The Role of Alpha-2 Adrenergic Receptors in the Anti-ulcerative Activity of Famotidine and Omeprazole in Rats and its Relationship with Oxidant-antioxidant Parameters

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Abstract: Alpha-2 adrenergic receptors have antinulcer activity. Omeprazole and famotidine are anti-ulcerative drugs. In this study the role of alpha-2 adrenergic receptors in the antulcerative activity of famotidine and omeprazole was examined pharmacologically and biochemically. Anti-ulcerative effects of all drugs (famotidine, omeprazole, histamine and yohimbine) were investigated on indomethacin-induced gastric ulcer model in rats. At first, mentioned drugs were given to 24 h-fasted rats. Then, 25 mg kg⁻¹ indomethacin was given to induce gastric ulcer. Six hours after the administration of indomethacin, the animals were euthanized with high-dose anesthetic. The stomachs of the animals were removed and the ulcerative focuses located on the surface of the stomach were macroscopically evaluated. Thereafter, all the stomachs were sent to the laboratory for the measurement of the levels of total glutathione, malondialdehyde and the activity of superoxide dismutase, myeloperoxidase. Mean area of ulcer was 31.6, 1.08 and 0.83 mm² in the rats of the control, famotidine and omeprazole groups treated with indomethacin, respectively. It was 29.3 mm² in the indomethacin control group and, respectively, 24.5 and 26.1 mm² in the famotidine and omeprazole that received yohimbine groups. It was 30 mm² in the indomethacin control group, in the famotidine and omeprazole + histamine group it was 0.91 and 0.16 mm². In the histamine and yohimbine alone groups, ulcer was not observed. It was found that alpha-2 adrenergic receptors had a role in anti-ulcer mechanism of famotidine and omeprazole. Moreover, it was seen that there was a direct relation between alpha-2 adrenergic receptors and oxidant-antioxidant parameters.

Key words: Famotidine, omeprazole, yohimbine, alpha-2 adrenergic receptors, histamine

INTRODUCTION

As known, famotidine and omeprazole suppress the release of gastric acid, by blocking blocking H2 receptors in the gastric parietal cells and by inhibiting ATPase (Ramachandran et al., 2011; Yan, 2006). It was demonstrated that famotidine and omeprazole show an anti-ulcerative activity by increasing Prostaglandin E2 (PGE₂), bicarbonate and mucus secretion and thereby, by inhibiting the acid secretion in the stomach (Hooderwerf and Pasricha, 2006). It is claimed that indomethacin which is used to form an ulcer model (Olaleye et al., 2006), leads to a damage of gastric tissue, by inhibiting PGE₂, bicarbonate and mucus production and by increasing acid secretion (Suleyman et al., 2010; Rıfat-uz-Zaman et al., 2005).

However, it was demonstrated that when aspirin that inhibits PGE₂ is given via intraperitoneal route, it prevented the indomethacin-induced gastric ulcer (Koçkay et al., 2002). In addition, it was reported that lansoprazole showed an anti-ulcerative effect, without influencing gastric PGE₂ production (Fukuda et al., 1995). It was found that, although morphine decreases gastric acid secretion, it is not able to prevent the indomethacin-induced gastric ulcer (Tazi-Saad et al., 1991). Moreover, it was reported that atropine decreased gastric acid secretion by blocking M receptors in parietal cells but its anti-ulcerative activity disappeared following bilateral surgical vagotomy (Karadı et al., 2001). Data obtained showed that while some alkaloids show an anti-ulcerative effect without influencing mucus secretion, famotidine show an anti-ulcerative effect without influencing bicarbonate secretion (Li et al., 2006; Minaki et al., 2001).

These literature data show that no convincing correlation exists between the stimulation of
Prostaglandin (PG), bicarbonate and mucus production, the inhibition of acid secretion and anti-ulcerative effect (Brodie, 1962, 1968; Fukuda et al., 1995; Waisman et al., 1985).

In the recent studies conducted during the last years, it was claimed that alpha-2 adrenergic receptors play the main role in the mechanism that leads to the formation and the prevention of gastric ulcers (Suleymán et al., 2010). In the literature, some studies show that alpha-2 adrenergic receptors account for the gastro protective effect (Fülöp et al., 2005). It was seen that alpha-2 adrenergic receptors blockade via the elimination of protective effect although stimulation of alpha-2 adrenergic receptors caused gastroprotective effect. Moreover, the stimulation of alpha-2 adrenergic receptors with the use of prednisolone (by adrenerectomy) led to gastro protective effect (Suleyman et al., 2007). It was demonstrated that estrogen and Luteinizing Hormone (LH) provided a gastro protective effect via alpha-2 adrenergic receptors (Borekci et al., 2009). Estrogen and LH could not maintain these anti-oxidant features in the gastric tissue of the animals treated with yohimbine (Kumtepe et al., 2009).

These data obtained from the literature indicate that anti-ulcerative activity has no direct correlation with the stimulation of PG, bicarbonate and mucus secretion and the inhibition of acid secretion. In addition, it suggests that alpha-2 adrenergic receptors play a role in the mechanism of action of the classical anti-ulcerative drugs, such as famotidine and omeprazole. Therefore, this study aimed to investigate whether alpha-2 adrenergic receptors have a role in the anti-ulcerative activity of famotidine and omeprazole and to examine its correlation with oxidant-antioxidant parameters.

**MATERIALS AND METHODS**

**Experimental animals:** In this study, a total of 84 Albino Wistar male rats, with a weight ranging between 220-230 g, were used. Animals were provided by Medical Experimental Research and Application Center of Ataturk University. Before the study, the animals sheltered and fed under normal laboratory conditions, as groups. This study was held on 14.10.2010.

**Study design and indomethacin-induced gastric ulcer test:** In this experiment, anti-ulcerative effects of famotidine and omeprazole were investigated on an indomethacin-induced gastric ulcer model in rats (Guidobono et al., 1997). Of rats that were starved for 24 h, one group was given famotidine 20 mg kg⁻¹ and other group was given omeprazole 20 mg kg⁻¹ by oral route using gavage. All rat groups were given indomethacin 25 mg kg⁻¹ via oral route, 5 min after the administration of the drugs. Six hours after the administration of indomethacin, the animals were euthanized with high-dose anesthetic (Tiopental sodium 50 mg kg⁻¹). The stomachs of the animals euthanized were removed and the ulcerative foci located on the surface of the stomach were macroscopically evaluated. The widths of the ulcerative areas on the surface of the stomach were measured on millimeter paper. For famotidine and omeprazole, anti-ulcerative activity was evaluated by comparing the results with those obtained from the control group. Thereafter, all the stomachs were sent to the laboratory of Faculty of Medicine, Department of Biochemistry for the measurement of the levels of tGSH, MDA and the activity of SOD, MPO.

**Indomethacin-induced gastric ulcer test using yohimbine:** In this series of our experiment, we investigated the effect of famotidine and omeprazole on the indomethacin-induced gastric ulcers in the rats treated with yohimbine (Guidobono et al., 1997).

**Indomethacin-induced gastric ulcer test using histamine:** In this series of our experiment, we investigated the effect of famotidine and omeprazole on the indomethacin-induced gastric ulcers in the rats treated with histamine (Guidobono et al., 1997).

**Biochemical analysis of gastric tissue:** In this part, 0.2 mg of whole gastric tissue (damaged and healthy parts together) was weighed for each stomach. The samples were homogenized in ice with 2 mL buffers (consisting of 0.5% HDTMAB [0.5% hexadesil tri methyl ammonium bromide] pH = 6 potassium phosphate buffer for myeloperoxidase analyze, consisting of 1.15% potassium chloride solution for malondialdehyde analysis and pH = 7.5 phosphate buffer for the other analyses). Then, they were centrifuged at 4°C, 10000 rpm for 15 min. The supernatant part was used as the analysis sample.

**Total Glutathione (tGSH) analysis:** The amount of tGSH in the total homogenate was measured according to the method of Sedlak and Lindsay with some modifications (Sedlak and Lindsay, 1968).

**Superoxide Dismutase (SOD) analysis:** Measurements were performed according to Sun et al. (1988).

**Malondialdehyde (MDA) analysis:** The concentrations of ovarian lipid peroxidation were determined by estimating MDA using the thiobarbituric acid test (Ohkawa et al., 1979).
Myeloperoxidase (MPO) analysis: The activity of MPO in the total homogenate was measured according to the method of Wei and Frenkel with some modifications (Wei and Frenkel, 1991).

Statistical analyses: All of the statistical analyses were performed in SPSS 13.0 statistical program by using one-way ANOVA. Differences between groups were obtained using LSD option and the significance was implied at p<0.05. Results were expressed as Mean±Standard error of the mean.

RESULTS

Effects of famotidine and omeprazole on indomethacin-induced gastric ulcers: As seen in Table 1 and Fig. 1 mean area of ulcer was 31.6 mm² in the rats of the control group treated with indomethacin and respectively, 1.08 and 0.83 mm² in the rats that received famotidine and omeprazole.

Effects of famotidine and omeprazole on indomethacin-induced gastric ulcers treated with yohimbine: Mean area of ulcer was 29.3 mm² in the control group treated with indomethacin and respectively, 245 and 26.1 mm² in the groups that received famotidine and omeprazole that received yohimbine. Ulcer was not observed in the stomachs of the rats that received yohimbine alone (Table 2, Fig. 2).

Effects of famotidine and omeprazole on indomethacin-induced gastric ulcers treated with histamine: In the control group that received indomethacin, mean area of ulcer was found to be 30 mm². In the famotidine and omeprazole group that received histamine, mean area of ulcer was found to be, respectively, 0.91 and 0.16 mm². In the stomachs of the rats that received histamine alone, ulcer was not observed (Table 3, Fig. 3).

Biochemical results: Effects of famotidine and omeprazole on the levels of IGF-I, SOD, MDA and MPO in the gastric tissue treated with indomethacin.

Table 2: Effects of famotidine and omeprazole on indomethacin-induced gastric ulcers treated with yohimbine

<table>
<thead>
<tr>
<th>Drugs</th>
<th>N</th>
<th>Ulcer area (mm²)</th>
<th>Antiulcer effect (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Famotidine (20 mg kg⁻¹)</td>
<td>6</td>
<td>1.08±0.27</td>
<td>96.59</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>+ Indomethacin (25 mg kg⁻¹)</td>
<td>6</td>
<td>0.83±0.16</td>
<td>97.30</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Omeprazole (20 mg kg⁻¹)</td>
<td>6</td>
<td>24.5±3.08</td>
<td>16.4</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>+ Indomethacin (25 mg kg⁻¹)</td>
<td>6</td>
<td>26.1±1.4</td>
<td>11</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Healthy Control</td>
<td>6</td>
<td>29.3±1.05</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Yohimbine (10 mg kg⁻¹)</td>
<td>6</td>
<td>31.6±1.2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Yohimbine (10 mg kg⁻¹)</td>
<td>6</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Fig. 1(a-d): Gastric tissue of Famotidine 20 mg kg⁻¹ + Indomethacin (25 mg kg⁻¹) group (a), Omeprazole 20 mg kg⁻¹ + Indomethacin (25 mg kg⁻¹) group (b), Indomethacin 25 mg kg⁻¹ (control) (c) and healthy (d) given rats

Fig. 2(e-h): Gastric tissue of Yohimbine (10 mg kg⁻¹) + Famotidine (20 mg kg⁻¹) + Indomethacin (25 mg kg⁻¹) group (e), Yohimbine (10 mg kg⁻¹) + Omeprazole (20 mg kg⁻¹) + Indomethacin (25 mg kg⁻¹) group (f), Yohimbine (10 mg kg⁻¹) + Indomethacin (25 mg kg⁻¹) (control) group (g), Yohimbine (10 mg kg⁻¹) group (h) given rats
Fig. 3(i-l): Gastric tissue of Histamine (1 mg kg⁻¹) +Famotidine (20 mg kg⁻¹) +Indomethacin (25 mg kg⁻¹) group (i), Histamine (1 mg kg⁻¹) +Omeprazole (20 mg kg⁻¹) +Indomethacin (25 mg kg⁻¹) group (j), Histamine (1 mg kg⁻¹) +Indomethacin (25 mg kg⁻¹) (control) group (k), Histamine (1 mg kg⁻¹) group (l) given rats

Table 3: Effects of famotidine and omeprazole on indomethacin-induced gastric ulcers treated with histamine

<table>
<thead>
<tr>
<th>Drugs</th>
<th>N</th>
<th>Ulcer area (mm²)</th>
<th>Artiflucker effect (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histamine (1 mg kg⁻¹)</td>
<td>6</td>
<td>0.91±0.2</td>
<td>97</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>+Famotidine (20 mg kg⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+Indomethacin (25 mg kg⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histamine (1 mg kg⁻¹)</td>
<td>6</td>
<td>0.16±0.2</td>
<td>99</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Omeprazole (20 mg kg⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+Indomethacin (25 mg kg⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histamine (1 mg kg⁻¹) +</td>
<td>6</td>
<td>30±1.4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Indomethacin (25 mg kg⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histamine (1 mg kg⁻¹)</td>
<td>6</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Healthy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 4 shows the levels of tGSH, MDA and the activity of SOD, MPO were respectively 3.8 nmol g⁻¹ protein, 2.4 µmol g⁻¹ protein, 5.6 and 2 µg⁻¹ in the gastric tissue of the healthy rats and 1.3 nmol g⁻¹ protein, 7.4 µmol g⁻¹ protein, 3.2 and 4.7 µg⁻¹ in the control group that was given indomethacin. In the group that was given famotidine, the levels of tGSH, MDA and the activity of SOD, MPO were found to be 4.3 nmol g⁻¹ protein, 2.2 µmol g⁻¹ protein, 6.0 µg⁻¹ and 1.7 µg⁻¹, respectively. In the group that was given omeprazole, these parameters were respectively, 4.1 nmol g⁻¹ protein, 1.9 µmol g⁻¹ protein, 6.2 µg⁻¹ and 1.9 µg⁻¹.

Yohimbine test: Figure 5 shows the levels of tGSH, MDA and the activity of SOD, MPO were, respectively 3.6 nmol g⁻¹ protein, 2.5 µmol g⁻¹ protein, 5.3 and 2.3 µg⁻¹ in the gastric tissue of the healthy rats. The levels of tGSH, MDA and the activity of SOD, MPO were measured as 1.1 nmol g⁻¹ protein, 7.6 µmol g⁻¹ protein,
indomethacin. They were, respectively measured as 3.7 protein, 2.4 nmol g⁻¹ protein, 5.4 and 2.1 μg⁻¹, in the gastric tissue of the rats that received histamine.

**DISCUSSION**

This experimental results showed that famotidine and omeprazole prevented indomethacin-induced gastric ulcers. It is known that famotidine and omeprazole prevent indomethacin-induced gastric ulcers in rats (Albayrak et al., 2010; Aydınlı et al., 2007). As cited above, indomethacin leads to a damage of gastric tissue, by inhibiting the production of PGE2 (Suleyman et al., 2010). However, the healing of mucosal lesion observed despite the large decrease of PGE2 formation with the chronic administration of NSAIDs (Brzoskowski et al., 2005) and in the rats with adrenalectomy, the transformation of the gastric damage which is one of the serious side effects of glucocorticoids (due to PG inhibition), to anti-ulcerative activity. Bailey (1991) and Suleyman et al. (2007) showed that the theory of PG inhibition in the pathogenesis of ulcer is not convincing. These literature data reveal that there is no a direct correlation between PG inhibition and GIS damage.

The result of this study has shown that while the anti-ulcerative activity of famotidine did not change in those that received histamine, it disappeared in those that received yohimbine. These results suggest the role of alpha-2 adrenergic receptors, rather than H2 histaminergic receptors, in the anti-ulcerative activity of famotidine.

As stated above, it was shown that prednisolone has anti-ulcer effect via alpha-2 adrenergic receptors (Suleyman et al., 2007). But in the literature, some studies asserting that glucocorticoids shows gastroprotective effects via own receptors are available (Filaretova et al., 2002).

It is known that yohimbine is the selective blockers of alpha-2 adrenergic receptors (Cherian et al., 2010).

It was shown that pre-synaptic alpha-2 adrenergic receptors play a role in the inhibition of the ulcers induced by the binding of pylorus, indomethacin, aspirin, ethanol and stress (Del Soldato, 1986; Dülşöpeh et al., 1987). It was reported that anti-ulcerative activity was experimentally blockaded with yohimbine (Suleyman et al., 2007). These literature data obtained and our study results suggest the role of alpha-2 adrenergic receptors in the anti-ulcerative activity of famotidine. Similar blockade of anti-ulcerative activity of omeprazole by yohimbine indicates that both drugs show their effect via alpha-2 adrenergic receptors.
There are many studies that demonstrated that, in the gastric tissue damaged by indomethacin, antioxidant parameters were decreased and oxidant parameters were increased. In the gastric tissue of the animals that were given indomethacin, the levels of MPO and MDA were increased. On the other hand, enzymatic and non-enzymatic antioxidant parameters, such as total glutathione (tGSH), Glutathione-S-Transferase (GST), Superoxide Dismutase (SOD), Catalase (CAT) and Glutathione Peroxidase (GPx) were decreased (Naito et al., 1998).

Therefore, it is claimed that indomethacin-induced gastric ulcers may be eliminated with antioxidant effect. It was demonstrated that the increase of mucosal oxidants and the decrease of enzymatic and non-enzymatic antioxidants, induced by indomethacin, may be reversed with the use of famotidine and omeprazole (Naito et al., 1998). Again, some studies show that the gastroprotective effects of anti-ulcerative drugs are not associated with the mechanisms, such as the inhibition of gastric acid and the prevention of oxidative tissue damage (Naito et al., 1998).

In this study, the decrease of enzymatic and non-enzymatic oxidant parameters (compared to control group that received indomethacin) and the increase of antioxidant parameters observed in the gastric tissue of the animals that received famotidine and omeprazole overlap with the information obtained from the literature. As specified above (Introduction), the antioxidants which were increased with the administration of anti-ulcerative drugs, were decreased with the use of yohimbine (Borekci et al., 2009; Kumtepe et al., 2009). In this study, famotidine and omeprazole increased the levels of tGSH and SOD compared to control (indomethacin) group and could not prevent the decrease of these parameters in those treated with yohimbine. It is claimed that these antioxidants are a protective factor in the control of indomethacin-induced damage. tGSH and other antioxidants prevent the tissue damage, by maintaining the amount of ROS at specific concentrations and at lower levels in the cells. SOD is the antioxidant enzyme which catalyzes the conversion of superoxide free radical (O$_2^-$) to hydrogen peroxide (H$_2$O$_2$) and to molecular oxygen (O$_2$). SOD and endogenous antioxidant enzymes render the free radicals harmless and protects the tissues from the harmful effects of free radicals and active oxygen species. When these antioxidant defense mechanisms are inadequate, free radicals lead to serious damage in the tissues (Bast et al., 1991). The most important and most harmful effect that the free radicals trigger in the cell is lipid peroxidation.

MDA is the end product of lipid peroxidation and leads to further damage in the cells (Slater, 1984). In the animal group that was given famotidine and omeprazole, both MDA and MPO levels showed significant decrease. Famotidine and omeprazole could not prevent the elevation of MDA level nor MPO level in the gastric tissue of the animals that received yohimbine. MPO is present in phagocytic cells (PNL). MPO is the enzyme that catalyzes the production of toxic hypochlorous acid (HOCl) from HClO. PNLs lead to uncontrolled, excessive production of superoxide anion (O$_2^-$) and hydroxyl radical (OH$^-$) which are free oxygen radicals. Excessive production of MPO and other reactive radicals causes oxidative damage (Hiraishi et al., 1994; Suzuki et al., 1996).

**CONCLUSION**

Consequently, we learned that alpha-2 adrenergic receptors play a role in the anti-ulcerative activity of omeprazole, inhibitor of ATPase and famotidine, a H$_2$ receptor blocker. Furthermore, we observed a direct correlation between alpha-2 adrenergic receptors and oxidant-antioxidant parameters. In addition, it was shown that there was a direct relationship between alpha-2 adrenergic receptors and oxidant-antioxidant parameters. It was also determined that stimulation of alpha-2 adrenergic receptors caused a decrease in enzymatic and non enzymatic antioxidant parameters and an increase in the antioxidant parameters. Alpha-2 adrenergic receptors have been shown to play an important role in the pathogenesis of gastric ulcer. For this reason it was thought that use and design of more selective alpha-2 adrenergic agonists may be useful in the prevention of gastric damage.

**REFERENCES**


