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Cyclo-oxygenase-2 expression and prostaglandin E_2 production in experimental chronic gastric ulcer healing

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Abstract

Prostaglandin, a key molecule that stimulates the complex array of ulcer healing mechanism, gets synthesized in the mucosal cells by cyclooxygenase (COX) enzymes: COX-1 and COX-2. High expression level of COX-2 protein at healing ulcer margins highlights its role in ulcer healing and hypothesized to be an important contributing factor in healing mechanism of anti-ulcer drugs. In the present study we have compared the expression profile of COX-2 protein, prostaglandin E_2 (PGE_2) levels and myeloperoxidase activity in acetic acid induced chronic gastric ulcer model in rats treated with omeprazole, misoprostol and COX-2 selective nonsteroidal anti-inflammatory drug (NSAID) celecoxib. Both COX-2 expression and PGE2 level have shown differential pattern in different treated groups parallel to the differential effects of these drugs on ulcer healing. Omeprazole has significantly elevated the expression level of COX-2 protein, PGE_2 level (19.37%), and decreased myeloperoxidase activity (81.92%), thereby causing the most effective ulcer healing (89.74%). Similar trend was observed with misoprostol, but with relatively less pronounced ulcer healing and COX-2 expression. Celecoxib has retarded COX-2 expression and delayed ulcer healing. Therefore, induction of COX-2 expression leading to higher level of prostaglandin appears to be an important contributing factor in drug mediated ulcer healing apart from the respective mechanisms of different drugs.

Keywords: COX-2; Prostaglandin; Ulcer healing; Omeprazole; Misoprostol; Celecoxib

1. Introduction

Cyclooxygenase (COX) is an important enzyme family that comprises of enzymes responsible for the synthesis of prostaglandins from arachidonic acid that plays varied range of roles in various physiological and pathological conditions. COX exists in two isoforms: COX-1 and COX-2. Both enzyme catalyses the same reaction, share 60% homology within a given species and exhibit remarkable structural homology at the molecular level (FitzGerald and Loll, 2001). They are encoded by different genes located on different chromosomes and seem to subserve different functions even within the same cell type (Smith and Langenbach, 2001).

According to the classical hypothesis, constitutively expressed COX-1 produces prostanoids involved in physiological and housekeeping functions such as protection of gastrointestinal mucosa, regulation of renal haemodynamics and stimulation of platelet aggregation, whereas the other form COX-2 is inducible in most of the cells in response to tissue injury by pro-inflammatory or mitogenic agents (Halter et al., 2001) and has a leading role in inflammatory process. However, recent findings reveal that this classical hypothesis is oversimplistic and COX-2 plays more complex and wider biological role than mere involvement in inflammation and pain (Peskar, 2001). Important among these roles are ulcer healing process, renal development, regulation of homeostasis in cardiovascular system, ovulation and

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implantation (Schmassmann et al., 1998; Parente and Perretti, 2003). More recently, a new isoforms of COX enzyme-COX-3 has been reported (Chandrasekharan et al., 2002), which is a variant of COX-1 enzyme (COX-1b) but completely lacks normal COX activities that leads to eicosoinds production (Botting and Ayoub, 2005).

Role of COX-2 in ulcer healing remained debated over the years and was considered from being useless to mandatory in different reports. Ulcer healing being an active and complicated process of reconstruction of mucosal architecture involves filling of the mucosal defect with proliferating and migrating epithelial cells and connective components (Perini et al., 2003), whereas prostaglandins play a key role in triggering the cell proliferation and promoting angiogenesis along with several other functions required to restore the mucosal integrity. Therefore, COX enzymes, both COX-1 and COX-2, being the architect of prostaglandin synthesis become an obvious target for the treatment modalities of gastric ulcer and continues to arrest the attentions of both researchers and clinicians.

Classical COX hypothesis leads to the development of most of the recent nonsteroidal anti-inflammatory drugs (NSAIDs) like selective COX-2 specific inhibitors (celecoxib, rofecoxib, etc.) that have been developed as anti-inflammatory agents, which avoids the adversity of gastrointestinal bleeding by sparing the production of prostaglandins E_2 (PGE_2), important for maintenance of mucosal integrity (Berenguer et al., 2002; Coppeli et al., 2004). However, with a shift in classical hypothesis, it was also evident that COX-2 not only plays important regulative roles in gastric mucosal defence mechanism but is also pivotal in the process of gastric ulcer healing (Shigeta et al., 1998; Brzozowski et al., 2001) and produces prostaglandin, which exerts anti-inflammatory actions (Gilroy et al., 1999). These findings diluted the utility and significance of selective COX-2 inhibitor NSAIDs and it became clearer that although these NSAIDs prevent ulcerogenesis but also intensifies the pre-existing ulcers by delaying the healing process. Researchers have shown that selective COX-2 inhibitors aggravate gastric lesions induced by ischemia–reperfusion (Brzozowski et al., 1999; Maricic et al., 1999), delays ulcer healing in rats and mice (Mizuno et al., 1997; Lesch et al., 1998; Brzozowski et al., 2001) and inhibit angiogenesis, epithelial cell proliferation and maturation of granulation tissue in chronic gastric ulcers (Schmassmann et al., 1998). These observations lead to the notion that COX-2 inhibition is not associated with gastric damage in normal mucosa, but it can de detrimental when gastric mucosal defense is impaired.

The recent view is further supported by reports which suggests that COX-2 protein is over expressed during the healing of gastric lesions while COX-2 specific inhibitors cause a delay in ulcer healing in rats, highlighting the pivotal role of this isozyme in gastric ulcer healing (Tsuji et al., 2002; Motilva et al., 2005). As researchers reached on a consensus regarding the importance of COX-2 in ulcer healing, another important concern that emerged is whether COX-2 enzyme plays an equally effective role in both normal and drug mediated ulcer healing or there occurs a differential expression of COX-2 in different drug treatments that varies both in their acting mechanisms and healing effect. In the present study, we have tried to address this question by comparing the expression profile of COX-2 protein, PGE_2 levels and Myeloperoxidase activity during normal and drug mediated ulcer healing after inducing the chronic gastric ulcers by acetic acid in rat model. The two anti-ulcerogenic drugs studied act through different mechanisms, omeprazole is a proton pump inhibitor and misoprostol is a PGE_1 analog while COX-2 selective NSAID celecoxib was used as negative control.

2. Materials and methods

2.1. Animals, drugs and doses

Naïve Sprague–Dawley rats of either sex, weighing 180–200 g, were used. They were housed three to four animals per cage at temperature 22±2 °C and 12 h light/dark (8.00 A.M. to 8.00 P.M.) under controlled environment. Animals were fed with standard laboratory food (Pellet) and water was given ad libitum. All animals were deprived of food but not of water for 18 h before getting subjected to ulcerogen and were randomly allocated to different experimental groups. Experimental protocols were approved by ethical committee of Central Drug Research Institute, Lucknow that follows guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals), which complies with international norms of INSA. All experiments were in accordance with the recommendation of European Union.

The study has been designed in such a manner that 4 experimental groups have been used in all the experiments corresponding to the control (no drug treatment after ulcer induction), omeprazole treated rats, misoprostol treated rats and negative control (celecoxib treated rats). Total 42 animals were used in each group, out of which 24 animals were used for assessment of ulcer size and healing (6 animal each for day 0, 5, 10 and 14) and 6 animals each were used for measuring myeloperoxidase activity, PGE_2 level and COX-2 protein expression respectively in each of the group on the day 14th of treatment.

All the chemicals and drugs were purchased from Sigma (St. Louis, MO) unless otherwise specified. Omeprazole, misoprostol and celecoxib (gift from Dr. Reddy's lab) were prepared in 1% sodium carboxymethylcellulose suspension as vehicle and administered orally once in a day at a volume of 1 ml/200 g of body weight. Control group of animals was treated only with the vehicle similar to experimental groups.

The selection of a specific dose for each of the drugs was based on our earlier finding and reports from other groups worked on the rat models (Penney et al., 1994; Dharmani et al., 2003). The ulcer index and ulcer healing analysis of our preliminary study has revealed that the most effective dose was 10 mg/kg body weight for omeprazole, 100 μg/kg body weight for misoprostol and 10 mg/kg body weight for celecoxib respectively.

2.2. Induction of chronic gastric ulcer

Chronic gastric ulcers were induced experimentally in rats according to the method of Konturek et al. (2003), which follows the technique of Okabe et al. (1987) with few modifications. Under anesthesia with pentobarbitone (35 mg/kg body weight, i.p.), the abdomen was opened by midline incision. A plastic tube of 6 mm, opened at both the ends was applied tightly to the serosal surface of anterior wall of the stomach just proximal to antral gland area. Onto the surface of stomach, 60 μl of 40% acetic acid was poured through a tube for 90 s. This produced immediate necrosis of the entire mucosa and sub-mucosa within the area where the acetic acid was applied. Acetic acid remaining on the surface was then wiped off with a filter and the opened abdomen was closed. Neosporin powder was sprayed at the site of operation in rats in order to avoid post surgical infections.

Our previous studies (Dharmani et al., 2003, 2004) documented that this procedure produces histologically well characteristic ulcers in 3 days after acetic acid exposure and heals completely within 3 weeks without perforation or penetration to the surrounding organs. Animals were then divided into four different groups mentioned above. The treatment with omeprazole, misoprostol and celecoxib was started, once the ulcer is fully formed, i.e. from the 3rd day of surgery considering it as day 1 of treatment, for the next 14 days.

2.3. Assessment of ulcer size and percentage healing

Total ulcer area and percentage ulcer healing was assessed in rats of all the four groups on 0, 5th, 10th and 14th day of treatment. Six animals of each group were sacrificed with overdose of ether and stomachs were rapidly dissected out. The stomach was cut along the greater curvature and area of ulcer (mm^2) was measured with Biovis image analysis software coupled with zoom stereomicroscope (Olympus). The percentage healing was calculated as

$$\text{Percentage healing} = \frac{\text{Ulcer area of control} - \text{Ulcer area of treated}}{\text{Ulcer area of control}} \times 100.$$

2.4. Histopathological study

Histological studies were performed according the method previously described by Ogihara and Okabe (1993). Briefly, ulcer crater of each stomach was cut out and was fixed for 4 h in paraformaldehyde (40%), then dehydrated gradually in ethanol and embedded in paraffin, using xylene as intermediate solvent. Serial sections of 5 μm were obtained in an automated microtome. Ulcerated sections were stained with haematoxylin and eosin (HE) before evaluation of histological changes during healing.

2.5. Western blot analysis of COX-2

COX-2 and β-actin (internal standard) protein analysis was carried out in the gastric mucosa of intact tissue as well as of those with gastric ulcer after the 14th day of treatment by Western blot analysis. Six rats from each of the treated group were sacrificed. Tissues from ulcer area, including ulcer margin and the remaining, i.e. non-ulcerated mucosa, adjacent glandular mucosa were scrapped on ice using a glass slide and then immediately snap frozen in liquid nitrogen. Sample of the stomachs were taken from all treated groups and were homogenized in phosphate buffer saline (pH 7.4) containing 1% nonidet-40, 0.5% sodium deoxycholate and 0.1% sodium dodecyl sulphate (SDS). Protein concentration was measured with the method of Lowry et al. (1951) and 60 μg of protein was size fractionated on 10% SDS-polyacrylamide gel electrophoresis. Specific COX-2 marker of ovine origin (72 kDa) was also electrophoresed along with the samples. The electrophoresed proteins were then transferred to nitrocellulose membrane using a semidry transfer cell (Hofer). The membranes were blocked with phosphate buffer saline containing 5% non-fat milk and 0.1% tween-20. Membranes were incubated with primary antibody for COX-2 (1:200) (Santa Cruz). The horse reddish peroxidase linked mouse anti goat IgG antibody was used as secondary antibody (1:5000). Immunodetection was performed using enhanced chemiluminescence (Amersham Biosciences). Quantitation was carried out by a video densitometer. Results were expressed as the ratio of COX-2 protein to that of β-actin protein in all the treated groups.

2.6. Estimation of prostaglandin E_2

The sample of oxyntic gland area were taken by biopsy (about 100 mg) immediately after six animals of each of the treated group has been sacrificed to determine the mucosal generation of PGE_2 by competitive enzyme immuno assay using enzyme immuno assay kit for prostaglandin estimation (Cayman Chemicals). PGE_2 estimation was carried out in both intact and ulcerated mucosa of different treated groups. The gastric mucosa of different treated groups was excised and homogenized in an ice-cold Tris/HCl buffer containing 50 mM Tris/HCl (pH 7.4), 100 mM sodium chloride, 1 mM calcium chloride, 1 mg/ml D-glucose and 28 μM indomethacin according to the method of Guo et al. (2002). The protein concentration of homogenate was measured by the method of Lowry et al. (1951). Homogenate was centrifuged at 12,000×*g* for 30 min at 4 °C for the determination of PGE_2 concentration. The supernatant was transferred in separate vial and kept at − 70 °C until assayed. The concentration of PGE_2 present in the supernatant was measured in duplicates with PGE_2 enzyme immuno assay kit. The assay was performed in a total volume of 150 μl with the following components being added in 50 μl volumes: standards or homogenate, enzymatic tracer and specific antiserum. After overnight incubation at 4 °C, the plates were washed and 200 μl Ellman's reagents were dispensed into each well. After 1 h absorbance at 412 nm each well was measured. Results are expressed as ng of PGE_2 per mg of protein.

2.7. Estimation of myeloperoxidase activity

Myeloperoxidase activity was assessed as a marker of neutrophil infiltration by the method of Gilbert et al. (1974). Six animals of each of the treated group were sacrificed and the tissue was homogenized in 5 volumes of hexadecyltrimethylammonium bromide buffer (0.5% hexadecyltrimethylammonium bromide in 50 mM phosphate buffer, pH 6.0). The homogenate was subjected to sonication for 15 s followed by centrifugation at 2400×*g* at 4 °C. The supernatant was shock froze and thawed. A

sample of 100 μl was mixed with 1 ml of 0.22% aqueous guaiacol and 2 ml of 10 mM phosphate buffer (pH 6.0). 20 μl of 0.3% hydrogen peroxide was added to start the reaction. Absorbance at 470 nm was recorded with a Beckman spectrophotometer at 15 s intervals for 2 min. As a standard, 100 μl of horseradish peroxidase (0.975 U/ml) was combined with 1 ml of guaiacol, 2 ml of 10 mM phosphate buffer (pH 6) and 5, 10 and 20 μl of 0.3% hydrogen peroxide was added to start the reaction. The absorbance change at 470 nm for 1 μmol peroxide/min was calculated from the standard curve, which equals 1 unit of myeloperoxidase activity. Protein concentrations were measured in the supernatant according to Lowry et al. (1951), and the results were expressed as units of myeloperoxidase per mg tissue protein.

2.8. Statistical analysis

Values are given as mean±S.E.M. The significance of differences between means was evaluated using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. $P<0.05$ was considered significant.

3. Results

3.1. Effect of the drugs on ulcer healing

Chronic gastric ulcers were developed with an incidence of 100% after 3 days of serosal application. The gastric ulcer showed progressive healing in control, omeprazole and misoprostol treated groups of animals where ulcer area started reducing in size on day 5 showed a gradual decrease on day 10 and exhibited maximum healing on day 14 in each of the group. As previously reported celecoxib showed a delayed ulcer healing in comparison to control on all the days of evaluation. No significant difference was observed in reduction of ulcer area in the four groups on day 5 (Fig. 1A), but as the healing progressed, it became evident that omeprazole and misoprostol were causing significant increase in ulcer healing as compared to control ($P<0.001$) on day 10. Although celecoxib delayed the ulcer healing, however, the difference was not significant from control ($P>0.05$). Similar trend were observed on day 14 when nearly complete ulcer healing was evident as restoration of surface epithelium and extensive regeneration was recorded. It was on day 14 when clearer differentiation in the effect of the three drugs on ulcer healing was observed. After 14 days of the drug treatment, the area of ulcer in control group was 19.5±2.72 mm^2, whereas that in the omeprazole, misoprostol and celecoxib treated group was 2±00, 4.2±0.34 and 23.25±1.11 mm^2 respectively. Therefore, percentage healing shown by omeprazole, misoprostol and celecoxib was 89.74%, 78.46% and − 12.2% respectively on day 14 (Fig. 1B).

3.2. Histological examination of ulcerated tissue

Histologically, control rats showed reduced inflammatory exudates along with some extent of mucosal regeneration, glandular organization and reduced size of ulcer (Fig. 2A). Omeprazole treated rats showed clear evidence of restoration of mucosal epithelium (re-epithelization), almost complete clearance

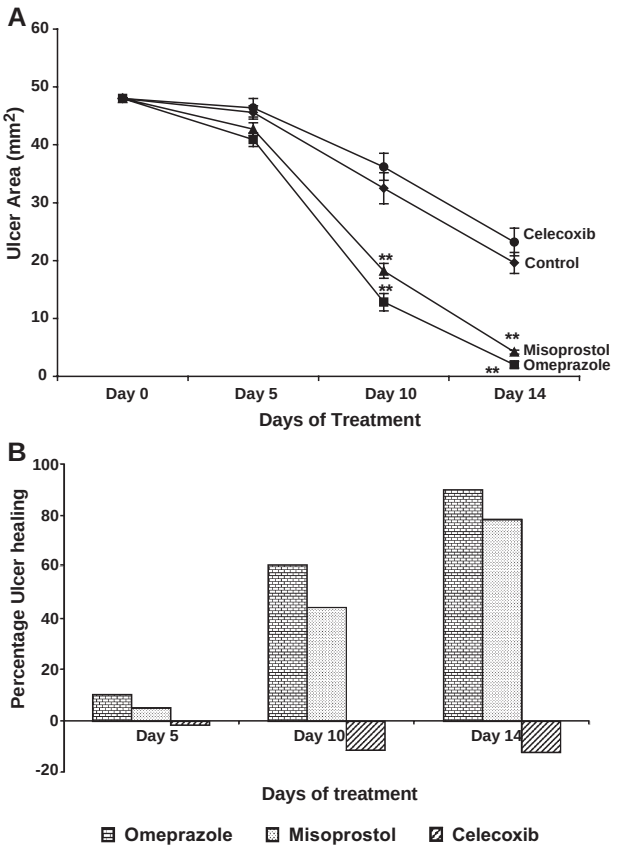

Fig. 1. (A) Effect of omeprazole, misoprostol and celecoxib at doses of 10 mg/kg, 100 μg/kg and 10 mg/kg body weight respectively on ulcer area in acetic acid induced chronic ulcer model after 0, 5, 10 and 14 days of treatment. Values are presented as mean±S.E.M. *$P<0.05$ and **$P<0.001$ when compared to control ($n=6$ in each group). (B) Effect of omeprazole, misoprostol and celecoxib at doses of 10 mg/kg, 100 μg/kg and 10 mg/kg body weight respectively percentage healing in acetic acid induced chronic ulcer model after 5, 10 and 14 days of treatment *$P<0.05$ and **$P<0.001$ when compared to control ($n=6$ in each group).

of inflammatory exudates and good secretory activity of normally arranged glands. Some superficial remodeling was also observed (Fig. 2B). In misoprostol treated rats, the picture was almost similar as in omeprazole treated rats, with minimal inflammatory exudates and proper organizing glands, however the re-epithelization of mucosa was still not completed (Fig. 2C). The celecoxib treated group has not shown any sign of ulcer healing and has shown more inflammatory exudates, disorganized glands and a bigger size of ulcer than that of the controls (Fig. 2D).

3.3. Effect of the drugs on myeloperoxidase activity

Myeloperoxidase activity was assessed in intact tissue sample as well as in ulcerated samples after the 14th day of treatment. No change in the activity was found in intact tissues but in omeprazole, misoprostol and celecoxib treated group the activity was found 4.5±0.17, 9.8±1.13 and 26.33±1.38 with respect to control of 24.9±1.77 U/mg tissue protein. Therefore the overall percentage decrease in myeloperoxidase activity was 81.92%, 71.64% and − 5.7% respectively for omeprazole, misoprostol and celecoxib treated groups (Fig. 3).

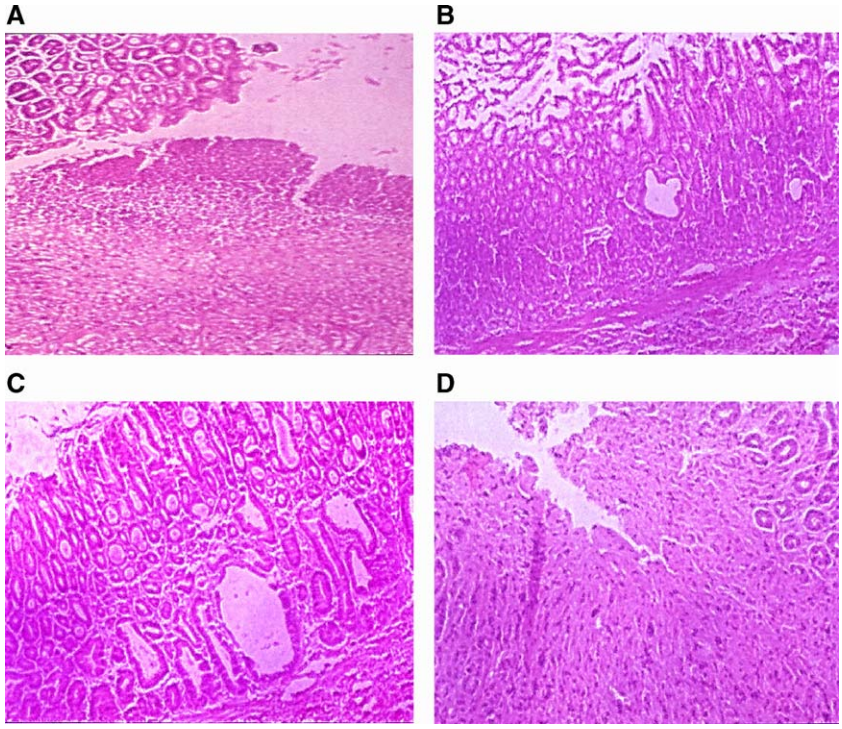

Fig. 2. (A) Sections of ulcerated stomach obtained from rats of control groups acetic acid induced chronic ulcer model in rats after 14 days of treatment ($n=6$ in each group) HE×100. (B) Sections of ulcerated stomach obtained from rats of omeprazole groups in acetic acid induced chronic ulcer model in rats after 14 days of treatment ($n=6$ in each group) HE×100. (C) Sections of ulcerated stomach obtained from rats of misoprostol groups in acetic acid induced chronic ulcer model in rats after 14 days of treatment ($n=6$ in each group) HE×100. (D) Sections of ulcerated stomach obtained from rats of celecoxib groups in acetic acid induced chronic ulcer model in rats after 14 days of treatment ($n=6$ in each group) HE×100.

3.4. Differential COX-2 expression in different treated groups

Western blot analysis reveals that COX-2 protein was undetectable in the intact mucosa but a well characterized, albeit low signal of COX-2 band signifying lesser expression of COX-2 protein was observed in ulcerated portion of control group on day 14 (Fig. 4A). The expression of house keeping β-actin was well preserved in gastric mucosal sample from both

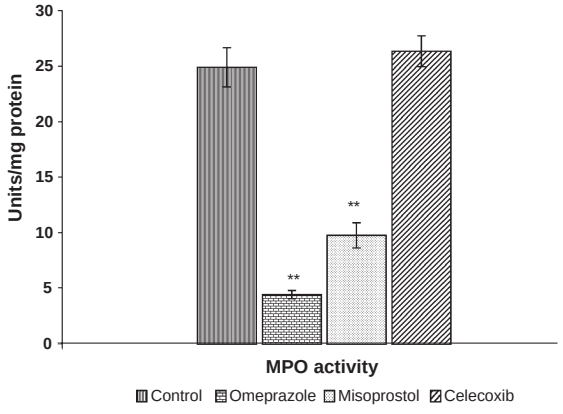

Fig. 3. Effect of omeprazole, misoprostol and celecoxib at doses of 10 mg/kg, 100 μg/kg and 10 mg/kg body weight respectively, on myeloperoxidase activity in acetic acid induced chronic ulcer model after 14 days of treatment. Values are presented as mean±S.E.M. *$P<0.05$ and **$P<0.001$ when compared to control ($n=6$ in each group).

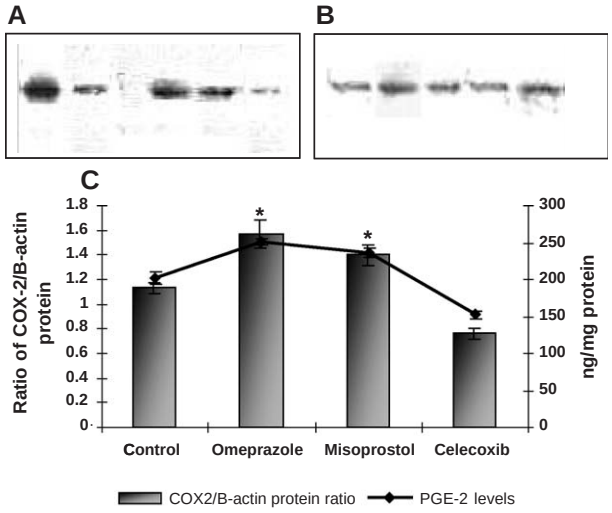

Fig. 4. (A) Radiogram showing different levels of COX-2 expressions obtained on Western blotting in ulcer tissues from different study groups; Lane 1: Ovine COX-2 marker (72 kDa), Lane 2: normal ulcer tissue, Lane 3: Non-ulcerated region, Lane 4: omeprazole treated ulcer tissue, Lane 5: misoprostol treated ulcer tissues and Lane 6: celecoxib treated ulcer tissues. (B) Radiogram showing expression levels of β-actin obtained on Western blotting in normal and ulcerated tissues from different study groups; Lane 1: Normal ulcer tissue, Lane 2: Non-ulcerated region, Lane 3: omeprazole treated ulcer tissue, Lane 4: misoprostol treated ulcer tissues and Lane 5: celecoxib treated ulcer tissues. (C) Ratio of COX-2/β-actin protein and PGE_2 levels detected in ulcerated tissue of different treated groups. Data are expressed mean±S.E.M. *$P<0.05$ when compared to control.

Table 1
Effect of omeprazole, misoprostol and celecoxib on prostaglandin E_2 production in ulcerated and intact mucosa in acetic acid induced chronic ulcer model

Group	Prostaglandin E_2 (ng/mg protein) in ulcerated mucosa	Prostaglandin E_2 (ng/mg protein) in intact mucosa
Control	202.2±9.36	186±3.05
Omeprazole	250.8±3.77[a]	193±1.05
Misoprostol	236.3±11.71[b]	190.5±1.628
Celecoxib	152.5±5.11[a]	188.5±2.75

Data are represented as mean±S.E.M. Statistical analysis was done by one-way ANOVA followed by Dunnett's multiple comparison test. [a]$P<0.001$ and [b]$P<0.05$ as compared to vehicle treated control ($n=6$ in each group).

intact and ulcerated tissue (Fig. 4B). Both misoprostol and omeprazole treated group showed significantly higher expression of COX-2 protein than controls with its highest expression observed in omeprazole treated group. In celecoxib treated animals a faint band of COX-2 was found (Fig. 4A). Ratio of COX-2/β-actin protein confirmed that COX-2 expression peaks in omeprazole treated group (1.57 in comparison to 1.13 to that of control) while its least expression was observed in celecoxib treated group (0.76 in comparison to 1.13 to that of control) (Fig. 4C).

3.5. Differential PGE_2 synthesis in different treated groups

The PGE_2 generation in the intact gastric mucosa averaged 186±3.05 ng/mg tissue protein, whereas in ulcerated portion of control group the value was higher—202±7.67 ng/mg tissue protein. Although the differences were not significant on day 14 but during time course of ulcer healing, this difference in PGE_2 level of ulcerated and intact tissue was more evident on day 10 (data not shown). In omeprazole and misoprostol treated group, significantly higher ($P<0.001$) PGE_2 value of 250.8±3.77, 236.3±6.13 ng/mg protein was found in comparison to control. The results were in agreement with the COX-2 expression analysis. Selective COX-2 inhibitor celecoxib has significantly reduced the PGE_2 level to 152.5±5.11 ng/mg proteins that accounts for capability of inhibiting eicosanoid production by blocking COX-2 activity (Table 1 and Fig. 4C).

4. Discussion

Ulcer healing is a cumulative effect of several physiological and constitutive processes that occurs in tandem and requires a high level of co-ordination and regulation where numerous factors like prostaglandins and growth factors play an essential role. Prostaglandins, synthesized by COX enzymes, has a key role in ulcer healing process as they act through different mechanism like stimulation of mucosal bicarbonate secretion, increase in blood flow, prevention of disruption of mucosal barrier, acceleration of cell proliferation and enhancement of angiogenesis (Robert, 1979; Miller, 1983; Isenberg et al., 1985; Scheiman, 1996).

The present study confirmed that chronic gastric ulcers healed in a progressive manner with maximum healing observed on the 14th day of treatment in all treated and untreated group except that of celecoxib treated animals. Ulcer area showed progressive decline from day 5 to 14. Regeneration of surface epithelial cells and restoration of gastric mucosa were clearly observed on day 14. Omeprazole depicts the fastest ulcer healing on all the three days of evaluation followed by misoprostol. Histologically, omeprazole-treated rats exhibited a flat ulcer margin and this may have been due to the protection of the newly formed epithelial cells in the ulcer margin and granulation tissue (including microvessels in the ulcer bed) from direct gastric acid-pepsin damage (Shigeta et al., 1998). Proton pump inhibitors also promote re-epitheliazation (Folkman et al., 1991). Omeprazole increased the rate of regeneration of the ulcerated mucosa. In general gastrin is known to have a trophic action on the gastric mucosa (Johnson and Guthrie, 1994). As omeprazole increases serum gastrin level, the accelerated regeneration of the ulcerated mucosa is likely due to an increase in serum gastrin level. Misoprostol along with accelerating regeneration of mucosa stimulates formation of granulation tissue, resulting in the thickness of the ulcer base. Celecoxib delays healing procedure by decreasing epithelial cell proliferation, angiogenesis and maturation of granulation tissue in ulcer margin, that is why it delays the healing of ulcer.

The most interesting finding of the present study is the importance of COX-2 enzyme and prostaglandin-E_2 in the process of normal as well as drug mediated ulcer healing. Furthermore, it was also evident that the up-regulation the COX-2 expression followed a differential pattern in drugs showing differential profile of ulcer healing despite of the fact that the underlying mechanism of action of these drugs is independent of COX-2 expression. Significantly, the drug that inhibits COX-2 activity has also been shown to down-regulate the COX-2 expression as well as hinder the process of ulcer healing. These results comply with the previous reports where higher amounts of PGE_2 are detected at the site of ulceration than in non-ulcerated mucosa (Lesch et al., 1998). Mizuno et al. (1997) have also demonstrated that COX-2 expression is up-regulated in the margins of healing of gastric ulcers highlighting that COX-2 represents an important aspect of defense necessary for maintenance of mucosal integrity and healing.

Omeprazole, the most effective ulcer healing drug found in the present study is known to accelerate ulcer healing by inhibiting acid secretion and augmenting luminal pH (Dent and Chir, 1998), thereby decreasing acid and pepsin damage to the mucosa. However, omeprazole is also known to exert its cytoprotective effects due to prostaglandins (Okabe et al., 1986). Increased expression of COX-2 protein and elevated levels of PGE_2 augment strong healing depicted by omeprazole. Similar effect was shown by Tsuji et al. (2002), where another proton pump inhibitor, lansoprazole, was found promoting mucosal protection by up-regulating COX-2 expression and PGE_2 levels in rats during ulcer healing. Authors have suggested that the gastrin dependent pathway is responsible for the up-regulation of COX-2

expression and elevated levels of PGE_2, as when lansoprazole was used along with a gastrin receptor antagonist AG-04R, significant reduction in COX-2 expression as well as PGE_2 level was observed. Present study also compliment the findings of Tsuji et al. (2002) and the possible explanation could be that the particular dose of omeprazole might be having an acid inhibitory effect as we have also demonstrated in some of our earlier studies (Dharmani et al., 2003, 2004, 2005a,b), but the parallel increase in the COX-2 expression and PGE_2 levels with ulcer healing signifies that omeprazole along with it anti-secretory effects also stimulates cytoprotective mechanism by up-regulating the COX-2 expression and prostaglandin synthesis.

Another anti-ulcerogenic drug, misoprostol, (an exogenous PGE) has also shown a rapid increase in healing through enhancement of endogenous PGE_2 and elevation in COX-2 expression but the relative delay as compared to that of omeprazole could be because of the fact that the reported effective dose of misoprostol that inhibits acid secretion is much higher and known to posses severe side effects. Stimulation of COX-2 expression and endogenous PGE_2 synthesis is another important finding as there is contradiction regarding the role of an exogenous PGE_2 analogue in promoting the synthesis of endogenous PGE_2. Some authors suggest that supply of exogenous PGE_2 retards the production of endogenous PGE_2. However, there are several reports available in recent times that categorically states misoprostol or any other exogenous PGE_2 analogue (dimethylprostaglandin E_2) up-regulates the expression of COX-2 m-RNA enzyme and elevates the levels of endogenous PGE_2 both in vivo (Wang et al., 1989; Tjandrawinata et al., 1997; Buluc et al., 2002) and in vitro (Minghetti et al., 1997; Bonazzi et al., 2000; Hinz et al., 2000).

Furthermore, a delay in ulcer healing by COX-2 inhibitor celecoxib drug provides further line of support. Celecoxib was found suppressing the prostaglandin production significantly by inhibiting COX-2 activity as well as down-regulating COX-2 expression. Some of earlier reports have also shown that selective COX-2 inhibitors along with suppressing the COX activity also results in retardation of its expression, however no possible mechanism has been suggested for this effect (Tanaka et al., 2002). Recently, Yokota et al. (2005) have also showed that indomethacin, a non-selective NSAID, induces COX-2 expression in intestine but not in stomach. We postulate that the failure of COX-2 enzyme in producing prostaglandin due to coxib activity might be having a plummeting effect on its expression.

Biochemical analysis showed that omeprazole decreased the inflammatory infiltrate most significantly after 14 days. The selective inhibition of COX-2 by celecoxib that resulted in pronounced reduction in ulcer healing was accompanied by lesser decrease in granulocyte infiltration as measured by myeloperoxidase activity. According to Fujita et al. (1998), the delay in ulcer healing may be due to a decrease in antichemotactic activity, characteristic of normal mucosa, and also due to increase in chemotactic activity in ulcerated tissue, leading to persistent neutrophil infiltration. Infiltration of inflammatory cells such as neutrophil and mononuclear cells play a key role in the recurrence of ulcers (Arakawa et al., 1998).

Conclusively, our results confirm the essential role of COX-2 enzyme in the resolution of chronic gastric inflammatory process with PGE_2 being the principal product. Results indicate that gastric mucosal production of prostaglandins by enhancing expression of COX-2 aggravate the healing effect of drugs that act through other mechanisms like a combination of acid inhibitory effect and increased prostaglandin synthesis via COX-2 expression account for the highest healing effect of omeprazole.

Induction of COX-2 protein expression that controls the reparative mechanism through angiogenesis and adaptive cytoprotection along with elevation in the synthesis of prostaglandin seems to be the certain candidates for the ulcer healing modalities. Endogenous prostaglandins (PGE_2), having modulating roles on COX-2 expression, are an additional aspect. Drugs that can lead to an elevation in the expression level of COX-2 remain more successful in ulcer healing while its inhibition produces a downward effect.

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