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THE EFFECT OF ATORVASTATIN ON GASTRIC ULCERS INDUCED BY INDOMETHACIN IN MICE

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ABSTRACT
Statins are a class of hypolipidemic drugs, which commonly prescribed for elderly people and those with heart-related conditions, often in combination with Aspirin and other Non-Steroidal Anti-Inflammatory Drugs (NSAIDs). The use of NSAIDs is one of the primary causes in the development of gastric ulcers, however studies about the effect of statins on gastric ulcers have presented conflicting results. Therefore, this study aims to investigate the effect of Atorvastatin on gastric ulcers induced by Indomethacin in mice, thus evaluating the safety of this combination. Animals were divided into 4 groups (n= 7 in each): group 1 (Control), group 2 (Indomethacin), group 3 (Atorvastatin 16.4 mg/kg), group 4 (Famotidine 8.3 mg/kg). Drugs were given orally for 26 days, and then gastric ulcers were induced by a single oral dose of Indomethacin (300 mg/kg) in the last day of the experiment. Histological findings revealed that the group of animals that received Indomethacin experienced ulceration of the entire mucosa, while pre-treatment with Atorvastatin prevented ulceration and reduced the depth of lesions in comparison to the Indomethacin group. There was no statistically significant difference observed between the Atorvastatin group and the Famotidine group.

KEYWORDS: Atorvastatin, gastric ulcers, NSAIDs, Famotidine, mice.

INTRODUCTION

Gastric ulcer is a common health problem that affects a lot of the population all over the world.[1] There is damage in the mucosal layer that penetrates the muscularis mucosa, and this damage is a result of an imbalance between aggressive and protective factors in the stomach. The imbalance can be caused by various factors; however, Helicobacter pylori and Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) account for the majority of the disease etiology.[2, 3]

NSAIDs widely used in the treatment of pain, fever and inflammation.[4] The main mechanism of NSAID-associated gastric ulcers is the systemic inhibition of constitutively expressed cyclooxygenase-1 (COX-1), which is responsible for the synthesis of prostaglandins that play an essential role in the maintenance of gastric mucosal integrity via stimulating the synthesis and secretion of mucus and bicarbonate, providing an adequate mucosal blood flow, and promoting epithelial proliferation.[5, 6]

Statins are a class of hypolipidemic drugs that lower the blood cholesterol levels by inhibiting 3-hydroxy-3-methylglutaryl coenzyme A (HMG-COA) reductase enzyme in hepatocytes.[4]

Previous studies have established that Atorvastatin, a drug from the statins family, has gastro-protective effects in Indomethacin-induced gastric ulcers.[7] Nonetheless, one research has shown that Atorvastatin considerably triggered gastric ulcers caused by Indomethacin.[6]

Statins are usually used in elderly people and individuals with cardiovascular diseases, together with Aspirin and other NSAIDs.[4] Therefore, the purpose of our study was to investigate the effect of Atorvastatin on gastric ulcers induced by Indomethacin in mice, thus evaluating the safety of this combination.

MATERIALS AND METHODS

Drugs and chemicals

Atorvastatin and Famotidine were obtained from Alpha

Company for pharmaceutical industries (Syria), while Indomethacin was obtained from Medco Labs for pharmaceutical industries (Syria). All drugs were suspended in Carboxymethyl cellulose (CMC) and were given orally. Atorvastatin and famotidine were suspended in CMC (0.5%), while Indomethacin was suspended in CMC (1%).

Animals

In this experiment, 28 female Balb/c mice weighing 25-30 g were used. The animals were housed in a temperature-controlled room at 25 ℃ with a 12 h light/ dark cycle and free access to food and water. All experimental procedures followed regulatory guidelines for the care and use of laboratory animals.

Experimental design

The mice were fasted for 24 hours in mesh-bottomed cages to minimize coprophagia, and had free access to water except for the last 6 h before we performed the anatomy. The experiment lasted 26 days in which all drugs were administered during the same time of the day to avoid changes due to diurnal rhythms of putative regulators of gastric functions.

The mice were classified into 4 groups with 7 members included in each group:

1. Control group: in which animals received CMC (0.5%).
2. Indomethacin group: in which animals received CMC (0.5%).
3. Atorvastatin+ Indomethacin group: in which animals were treated with 16.4 mg/kg of Atorvastatin.
4. Famotidine + Indomethacin group: in which animals received 8.3 mg/kg of Famotidine.

In all groups (except for the Control), gastric ulcers were induced by a single dose of Indomethacin (300 mg/kg) in the last day of the experiment (day 26).

After 6 hours from inducing ulcers, animals of all groups were sacrificed with an overdose of ether, their stomachs were removed, opened along the greater curvature and washed with saline to remove gastric contents and blood clots. The stomachs were fixed in 10% formalin for subsequent histological evaluation.

Tissue processing and histological evaluation

The stomachs were dehydrated in ascending alcohol series and embedded in paraffin. For each animal, 5µm-thick sections were obtained and stained with hematoxylin and eosin (H&E) prior to the study under a light microscope. All tissue sections were examined by an experienced histologist who was unaware of the treatment groups.

Based on the depths of lesions, the gastric injury was measured using a scale ranging from 0 to 5 (Table 1).

Table 1: Measuring system.

Score	Changes in the gastric mucosa
0	normal gastric mucosa
1	superficial erosions of mucosa
2	medium depth lesions of mucosa
3	deep lesions of mucosa with muscularis mucosa intact
4	deep lesions of the mucosa with damage to the muscularis mucosa but without penetration
5	Ulceration of the entire mucosa (there is penetration of the muscularis mucosa into the submucosa)

Assessment of ulcer index and protective ratio

Ulcer index (UI) was used to asses gastric mucosal lesions according to the method of Khallouf *et al.*[8], and was estimated according to the formula:

Ulcer Index (UI) = mean of intensity in a group + [number of ulcer positive animals/total number of animals] × 2

The percentage protective ratio was estimated according to the formula:[9] Protective ratio = 100 - (UI pretreated group/ UI control group) × 100

Statistical analysis

The Kruskal-Wallis test was used to analyze the data followed by the Mann–Whitney U test for multiple comparisons. The values were represented as mean ± SD. All statistical analysis was done using IBMM SPSS Statistic 20. The differences were considered significant when the calculated P value was less than 0.05.

RESULTS

Effect of Atorvastatin on histological picture

The Control group showed no gastric lesions or ulcers with normal gastric mucosa epithelium (Figure 1A).

On the other hand, the animal group that received Indomethacin showed ulceration of the entire mucosa. The ulcers were multiple and extensive and severe inflammatory infiltrates were observed (Figure 1B).

In comparison, no ulceration was observed in the Famotidine group, and the lesions were few and focal. Inflammatory infiltrates were absent or mild if present (Figure 1C).

The results in the Atorvastatin group were similar to the Famotidine group whereas pre-treatment with Atorvastatin prevented ulceration and decreased the depth of lesions compared to the Indomethacin group. The lesions were also few and focal and the inflammatory infiltrates, if present, were mild (Figure 1D).

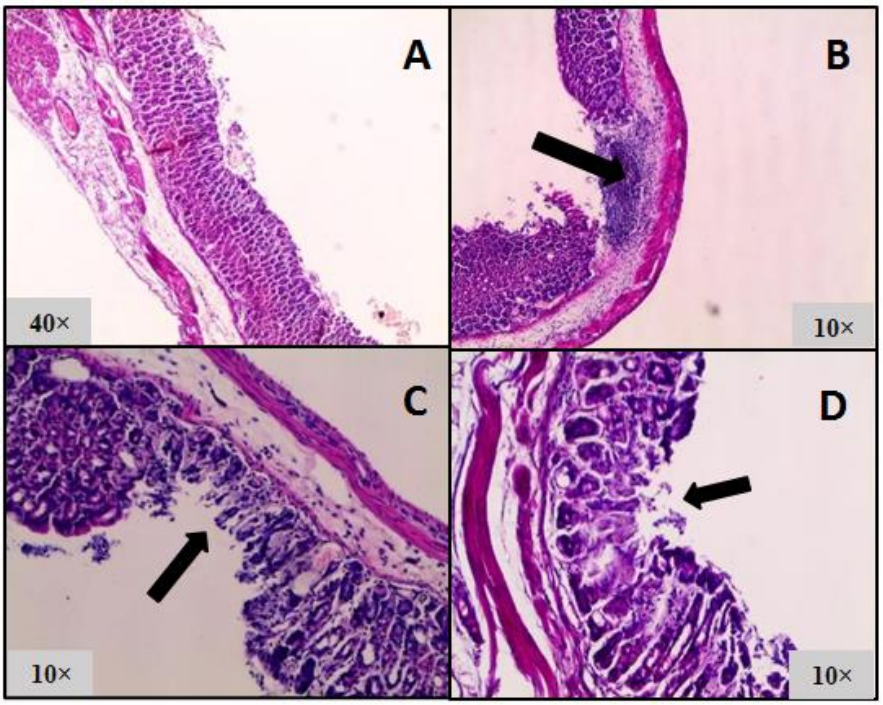

Figure 1: Histological assessment of gastric tissues using H&E stain. (A) Control group showed normal mucosa without lesions or ulcers. (B) Indomethacin group showed ulceration of the entire mucosa. (C) Famotidine group and (D) Atorvastatin group showed shallow mucosal lesions without any ulcer.

Effect of Atorvastatin on ulcer score, ulcer index and protective ratio

There were statistically significant differences in ulcer scores between the Control group and both the Famotidine group and Atorvastatin group. Pre-treatment with Atorvastatin significantly decreased ulcer score compared to Indomethacin group, and no statistically significant difference was measured in ulcer score between Atorvastatin group and Famotidine group (Table 2) (Figure 2).

Table 2: Effect of Atorvastatin on Indomethacin- induced gastric ulcer in mice.

Parameter / Group	Ulcer score Mean ± SD	Ulcer index	Protective ratio
Control	0.00±0.000	0.00	100
Indomethacin	4.57±0.78	5.99	-
Atorvastatin	1.57±0.53	1.57	73.78
Famotidine	2.00± 1.15	2.00	66.61

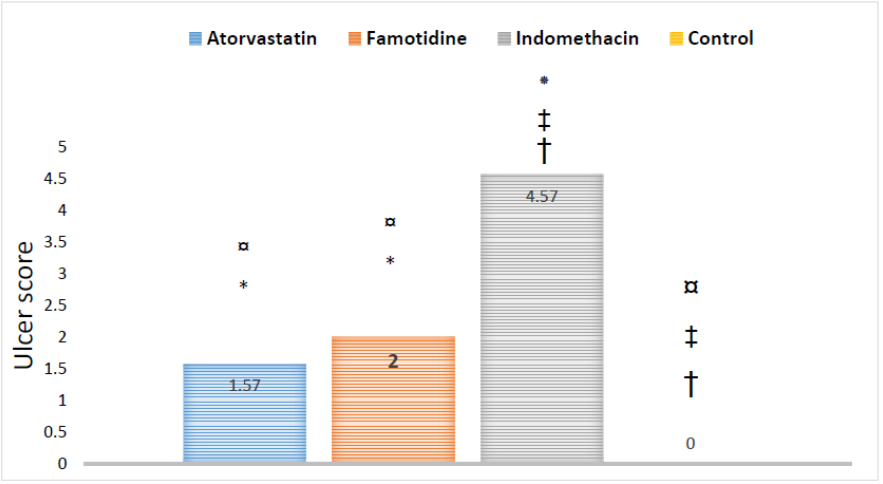

Figure 2: Effect of Atorvastatin on the ulcer score in an Indomethacin-induced gastric ulcer model. p<0.05*compared to the Control group, p<0.05¤ compared to the Indomethacin group, p<0.05† compared to the Famotidine group, p<0.05‡ compared to the Atorvastatin group0

DISCUSSION

Numerous studies have indicated the antioxidant, anti-inflammatory, and immunomodulatory effects of statins, and due to these effects, they exhibit therapeutic properties in gastrointestinal disorders, such as colitis, inflammatory bowel disease and intestinal and gastric ulcers.[6] However, some studies about the effect of statins on gastric ulcers have presented conflicting outcomes.[6,7] The use of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) is one of the primary causes of the development of gastric ulcers, as they inhibit the production of prostaglandins, which are crucial for maintaining the integrity of the gastric mucosa, thereby restricting their clinical use in the treatment of pain, inflammation, and fever.[5] Statins are commonly prescribed for elderly people and those with heart-related conditions, often in combination with Aspirin and other NSAIDs.[4]

In the present study, we evaluated the effect of Atorvastatin on the experimentally Indomethacin-induced gastric ulcer model. The histopathological examination revealed that Indomethacin caused ulceration of the entire mucosa and pre-treatment with Atorvastatin (at the 16.4 mg/kg dose) for 26 days prevented ulceration and decreased the depth of lesions caused by this medication.

EL-Sheikh *et al.*[7] and Ibrahim *et al.*[1] reported that pre-treatment with Atorvastatin (10 mg/kg, orally, for 7 days) showed a gastro-protective impact on an Indomethacin-induced gastric ulcer model (40 mg/kg) and this effect is mediated by a decrease in oxidative stress, the pro-inflammatory cytokine TNF-α and gastric acidity along with an increase in mucosal NO, PGE2 and mucin.

Furthermore, a study utilizing the Taiwan National Health Insurance Research Database (NHIRD) indicated that the treatment with statins lowered the risk of developing peptic ulcer disease.[10]

In contrast to the experimental and clinical results mentioned above, which provides promising evidence for the protective effect of Atorvastatin against gastric ulcers, Yildirim *et al.*[6] assessed the effects of increasing doses of Atorvastatin (0.5, 5, and 50 mg/kg) in both single (acute) and multiple (sub-chronic, 5 days) administrations in gastric ulcer model induced by Indomethacin (30 mg/kg). They found that the high dose of Atorvastatin (50 mg/kg) aggravated the gastric mucosal injury caused by Indomethacin in both acute and sub-chronic treatments, while the doses of 0.5 and 5 mg/kg showed no significant effects. The differences observed in the last study compared to our research could be related to the experimental protocols, such as the duration of drug administration and the used dosages.

We administered a dose of Atorvastatin equivalent to the maximum daily dose typically used in humans, whereas they used a significantly higher dose of Atorvastatin than what was utilized in our research. High doses of statins may cause hepatotoxicity[11], which in turn negatively affects the metabolism of various medications, including Indomethacin.[12,13] When the metabolism of Indomethacin is inhibited, it remains in the stomach for a longer period, thereby continuing to harm the gastric mucosa. What supports this hypothesis is that in the same research, they found no damaging effects on the gastric mucosa from Atorvastatin at different administered dosages (0.5, 5, and 50 mg/kg) when Indomethacin was not present, and they noted such effects only when Atorvastatin was combined with Indomethacin, particularly at a dosage of 50 mg/kg. Furthermore, in comparison to our study, Atorvastatin was administered in this research for a considerably shorter period, which resulted in Atorvastatin not showing its protective effect significantly.

CONCLUSION

Our study shows that Atorvastatin provides a protective effect against Indomethacin-induced gastric ulcers in mice. Therefore, it can be safely given in therapeutic doses combined with medications that may cause gastric ulcers. To precisely determine the mechanisms behind this effect, we recommend performing further studies with biochemical analysis.

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