Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmacology 485 (2004) 275–281
www.elsevier.com/locate/ejphar

Dexamethasone delays ulcer healing by inhibition of angiogenesis in rat stomachs

Jiing C. Luo[a], Vivian Y. Shin[b], Edgar S.L. Liu[b], Yi N. Ye[b], William K.K. Wu[b], Wallace H.L. So[b], Full Y. Chang[a], Chi H. Cho[b,*]

[a] *Division of Gastroenterology, Department of Medicine, Taipei Veterans General Hospital and National Yang-Ming University, School of Medicine, Taiwan*
[b] *Department of Pharmacology, Faculty of Medicine, The University of Hong Kong, L2-55 Laboratory Block, Faculty of Medicine Building, 21 Sassoon Road, Pokfulam, Hong Kong SAR, China*

Received 14 August 2003; received in revised form 7 November 2003; accepted 13 November 2003

Abstract

Using the non-ulcerogenic doses of dexamethasone, we explored the action of glucocorticoids on ulcer healing and its relationship with angiogenic factors in the gastric mucosa. We applied dexamethasone (0.1 or 0.2 mg/kg/day) intragastrically in rats with acetic acid-induced gastric ulcer. The mucosal prostaglandin E_2 level and protein expressions of basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF) at the ulcer margin were determined. Ulcer induction significantly increased protein expressions of bFGF, VEGF, and prostaglandin E_2 level at the ulcer margin together with angiogenesis at the ulcer margin and base. The non-ulcerogenic doses of dexamethasone inhibited angiogenesis at the ulcer margin and ulcer base and delayed ulcer healing. These were associated with a significant decrease of prostaglandin E_2 level and VEGF expression, but not the bFGF expression. Supplementation with prostaglandin E_2 attenuated the inhibitory action of dexamethasone on VEGF expression and reversed the adverse effects of dexamethasone on angiogenesis and ulcer healing, without influencing bFGF expression. We concluded that dexamethasone given at non-ulcerogenic doses could decrease angiogenesis and delay acetic acid-induced ulcer healing; these actions were at least, in part, due to depletion of prostaglandin E_2 level followed by down-regulation of VEGF at the ulcer margin of the stomach.

Keywords: Angiogenesis; bFGF (basic fibroblast growth factor); Dexamethasone; Gastric ulcer; Prostaglandin E_2; VEGF (vascular endothelial growth factor)

1. Introduction

Glucocorticoids are reported to be ulcerogenic in the stomach (Nobuhara et al., 1985; Wallace, 1987; Filep et al., 1992) and delay gastric ulcer healing (Kuwayama et al., 1991; Carpani de Kaski et al., 1995). Both actions could be interrelated and cannot be differentiated if ulcerogenic doses of glucocorticoids are given. It is hard to define whether this type of drug indeed can delay ulcer healing in the stomach. It is therefore necessary to delineate such action using a non-ulcerogenic dose of corticosteroid. With this approach, we can elucidate not only the adverse action of corticosteroids but also the mechanisms for ulcer repair in the stomach.

Following acute gastric mucosal necrosis such as erosions or ulcers, most of the mucosal components, including microvessels, are destroyed within the focal lesion area. Healing of such lesions requires a reconstruction of the surface epithelium, glandular epithelial structures, restoration of the lamina propria, and, most importantly, reconstruction of the microvascular network essential for delivery of oxygen and nutrients to the healing site. Angiogenesis within granulation tissue is considered to be one of the most important processes in ulcer healing. The growth of new microvessels through angiogenesis is promoted by angiogenic growth factors such as basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), platelet-derived growth factor, and angiopoietin (Tarnawski, 2002).

The two heparin-binding angiogenic factors, bFGF and VEGF, which are the important angiogenic mediators, are examined in this study. bFGF is a direct mitogen for vascular

* Corresponding author. Tel.: +852-2819-9252; fax: +852-2817-0859.
E-mail address: chcho@hkusua.hku.hk (C.H. Cho).

0014-2999/$ - see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2003.11.038

endothelial cells, fibroblast, and smooth muscle cells (Shing et al., 1984; Folkman et al., 1988). bFGF expression is up-regulated in the submucosa during the early ulcer healing stage (Pohle et al., 1999) and its effect in ulcer healing via stimulating angiogenesis has been well established (Folkman et al., 1991; Szabo et al., 1994). VEGF expression was elevated during granulation tissue formation in a skin wound repairing model (Frank et al., 1995). This growth factor acts specifically on vascular endothelial cells to increase vascular permeability, and stimulates endothelial cell proliferation, migration and tube formation (Szabo et al., 2000). VEGF significantly accelerates gastric ulcer healing by enhancing angiogenesis at the ulcer site (Jones et al., 2001).

It has been shown that corticosteroids affect prostaglandin synthesis in tissues (Flower, 1998; Izhar et al., 1992) and its analog prostaglandin E_2 causes vasodilatation and stimulates angiogenesis (Form and Auerbach, 1983; Diaz-Flores et al., 1994). It is therefore suggested that depletion of prostaglandins could be the major detrimental factor contributing to the adverse action of corticosteroids on ulcer repair through the suppression of angiogenesis at the ulcer site. This action could act in line with the expression of the abovementioned growth factors in the course of ulcer healing process. Studying the interrelationship between prostaglandin E_2 and other angiogenic factors including bFGF and VEGF in the induction of angiogenesis and ulcer healing is interesting and should be important to understand the mechanisms for tissue repair in the stomach.

In this study, we would like to apply the non-ulcerogenic doses of dexamethasone, a potent corticosteroid, to explore its action on angiogenesis and further on ulcer healing in connection with prostaglandin E_2 and the angiogenic factors bFGF and VEGF in rat stomachs.

2. Materials and methods

2.1. 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 with tap water. They were kept in a room where temperature (22 ± 1 °C), humidity (65–70%), and day/night cycle (12:12-h light/dark) were controlled. Rats were fasted for 24 h but had free access to water before being subjected to acetic acid to induce gastric ulcer.

2.2. Chemicals and drugs

Chemicals and drugs were purchased from Sigma unless otherwise stated. Dexamethasone was prepared in 1% ethanol vehicle for intragastric administration. Prostaglandin E_2 was dissolved in 0.01M phosphate-buffered saline for intraperitoneal injection.

2.3. Dexamethasone treatment and mucosal damage

To determine the non-ulcerogenic dose of dexamethasone, rats were given dexamethasone intragastrically at the dose of 0.2 mg/kg once daily for a period of 9 days. They were sacrificed on day 10 and gastric mucosal damage was assessed by morphological observation under magnifying glass and histological examination with a microscope. The specimens with hematoxylin–eosin stain were assessed for mucosal damage according to the criteria of Whittle et al. (1990) with modification: (1) epithelial cell damage and glandular disruption, (2) hemorrhagic damage in the mucosa, and (3) deep necrosis and ulceration.

2.4. Dexamethasone treatment and ulcer healing

2.4.1. 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 exposed. The anterior and posterior walls of the stomach were clamped together with a pair of forceps with a round ring (i.d. 10 mm) 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. Forty-five seconds later, the acid solution was removed and the abdomen was closed. Thereafter, rats were fed with a standard diet and given with tap water. For further comparisons in this study, we also collected mucosa from normal rats without any treatment as the normal group.

2.4.2. 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 treated 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 on day 4, 7, or 10 after ulcer induction. The ulcers size (mm^2) in the anterior and posterior walls were determined and summed in each stomach. After measuring the ulcers, 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.

2.4.3. 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.

2.4.4. Measurement of mucosal prostaglandin E_2 level

Gastric tissues were homogenized with homogenizing buffer (0.05 M Tris–HCl at pH 7.4, 0.1 M NaCl, 0.001 M $CaCl_2$, 1 mg/ml D-glucose, 28 μM indomethacin to inhibit further prostaglandin E_2 formation) for 30 s. Then, they were centrifuged at 12000 rpm for 15 min at 4 °C. The supernatants were assayed by using a commercial available prostaglandin E_2 enzyme-linked immunosorbent assay kit (Quantikine, R&D systems, Minneapolis, MN). The assay procedures were in accordance to the protocol suggested in the kit. The 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 mucosal prostaglandin E_2 level was expressed as ng/mg protein.

2.4.5. Western blotting for bFGF and VEGF expressions over the ulcer margin

Gastric tissues were homogenized (Ultra-Turrax, Janke & Kunkel, Staufen, Germany) with radioimmunoprecipitation assay buffer (50 mM Tris–HCl, pH 7.5, 150 mM NaCl, 0.1% sodium dodecyl sulfate, 0.5% α-cholate, 2 mM EDTA, 1% Triton-X, 10% glycerol) and then centrifuged (J2-21, Beckman Instrument, California) to take the supernatants. Protein concentration was measured using a protein assay kit with bovine serum albumin as a standard (Bio-Rad Laboratories, Hercules, CA). Proteins were separated by electrophoresis on a 15% sodium dodecyl sulfate-acrylamide gel, and then transferred to Hybond C nitrocellulose membranes (Amersham International, Amersham, UK). The membranes were probed with antibodies against bFGF and VEGF (Santa Cruz Biotechnology, Santa Cruz, CA) overnight at 4 °C and incubated for one h with secondary antibodies conjugated with peroxidase. The membrane were developed by the enhanced chemiluminescence system (Amersham Biosciences, Piscataway, NJ) and exposed to an X-ray film (Fuji Photo Film, Tokyo, Japan). Quantitation was carried out by a video densitometer (Scan Marker III, Microtek, Carson, USA).

2.5. Prostaglandin E_2 and dexamethasone treatments on ulcer healing, angiogenesis and expression of bFGF and VEGF

One day after ulcer induction, rats were given prostaglandin E_2 (intraperitoneal injection at 100 or 200 μg/kg/day) or its vehicle (0.01 M phosphate-buffered saline solution) immediately after each dexamethasone treatment applied intragastrically at dose of 0.2 mg/kg once daily for 3 or 6 days in the same batch of rats. Likewise, prostaglandin E_2 treatment did not affect the physical health of the animals. Ulcer sizes, blood vessel count at the ulcer margin and base were determined. Western blotting for bFGF and VEGF expressions at the ulcer margin were measured.

2.6. Statistical analysis

The results were expressed as means ± S.E.M. The number of animals in each group used was ranging from six to eight. Differences between the 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.

3. Results

3.1. Effects of dexamethasone on gastric mucosal damage

Dexamethasone treatment at the dose of 0.2 mg/kg for 9 days did not produce any observable petechiae or erosions in the gastric glandular mucosa. Microscopically, the epithelium of mucosa and glandular architecture were intact and there was no sign of hemorrhage in the mucosal and submucosal layers of the stomach.

3.2. Effects of dexamethasone on gastric ulcer healing

3.2.1. Effects of dexamethasone on gastric ulcer sizes

Ulcers healed spontaneously in a time-dependent manner (Table 1). Dexamethasone treatment did not affect the ulcer sizes on day 4 after ulcer induction. However, the ulcer sizes were significantly bigger in the higher dose of dexamethasone-treated group when compared with the control group on day 7 and also on day 10 after ulcer induction, implicating that dexamethasone could delay ulcer healing (Table 1).

3.2.2. Effects of dexamethasone on angiogenesis at the ulcer margin and base

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 of dexamethasone significantly decreased numbers of microvessels at the ulcer base and

Table 1
Effect of dexamethasone (Dex) on gastric ulcer healing

Days after ulcer induction	Ulcer size (mm^2)		
	Day 4	Day 7	Day 10
Control group	42.4 ± 2.3	15.8 ± 1.7	9.8 ± 0.9
Dex 0.1 mg/kg/day	40.1 ± 2.4	19.0 ± 1.7	14.0 ± 2.1
Dex 0.2 mg/kg/day	39.1 ± 1.9	26.2 ± 2.9[a]	19.0 ± 1.8[a]

Values are means ± S.E.M of six to eight rats per group.
[a] $P<0.01$ when compared with the respective control group.

Table 2
Effect of dexamethasone (Dex) on angiogenesis of ulcer margin and ulcer base

	Numbers of microvessels/mm^2	
	Ulcer margin	Ulcer base
(A) 4 days after ulcer induction		
Control	31.6 ± 1.9[a]	16.2 ± 1.7[b]
Dex 0.1 mg/kg/day	24.6 ± 1.6[c]	10.5 ± 0.8[c]
Dex 0.2 mg/kg/day	23.1 ± 1.3[d]	9.7 ± 1.1[d]
(B) 7 days after ulcer induction		
Control	36.4 ± 2.3[a]	22.9 ± 1.9[a]
Dex 0.1 mg/kg/day	33.4 ± 1.6	19.1 ± 1.8
Dex 0.2 mg/kg/day	28.4 ± 1.8[c]	16.8 ± 1.4[c]
Normal group	6.8 ± 1.1	

Values are means ± S.E.M of six to eight rats per group.
[a] $P<0.01$ when compared with the normal group.
[b] $P<0.001$ when compared with the normal group.
[c] $P<0.05$ when compared with the respective control group.
[d] $P<0.01$ when compared with the respective control group.

ulcer margin at these time points in a dose-related manner when compared with those of the respective control group (Table 2).

3.2.3. Effects of dexamethasone on mucosal prostaglandin E_2 level

There was more than 10-fold increase in mucosal prostaglandin E_2 level at ulcer margin when compared with those of the normal mucosa. The increase was more drastic on day 7 after ulcer induction ($P<0.001$). Again, dexamethasone treatment dose-dependently decreased mucosal prostaglandin E_2 level at the ulcer margin on day 4 and 7 after ulcer induction when compared with those of the respective control group (Table 3).

3.2.4. Effects of dexamethasone on protein expressions of bFGF and VEGF at the ulcer margin

The VEGF protein expression was weak in the intact normal gastric mucosa. Ulcer induction markedly increased its expression by more than threefold when compared with the normal mucosa ($P<0.001$). Dexamethasone dose-dependently decreased the VEGF protein expression at the ulcer margin on day 4 and day 7 after ulcer induction when compared with the respective control group (Fig. 1). Significant effect was more observed at the higher dose of dexamethasone. The bFGF protein expression in the gastric mucosa was significantly increased but to a lesser extent (only about 20% increase) after ulcer induction when compared with those of normal group ($P<0.01$); there

Table 3
Effect of dexamethasone (Dex) on mucosal prostaglandin E_2 (PGE_2) level of ulcer margin

Days after ulcer induction	Mucosal PGE_2 (ng/mg protein)	
	Day 4	Day 7
Control	3.60 ± 0.68[a]	6.76 ± 0.54[b]
Dex 0.1 mg/kg/day	3.04 ± 0.24	5.74 ± 0.96
Dex 0.2 mg/kg/day	1.67 ± 0.37[c]	3.08 ± 0.56[d]
Normal group	0.23 ± 0.02	

Values are means ± S.E.M of six to eight rats per group.
[a] $P<0.01$ when compared with the normal group.
[b] $P<0.001$ when compared with the normal group.
[c] $P<0.05$ when compared with the respective control group.
[d] $P<0.01$ when compared with the respective control group.

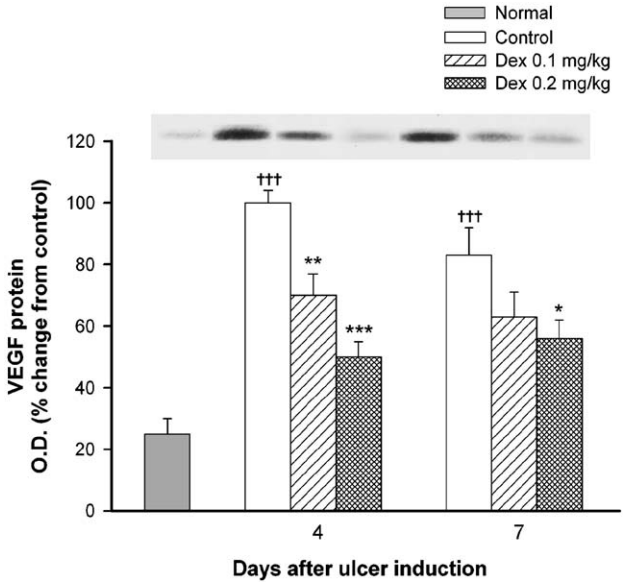

Fig. 1. Effect of dexamethasone (Dex 0.1 or 0.2 mg/kg given intragastrically once daily) on VEGF protein expression at the ulcer margin on day 4 and day 7 after ulcer induction. Values are means ± S.E.M. of six to eight rats per group. $^{\dagger\dagger\dagger}P<0.001$ when compared with the normal mucosa. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ when compared with the respective control group.

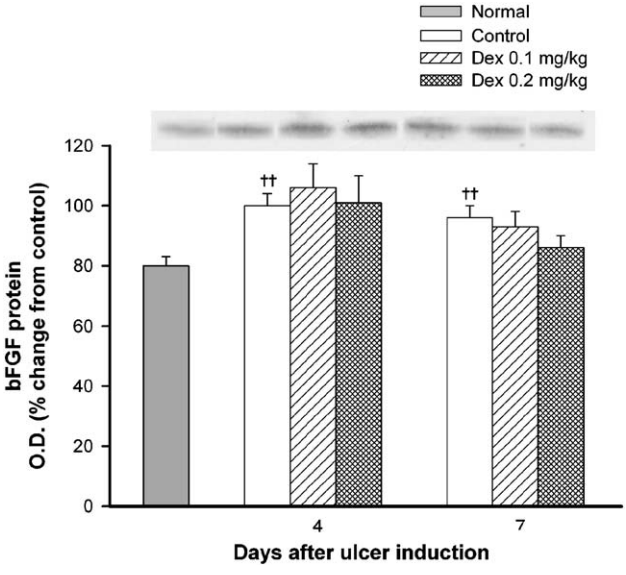

Fig. 2. Effect of dexamethasone (Dex 0.1 or 0.2 mg/kg given intragastrically once daily) on bFGF protein expression at the ulcer margin on day 4 and day 7 after ulcer induction. Values are means ± S.E.M. of six to eight rats per group. $^{\dagger\dagger}P<0.01$ when compared with the normal mucosa.

Table 4
Effect of prostaglandin E_2 (PGE_2) treatment on the inhibitory action of dexamethasone (Dex) on ulcer healing

Days after ulcer induction	Ulcer size (mm^2)	
	Day 4	Day 7
Control group	43.0 ± 2.7	18.3 ± 2.0
Dex 0.2 mg/kg/day	39.5 ± 1.4	27.6 ± 3.9[a]
Dex 0.2 mg/kg/day + PGE_2 100 μg/kg/day	35.4 ± 2.2	18.2 ± 2.3
Dex 0.2 mg/kg/day + PGE_2 200 μg/kg/day	35.0 ± 2.6	14.6 ± 0.9[b]

Values are means ± S.E.M. of six to eight rats per group.
[a] $P<0.05$ when compared with the control group.
[b] $P<0.05$ when compared with the respective Dex-treated group.

was no significant different among the control group, and the dexamethasone-treated groups (Fig. 2).

3.3. Reversal of prostaglandin E_2 treatment on the effects of dexamethasone on ulcer healing, angiogenesis and expression of VEGF

Prostaglandin E_2 administration at the doses of 100 or 200 μg/kg significantly and dose-dependently reversed the adverse action of dexamethasone on the delay of ulcer healing on day 7 after ulcer induction (Table 4). The same doses of prostaglandin E_2 also significantly attenuated the inhibitory action of dexamethasone on the number of blood vessels at the ulcer margin and base (Table 5). In stomachs with ulcers on day 4 after ulcer induction, again dexamethasone treatment alone did not affect the protein expression of bFGF when compared with the control (control: 100 ± 1% vs. dexamethasone: 103 ± 5%). Prostaglandin E_2 treatment together with dexamethasone administration did not significantly change the mucosal bFGF expression when compared with that of the dexamethasone-treated group (prostaglandin E_2 100 μg/kg plus dexamethasone: 89 ± 5%, prostaglandin E_2 200 μg/kg plus dexamethasone: 90 ± 4%). Similar findings were found on day 7 after ulcer induction (control: 100 ± 3%, dexamethasone: 98 ± 5%, prostaglandin E_2 100 μg/kg plus dexamethasone: 101 ±6%, prostaglandin E_2 200 μg/kg plus dexamethasone: 95 ± 4%). However, supplementation with prostaglandin E_2 dose-dependently but partially reversed the inhibitory action of dexamethasone on VEGF expression and reached a significant difference at the higher dose group (Fig. 3).

Table 5
Effect of prostaglandin E2 (PGE2) treatment on the inhibitory action of dexamethasone (Dex) on angiogenesis

	Number of microvessels/mm^2	
	Ulcer margin	Ulcer base
(A) 4 days after ulcer induction		
Control	37.3 ± 2.3	14.3 ± 1.7
Dex 0.2mg/kg/day	24.8 ± 2.1[a]	9.1 ± 1.3[b]
Dex 0.2 mg/kg/day + PGE_2 100 μg/kg/day	31.4 ± 2.8	13.9 ± 0.8[c]
Dex 0.2 mg/kg/day + PGE_2 200 μg/kg/day	33.8 ± 2.2[d]	16.7 ± 1.4[c]
(B) 7 days after ulcer induction		
Control	39.8 ± 1.7	20.2 ± 0.9
Dex 0.2mg/kg/day	33.5 ± 2.2[b]	16.4 ± 1.3[b]
Dex 0.2 mg/kg/day + PGE_2 100 μg/kg/day	39.7 ± 1.1[d]	21.2 ± 2.7
Dex 0.2 mg/kg/day + PGE_2 200 μg/kg/day	44.9 ± 3.1[d]	22.8 ± 1.4[c]

Values are means ± S.E.M of six to eight rats per group.
[a] $P<0.05$ when compared with the respective control group.
[b] $P<0.01$ when compared with the respective control group.
[c] $P<0.01$ when compared with the respective Dex-treated group.
[d] $P<0.05$ when compared with the respective Dex-treated group.

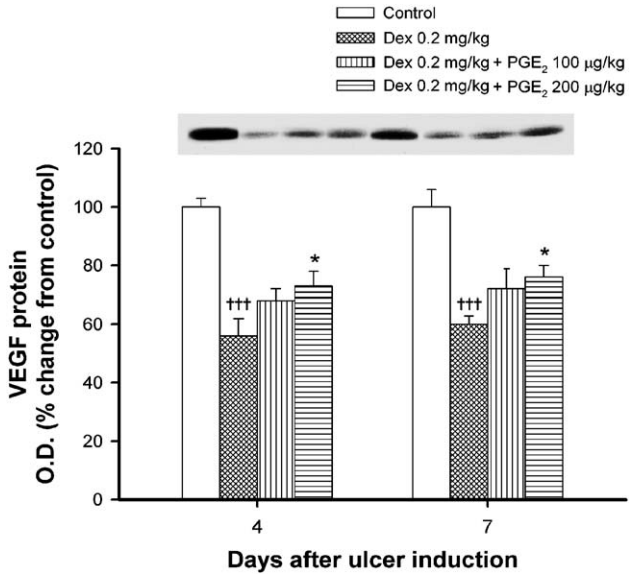

Fig. 3. Effect of prostaglandin E_2 treatment (PGE_2 100 or 200 μg/kg injected intraperitoneally once daily) on the inhibitory action of dexamethasone (Dex 0.2 mg/kg given intragastrically once daily) on VEGF expression measured at the ulcer margin on day 4 and day 7 after ulcer induction. Values are means ± S.E.M. of six to eight rats per group. †††$P<0.001$ when compared to the control group; *$P<0.05$ when compared with the respective dexamethasone-treated group.

4. Discussion

Ulcer healing is an active and complicated process of filling the mucosal defect with proliferation and migration of epithelial cells and stromal cells of connective tissues. In order to promote tissue repair, induction of angiogenesis leading to microvascular reconstruction within the granulation tissue is also an important component in the ulcer healing process. There are a number of angiogenic factors which contribute to the angiogenic process including endothelial cell organization and subsequently vascularization and tubular formation. Cyclooxygenase (COX) derived prostaglandin E_2 and angiogenic factors such as bFGF and VEGF are actively involved in these biological events (Tarnawski, 2002).

The current study showed that ulcer induction significantly increased the protein expression of bFGF and VEGF, and

also prostaglandin E_2 level at the ulcer margin and angiogenesis at the ulcer margin and base. All these findings were compatible with others' reports (Suzuki et al., 1998; Pohle et al., 1999; Guo et al., 2002). However, the increased expression of bFGF was significantly less as compared with those of VEGF and prostaglandin E_2 in our study. We also demonstrated that non-ulcerogenic doses of dexamethasone inhibited angiogenesis at the ulcer margin and base and delayed gastric ulcer healing. These were associated with a significant decrease of prostaglandin E_2 level and VEGF expression, but not the bFGF expression at the ulcer margin. Supplementation with prostaglandin E_2 significantly attenuated the inhibitory action of dexamethasone on VEGF expression without influencing bFGF expression. These findings are coincided with the reports showing that dexamethasone did not inhibit bFGF production in cultured synoviocytes (Nagashima et al., 2000) and prostaglandin E_2 stimulated VEGF expression in rat gastric microvascular endothelial cells and in carrageenin-induced granulation tissue in rats (Ghosh et al., 2000; Pai et al., 2001). However, previous studies showed that bFGF and VEGF are inducers of cyclooxygenase with subsequent production of prostaglandin synthesis in endothelial cells (Kage et al., 1999; Hernandez et al., 2001; Tamura et al., 2002). Blockade of cyclooxygenase activity by non-selective cyclooxygenase inhibitor inhibited 50% action of bFGF- and VEGF-induced angiogenesis (Salcedo et al., 2003). These findings indicated that prostaglandin seems to be on the downstream of bFGF and VEGF in the induction of microvessels formation during the ulcer healing process in the stomach. All these findings suggested that both VEGF and prostaglandin could co-regulate each other in the induction of angiogenesis and promote ulcer healing in the stomach. This mechanistic pathway is particular sensitive to the action of dexamethasone in rat stomachs.

The anti-inflammatory actions of glucocorticoids involve (1) inhibiting proinflammatory activities of transcription factors, in particular nuclear factor κB, (2) altering T helper cell type 1/T helper cell type 2 cytokine balance, (3) inducing annexin 1 (lipocortin 1) synthesis, hence suppressing arachodonic acid release via antagonizing phospholipase A_2 activity, and (4) affecting prostaglandin synthesis via decreasing cyclooxygenase-2 expression (Almawi et al., 1999; Roviezzo et al., 2002; Yang and Lichtenstein, 2002). These are different from the anti-inflammatory actions of non-steroidal anti-inflammatory drugs (NSAIDs), which inhibit cyclooxygenase-1 and/or cyclooxygenase-2 activity and prostaglandin synthesis. Animal studies demonstrated that NSAIDs delayed experimental gastric ulcer healing, in part, by significantly inhibiting angiogenesis in granulation tissue (Halter et al., 2001) and it was closely related to the suppressive action on bFGF expression at the ulcer margin (Guo et al., 2002). These findings implicate that although the ultimate effect of corticosteroids and NSAIDs on ulcer healing is the same, the anti-angiogenic mechanism of the two drugs in relationship with growth factors is different.

In this study although prostaglandin E_2 treatment only partially abrogated the inhibitory action of dexamethasone on VEGF expression at the ulcer margin, it could completely reverse the action on angiogenesis in the ulcer margin and base. This result implicates that the stimulatory action of prostaglandin E_2 on new microvessel growth at the ulcer site may be mediated through other mechanisms in addition to VEGF expression (Salcedo et al., 2003). On the other hand, although prostaglandin E_2 treatment can completely reverse the adverse effect of dexamethasone on ulcer healing, the action of prostaglandin E_2 on angiogenesis is not the only contributor to tissue repair in the stomach. It has been shown that prostaglandin E_2 can also increase epithelial cell proliferation, which is also important for ulcer healing (Carpani de Kaski et al., 1995).

In conclusion, ulcer induction by acetic acid activates angiogenesis, which is one of the most important repairing systems, to promote ulcer healing in the stomach. Dexamethasone given at non-ulcerogenic doses could deter this repairing mechanism and delay ulcer healing. These actions were at least, in part, due to depletion of prostaglandin E_2 level and down-regulation of VEGF in the granulation tissue at the ulcer site of the stomach.

Acknowledgements

Jiing-Chyuan Luo is the recipient of Physician Scientist Award from the National Health Research Institutes of Taiwan.

The project was also partly supported by the Hong Kong Research Grant Council (HKU 7397/03M) and CRGC grant from the University of Hong Kong.

We also thank Miss Pui-J. Lee (Department of Medicine, Taipei Veterans General Hospital) for her help in figures editing.

References

Almawi, W.Y., Melemedjian, O.K., Rieder, M.J., 1999. An alternate mechanism of glucocorticoid anti-proliferative effect: promotion of a Th2 cytokine-secreting profile. Clin. Transplant. 13, 365–374.

Augustin, H.G., Braun, K., Telmenakis, I., Modlich, U., Kuhn, W., 1995. Ovarian angiogenesis: phenotypic characterization of endothelial cells in a physiological model of blood vessel growth factor and regression. Am. J. Pathol. 147, 339–351.

Carpani de Kaski, M., Rentsch, R., Levi, S., Hodgson, H.J., 1995. Corticosteroids reduce regenerative repair of epithelium in experimental gastric ulcers. Gut 37, 613–616.

Diaz-Flores, L., Gutierrez, R., Varela, H., 1994. Angiogenesis: an update. Histol. Histopathol. 9, 807–843.

Filep, J.G., Herman, F., Foldes-Filep, E., Schneider, F., Braquet, P., 1992. Dexamethasone-induced gastric mucosal damage in the rat: possible role of platelet-activating factor. Br. J. Pharmacol. 105, 912–918.

Flower, R.J., 1998. Lipocortin and the mechanisms of action of the glucocorticoids. Br. J. Pharmacol. 94, 987–1015.

Folkman, J., Klagsbrun, M., Sasse, J., Wadzinski, M., Ingber, D., Vlodavsky, I., 1988. A heparin-binding angiogenic protein—basic fibroblast

growth factor—is stored within basement membrane. Am. J. Pathol. 130, 393–400.

Folkman, J., Szabo, S., Stovroff, M., McNeil, P., Li, W., Shing, Y., 1991. Duodenal ulcer. Discovery of a new mechanism and development of angiogenic therapy that accelerates healing. Ann. Surg. 214, 414–427.

Form, D.M., Auerbach, R., 1983. PGE_2 and angiogenesis. Proc. Soc. Exp. Biol. Med. 172, 214–218.

Frank, S., Hubner, G., Breier, G., Longaker, M.T., Greenhalgh, D.G., Werner, S., 1995. Regulation of vascular endothelial growth factor expression in cultured keratinocytes. Implications for normal and impaired wound healing. J. Biol. Chem. 270, 12607–12613.

Ghosh, A.K., Hirasawa, N., Niki, H., Ohuchi, K., 2000. Cyclooxygenase-2 mediated angiogenesis in carrageenin-induced granulation tissue in rats. J. Pharmacol. Exp. Ther. 295, 802–809.

Guo, J.S., Cho, C.H., Liu, E.S.L., Edgar, S.L., Choy, H.T., Wang, J.Y., Koo, M.W.L., Marcel, W.L., 2002. Antiangiogenic effect of a highly selective cyclooxygenase-2 inhibitor on gastric ulcer healing in rats. Toxicol. Appl. Pharmacol. 183, 41–45.

Halter, F., Tarnawski, A.S., Schmassmann, A., Peskar, B.M., 2001. Cyclooxygenase 2 implications on maintenance of gastric mucosal integrity ulcer healing: controversial issues and perspectives. Gut 49, 443–453.

Hernandez, G.L., Volpert, O.V., Iniguez, M.A., Lorenzo, E., Martinez-Martinez, S., Grau, R., Fresno, M., Redondo, J.M., 2001. Selective inhibition of vascular endothelial growth factor-mediated angiogenesis by cyclosporin A: roles of the nuclear factor of activated T cells and cyclooxygenase 2. J. Exp. Med. 193, 607–620.

Izhar, M., Pasmanik, M., Marcus, S., 1992. Dexamethasone inhibition of cyclooxygenase expression in bovine term placenta. Prostaglandins 43, 239–254.

Jones, M.K., Kawanaka, H., Baatar, D., Szabo, I.L., Tsugawa, K., Pai, R., Koh, G.Y., Kim, I., Sarfeh, I.J., Tarnawski, A.S., 2001. Gene therapy for gastric ulcers with single local injection of naked DNA encoding VEGF and angiopoietin-1. Gastroenterology 121, 1040–1047.

Kage, K., Fujita, N., Oh-hara, T., Ogata, E., Fujita, T., Tsuruo, T., 1999. Basic fibroblast growth factor induce cyclooxygenase-2 expression in endothelial cells derived from bone. Biochem. Biophys. Res. Commun. 254, 259–263.

Kuwayama, H., Matsuo, Y., Eastwood, G.L., 1991. Effects of prostaglandins on hydrocortisone-induced delayed healing of chronic gastric ulcers in the rats. J. Clin. Gastroenterol. 13 (Suppl. 1), S54–S57.

Nagashima, M., Wauke, K., Hirano, D., Ishigami, S., Aono, H., Takai, M., Sasano, M., Yoshino, S., 2000. Effects of combinations of anti-rheumatic drugs on the production of vascular endothelial growth factor and basic fibroblast growth factor in cultured synoviocytes and patients with rheumatoid arthritis. Rheumatology 39, 1255–1262.

Nobuhara, Y., Ueki, S., Takeuchi, K., 1985. Influence of prednisolone on gastric alkaline response in rat stomach. A possible explanation for steroid-induced gastric lesion. Dig. Dis. Sci. 30, 1166–1173.

Pai, R., Szabo, I.L., Soreghan, B.A., Atay, S., Kawanaka, H., Tarnawski, A.S., 2001. PGE_2 stimulates VEGF expression in endothelial cells via ERK2/JNK1 signaling pathways. Biochem. Biophys. Res. Commun. 286, 923–928.

Pohle, T., Shahin, M., Domschke, W., Konturek, J.W., 1999. Effect of basic fibroblast growth factor on gastric ulcer healing and its own mRNA expression. Aliment. Pharmacol. Ther. 13, 1543–1551.

Roviezzo, F., Getting, S.J., Paul-Clark, M.J., Yona, S., Gavins, F.N.E., Perretti, M., Hannon, R., Croxtall, J.D., Buckingham, J.C., Flower, R.J., 2002. The annexin-1 knockout mouse: what it tell us about the inflammatory response. J. Physiol. Pharmacol. 53, 541–553.

Salcedo, R., Zhang, X., Young, H.A., Michael, N., Wasserman, K., Ma, W.H., Martins-Green, M., Murphy, W., Oppenheim, J.J., 2003. Angiogenic effects of prostaglandin E_2 are mediated by up-regulation of CXCR4 on human microvascular endothelial cells. Blood 102, 1966–1977.

Shing, Y., Folkman, J., Sullivan, R., Butterfield, C., Murray, J., Klagsbrun, M., 1984. Heparin affinity: purification of a tumor-derived capillary endothelial cell growth factor. Science 223, 1296–1299.

Suzuki, N., Takahashi, S., Okabe, S., 1998. Relationship between vascular endothelial growth factor and angiogenesis in spontaneous and indomethacin-delayed healing of acetic acid-induced gastric ulcers in rats. J. Physiol. Pharmacol. 49, 515–527.

Szabo, S., Folkman, J., Vattay, P., Morales, R.E., Pinkus, G.S., Kato, K., 1994. Accelerated healing of duodenal ulcers by oral administration of a mutein of basic fibroblast growth factor in rats. Gastroenterology 106, 1106–1111.

Szabo, S., Khomenko, T., Gombos, Z., Deng, X.M., Jadus, M.R., Yoshida, M., 2000. Review article: transcription factors and growth factors in ulcer healing. Aliment. Pharmacol. Ther. 14 (suppl 1), 33–43.

Tamura, M., Sebastian, S., Gurates, B., Yang, S., Fang, Z., Bulun, S.E., 2002. Vascular endothelial growth factor up-regulates cyclooxygenase-2 expression in human endothelial cells. J. Clin. Endocrinol. Metab. 87, 3504–3507.

Tarnawski, A.S., 2002. Role of angiogenesis and angiogenic growth factors in mucosal repair and ulcer healing. In: Cho, C.H., Wang, J.Y. (Eds.), Gastrointestinal mucosal repairing and experimental therapeutics, 1st ed. Karger, Basel, Switzerland, pp. 101–116.

Tsukimi, Y., Okabe, S., 1994. Validity of kissing gastric ulcers induced in rats for screening of antiulcer drugs. J. Gastroenterol. Hepatol. 9, S60–S65.

Wallace, J.L., 1987. Glucocorticoid-induced gastric mucosal damage: inhibition of leukotriene, but not prostaglandin biosynthesis. Prostaglandins 34, 311–323.

Whittle, B.J.R., Lopez-Belmonte, J., Moncads, S., 1990. Regulation of gastric mucosal integrity by endogenous nitric oxide: interaction with prostanoids and sensory neuropeptide in the rat. Br. J. Pharmacol. 99, 607–611.

Yang, Y.X., Lichtenstein, G.R., 2002. Corticosteroids in Crohn's disease. Am. J. Gastroenterol. 97, 803–830.