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The Effect of Lacidipine on Indomethacin Induced Ulcers in Rats

¹Bahadır Suleyman, ¹Zekai Halici, ²Fehmi Odabasoglu and ¹Fatma Gocer

¹Department of Pharmacology, Faculty of Medicine,
Ataturk University, 25240 Erzurum, Turkey

²Department of Biochemistry, Faculty of Pharmacy,
Ataturk University, 25240 Erzurum, Turkey

Abstract: In this study, the antiulcer activity of lacidipine was investigated on indomethacin-induced gastric ulcer model and it was examined whether the antiulcer effect of lacidipine was related to oxidant/antioxidant parameters in rats. Anti-ulcerative effects of lacidipine were investigated on an indomethacin-induced gastric ulcer model in rats. The antiulcer capability of lacidipine was compared to 20 mg kg⁻¹ famotidine. Results showed that lacidipine prevented the formation of indomethacin-induced ulcers at 2 and 4 mg kg⁻¹ doses significantly. Enzymatic and non-enzymatic antioxidant parameters, such as total glutathione, superoxide dismutase and glutathione peroxidase, were found low and enzymatic and non-enzymatic oxidant parameters, such as myeloperoxidase and malondialdehyde, were found high in the gastric tissues of indomethacin-given rats. Indomethacin acts through not only the inhibition of the synthesis of cytoprotective prostaglandins but also by affecting enzymatic and non-enzymatic, oxidant and anti-oxidant mechanisms.

Key words: Lacidipine, indomethacin, ulcer, oxidant and antioxidant parameters, rat

INTRODUCTION

Lacidipine was first created as an anti-hypertensive drug and it was later discovered that it had anti-aggregative and anti-atherogenic effects. Lacidipine is a vasoselective drug and is a derivative of dihydropyridine (DHP). Lacidipine selectively blocks L-type calcium channels of smooth muscle in blood vessels (Haller *et al.*, 2002).

Calcium is required for the stimulation of gastric mucosal oxyntic cells as well as many other excitable cells. And changes in calcium balance play an important role in the pathogenesis of gastric ulcer. It was shown that calcium induces gastric acid secretion both *in vitro* and *in vivo* (Aadland and Berstad, 1983). Experimental studies demonstrated that certain calcium channel blockers exert their gastro-protective effects through inhibition of acid secretion (Ghanayem *et al.*, 1987). In an *in vitro* study, it was reported that Verapamil and Gallopamil show their gastro-protective effects by inhibiting the proton pumps of (K⁺/H⁺ ATPase) in gastric parietal cells (Sewing and Hannemann, 1983). It was indicated that calcium channel blockers inhibit basal and induced gastric acid secretion and motility (Ghanayem *et al.*, 1987; Nielsen and Sulkowski, 1988). Furthermore, it was claimed that calcium channel blockers protect gastric mucosa also by

increasing gastric blood flow (Ghanayem *et al.*, 1987). Increase in the permeability of calcium ions in gastric mucosa results in accumulation of intracellular calcium and damage in gastric mucosa (Szabo *et al.*, 1985).

Although, etiology of ulcer is very diverse, the physiopathology in the onset of disease is similar. For instance, eventhough aggressive factors leading to ulcer vary the mechanism of gastric damage caused by all the aggressive factors involves an increase in the amount of Radical Oxygen Species (ROS) (Itoh and Guth, 1985). These data support the notion that ROS are involved in the pathogenesis of ulcer (Salim, 1994). The fact that the differences in the levels of oxidants and antioxidants in undamaged and damaged tissues are substantive shows the significance of these parameters in the onset and treatment of ulcer. All these previous data suggest that calcium channel blockers may have gastro-protective effects.

Our survey of the literature on the effects of calcium channel blockers on stomach failed to find any studies on the anti-ulcer effects of lacidipine. Therefore, the goal of this study was to investigate the anti-ulcer effects of lacidipine on rat Indomethacin ulcer model and to determine, whether these effects are related to oxidant-antioxidant parameters.

MATERIALS AND METHODS

Experimental animals: In this study male Wistar rats, whose body weights ranged between 200 and 210 g, were used. Animals were obtained from Ataturk University's Medical and Experimental Research and Practice Center. Prior to the experiments, animals were housed and fed at room temperature (22°C) for one week (7 days) in groups. In document B.30.2 ATA 0.23.85-109 dated 11 November 2009, Ataturk University Local Ethical Committee of Experimental Animals (AUDHADYEK) approved that all the steps of this study were compliant with ethical rules.

Indomethacin ulcer test: In this study, anti-ulcer effects of lacidipine were investigated in gastric ulcer model induced by indomethacin (Abd El-Kader *et al.*, 2011; Olaleye *et al.*, 2006; Rifat-uz-Zaman *et al.*, 2005). Lacidipine was administered through oral gavage at 2.4 mg kg⁻¹ dose to rats that were starved for 24 h. To compare the anti-ulcer effects of lacidipine, Famotidine (at 20 mg kg⁻¹) was administered to another group of rats through the same route. The control group was given distilled water in same volume. Five minutes after the administration of drugs, Indomethacin was applied to all the rat groups at 25 mg kg⁻¹ by oral route. Six hours after the administration of Indomethacin, animals were euthanized by giving an anesthetic at high dose (50 mg kg⁻¹ sodium thiopental). The stomachs of the euthanized animals were taken out and evaluated macroscopically. The areas of ulcer in the surface of the stomach were measured using a mm² sheet. The anti-ulcer activity of lacidipine was compared to that of 20 mg kg⁻¹ Famotidine as well as the results from the control group. Then, all the stomachs were sent to Biochemistry Department in Faculty of Pharmacy for the measurement of GSH, MDA, GPO and SOD levels.

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% hexa desil 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 GSH in the total homogenate was measured according to the method of Sedlak and Lindsay (1968) with some

modifications. The sample was weighed and homogenized in 2 mL of 50 mM Tris-HCl buffer containing 20 mM EDTA and 0.2 mM sucrose at pH 7.5. The homogenate was immediately precipitated with 0.1 mL of 25% trichloroacetic acid and the precipitate was removed after centrifugation at 4200 rpm for 40 min at 4°C. The supernatant was used to determine GSH using 5,5-dithiobis (2-nitrobenzoic acid) (DTNB). Absorbance was measured at 412 nm using a spectrophotometer.

Superoxide dismutase (SOD) analysis: Measurements were performed according to Sun *et al.* (1988). SOD estimation was based on the generation of superoxide radicals produced by xanthine and xanthine oxidase which react with Nitro Blue Tetrazolium (NTB) to form purple colored-formazan dye. The sample was centrifuged at 6000 rpm for 10 min and then the brilliant supernatant was used as assay sample. The supernatant was immediately reacted with xanthine oxidase. The assay tubes incubated for 1 min and formazan was then measured at 560 nm. As more of the enzyme exists, the least O₂⁻ radical that reacts with NBT occurs.

Glutathione peroxidase (GPO) analysis: GPx activity was determined according to a previously reported method (Lawrence and Burk, 1976). The absorbance at 340 nm was recorded for 5 min. The activity was determined by measuring the amount of oxidised NADPH as mM min⁻¹ mg⁻¹ tissue.

Myeloperoxidase (MPO) analysis: The activity of MPO in the total homogenate was measured according to the method of Wei and Frenkel (1991) with some modifications. The sample was weighed and homogenized in 2 mL of 50 mM phosphate buffer containing HDTMAB (0.5%) and centrifuged at 3500 rpm for 60 min at 4°C. The supernatant was used to determine MPO activity using 4-aminoantipyrine-2% phenol solution. 4-aminoantipyrine-2% phenol solution and H₂O₂ were added and equilibrated for 3-4 min. After establishing the basal rate, a 0.2 mL sample suspension was added and quickly mixed. Increases in absorbance at 510 nm for 4 min at 0.1 min intervals were recorded. Protein concentration was assayed with bicinchoninic acid absorbance was measured at 412 nm using a spectrophotometer.

Malondialdehyde (MDA) analysis: The concentrations of gastric mucosal lipid peroxidation were determined by estimating MDA using the thio barbituric acid test (Ohkawa *et al.*, 1979). The rat stomachs were promptly excised 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⁻¹ KCl. The homogenate (0.5 mL) was added to a solution containing 0.2 mL of 80 g L⁻¹ sodium lauryl sulfate, 1.5 mL of 200 g L⁻¹ acetic acid, 1.5 mL of 8 g L⁻¹ 2-thiobarbiturate and 0.3 mL distilled water. The mixture was incubated at 98°C for 1 h. Upon cooling, 5 mL of n-butanol:pyridine (15:1) was added. The mixture was vortexed for 1 min and centrifuged for 30 min at 4000 rpm. The absorbance of the supernatant was measured at 532 nm. The standard curve was obtained by using 1,1,3,3-tetramethoxypropane.

Statistical analyses: Results obtained from the experiments were expressed as “mean value±standard deviation”. Significance of the differences between the groups was determined by one-way ANOVA test. Then, Fisher’s post-hoc LSD (Least Significant Differences) was performed. All the statistical calculations were done using “SPSS for Windows, 13.0” software and p<0.05 values were interpreted as being statistically significant.

RESULTS

Effects of lacidipine on indomethacin induced ulcers:

Macroscopic observations showed that all the animals used in this study developed ulcer in their stomach tissues. Ulcer nodes appeared in various forms and sizes throughout the surface of the stomach. Ulcers were composed of round, oval and irregular mucosal defects that had varying radii and depth. Borders of ulcers were noticeable. The ulcers in the stomach of animals that got lacidipine and Famotidine were less in number and had smaller surface area than those of the control group. Severe hyperaemia was seen in the control group that was given Indomethacin. As seen in Table 1, while the average area of gastric ulcers in the control group which got Indomethacin, was 27.0±2.16 mm², average area of ulcers in rats that received 2 or 4 mg kg⁻¹ doses of lacidipine or 20 mg kg⁻¹ Famotidine was 1.5±0.67, 0.17±0.17 and 0.83±0.54 mm², respectively.

Biochemical analyses: As seen in (Fig. 1) tGSH levels in the gastric tissues of the rats that received 2 and 4 mg kg⁻¹ lacidipine were 4.31±0.03 and 4.48±0.03 nmol mg⁻¹ tissue; whereas the levels in Famotidine group and the control group that received Indomethacin were 4.10±0.08 and 3.74±0.03 nmol mg⁻¹ tissue, respectively. tGSH levels in stomachs of healthy rats were 4.36±0.03 nmol kg⁻¹ tissue.

SOD levels in the gastric tissues of rats that were dosed with 2 and 4 mg kg⁻¹ lacidipine were measured as

Table 1: Effects of lacidipine and famotidine on indomethacin-induced gastric ulcers in rats

Drugs	Dose (mg kg ⁻¹)	N	Ulcer area (mm ²)	Anti-ulcer effect (%)	p
Lacidipine	2	6	1.50±0.67	94.45	<0.001
Lacidipine	4	6	0.17±0.17	99.37	<0.001
Famotidine	20	6	0.83±0.54	96.92	<0.001
Indomethacin (Control)	25	6	27.00±2.16	-	-

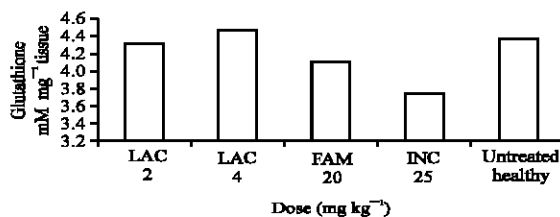


Fig. 1: Effects of lacidipine (LAC) and famotidine (FAM) on total glutathione (tGSH) level in indomethacin (IND) -induced ulcer model in rat gastric tissue

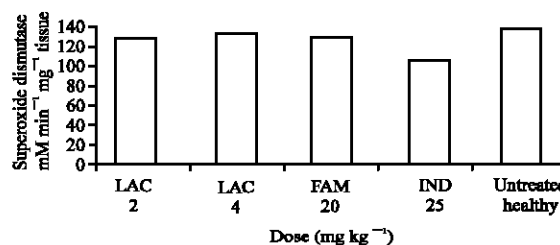


Fig. 2: Effects of lacidipine (LAC) and famotidine (FAM) on super oxide dismutase (SOD) enzyme activity in indomethacin (IND) -induced ulcer model in rat gastric tissue

122.3±4.9 and 128.0±1.5 nmol min⁻¹ mg⁻¹ tissue. The levels in Famotidine, control and healthy (intact) rat groups were evaluated as 122.9±3.0, 98.71±5 and 131.32±2.0 nmol min⁻¹ mg⁻¹ tissue, respectively (Fig. 2).

GPO activity in the control group which received Indomethacin, was 2.52±0.17 μM min⁻¹ mg⁻¹ tissue, whereas, the activities in the rats dosed with lacidipine (2, 4 mg kg⁻¹) or Famotidine (20 mg kg⁻¹) were 4.06±0.24, 6.90±0.23 and 4.17±0.05 μM min⁻¹ mg⁻¹ tissue, respectively. On the other hand, GPO activity in healthy (intact) rat group was detected as 6.63±0.36 μM min⁻¹ mg⁻¹ tissue (Fig. 3).

MPO activity was significantly lowered in the gastric tissues of rats dosed with lacidipine, compared to the activity in the control group. MPO activity was 16.01±0.22 36 μM min⁻¹ mg⁻¹ tissue in the control group which was given Indomethacin. MPO activities in lacidipine (2, 4 mg kg⁻¹), Famotidine and healthy (intact)

DISCUSSION

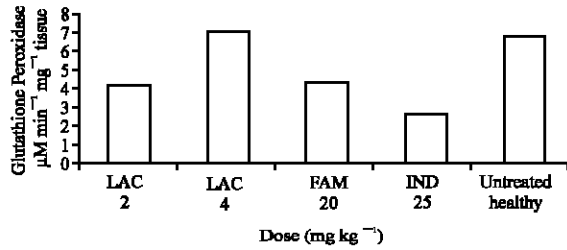


Fig. 3: Effects of lacidipine (LAC) and famotidine (FAM) on glutathione peroxidase (GPO) enzyme activity in indomethacin (IND) -induced ulcer model in rat gastric tissue. (INT: Intact)

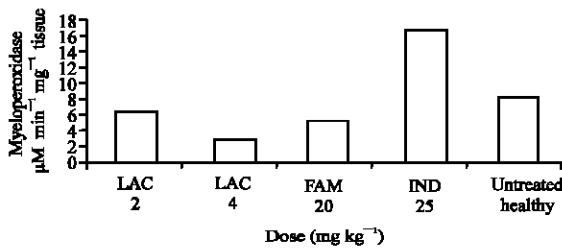


Fig. 4: Effects of lacidipine (LAC) and famotidine (FAM) on myeloperoxidase (MPO) enzyme activity in indomethacin (IND) -induced ulcer model in rat gastric tissue. (INT: Intact)

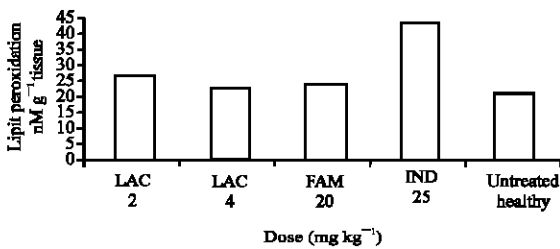


Fig. 5: Effects of lacidipine (LAC) and famotidine (FAM) on lipid peroxidation (LPO) level in indomethacin (IND) -induced ulcer model in rat gastric tissue. (INT: Intact)

group were measured as 6.22±0.30, 2.74±0.18, 5.14±0.08 and 7.88±0.13 μmol min⁻¹ mg tissue, respectively (Fig. 4).

While MDA levels in the gastric tissues of rats that were given lacidipine (2, 4 mg kg⁻¹) and Famotidine (20 mg kg⁻¹) were 25.67±0.60, 21.39±0.43 and 23.35±0.86 nmoL g⁻¹ tissue, the levels in healthy (intact) and control (Indomethacin-dosed) groups were 20.28±0.29 and 41.97±0.47 nmoL g⁻¹ tissue (Fig. 5).

In this study, the effects of lacidipine on Indomethacin-induced ulcer model in rat were investigated and whether anti-ulcer activity was related to oxidant-antioxidant parameters in gastric tissues was determined. The results of the experiment demonstrated that lacidipine at 2 and 4 mg kg⁻¹ doses significantly prevented indomethacin induced ulcer formation. The anti-ulcer activity of lacidipine was found to be approximately equal to that of Famotidine. However, gravimetric anti-ulcer effects of both doses of lacidipine were seen to be higher than that of Famotidine. The difference between the two doses of lacidipine was statistically insignificant.

It is suggested that the Indomethacin used in the ulcer model acts through the inhibition of PGE₂, bicarbonate and mucus production and also through increasing the levels of oxidant parameters while decreasing the levels of antioxidant parameters (Khotimchenko *et al.*, 2006; Sabina and Rasool, 2007). Classical anti-ulcer medicines prevent Indomethacin-induced ulcers by acting in the opposite direction of Indomethacin (i.e. increasing PGE₂, bicarbonate and mucus production, inhibiting acid secretion, decreasing the levels of oxidant parameters and increasing the levels of anti-oxidant parameters) (Suleyman *et al.*, 2010). As mentioned earlier, our review of the literature did not yield any studies on the anti-ulcer effects and anti-ulcer mechanism of lacidipine. Therefore, to reveal the anti-ulcer mechanism of lacidipine, oxidant (MPO, LPO) and antioxidant (tGSH, SOD, GPO) levels were measured (Ogunlade *et al.*, 2012) in gastric tissues of rats that were given lacidipine. Our results showed that lacidipine, at doses that prevent Indomethacin ulcers, significantly increases tGSH levels compared to the levels in control group. Levels of tGSH were higher in gastric tissues of rats that got 4 mg kg⁻¹ lacidipine than gastric tissues of health rats. This finding suggests that lacidipine both prevents the decrease of tGSH levels by Indomethacin and increases the production of tGSH. Also, it was previously shown that the difference in the levels of tGSH in damaged and undamaged tissues was significant (Dursun *et al.*, 2009; Suleyman *et al.*, 2009). GSH is a tripeptide that is composed of L-glutamate, L-cysteine and L-glycine. Endogenous GSH plays an important role in the sustenance of gastric mucosal integrity. The antioxidant properties of GSH are due to the cysteine thiol in its structure. A decrease in GSH levels in gastric tissue results in gastric damage, while an increase in GSH levels has gastro-protective effects (Kisaoglu *et al.*, 2011). GSH

reacts with peroxides and toxic oxygen radicals such as OH and singlet oxygen (1O_2) and protects cells from damage. Furthermore, it keeps -SH groups in proteins reduced and prevents their oxidation (Walker *et al.*, 1995). Decreasing gastric tGSH levels by giving animals diethyl malate, a GSH antagonist, causes damage to the gastric tissues (Naito *et al.*, 1993). On the other hand, application of reduced glutathione prevents the damage formation. This finding establishes that GSH is an important endogenous anti-ulcer factor.

In this study, it was also determined that SOD activity in the gastric tissues of control group rats that only received Indomethacin was lower compared to that in intact rat group. SOD activity in gastric tissues of rats that were given lacidipine was higher than that of the control group. In this part of the experiment, 4 mg kg⁻¹ dose of lacidipine was the dose that was most efficacious in prevention of the inhibition of SOD activity. It is known that SOD activity is low in damaged tissues (Adebayo *et al.*, 2009). The decrease of SOD activity in the gastric tissues treated with Indomethacin correlates well with the damage in these tissues (Karakus *et al.*, 2009; Odabasoglu *et al.*, 2008). There is experimental evidence suggesting that diethyldithiocarbamate, an SOD inhibitor, inhibits secretion of bicarbonate and this inhibition can be abolished by SOD (Takeuchi *et al.*, 1996). This shows that SOD has gastro-protective effects and that there is a direct relationship between SOD and gastro-protection.

In this study, both doses of lacidipine significantly prevented Indomethacin-induced inhibition of GPO activity in gastric tissues of rats. The highest GPO activity was seen in the rat group that received 4 mg kg⁻¹ lacidipine. The fact that GPO activity in gastric tissues receiving lacidipine was higher than that in control group indicated the strong correlation between the inhibition of GPO activity and gastro-toxic effects. The significant difference in GPO activity between damaged and undamaged gastric tissues was also corroborated by previous findings (Mohammadirad and Abdollahi, 2011; Vasanthkumar *et al.*, 2010).

Lacidipine, at the used doses, also decreased MPO activity in gastric tissues of rats. A dose of 4 mg kg⁻¹ lacidipine significantly decreased MPO activity compared to Famotidine and the low dose (2 mg kg⁻¹). Sener-Muratoglu *et al.* (2001) previously reported that MPO played a role in the anti-ulcer mechanism of Famotidine. Many studies demonstrated an increase of MPO activity in damaged gastric tissues (Zinkievich *et al.*, 2010; Guha *et al.*, 2010; Zhang *et al.*, 2008). MPO is an enzyme found in phagocytic cells (PNL)

(Biasucci *et al.*, 1996). Indomethacin activates the PNLs in gastric mucosa (Asako *et al.*, 1992). Activation of PNLs results in the secretion of O_2^- , OH, H_2O_2 and MPO which are known as cytotoxic radicals. Hypochlorous acid and N-chloramines, formed by the reaction of the above mentioned radicals, Cl^- and MPO, initiate the cytotoxic effect in tissues (Karmeli *et al.*, 1996).

In this study, MDA levels increased in the gastric tissues damaged by Indomethacin. Two hours post Indomethacin administration, a sudden increase in the production of toxic oxygen species is seen in gastric mucosa (Hassan *et al.*, 1998). This increase shows that toxic radicals result in (oxidative) damage in the stomach. MDA, the final product of lipid peroxidation, is used to determine the levels of lipid peroxidation (Valenzuela, 1991). Lacidipine and famotidine, at the doses used, significantly reduce MDA levels compared to the control group. Lacidipine dosed at 4 mg kg⁻¹ reduces MDA levels more than the low lacidipine dose (2 mg kg⁻¹). There are studies showing that the increasing MDA levels in damaged tissues are repressed by anti-ulcer reagents (Zhao *et al.*, 2009; Prabha *et al.*, 2009). Results from our experiments and previous studies establish the role of MDA levels in the anti-ulcer mechanism of lacidipine.

As a result, at 2 and 4 mg kg⁻¹ doses, lacidipine was shown to significantly prevent gastric damage induced by Indomethacin in rats. Indomethacin acts through not only the inhibition of the synthesis of cytoprotective PG but also by affecting enzymatic and non-enzymatic, oxidant and anti-oxidant mechanisms such as GSH, SOD, GPO, MPO and MDA. On the other hand, lacidipine is shown to exhibit anti-ulcer effects by reversing the effects of Indomethacin on oxidant and anti-oxidant parameters.

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