Non-Ulcerogenic Dose of Dexamethasone Delays Gastric Ulcer Healing in Rats

JIING C. LUO, VIVIAN Y. SHIN, EDGAR S. L. LIU, WALLACE H. L. SO, YI N. YE, FULL Y. CHANG, and CHI H. CHO
Division of Gastroenterology, Department of Medicine, Taipei Veterans General Hospital and National Yang-Ming University, School of Medicine, Taipei, Taiwan (J.C.L., F.Y.C.); and Department of Pharmacology, Faculty of Medicine, the University of Hong Kong, Hong Kong, China (V.Y.S., E.S.L.L., W.H.L.S., Y.N.Y., C.H.C.)

Received June 3, 2003; accepted July 29, 2003

ABSTRACT
Although the ulcerogenic action of corticosteroids in the stomach is controversial, its action on ulcer healing has not been defined. In this study, we used non-ulcerogenic doses of dexamethasone (0.1 or 0.2 mg/kg/day) to explore the adverse effect on ulcer healing as well as its pathological mechanisms in rat stomach. In this regard, we measured ulcer size, mucus thickness, epithelial cell proliferation and apoptosis, and angiogenesis at the ulcer site at different time points after ulcer induction. Protein expressions of cyclooxygenase-1 and -2 (COX-1 and COX-2) and cytosolic phospholipase A2 (cPLA2) over the ulcer margin were evaluated, and the mucosal prostaglandin E2 (PGE2) level was also determined. Dexamethasone treatment in the current doses did not produce mucosal damage in intact animals. However, the drug dose-dependently delayed gastric ulcer healing. It also decreased mucus content and epithelial cell proliferation at the ulcer margin as well as angiogenesis at the ulcer margin and base. These were associated with a significant decrease of COX-2 expression and PGE2 level but not COX-1 at the ulcer margin. The drug only marginally reduced the cPLA2 expression without affecting the apoptosis at the ulcer margin. PGE2 treatment reversed the adverse effects of dexamethasone on ulcer healing. It is concluded that non-ulcerogenic doses of dexamethasone can delay ulcer repair via depression of COX-2 expression and PGE2 formation in the gastric mucosa.

Corticosteroids manifest anti-inflammatory and immunosuppressive actions and have been used to treat various diseases for more than 50 years. Although it is known that long-term application of corticosteroids can cause various side effects in the body, it is still uncertain whether they are ulcerogenic to the gastrointestinal tract. Clinical studies showed inconsistent results regarding the association of corticosteroid usage and peptic ulcer formation (Conn and Blitzer, 1976; Messer et al., 1983). Administration of corticosteroids to experimental animals resulted in acute gastric erosions (Nobuhara et al., 1985; Wallace, 1987; Filep et al., 1992), but other studies showed that dexamethasone, a potent corticosteroid, did not induce gastric lesions (Akiba et al., 1998; Gretzer et al., 2001).

In fact, peptic ulcer formation is a dynamic state of imbalance between aggressive and protective factors. It has been suggested that the mechanisms responsible for gastric mucosal damage induced by corticosteroids include inhibition of gastric mucus synthesis, enhancement of gastrin and parietal cell hyperplasia with augmented acid secretion, and suppression of arachidonic acid metabolism and prostaglandin (PG) synthesis (Menguy and Masters, 1963; Delaney et al., 1979; Bandyopadhyay et al., 1999; Wolfe et al., 1999).

The mechanism for ulcer repair represents a different entity of process including the balance of cell damage and repair at the ulcer site. Corticosteroids given in ulcerogenic doses could not only damage the mucosa but also affect the regenerative system in the gastric mucosa; both of which could delay ulcer healing in the stomach (Carpani de Kaski et al., 1995). It is difficult to differentiate the two actions on ulcer repair. There is no detailed mechanistic study reporting the direct action of corticosteroids on ulcer healing in the stomach. Thus, it is interesting to investigate whether a non-ulcerogenic dose of corticosteroid could indeed affect cell proliferation, angiogenesis, and apoptosis at the ulcer site during ulcer healing. All these parameters have been shown to be important in tissue repair in the stomach (Carpani de Kaski et al., 1995; Li et al., 1999; Wang et al., 2000).

Corticosteroids can affect PG synthesis in tissues (Flower, 1988; Izhar et al., 1992), and PGs play a significant role in the prevention of ulcer formation and improvement of ulcer healing (Cho et al., 1990; Kuwayama et al., 1991; Carpani de...
Kaski et al., 1995). It is therefore suggested that depletion of PGs could be the major detrimental factor contributing to the action of corticosteroids on ulcer repair in the stomach. In this regard, enzymes involved in PG synthesis including phospholipase A\(_2\) (PLA\(_2\)), which influences the production of arachidonic acid, and cyclooxygenases (COXs), which contribute to the formation of different forms of PG, play a significant role in the action of corticosteroids on ulcer healing.

In this study, we apply the non-ulcerogenic doses of dexamethasone, a potent corticosteroid, to explore its action on ulcer healing and the mechanistic pathway related to PG synthesis in a rat gastric ulcer model.

**Materials and Methods**

**Animals**

The use of animals in this study was approved by the Committee on the Use of Live Animals in Teaching and Research of The University of Hong Kong. Male Sprague-Dawley rats (200–220 g) were reared on a standard laboratory diet and given tap water. They were kept in a room where temperature (22° ± 1°C), humidity (65–70%), and day/night cycle (12 h light-dark) were controlled. Rats were fasted for 24 h but had free access to water before being subjected to acetic acid to produce gastric ulcer.

**Chemicals and Drugs**

Chemicals and drugs were purchased from Sigma-Aldrich (St. Louis, MO) unless otherwise stated. Dexamethasone was prepared in 1% ethanol vehicle for intragastric administration. PGE\(_2\) was dissolved in 0.01 M phosphate-buffered saline for intraperitoneal injection.

**Part I: Dexamethasone Treatment and Mucosal Damage.** To determine the non-ulcerogenic dose of dexamethasone, rats were given dexamethasone intragastrically at the maximal dose of 0.2 mg/kg once daily for a period of 9 days. They were sacrificed on day 10 and the gastric mucosa damage was assessed by morphological observation under magnifying glass and histological examination with a microscope. The specimens with hematoxylin-eosin stain were assessed according to the criteria of Whittle et al. (1990) with modification. The microscopic scoring was defined as follows: 1) epithelial cell damage and glandular disruption; 2) hemorrhagic damage in the mucus; and 3) deep necrosis and ulceration. For further comparisons in this part with the other portions of the study, we also collected mucosa from normal rats without any treatment as the normal group.

**Part II: Dexamethasone Treatment and Ulcer Healing**

**Induction of gastric ulcer.** Gastric kissing ulcers were produced by luminal application of acetic acid solution to rats as previously described with modification (Tsukimi and Okabe, 1994). Briefly, the abdomen was opened under ether anesthesia, and the stomach was exposed. The anterior and posterior walls of the stomach were clamped together with a pair of forceps with a round ring (10 mm i.d.) situated between the two arms of the forceps. A 60% acetic acid solution of 0.12 ml was injected into the clamped portion through the forestomach via a 21-gauge needle. After 45 seconds, the acid solution was removed and the abdomen was closed. Thereafter, rats were fed a standard diet and given tap water.

**Drug treatment and measurement of gastric ulcer.** One day after ulcer induction, rats were given dexamethasone intragastrically at doses of 0.1 or 0.2 mg/kg once daily for 3, 6, or 9 days to observe the ulcer-healing effect. Rats receiving 1% ethanol solution were regarded as vehicle control. There were no observable differences in the daily physical activities and body weight gain between the control and the dexamethasone-treated groups during the experimental period. After treatment, rats were sacrificed at day 4, 7, or 10 after ulcer induction. The ulcer size (mm\(^2\)) on both the anterior and posterior walls was determined in each stomach. After measuring the ulcer areas, gastric tissues were excised for immunohistochemical analysis. Gastric mucosa and submucosa over the ulcer margins were removed by scraping with a glass slide and immediately frozen in liquid nitrogen and stored at −70°C until determinations for different parameters.

**Assessment of mucosal mucus content.** After fixation in buffered formalin and immersion in wax, 5-μm sections were made and stained with periodic acid-Schiff technique. Finally, they were counterstained with Harris hematoxylin and mounted in Permount. The mucus contained in the cells was stained purple-red. The amount of the mucus content was assessed by measuring the thickness of the mucus-secreting layer under an image analyzer (Q500IW; Leica Image Systems, Cambridge, England) in three consecutive fields of each side of the ulcer crater. The results were averaged from both sides of ulcer margins and expressed as the ratio of the thickness of the mucus layer to the thickness of the total mucosa (Ma et al., 2000).

**Assessment of epithelial proliferation at ulcer margin.** To determine cell proliferation, a single dose of 100 mg/kg 5-bromo-2′-deoxyuridine (BrdU) was injected intraperitoneally 1 h before animals were sacrificed. The cell proliferation was assessed by immunohistochemical staining with anti-BrdU antibody as described previously (Lacy et al., 1991). The sections were counterstained with Mayer’s hematoxylin. The cells labeled with BrdU at a field of 0.899 mm\(^2\) (200×) were counted in both sides of the ulcer margin of the ulcer crater for each rat and expressed by taking the average of both sides of the ulcer margin.

**Determination of angiogenesis at ulcer margin and base.** The microvessels at the ulcer margin and base in the granulation tissue of the submucosa was identified by immunohistochemical staining with von Willebrand factor antibody (DAKO, Glostrup, Denmark) (Augustin et al., 1995). The microvessels stained with the antibody were quantified at the two sides of the ulcer margin and at the base of ulcer crater in a microscopic field of 0.899 mm\(^2\) (200×). The number of blood vessels at the ulcer margin was expressed by taking the average of both sides of ulcer margin.

**Assessment of mucosal cell apoptosis at ulcer margin.** Terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) method was used to stain apoptotic cells, as described previously (Gavrieli et al., 1992). The number of apoptotic cells was counted in a microscopic field of 0.899 mm\(^2\) (200×) and was expressed by taking the average of both sides of ulcer margin.

**Western blotting for COX-1, COX-2, and cPLA\(_2\) expressions.** Gas-
tric tissues were homogenized (Ultra-Turrax; Janke and Kunkel Co., Staufen, Germany) with radioimmunoprecipitation assay buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.1% SDS, 0.5% α-cholate, 2 mM EDTA, 1% Triton-X, 10% glycerol) and then centrifuged (J2–21; Beckman Coulter, Inc., Fullerton, CA) for supernatants. Protein concentration was measured using a protein assay kit, and the mucosal PGE2 level was expressed as picogram per milligram of protein.

Measurement of mucosal PGE2 level. Gastric tissues were homogenized with homogenizing buffer (0.05 M Tris-HCl at pH 7.4, 0.1 M NaCl, 0.001 M CaCl2, 1 mg/ml D-glucose, 28 μM indomethacin to inhibit further PGE2 formation) for 30 s. They were then centrifuged at 12,000 rpm for 15 min at 4°C. Supernatants were assayed by using a commercially available PGE2 enzyme-linked immunosorbent assay kit (Quantikine; R&D Systems, Minneapolis, MN). The assay procedure was in accordance with the protocol suggested by the manufacturer. Optical densities were determined by the MRX microplate reader (Dynex Technologies, Chantilly, VA) at 405 nm. The amount of protein in the sample was determined by a protein assay kit, and the mucosal PGE2 level was expressed as picogram per milligram of protein.

**PGE2 and Dexamethasone Treatments on Ulcer Healing.** One day after ulcer induction, rats were given PGE2 (intraperitoneal injection at 100 or 200 μg/kg/day) or vehicle (0.01 M phosphate-buffered saline solution) immediately before each dexamethasone treatment applied intragastrically at the dose of 0.2 mg/kg once daily for 3 or 6 days in the same batch of rats. Likewise, PGE2 treatment did not affect the physical health of the animals. Ulcer sizes, cell proliferation, and blood vessel count at the ulcer margin and base were determined.

---

**TABLE 1**

Effect of dexamethasone on mucus thickness at the ulcer margin on day 4 and day 7 after ulcer induction

Field 1 means at the ulcer margin; fields 2 and 3 are the areas away from the ulcer margin to the adjacent normal mucosa. Values are means ± S.E.M. of 8 to 12 rats per group.

<table>
<thead>
<tr>
<th>Field 1</th>
<th>Field 2</th>
<th>Field 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>10.5 ± 3.6</td>
<td></td>
</tr>
<tr>
<td>Day 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>39.7 ± 2.8***</td>
<td>30.4 ± 1.3</td>
</tr>
<tr>
<td>Dexamethasone 0.1 mg/kg</td>
<td>29.0 ± 1.8**</td>
<td>22.3 ± 1.5**</td>
</tr>
<tr>
<td>Dexamethasone 0.2 mg/kg</td>
<td>24.9 ± 0.8***</td>
<td>18.0 ± 1.3***</td>
</tr>
<tr>
<td>Day 7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>34.9 ± 2.3***</td>
<td>27.1 ± 2.5</td>
</tr>
<tr>
<td>Dexamethasone 0.1 mg/kg</td>
<td>30.4 ± 3.2</td>
<td>20.7 ± 2.5</td>
</tr>
<tr>
<td>Dexamethasone 0.2 mg/kg</td>
<td>23.2 ± 2.0*</td>
<td>15.9 ± 2.8**</td>
</tr>
</tbody>
</table>

* p < 0.05, ** p < 0.01, *** p < 0.001 when compared with the respective control group; ††† p < 0.001 when compared with the normal group.

---

**Fig. 2.** Effect of dexamethasone (Dex, 0.1 or 0.2 mg/kg given intragastrically once daily) on epithelial cell proliferation measured from the edge of ulcer margin toward the normal mucosa (i.e., from the first to the third field) on day 4 and day 7 after ulcer induction. Values are means ± S.E.M. of 8 to 12 rats per group. † p < 0.05, †† p < 0.01 when compared with the normal mucosa; ††† p < 0.001 when compared with the respective control group.

**Fig. 3.** Effect of dexamethasone (Dex, 0.1 or 0.2 mg/kg given intragastrically once daily) on angiogenesis measured at the ulcer margin and ulcer base on day 4 and day 7 after ulcer induction. Values are means ± S.E.M. of 8 to 12 rats per group. †† p < 0.01, ††† p < 0.001 when compared with the normal mucosa; * p < 0.05, ** p < 0.01 when compared with the respective control group.
the ulcer margin and base was markedly increased 4 and 7 days after ulcer induction ($p < 0.01$). Administration of dexamethasone significantly decreased numbers of microvessels at ulcer base and ulcer margin on day 4 and day 7 after ulcer induction in a dose-related manner when compared with those of the respective control group (Fig. 3).

**Effect of Dexamethasone on Apoptosis at the Ulcer Margin.** The number of apoptotic cells in the epithelium was significantly increased at the ulcer margin only on day 7 after ulcer induction when compared with that of the normal mucosa ($p < 0.05$). Dexamethasone did not significantly affect the number of apoptotic cells in the gastric epithelium at the ulcer margin 4 and 7 days after ulcer induction (Table 2).

**Effects of Dexamethasone on Protein Expressions of COX-1, COX-2, and cPLA2 at the Ulcer Margin.** COX-2 protein expression was very weak in intact normal gastric mucosa. Ulcer induction markedly increased the COX-2 protein expression by more than 5-fold when compared with the normal mucosa ($p < 0.001$). Dexamethasone dose-dependently decreased COX-2 protein expression at the ulcer margin on day 4 and day 7 after ulcer induction when compared with the respective control group (Fig. 4A). Again, significant effect was observed at the higher dose of dexamethasone. However, COX-1 protein expression in the gastric mucosa was not significantly different among the normal group, control group, and the dexamethasone-treated groups (Fig. 4B).

Regarding the cPLA2 protein expression, ulcer induction did not significantly alter the expression of this protein in the gastric mucosa, although dexamethasone had a tendency to reduce the protein level on day 4 and day 7 after ulcer induction (Fig. 4C).

**Effect of Dexamethasone on Mucosal PGE2 Level.** Dexamethasone treatment alone in normal rats without ulcer did not significantly affect mucosal PGE2 level when compared with the normal control group (normal control, 233 ± 71 pg/mg of protein versus dexamethasone (0.2 mg/kg)-treated group, 318 ± 64 pg/mg of protein, $p = 0.394$). There was more than a 10-fold increase in mucosal PGE2 level at ulcer margin when compared with those of the normal mucosa. The increase was more prominent at day 7 after ulcer induction ($p < 0.001$). Dexamethasone treatment dose-dependently decreased mucosal PGE2 level at the ulcer margin on day 4 and day 7 after ulcer induction when compared with those of the respective control group (Fig. 5).

**Table 2**

Effect of dexamethasone on epithelial cell apoptosis at the ulcer margin on day 4 and day 7 after ulcer induction

<table>
<thead>
<tr>
<th>Field 1</th>
<th>Field 2</th>
<th>Field 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>2.2 ± 0.7</td>
<td>2.5 ± 0.6</td>
</tr>
<tr>
<td>Day 4</td>
<td>3.0 ± 0.7</td>
<td>2.7 ± 0.5</td>
</tr>
<tr>
<td>Control</td>
<td>3.0 ± 0.6</td>
<td>2.5 ± 0.6</td>
</tr>
<tr>
<td>Dexamethasone 0.1 mg/kg</td>
<td>3.4 ± 0.4</td>
<td>3.0 ± 0.5</td>
</tr>
<tr>
<td>Dexamethasone 0.2 mg/kg</td>
<td>3.0 ± 0.6</td>
<td>2.8 ± 0.6</td>
</tr>
<tr>
<td>Day 7</td>
<td>4.3 ± 0.7†</td>
<td>3.2 ± 0.6</td>
</tr>
<tr>
<td>Control</td>
<td>4.3 ± 0.6</td>
<td>3.0 ± 0.5</td>
</tr>
<tr>
<td>Dexamethasone 0.1 mg/kg</td>
<td>3.4 ± 0.4</td>
<td>3.2 ± 0.5</td>
</tr>
<tr>
<td>Dexamethasone 0.2 mg/kg</td>
<td>3.0 ± 0.6</td>
<td>2.8 ± 0.6</td>
</tr>
</tbody>
</table>

† $p < 0.05$ when compared with the normal group.

**Statistical Analysis**

The results were expressed as means ± S.E.M. The number of animals in each group used ranged from eight to ten. Differences between means were analyzed with Student’s $t$ test and one-way analysis of variance when appropriate. Values of $p < 0.05$ were considered statistically significant. To avoid subjective bias on the assessment of histological parameters measured in this study, samples were blinded from the observers when they were determined.

**Results**

**Effect of Dexamethasone on Gastric Mucosal Damage.** There were no observable petechiae or erosions found in the gastric glandular mucosa after 9 days of 0.2 mg/kg dexamethasone treatment. Microscopically, the epithelium of mucosa and glandular architecture were intact, and there was no sign of hemorrhage or inflammatory cells in the mucosal and submucosal layers of the stomach.

**Effect of Dexamethasone on Gastric Ulcer Healing.** The average ulcer sizes in the control were similar to those of the dexamethasone treatment groups in day 4 after ulcer induction. On day 7 and day 10, ulcer sizes were dose-dependently increased in the dexamethasone-treated groups. Significant effect was indicated in the higher dose of dexamethasone when compared with the control group on day 7 and also on day 10 after ulcer induction, implicating that dexamethasone could delay ulcer healing (Fig. 1).

**Effect of Dexamethasone on Thickness of Gastric Mucus Layer.** The thickness of mucus layer at the ulcer margin was the highest and significantly decreased from there to the adjacent normal mucosa. The thickness of mucus layer in the gastric mucosa was higher on day 4 than on day 7 after ulcer induction. Dexamethasone treatment dose-dependently decreased the thickness of mucus layer at all parts of the mucosa, especially at the ulcer margin on day 4 and day 7 after ulcer induction when compared with those of the control group (Table 1).

**Effect of Dexamethasone on Epithelial Cell Proliferation at the Ulcer Margin.** Epithelial cell proliferation in the control group was increased significantly at the ulcer margin after ulcer induction when compared with those of normal mucosa ($p < 0.01$). Dexamethasone treatment dose-dependently decreased epithelial cell proliferation at the ulcer margin on day 4 and day 7 after ulcer induction when compared with those of the respective control group (Fig. 2).

**Effect of Dexamethasone on Angiogenesis at the Ulcer Margin and Base.** Again, the number of blood vessels at day 7 after ulcer induction when compared with the respective control group (Fig. 5).
Reversal of PGE₂ Treatment on the Effects of Dexa-methasone on Ulcer Healing. PGE₂ administration at the doses of 100 or 200 g/kg dose-dependently reversed the adverse action of dexamethasone on the delay of ulcer healing on day 7 after ulcer induction (Fig. 6). The same doses of PGE₂ also significantly attenuated the inhibitory actions of dexamethasone on the number of proliferative cells at the ulcer margin (Fig. 7) and also the number of blood vessels at the ulcer margin and base (Fig. 8).

Fig. 4. A, effect of dexamethasone (Dex, 0.1 or 0.2 mg/kg given intragastrically once daily) on COX-2 protein expression at the ulcer margin on day 4 and day 7 after ulcer induction. Values are means ± S.E.M. of 8 to 12 rats per group. †††, p < 0.001 when compared with the normal mucosa. *, p < 0.05, **, p < 0.01 when compared with the respective control group. B, effect of dexamethasone (Dex, 0.1 or 0.2 mg/kg given intragastrically once daily) on COX-1 protein expression at the ulcer margin on day 4 and day 7 after ulcer induction. Values are means ± S.E.M. of 8 to 12 rats per group. C, effect of dexamethasone (Dex, 0.1 or 0.2 mg/kg given intragastrically once daily) on cPLA₂ protein expression at the ulcer margin on day 4 and day 7 after ulcer induction. Values are means ± S.E.M. of 8 to 12 rats per group. *, p < 0.05 when compared with the respective control group.

Discussion

In the current study, we demonstrated for the first time that dexamethasone given at non-ulcerogenic doses 0.1 or 0.2 mg/kg/day relevant to those used in different clinical conditions ranging from 0.05 to 0.2 mg/kg/day or its equivalent dose (Vecht, 1998; Chatham and Kimberly, 2001; Singh et al., 2002; Yang and Lichtenstein, 2002), delayed gastric ulcer healing. To further explore this problem, we used the gastric ulcer model to investigate the pathological mechanism of dexamethasone on ulcer healing.

In this study, the highest dose of dexamethasone 0.2 mg/kg/day for 9 days did not affect the basal mucosal PGE₂ level; the expression of COX-2 was extremely low and the COX-1 expression was unaffected (Fig. 4). These results demon-
strated that dexamethasone at this dosage did not inhibit COX-1 protein expression and its biological activity. This finding also coincided with our finding that dexamethasone under this dosage did not induce mucosal damage because inhibition of both COX-1 and COX-2 was essential for gastric injury in animals (Wallace et al., 2000). However, previous studies showed discordant results regarding glucocorticoid-associated mucosa damage (Nobuhara et al., 1985; Wallace, 1987; Filep et al., 1992; Akiba et al., 1998; Gretzer et al., 2001). These dichotomous findings may be due to different dosages and administration frequency of corticosteroid at different time intervals in evaluating mucosal injury. Our approaches in the selection of dosages and the duration of drug treatment were more relevant to those in clinical situations. Therefore, the current findings with dexamethasone on ulcer healing could have significant implications in patients with gastric ulcer.

Our study also showed that COX-2 protein expression and PGE₂ formation were significantly increased at the ulcer margin at day 4 and day 7 after ulcer induction when compared with those of the normal group, but the protein expression of COX-1 and cPLA₂ were unaffected. These findings implicated that the increase of PGE₂ in the gastric mucosa after ulcer induction was largely derived from the activation of COX-2. Furthermore, dexamethasone dose-dependently decreased COX-2 expression and PGE₂ formation without affecting COX-1 and cPLA₂ expressions, which confirmed further the above phenomenon. These results were consistent with previous reports stating that COX-1 was constitutive and COX-2 was inducible by cytokines and growth factors during ulceration. Dexamethasone only decreased COX-2 expression and PGE₂ formation by this enzyme (Wolfe et al., 1999; Fernandez-Morata et al., 2000; Gretzer et al., 2001). Previous studies showed that glucocorticoid inhibited PLA₂ activity, which influenced the liberation of arachidonic acid, a precursor for PG synthesis (Flower, 1988; Goppel-Struede et al., 1989). Our result revealed that ulcer induction did not affect the expression of cPLA₂, suggesting that the amount of arachidonic acid in the gastric mucosa could be sufficient to support the elevated COX-2 activity due to ulceration that produces more PGE₂ in the tissue. Therefore, it suggests that cPLA₂ is not the limiting enzyme for PGE₂ synthesis during ulceration in the gastric mucosa.

Ulcer induction initiated epithelial cell proliferation and angiogenesis at the ulcer margin. Both of them play a pivotal role in ulcer healing (Tarnawski et al., 2002; Wang, 2002). At the non-ulcerogenic doses of dexamethasone, it significantly inhibited cell proliferation and angiogenesis at the ulcer margin. As discussed before, dexamethasone decreased COX-2 expression and PGE₂ formation at the same site, both of which are important factors for gastric ulcer healing (Mizuno et al., 1997). COX-2-derived PGE₂ increases vascular endothelial growth factor expression, which stimulates endothelial cell proliferation and angiogenesis (Ghosh et al., 2000; Szabo et al., 2000; Pai et al., 2001). This may be the reason why dexamethasone decreased angiogenesis at the ulcer margin and ulcer base. PG also can induce the expression of hepatocyte growth factor, which strongly stimulates proliferation of gastric epithelial cells (Takahashi et al., 1996). In addition, PGE₂ rapidly phosphorylates epidermal growth factor receptor and triggers the extracellular signal-regulated kinase 2-mitogenic signaling pathway in normal gastric epithelial cell lines (Pai et al., 2002). All of these could lead to more cell proliferation at the ulcer margin. This might explain why depletion of PGE₂ by dexamethasone could decrease epithelial cell proliferation as well as angiogenesis at
the ulcer margin and thereby delay ulcer healing in
the stomach, whereas supplementation with PGE₂ dose-depen-
dently resumed these ulcer-healing processes back to normal
rates in the gastric mucosa.

In this study, we also found that mucous thickness at the
ulcer margin was increased profoundly after ulcer induction.
This could be a positive biological feedback mechanism to
preserve the ulcer from further ulceration and promote ulcer
healing (Ma et al., 2000). Dexamethasone administration
markedly decreased gastric mucous thickness and weakened
the defensive mechanism in the gastric mucosa. In fact, dexa-
methasone could down-regulate gastric mucin gene expres-
sion and thereby decrease mucin biosynthesis (Okazaki et
al., 1998). PG deficiency also impairs gastric mucous produc-
tion (Menguy and Masters, 1963). It is likely that the reducti-
on of mucus layer induced by dexamethasone could be the
result of its direct action on mucin gene expression in the
nucleus and also indirectly through the depletion of PG in the
gastric mucosa.

In conclusion, ulcer induction activates the repair sys-
tem in the gastric mucosa. This system includes mucous se-
cretion, epithelial cell proliferation, and angiogenesis at the
ulcer margin to promote ulcer healing in the stomach. Dexam-
ethasone given at non-ulcerogenic doses could deter such
defensive mechanisms at the early stage of the regenerative
mechanism. These adverse actions were probably due to the
down-regulation of COX-2 and depletion of PGE₂ in the
gastric mucosa. The present study reports for the first time that
dexamethasone given at non-ulcerogenic doses similar to
those in humans could worsen the ulcer healing process
through a defined mechanism in rat stomachs.

References
Akiba Y, Nakamura M, Mori M, Suzuki H, Oda M, Kimura H, Miura S, Tsuchiya M,
and Ishii H (1998) Inhibition of inducible nitric oxide synthase delays gastric ulcer
angiogenesis. Phenotypic characterization of endothelial cells in a physiological
Bandyopadhyay U, Biswas K, Bandyopadhyay D, Ganguly CK, and Banerjee RK
(1998) Dexamethasone makes the gastric mucosa susceptible to ulceration by
inhibiting prostaglandin synthase and peroxidase–two important gastroprotective
reduce regenerative repair of epithelium in experimental gastric ulcers. Gut 37:
613–616.
Lupus 10:140–147.
Cho CH, Chen BW, Hui WM, Luk CT, and Lam SK (1990) Endogenous prostag-
landins: its role in gastric mucosal blood flow and ethanol ulceration in rats.
Prostaglandins 40:397–403.
Conn HO and Blitzer BL (1976) Nonassociation of adrenocorticosteroid therapy and
Gavriel Y, Sherman Y, and Ben-Sasson SA (1992) Identification of programme
cell death in situ via specific labeling of nuclear DNA fragmentation. J Cell Biol
angiogenesis in carrageenan-induced granuloma tissue in rats. J Pharmac Exp
Ther 295:802–809.
Goppi-Mestre M, Wolter D, and Resch K (1989) Glucocorticoids inhibit prostag-
landin synthesis not only at the level of phospholipase A2 but also at the level of
specific inhibition of cyclooxygenase-1 and cyclooxygenase-2 in the rat stomach
inhibition of cyclooxygenase expression in bovine term placenta. Prostaglandins
Kawayama H, Matsuyu Y, and Eastwood GL (1991) Effects of prostaglandins on
hydrocortisone-induced delayed healing of chronic gastric ulcers in the rat. J Clin
Lucy ER, Kawayama H, Court WS, Deutz AH, and Sistrunk S (1991) A rapid, accurate,
immunohistochemical method in endothelial proliferating cells in the
digestive tract. A comparison with tritiated thymidine. Gastroenterology 100:259–
262.
growth factor, epidermal growth factor, and constitutive nitric oxide synthase on
Menguy B and Masters YF (1963) Effect of cortisol on mucoprotein secretion by
Messer J, Reitman D, Sacks HS, Smith HJ, and Chalmers TC (1983) Association of
Minowa Y, Komoto C, Matsuda K, Wada K, Uchida T, Noguchi H, Akamatsu T,
and Kasuga M (1997) Induction of cyclooxygenase-2 in gastric mucosal lesions and
its inhibition by the specific antagonist delays healing in mice. Gastroenterology
113:257–267.
alkaline response in rat stomach. A possible explanation for steroid-induced gas-
Okazaki K, Chiba T, and Hajiri K (1998) Downregulation of gastric mucin gene expres-
sion and its biosynthesis by dexamethasone in the human. J Clin Gastro-
Prostaglandin E2 transactivates EGF receptor: a novel mechanism for promoting
PGE₂ stimulates VEGF expression in endothelial cells via ERK2/JNK1 signaling
for patients with acute exacerbations of chronic obstructive pulmonary disease: a
Review article: transcription factors and growth factors in ulcer healing. Aliment
Takahashi M, Ota S, Hata Y, Mikami Y, Azuma N, Nakamura T, Terano A, and
Oomura Y (1996) Hepatocyte growth factor as a key to modulate anti-ulcer action
Tarnawski AS, Jones MR, Baatar D, and Pai R (2002) Role of angiogenesis and
angiogenesis growth factors in mucosal repair and ulcer healing, in Gastrointesti-
nal Medical Repair and Experimental Therapies (Cho CH and Wang JY eds) pp
101–116, Karger, Basel, Switzerland.
Tsukimi Y and Okabe S (1994) Validity of kissing gastric ulcers induced in rats for
Wallace JL (1987) Glucocorticoid-induced gastric mucosal damage: inhibition of
leukotriene, but not prostaglandin biosynthesis. Prostaglandins 34:311–323.
Wallace JL, McLaughlin R, and Vargnolle N (2000) NSAID-induced gastric damage
in rats: requirement for inhibition of both cyclooxygenase 1 and 2. Gas-
troenterology 118:706–714.
apoptosis in the rat gastric mucosa through a reactive oxygen species-mediated
mucosal injury, in Gastrointestinal Medical Repair and Experimental
Therapies (Cho CH and Wang JY eds) pp 62–100, Karger, Basel, Switzerland.
integrity by endogenous nitric oxide: interactions with prostanoids and sensory
Wolf MM, Lichtenstein DR, and Singh G (1999) Gastrointestinal toxicity of nonste-
Gastroenterol 97:803–830.
Address correspondence to: Prof. C. H. Cho, Department of Pharmacology,
Faculty of Medicine, 2nd Floor, Laboratory Block, The University of Hong
Kong, 21 Sassoon Rd., Hong Kong, China. E-mail: chcho@hkusua.hku.hk.