Mechanism of Seratonin-Induced Gastric Mucosal Injury in Rats: Role of Acid-Back Diffusion

SIÇANLARDA SERATONİNİN NEDEN OLDUĞU MİDE MUKOZA HASARINDA ASİD-GERİ EMİLİMİNİN ROLÜ

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Abstract

Objective: It is known that, in rats, seratonin (5-HT) causes acute gastric mucosal injury due to decreased gastric mucosal blood flow (GMBF) resulting from vasoconstriction. Decreased gastric acid secretion in 5-HT-induced gastric injury has been described. The purpose of this study was to investigate whether the increase in acid back diffusion had an effect on 5-HT-induced gastric mucosal injury, in addition to the known effect on GMBF.

Material and Methods: Acute gastric mucosal injury was induced by the intra-peritoneal administration of 5-HT in 29 Wistar male rats. The animals were divided into four groups, the first control (5-HT only, n=7), the three others who were treated with either 10% polyethylene glycol, 2 µmol/kg omeprazole (n=8) or 10µmol/kg omeprazole (n=8) injected into the femoral vein one hour before 5-HT administration. The stomachs were subsequently removed and the severity of ulcers, if present was assessed using a previously validated scale, the histological ulcer index (HUI).

Results: In the group receiving 10µmol/kg of omeprazole, the HUI was significantly (p= 0.001) less than that seen with 5-HT alone. However, low dose omeprazole and PEG reduced HUI to a similar degree.

Conclusion: These results suggest that high doses of omeprazole decrease 5-HT-induced gastric injury and this might be related to the inhibition of the proton pump by omeprazole, which decreases H^+ output and thus, indirectly prevents acid back diffusion.

Key Words: Rat, serotonin, omeprazole, mucosal injury, acid-back diffusion

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Özet

Amaç: Seratonin'in (5-HT) sıçanlarda vazokonstriksiyon sonucu mide mukoza kan akımını azaltarak, akut mide mukoza hasarına neden olduğu bilinmektedir. 5-HT 'nin neden olduğu mide hasarında, mide asid sekresyonunun azaldığı tanımlanmaktadır. Bu çalışmanın amacı; 5-HT'nin indüklediği mide hasarında, mide mukozası kan akımı üzerindeki bilinen etkisine ek olarak, asid-geri emiliminde neden olduğu artışın bir etkisinin olup olmadığının araştırılmasıdır.

Gereç ve Yöntemler: 29 adet erkek Wistar sıçanda, akut mide mukoza hasarı, 5-HT'nin intraperitoneal uygulanması ile oluşturuldu. Hayvanlar 4 gruba ayrıldı: 1. Kontrol grubuna (n=7) yalnız 5-HT uygulandı, diğer 3 gruba sırasıyla 5-HT uygulanmadan 1 saat önce sırasıyla %10 polietilen glikol (2. kontrol grubu, n=6), 2 µmol/kg (n=8) veya 10 µmol/kg omeprazol (n=8) femoral venden uygulandı. Hayvanların mideleri çıkarıldı ve varolan ülserlerin şiddeti, daha önceden belirlenmiş bir skala ile değerlendirildi.

Bulgular: 10 µmol/kg omeprazol uygulanan grupta histolojik ülser indeksi (HÜİ), yalnız 5-HT uygulanan gruptan anlamlı olarak düştü (p=0.001). Düşük doz omeprazol ve PEG, HÜİ'ni aynı derecede düşürdü.

Sonuç: Bu sonuç, yüksek doz omeprazolün, 5-HT'nin indüklediği mide hasarını azalttığını göstermektedir. Omeprazolün bu etkisinin proton pompasını inhibe etmesine bağlı olarak, H^+ çıkışını azaltması ve indirek olarak asid-geri emilimini önlemesi ile ilişkili olabileceğini düşündürmektedir.

Anahtar Kelimeler: Sıçan, serotonin, omeprazol, mukoza hasarı, asid-geri emilimi

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Serotonin (5-hydroxytryptamine, 5-HT) has a number of effects on a variety of different cells, as well as causing changes in the gastric function.[1] It is known that, in rats, 5-HT causes vasoconstriction thus reducing gastric mucosal blood flow (GMBF) resulting in acute injury.[2-5] GMBF is an important defensive mechanism of the gastric mucosa. The relation between H^+ back diffusion, tissue acidification and ulceration has been well described.[6] There is also a linear relationship between H^+ output and GMBF.[7] When acid back diffusion occurs, the gastric mucosal blood flow increases (protective hyperemia). It has been suggested therefore that the use of systemic 5-HT might decrease gastric acid output.[8]

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Omeprazole is a potent inhibitor of gastric acid secretion. It's mechanism of action is direct inhibition of the gastric proton pump hydrogen-potassium stimulated adenosine triphosphatase (H^+ - K^+ ATP ase) on the luminal surface of the parietal cells.[9-11] However, reporters suggest that it does not have a significant effect on GMBF.[12]

The purpose of this study was to investigate whether the increase in acid back diffusion has an effect on 5HT–induced gastric mucosal injury, along with the known effect on GMBF.

Material and Methods

After approval from the Ethics Committee of Research of Laboratory animals of Dokuz Eylül University Medical School, the study was performed on 29 Wistar male rats. All animal procedures were performed according to the Guide for the Care and Use of Laboratory Animals of the National Institute of Health.

Animals

Male Wistar rats weighing 180-200 g, were allowed to become acustomed to the enviroment at least 3 days before the start of the experiments. They were kept under standardized conditions of temperature (21-22°C) and illumination (12 hours light/ 12 hours darkness). All rats were kept in cages with mesh bottoms and free access to tap water and pelleted food. The animals were fasted for 18-24 hours before the experiment, but had free access to water right up to the beginning of the experiment. All experiments were carried out in a tranquil environment and at the same time period of the day to minimize circadian variations. The rats were anaesthetized by diethyl ether inhalation. The animals were divided into four groups.

Omeprazole Administration

The Na^+ salt of omeprazole was dissolved in 10% polyethylene glycol 400 in a 2,5 mM phosphate buffer and adjusted to pH 8 with 10 mM HCl. Serotonin creatinine sulfate alone at a dose of 20 mg/kg was administered intraperitoneally (i.p.) to Group 1 (1^{st} control group, n=7). 10% polyethylene glycol was administered i.v. to Group 2 (2^{nd} control group, n=6). A bolus injection, 2 and 10 μmol/kg omeprazole in a volume of 1 ml/kg, of this solution was given intravenously into the femoral vein over a period of 2 minutes to Groups 3 and 4 (n=8, n=8), respectively. 5-HT was administered intraperitoneally to all of the three groups 1 hour after omeprazole or vehicle injection.

Pathological-Histological Examination

Four hours after administration of 5-HT, all rats were sacrificed and their stomachs were rejected. The rejected stomachs were opened along the greater curvature and after being washed under tap water fixed in 10% formalin solution. Three samples from comparable region of each stomach were excised and routinely processed followed by paraffin embedding. Sections (5 μm thick) were stained with haematoxylin and eosin and examined under a light microscope. One- centimeter lengths of each histological section was divided into three fields. Each field was scored histologically on a 0-4 scale according to previously described criteria: 0, normal; 1, epithelial cell damage; 2, glandular disruption, vasocongestion or edema in the upper mucosa; 3, mucosal disruption, vasocongestion or edema in the mid-lower mucosa; 4, extensive mucosal disruption involving the full thickness of the mucosa.[13] The overall mean value of the scores for each of the fields was taken as the histological ulcer index (HUI) for that section. All determinations were performed in a randomized manner and histological sections were coded to eliminate observer bias.

Drugs

5-hydroxytryptamine creatinine sulphate complex (Sigma Chemical Company, St. Louis), was prepared daily in deionized distilled water and kept on ice during the course of the experiments. The Na^+ salt of omeprazole (Ilsan-Iltas Company, Turkey) was dissolved in 10% polyethylene glycol 400 in a 2, 5 mM phosphate buffer and adjusted to pH 8 with 10 mM HCl.

Statistical Analysis

All data are expressed as mean±SEM. Kruskal-Wallis variance analysis was used to compare dif-

Table 1. Effect of intravenously administered Omeprazole and Vehicle on HUI.

Drug/dose	n	HUI	p
Serotonin 20mg/kg i.p.	7	3.2±0.37	
Polyethylene glycol 1ml/kg i.v. + Serotonin 20mg/kg i.p.	6	2.1±0.18*•	0.002
Omeprazole 2µmol/kg i.v. + Serotonin 20mg/kg i.p.	8	2.0±0.45*•	0.001
Omeprazole 10µmol/kg i.v. + Serotonin 20mg/kg i.p.	8	1.66±0.28*	0.001

*Indicates statistical significance compared with 5-HT.
•Group 2 (PEG) vs Group 3 (Omeprazole 2 µmol/kg), p=0.203

ferences between groups; paired comparisons were performed using the Mann Whitney U test to find out which group caused the difference. Differences between groups were considered significant if $p<0.05$.

Results

The mean HUI for each group of animals are summarized in Table 1.

Treatment with 20mg/kg, i.p. of 5-HT alone (1st control group) led to hemorrhagic lesions in all animals. The mean histological ulcer index for this group was 3.2±0.37 (Figure 1a, 1b). In the 2nd control group (10% polyethylene glycol) and the group receiving 2µmol of omeprazole, the HUI were 2.1±0.18 and 2.0±0.45, respectively. These values were less than 1st control group but were not significant ($p>0.05$, Table 1). However, pre-treatment with omeprazole at a dose 10µmol/kg significantly decreased HUI (1.66±0.28) ($p<0,05$, Table 1, Figure 2). Only epithelial cell damage and certain vasocongestion or edema in the upper mucosa was found in the group receiving 10µmol/kg of omeprazole.

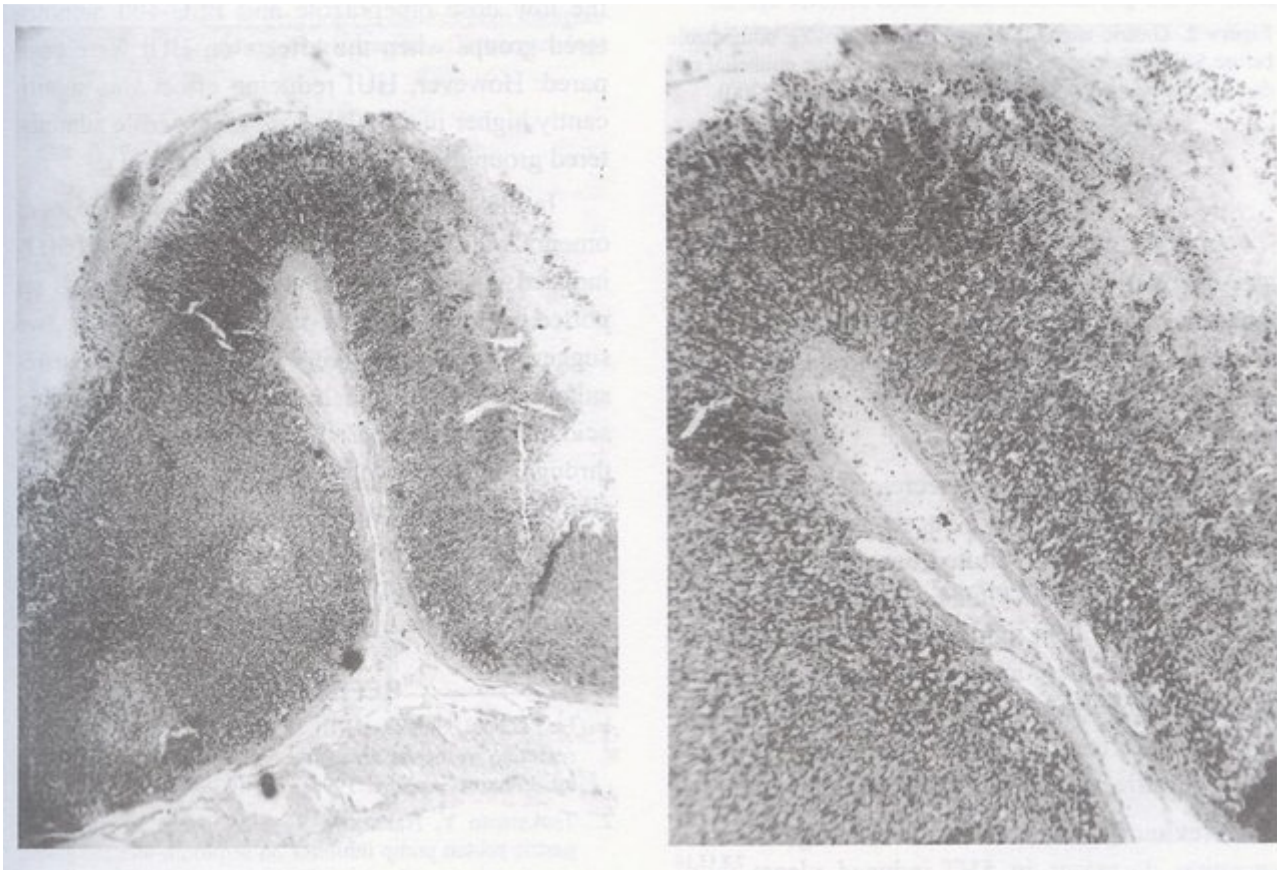

Figure 1a, 1b. Gastric mucosa exposed to i.p. 5-HT is seen. This micrograph was specifically selected to demonstrate the severe damaging effects of 5-HT. (Haematoxylin-eosin; original magnification X40, X100, respectively)

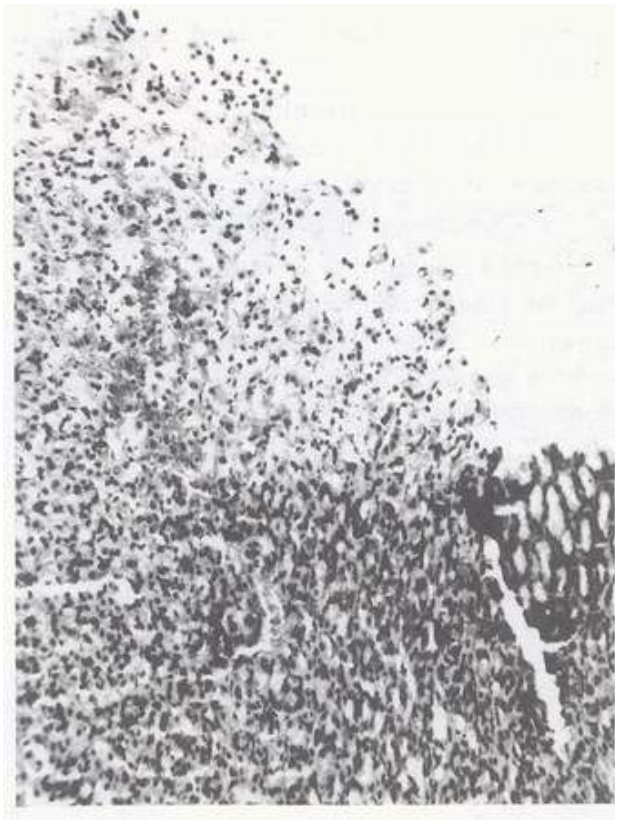

Figure 2. Gastric mucosa exposed to 10µmol/kg omeprazole before 5-HT administration is seen. There is some epithelial cell damage. (Haematoxylineosin; original magnificationX200)

Discussion

In this study, omeprazole was used to investigate the relationship between acid output and 5HT-induced gastric injury. We found that a high dose of omeprazole reduces 5HT-induced gastric mucosal injury, whereas a low dose of omeprazole has less effect.

Omeprazole is an antisecretory drug that inhibits gastric secretion induced by a variety of substances. It is a substituted benzimidazol, which inhibits the H^+-K^+ ATP ase localized in gastric parietal cells.[9,10] The inhibitory effect of omeprazole on gastric secretion is well known. However, it has been reported that administration of omeprazole has no effect on GMBF or, has very little effect on gastric mucosal microcirculation in rats.[12]

Previously it has been shown that gastric acid secretion decreases in 5HT-induced ulcers.[2,8,15,16] This decrease has been attributed to an increase in acid back diffusion.[2] In addition, 5HT decreases GMBF which in turn influences H^+ output.[7,17] It is also known that in acid back diffusion there is a protective increase in gastric mucosal blood flow (protective hyperemia). Although it is evident that 5-HT causes acute gastric mucosal injury in rats, the ability of proton pump inhibitors to protect the gastric mucosa from this particular type of injury has not been previously demonstrated.

Omeprazole was dissolved in 10% polyethylene glycol 400 and we have observed the slight HUI reducing effect of PEG-400 when administered alone in the present study. There are a few articles by Gutierrez-Cabano, showing the effects of polyethylene glycol 400 (PEG-400) on gastric function mentioning that PEG-400 has protected the rat gastric mucosa from ethanol-induced damage in a dose dependent manner.[18,19] Therefore, we can state that this effect of PEG-4000 on gastric mucosa may be expected.

No statistical difference was detected between the low dose omeprazole and PEG-400 administered groups when the effects on HUI were compared. However, HUI reducing effect was significantly higher in the high dose omeprazole administered group than PEG-400 group (Table 1).

In this study, we have found that high doses of omeprazole significantly reduced the level of 5HT-induced gastric injury. Since omeprazole was reported to have little or no effect on GMBF, we suggest that a more likely explanation for our results may be the role of omeprazole in reducing acid back diffusion by decreasing H^+ output through inhibition of proton pumps. Because of similar level of decresing in HUI at low dose omeprazole and PEG may be explain that low dose omeprazole does not have sufficient effect on acid back diffusion such as high dose omeprazole.

REFERENCES

1. Le Pard KJ, Stephens JRL. Serotonin inhibits gastric acid secretion in the rat through a 5-HT–1 like receptor in the rat. J Pharm Exp Ther 1994; 270: 1139-44.
2. Tsukamoto Y, Nakazawa S, et al. The effect of a new gastric proton pump inhibitor on serotonin-induced gastric mucosal lesions in rats. Gastroenterol Jpn 1988; 23(5): 501-5.

3. Salim AS. Gastric mucosal cytoprotection in the by naftidrofuryl oxalate. J Pharm Pharmacol 1990; 42: 125-7.
4. Wong SH, Cho CH, Ogle CW. The role of serotonin in ethanol-induced gastric glandular damage. Digestion 1990; 45:52-60.
5. Cho CH, Mei KB, Kaan SK. The intraperitoneal effects of 5-hydroxytryptamine on haemodynamics, gastric mucosal blood flow and platelet aggregation. Eur J Pharmacol 1994; 256(1): 9-15.
6. Kıviliaakso E, Barzilai A, Schiesswel R, Fromm D, Silen W. Experimental ulceration of rabbit antral mucosa. Gastroenterol 1981; 80(1): 77-83.
7. Tashima K, Korolkiewicz R, Kubomi M, Takeuchi K. Increased susceptibility of gastric mucosa to ulcerogenic stimulation in diabetic rats: role of capsaicin-sensitive sensory neurons. Br J Pharmacol 1998; 124(7): 1395-402.
8. Le Pard KJ, Chi J, Mohammed JR, Gidener S, Stephens RJ. Gastric antisecretory effect of serotonin: quantitation of release and site of action. Am J Physiol 1996; 271(4-1): E669-77.
9. Wallmark B, Jaresten BM, Larsson H, Ryberg B, Brandstroad A, Fellenius E. Differentiation among inhibitory actions of omeprazole, cimetidine, and SCN on gastric acid secretion. Am J Physiol 1983; 245:64-71.
10. Sachs G, Wallmark B. The gastric H+, K+ ATPase: the site of action of omeprazole. Scand J Gastroenterol 1989; 24(166): 3-11.
11. Synnerstad I, Holm L. Omeprazole induces high intraglanduler pressure in the rat gastric mucosa. Gastroenterology 1997; 112: 1221-30.
12. Holm-Rutili L. Effects of omeprazole on gastric mucosal microcirculation and acid secretion in the rat. Gastroenterol 1987; 92: 716-23.
13. Esplugues JV, Whittle BJR. Gastric damage following local intraarterial administration of reaction oxygen metabolites in the rat. Br J Pharmacol 1989; 97: 1085-92.
14. Larsson H, Carlsson E, Junggren U, Olbe L, Sjöstrand SE, Skanberg I, Sundell G. Inhibition of gastric acid secretion by omeprazole in the dog and rat. Gastroenterol 1983; 85: 900-7.
15. Salim AS. Role of 5-hydroxytriptamine (5-HT) in the mechanism of reserpine-induced stimulation of H^+ output in the rat. Clin Physiol Biochem 1992; 9(4): 132-7.
16. Gidener S, Kalkan S, Kupelioglu A, Gure A. The effect of acute bilateral adrenalectomy on serotonin-induced inhibition of gastric acid secretion and acute gastric mucosal injury in rats.Int J Exp Path 1996; 77: 163-6.
17. Guth PH. Current concepts in gastric microcirculatory pathophysiology. Yale J Biol Med 1992; 65(6): 677-88.
18. Gutierrez-Cabano CA. Intragastric polyethylene glycol-400 protects against ethanol-induced gastric mucosal lesions despite pretreatment with indomethacin or iodoacetamide. Dig Dis Sci 1995; 40(9): 2029-36.
19. Gutierrez-Cabano CA. Protection by intragastric polyethylene glycol-400 in rat stomach against ethanol damage involves alpha-2 adrenoceptors. Dig Dis Sci 2000; 45(1): 105-9.