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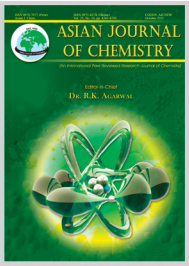

Effect of Sertraline in Indomethacin-Induced Gastric Mucosal Damage

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The antiulcerative effect of sertraline was investigated using an indomethacin-induced ulcer model in rats. After the macroscopic analyses, malondialdehyde and glutathione amounts and superoxide dismutase, glutathione-S-transferase and glutathione peroxidase enzyme activities were determined in stomach tissues. It is presumed that the sertraline has positive effect in the treatment of gastric mucosal damages and this effect may be related to the decrease of oxidative stress and activation of antioxidant mechanisms by sertraline. For clinical use of sertraline by this effect, more prospective randomized studies should be done.

Key Words: Sertraline, Depression, Ulcer, Oxidant, Antioxidant, Rat.

INTRODUCTION

Ulcer was defined as a damage, which pass over the muscular layer in gastrointestinal mucosa[1]. An imbalance between protective and aggresive factors in mucosa plays an important role in ulcer formation[2]. Non-steroidal antiinflammaory drugs (NSAID's), stress and various enviromental factors are some of the aggressive factors in ulcer formation[3].

It is known that reactive oxygen species (ROS) are pathogen factors in mucosal damage caused by stress, burnt, alcohol, NSAID's and especially ischemia/reperfusion[4]. Detoxification of reactive oxygen species directly or indirectly is essential for the health of the organism and organs[5]. It is thought that various diseases such as atherosklerosis, cardiovascular disorders, cancer, aging, otoimmun diseases, infections and ulcer occur when reactive oxygen species increse and/or antioxidant systems fall short[6,7].

At the present time, mechanism of gastric mucosal damage caused by reactive oxygen species is not explained clearly yet. It is well known that serious damage occurs in cell membrane integrity at the end of reactive oxygen species originated lipid peroxidation (LPO)[8,9]. Lipid peroxidation can damage barrier characterization of epithelium and endothel by changing vital features of the membrane. As a result of membrane peroxidation, some changes occur in membrane liquid fluence and permeability, protein ratio of the membrane changes and finally the cell dies[5,9].

In experimental studies, which performed to define the role of reactive oxygen species and LPO in indomethacin induced ulcers, it is showed that antioxidants such as superoxide dismutase (SOD), glutathione S-transferase (GST), glutathione peroxidase (GPx), glutathione (tGSH), catalase (CAT) and dimethyl sulfoxide reduce gastric mucosal lesions[4,9]. In recent years, reactive oxygen species is one of the most popular scientific topic and the role of reactive oxygen species in pathogenesis of various clinical case such as ulcer which ends by tissue damage emphasizes so much[10].

Reactive oxygen species have an important roles in the ulcer formation. Reactive oxygen species composes as a consequence of normal metabolic events of the organism. At the same time the cell needs to reactive oxygen species to provide normal function[5]. Antioxidant systems such as superoxide dismutase, catalase, glutathione S-transferase and glutathione peroxidase inhibit the accumulation of reactive oxygen species in the organism. An irregularity of these systems causes reactive oxygen species accumulation and tissue damage. Reactive oxygen species leads to lipid peroxidation and tissue damage[9]. Many papers have already published about positive effects of tricyclic antidepressants in healing process of peptic ulcer. But the mechanism is not clear and there is not enough clinical studies on this topic[11-13].

It has shown that selective serotonin reuptake inhibitors has therapeutic effect in many psychiatric disorders such as depression and anxiety. In present studies that weree conducted

on animals and humans, it was reported that when serotonine level decreased, affective aggregation was happened and sleeping arragement, eating habits and pain sensitivity were impaired. Conversely, aggressive behaviours were decreased when serotonine level increased[14].

In the light of these knowledges, we aimed to study whether sertraline, a tricyclic antidepressan, had a protective effect, which may be useful clinically in indomethacin induced ulcer and to investigate the relationship between oxidant/ antioxidant parameters and the probable protective effect of sertraline.

EXPERIMENTAL

Animals: A total of 36 male Sprague Dawley rats obtained from the Medical Experimental Research Centre, Ataturk University, weighing between 200 and 220 g, were used in this study. The animals were fed under normal conditions (22 ºC) in separate groups and divided into 6 groups. Animal experiments were performed in accordance with the national guidelines for the use and care of laboratory animals and approved by the local animal care committee of Ataturk University, Turkey.

Chemicals: For laboratory experimentation, indomethacin, famotidinee and sodium thiopental were obtained from Deva Drugs Istanbul, Turkey, Fako Drugs Istanbul, Turkey and IE-Ulagay Drugs Istanbul, Turkey respectively, while sertraline was purchased from Pfiser Drugs, Istanbul, Turkey. The other chemicals for biochemical assays were provided from Sigma Chem, Germany and Merck Chem, USA.

Test for effect of sertraline on indomethacin-induced ulcer in rats: For this part of our experiment, the antiulcerative effect of sertraline was investigated using an indomethacin-induced ulcer model in rats[15]. Sertraline was administered to first, second and the third groups of rats fasted for 24 h orally at 10, 20 and 40 mg/kg dosages. Famotidinee was given to the 24 h fasted-fourth group at 20 mg/kg dose. After 5 min, 25 mg/kg indomethacin was given to first five groups of rats by oral gavage. Distilled water was given to the healthy group (sixth) at the same volume as the vehicle. 6 h after the indomethacin administration, all groups were sacrificed by high dose (50 mg/kg) thiopental anesthesia. The stomachs of the rats were removed and ulcerous regions were examined macroscopically. Ulcerous areas were measured on millimeter paper. The antiulcerative activity of sertraline was evaluated by comparison with the results obtained from the control and the famotidinee (20 mg/kg) groups.

Biochemical analyses: After the macroscopic analyses, malondialdehyde and gluathione amounts and superoxide dismutase, glutathione S-transferase and glutathione peroxidase enzyme activities were determined in stomach tissues. To prepare the tissue homogenates, stomach tissues were ground with liquid nitrogen in a mortar. About 0.5 g was weighed for each group and then treated with 4.5 mL of appropriate buffer. This mixture was homogenized on ice using an ultra-turrax homogenizer for 15 min. Homogenates were filtered and centrifuged by using a refrigerator centrifuge at 4 ºC. Then, the supernatants were used for the determination of the enzymatic activities. All assays were carried out at room temperature.

Determination of malondialdehyde: The concentrations of gastric mucosal lipid peroxidation were determined by estimating malondialdehyde using the thiobarbituric acid test[16]. Namely, the rat stomachs were promptly excized and rinsed with cold saline. To minimize the possibility of interference of hemoglobin with free radicals, any blood adhering to the mucosa was carefully removed. The corpus mucosa was scraped, weighed and homogenized in 10 mL of 100 g/L KCl. The homogenate (0.5 mL) was added with a solution containing 0.2 mL of 80 g/L sodium lauryl sulfate, 1.5 mL of 200 g/L acetic acid, 1.5 mL of 8 g/L 2-thiobarbiturate and 0.3 mL distilled water. The mixture was incubated at 98 ºC for 1 h. Upon cooling, 5 mL of *n*-butanol:pyridine (15:l) was added. The mixture was vortexed for 1 min and centrifuged for 0.5 h at 4000 rpm. The supernatant was measured at 532 nm.

Total glutathione (GSH) determination: The amount of GSH in the gastric mucosa was measured according to the method described by Sedlak and Lindsay[17]. The mucosal surface of the stomach was collected by scraping, weighed and homogenized in 2 mL of 50 mM *tris*-HCl buffer containing 20 mM EDTA and 0.2M sucrose, pH 7.5. The homogenate was centrifuged. After centrifugation at 4200 rpm for 40 min at 4 1C, the supernatant was used to determine the GSH amount using DTNB [5,50-Dithiobis(2-nitrobenzoic acid)]. Absorbance was measured at 412 nm using a spectrophotometer.

Superoxide dismutase activity: It was measured according to Sun *et al*.[18]. Superoxide dismutase estimation was based on the generation of superoxide radicals produced by xanthine and xanthine oxidase, which reacts with nitro blue tetrazolium (NBT) to form formazan dye. Superoxide dismutase activity was then measured at 560 nm by the degree of inhibition of this reaction.

Glutathione-S-transferase activity: Total glutathione-S-transferase activity was determined as described by Habig and Jakoby[19]. Briefly, the enzyme activity was assayed spectrophotometrically at 340 nm in a 4 mL cuvette containing 0.1 M PBS (pH 6.5), 30 mM glutathione, 30 mM 1-chloro-2,6-dinitrobenzene and tissue homogenate.

Glutathione peroxidase activity: Glutathione peroxidase activity was determined according to the method of Lawrence and Burk[20]. The absorbance at 340 nm was recorded for 5 min.

Statistical analyses: All data were subjected to one-way ANOVA using SPSS 13.0 software. Differences among groups were attained using the LSD option and significance was declared at $P < 0.05$.

RESULTS AND DISCUSSION

Indomethacin ulcer test: In macroscopic examination, ulcerous areas were shown in the rat stomachs of all sertraline groups and the control group (25 mg/kg indomethacin group). Different numbers and sizes of ulcerous areas were determined. The ulcer focus was composed of mucosal defects that were circular and/or oval shaped and dispersed to all stomach surfaces. The ulcer edges were clear and a blister was seen on the edge. Hyperemia in the stomachs of the control group was clearer than in all the chronic indomethacin groups. As seen in Table-1, the average ulcerous areas in the 10, 20 and 40 mg/kg sertraline groups were 17 ± 3.4, 14 ± 4.3 and 28.5 ±

TABLE-1
ANTIULCER EFFECT OF SERTRALINE IN INDOMETHACIN INDUCED ULCER IN RATS

Drug	Dose (mg/kg)	Number of animal (n)	Ulcer area (mm^2)	Antiulcer effect (%)	p
Sertraline	10	6	17 ± 3.4	48.5	< 0.0001
	20	6	14 ± 4.3	57.6	< 0.0001
	40	6	28.5 ± 1.8	13.6	< 0.05
Famotidine	20	6	0.98 ± 0.2	97.0	< 0.0001
Indomethacine	25	6	33 ± 3.9	-	-
Sham	-	6	0.0 ± 0.0	-	-

1.8 mm^2, respectively. The ulcers measured 33 ± 3.9 mm^2 in the indomethacin control group and 0.98 ± 0.2 mm^2 in the famotidine group.

Biochemical results

Malondialdehyde and glutathione S-transferase levels: Lipid peroxidation product, malondialdehyde was the lowest in the sham group (0.37 µmol/g) and the highest in indomethacin group (1.32 µmol/g). The difference between these two groups were significant statistically ($p < 0.0001$). In sertraline groups, the highest malondialdehyde level was in 40 mg/kg dose and 0.68 µmol/g and it was significant when compared to indomethacin groups ($p < 0.0001$). In famotidine groups, this level was close to sham group as 0.45 µmol/g and also significant *versus* indomethacin group ($p < 0.0001$) (Fig. 1).

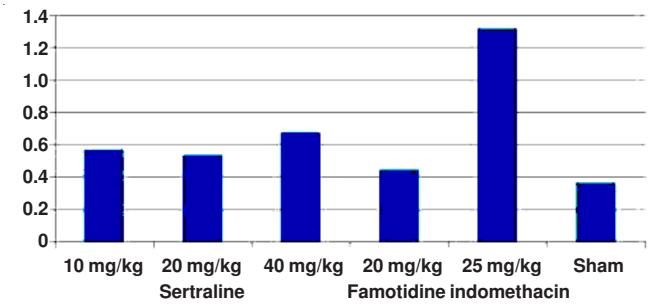

Fig. 1. Effects of sertraline and famotidine treatments on the level of malondialdehyde (MDA) in indomethacin-induced ulcer model in rat gastric tissue. Results are means ± SE of three measurements. N:6 (the number of rats)

An antioxidant, glutathione S-transferase level was the highest (50.5 U/g) in sham group and the lowest F (20.83 U/g) in indomethacin group. The difference between the groups was significant ($p < 0.001$). In sertraline groups, the highest glutathione S-transferase level was measured in 20 mg/kg sertraline dose as 45.31 U/g. In famotidine group, average of the glutathione level was 47.53 U/g. These two groups were significant statistically when compared to indomethacin groups ($p < 0.001$) (Fig. 2).

Superoxide dismutase, glutathione S-transferase and glutathione peroxidase enzyme activity: As seen in Figs. 3-5, superoxide dismutase, glutathione S-transferase and glutathione peroxidase enzyme activities were the highest level in sham group (13.80, 123.4 and 81.08 U/mg respectively) and the lowest in indomethacin group (6.6, 58.1 and 16.5 U/mg, respectively). The difference between these two groups were significant statistically ($p < 0.0001$). In sertraline groups, the least superoxide dismutase, glutathione S-transferase and glutathione peroxidase enzyme activities were measured in 40 mg/kg sertraline group (8.7, 79.3 and 29.1 U/mg respectively). For these parameters, the difference between 40 mg/kg sertraline and indomethacin group was significant statistically ($p < 0.01$). In famotidine group, average of superoxide dismutase, glutathione S-transferase and glutathione peroxidase enzyme activities was 11.4 U/g, 118.7 U/g and 78.7 U/mg respectively. These two groups were significant statistically when compared to indomethacin groups ($p < 0.001$).

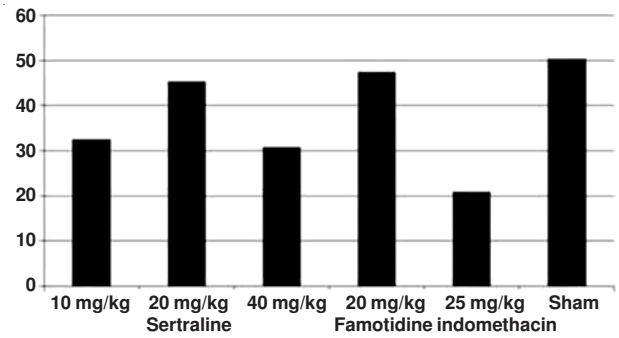

Fig. 2. Effects of sertraline and famotidine treatments on the level of total glutathione (tGSH) in indomethacin-induced ulcer model in rat gastric tissue. Results are means ± SE of three measurements. N:6 (the number of rats)

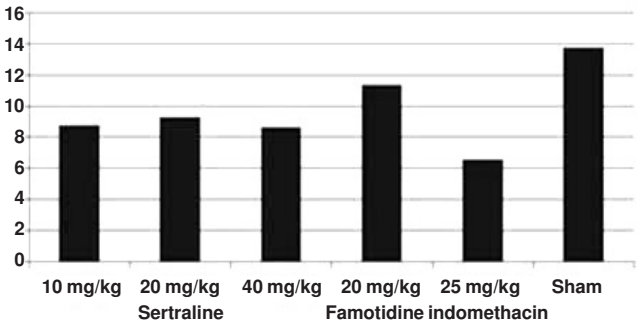

Fig. 3. Effects of sertraline and famotidine treatments on the activity of superoxide dismutase (SOD) in indomethacin-induced ulcer model in rat gastric tissue. Results are means ± SE of three measurements. N:6 (the number of rats)

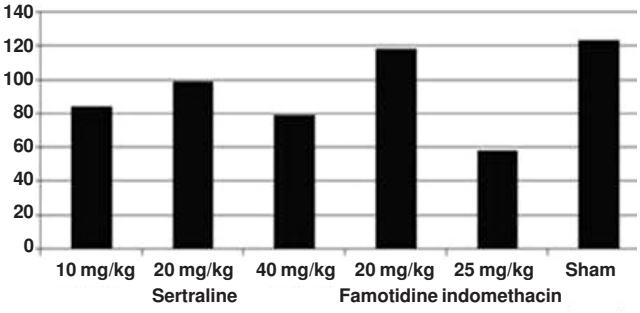

Fig. 4. Effects of sertraline and famotidine treatments on the activity of glutathione-S-transferase (GST) in indomethacin-induced ulcer model in rat gastric tissue. Results are means ±SE of three measurements. N:6 (the number of rats)

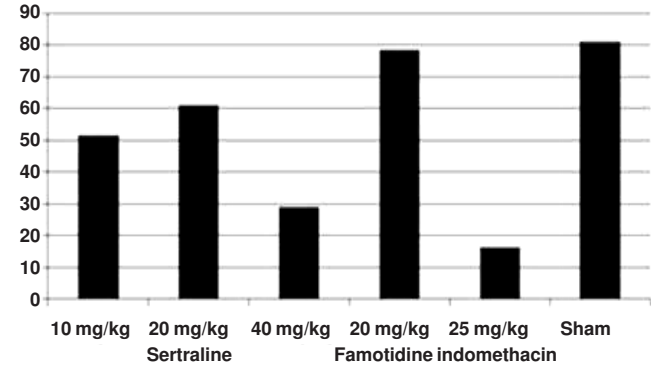

Fig. 5. Effects of sertraline and famotidine treatments on the activity of glutathione peroxidase (GPx) in indomethacin-induced ulcer model in rat gastric tissue. Results are means ± SE of three measurements. N:6 (the number of rats)

Mucosal damage in gastrointestinal system (especially in stomach and duodenum) and ulcer formation is still a serious problem in Turkey. All over the world, 5-10 % of people face with this problem in any period of their life[1].

In mucosa, an imbalance between protective and aggresive factors plays an important role in ulcer formation[2,3]. Uncontrolled drug consumption is one of the foremost factors, which cause this imbalance. NSAIDs are one of the most prescribing drugs and used in the treatment of pain, fever and inflammation widely[21]. But these drugs have common side effects such as gastric mucosal lesions, ulcer, bleeding and perforation in gastrointestinal system[22]. Because of these well-known ulcerative effect, in this study indomethacin is used as an experimental model to induce ulcer. While no ulcerative area was observed in sham group, the most common ulcerative areas were in indomethacin group significantly.

Most drugs were used against these common pathological diseases and many patients were treated with the surgery. In 1980, by the discovery of H_2 receptor antagonists and then proton pump inhibitors, a great success was gained in the treatment. In this study, a H_2 receptor antagonist, famotidine was used as an antiulcer agent and its antiulcer activity was 97 %. Antiulcer activity of famotidine was higher and statistically significant than other groups. Although these drugs have high efficacy, patients' complaints could not be resolved completely and permanently. Therefore, therapy was entered into a new quest. Researchers are still continuing their researches on the basis of the disease which can not be known. At the beginning of possible causes, psychological aspects of patients is of great importance. This stuation means that common depression in society might have an important role in the etiology of mucosal damage.

Enteric nervous system and central nervous system directly affect each other. For example, it is well-known that stimulation of gastrointestinal system by release of neuropeptides and neurotransmitters in the presence of stress, various gastrointestinal responses occur[14]. This case supports the existence of a mechanism between gastrointestinal dysfunction and physico-emosyonel situation[23]. In various studies it has revealed that patients with depression complain from both gasrointestinal ulcer formation and psychic and somatic complaints[24,25]. Clinical studies in this regard have shown that use of anxiolytic and antidepressant drugs have beneficial effects in patients with ulcer[24,26]. For this purpose different antidepressants have used in the treatment of ulcer stages.

Tricyclic antidepressants are particularly useful in the treatment of endogenous depression. Antidepressants prevent the serotonin and norepinephrine reuptake in nerve terminals in a powerful way[24]. In addition, in various studies significant antiulcer effects of antidepressants have also showed[24,27-30]. Otaka *et al*.[14] have reported that addition of an antidepressant, amityptiline to the treatment of patients whose functional dyspepsia can not be resolved will be beneficial. However, many gastrointestinal side effects of antidepressants are well-known[31]. Antiulcerogenic effect was observed in the stomachs of all of the rats received sertraline. This effect was the highest in 20 mg/kg sertraline dose. When the antiulcer effect of all sertraline doses was compared to indomethacin group, the difference between sertraline groups and indomethacin group was significant in ulcer treatment ($p < 0.05$). The antiulcer effect of 20 mg/kg sertraline is lower than famotidine group but the fact that all of the doses has antiulcer effect shows that sertraline might be an antiulcerative drug. For this reason, if an antidepressant drug has to use in second or thirth step in the resistant patients, sertraline may be an alternative.

It is not known that antidepressants show their antiulcer effect by which mechanisms. Naito *et al*.[32] have reported that reactive oxygen species (ROS), which compose as a product of lipid peroxidation play an important role in the indomethacin induced gastric ulcer. The common image is that reactive oxygen species are pathogen factors in stress, alcohol, NSAIDs and ischemia/reperfusion related gasric mucosal damage[9,33]. In the light of these knowledge, lipid peroxidation products and some antioxidant parameters were investigated in the indomethacin induced mucosal damage mechanism.

At the end of the cellular metabolic reaction, reactive oxygen radicals like H_2O_2, O_2^- and OH ocur in the organism. The production of reactive oxygen radicals increase and activities of antioxidant enzymes decrease in stress-exposed organism[34]. Similar alterations were also observed in our study. Harmful effects are observed when the balance between the production and detoxification of oxygen radicals impairs.

As an indicator of lipid peroxidation in experimental model, malondialdehyde (MDA) levels were measured in rat gastric tissue. In normal conditions, malondialdehyde exists in sham group but in very low levels. In all of the other groups, malondialdehyde level has increased. This increase was the highest in indomethacin group. The increase in the sertraline group was higher than famotidine group and the least increase was observed in 20 mg/kg sertraline groups. The malondialdehyde level of 20 mg/kg sertraline was low significantly when compared to indomethacin groups. This measurements showed that experimental model led to lipid peroxidation and sertraline decresed this peroxidation.

It is suggested that endogen defence meshanisms are very important in the preventation of acute gastric mucosal damage[35]. Organisms have defence mechanisms both enzymatic and non-enzymatic to prevent toxicity of oxygen radicals and tissue damage[36]. Glutathione S-transferase, superoxide dismutase, glutathione S-transferase and glutathione peroxidase

are prominent antioxidants, which play role in the preventation of these negative effects[26,37].

Especially in human and rat gastric mucosa, the tripeptide structured- glutathione S-transferase is found in high concentrations, it is also a natural superoxide radicals collector and protects protein-thiol groups, which is necessary for the cellular integrity against oxidation[9]. In the study, it was determined that the level of glutathione S-transferase decreased in all of the drug groups when compared to sham group. The most decrease was measured in the indomethacin group. This shows that antioxidant activities of 20 mg/kg sertraline group is very close to the famotidine group.

Superoxide dismutase is one of the enzymes that compose enzymatic defense mechanisms. It prohibits the damage of superoxide oxygen radicals by inhibiting lipid peroxidation[38]. Another antioxidant, glutathione S-transferase catalyzes GSH reactions[39]. Glutathione S-transferase has a vital importance because it is found in all cells and it has catalytic and non-catalytic functions[10]. The results are similar to the previous studies. Indomethacin decreased superoxide dismutase and glutathione S-transferase enzyme activities in rat gastric tissue[9,36]. In the drug groups, superoxide dismutase and glutathione S-transferase enzyme activities diminished *versus* sham group. While the most decrease was observed in indomethacin group, the least decrease was seen in the famotidinee group. In drug groups, the least decrease was occurred in 20 mg/kg sertraline given rats. The difference between indomethacin group and sertraline group was significant statistically. The data supports, that defense mechanisms about superoxide dismutase and glutathione S-transferase is active and with this way, a decrease occurs in enzyme activities. It is though that superoxide dismutase and glutathione S-transferase play an important role in the decrease of gastric mucosal injury by preventing oxidative damage.

In glutathione peroxidase activity, there were also similar changes to the other antioxidant enzymes. Yoshikawa *et al.*[40] showed a decrease in rat gastric tissue superoxide dismutase, GSH and glutathione peroxidase enzyme activity after administration of indomethacin. We also found a decrease in these enzyme activities. In glutathione peroxidase activity, the most decrease was observed in indomethacine group. The levels in famotidine and sham group were close to each other. The least decrease was seen in 20 mg/kg sertraline group. The activity of glutathione peroxidase was high in 20 mg/kg sertraline group when compared to indomethacin group statistically. These data was thought that glutathione peroxidase enzyme mechanism was similar to the other enzymes.

In conclusion, it is thought that sertraline has positive effect in the treatment of gastric mucosal damages and this effect may be related to the decrease of oxidative stress and activation of antioxidant mechanisms by sertraline. For clinical use of sertraline by this effect, more prospective randomized studies should be done.

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