which is a modification of Ellman procedure [23]. Lipid peroxide levels are expressed in terms of MDA equivalents as nmol MDA/g tissue [24].

Measurement of MPO activity

Gastric MPO activity- an indicator of neutrophil accumulation- was assessed by measuring the H_2O_2-dependent oxidation of o-dianizidine 2HCl. One unit of enzyme activity was defined as the amount of MPO present that causes a change in absorbance of 1.0 unit min at 460 nm and 37 ^{0}C and expressed in units per g tissue [25].

CL assay

Chemiluminescence assay is a direct noninvasive method for measuring reactive oxygen species. Due to limitations, i.e., potential variability and low intensity of native CL, luminol and lucigenin can be used as enhancers. Due to their high quantum efficiency after oxidation, they function as bystander-substrates for oxygenation and form high levels of excited-state products and CL, when added to an in vitro biological system. The excited electrons in these compounds revert to their ground state with the emission of energy as light (CL) and can be detected by a luminometer.

In this study, CL of the gastric samples was recorded at room temperature using Mini Lumat LB 9506 luminometer (EG&G Berthold, Germany) in the presence of luminol or lucigenin probes 0.2 mM each. All counts were obtained at 15 s intervals for 5 min and the results were expressed as area under the curve (AUC) of relative light unit (rlu) for 5 min per mg tissue [26]. The calculation was based on the integration of the curve by the trapezoidal rule (a linear approximation).

Statistical analysis

All data are expressed as means ± S.E. The histological data were compared by Mann-Whitney U non-parametric test and other parameters were compared by two-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison tests. Values of p <0.05 were regarded as significant. Calculations were done using Instat statistical analysis package (GraphPad Software, San Diego, CA, USA).

Results

Evaluation of the severity of gastric lesions

Cold-restraint stress resulted in production of gastric lesions. Although it did not reach a statistically significant level, the lesion index of the stress group (6.16±2.28 mm) showed a marked decrease by SIM pretreatment (2.32±1.74 mm). The ulcer index did not change significanty in SIM+stress groups treated with L-NAME or Indo in comparison to SIM+stress group. However, L-NAME or Indo treatment *per se* augmented the stress-induced lesion formation (Figure 1).
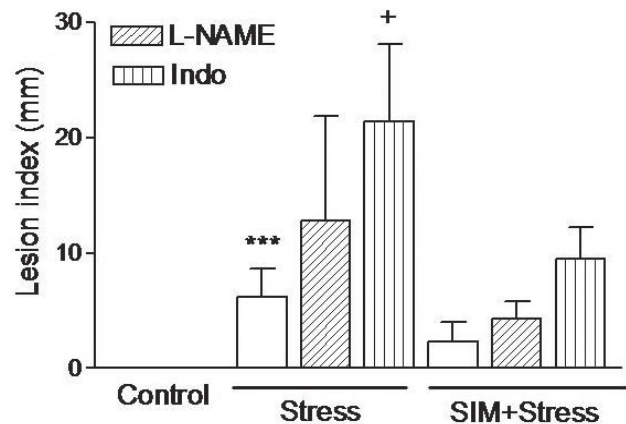

Figure 1. The macroscopic evaluation of the gastric lesions in experimental groups. ***p<0.001, compared to control group; +p<0.05, compared to untreated stress ulcer group.

In accordance with macroscopic examination, SIM pretreatment was also found to be beneficial on the severity of the lesions upon microscopic evaluation when compared with the untreated stress group (6.71±0.28 vs. 9.33±0.55; p<0.01). However, this effect did not seem to be modified by L-NAME or Indo administration along with SIM (Figure 2). As seen in Figure 3, the gastric samples of the stres group were characterized with severely damaged epithelium with diffuse hemorrhage and inflammatory cell infiltration. In SIM-treated stress group, the epithelial layer had regular morphology with mild glandular epithelial cell damage, mild vascular congestion and less inflammatory cell infiltration in comparison to the untreated stress group. L-NAME- or Indo-treated SIM groups revealed a similar gastric morphology when compared with SIM-treated stress group. L-NAME or Indo treatments alone did not change the severity of the mucosal lesions of the stress group (Figure 3).
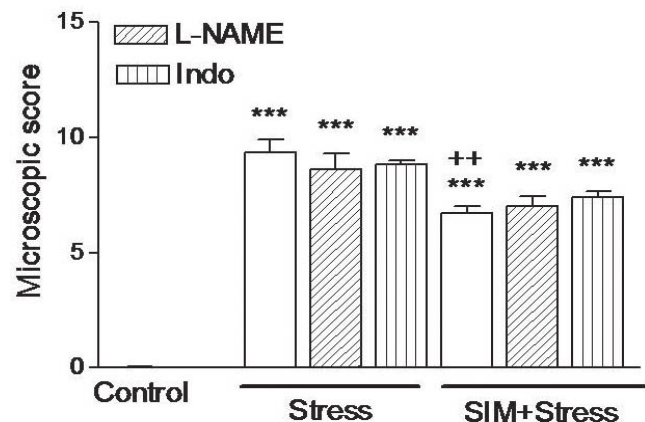

Figure 2. The microscopic evaluation of the gastric lesions in experimental groups. ***p<0.001, compared to control group; ++p<0.01, compared to untreated stress ulcer group.
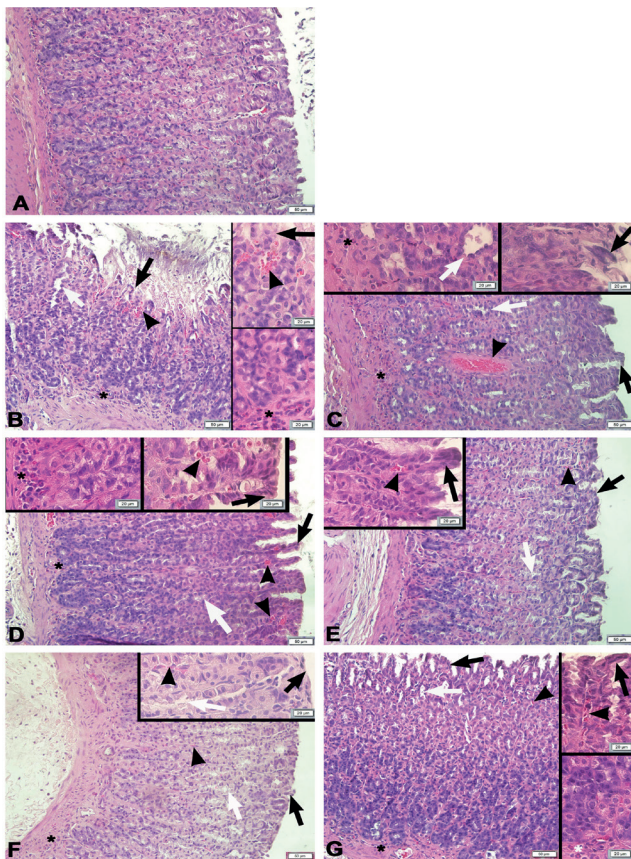
Figure 3. Photomicrographs depicting the histological characteristics of gastric samples representing the experimental groups. *Control group (A):* Surface epithelium and glandular epithelium with regular morphology; *Stress group (B)*: Severily damaged surface epithelium (black arrow) and glandular epithelium (white arrow), hemorrhage (arrow head) and inflammatory cell infiltration (*); *Indo+Stress group (C):* Desquamation of the surface epithelium (black arrow), damaged glandular epithelium (white arrow), vascular congestion (arrow head) and inflammatory cell infiltration (*); *L-NAME+Stress group (D):* Partially damaged surface epithelium (black arrow) and glandular epithelium (white arrow), vascular congestion (arrow head) and inflammatory cell infiltration *(*); SIM+Stress group (E):* Surface epithelium with regular morphology (black arrow), mild damage of the glandular epithelium (white arrow), mild vascular congestion (arrow head) and mild inflammatory cell infiltration (*); *Indo+SIM+Stress group (F):* Mild damage of the surface epithelium (black arrow) and glandular epithelium (white arrow), mild vascular congestion (arrow head) and mild inflammatory cell infiltration (*); *L-NAME+SIM+Stress group (G):* Surface epithelium with regular morphology (black arrow) and), mild damage of the glandular epithelium (white arrow), mild vascular congestion (arrow head) and mild inflammatory cell infiltration (*). Hematoxylin and eosin staining; original magnifications: X200, inserts X400.

Evaluation of gastric MDA and GSH levels

Gastric MDA levels did not show any statistically significant difference among the experimental groups (Table I).

Table I. The gastric malondialdehyde (MDA) and glutathione (GSH) data of the experimental groups.

	MDA (nmol/g)	GSH (mmol/g)
Control group	10.16±1.34	13.24±1.01
Stress ulcer group	11.01±1.81	9.67±0.58[*]*
Stress ulcer + treatment groups		
SIM+Stress	10.78±0.42	9.58±0.64[**]
L-NAME+SIM+Stress	11.16±1.37	9.09±1.01[**]
Indo+SIM+Stress	13.93±2.43	7.91±0.24[**]
L-NAME+Stress	11.01±1.81	10.85±0.97
Indo+Stress	8.22±1.40	10.23±1.17

**p<0.01, compared to control group.

Gastric GSH content of the control group (13.24±1.01 μmol/g) decreased markedly in the stress group (9.67±0.58 μmol/g; p<0.01). However, SIM pretreatment alone or along with L-NAME or Indo did not cause a significant change in the gastric GSH content of the stress group (Table I).

Evaluation of gastric MPO activity

Cold-restraint stress caused a significant increase in gastric MPO activity in comparison to control level (145.80±11.48 U/g vs. 102.40±8.64 U/g; p<0.01). In SIM-treated stress group, the gastric MPO activity was not significantly different from that of the untreated stress group (188.80± 20.82 U/g) (Figure 4).
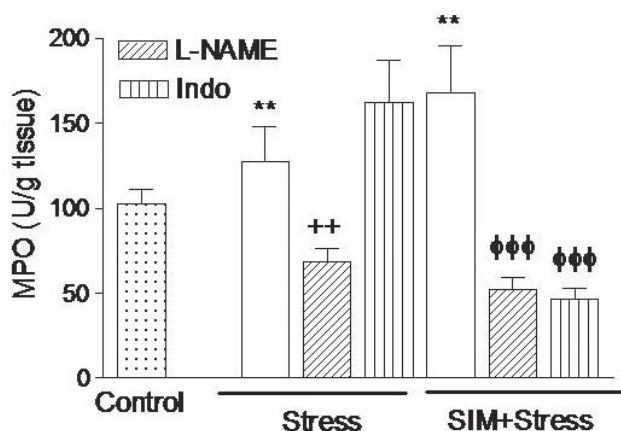

Figure 4. The gastric myeloperoxidase (MPO) activity of the experimental groups. **p<0.01, compared to control group; ++p<0.01, compared to untreated stress ulcer group; ϕϕp<0.001, compared to SIM+Stress ulcer group.

Evaluation of gastric luminol and lucigenin CL levels

As demonstrated in Table II, cold-restraint stress caused marked elevations in both luminol and lucigenin CL levels which were reversed by SIM pretreatment. The beneficial effect of SIM on luminol CL levels continued in animals pretreated with L-NAME or Indo prior to SIM.

Table II. The gastric chemiluminescence (CL) data of the experimental groups.

	Luminol CL (rlu/mg)	Lucigenin CL (rlu(mg)
Control group	6.77±0.77	12.38±0.79
Stress ulcer group	26.50±2.37***	31.70±2.15***
Stress ulcer + treatment groups		
SIM+Stress	11.60±1.06**+++	12.84±1.25+++
L-NAME+SIM+Stress	8.57±0.95	20.05±1.66ϕϕ
Indo+SIM+Stress	8.60±1.84	11.92±1.03
L-NAME+Stress	10.26±1.32+++	12.80±0.70+++
Indo+Stress	6.42±1.06+++	14.58±1.75+++

***$p<0.001$, compared to control group; +++$p<0.001$, compared to stress ulcer group; ϕϕ$p<0.01$, SIM+Stress group.

Discussion

The cold-restraint stress model in rats mimics clinical acute gastric lesions, that may appear in the gastric mucosa as a consequence of major trauma, surgery or sepsis and it is widely accepted for studying the mechanism of stress-induced gastric lesions [27,28]. An acute inflammation of the gastric mucosa plays an important role in the pathogenesis of stress-induced ulcerogenesis, being responsible for the enhanced permeability of blood vessels to activated neutrophils resulting in an excessive infiltration of gastric mucosal tissue [29-31]. In the present study, the cold-restraint stress model caused severe mucosal injury in the stomach of rats along with increased neutrophil infiltration and consumption of tissue glutathione content. Increased gastric luminol- and lucigenin-enhanced CL levels in cold-restraint animals revealed the involvement of oxidants in the pathogenesis of gastric injury.

The anti-inflammatory actions of the statins were substantiated by in vivo studies. In a murine model of acute inflammation, oral administration of SIM (3 mg/kg) resulted

in a significant decrease in foot-pad edema, with a potency and effectiveness comparable to that of indomethacin [32]. In a previous study performed in our laboratory, both SIM and fluvastatin were found to be beneficial in trinitrobenzene sulfonic acid-induced colonic inflammation in rats [33]. A study by Heeba et al. [11] comfirmed that SIM, at a dose of 10 mg/kg for 2 weeks, is gastroprotective against indomethacin-induced ulcer formation via scavanging free radicals, increasing NO and prostaglandin E2 levels, and increasing gastric juice mucin production. In line with these observations, in our study, SIM pretreatment (10 mg/kg for 14 days) reduced the severity of the gastric mucosal lesions induced by cold-restraint, as confirmed by macroscopic and microscopic examination. Although SIM pretreatment did not seem to affect the endogenous antioxidant glutathione consumption and neutrophil infiltration due to cold-restraint, there was marked suppression of oxidant production in the gastric samples. These findings may imply that SIM shows a beneficial effect on stress-induced gastric ulcer formation via scavanging oxidants which may derived from sources other than neutrophils (e.g. macrophages, endothelial cells, epithelial cells, or mast cells).

Stress ulcer increases the formation of reactive oxygen metabolites and promotes inhibition of prostaglandin synthesis, leading to alterations in gastric NO levels (34). NO is one of the most efficient mediators of gastric defense mechanism. Previous studies have shown that gastric lesions induced by various chemical agents are attenuated by administration of NO donors or augmented by suppression of the NO pathway [35]. However, in our study, the non-selective NOS inhibitor L-NAME given alone or prior to SIM to animals with cold-restraint ulcer did not show a statistically significant effect on the severity of the gastric lesions, gastric GSH content and gastric CL levels.

On the other hand, prostaglandins also play an essential role in gastric mucosal defense. This effect is dependent on the prostaglandin-induced stimulation of bicarbonate and mucous secretion, inhibition of gastric acid secretion, and regulation of maintaining epithelial cell restitution and mucosal blood flow [36]. Early studies have shown that inhibition of COX activity by non-selective nonsteroidal anti-inflammatory drugs (NSAIDs) leads to gastric ulcers and delays healing of the gastric mucosa [36,37]. In the present study, we used Indo- the non-selective inhibitor of COX-1 and COX-2- to examine if the actions of SIM are mediated via the COX-derived prostaglandins. According to our data, the suppression of the COX pathway by Indo did not cause a statistically significant change on the extent of

gastric lesions and gastric CL levels of the SIM-treated cold-restraint group.

In conclusion, pretreatment of rats with cold-restraint-induced gastric ulcers with SIM showed partial protection against gastric lesion formation which might be attributed to its effect to supress the generation of oxidants derived from sources other than neutrophils and that NOS and COX systems do not take part in this process.

Conflict of interest: The authors have declared that there is no conflict of interest.

References

1. Corsini A, Maggi FM, Catapano AL. Pharmacology of competitive inhibitors of HMG-CoA reductase. Pharmacol Res 1995;31:9–27. doi:10.1016/1043-6618(95)80042-5

2. Simes J, Furberg CD, Braunwald E, Davis BR, Ford I, Tonkin A, Shepherd J. Effects of pravastatin on mortality on patients with and without coronary heart disease across a broad range of cholesterol levels. Eur Heart 2002;23:207–15.

3. The Scandinavian Simvastatin Survival Study Group. Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). Lancet 1994;344:1383–9.

4. Downs JR, Clearfield M, Weis S, Whitney E, Shapiro DR, Bere PA, Langendorfer A, Stein EA, Kruyer W, Gotto AM Jr. Primary prevention of acute coronary events with lovastatin in men and women with average cholesterol levels: results of AFCAPS/TexCAPS. Air Force/Texas Coronary Atherosclerosis Prevention Study. JAMA 1998;279:1615–22. doi:10.1001/jama.279.20.1615.

5. Liao JK. Effect of statins on 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibition beyond low-density lipoprotein cholesterol. Am J Cardiol 2005;96:24F–33F. doi:10.1016/j.amjcard.2005.06.009

6. Werner N, Nickeniq G, Laufs U. Pleiotropic effects of HMG-CoA reductase inhibitors. Basic Res Cardiol 2002;97:105–16.

7. Trochu JN, Mital S, Zhang Xp, Xu X, Ochoa M, Liao JK, Recchia FA, Hintze TH. Preservation of NO production by statins in treatment of heart failure. Cardiovasc Res 2003;60:250-8. doi: http://dx.doi.org/10.1016/j.cardiores.2003.08.003.

8. Jiang JL, Jiang DJ, Tang YH, Li NS, Deng HW, Li YJ. Effect of simvastatin on endothelium-dependent vaso-relaxation and endogenous nitric oxide synthase inhibitor. Acta Pharmacol Sin 2004;25:893-901.

9. Dobrucki LE, Kalinowski L, Dobrucki IT, Malinski T. Statin-stimulated nitric oxide release from endothelium. Med Sci Monit 2001;7:622-7.

10. Schror K, Lobel P, Steinhagen-Thiessen E. Simvastatin reduces platelet thromboxane formation and restores normal platelet sensitivity against prostacyclin in type IIa hypercholesterolemia. Eicanosoids 1989;2:39-45.

11. Scalia R, Gooszen ME, Jones SP, Hoffmeyer M, Rimmer DM 3rd, Trocha SD, Huang PL, Smith MB, Lefer AM, Lefer DJ. Simvastatin exerts both anti-inflammatory and cardioprotective effects in apoliprotein E-deficient mice. Circulation 2001;103:2598–603. doi: 10.1161/01.CIR.103.21.2598.

12. Pruefer D, Scalia R, Lefer AM. Simvastatin inhibits leukocytes– endothelial cell interactions and protects against inflammatory processes in normocholesterolemic rats. Arterioscler Thromb Vasc Biol 1999;19:2894–900. doi: 10.1161/01.ATV.19.12.2894.

13. Ungureanu D, Filip C, Artenie A, Artenie R. Evaluation of simvastatin antioxidant effects. Rev Med Chir Soc Med Nat 2003;107:66–71.

14. Naidu BV, Woolley SM, Farivar AS, Thomas R, Fraga C, Mulligan MS. Simvastatin ameliorates injury in experimental model of lung ischemia-reperfusion. J Thorac Cardiovasc Surg 2003;126:482-9. doi:10.1016/S0022-5223(03)00699-8.

15. Inman SR, Davis NA, Olson KM, Lukaszek VA. Simvastatin attenuates renal ischemia/reperfusion injury in rats administered cyclosporine A. Am J Med Sci 2003;326:117-21.

16. Rendig SV, Symons JD, Amterdam EA. Effects of statins on myocardial and coronary artery response to ischemia-reperfusion. Can J Physiol Pharmacol 2003;81:1064-71. doi: 10.1139/y03-105.

17. Shabanzadeh AP, Shuaib A, Wang CX. Simvastatin reduced ischemic brain injury and perfusion deficits in an embolic model of stroke. Brain Res 2005;1042:1-5. doi:10.1016/j.brainres.2005.01.105

18. Tariq M, Khan HA, Elfaki I, Arshaduddin M, Al Moutaery M, Al Rayes H, Al Swailam R. Gastric antisecretory and antiulcer effects of simvastatin in rats. J Gastroenterol Hepatol 2007;22:2316–23. doi: 10.1111/j.1440-1746.2007.05021.x.

19. Heeba GH, Hassan MKA, Amin RS. Gastroprotective effect of simvastatin against indomethacin-induced gastric ulcer in rats: role of nitric oxide and prostaglandins. Eur J Pharmacol 2009;607:188–93. doi: 10.1016/j.ejphar.2009.02.008.

20. Yeomans N. The ulcer sleuths: the search fort he cause of peptic ulcers. J Gastroenterol Hepatol 2011;26:35-41. doi: 10.1111/j.1440-1746.2010.06537.x.

21. Senay EC, Levine RJ. Synergism between cold and restraint for rapid production of stress ulcer in rats. Proc Soc Exp Biol Med 1967;124:1221-3.

22. Jahovic N, Erkanli G, Işeri S, Arbak S, Alican I. Gastric protection by alpha-melanocyte-stimulating hormone against ethanol in rats: involvement of somatostatin. Life Sci 2007;80(11):1040-5. doi:10.1016/j.lfs.2006.11.036.

23. Aykac G, Uysal M, Yalcin AS, Kocak-Toker N, Sivas A, Oz H. The effect of chronic ethanol ingestion on hepatic lipid peroxide, glutathione peroxidase and glutathione transferase in rat. Toxicology 1985:46:71–6.

24. Casini A, Ferrali M, Pompella AS, Maellaro E, Comporti M. Lipid peroxidation and cellular damage in extrahepatic tissues of bromobenzene intoxicated mice. Am J Pathol 1986: 123:520–31.

25. Bradley PP, Preibat D, Christerser RD, Rothstein G. Measurement of cutaneous inflammation, estimation of neutrophil content with an enzyme marker. J Invest Dermatol 1982:78:206–9.

26. Haklar G, Ulukaya-Durakbasa C, Yuksel M, Dagli T, Yalcin AS. Oxygen radicals and nitric oxide in rat mesenteric

ischemia-reperfusion: modulation by ʟ-arginine and N-nitro-L-arginine methyl ester. Clin Exp Pharmacol Physiol 1998;25:908–12. doi: 10.1111/j.1440-1681.1998.tb02342.x.

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

28. Brzozowski T, Konturek PC, Pajdo R, Ptak-Belowska A, Kwiecien S, Pawlik M, Drozdowicz D, Sliwowski Z, Brzozowski B, Konturek SJ, Pawlik WW. Physiological mediators in nonsteroidal anti-inflammatory drugs (NSAIDs)-induced impairment of gastric mucosal defense and adaptation. Focus on nitric oxide and lipoxins. J Physiol Pharmacol 2008;59:89-102.

29. Kwiecień S, Brzozowski T, Konturek PC, Pawlik MW, Pawlik WW, Kwiecień N, Konturek SJ. Gastroprotection by pentoxyfilline against stress-induced gastric damage. Role of lipid peroxidation, antioxidizing enzymes and proinflammatory cytokines. J Physiol Pharmacol 2004;55:337-55.

30. Kwiecień S, Brzozowski T, Konturek PC, Pawlik MW, Pawlik WW, Kwiecień N, Konturek SJ. The role of reactive oxygen species and capsaicin-sensitive sensory nerves in the pathomechanism of gastric ulcers induced by stress. J Physiol Pharmacol 2003;54:423-37.

31. Konturek SJ, Konturek PC. Role of nitric oxide in digestive system. Digestion 1995;56:1-13.

32. Sparrow CP, Burton CA, Hernandez M, Mundt S, Hassing H, Patel S, Rosa R, Hermanowski-Vosatka A, Wang PR, Zhang D, Peterson L, Detmers PA, Chao YS, Wright SD. Simvastation has anti-infallamtory and antiatherosclerotic activities independent of plasma cholesterol lowering. Arterioscler Thromb Vasc Biol 2001;21:115-21. doi: 10.1161/01.ATV.21.1.115.

33. Jahovic N, Gedik N, Ercan F, Şirvancı S, Yüksel M, Şener G, Alican İ. Effects of statins on experimental colitis in normocholesterolemic rats. Scand J Gastoenterol 2006;41:954-62. doi: 10.1080/00365520600554444.

34. Bjarnason I, Scarpignato C, Takeuchi K, Rainsford KD. Determinants of the short-term gastric damage caused by NSAIDs in man. Aliment Pharmacol Ther 2007;26(1):95-106. doi: 10.1111/j.1365-2036.2007.03348.x.

35. Lopez-Belmonte J, Whittle BJ, Moncada S. The actions of nitric oxide donors in the prevention or induction of injury to the rat gastric mucosa. Br J Pharmacol 1993;108(1):73-8. doi: 10.1111/j.1476-5381.1993.tb13442.x.

36. Matsui H, Shimokawa O, Kaneko T, Nagano Y, Rai K, Hyodo I. The pathophysiology of non-steroidal antiinflammatory drug (NSAID)-induced mucosal injuries in stomach and small intestine. J Clin Biochem Nutr 2011;48:107-11. doi: http://doi.org/10.3164/jcbn.10-79.

37. Konturek SJ, Piastucki I, Brzozowski T, Radecki T, Dembińska-Kieć A, Zmuda A, Gryglewski R. Role of prostaglandins in the formation of aspirin-induced gastric ulcers. Gastroenterology 1981;80:4-9.