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Role of free radicals in peptic ulcer and gastritis

Serbest radikallerinin peptik ülser ve gastritteki rolü

Süleyman DEMİR[1], Mustafa YILMAZ[2], Mehmet KÖSEOĞLU[1], Nalan AKALIN[1], Diler ASLAN[1], Ahmet AYDIN[3]

Pamukkale University Medical Faculty, Departments of Biochemistry[1] and Gastroenterology[2], Denizli

Background/aims: *It has been suggested that the free radicals are closely related with peptic ulcer disease and gastritis. Although many studies have been undertaken to clarify the role of oxygen-derived free radicals, most of them were carried out in animal models. The aim of this study was to assess the reactive oxygen species activity and the damage in Helicobacter pylori-infected gastric mucosa in humans.* ***Methods:*** *In a total group of 42, there were fifteen cases of peptic ulcer, 14 cases of gastritis and!2 control subjects. Measurement of gastric mucosal malondialdehyde concentrations, which is the end - product of lipid peroxidation, was used to assess oxidative damage to membranes in patients with peptic ulcer and gastritis. Mucosal reduced glutathione glutathione concentrations were also measured in order to study whether reactive oxygen species generation affects levels of the antioxidant peptide. malondialdehyde and glutathione content was then measured in biopsies taken from the gastric antrum.* ***Results:*** *Tissue levels of glutathione were significantly ($p < 0.001$) and malondialdehyde was higher ($p<0.001$) in patients with peptic ulcer compared to controls. In patients with gastritis, glutathione was also lower ($p<0.001$) and malondialdehyde higher ($p<0.01$).* ***Conclusions:*** *Depletion of gastric mucosal glutathione in cases with H.pylori positive peptic ulcer and gastritis may be caused by accumulation of free radicals that can initiate membrane damage by lipid peroxidation.*

Key words: Peptic ulcer, gastritis, malondialdehyde, glutathione, lipid peroxidation

Amaç: *Serbest oksijen radikallerinin peptik ülser ve gastrit patogenezi ile yakından ilişkili olabileceği düşünülmektedir. Serbest oksijen radikallerinin bu patolojilerdeki rolünü açıklayabilmek için çok sayıda çalışma vardır fakat bunların büyük çoğunluğu hayvan modellerinde yapılmıştır. Bu çalışma H.pylori ile infekte mide mukozasında serbest oksijen radikallerinin aktivitesi ve mukozal hasarın değerlendirilmesi amacıyla yapılmıştır.* ***Yöntem:*** *Çalışma 15'i peptik ülser (duodenal), 14'i gastrit ve 12'si sağlıklı kontrol grubunda yapıldı. Peptik ülser ve gastritti hastalarda, mide mulozasında oksidatif hasarın etkisini belirlemek için lipid peroksidasyonunun son ürünü olan malondialdehyde düzeyini araştırdık. Ayrıca reaktif oksijen radikallerinin antioksidan peptit düzeyini etkileyip etkilemediğini ortaya koymak için de mukozal kaynaklı glutathione konsantrasyonunu ölçtük, malondialdehyde ve glutathione düzeyleri mide antrumundan alınan biopsi örneklerinde ölçüldü.* ***Bulgular:*** *Kontrol grubu ile karşılaştırıldığında peptik ülserli hastalarda glutathione doku düzeyi belirgin olarak düşük ($p<0.001$), MDA düzeyinin ise yüksek olduğu ($p<0.001$) saptandı. Gastritti hastalarda da GSH düşük ($p<0.001$), malondialdehyde (MDA) yüksek bulundu ($p<0.01$).* ***Sonuç:*** *H pylori pozitif peptik ülser ve gastritti hastalardaki gastrik mukozal glutathione (GSH) düzeyinde azalma lipid peroksidasyonu yoluyla memran hasarını başlatabilen serbest oksijen radikallerinin artmasının bir sonucu olabilir.*

Anahtar kelimeler: Peptik ülser, gastritis, malondialdehyde, glutathione, lipid peroksiasyonu

INTRODUCTION

Although Helicobacter pylori *(H. Pylori)* is etiologically linked to several major gastroduodenal diseases, the mechanism of its action has not been fully explained. However, it has been suggested that free radicals are closely related with peptic ulcer and gastritis (1).

Oxygen free radicals are detrimental to the integrity of biological tissues and mediate their injury. The mechanism of damage involves lipid peroxidation, which destroys cell membranes with the release of intracellular components, such as lysosomal enzymes, leading to further tissue damage. The radicals also promote mucosal damage by causing degradation of the epithelial basement membrane components, complete alteration of the cell metabolism and DNA damage (2). The generation of the superoxide anion as a mechanism of damage is well established in different models of acute and chronic injury, but it has not been clarified whether this radical is involved in gastric mucosal damage (3-6).

Address for correspondence: Dr. Mustafa YILMAZ
Pamukkale Üniversitesi Tıp. Hastanesi Doktorlar Cad No:42
20100, Denizli - Turkey
Tel: +90 258 2410034 - Fax: +90 258 2410040
E-mail kmustyil@yahoo.com

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The body has developed several endogenous antioxidant systems to deal with the production of reactive oxygen species. These systems can be divided into enzymatic and nonenzymatic groups. The enzymatic antioxidants include superoxide dismutase (SOD), which catalyzes the conversion of 02- to H2 Oa and Efo O, catalase, which then converts Ha Oa to Ha O and Oa and glutathione peroxidase, which reduces HaOa to HaO by reduced glutathione (GSH). Re-reduction of the oxidized form of glutathione (GSSG) is then catalyzed by glutathione reductase. These enzymes also require trace metal co-factors for maximum efficiency, including selenium for glutathione peroxidase, copper, zinc or manganese for SOD and iron for catalase.

Ha Oa + 2 GSH -^GSSG + 2 Ha O

GSSG + NADPH + H+ -»2 GSH + NADP+

The nonenzymatic antioxidants include the lipid-soluble vitamins, vitamin E and vitamin A or provitamin A (beta-carotene), and the water-soluble vitamin C and GSH. Glutathione, which is synthesised intracellularly from cysteine, glycine, and glutamate, is capable of scavenging reactive oxygen species either directly or enzymatically via glutathione peroxidase. In addition, GSH is crucial to the maintenance of enzymes and other cellular components in a reduced state. The majority of GSH is synthesised in the liver. Its biologic role is believed to be a defence against dietary xenobiotics and lipid peroxidation (7). Changes in antioxidative molecule levels may be an important factor in ulcer generation.

Many studies have attempted to clarify the role of oxygen-derived free radicals in peptic ulcer and gastritis. However, most of them were carried out in animal models (8-11).

In this study, measurement of gastric mucosal malondialdehyde (MDA) concentration, which is the end-product of lipid peroxidation, was used to assess oxidative damage to membranes in patients with peptic ulcer and gastritis. Mucosal GSH concentrations were also measured in order to assess whether reactive oxygen species generation affects levels of the antioxidant peptide.

MATERIALS AND METHODS

Patients were recruited from those undergoing upper gastrointestinal endoscopy for dyspepsia in the Department of Gastroenterology, Ege University and all patients gave their informed consent to be included in the study. Gastric biopsy specimens of 41 patients undergoing endoscopy for dyspepsia were obtained for analysis. One of the biopsy specimens was examined histologically using hematoxylin and eosin stains and diagnosed as chronic active gastritis. In addition, one antral biopsy specimen was used for urease testing (CLO test) for *H.pylori.* The specimens with normal histologic features and which were *H.pylori* negative in urease testing were selected as controls. The other biopsy specimen was used for mucosal MDA and GSH concentration measurement.

Malondialdehyde Measurement

Biopsy specimens were stored at -7O^{0}C and assayed by the procedure of Ohkawa (12). Briefly, each sample was weighed after thawing. They were homogenised in 0.15 M KCl solution. One ml of homogenate, 1.5 ml thiobarbituric acid, 1.5 ml acetic acid (pH 3.5) and 0.2 ml sodium dodecyl sulphate were mixed and a set of malondialdehyde standards was also freshly prepared. After mixing, all samples and standards were heated at 100°°C for one hour. The samples and standards were cooled on ice and MDA extracted by adding 5 ml 1-butanol to each. Each tube was centrifuged at 2000 rpm for 10 minutes to separate the aqueous and organic phases. The absorbance of 1-butanol phase was then recorded at 532 nm and compared with those obtained from malondialdehyde standards.

Reduced Glutathione Measurement

GSH concentrations were determined by the procedure of Ellman (13). Briefly, 0.5 ml homogenate, 1.5 ml 0.15 M KCl and 3 ml deproteinisation solution were mixed. Each sample was centrifuged at 3000 rpm for 10 minutes and supernatant was removed following which 2 ml phosphate solution and 0.5 ml DTNB was added to the 0.5ml supernatant, absorbance was read at 412 nm and compared with glutathione standards.

Statistical analysis

Data were expressed as mean ± SD, and statistical analyses were performed by Mann-Whitney U test.

RESULTS

There were 41 patients (29 male, 12 female) with a mean age of 47 years (range 32-60), of whom 15

had duodenal ulcer, 14 *H.pyloriassociated* chronic active gastritis and 12 normal histology. In histologic examination, it was found that all specimens from patients with peptic (duodenal) ulcer and gastritis were *H.pylori* pylori positive. Gastric mucosal MDA and GSH levels are shown in Figure 1 and Figure 2. Tissue levels of GSH were significantly lower [728±272 nmol/g w.t versus 2454±1255 nmol/g w.t (p<0,001)] and MDA levels higher [958±172 nmol/g w.t versus 314±164 nmol/g w.t (p<0,001)] in patients with peptic ulcer compared to controls. In patients with gastritis, GSH levels were also lower [672±142 nmol/g w.t (p<0.001)] and MDA levels higher [470±133 nmol/g w.t (p<0.01)]. The differences in MDA level between patients with peptic ulcer and gastritis were statistically significant (p<0.01).

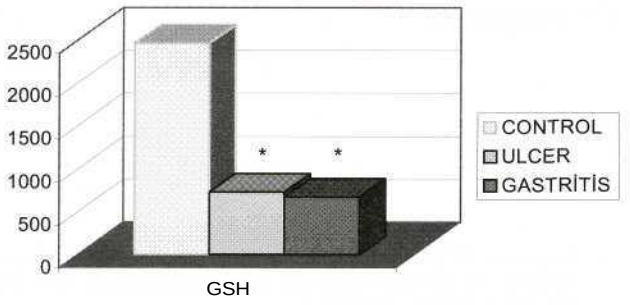

Figure 1. Gastric mucosal GSH levels in patients with ulcer and gastritis. (*P<0.001 ulcer and gastritis groups vs control, P> 0.05 gastritis vs ulcer)

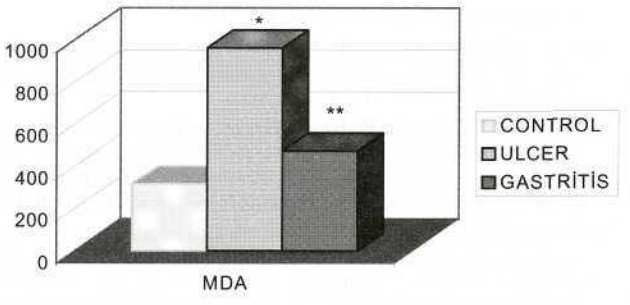

Figure 2. Gastric mucosal MDA levels in patients with ulcer and gastritis. (*P<0.001 ulcer vs control and gastritis, **P<0.01 gastritis vs control)

DISCUSSION

Peptic ulcer and gastritis have multietiopathogenetic factors. It is widely accepted that a major underlying factor of this disorder is the generation of free radicals. There is substantial evidence that oxygen derived free radicals play an important role in the pathogenesis of the injury of various tissues, including the digestive system (14,15). In addition, involvement of oxygen derived free radicals such as the superoxide anion, hydrogen peroxide, and hydroxyl radical are well established in the pathogenesis of ischaemic injury of gastrointestinal mucosa and in other models of mucosal damage induced by non-steroidal anti-inflammatory drugs (16), ethanol (9), feeding rectriction stress (8), *and H.pylori* (14,17). In this study, high gastric mucosal MDA levels in patients with peptic ulcer and gastritis were determined. Levels of MDA are thought to reflect free radical mediated cell membrane damage. It is known that radical scavengers, such as alfa tocopherol, carotenoids and glutathione redox system, play a significant role in protecting membranes from oxidative damage. Depletion of gastric mucosal GSH may result in the accumulation of free radicals that can initiate membrane damage by lipid peroxidation. The present authors found lower gastric mucosal GSH levels in patients with peptic ulcer and gastritis.

Salim et al. investigated the influence of free radical scavengers on the healing of gastric and duodenal ulcers resistant to therapy and found that antioxidative therapy stimulates the healing of therapy resistant ulcers (18). Santra et al. have shown that infection with *H.pylori* is associated with generation of reactive oxygen molecules, which leads to oxidative stress in the gastric mucosa (14). They found that gastric mucosal GSH levels were significantly lower and MDA levels higher in duodenal ulcer patients with or without *H.pylori* infection. Galaktinova et al. reported that total oxidative activity and plasma concentrations of lipid *H.pylori and* MDA were higher in the patients with *H.pylori* infection (19), while Ferrinati et al. found that gastric MDA levels were significantly increased by gastritis and that glutathione turnover was higher than normal in gastritis (5). Maity et al. suggested that glutathione plays a major role in the cytoprotection against ulceration (20). Our findings are concordant with these observations.

Potential sources of free radicals may be the activated inflammatory cells, the hypoxanthine - xan-

thine oxidase system, the disrupted mitochondrial electron transport system, the metabolism of arachidonate via the lipoxygenase pathway, and vascular endothelial cells (21). The lipoxygenase pathway and the activated inflammatory cells could be involved in the pathogenesis of mucosal damage. Gastric mucosal cells metabolise arachidonic acid via both the cyclo-oxygenase and lipoxygenase pathways and the presence of inflammatory cell infiltrates in the gastric mucosa (17). Inflammation could be activated by *H.pylori.* In *H.pylori* - infected gastric mucosa and duodenal gastric metaplasia, active inflamation , with infiltration of neutrophils in the acute stage of the infection and of macrofages/monocytes, lymphocytes and plasma cells in the chronic stage, is observed in the lamina propria of the stomach. These neutrophils and macrophage/monocytes produce a large quantity of oxygen derived free radicals that could cause cells damage and lead ultimately to mucosal injury (22,23). Previous studies have shown that *H.pylori* strains from duodenal ulcer patients have increased neutrophil activity compared with gastritis strains (24). In this study, higher gastric mucosal MDA levels were determined in the patients with peptic ulcer than in patients with gastritis and in the *H.pylori* negative control group. However, it was not possible to assess *H.pylori* strains and neutrophil density in the gastric mucosa. The GSH level was lower in patients with peptic ulcer than gastritis but no significant difference was found. The present authors consider that the presence of lower mucosal GSH levels and higher mucosal MDA levels may indicate increased neutrophil activitiy in our patients with peptic ulcer even though it was not possible to quantify neutrophil activitiy.

In conclusion, the findings of this study suggest that oxygen-derived free radicals play a pathological role in peptic ulcer and gastritis.

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