ORIGINAL ARTICLES

Role of Copper-Albumin Complex in Treatment of Gastric Ulcer in Rats

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ABSTRACT

The main goal of this submitted work was to investigate the effect of copper-albumin complex on serum and mucosal oxidative stress for gastric ulcer treatment. Forty seven 8-week-old rats were classified into five groups as follow: G1 (control, n=7), G2 (Experimental control, n=10) was left without treatment, G3 (Mucogel group, n=10), G4 (Mucogel-plus group, n=10) and G5 (Omeprazole group, n=10). Treatment has been started six hour after Induction of Ulcers and continued till the 6th day. Ulcer index were reduced more than 30% in G3 and about 90% in G4 more than that of G5 in comparison to G2. The immunostained fundic tissue showed a marked reduction of iNOS activity in G4 more than in G3 and G5. Nitric oxide (NO) was slightly reduced in serum of G3 but it was somewhat at the level of reference control by treatment in G4 and G5 which recorded the strongest reduction of mucosal NO level followed by G4 then G3 compared to G1. Moreover, serum and mucosal total peroxide and oxidative stress index (OSI) were highly elevated in G2 while G4 recorded the strongest reduction followed by G5 then G3 compared to G1. On the other hand, Serum and mucosal superoxide dismutase (SOD) activity showed a significant reduction in G2 and G3 from that of G1 but significantly increased in G4 followed by G5. We also noted that total antioxidant activity (TAO) levels in both Serum and mucosa was almost normalized in G4 followed by G5 then the serum TAO in G3 but mucosal TAO level was non-significantly different comparing G2 and G3. Thus, co treatment of copper-albumin complex seems to be useful for gastric ulcer treatment by decreasing oxidative stress.

Key words: gastric ulcer, copper-albumin complex, oxidative stress.

Introduction

Gastric ulcer is a deep defect in gastric wall penetrating the entire mucosal thickness and the muscularis mucosa (Tarnawski, A.S., 2005). It is one of the most common gastrointestinal tract diseases, and has affected humans for centuries. Helicobacter pylori (H. pylori) infection from the known factors causing gastric ulcers where it weaken the protective mucous coating of the stomach and duodenum, allowing acid to get through to the sensitive lining beneath. The acid and bacteria irritate the lining to cause a sore or gastric ulcer to develop in the area. Also in rare cases, stomach tumors or pancreatic tumors can cause ulcers. Although smoking doesn’t cause gastric ulcers, it increases chances of getting an ulcer and slows down the healing of existing ulcers. Coffee, spicy foods and emotional stress can increase secretion of stomach acid causing the pain of an existing ulcer to be worse (Hui, A.J., et al., 2004; Chi, C., 2009), using of drugs such as non-steroidal anti-inflammatory drugs (NSAIDs), known to be aggressive agents for gastric ulcer development (Karakaya, K., et al., 2009). Through inhibition of Cyclooxygenase (COX) is an enzyme which plays a role in the PG pathway during synthesis from arachidonic acid (Polat, B., et al., 2010). Also others (Suleyman, H., et al., 2009) demonstrated that antioxidant parameters reduced in gastric tissue with indomethacin induced damage. Several drugs are extensively used for management of gastric ulcer such as antacids, Hydrogen receptor (H₃R) antagonists and proton pump inhibitors (PPIs) (Barksdale, A.R. and R.W. Schwartz, 2000). Antacids are medications that neutralize hydrochloric acid in the lumen of the stomach where they reduce hydrogen ion concentration (gastric acidity) and increase intragastric pH (Richardson, C.T., 2004). H₂ receptor antagonists, recently, have become first-line therapy for acid-related peptic disease, leading to a marked improvement in the quality of life for a large number of patients with a dramatic reduction in the use of surgical intervention for ulcer treatment (Aihara, T., et al., 2003).

Proton pump inhibitors are extremely popular and prevalent medications. Owing to their proven efficacy and safety, they are frequently prescribed both in the hospital and on an outpatient basis. Proton pump inhibitors are used to treat acid-related diseases, including gastroesophageal reflux disease (GERD), Barrett’s esophagus,
peptic ulcer disease (PUD), Zollinger-Ellison syndrome, gastrinomas, and esophagitis gastritis (Robinson, M., 2005). While recently another studies concluded that some serious potential risks of long-term PPIs therapy include cancer, infection, nutritional deficiencies, fractures, pneumonia and acute interstitial nephritis (Williams, C. and K.E. McColl, 2006).

Copper complexes can biochemically affect the cells and impinge upon metabolic pathways especially highly effective copper dependent enzymes, such as superoxide dismutase (SOD). It is predicted also that copper containing vitamins or biochemically active organic compounds such as amino acids and peptides or synthetic organic compounds in complexes can effectively scavenge superoxide radical. Previous studies confirmed such activity for copper nicotinate and copper glycinate complex in rats as anti-inflammatory agents against gastric ulcer. Both of them very efficiently reduced the ulcer index and severity, although through different mechanisms of action at the Prostaglandin E2 and Thromboxane A2 levels (El-Saadani, M.A., et al., 1993).

This submitted work has been designed to evaluate a copper chelating complex consisting of egg albumin and copper as one of the copper peptides that can be used as anti-inflammatory agent and effective in gastric ulcer treatment that could be added to raise the efficacy of the currently used simple and cheap gastric anti-inflammatory drug mucogel to be approached or equalized to the beneficial omeprazole effect as a substitute especially in cases that omeprazole should be prevented or minimized.

Materials and Methods

A- Animals:

In this study 47 male Wister Albino rats weighted 120 to 150 gm. and aged approximately 8 weeks. Standard commercial pellets for feeding, water ad libitum and other animal health conditions during all time course of the experimentation were provided.

B- Chemicals:

1- Liometacin:

(Indomethacin): It is an ulcerogenic non-steroidal anti-inflammatory drug.

2- Mucogel:

It is an antacid composed of dried aluminum hydroxide gel, magnesium hydroxide and oxethazaine.

3- Risek:

(Omeprazole): It is a proton pump inhibitor for intravenous infusion.

4- Copper albumin complex:

Copper-albumin complex was obtained from Prof. Dr. Ahmed Yassein Nassar - professor of biochemistry, faculty of Medicine, Assiut University, Assiut, Egypt as patent cooperation treaty (PCT) in the international Bureau of World Intellectual Property Organization (WIPO), Geneva, Switzerland / World Organization (WO) 2008 / 028497.

5- Mucogel-plus (30%):

30 gm of copper albumin complex was mixed uniformly with mucogel to 100 mL.

C- Induction of Ulcers:

Ulceration in the experimental groups was induced with indomethacin (25 mg/kg body weight, oral intubation) (Dursun, H., et al., 2009). Rats were deprived of food but had free access to tap water 24 h before ulcer induction.

D- Experimental design:

I- Reference control group (G1):
Served as normal reference group and consisted of 7 rats. They received distilled water – the vehicle for the treatments used.

II- Experimental control group (G2):

Consisted of 10 rats were sacrificed after 7 days from indomethacin administration.

III- Mucogel group (G3):

Consisted of 10 rats and treated with mucogel (2 mL/rat once daily, oral intubation) after ulcer induction and continued till the 6th day.

IV- Mucogel-plus group (G4):

Consisted of 10 rats and treated with mucogel-plus (1 mL/rat once daily, oral intubation) after ulcer induction and continued till the 6th day.

V- Omeprazole group (G5):

Consisted of 10 rats and treated with omeprazole (20 mg/rat once daily, oral intubation) after ulcer induction and continued till the 6th day (Hussain, F., et al., 2008).

E- Sampling:

Serum:

At time of sacrifice at the 7th day, blood sample from each rat was taken, left to clot at room temperature for 15 minutes and centrifuged at 3000 round per minute (rpm) for 10 minutes. The obtained serum was aliquotted and kept at -40 °C till time of analysis.

Stomach mucosal homogenate:

After scoring the ulcer index (see below), the glandular mucosa was scraped with scalpel and the tissue was homogenized in phosphate buffer, pH 7.4 (1:5 w/v) then centrifuged at 3000 rpm for 15 minutes to get rid of intact and course tissues. The supernatant was collected aliquotted and kept at -40 °C till time of analysis.

F- Ulcer scoring:

The stomach was opened along its greater curvature and expanded flat on its serosal surface on aluminum foiled frozen ice block. The mucosal surface was washed with cold saline solution (0.9 gm/dL NaCl). According to the technique of (Peskar, B.M., et al., 1986), the mucosal ulcerations restricted to the glandular area were rapidly scored. The number of ulcerated bands of more than 4 mm length was multiplied by a severity factor, 3; lesions of 2-4 mm length were multiplied by the severity factor, 2 while, lesions of less than 2 mm length were multiplied by a severity factor, 1. The ulcer index was calculated as the total number of lesions multiplied by the corresponding severity factor.

G- Histochemical examination:

For inducible nitric oxide synthase (iNOS): By using immunohistochemical staining for (iNOS) (Zhang, J., et al., 2008).

H- Biochemical measurements:

I- Statistical analysis:
  Statistical analyses for various variables were performed, using (SAS), 1987. The differences were considered significant at P≤0.05.

Table 1: The cumulative changes in mucosa of the studied groups of indomethacin-induced experimental gastric ulceration in rats.

<table>
<thead>
<tr>
<th>Item</th>
<th>Ulcer Index</th>
<th>Mucosal NO</th>
<th>Mucosal peroxide</th>
<th>Mucosal SOD</th>
<th>Mucosal TAO</th>
<th>Mucosal OSI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference control (G1)</td>
<td>0.00±0.00b</td>
<td>1.34±0.08b</td>
<td>754.20±26.51b</td>
<td>1.24±0.03ab</td>
<td>0.044±0.002a</td>
<td>81.32±3.473c</td>
</tr>
<tr>
<td>Experimental control (G2)</td>
<td>16.52±2.82a</td>
<td>1.73±0.09a</td>
<td>859.80±22.94a</td>
<td>1.17±0.03b</td>
<td>0.028±0.001c</td>
<td>156.7±5.675a</td>
</tr>
<tr>
<td>Mucogel (G3)</td>
<td>5.15±0.94b</td>
<td>1.52±0.09b</td>
<td>829.00±13.47b</td>
<td>1.20±0.02b</td>
<td>0.027±0.001c</td>
<td>149.3±5.144a</td>
</tr>
<tr>
<td>Mucogel-plus (G4)</td>
<td>1.48±0.31b</td>
<td>1.39±0.08b</td>
<td>819.60±14.41b</td>
<td>1.29±0.02a</td>
<td>0.034±0.002b</td>
<td>117.9±5.855b</td>
</tr>
<tr>
<td>Omeprazole (G5)</td>
<td>2.89±0.70b</td>
<td>1.29±0.09b</td>
<td>827.70±11.93b</td>
<td>1.23±0.02ab</td>
<td>0.035±0.001b</td>
<td>125.0±6.025b</td>
</tr>
</tbody>
</table>

Means within the same column carrying different letters are significantly different (P<0.05)

Table 2: The cumulative changes in serum of the studied groups of indomethacin-induced experimental gastric ulceration in rats.

<table>
<thead>
<tr>
<th>Item</th>
<th>Serum NO</th>
<th>Serum peroxide</th>
<th>Serum SOD</th>
<th>Serum TAO</th>
<th>Serum OSI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference control (G1)</td>
<td>31.72±1.515b</td>
<td>7.025±0.782b</td>
<td>8.949±0.267a</td>
<td>0.637±0.034a</td>
<td>11.52±1.913b</td>
</tr>
<tr>
<td>Experimental control (G2)</td>
<td>46.94±2.682a</td>
<td>10.51±0.659b</td>
<td>7.517±0.134b</td>
<td>0.451±0.028b</td>
<td>25.33±2.034a</td>
</tr>
<tr>
<td>Mucogel (G3)</td>
<td>40.66±2.704ab</td>
<td>9.137±0.357b</td>
<td>7.382±0.158b</td>
<td>0.557±0.024ab</td>
<td>17.67±1.187b</td>
</tr>
<tr>
<td>Mucogel-plus (G4)</td>
<td>29.27±1.676b</td>
<td>8.308±0.326b</td>
<td>8.544±0.128a</td>
<td>0.703±0.009b</td>
<td>11.84±0.463b</td>
</tr>
<tr>
<td>Omeprazole (G5)</td>
<td>28.49±2.022b</td>
<td>8.858±0.315b</td>
<td>8.297±0.102a</td>
<td>0.679±0.013b</td>
<td>13.09±0.5b</td>
</tr>
</tbody>
</table>

Means within the same column carrying different letters are significantly different (P<0.05)

Fig. 1: A part of the fresh stomach of G2 shows multiple ulcer patches (arrows) in the fundic mucosa.

Fig. 2: A part of the fresh stomach of G1 shows healthy fundic mucosa.
Fig. 3: A photomicrograph in the stomach fundus of the G1 immunostained with iNOS shows positive reaction (arrows) in few scattered cells especially in the basal part of the gland (x400).

Fig. 4: A photomicrograph in the stomach fundus of the G2 immunostained with iNOS shows marked increase in the positive reaction that observed in all cells of the fundic gland (x400).

Fig. 5: A photomicrograph in the stomach fundus of the G3 immunostained with iNOS shows a relative reduction in the reaction (arrows) in comparison to G2 (x400).

Fig. 6: A photomicrograph in the stomach fundus of the G4 immunostained with iNOS shows a marked reduction in the reaction (arrows) in few scattered cells especially in comparison to G2 (x400).
Fig. 5: A photomicrograph in the stomach fundus of the G5 immunostained with iNOS shows a relative reduction in the reaction (arrows) in comparison to G2 (x400).

Results and Discussion

Ulceration and ulcer index:

As it is shown in Table (1) and Fig. (1, 2) Experimental control rats (G2) showed extensive glandular gastric ulceration with high ulcer index. In comparison, treatment of mucogel reduced more than thirty percent of that of the untreated group (G2) such a result indicates that mucogel is a good effective treatment. Reducing the ulcer index was most significantly in G4 where the topical nature mucogel-plus reduced this parameter by about ninety percent which denotes that the additive copper-albumin complex elevated the protective effect of mucogel than that of omeprazole in (G5).

Since the antacid mucogel treatment that was proved where neutralizing effect of aluminum hydroxide against the aggressive action of the gastric HCl (Richardson, C.T., 2004), and the omeprazole significantly decreased the macroscopic and histologic damage induced by indomethacin in the rats small intestine (Pozzoli, C., et al., 2007). Mucogel-plus could be accepted that the topical route of treatment which is a highly effective antacid and gave the most biological curative response.

Histochemical changes:

Induction of gastric ulcer by indomethacin increased the iNOS activity several times more than the normal healthy control, the result which is inconsistency with others. Oxidative stress indicated by high mucosal immunostaining for iNOS, catalase and SOD associating H. pylori-infected asymptomatic human gastric biopsies decreases after H. pylori eradication (Felley, C.P., et al., 2002). Other authors (Piotrowski, J., et al., 1999) found that increased mucosal iNOS was highly detected as a common features of the damage induced by deleterious agents such as NSAIDS. Chow et al., 1998 and others found that inhibition of iNOS reduces the level of gastric damage in many of experimental models of injury. Whatever the origin of this iNOS, it is clear that gastric ulcer is firmly associated by increase of iNOS as well as nitric oxide content in the injured inflamed gastric fundus.

Basically, increment of scattered spots of iNOS in the injured tissue which is relative to the level of injury in gastric fundic tissue, the treatment with mucogel-plus (co-treatment of copper-albumin complex) resulted in the best sign of healing up where distribution of iNOS spots more or less was similar to the healthy control tissue. Accordingly, such a histochemical parameter further confirms the prediction of utilization of copper-albumin complex as a co-treatment with mucogel as a mucogel-plus could be highly beneficial treatment for gastric ulcer.

Biochemical changes:

1- Nitric oxide:

Nitric oxide is a mediator of gastrointestinal mucosal defense but paradoxically, it also contributes to mucosal damage. The synthesis of nitric oxide is mediated by the enzyme NOS, which is present in gastric mucosa in two constitutive (cNOS) isoforms, namely endothelial NOS and neuronal NOS [26]. The inducible (iNOS) enzyme is found in macrophages and neutrophils (Wallace, J.L. and M.J. Miller, 2000).

In the present study, serum and mucosal level of nitric oxide showed highly significant increase by induction of gastric ulcer during the time course of the experiment, the serum level that was slightly reduced in
G3 but it was somewhat at the level of reference control (G1) by treatment in G4 and G5 but the G5 recorded the strongest treatment in reduction of mucosal NO level followed by G4 then G3 compared to G1. Anyhow it is clear that indomethacin effect increased the level of nitric oxide biosynthesis as an inflammatory respond (Morsy, M.A. and A.A. Fouad, 2008). The effect that was compensated by treatment especially by mucogel-plus and omeprazole. Also other authors (Lopez-Belmonte, J., 1993) reported that Nitric oxide has dual role with respect to mucosal integrity could be achieved by the use nitric oxide donors to be protective when used in low doses and can cause direct damage and worsening exogenously induced injury at large doses.

In the digestive system, nitric oxide produced by eNOS is cytoprotective and nitric oxide produced by iNOS is cytotoxic (Motawi, T.K., et al., 2007). This protective effect of nitric oxide is because of the inhibition of activation, adhesion and migration of leucocytes in the inflammatory area (Banick, P.D., et al., 1997), While Nitric oxide produced from activation of iNOS reacts directly with superoxide to form peroxynitrite, a potent cytotoxic oxidant that causes gastric damage (Lanas, A., 2008). Simultaneously, indomethacin causes up-regulation of endothelin-1 that leads to decreased production of gastric mucosal eNOS leading to suppression in the cytoprotective nitric oxide level in gastric mucosa which further adds to the neutrophil infiltration (Slomiany, B.L. and A. Slomiany, 2000).

2- Total peroxide:

Measurement of lipid peroxidation levels is used to determine oxidative damage (Peralta, C., et al., 2001) where it is an important reason for cell membrane damage as well as peroxides are the main prominent production of the active oxygen free radical in the organic systems (Nielsen, F., et al., 19997).

The present data shows that (G2) was associated with significant elevation in the cumulative serum and mucosal total peroxide. Such an elevation was strongly reduced in (G4) followed by (G5) then (G3) compared to (G1) during the time course of the experiment. This could be attributed to the potent antioxidant activity of omeprazole and mucogel-plus that contains copper-albumin complex in reduction of peroxidation as well as gastric tissue damage caused by indomethacin. These results were correlated with that of others authors who reported that even one single dose of indomethacin (25mg/kg) in experimental animals results in a significant increase in toxic oxygen radicals that produce largely oxidative stress exposed tissues which stimulates lipid peroxidation, the statements that interpret the herein results (Polat, B., et al., 2010) Furthermore, many authors (Pozzoli, C., et al., 2007; Kianbakht, S. and K. Mozaffari, 2009) confirmed that omeprazole (30 mg/kg, p.o.) has prevented the gastric lesions and increasing of lipid peroxidation induced by indomethacin. Accordingly, it could be considered that mucogel-plus results in the same effect of omeprazole a result coincided by others who found that Various copper (II) complexes protected gastric mucosa against damage in different animal models including gastric ulceration induced by a necrotic agent such as 0.6 N HCl, indomethacin and intragastric distension (Frechilla, D., et al., 1991).

3- Superoxide dismutase:

The biological antioxidant guard serum and mucosal SOD level showed a significant reduction in (G2) and (G3) from that of (G1) but significantly increased in (G4) followed by (G5). Such a discrepancy in mucogel treated alone group may be interpreted due to conversion of aluminum hydroxide into aluminum chloride by gastric HCl (Richardson, C.T., 2004) the compound that is found to exhibit a reducing effect on SOD (Rui, D. et al., 2007) and the amide form of niacin or vitamin B3, nicotinamide (Chlopicki, S., et al., 2007).

Our results are in accordance with results of other authors who stated that indomethacin reduced the SOD activity in gastric tissues. The theory that SOD is a protective factor in indomethacin-induced damage (Bishnoi, M., et al., 2007; Motawi, et al., 2007).

4- Total antioxidants (TAO):

Most species are produced at low levels during normal physiological conditions and are scavenged by endogenous antioxidant systems that include superoxide dismutase (SOD), glutathione peroxidase, catalase, and small molecule substances such as vitamins C and E. Other closely linked pathways to oxidative stress may be tempered by different vitamins, such as vitamin D3 (Regulska, et al., 2007) and the amide form of niacin or vitamin B3, nicotinamide (Chlopicki, S., et al., 2007).

In the present study, the systemic blood and mucosal total antioxidants showed a significant reduction in induced gastric ulcer (G2) compared to (G1). In comparison, serum and mucosal TAO was almost normalized in treatment groups with highest level noticed for (G4) followed by (G5) then the serum TAO in (G3) but mucosal
TAO level was non-significantly different comparing the (G2) and (G3). These results are in agreement with previous findings (El-Missiry, et al., 2001) who found that copper complexes pretreatment was gastroprotective by preventing decline in activity of antioxidant enzymes (SOD, catalase, and glutathione-S-transferase), glutathione content and lipid peroxidation. Moreover, it was previously reported that copper nicotinate preventing the decline in the activity of antioxidants whereby fighting each of peroxide elevation and subsequently lipid peroxidation, affecting enzymes activity both as a cofactor and as a prosthetic component of several cuproenzymes controlling oxidation-reduction reactions including particularly copper-zinc SOD enzyme (Jaber, M.F., et al., 2009).

Similarly, a previous studies (Fornai, M., et al., 2005) reported that the curative signs as well as biochemical parameters especially those of total antioxidants were found to be significantly increased in treated animals with different routes of treatment as well as omeprazole derivatives. Originally, some theorized that this was the result of the acidic properties of NSAIDs and that the damage was topical in nature, but much work has been done to elucidate the mechanism of the formation of ulcers related to NSAID use (Gustafson, J. and D. Welling, 2009). This could be attributed to the systemic prostaglandin inhibition caused by NSAID use decreases epithelial mucus, mucosal blood flow, epithelial proliferation, bicarbonate secretion, and mucosal resistance to injury (Schoen, R.T. and R.J. Vender, 1989).

5- Oxidative stress index (OSI):

Oxidative stress is a major cause of gastric ulceration and subsequent lipid peroxidation is mainly due to glutathione and alpha-tocopherol depletion (Dotan, Y., et al., 2004) so it is considered a good biochemical index that determines the ratio between the two antagonistic actions; total peroxide over the antioxidant as a good parameter that denotes the biochemical collapse in injured tissue. In the present study, Tables (1, 2) represent the serum and mucosal OSI in which was highly elevated by induction of the gastric ulcer (G2) although (G4) recorded the strongest treatment in reduction of OSI level followed by (G5) then (G3) compared to (G1). These results are in agreement with previous findings (Ohta, Y., et al., 2004; Ohta, Y., et al., 2006) showing that treatment with the anti-ulcer drug teprenone or vitamin E or SOD + Catalase prevented compound 48/80-induced rat acute gastric mucosal lesion by suppressing oxidative stress in the gastric mucosal tissue. Oxidative stress leads to the destruction of multiple cell types through apoptotic pathways (De Felice, et al., 2007; Verdaguer, et al., 2007) and the oxidative stress indicated by significant decreased gastric mucosal protein and non-protein sulfhydryls and increased molondialdehyde (MDA) was detected 3 hr after stress-induced gastric lesion (Huang, J., et al., 1998). In addition, mucus performed an important structural role in creating an unstirred layer that retains bicarbonate secreted by surface epithelial cells on the mucosal surface which supports maintenance of a near-neutral pH at that surface as well as acting as a physical barrier against luminal pepsin and thus proteolytic digestion of the surface epithelium (Allen, A. and G. FlemstrÖm, 2005).

Recommendation:

From the previous conclusion, it could be recommended for the centers of advancement of drug researches to deal with such a complex to prepare mucogel-plus or similar drug as an antigastric ulcer therapy for its low price, safety and simplicity of synthesis and preparation for biological use as early as possible since the structure is registered as an international PCT for Egyptian authors that could be easily designed to get an Egyptian therapeutic drug for a very common popular disease.

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