Dexamethasone Inhibits Epidermal Growth Factor-Stimulated Gastric Epithelial Cell Proliferation

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ABSTRACT

Epidermal growth factor (EGF) is essential to heal gastric ulcers, whereas glucocorticoid delays rat gastric ulcer healing. We found that dexamethasone inhibited EGF-stimulated rat gastric epithelial cell (RGM-1) proliferation by cell count and DNA synthesis analysis of flow cytometry and attempted to elucidate the possible mechanistic pathway via Western blot analysis. EGF (10 ng/ml) treatment for 24 h significantly increased RGM-1 cell proliferation, and dexamethasone (10^{-8} and 10^{-6} M) markedly suppressed EGF-stimulated cell proliferation. Western blotting results demonstrated that the phosphorylated extracellular signal-regulated kinase (pERK) (pERK1/pERK2) significantly increased at 10 min after EGF treatment. This was followed by increase of cyclooxygenase (COX)-2 expression at 3 h after EGF treatment. The continued increase of COX-2 (up to 18 h) resulted in increased intracellular prostaglandin E_2 and cyclin D1 expression significantly after 8 and 12 h of EGF treatment. Dexamethasone substantially reduced EGF-stimulated COX-2 expression at 3 and 6 h and cyclin D1 expression at 8 and 12 h. Pretreatment of RGM-1 cells with dexamethasone or 2’-amino-3’-methoxyflavone (PD98059)-mitogen-activated protein kinase inhibitor (5 × 10^{-5} M) significantly reduced EGF-stimulated pERK1/pERK2 expression. Simultaneous treatment of RGM-1 cells with PD98059 and EGF also markedly decreased EGF-stimulated COX-2 expression at 6 h. These findings indicate that dexamethasone significantly suppresses EGF-stimulated gastric epithelial cell proliferation, and one of the pathways involved is via inhibiting activation of ERK1/ERK2, followed by inhibition of COX-2, cyclin D1 expression, and finally DNA synthesis.

Ulcer healing is a programmed repair process, including inflammation, cell proliferation, granulation tissue formation, and angiogenesis. Growth factors such as epidermal growth factor (EGF) and basic fibroblast growth factor, which are produced at the injured gastric mucosa or ulcer margins, may regulate the process of mucosal healing by coordinating the timing of early- and late-phase responses (Szabo et al., 2000; Tarnawski et al., 2001; Yoo et al., 2002). The early-phase response occurs in the absence of cellular proliferation and is called restitution (cell migration) (Silen and Ito, 1985; Yoo et al., 2002). The late-phase response consists of cell division and cell proliferation to fill in the defect and restore mucosal architecture (Szabo et al., 2000; Tarnawski, 2005). EGF, a well studied growth factor, plays a critical role in ulcer healing and mucosal protection via mitogenic and non-mitogenic actions (Uribe and Barrett, 1997; Tarnawski and Jones, 1998).

The role of EGF and its receptor in regulating gastric mucosal cell proliferation has been well documented (Tarnawski et al., 1992; Konturek et al., 1995; Tarnawski and Jones, 1998). Binding of growth factors, such as EGF and...
transforming growth factor-α, to their common receptor produces the autophosphorylation of receptor tyrosine kinase on tyrosine residues, inducing cascading events that lead to activation of mitogen-activated protein kinases (MAPKs) (Lemmon and Schlessinger, 1994; Heldin, 1995). These MAPKs have a central role in the signal pathways that regulate cell proliferation, migration, and differentiation (Blenis, 1993; Vietor et al., 1993). Of the several MAPK family members, extracellular signal-regulated kinase (ERK1 and 2 have been shown to play crucial roles in healing of ulcerated or injured mucosa (Pai et al., 1998). Once activated, MAPKs translocate into the nucleus, where they induce transcription factors, including proto-oncogenes such as c-fos, c-myc, and c-jun, and cell cycle activator cyclin D1 (Blenis, 1993; Hill and Treisman, 1995; Lavoie et al., 1996; Torada et al., 1999). EGF also up-regulates cyclooxygenase (COX)-2 expression via ERK and p38 MAPK signaling in rat gastric ulcerated mucosa and in other cell lines (Brezowski et al., 2001; Huh et al., 2003; Sasaki et al., 2003), COX-2 expression via ERK and p38 MAPK signaling in rat gastric ulcer healing by inhibiting epithelial cell proliferation; and 4) affecting prostaglandin synthesis via decreased prostaglandin (PG) formation is essential for cell proliferation and ulcer healing (Peskar et al., 2001; Luo et al., 2003).

The pharmacological actions of glucocorticoids involve 1) inhibiting proinflammatory activities of transcription factors, in particular nuclear factor-κB; 2) altering T-helper cell type 1/Thelper cell type 2 cytokine balance; 3) inducing annexin 1 (lipocortin 1) synthesis, thereby suppressing arachidonic acid release via antagonizing phospholipase A2 activity; and 4) affecting prostaglandin synthesis via decreasing COX-2 expression (Almawi et al., 1999; Roviezo et al., 2002; Yang and Lichtenstein, 2002). Animal studies demonstrated that dexamethasone, which is a potent corticosteroid, delayed rat gastric ulcer healing by inhibiting epithelial cell proliferation and angiogenesis at the ulcer margin and base (Carpani de Kaski et al., 1995; Luo et al., 2003, 2004). These adverse actions of glucocorticoids on gastric ulcer healing are due to their inhibiting COX-2 expression and PGE2 formation (Luo et al., 2003, 2004).

This in vitro study identified that dexamethasone significantly inhibited EGF-stimulated gastric epithelial cell proliferation in normal gastric epithelial cell line RGM-1. The study also further elucidated the possible mechanistic pathway through which dexamethasone inhibits EGF-stimulated gastric cell proliferation.

**Materials and Methods**

**Study Chemicals and Preparations.** All chemicals studied were purchased from Sigma-Aldrich (St. Louis, MO) unless otherwise specified. Glucocorticoid receptor antagonist mifepristone, COX-2-selective inhibitor NS-398, and MEK inhibitor PD98059 were dissolved in dimethyl sulfoxide; prostaglandin E2 was dissolved in pure ethanol. EGF (recombinant human epidermal growth factor) was purchased from Invitrogen (Carlsbad, CA), 100 μg of EGF was dissolved in distilled water with 0.2% bovine serum albumin (BSA) as a stock solution.

**Cell Culture and Cell Number Determination.** Gastric epithelial cells obtained from the rat gastric mucosal epithelial cell line RGM-1 (RCB-0876; Riken Cell Bank, Tsukuba, Japan) were grown in Dulbecco’s modified Eagle’s medium (DMEM/F-Ham’s F-12 medium (Invitrogen) supplemented with 100 U/ml penicillin G, 100 μg/ml streptomycin, and 20% fetal bovine serum (FBS) (Invitrogen) in an incubator at 37°C and 5% carbon dioxide.

Cells were detached from the wells, using 0.25% trypsin EDTA. Trypsan blue was added to the cell suspension to assess cell viability. Cell numbers were counted using Burker hemocytometer (Marienfeld GmbH, Marienfeld, Germany).

**Flow Cytometry for DNA Synthesis Analysis.** Cellular DNA replication was analyzed by flow cytometry. The two peaks of DNA contents corresponding to G1 and G2/M phase cells, respectively, and the intermediate amount of DNA corresponding to S-phase cells were counted. RGM-1 cells were seeded in a 10-cm dish at roughly 10^5 cells/ml and were allowed to grow in DMEM/Ham’s F-12 medium containing 20% FBS for 24 h. Theretofore, cell growth was arrested in the same medium with 1% FBS for a further 24 h to synchronize cell cycles. Cells were then treated for 24 h with 1% FBS DMEM/Ham’s F-12 medium containing either 10 ng/ml EGF, 10^-6 M dexamethasone, or 10^-6 M mifepristone, the glucocorticoid receptor antagonist. The following combinations were also applied: 10 ng/ml EGF with 10^-6 or 10^-8 M dexamethasone in the presence or absence of 10^-6 M mifepristone; 10 ng/ml EGF with 10^-6 M dexamethasone; 10^-5 M NS-398; or a combination of both. After treatment, cells (~2 x 10^6 each dish) were trypsinized, pelleted, washed with phosphate-buffered saline, resuspended with lysis buffer (0.5% Triton X-100, 0.2 μg/ml Na2EDTA, 2 mM H2O, and 1% BSA) for 15 min. Cells were fixed in 80% cold methanol at −20°C overnight. The fixed cells were centrifuged, washed with phosphate-buffered saline, pre-treated with RNase (5 Konitz U/ml) at 37°C for 30 min, and then reacted with 50 μl of propidium iodide. Cells were analyzed using a FACSCalibur flow cytometer (BD Biosciences, San Jose, CA). Data were analyzed using ModFit and CellQuest software (BD Biosciences) (Huang et al., 2004).

**Western Blot Analysis for ERK1/2, pERK1/2, p38 MAPK, pp38 MAPK, c-Jun NH2-Terminal Kinase 1, pJNK1, Phospholipase A2, COXs, and Cyclin D1.** After synchronization as described above, cells were treated with 1% FBS DMEM/Ham’s F-12 containing 10 ng/ml EGF in the presence or absence of 10^-6 or 10^-8 M dexamethasone or 10^-6 M dexamethasone alone for different periods. In a second set of experiments, cells were pretreated with PD98059 (MEK inhibitor) (10^-5 and 5 x 10^-5 M) or dexamethasone (10^-8 and 10^-6 M) for 2 to 3 h, followed by 10 ng/ml EGF. Cells were then collected in radioimmunoprecipitation assay buffer for Western blot analysis. Following sonication and centrifugation, protein concentration was measured using a protein assay kit (Bio-Rad, Hercules, CA). Proteins were separated by SDS-polyacrylamide gels electrophoresis overlaid with a 5% acrylamide stacking gel and then transferred to Hyperb C nitrocellulose membranes (GE Healthcare, Little Chalfont, Buckinghamshire, UK). Membranes were probed with antibodies against ERK1/2, pERK1/2, p38 MAPK, JNK1, JNK2, cyclin D1, cytosolic phospholipase A2 (cPLA2), COX-1, and COX-2 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) overnight at 4°C and incubated for 1 h with secondary antibodies conjugated with peroxidase. The membrane was developed using the **TABLE 1**

<table>
<thead>
<tr>
<th>Treatment Duration</th>
<th>Cell Count (10^5/ml)</th>
<th>Cell in S Phase of Cell Cycle (% Change from Control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 h</td>
<td>Control</td>
<td>10 mg/ml EGF</td>
</tr>
<tr>
<td>3 h</td>
<td>5 ± 1</td>
<td>100 ± 12</td>
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<tr>
<td>6 h</td>
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<td>100 ± 12</td>
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<tr>
<td>12 h</td>
<td>14 ± 2</td>
<td>100 ± 18</td>
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<td>18 h</td>
<td>24 ± 3</td>
<td>100 ± 17</td>
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<tr>
<td>24 h</td>
<td>36 ± 3</td>
<td>100 ± 16</td>
</tr>
<tr>
<td>36 h</td>
<td>38 ± 2</td>
<td>100 ± 15</td>
</tr>
</tbody>
</table>

**P < 0.01 compared with the control group.**
enhanced chemiluminescence system (Amersham Biosciences) and was exposed to an X-ray film (Fuji Photo Film, Tokyo, Japan). Quantitation was performed using a densitometer (Scan Marker III, Microtek, Carson, NV).

**Measurement of Intracellular PGE₂ Level.** Following treatment with 10 ng/ml EGF in the presence or absence of dexamethasone (10⁻⁸ and 10⁻⁶ M) or 10⁻⁸ M dexamethasone alone for 8 and 12 h, respectively, cells were homogenized with homogenizing buffer for 30 s (0.05 M Tris-HCl, pH 7.4, 0.1 M NaCl, 0.001 M CaCl₂, 1 mg/ml B-glucose, and 28 μM indomethacin). Then, cells were centrifuged at 12,000 rpm for 15 min at 4°C. Supernatants were assayed by using a PGE₂ enzyme-linked immunosorbent assay kit (Quantikine; R&D Systems, Minneapolis, MN). Assay procedures were performed in accordance with the manufacturer's instructions. Optical densities were determined with 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 medium PGE₂ level was expressed as picograms per milligram of protein.

**Statistical Analysis.** Analytic results are expressed as means ± S.D. There were six samples in each group. Differences between the means were analyzed with the Student’s t test when appropriate. Bonferroni correction was performed to adjust for the fact that multiple comparisons were done in each experiment. A gross P < 0.05 was considered statistically significant.

**Results**

**Proliferation of RGM-1 Cells.** Treatment with 10 ng/ml EGF for 3, 6, and 12 h did not significantly stimulate growth of rat gastric epithelial RGM-1 cells (Table 1), but treatment with EGF for 18, 24, and 36 h significantly stimulated RGM-1 cell proliferation (Table 1). Dexamethasone (10⁻⁸ and 10⁻⁶ M) significantly inhibited EGF-stimulated cell proliferation (Fig. 1A). This is a specific inhibition because mifepristone (10⁻⁶ M) completely blocked the inhibitory action of dexamethasone (10⁻⁶ M) on EGF-stimulated cell proliferation. Neither dexamethasone (10⁻⁶ M) nor mifepristone (10⁻⁶ M) alone had impact on cell proliferation compared with that in the control group (Fig. 1A). When analyzed further by flow cytometry, 10 ng/ml EGF treatment for 24 h markedly increased S-phase cells compared with the control group (Fig. 1A). When analyzed by flow cytometry, 10 ng/ml EGF for 24 h markedly increased S-phase cells compared with the control group (Fig. 1A). When analyzed further by flow cytometry, 10 ng/ml EGF treatment for 24 h markedly increased S-phase cells compared with the control group (Fig. 1A).

**Effect of EGF and dexamethasone (Dexa) on cell proliferation of the RGM-1 cells.** A, cell numbers were counted using hemocytometer. Cells were incubated with EGF, Dexa, and mifepristone (Mif) for 24 h. Value are means ± S.D. for six samples per group. +, P < 0.01 compared with the control group; ++, P < 0.01 compared with the EGF-treated group; and ###, P < 0.001 compared with the control group. Dexa-treated (10⁻⁶ M) significantly reduced EGF-induced response, and mifepristone blocked the dexamethasone inhibitory effect (Fig. 1, B and C). When the RGM-1 cells were treated with 10 ng/ml EGF for a longer time (1–18 h), it was observed that the expression of COX-2 increased significantly at 3 h, and the response lasted up to hour 18. In addition, cyclin D1 expression also increased significantly at 8 and 12 h (Fig. 2B). In contrast, the expression of COX-1 and cPLA₂ was similar to that of the

**Signal Transduction after EGF Treatment.** After cells were treated with 10 ng/ml EGF for 0 (control) to 120 min, it was found that 10 ng/ml EGF markedly increased the expression of pERK1/ pERK2 at 10 min, but the expression of total ERK1/ERK2 at 10 to 120 min remained the same (Fig. 2A). When the RGM-1 cells were treated with 10 ng/ml EGF for a longer time (1–18 h), it was observed that the expression of COX-2 increased significantly at 3 h, and the response lasted up to hour 18. In addition, cyclin D1 expression also increased significantly at 8 and 12 h (Fig. 2B). In contrast, the expression of COX-1 and cPLA₂ was similar to that of the
control (data not shown). The expression of another two activators of COX-2, namely, p38 MAPK/pp38 MAPK and JNK1/pJNK1 did not show any change following EGF treatment for 10 to 120 min, respectively (Fig. 2A).

**Signal Transduction after EGF Cotreated with Dexmethasone.** Incubation of RGM-1 cells with 10 ng/ml EGF and dexamethasone combined (10^{-8} and 10^{-6} M) for 10 min did not alter expression of ERK1/ERK2, pERK1/pERK2, p38 MAPK/pp38 MAPK, or JNK1/pJNK1 compared with the EGF-treated group (Fig. 3). However, pretreatment of RGM-1 cells with dexamethasone (10^{-8} and 10^{-6} M) for 3 h significantly reduced EGF-stimulated expression of pERK1/pERK2 (Fig. 4), and it did not influence expression of ERK1/ERK2, p38 MAPK/pp38 MAPK, or JNK1/pJNK1 compared with those in the EGF-treated group at 10 min (data not shown). In contrast, incubation of RGM-1 cells with 10 ng/ml EGF and dexamethasone combined (10^{-8} and 10^{-6} M) for 3 and 6 h significantly decreased EGF-stimulated COX-2 expression (Fig. 5, A and B) and not COX-1 and cPLA₂ expression compared with the 10-ng/ml EGF group. Incubation of RGM-1 cells with 10 ng/ml EGF and 10^{-8} and 10^{-6} M dexamethasone for 8 and 12 h decreased EGF-stimulated cyclin

![Fig. 2. A, time-course effect of EGF on ERKs activities (phosphorylated ERK1/ERK2), p38 MAPK activity, and JNK1 activity. Cells were incubated with 10 ng/ml EGF for 0 (control), 10, 20, 30, 60, and 120 min, respectively. Western blot analysis was performed; bands of ERK1/ERK2, phosphorylated form of ERK1/ERK2, p38MAPK, phosphorylated form of p38MAPK, JNK1, and phosphorylated form of JNK1 are the representative of three separate assays. B, time course effect of EGF on COX-2 and cyclin D1 expression. Cells were incubated with 10 ng/ml EGF for 0 min (control), 1, 3, 6, 8, 12, and 18 h, respectively. Western blot analysis was performed; the bands shown COX-2 and cyclin D1 are the representative of three separate assays.](image-url)

![Fig. 3. Effect of EGF, EGF plus Dexa, and Dexa alone on expression of ERK1/ERK2, pERK1/pERK2, p38 MAPK, pp38 MAPK, JNK1, and pJNK1 in the RGM-1 cells. Cells were incubated with 10 ng/ml EGF in the presence or absence of Dexa (10^{-8} and 10^{-6} M), or Dexa (10^{-6} M) for 10 min. Western blot analysis was performed; the bands shown ERK1/ERK2, pERK1/pERK2, p38 MAPK, pp38 MAPK, JNK1, and pJNK1 are the representative of three separate assays.](image-url)

![Fig. 4. Effect of EGF and EGF pretreated with Dexa on the expression of phosphorylated ERK1/ERK2 in the RGM-1 cells. Cells were first incubated with Dexa (10^{-8} and 10^{-6} M) for 3 h, followed with 10 ng/ml EGF in the presence or absence of Dexa (10^{-8} and 10^{-6} M) for 10 min. Western blot analysis was performed; quantitation was via a video densitometer. Values are means ± S.D. for six samples per group. +++ P < 0.001 compared with the control group; and ++, P < 0.01 and +++ P < 0.001 compared with the EGF-treated group.](image-url)
D1 expression compared with that in the 10-ng/ml EGF group (Fig. 6, A and B).

**Signal Transduction after EGF Cotreated with PD98059.** Incubation of RGM-1 cells with 10 ng/ml EGF and PD98059 (MEK inhibitor at 10⁻⁵ M and 5 × 10⁻⁶ M) combined for 10 min did not affect expression of ERK1/ERK2 and pERK1/pERK2 compared with those in the EGF-treated group (Fig. 7). Pretreatment of RGM-1 cells with 5 × 10⁻⁵ M PD98059 for 2 h significantly reduced EGF-stimulated expression of pERK1/pERK2 compared with that in the EGF-alone group at 10 min (Fig. 7). However, PD98059 (10⁻⁵ and 5 × 10⁻⁵ M) significantly decreased EGF-stimulated COX-2 expression at 6 h (Fig. 5B). These experimental results indicate that EGF-induced expression of phosphorylated ERK1/ERK2, COX-2 and cyclin D1 and not that of phosphorylated p38 MAPK and JNK1.

**The PGE₂ Concentration following EGF Cotreated with Dexamethasone.** Treatment of RGM-1 cells with 10 ng/ml EGF for 8 and 12 h significantly increased the intracellular PGE₂ concentration (Fig. 8, A and B). Again, dexamethasone (10⁻⁸ and 10⁻⁶ M) significantly reduced EGF-induced PGE₂ levels compared with the EGF-treated group.

**Effect of COX-2 Inhibitor and Dexamethasone on EGF-Stimulated Cell Proliferation.** COX-2-selective inhibitor NS-398 at 10⁻⁵ M alone did not affect the basal cell proliferation (Fig. 9). Same as 10⁻⁸ M dexamethasone treatment, NS-398 treatment significantly decreased the stimulatory action of EGF on cell proliferation, and the inhibitory action was slightly less than that of 10⁻⁶ M dexamethasone. Combination of 10⁻⁵ M dexamethasone and 10⁻⁵ M NS-398 did not have an additive effect in the inhibition of EGF-stimulated cell proliferation compared with the individual drug-treated group (Fig. 9).

**Discussion**

This study, for the first time, demonstrated that dexamethasone strongly inhibited EGF-stimulated gastric epithelial
cell proliferation partially by inhibiting phosphorylation of ERK1/ERK2, followed by inhibition of COX-2 and DNA synthesis. It is well accepted that EGF promotes gastric epithelial cell proliferation and gastric ulcer healing by activating the Ras/Raf/MAPK signal pathway (Pai et al., 1998; Tarnawski, 2005). Furthermore, EGF up-regulated gastric epithelial COX-2 expression, which is important for ulcer healing, in in vivo and in vitro studies (Brzozowski et al., 2001; Sasaki et al., 2003). This study indicated that EGF activated ERK1/ERK2, then increased COX-2 expression and PGE2 synthesis, finally promoting RGM-1 cell proliferation; dexamethasone, a glucocorticoid frequently used clinically, suppressed EGF-induced pERK1/pERK2, COX-2 expression, and then decreased PGE2 synthesis and cell proliferation in the RGM-1 cells. Current experimental findings are coincident with those obtained in our previous in vivo study, which demonstrated that dexamethasone at 0.1 and 0.2 mg/kg/day delayed ulcer repair via limiting epithelial cell proliferation at the ulcer margin by suppressing COX-2 expression and PGE2 formation in rat stomachs (Luo et al., 2003). Studies by Filaretova demonstrated that glucocorticoid had a gastric protective role during stress and administration of nonsteroidal anti-inflammatory drugs in rats (Filaretova et al., 1998, 2002). In our opinion, our results and their observations on the functional role of glucocorticoid on ulcer healing are not contradictory since endogenous glucocorticoids may have a physiologically protective role (Filaretova et al., 1998, 2002), whereas its administration in pharmacological doses would in turn delay ulcer repair and wound healing (Carpani de Kaski et al., 1995; Luo et al., 2003; Lee et al., 2005).

MAPK signal transduction pathways are important in regulating cell proliferation, migration, and differentiation (Blenis, 1993; Vieror et al., 1993). Previous studies have shown that COX-2 expression is regulated by MAPK subtypes, such as ERK1/ERK2, p38 MAPK, and JNK, depending on the types of extracellular stimuli and cells (Sheng et al., 1998; Matsuura et al., 1999; Lasa et al., 2000). Our results clearly showed that EGF activated ERK1/ERK2 in RGM-1 cells within 10 to 20 min (Fig. 2), but it did not affect expression of the phosphorylated forms of p38 MAPK and JNK1 from 10 to

![Fig. 7](image1.png)

**Fig. 7.** Effect of EGF, EGF plus PD98059, and PD98059 alone on the expression of phosphorylated ERK1/ERK2 in the RGM-1 cells. Cells were incubated with 10 ng/ml EGF in the presence or absence of PD98059 (10⁻⁵ and 5 × 10⁻⁵ M) for 10 min or pretreated with PD98059 (10⁻⁵ and 5 × 10⁻⁵ M) for 2 h and then cotreated with 10 ng/ml EGF and PD98059 (10⁻⁵ and 5 × 10⁻⁵ M) for 10 min. Western blot analysis was performed; quantitation was via a video densitometer. Values are means ± S.D. for six samples per group. **+, P < 0.01 compared with the control group; +, P < 0.05 and **, P < 0.01 compared with the EGF-treated group.

![Fig. 8](image2.png)

**Fig. 8.** Effect of EGF and Dexa on PGE₂ synthesis in RGM-1 cells. Cells were incubated with 10 ng/ml EGF in the presence or absence of Dexa (10⁻⁶ and 10⁻⁵ M) and Dexa alone for 8 (A) and 12 h (B), respectively. The intracellular PGE₂ level was measured with an enzyme-linked immunosorbent assay kit. Values are means ± S.D. of six samples per group. *, P < 0.05 compared with the control group; and **, P < 0.01 compared with the EGF-treated group.

![Fig. 9](image3.png)

**Fig. 9.** Effect of combined EGF and COX-2 inhibitor NS-398 (10⁻⁵ M) on cell proliferation (cell cycle S phase) in RGM-1 cells. Cells were incubated with 10 ng/ml EGF, 10 ng/ml EGF with 10⁻⁵ M Dexa, EGF with 10⁻⁵ M NS-398, and EGF with Dexa and NS-398 for 24 h. The S phase of cell cycle of the RGM-1 cells was assessed by flow cytometry, and the data were evaluated using ModFit and CellQuest software. Value are means ± S.D. for six samples per group. ***, P < 0.001 compared with the control group; and ++, P < 0.01 compared with the EGF-treated group.
120 min. This reaction was followed by increased COX-2 expression at 3 h after EGF treatment (Fig. 5). Moreover, dexamethasone suppressed EGF-induced COX-2 expression by reducing ERK1/ERK2 phosphorylation and not reducing phosphorylated forms of p38 MAPK and JNK1. However, it is interesting that an effective dose of PD98059 (5 × 10^{-5} M) reduced ERK activation by EGF (Fig. 7), yet it did not completely block EGF-induced COX-2 expression (Fig. 5B). This suggests that EGF-induced COX-2 activation may involve mechanisms other than ERK pathway.

Our results demonstrated that dexamethasone alone had no inhibitory effect on RGM-1 cells growth (Fig. 1), but the EGF-induced ERK response was blocked only when RGM-1 cell were pretreated for 3 h (Fig. 4) and not by simultaneous treatment with dexamethasone and EGF (Fig. 3). Similar to our results, a study by Xu et al. (2005) showed that gene 33, an adaptor protein, suppressed EGF receptor autophosphorylation and subsequent activation of downstream signaling in Rat 2 cells. In that study, dexamethasone induced gene 33 expression and 2-h pretreatment with dexamethasone similarly limited EGF-induced EGF receptor phosphorylation. These findings suggest that dexamethasone may have indirectly interfered with EGF binding to its receptor or EGF activation of ERK1/ERK2.

The fact that the glucocorticoid-receptor antagonist mifepristone significantly and completely reversed the action of dexamethasone on EGF-stimulated cell proliferation suggested that the inhibitory action of dexamethasone was glucocorticoid receptor-mediated. The current study also revealed that combination of dexamethasone and COX-2-selective inhibitor did not have an additive effect in the inhibition of EGF-stimulated cell proliferation compared with the individual drug-treated group (Fig. 9). The findings suggested that the main inhibitory effect of dexamethasone on EGF-stimulated cell proliferation was likely through blocking COX-2 activity.

Cyclin D1 is linked to the cell cycle progression by shortening the G_{1} phase and predisposition of cells to enter the S phase (Jiang et al., 1993; Resnitsky et al., 1994). Cyclin D1 accumulation via ERK activation is needed to pass the G_{1} restriction point and enter the S phase (Talarmin et al., 1999; Terada et al., 1999). Results from this study clearly indicated that EGF induced ERK1/ERK2 activation within minutes and then activated COX-2 function 3 to 6 h later, leading to increased cyclin D1 expression at 8 and 12 h, and finally it promoted DNA synthesis in RGM-1 cells at 24 h.

In conclusion, EGF enhanced gastric ulcer healing by stimulating epithelial cell proliferation, whereas glucocorticoid delayed ulcer healing partially via inhibiting epithelial cell proliferation. The study demonstrated that dexamethasone significantly suppressed EGF-stimulated gastric epithelial cell proliferation partially by inhibiting EGF-induced activation of ERK1/ERK2, followed by inhibition of COX-2, cyclin D1 expression, and DNA synthesis in a rat gastric epithelial cell line.

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