Effects of omeprazole on healing of non-steroidal anti-inflammatory drug (NSAID)-induced peptic ulceration in rats and protective role of indole-3-carbinol

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Abstract:
Gastric hyperacidity and gastroduodenal ulcer is a very common global problem today. It is now generally agreed that gastric lesions develop when the delicate balance between some gastroprotective and aggressive factors are lost. The objective of present study is to evaluate the effects of omeprazole on healing of non-steroidal anti-inflammatory drug (aspirin) induced peptic ulceration in adult male albino rats and gastroprotective role of indole-3-carbinol. Male albino rats were randomly divided into two main groups; control group administrated distilled water and ulcerated group administrated aspirin at a dose of 500 mg/kg/body weight for seven consecutive days. Aspirin administration was stopped after 7 days representing the initial duration for the experiment and was followed by the beginning of different experimental regimens for a total experimental duration of 26 days. Results of the present study showed that, groups treated with omeprazole alone accelerated ulcer healing by inhibiting gastric acid secretion but indole-3-carbinol (I3C) possessed protective activity possibly as evidenced by the reduction of histopathological alteration of stomach and duodenum tissues and inhibition of gastric acid secretion. This study provides a strong evidence of indole-3-carbinol which showed significant gastroprotective and antioxidant activities against aspirin induced peptic ulceration in male albino rats.

Key words: Omeprazole, Histopathological, Aspirin, Peptic ulceration, Indole-3-carbinol, Rats.
Introduction:

The basic physiopathological of peptic ulceration results from an imbalance between some endogenous aggressive factors (hydrochloric acid, pepsin, refluxed bile, leukotrienes, reactive oxygen species (ROS) and cytoprotective factors, which include the function of the mucus-bicarbonate barrier, surface active phospholipids, prostaglandins (PGs), mucosal blood flow, cell renewal and migration, non-enzymatic and enzymatic antioxidants, and some growth factors (Konturek et al., 2005 and Mota et al., 2008). Peptic ulcer disease (PUD) is one of the oldest diseases known to human kind. The term PUD generally refers to spectrum of disorders that includes gastric ulcer, pyloric channel ulcer, duodenal ulcer and postoperative ulcers at or near the site of surgical anastomosis (Zimaity, 2007). Gastric ulcer associated with the use of aspirin is a major problem. Many factors such as gastric acid and pepsin secretion, gastric microcirculation, prostaglandin E2 (PGE2) content and proinflammatory cytokines interleukin (IL)-1 and tumor necrosis factor α (TNF α) play important roles in the genesis of gastric mucosal damage, and its subsequent development (Wang et al., 2007 and Wallace, 2008). It has been reported that increases in NO synthase (NOS) activity is involved in the gastrointestinal mucosal defense and also in the pathogenesis of mucosal damage (Wallace et al., 2000).

Non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin are widely used as anti-inflammatory, analgesic drugs and in the prevention of cardiovascular events (Weisman and Graham, 2002). However, the major limitations of their clinical application are serious gastrointestinal side effects, especially peptic ulcerations and gastrointestinal bleeding (Lanas, 2006). Some NSAIDs, particularly those of acidic nature, can directly kill epithelial cells. NSAIDs can also reduce mucus and bicarbonate secretion, thereby decreasing the effectiveness of the juxtamucosal pH gradient in protecting the epithelium (Wallace, 2008). Aspirin (ASA) often used as an analgesic to relieve minor aches and pains, as an antipyretic to reduce fever and as an anti-inflammatory medication. Aspirin also has an antiplatelet or anti-clotting effect and is used in long-term at low doses to prevent heart attacks, strokes and blood clot formation in people at high risk for developing blood clots (Julian et al., 1996). Despite the cardiovascular benefits of ASA, a potential gastrointestinal harm has been noted in several clinical and preclinical studies. The main undesirable side effects of ASA are gastrointestinal ulcers, stomach bleeding, and tinnitus, especially in higher doses. The induction of ASA is characterized by infiltration of neutrophils, growth factor inhibition and elevation of cytokines, which is produced by activated macrophages (Konturek et al., 2004 and Sanchez-Fidalgo et al., 2004).

Drug treatment of peptic ulcers is targeted at either counteracting aggressive factors (acid, pepsin, active oxidants, platelet aggravating factor, leukotrienes, endothelins, bile or exogenous factors including NSAIDs) or stimulating the mucosal defenses (mucus, bicarbonate, normal blood flow, prostaglandins, nitric oxide (AL-Yahya et al., 1990). The goals of treating peptic ulcer disease are to relieve pain, heal the ulcer and prevent ulcer recurrence. Omeprazole (OMP) is an effective agent in the treatment of peptic ulcer disease. The effects of OMP against gastric mucosal injuries have been thought to depend on its inhibitory action on gastric acid secretion (Kobayashi et al., 2002). However, clinical evaluation of this drug have shown relapse in the long run (Szabo and Vincze, 2000), side effects and drug interactions (Abdul-
Aziz, 2011). This has been rational for the development of innovative drug that reduces the offensive factors and is proved to be safe, clinically effective, having better patient tolerance, relatively less expensive and globally competitive. Plant extracts, however, are some of the most attractive sources of new drugs and have been shown to produce promising results in the treatment of ulcers (Sunilson et al., 2008). A number of natural products found in fruits and vegetables are known to possess anti-mutagenic and anti-carcinogenic properties (Boone et al., 1990 and Ahmad et al., 2011). Cruciferous vegetables are a rich source of many phytochemicals, including indole derivatives, dithiolthiones, and isothiocyanates. Indole-3-carbinol (I3C) is an indole found in some fruits and vegetables, including members of the cruciferous family and, particularly, in members of the genus Brassica including broccoli, brussels sprouts, cabbage, cauliflower, collard greens, kale, kohlrabi, mustard greens, radish, rutabaga and turnip. These vegetables are rich in glucosinolate, one of the anti-cancer agents in cruciferous vegetables (Holst and Williamson, 2004).

**Aim of study:**

The aim of the present work is to study the effects of I3C as a new safer cytoprotective and antioxidant activities compound found in cruciferous vegetables, which protects the peptic ulceration. In addition, to investigate the effects of treatments of OMP and I3C either alone or combined with each others on the acute phase of inflammation, models induced by aspirin as one of the NSAIDs.

**Material & Methods:**

**Drugs:**

1- Indole-3-carbinol (I3C) was purchased from Sigma-Aldrich Chemical Company U.S.A. (Cairo, Egypt).
2- Aspirin (ASA) tablets (Bayer AG, Germany) were obtained from pharmacy.
3- Omeprazole (OMP) tablets (European Egyptian Pharmaceutical Industries, Cairo Egypt) were obtained from pharmacy

**Experimental animals:**

Adult male albino rats weighing about 150-200 g were used throughout the experiment. The animals were housed in polypropylene cages with sterile, inert husk materials as bedding. The experimental animals were maintained under controlled environment conditions of light and dark cycle (12h light/12 h dark, temperature 23±2 °C). They were allowed to acclimatize for 10 days and were provided a free access to standard pellet diet and water ad libitum.

**Experimental groups:**

Adult male albino rats were randomly divided into two main groups: Control groups administrated distilled water and ulcerated groups administrated aspirin (ASA) at a dose of 500 mg/kg/body weight for seven consecutive days. ASA administration was stopped after 7 days representing the initial duration for the experiment and was followed by the beginning of different experimental regimens for a total experimental duration of 26 days. Control group was further divided into four subgroups of six animals each which are normal control group (NC), indole3-carbinole (I3C) group
receiving a dose of 50 mg I3C /kg/body weight, omeprazole (OMP) group receiving a dose of 20 mg OMP /kg body weight and OMP+I3C group receiving both treatments of OMP and I3C. Ulcerated group was further divided into another four subgroups of six animals in each group which are ulcer group (U), U+ I3C group, U+ OMP group and U+I3C+OMP group. All chemicals were given to rats by stomach tube. Dissection to all rats was done after an experimental period of 26 days.

Measurement of total gastric acid (total acidity) and pH value:

After dissection, rats’ stomachs were ligated from its two ends; the pylorus and the lower esophagus, and injected with 2ml distilled water. A small incision was made for each fore stomach, and the stomach contents were expelled (Hsu et al., 2009). Gastric contents were collected in tubes then measured. Gastric juice was centrifuged at 3500 rpm for 15 min and the supernatant was used for determining total acidity. One ml of the supernatant liquid was pipetted out and diluted to 10 ml with distilled water. The solution was titrated against 0.01N NaOH using phenolphthalein reagent as indicator, to the endpoint when the solution turned to pink color. The volume of NaOH required was denoted to calculate the total acidity. The total acidity and pH of the supernatant were calculated according to Vinothapooshan and Sundar, (2010) and Sadler and Murphy, (2010) respectively.

Histopathological examination:

Pieces of the stomach and duodenum were immersed in 10% formalin solution, embedded in paraffin. The sections were made at a thickness of 5μm and stained with hematoxilin and eosin (Lillie, 1954). Histopathological examination was performed under light microscopy.

Statistical analysis:

Results were expressed as the mean ± standard error (SE). Statistical analysis was performed using the statistical package for social science (SPSS) version 17.0 statistical analysis package. Parameters were analyzed using significance by one way analysis of variance (ANOVA) followed by Dennett’s T3 multiple comparison test significance levels were analyzed at p < 0.001, p < 0.05.

Results:

Evaluation of total gastric acid (total acidity) and pH value of the gastric juice:-

Data recorded for total gastric acid (total acidity) and pH value of the gastric juice were presented in (fig. 1&2). Normal control animals signed more or less constant varying levels of total acidity and pH value of the gastric juice throughout the experimental period. Data from figures indicated significant increase (p <0.001 and p<0.05) in total acidity and decrease in pH value in ASA ulcerated rats (U) when compared to the normal control group. The acidity began to decrease by the concurrent treatment of I3C or OMP either alone or together for 26 days compared to the U group. In addition, pH values increased significantly in all ASA treated groups. The most significant (p <0.001) increase was recorded in the U+I3C+OMP compared to ASA group.
Figure (1): The mean values of total gastric acid (total acidity) in control and experimental groups.

Figure (2): The mean values of pH level in control and experimental groups.

Histopathological results:
All animals administered of I3C, OMP and I3C+OMP at 26 days there were no evident microscopical changes in stomach [fig.3(e.g &i)] throughout the experimental time as compared to normal control group [fig. 3(a&b)] at the same period. Histopathology of ulcerated stomach of male albino rats receiving distilled water for 26 days after ulcer induction, stomach revealed many histological alterations. This was established in the form of many eroded areas in the gastric tissue. The surface epithelium manifested exfoliation and sloughing of the surface mucosal cells, which were aggregated in the lumen of the stomach forming debris of the damaged tissue. The connective tissues localized between gastric pit and muscularis mucosa was damaged with marked aggregation of inflammatory cells mainly eosinophils, plasma cells and lymphocytes (fig3. c). Moreover, the blood vessels localized in these regions were dilated, these signs of haemorrhages were observed as the result of extravasted blood vessels. Also, the picture of chronic superficial gastritis appeared in many areas of the mucosa where the glands were tortuous (fig3. d). Microscopically, stomach sections from ulcer rats group treated with indole-3-carbinol manifested minimal histological lesions in the gastric mucosal cells through the 26 days. This was apparent in the limit areas in the form of the divergence of parietal oxyntic cells at the gastric gland where chief peptic cells were paucity (fig3. f). Animals in U+OMP group were orally given omeprazole for 26 days after ulcer induction. The mucosal gastric tissue of this group was fairly protected even though; few areas revealed loss of more glandular cells, some parietal and chief cells appeared degenerated, vacuolated and detached from their basement membrane with pyknotic and the glands were markedly destroyed with loss of their normal architecture (fig3. h). Following 26 days, treatment with I3C and OMP, few pathological consequences in the gastric mucosal cells was revealed. The surface mucous cells have designated noticeable damage and erosion. The mucous neck cells displayed prominent vacuolation of their cytoplasm with marked increase of mitotic figures in some areas (fig3. k).
In comparison with control (Fig. 4a), histopathological examination of duodenal mucosal tissue of ulcerated rats administrating aspirin for 7 days and left for 26 days and OMP-treated ulcerated rats showed, inflammatory cells infiltration in the lamina
propria of the mucosa (Fig. 4b&f). On the other hand, the sections of duodenum from animals treated with I3C or I3C+OMP manifested more or less similar features as in NC group during the experimental duration i.e. 26 days (Fig. 4c, e & g). Furthermore, no evident histopathological changes were seen in duodenum of ulcerated rats treated with I3C or I3C+OMP as compared to control group (Fig. 4d & h).
Discussion:

Free radicals are produced basically during cellular metabolism and some functional activities and have essential roles in cell signaling, apoptosis and gene expression. On the other hand, excessive free radical attack can damage DNA, proteins and lipids, resulting in very important diseases. Antioxidants can decrease the oxidative damage by reacting with free radicals or by inhibiting their activity (Tan et al., 1993). Moreover, Antioxidants could help to protect cells from damage caused by oxidative stress and enhanced the body’s defense systems against degenerative diseases. Administration of antioxidants inhibits ASA-induced tissue injury in rat (Sathish et al., 2011). In addition, Potrich et al. (2010) proposed that reactive oxygen species (ROS) are involved in the development of gastric ulcers induced by non-steroidal anti-inflammatory drugs.

Peptic ulcers are open areas in mucous lining of the stomach and duodenum. NSAIDs (from which is aspirin) stimulate HCl secretion and cause weakness of mucous gel layer which acts as barrier by decreasing mucin production and increasing the secretion of bicarbonate from gastric and duodenal mucosa. Aspirin causes mucosal damage by interfering with prostaglandin synthesis, increasing acid secretion and blocking the diffusion of H+ ions (Mabrouk et al., 2009). So, the increase in total acidic activity and decrease in pH value of the ulcer group is undoubtedly due to the increased production of HCl as is evident from the total acidity of the gastric juice. Also, it is well documented that ASA administration causes inhibition of prostaglandins (Potrich et al., 2010). Prostaglandins are important cytoprotective agents in the gastrointestinal tract because they increase mucosal blood flow. Inhibition of prostaglandin synthesis by aspirin causes damage to the cell membrane of mucosal, parietal and endothelial cells (Agnelarul et al., 2010). Besides, ASA causes decreased pH value resulting in mucosal injury due to its direct irritant effect and by permitting back diffusion of hydrogen ion through the mucosa (Bharti et al., 2010 and El-Shinnawy et al., 2012). Moreover, aspirin induces gastric damage followed by a multistage pathogenetic event in which ROS, vascular permeability, luminal contents, neutrophils, gastric motility and microcirculation all play a role in the development of inflammation and ulcers (Kato et al., 2002). Gastric mucosal lipid peroxidation, mediated by oxygen free radicals, is an important cause of destruction and damage to mucosal cells and gastric mucosal integrity (Jainu and Devi 2005). Also, Bharti et al., (2010) confirmed that the
exposure of gastric mucosa to ASA has been shown to affect cellular integrity and such changes are associated with oxidative stress and mitochondrial damage.

In ulcer group the gastric mucosal lesions persisted even after 26 days of stopping aspirin administration. Ulcers are defined histologically as a breach in the mucosa of the alimentary tract that extends through the muscularis mucosa into the submucosa or deeper and showed, inflammatory cells (eosinophils, plasma cells and lymphocytes). Moreover, the blood vessels localized in these regions were dilated. Although they may occur anywhere in the alimentary tract, none are as prevalent as the peptic ulcers that occur in the stomach (Jaikumar et al., 2010). Ulcer formation as a result of ASA administration may involve several mechanisms which are reducing gastric blood flow, thereby contributing to the development of hemorrhage and solubilization of mucus constituents in stomach. These actions result in an increased pepsin secretion and flux of Na⁺ and K⁺, with a decrease in histamine and H⁺ ions into the lumen (Tour and Talele, 2011). In addition, ASA penetrates the gastric mucosa, promoting membrane damage, erosion and ulcer formation through destruction of the mucus barrier, increases in vascular permeability and decreases in non-proteic sulphydrilic groups (NP-SH) of the gastric mucosa (Repetto and Llesuy, 2002 and Siegmund et al., 2003).

Additionally, the histopathological examination of duodenal mucosal tissue of ulcerated group revealed inflammatory cells infiltration. These consequences may be related to the back-diffusion of acid into the mucosa, which directly leads to vascular leakage and aggressive damaging effect in the basement membrane of both epithelial and mucosal cells in the duodenal wall (Jainu et al., 2006). Furthermore, inflammation in gastric mucosa and duodenal mucosa by aspirin is accompanied by increased production of TNFα, which augments neutrophil-derived superoxide generation and stimulates production of IL-1 leading to neutrophil accumulations (Kokura et al., 2000). Cytotoxic effects of ASA that affect the mucosal integrity of gastrointestinal tract are manifested by the disturbances of NOS, a key mediator of signaling events linked to apoptotic cell death (Slomiany et al., 1998). The enhanced expression of NOS upon aspirin administration results in the formation of nitric oxide (NO) related species, which exert a direct inhibitory effect on nuclear factor-kappa B (NFκB), (a zinc-dependent transcription factor) which appears to play a key role in the process of ulcer healing, because its activation is up regulated in rat gastric ulcers and the blockade of NFκB activation results in impairment of ulcer repair (Mei et al., 2009 and El-Shinnawy et al., 2012). Inhibition of NFκB leads to up regulation of proinflammatory cytokine production, reactive oxygen species and enhanced rate of apoptosis (Slomiany and Slomiany, 2001).

Data from the present study clearly proves the amelioration of all the above tested biochemical parameters produced by concomitant treatments of I3C and OMP to ulcerated rats either alone or combined with each other which produced a significant decrease in total acidic activity with an increase in pH level. Nevertheless, combined treatments produced the best results.

Omeprazole, a proton pump inhibitor (PPI) offered a fair protection to the gastric mucosa while I3C produced a more invasive protective effect on the rat gastric and duodenal mucosa. The results of this study indicated that the effect of I3C is almost similar to that of OMP, this suggests a possible inter-relationship in their mechanism of
action. PPIs are capable of producing almost complete suppression of acid secretion. The mechanism of action of OMP is such that it binds very specifically to a single subunit of the H\(^+\), K\(^+\)-ATPase at the secretory surface of parietal cell causing its inactivation (Munson et al., 1995). It reduces acid secretion regardless of the source of secretory stimulation by increasing intragastric pH through inhibition of acid secretion. Besides, PPIs inhibit activation of pepsin (Schneeweiss et al., 2006). Even though most antulcer drugs require prolonged periods of intake, yet ulcer relapse is of common occurrence (Munson et al., 1995). Many have limited efficacy with various adverse effects (Lehne, 1998) and no drug proves solely effective in treating peptic ulcer (Ode et al., 2011). A rapid progress in the field of gastroenterology has led to the identification of several potential supportive drugs from plant-based phytomedicines (Akah et al., 1998). I3C is found in cruciferous vegetables. These vegetables lower the risk of cancer incidence, which may be due to their high contents of glucosinolates and their derivatives, including indole-3-carbinol (Wang et al., 2012). The protective effects of I3C against increased acidic activity and decreased pH value could be related to the increase in prostaglandins that normally protect the gastrointestinal mucosa from damage by maintaining blood flow and increasing mucosal secretion of mucous and bicarbonate (Voutilainen et al., 2001). These findings support the hypothesis that I3C attenuates aspirin-induced neutrophil accumulation by inhibiting production of proinflammatory cytokines. I3C decreased the mucosal NOS activity when compared to ulcerated rat, which suggest that gastroprotective effect of I3C may be due to the reduction of ROS and NO toxicity (Jainu et al., 2006 and El-Shinnawy et al., 2012). The anti-inflammatory activity of I3C was associated with its ability to inhibit the production of pro-inflammatory cytokines such as IL-1 or TNFα and inducible NO synthase. I3C preserves erythrocytes against oxidative stress and exhibit strong antioxidant activity in vitro and in vivo model. The ability of I3C to enhance antioxidant enzymes demonstrates its possible preventative value in the inhibition of ulcerogenesis involving free radical reactions. This may be possible by blocking oxidative damage through lipid peroxidation. In addition, I3C prevents loss of membrane permeability and dysfunction of cellular proteins, leading to survival of the functionally active cells (Chen et al., 2003 and Tsai et al., 2010). I3C could have a unique capacity to block this oxidative damage similar to that shown by H\(_2\)O\(_2\) scavenger, catalase, indicating its potent antioxidant role to protect DNA from the attack of ROS. Moreover, by the suppression of proinflammatory cytokine production and NOS activity that would greatly facilitate various healing mechanisms such as increased epithelial cell proliferation and decreased epithelial apoptosis in ASA ulcerated animals. Another possible protective mechanism of action of I3C as an antiinflammatory drug is by acting on first phase by inhibiting the mediator of inflammation, probably by inhibiting the platelets activating factor receptors present in the proinflammatory cells like mast cells and neutrophils (Tour and Talele, 2011). The results of histological investigation revealed that I3C absolutely inhibited aspirin-induced lesions of rat gastric and duodenal mucosa. I3C treatment was found to preserve the functional cytoarchitecture of the entire gastric and duodenal mucosa. These findings confirm the cytoprotective nature of I3C.
Conclusion:

In conclusion, it was found demonstrated that rats treated with indole-3-carbinol manifested no abnormal signs. I3C could significantly protect the peptic tissue against ASA induced gastrointestinal injury by the inhibition of histopathological alteration and showed significant decrease in secretion of gastric juice. This study provides evidence that, I3C possesses as an effective gastroprotective, anti-inflammatory and antioxidant activities against aspirin-induced peptic ulceration in animals.

References:


