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

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Nonstandard abbreviations: PG: prostaglandin, cPLA2: cytosolic phospholipase A2, COX: cyclooxygenase

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ABSTRACT

While 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 stomachs. In this regard, we measured ulcer size, mucus thickness, epithelial cell proliferation and apoptosis and also angiogenesis at the ulcer site at different time points after ulcer induction. Protein expressions of cyclooxygenases (COX-1 and COX-2) and cytosolic phospholipase A2 (cPLA2) over the ulcer margin were evaluated and the mucosal prostaglandin E2 (PGE2) level were also determined. Dexamethasone treatment in the current doses did not produce mucosal damage in intact animals. The drug however 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 the 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 to have 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 A2 (PLA2) 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 would like to 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.
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 (200-220g) 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 (12h: 12h light/dark) were controlled. Rats were fasted for 24 hours 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 Chemical Co. unless otherwise stated. Dexamethasone was prepared in 1% ethanol vehicle for intragastric administration. PGE2 was dissolved in 0.01M phosphate buffer 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 and associates (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 mucosa, 3) deep necrosis and ulceration. For further comparisons
in this part with the other portions of the study, we also collected mucosae 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 (i.d.
10mm) 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
forty-five 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 were determined in each stomach. After measuring the ulcer areas, gastric tissues were excised for immunohistological 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 (PAS) technique. Finally, they were counterstained with Harris hematoxyline 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 one hour 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 mm² (200X) was counted in both sides of 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 0.899mm² (200X). 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 deoxy-transferase (TdT)-mediated dUTP-biotin 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 under a microscopic field of 0.899 mm² (200X) and was expressed by taking the average of both sides of ulcer margin.

Western blotting for COX-1, COX-2, and cPLA₂ expressions.

Gastric tissues were homogenized (Ultra-Turrax, Janke & Kunkel Co, Staufen, Germany) with RIPA 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 Instrument, CA) for supernatants. Protein concentration was measured using a protein assay kit with BSA as a standard (Bio-Rad Laboratories, Hercules, CA). Proteins were separated by electrophoresis on a 7.5% (COX-1, COX-2) or 5% (cPLA₂) SDS-acrylamide gel, and then transferred to Hybond C nitrocellulose membranes (Amersham International plc, Amersham, UK). Membranes were probed with antibodies against COX-1, COX-2, and cPLA₂ (Santa Cruz Biotechnology, Inc, Santa Cruz, CA) overnight at 4°C and incubated for 1 hour with secondary antibodies conjugated with peroxidase. Membranes were developed by the enhanced chemiluminescence system (Amersham Biosciences, Piscataway, NJ) and exposed to an X-ray film (Fuji Photo...
Measurement of mucosal PGE₂ level.

Gastric tissues were homogenized with homogenizing buffer (0.05M Tris-HCl at pH7.4, 0.1M NaCl, 0.001M CaCl₂, 1mg/mL D-glucose, 28µM indomethacin to inhibit further PGE₂ formation) for 30 seconds. They were then centrifuged at 12000 rpm for 15 minutes at 4 °C. Supernatants were assayed by using a commercial available PGE₂ ELISA kit (Quantikine, R&D systems Inc, Minneapolis, MN). The assay procedure was in accordance to 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 pg/mg protein.

Part III: 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 (0.01M phosphate buffer 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.

Statistical analysis.

The results were expressed as means ± S.E.M. The number of animals in each group used was ranging from eight to ten. Differences between means were analyzed with Student’s t-test and one-way analysis of variance (ANOVA) when appropriate. P values of <0.05 were considered statistically significant. In order 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 in day 7 and also in 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 in day 4 than in 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 in day 4 and day 7 after ulcer induction when compared to 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 to those of normal mucosa (p < 0.01). Dexamethasone treatment dose-dependently decreased epithelial cell proliferation at the ulcer margin in day 4 and day 7 after ulcer induction when compared to 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 the ulcer margin and base was markedly increased 4 and 7 days after ulcer induction (p < 0.01). Administration dexamethasone significantly decreased numbers of microvessels at ulcer base and ulcer margin in day 4 and day 7 after ulcer induction in a dose-related manner when compared to 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 to 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 cPLA$_2$ 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 five folds when compared to the normal mucosa ($p < 0.001$). Dexamethasone dose-dependently decreased COX-2 protein expression at the ulcer margin at day 4 and day 7 after ulcer induction when compared to 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 cPLA$_2$ 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 in 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 to the normal control group (normal control: 233 ± 71 pg/mg protein versus dexamethasone (0.2 mg/kg)-treated group: 318 ± 64 pg/mg protein, p=0.394). There was more than 10-fold increased in mucosal PGE₂ level at ulcer margin when compared to 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 in day 4 and day 7 after ulcer induction when compared to those of the respective control group (Fig. 5).

**Reversal of PGE₂ treatment on the effects of dexamethasone on ulcer healing.**

PGE₂ administration at the doses of 100 or 200 µg/kg dose-dependently reversed the adverse action of dexamethasone on the delay of ulcer healing at 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).
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 - 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, in which the expression of COX-2 was extremely low and the COX-1 expression was unaffected (Fig.4). These results demonstrated 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 were 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 dichotomy 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 gastric ulcer patients.

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 to 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, 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 the 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; Goppelt-Struebe 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 enough to support the elevated COX-2 activity due to ulceration to produce more PGE₂ in the tissue. It is therefore suggesting 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 PGE$_2$ formation at the same site, both of them are important factors for gastric ulcer healing (Mizuno et al., 1997). COX-2 derived PGE$_2$ 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, PGE$_2$ 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 these could lead to more cell proliferation at the ulcer margin. This might explain why depletion of PGE$_2$ 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 PGE$_2$ dose-dependently resumed these ulcer-healing processes back to normal rate in the gastric mucosa.
In this study, we also found that mucus 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 mucus thickness and weakened the defensive mechanism in the gastric mucosa. In fact, dexamethasone could down-regulate gastric mucin gene expression and thereby decrease mucin biosynthesis (Okazaki et al., 1998). PG deficiency also impairs gastric mucus production (Menguy and Masters, 1963). It is likely that the reduction 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 repairing system in the gastric mucosa. This system includes mucus secretion, epithelial cell proliferation and angiogenesis at the ulcer margin in order to promote ulcer healing in the stomach. Dexamethasone 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


Footnotes

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Figure legends

**Fig. 1.** Effect of dexamethasone (Dex, 0.1 or 0.2 mg/kg given intragastrically once daily) on gastric ulcer healing. Ulcer sizes were measured at day 4, 7 and day 10 after ulcer induction. Values are means ± S.E.M of 8-12 rats per group. **p < 0.01 when compared to the respective control 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 1st field to the 3rd field) at day 4 and day 7 after ulcer induction. Values are means ± S.E.M. of 8-12 rats per group. †p < 0.05, ††p < 0.01 when compared to the normal mucosa; *p < 0.05, **p < 0.01, ***p < 0.001 when compared to 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 at day 4 and day 7 after ulcer induction. Values are means ± S.E.M. of 8-12 rats per group. ††p < 0.01, †††p < 0.001 when compared to the normal mucosa; *p < 0.05, **p < 0.01 when compared to the respective control group.

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

**Fig. 4B.** Effect of dexamethasone (Dex, 0.1 or 0.2 mg/kg given intragastrically once daily) on cyclooxygenase-1 (COX-1) protein expression at the ulcer margin at day 4 and day 7 after ulcer induction. Values are means ± S.E.M. of 8-12 rats per group.

**Fig. 4C.** Effect of dexamethasone (Dex, 0.1 or 0.2 mg/kg given intragastrically once daily) on cytosolic phospholipase A2 (cPLA2) protein expression at the ulcer margin at day 4 and day 7 after ulcer induction. Values are means ± S.E.M. of 8-12 rats per group. *p < 0.05 when compared to the respective control group.

**Fig. 5.** Effect of dexamethasone (Dex, 0.1 or 0.2 mg/kg given intragastrically once daily) on mucosal prostaglandin (PG) E2 level at the ulcer margin at day 4 and day 7 after ulcer induction. Values are means ± S.E.M. of 8-12 rats per group. ††p < 0.01, †††p < 0.001 when compared to the normal mucosa. *p < 0.05, **p < 0.01 when compared to the respective control group.

**Fig. 6.** Effect of prostaglandin E2 treatment (PGE2, 100 or 200 µg/kg injected
intraperitoneally once daily) on the inhibitory action of dexamethasone (Dex) on ulcer healing.

Ulcer sizes were measured at day 4 and day 7 after ulcer induction. Values are means ± S.E.M. of 8-12 rats per group. †p < 0.05 when compared to the respective dexamethasone-treated group; *p < 0.05 when compared to the respective dexamethasone-treated group.

**Fig. 7.** Effect of prostaglandin E₂ treatment (PGE₂ 100 or 200 µg/kg injected intraperitoneally once daily) on the inhibitory action of dexamethasone (Dex) on epithelial cell proliferation measured from the edge of ulcer margin toward the normal mucosa (i.e. from the 1st field to 3rd field) at day 4 and day 7 after ulcer induction. Values are means ± S.E.M. of 8-12 rats per group. †p < 0.05, ††p < 0.01, †††p < 0.001 when compared to the respective dexamethasone-treated group; *p < 0.05, **p < 0.01 when compared to the respective dexamethasone-treated group.

**Fig. 8.** Effect of prostaglandin E₂ treatment (PGE₂ 100 or 200 µg/kg injected intraperitoneally once daily) on the inhibitory action of dexamethasone (Dex) on angiogenesis measured at the ulcer margin and ulcer base at day 4 and day 7 after ulcer induction. Values are means ± S.E.M. of 8-12 rats per group. †p < 0.05, ††p < 0.01 when compared to the respective dexamethasone-treated group; *p < 0.05, **p < 0.01 when compared to the respective dexamethasone-treated group.
Table 1. Effect of dexamethasone on mucus thickness at the ulcer margin at day 4 and day 7 after ulcer induction.

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<tr>
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<tr>
<td>Dexamethasone 0.2mg/kg</td>
<td>23.2±2.5*</td>
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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 8-12 rats per group. *p < 0.05, **p < 0.01, ***p < 0.001 when compared to the respective control group; †††p < 0.001 when compared to the normal group.
Table 2. Effect of dexamethasone on epithelial cell apoptosis at the ulcer margin at day 4 and day 7 after ulcer induction.

<table>
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<th>Field 1</th>
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<th>Field 3</th>
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<td>Normal</td>
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<tr>
<td>Day 4 Control</td>
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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 8-12 rats per group. †p < 0.05 when compared to the normal group.
Figure 1.

Days after ulcer induction

Ulcer size (mm²)

- Control
- Dex 0.1 mg/kg
- Dex 0.2 mg/kg

** p < 0.01
Figure 2.

Number of fields away from ulcer site

Day 4 after ulcer induction        Day 7 after ulcer induction
Figure 3.

Days after ulcer induction

**Number of microvessels/mm²**

- **Normal**
- **Control**
- **Dex 0.1 mg/kg**
- **Dex 0.2 mg/kg**

**Day 4**
- **Margin**
- **Base**

**Day 7**
- **Margin**
- **Base**

Statistical significance indicated by:
- * p ≤ 0.05
- ** p ≤ 0.01
- *** p ≤ 0.001
- **** p ≤ 0.0001
Figure 4.

(A) COX-2 protein (O.D. (% change from control))

(B) COX-1 protein (O.D. (% change from control))

(C) cPLA2 protein (O.D. (% change from control))

**Days after ulcer induction**
Figure 5.

Days after ulcer induction

- Normal
- Control
- Dex 0.1 mg/kg
- Dex 0.2 mg/kg

Mucosal PGE$_2$ (pg/mg protein)
Figure 6.

Days after ulcer induction

Ulcer size (mm²)

- Control
- Dex 0.2 mg/kg
- Dex 0.2 mg/kg + PGE₂ 100 μg/kg
- Dex 0.2 mg/kg + PGE₂ 200 μg/kg
Figure 7.

Number of fields away from ulcer site

Day 4 after ulcer induction          Day 7 after ulcer induction
Figure 8.

Days after ulcer induction