

Effects of statins in an indomethacin-induced gastric injury model in rats

Günnur ÖZBAKİŞ-DENGİZ¹, Aşkın HEKİMOĞLU², Nilüfer KANDEMİR³, Zehra KURCER¹

Departments of ¹Pharmacology and ³Pathology, Zonguldak Karaelmas University, School of Medicine, Zonguldak
 Department of ²Pharmacology, Dicle University, School of Medicine, Diyarbakır

Background/aims: Statins have additional pleiotropic effects beyond their lipid-lowering effects. In this study, the effects of statins were evaluated in an indomethacin-induced gastric injury model in rats. **Materials and Methods:** Animals were divided into eight groups. Distilled water (control group), omeprazole (30 mg/kg), atorvastatin (20 and 40 mg/kg), simvastatin (20 and 40 mg/kg), and rosuvastatin (20 and 40 mg/kg) were given orally (gavage). Thirty minutes later, indomethacin (25 mg/kg) was administered orally to all groups. Six hours later, the animals were sacrificed by decapitation. The mean ulcer indexes for each group were calculated, and the stomachs were evaluated histopathologically. **Results:** The ulcer indexes were as follows: control 1.72±0.16, omeprazole 0±0.00, and atorvastatin, simvastatin and rosuvastatin (at 20 and 40 mg/kg doses, respectively) 4.28±0.39, 4.99±0.96, 1.72±0.73, 1.90±0.48, 1.85±0.26, and 1.67±0.18. Atorvastatin significantly increased the indomethacin-induced ulcer index at both doses and the erosion score at 40 mg/kg dose. Although the 20 mg/kg dose of simvastatin inhibited mononuclear leukocyte infiltration, the 40 mg/kg dose induced hyperemia. Rosuvastatin did not decrease mononuclear leukocyte or neutrophil infiltrations at 20 mg/kg dose, and only neutrophil infiltration at the 40 mg/kg dose. **Conclusions:** In patients with gastric discomfort, statins must be used carefully. If statin therapy is needed, we recommend to avoid using atorvastatin and to use the other statins only in the minimum effective dose.

Key words: Atorvastatin, simvastatin, rosuvastatin, indomethacin, gastric ulcer

Sıçanlarda indometazin ile oluşturulan mide hasarı üzerine statinlerin etkileri

Amaç: Statinlerin lipid düşürücü etkilerinin yanısıra ilave pleiotropik etkileri vardır. Bu çalışmada, statinlerin sıçanlarda indometazinle oluşturulan mide hasarı üzerine etkileri değerlendirildi. **Gereç ve Yöntem:** Hayvanlar sekiz gruba ayrıldı. Kontrol grubuna distile su, diğer gruplara omeprazol (30 mg/kg), atorvastatin (20-40 mg/kg), simvastatin (20-40 mg/kg) ve rosuvastatin (20-40 mg/kg) oral yolla verildi. Otuz dakika sonra, bütün gruplara yine oral yolla indometazin (25 mg/kg) uygulandı. Altı saat sonra, hayvanlar dekapitasyon ile öldürüldü. Her grup için ortalama ülser indeksleri hesaplandı. Daha sonra, mideler histopatolojik olarak değerlendirildi. **Bulgular:** Ülser indeksleri kontrol grubunda 1.72±0.16, omeprazol grubunda 0±0.00, atorvastatin, simvastatin ve rosuvastatinin 20 ve 40 mg/kg dozlarında sırasıyla 4.28±0.39, 4.99±0.96, 1.72±0.73, 1.90±0.48, 1.85±0.26 ve 1.67±0.18 tespit edildi. Atorvastatin her iki dozda indometazinle oluşturulan ülser indeksini ve 40 mg/kg dozda erozyon skorunu anlamlı olarak artırmıştır. Simvastatinin 20 mg/kg dozu mononükleer lökosit infiltrasyonunu inhibe etmesine rağmen, 40 mg/kg dozu hiperemiye artırmıştır. Rosuvastatin 20 mg/kg dozda hem mononükleer lökosit hem de nötrofil infiltrasyonlarını, 40 mg/kg dozda ise sadece nötrofil infiltrasyonunu azaltmıştır. **Sonuç:** Mide rahatsızlığı olan hastalarda, statinler dikkatli kullanılmalıdır. Statin tedavisi mutlaka gerekiyorsa, atorvastatin kullanılmamasını ve diğer statinlerin de etkili en düşük dozlarda kullanılmasını tavsiye etmekteyiz.

Anahtar kelimeler: Atorvastatin, simvastatin, rosuvastatin, indometazin, mide ülseri

INTRODUCTION

Ulcers develop when the normal defense and repair mechanisms of gastric tissue are weakened.

Many drugs, especially aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) widely

Address for correspondence: Günnur ÖZBAKİŞ-DENGİZ
 Department of Pharmacology Zonguldak Karaelmas University
 School of Medicine, Zonguldak, Turkey
 E-mail: gunnurozbakis@ynet.com

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used in the treatment of pain, fever and inflammation, can cause gastric erosions and ulcers. NSAIDs inhibit the cyclooxygenase enzymes, which in turn diminishes the synthesis of cytoprotective endogenous prostaglandins and renders the mucosa vulnerable to noxious agents. The gastroduodenal mucosal injuries induced by NSAIDs vary from subtle microscopic injuries to gross macroscopic injuries (1). These agents break the mucosal barrier, provoke an increase in gastric mucosal permeability to H^+ and Na^+ ions, induce a drop in the transmucosal potential difference, and cause the formation of erosions and ulcers (2,3). NSAIDs also decrease the secretion of mucus, inhibit bicarbonate secretion, reduce the mucosal blood flow (4), cause microvascular injury, alter the microvascular structures (5), and increase acid levels, pepsinogen secretion and neutrophil infiltration (1).

Statins (3-hydroxy-3-methyl glutaryl-CoA reductase inhibitors) are a group of drugs designed to lower serum cholesterol levels. These drugs have been recognized as the most efficient drugs for the treatment of hyperlipidemia. Statins are usually used in elderly individuals with cardiovascular diseases, together with aspirin and other NSAIDs. Notably, statins have additional antioxidant, anti-inflammatory, immunomodulatory, antithrombotic, vascular protective, and neuroprotective pleiotropic effects beyond their lipid-lowering effects (6-9). In the literature, the effects of statins on gastric erosion and ulcer have been examined, and these studies have presented conflicting results (10,11). The purpose of our study was to investigate the effects at different doses of various statins (atorvastatin, simvastatin and rosuvastatin) in indomethacin-induced gastric injury, both macroscopically and histopathologically.

MATERIALS AND METHODS

Drugs and Chemicals

Atorvastatin (Lipitor®, Pfizer, Turkey), simvastatin (Zocor®, Merck Sharp & Dohme, Turkey), rosuvastatin (Crestor®, Astra Zeneca, Turkey), omeprazole (Omepral, Sandoz, Turkey), and indomethacin (Endol® Deva, Turkey) were used in this study. All drugs were dissolved in distilled water and were given orally (gavage) with a metal orogastric tube.

Animals

Male Wistar rats (150-200 g) were obtained from

Dicle University Health Sciences Practice and Research Center (Diyarbakır, Turkey). The animals were provided with standard laboratory conditions. Food was withdrawn 24 hours (h) before the experiment, but the animals were allowed free access to water. All experiments in this study were performed in accordance with the "Principles of Laboratory Animal Care" (NIH publication No. 86-23, revised 1984) and were approved by the Zonguldak Karaelmas University Animal Experiments Local Ethics Committee.

Procedure

The gastric injury model was established as described in our previous study (12). Animals were separated into eight groups: control (distilled water, 5 ml/kg), omeprazole (30 mg/kg), atorvastatin (20-40 mg/kg), simvastatin (20-40 mg/kg), and rosuvastatin (20-40 mg/kg). Distilled water, omeprazole and statins were given orally, and 30 minutes (min) later indomethacin (25 mg/kg – per oral) was administered to all the groups. Six hours later, the animals were sacrificed by decapitation. The stomachs were removed, opened along the great curvature, and washed with tap water to remove gastric contents. The stomachs were examined under a dissecting microscope with a square-grid eyepiece to assess the formation of ulcers. For each stomach, the ulcerated and total areas were measured as mm². The ulcer index for each stomach was calculated by the following formula:

Ulcer Index = [ulcerated area / total stomach area] X 100.

Pathological Analysis

The stomachs were fixed in 10% formalin solution and routinely processed for paraffin embedding. From each sample, 4 µm-thick sections were obtained and stained with hematoxylin-eosin for evaluation before investigation by light microscopy (DMLB, Leica, Germany). The histological slides were examined to determine the extent of mucosal hyperemia and erosion, the neutrophil count, and the degree of mononuclear leukocyte (MNL) infiltration (histopathologic score) (13).

Statistical Analysis

All data were expressed as the mean±SEM. The ulcer indexes and neutrophil counts of the groups were analyzed by one-way analysis of variance (ANOVA) and post-hoc LSD test. The erosion, hyperemia and MNL infiltration scores were analyzed by the Kruskal-Wallis test, and differen-

ces between the groups were evaluated by the Mann-Whitney U test. $p < 0.05$ was considered to be statistically significant.

RESULTS

Macroscopic Evaluation

The ulcer indexes and photographs of the stomachs in which belongs to all the groups are presented in Figure 1 and Figure 2 (a, b, c, d, e, f, g, h). The ulcer indexes were as follows: control 1.72 ± 0.16 , omeprazole 0 ± 0.00 , and atorvastatin, simvastatin and rosuvastatin (at 20 and 40 mg/kg doses, respectively) 4.28 ± 0.39 , 4.99 ± 0.96 ,

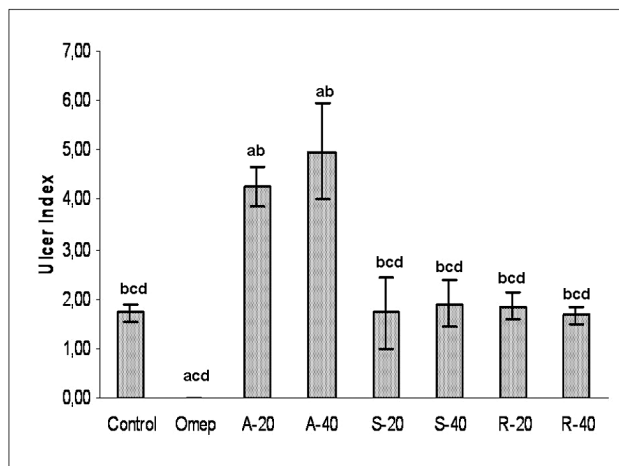


Figure 1. Effects of statins on the ulcer index in an indomethacin-induced gastric injury model. Data shown as mean values $\pm$ SEM (A-20: Atorvastatin 20 mg/kg. A-40: Atorvastatin 40 mg/kg. S-20: Simvastatin 20 mg/kg. S-40: Simvastatin 40 mg/kg. R-20: Rosuvastatin 20 mg/kg. R-40: Rosuvastatin 40 mg/kg).

a: $p < 0.05$, as compared with the control group
b: $p < 0.05$, as compared with omeprazole group
c: $p < 0.05$, as compared with A-20 group
d: $p < 0.05$, as compared with A-40 group

1.72 ± 0.73 , 1.90 ± 0.48 , 1.85 ± 0.26 , and 1.67 ± 0.18 . Omeprazole showed a protective effect in the indomethacin-induced ulcer model when compared to the control group ($p < 0.05$). Although pre-treatments with both doses of simvastatin and rosuvastatin did not change the indomethacin-induced ulcer indexes, atorvastatin pre-treatment (20 and 40 mg/kg) significantly increased ulcer indexes. The ulcer indexes of groups treated with atorvastatin were found to be higher than those of groups treated with simvastatin and rosuvastatin ($p < 0.05$) (Figure 1).

Histopathologic Evaluation

Histopathologic scoring is presented in Table 1 and histopathologic images in Figure 3 (a, b, c, d, e, f, g, h). Omeprazole reduced the hyperemia, erosion and MNL infiltration scores and the neutrophil count ($p < 0.05$) (Fig 3 b). MNL infiltration was observed to be lowest in the group treated with omeprazole ($p < 0.05$).

Atorvastatin treatment generally increased the histopathological damage (Fig 3 c, d). Only the erosion score for 40 mg/kg atorvastatin was found to be statistically significant. The neutrophil count was found to be higher in the 40 mg/kg atorvastatin group than all other groups except the control group ($p < 0.05$).

The erosion score was not increased by simvastatin or rosuvastatin in comparison to the control group. At high doses of these drugs, the erosion scores increased slightly, but these increases were not statistically significant. While the low dose of simvastatin inhibited MNL infiltration ($p < 0.05$), the high doses provoked hyperemia ($p < 0.05$). Both doses of rosuvastatin decreased the neutrophil count ($p < 0.05$), and the low dose reduced MNL infil-

Table 1. Histopathological effects of statins on indomethacin-induced gastric injury in rats

Group	n	Neutrophil	Erosion	Hyperemia	MNL infiltration
Control	6	24.28 ± 3.73 b	1.17 ± 0.48 b	1.83 ± 0.17 b	2.83 ± 0.17 b
Omeprazole	6	14.48 ± 1.58 ad	0.00 ± 0.00 ad	0.17 ± 0.17 acd	0.67 ± 0.21 acd
A-20	6	20.12 ± 2.51	2.50 ± 0.34 b	2.33 ± 0.21 b	2.50 ± 0.34 b
A-40	6	29.18 ± 2.32 b*	2.67 ± 0.21 ab	2.33 ± 0.21 b	2.33 ± 0.21 b
S-20	6	17.88 ± 1.58 cd	1.17 ± 0.54 d	1.33 ± 0.21 bcd	2.17 ± 0.17 ab
S-40	6	19.15 ± 2.57 d	2.00 ± 0.52 b	2.50 ± 0.22 ab*	2.33 ± 0.21 b
R-20	6	13.82 ± 0.84 ad	0.83 ± 0.31 bcd	1.50 ± 0.22 bcd	1.67 ± 0.21 abc
R-40	6	16.33 ± 2.20 ad	2.00 ± 0.45 b	2.17 ± 0.17 b*	2.50 ± 0.22 bd*

Data shown as mean values $\pm$ SEM (A-20: Atorvastatin 20 mg/kg. A-40: Atorvastatin 40 mg/kg. S-20: Simvastatin 20 mg/kg. S-40: Simvastatin 40 mg/kg. R-20: Rosuvastatin 20 mg/kg. R-40: Rosuvastatin 40 mg/kg). a: $p < 0.05$, as compared with the control group. b: $p < 0.05$, as compared with omeprazole group. c: $p < 0.05$, as compared with A-20 group. d: $p < 0.05$, as compared with A-40 group. *, difference between different doses of the same drug.

tration ($p < 0.05$). Simvastatin and rosuvastatin (20 mg/kg doses) reduced MNL infiltration significantly ($p < 0.05$).

DISCUSSION

In the present study, the effects of statins were studied in an indomethacin-induced gastric injury model. Macroscopically, while atorvastatin exhibited pro-ulcerogenic activity (Fig 2 c, d), simvastatin (Fig 2 e, f) and rosuvastatin (Fig 2 g, h) showed neither anti-ulcerogenic nor pro-ulcerogenic activities. The histopathological examination revealed that atorvastatin induced histopathologic damage (especially erosion at the 40 mg/kg dose) (Fig 3 c, d). Simvastatin at the low dose decreased MNL infiltration, while at the high dose, it induced hyperemia. While the low dose of rosuvastatin decreased MNL and neutrophil infiltrations, the high dose decreased only neutrophil infiltration.

NSAIDs may cause gastric ulcers due to an imbalance in aggressive and defensive factors in the stomach. These drug-induced ulcers are known to be related to the inhibition of cyclooxygenase,

which prevents prostaglandin biosynthesis. The inhibition of prostaglandin biosynthesis inhibits the release of mucus, a defensive factor against gastrointestinal damage (14), and decreases blood flow in the gastric mucosa (4). NSAIDs are commonly used as analgesic and antithrombotic drugs, especially in elderly patients. In these patients, statin usage also increases together with cardiovascular diseases. The correlation or not of statin usage with gastroduodenal ulcers or reflux esophagitis was investigated in a case-control study, and statin (hydrophilic or lipophilic) use was not associated with the risk of either disease. It was suggested that this therapy might be safe for patients with upper gastrointestinal disease (15). However, there is also a case report regarding atorvastatin-induced gastric ulcers, and in this patient, severe gastric ulceration was observed after three months of treatment with atorvastatin at 20 mg once daily for hypercholesterolemia. The patient had not been taking any pro-ulcerogenic drugs and had no evidence of *Helicobacter pylori* infection. El-Hajj et al. (16) could not explain the mechanism of this side effect. Atorvastatin

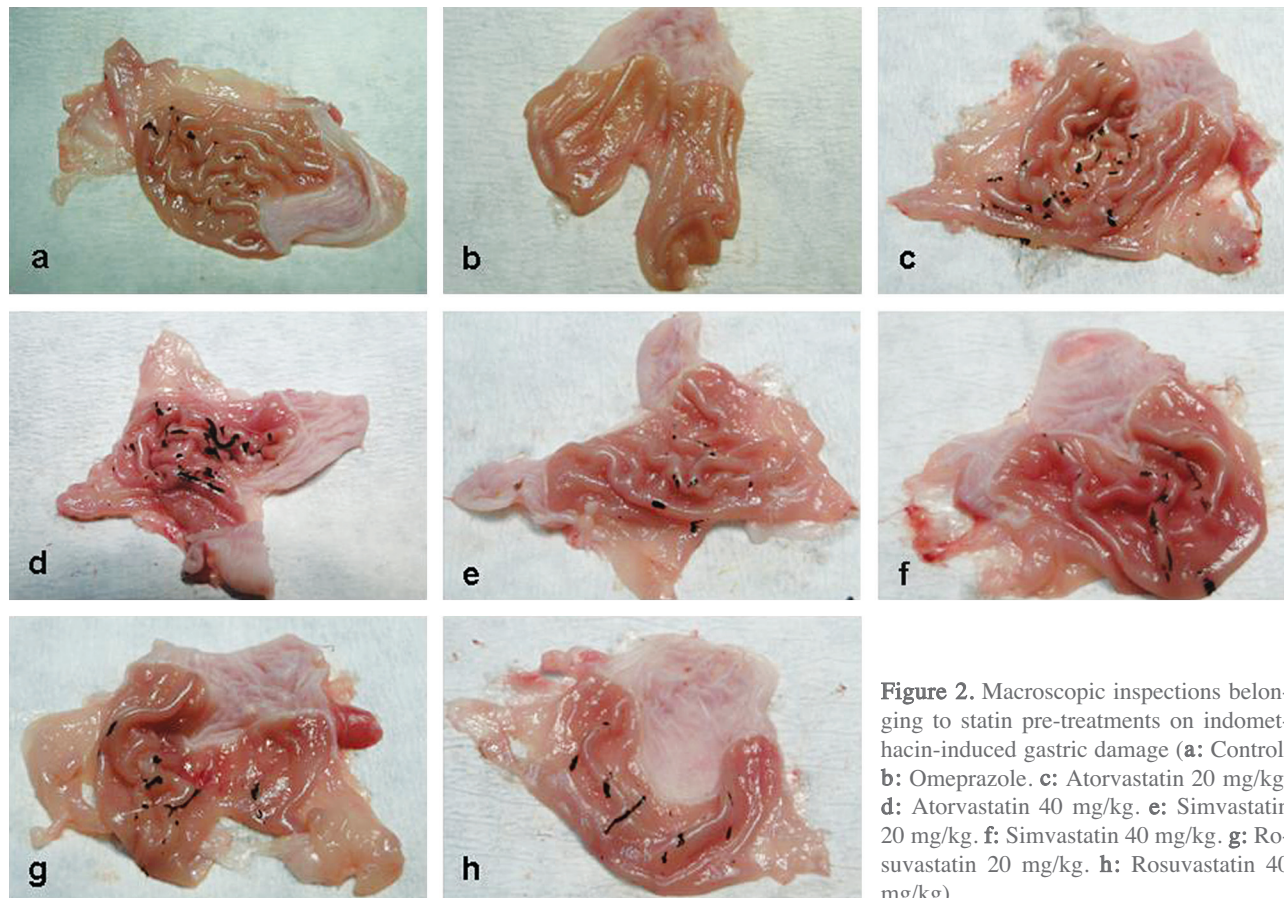


Figure 2. Macroscopic inspections belonging to statin pre-treatments on indomethacin-induced gastric damage (a: Control. b: Omeprazole. c: Atorvastatin 20 mg/kg. d: Atorvastatin 40 mg/kg. e: Simvastatin 20 mg/kg. f: Simvastatin 40 mg/kg. g: Rosuvastatin 20 mg/kg. h: Rosuvastatin 40 mg/kg).

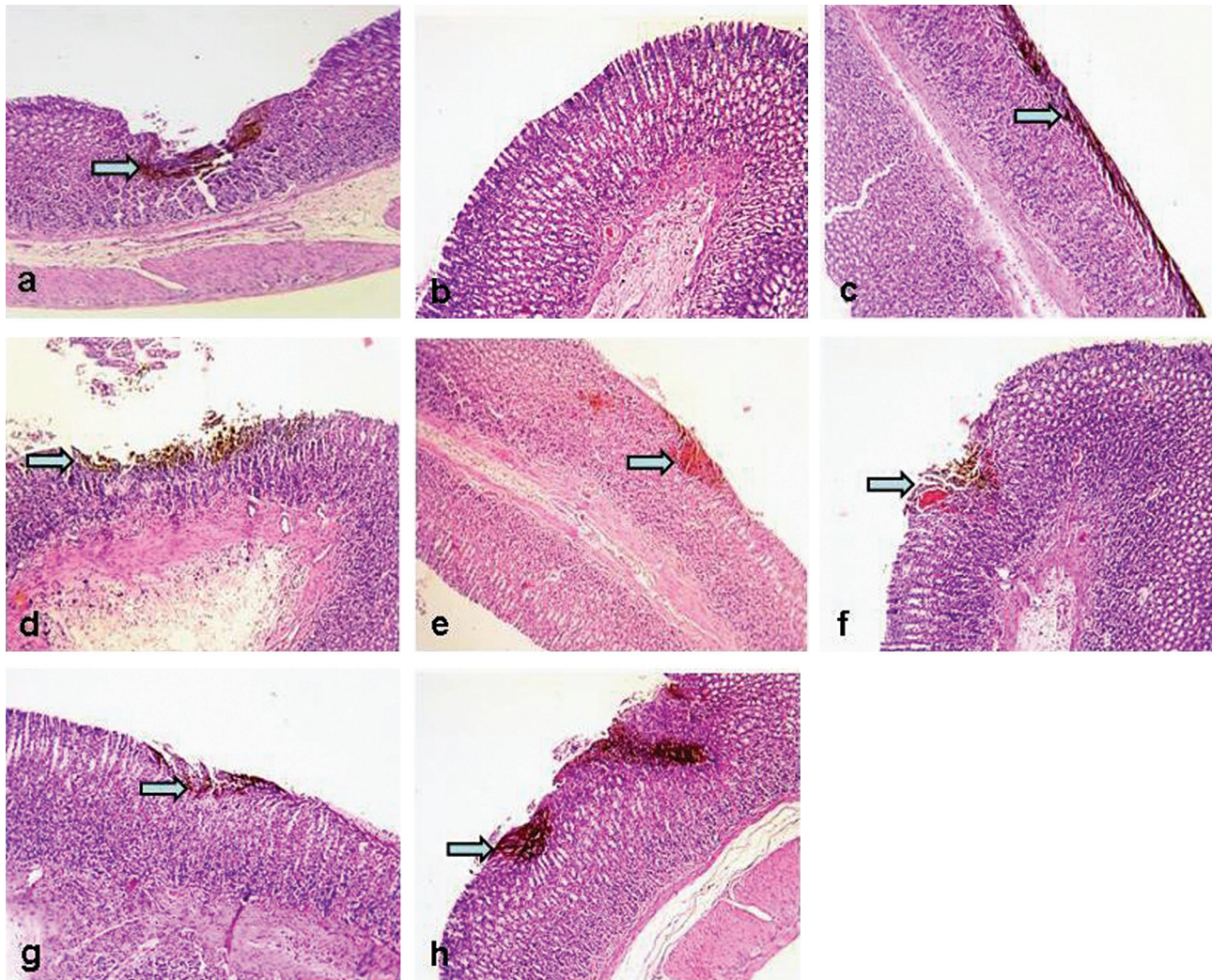


Figure 3. Histopathological evaluation of the gastric tissues of rats (A-F: hematoxylin and eosin, x100). **a)** In indomethacin group, ulcers causing a loss of surface epithelium and superficial glandular layer. **b)** In omeprazole group, regular gastric mucosa. **c)** In atorvastatin-20 group, widely superficial erosions. **d)** In atorvastatin-40 group, deep ulcer extending to the glandular layer. **e)** In simvastatin-20 group, superficial vascular congestion and erythrocyte extravasation in gastric mucosa. **f)** In simvastatin-40 group, superficial erosions and vascular congestion. **g)** In rosuvastatin-20 group, superficial ulcer. **h)** In rosuvastatin-40 group, ulcers affecting a portion of the glandular layer and surface epithelium.

was discontinued and the patient was put on proton pump inhibitor therapy for a total of six weeks. The patient experienced rapid relief from the symptoms. Simvastatin was started at a dose of 20 mg once daily with no adverse effects during a three-year follow-up. El-Hajj *et al.* (16) recommended that if a gastric ulcer is suspected, the drug should be stopped and replaced with another lipid-lowering drug to reduce the possibility of further damage to the gastric mucosa. In a study by Hagiwara *et al.* (17), fluvastatin repressed NSAID-induced ileal ulcer formation in rats, while atorvastatin and pravastatin did not. In our study, pretreatments with different statin preparations cau-

sed different results: while atorvastatin increased the ulcer index and erosion score, simvastatin and rosuvastatin did not change these measures.

In the literature, the gastroprotective effects of simvastatin in particular have been mentioned. Baraka *et al.* (18) reported that its administration (10 mg/kg, orally, for 7 days) caused a significant decrease in the acetic acid-induced ulcer area in diabetic rats, which could be attributed to a decrease in gastric levels of tumor necrosis factor- α , a pro-inflammatory cytokine. Heeba *et al.* (11) reported that simvastatin pre-treatment (10 mg/kg, orally, for 2 weeks) showed a gastroprotective effect, and that this effect is mediated by scavenging

free radicals, increasing nitric oxide and prostaglandin E2 levels and increasing gastric juice mucin production. In a study by Tariq et al. (10), simvastatin (20-40-60 mg/kg, orally, for 7 days) significantly and dose-dependently inhibited the volume of gastric secretion and its acidity. Simvastatin pre-treatment significantly reduced the formation of indomethacin- and ethanol-induced gastric lesions. The authors reported that simvastatin modified the gastric NO levels and reversed the ethanol-induced decrease in glutathione-S-transferase and increase in superoxide dismutase and catalase (10). However, in our study, simvastatin pre-treatment did not change the ulcer index, and only the low dose (20 mg/kg) reduced the MNL infiltration significantly while the high dose provoked hyperemia. These differences between our study and the other studies may be related to the experimental protocols, such as the duration of drug administration, doses and/or the experimental peptic ulcer model (ethanol, acetic acid, etc.). We administrated only one dose of simvastatin, while other investigators have used the drug for at least seven days. While the indomethacin dose was 30 mg/kg in other studies, we used a dose of 25 mg/kg in our study (10,11).

In addition, in our study, we evaluated whether rosuvastatin had effects on indomethacin-induced gastric injury. Rosuvastatin did not change the ul-

cer index, similar to simvastatin. While both doses of rosuvastatin decreased the neutrophil count, the low dose inhibited MNL infiltration. We found no published study related to the effects of rosuvastatin on experimental gastric injury. In one publication, it was reported that a 20 mg dose of rosuvastatin caused nausea in three of the 12 patients who participated in the study (19).

According to the results of our study, we drew the following conclusions:

- Atorvastatin may potentiate the ulcerogenic effect when it is used together with NSAIDs. In elderly patients, atorvastatin is usually used together with aspirin and other NSAIDs as a result of cardiovascular and rheumatic diseases. Because physicians prescribed proton pump inhibitors to these patients, the pro-ulcerogenic effects of atorvastatin may have been masked. In patients using NSAIDs, if statin therapy is necessary, we recommend the use of statins other than atorvastatin.
- Macroscopically, simvastatin and rosuvastatin did not induce ulcer formation. Although they did not change the erosion score, low doses had more beneficial effects than high doses on the hyperemia and MNL infiltration scores. Therefore, we recommend that these statins must be used at the minimum effective doses.

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