Effects of captopril on factors affecting gastric mucosal integrity in aspirin-induced gastric lesions in Sprague-Dawley rats

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Abstract

Introduction: Captopril is an angiotensin-converting enzyme inhibitor, which is used as an antihypertensive agent and has shown antioxidant properties. This study aims at determining the effects of captopril on factors affecting gastric mucosal integrity in aspirin-induced gastric lesions.
Material and methods: Eighteen male Sprague-Dawley (200-250 g) rats that were given aspirin (40 mg/100 g body weight) were divided into three groups: the control, captopril (1 mg/100 g body weight daily) and ranitidine (2.5 mg/100 g body weight twice daily) groups. Ranitidine and captopril were given orally for 28 days. Rats in all groups were sacrificed and the parameters measured.
Results: Captopril reduced gastric acidity, and increased gastric glutathione (GSH) and prostaglandin E_2 (PGE_2) significantly in comparison to the control group. Captopril also reduced malondialdehyde (MDA) and gastric lesions insignificantly compared to the control group. Ranitidine healed the lesions significantly compared to the control group. There was no difference between ranitidine and captopril on the severity of lesions, gastric acidity, MDA and GSH. Captopril increased PGE_2 compared to ranitidine ($p < 0.05$).
Conclusions: Captopril has desirable effects on the factors affecting gastric mucosal integrity (acidity, PGE_2 and GSH) and is comparable to ranitidine in ulcer healing.

Key words: captopril, ranitidine, aspirin, gastric lesions.

Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs), with their broad analgesic, anti-inflammatory and antipyretic effects, are among the most frequently used drugs because pain, inflammation, and pyrexia are so common. Approximately 30 million people worldwide are prescribed NSAIDs daily [1]. Additionally, the use of NSAIDs is gradually increasing, mostly because of the expanding aging population [2], which requires frequent administration of NSAIDs to treat conditions such as osteoarthritis, which is common in the elderly [3].

However, the non-selective NSAIDs are well known to cause gastrointestinal mucosal damage and of particular concern are patients with arthritic conditions. Statistics from the Western population have shown

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that about 2% to 4% of non-selective NSAID users develop serious gastric mucosal erosions [4] and about 20% of long-term NSAID users develop peptic ulcers [5]. In addition, about 1% to 8% of elderly NSAID users are hospitalized for complications of peptic ulcers within 1 year of initiating therapy [6].

Although the discovery of selective NSAIDs is indeed a breakthrough as it is devoid of gastrointestinal side effects, the use of non-selective NSAIDs is still popular due to their relatively low cost. Therefore, studies exploring the possibilities to preserve gastric mucosal integrity during treatment with non-selective NSAIDs are still much needed [7].

The mechanism of how NSAIDs induce gastric lesions remains unclear and cannot be explicitly explained. The most probable mechanism suggests disruption of gastric mucosal integrity via the production of free radicals [8]. The body has endogenous antioxidants, which under normal conditions are adequate to protect the organs. In situations that differ from normal such as exposure to noxious stimuli, vulnerable organs such as the lung, liver and stomach need a high level of endogenous antioxidants such as non-protein sulfhydryls (mainly reduced glutathione) to maintain their integrity. In such situations, exogenous antioxidants may prove to be beneficial [9]. The NSAIDs also inhibit the production of protective prostaglandins, which is another possible mechanism in the pathogenesis.

Captopril is the earliest angiotensin-converting enzyme (ACE) inhibitor [10]. It is commonly used as an antihypertensive drug. It contains a sulfhydryl group and recently this sulfhydryl containing ACE inhibitor is reported to exhibit the ability to scavenge free radicals and provide protection against free radical mediated oxidative damage [11]. In addition, captopril is also reported to be structurally similar to the enzyme kininase II [12]. Have to explain on kininase II in relation to gastric ulcer. As a result, captopril blocks the effect of this enzyme by competitive inhibition and causes a rise in plasma bradykinin levels. Accumulation of bradykinin is known to raise the production of prostaglandins, as reported in previous studies [13, 14].

In an attempt to find a new avenue to minimize gastric mucosal damage due to aspirin, this study was carried out to investigate the effects of captopril on the healing of gastric lesions, levels of gastric acidity, prostaglandin E_2 (PGE_2) and glutathione (GSH), as well as lipid peroxidation.

Material and methods

Eighteen male Sprague-Dawley rats (200-250 g) were randomly assigned to three groups. Group I (6 rats) was a control group, group II (6 rats) was a positive control treated with ranitidine, and group III (6 rats) was treated with captopril. At the beginning of the study, rats from all groups were challenged with a single dose of aspirin (40 mg/100 g body weight) to induce gastric lesions. Treatment was initiated 6 h after induction. The ranitidine group was given 2.5 mg/100 g body weight ranitidine twice daily and the captopril group was given 10 mg/kg body weight captopril once a day. After 28 days of treatment, all rats were deprived of food and housed in cages with a wide mesh wire bottom for 24 h to prevent coprophagy. The rats were killed on the 29th day and the severity of gastric lesions was assessed. The other parameters measured were gastric acidity, gastric tissue contents of malondialdehyde (MDA), PGE_2 and GSH. This study was approved by the Ethics Committee for animal studies, Faculty of Medicine, Universiti Kebangsaan Malaysia (UKM), Kuala Lumpur, Malaysia.

Measurement of gastric acidity

The lower end of the oesophagus and pylorus were clamped and the stomach removed. Samples of gastric juice were collected and centrifuged at 3000 r.p.m. for 10 min. Aliquots for each sample were titrated with 0.01 N NaOH to a pH of 7.0. The hydrogen ion concentration was calculated as described by Shay *et al.* [15].

Assessment of gastric lesions

The gastric mucosa was exposed by cutting the stomach along the greater curvature, washing with saline and laying it on a flat wooden board. The macroscopic assessment of aspirin-induced gastric lesions was performed by an independent examiner who was blinded to the supplementation that the rats had received. The assessment of lesions was made according to a semi-quantitative scale described earlier by Berry *et al.* [16] but which has since been modified by Ismail *et al.* [17]. The modified scale used was as follows: 5 = continuous lesions that occupied almost the entire length of the gastric fold, 4 = lesions which occupied almost 80% of the entire fold, 3 = presence of multiple lesions that measured 1-4 mm in length on 80% of the folds, 2 = presence of at least two lesions approximately 2 mm in length, 1 = presence of a single lesion with or without generalized erythema, 0.5 = presence of dot haemorrhages; and 0 = no visible damage.

Measurement of gastric malondialdehyde content

Tissue samples weighing 0.2 g from the corpus region were homogenized using a glass homogenizer (Potter S: B Braun, Germany). The content of gastric tissue MDA was then determined using the method described by Ledwozyw *et al.* [18]. The gastric tissue content of protein was determined by the Lowry method [19] and the MDA was expressed in terms of as nmol/mg protein.
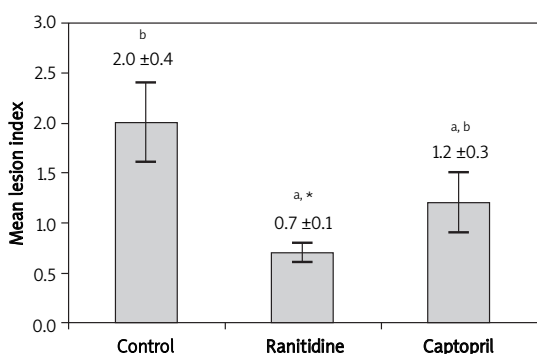
Nafeeza Mohd Ismail, Ibrahim Abdel Aziz Ibrahim, Najihah Binti Mohd Hashim, Kamsiah Jaarin

Figure 1. Effects of captopril and ranitidine treatments for 28 days on gastric lesion index in rats
[a, b]*The same letters of the alphabet indicate that the values are not significantly different, and different letters of the alphabet indicate that the values are significantly different*
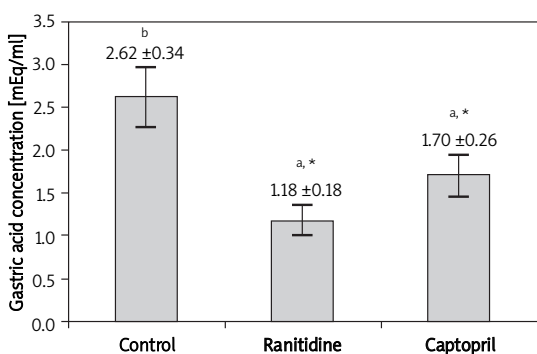

Figure 2. Effect of captopril and ranitidine on gastric acidity in rats subjected to aspirin-induced gastric lesions
[a, b]*Same letters of the alphabet indicate that the values are not significantly different, and different letters of the alphabet indicate that the values are significantly different*

Measurement of gastric prostaglandin E_2 content

Samples of gastric mucosal tissue were prepared for PGE_2 analysis according to the method described by Redfern *et al.* [20]. The tissue samples were homogenized in 20 volumes of 100% ethanol using a glass homogenizer on ice. Cold water was added to this mixture to make the concentration of 15% ethanol. The mixture was then centrifuged at 400 × G for 10 min. The supernatant obtained was transferred to another tube and 10 ml of acetic acid was added to make a pH of 3.0. The extraction of PGE_2 was performed using an Amprep C18 cartridge (Amersham International, UK) and the content was analyzed using a commercial kit (Prostaglandin E_2^{125} assay system: Amersham International, UK).

Measurement of gastric glutathione content

Gastric GSH content was measured using a well-established method [20]. The gastric tissue sam-

ples were homogenized in 4 volumes of (5% TCA/ 0.01 N) HCl and centrifuged at 17 000 × g for 15 min at 2°C. The supernatant was separated for GSH assay. The ratio of reduced glutathione to oxidized glutathione was calculated.

Statistical analysis

All results were expressed as mean ± SEM. Statistical analysis was performed using non-parametric tests such as Kruskal-Wallis and Mann-Whitney. A difference with a probability value of less than 5% ($p < 0.05$) was considered statistically significant for all parameters.

Results

Effect of captopril and ranitidine on gastric lesions

The effect of captopril and ranitidine on the severity of gastric lesions is shown in Figure 1. The ranitidine group exhibited a gastric lesion index of about 65% lower compared to the control group ($p < 0.05$). There was no difference between the captopril and the control groups although the gastric lesion index for the captopril group was numerically lower (40%) than the control group. There was also no difference between the captopril and the ranitidine groups.

Effect of captopril and ranitidine on gastric acidity

The effect of captopril and ranitidine on gastric acidity is shown in Figure 2. The ranitidine treatment significantly reduced the gastric acidity compared to the control group ($p < 0.05$). The ranitidine group showed 55% lower gastric acidity. There was also a significant reduction of gastric acidity in the captopril compared to the control group (35%; $p < 0.05$). The gastric acidity between the captopril and the ranitidine groups was comparable ($p > 0.05$).

Effect of captopril and ranitidine on gastric malondialdehyde content

The gastric MDA content for the ranitidine group was 45.7% lower than the control group ($p < 0.05$). There was no significant difference between the captopril and the control group. The gastric MDA content for the ranitidine group was 29% lower than the captopril group, but there was no significant difference between the groups, as shown in Figure 3.

Effect of captopril and ranitidine on gastric prostaglandin E_2 content

The effect of captopril and ranitidine on gastric PGE_2 content is shown in Figure 4. Gastric PGE_2 con-
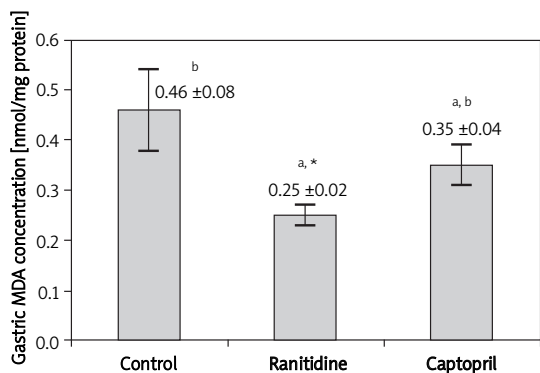
Figure 3. Effect of captopril and ranitidine on gastric MDA content in rats subjected to aspirin-induced gastric lesions
[a, b]*Same letters of the alphabet indicate that the values are not significantly different, and different letters of the alphabet indicate that the values are significantly different*

Figure 4. Effect of captopril and ranitidine on gastric PGE_2 content in rats subjected to aspirin-induced gastric lesions
[a, b]*Same letters of the alphabet indicate that the values are not significantly different, and different letters of the alphabet indicate that the values are significantly different*
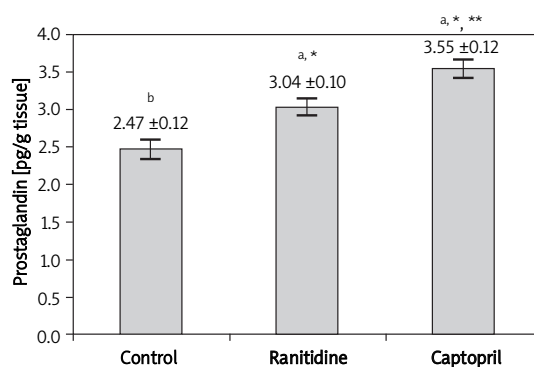
tent was significantly higher in the ranitidine group compared to the control group ($p < 0.05$). Captopril also had a higher gastric PGE_2 content ($p < 0.05$) compared to the control group. The gastric content of PGE_2 for the captopril group was 17% higher than the ranitidine group ($p < 0.05$).

Effect of captopril and ranitidine on gastric tissue content of glutathione

The effect of captopril and ranitidine on gastric GSH content is shown in Figure 5. There was a significant difference in the gastric content of GSH between the captopril and ranitidine groups compared to the control group ($p < 0.05$). Compared to the control group, the gastric GSH contents in the ranitidine and captopril groups were 26% and 41% higher, respectively. The GSH content for both the captopril and ranitidine groups were comparable.

Discussion

Many studies have been performed which show various antiulcer healing properties for aspirin-induced gastric lesions. In the present study, the effect of captopril on the gastric lesions, gastric acidity, lipid peroxidation, PGE_2 and GSH was investigated using an aspirin induction model, by comparing the efficacy of captopril with ranitidine. Based on our knowledge, there were no reports of the healing effect of captopril on aspirin-induced gastric lesions.

The present study shows that there was a trend toward healing in the captopril group; the gastric lesion index was numerically lower compared with the control group. The probable factors responsible for the lack of this effect could be the dosing frequency and the duration of the treatment. In this study, captopril was given once daily whereas the dosing frequency used in the treatment of hyper-

tension is twice a day. Our preliminary experiments showed that twice daily dosing of the dosage employed in this study caused the rats to become hypotensive. A daily dosing avoided the drop in blood pressure. Thus the latter dosing frequency was used. Further investigations on different dosages with a twice daily dosing are warranted. Also, the current study demonstrates that ranitidine promotes the healing of lesions from aspirin-induced gastric ulcer. This confirms and extends the previous finding that this histamine H_2 receptor antagonist is an effective antiulcer agent [21].

Malondialdehyde is one of the end-products of lipid peroxidation, and the extent of lipid peroxidation is most frequently measured by estimating MDA levels [22]. The current study shows that both ranitidine and captopril reduced the level of gastric MDA content. Surprisingly, only ranitidine was able reduce the MDA significantly compared to the con-
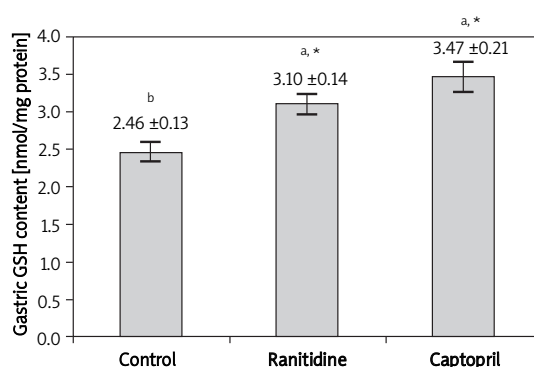

Figure 5. Effect of captopril and ranitidine on gastric GSH content in rats subjected to aspirin-induced gastric lesions
[a, b]*Same letters of the alphabet indicate that the values are not significantly different, and different letters of the alphabet indicate that the values are significantly different*

Nafeeza Mohd Ismail, Ibrahim Abdel Aziz Ibrahim, Najihah Binti Mohd Hashim, Kamsiah Jaarin

trol. A detailed explanation of this phenomenon is beyond the scope of the current study. Captopril, which exhibited free radical scavenging activities, was expected to retard the lipid peroxidation process, but this was not the case in our study. The probable factors responsible for the lack of this effect could again be the dosing frequency and the duration of the treatment. Furthermore, the free radicals generated by aspirin attacked not only lipid molecules but also DNA, protein and any molecule that has a double bond structure. As a result, measurement of MDA alone is not sufficient to demonstrate the antioxidant properties of ranitidine or to disprove the efficacy of captopril in free radical scavenging.

In contrast to the findings on MDA, we found that another indicator of antioxidant status, the gastric GSH content, was significantly higher in both ranitidine and captopril groups compared to the control group. This finding suggests that captopril, through its sulfhydryl group, is able to scavenge free radicals and this reduced the consumption of reduced GSH. On the other hand, the increment of gastric GSH content in the ranitidine group might be due to the ongoing healing process.

In this study, we found that captopril increased the gastric PGE$_2$ content. This observation is in agreement with previous studies, where most of the increment observed was in other organs such as the kidney [23, 24]. This finding could be due to the accumulation of bradykinin after the inhibition of ACE by captopril. Accumulation of bradykinin enhances biosynthesis of prostaglandins (PGs) [13]. Additionally, another study suggested that sulfhydryl group containing agents encourage the biosynthesis of PGs in the gastric mucosa [25]. Interestingly, we found that ranitidine also increased the gastric PGE$_2$ content. An explanation of this event is beyond the scope of the study. Studies to investigate the relation between ranitidine and prostaglandin have to be done.

Gastric acidity is an aggressive factor that ultimately leads to gastric lesions. In the present study, we found that both ranitidine and captopril reduced the gastric acidity effectively. This confirms and extends previous findings that ranitidine, a histamine H$_2$ receptor antagonist, is an effective antisecretory agent [26]. The inhibition of gastric acidity by captopril is via increasing gastric PGE$_2$ content. Captopril is reported to be structurally similar to enzyme kininase II. As a result, captopril blocks the effect of the enzyme by competitive inhibition and causes a rise in plasma bradykinin levels. Accumulation of bradykinin raises the production of prostaglandins. Finally, prostaglandin inhibits gastric acid secretion through the prostaglandin receptor on the membrane of parietal cells in the stomach [27, 28]. Besides that, the fall in gastric acidity in the captopril group might also be caused by the

presence of a sulfhydryl group in the structure of the compound, since agents containing such groups are capable of reducing gastric acidity [29].

In conclusion, we found that administration of captopril at 10 mg/kg/day for 28 days could not significantly heal the aspirin-induced gastric lesions but produced desirable effects on gastric acidity, PGE$_2$ and GSH. Its capability to increase gastroprotective parameters and at the same time reduce the aggressive acid factor optimises the balance between protective and aggressive factors. In view of this linkage, we strongly suggest captopril as a potential novel antiulcer agent.

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