Impaired Healing of Gastric Lesions in Streptozotocin-Induced Diabetic Rats: Effect of Basic Fibroblast Growth Factor

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ABSTRACT

We examined the influence of diabetes on the healing of HCl-induced gastric lesions and the healing promoting effect of basic fibroblast growth factor (bFGF) on these lesions under diabetic conditions induced in rats by streptozotocin (70 mg/kg, i.p.). The experiments were performed using 2-wk streptozotocin-diabetic rats with blood glucose levels of >300 mg/dl. After 18 hr fasting, these animals were given 1 ml of 0.6 N HCl by gavage, and 1 hr later they were fed normally before being killed on various days after HCl treatment. Recombinant human basic fibroblast growth factor (acid resistant recombinant human basic fibroblast growth factor mutein CS-23: 10 to 1000 ng/kg, i.p.) or insulin (4 U/rat, s.c.) was given 5 days after HCl treatment. Gastric lesions induced by HCl healed to quiescent state within 5 days both macro- and microscopically. Diabetic conditions did not affect the development of HCl-induced gastric lesions but significantly delayed the healing of these lesions. Daily administration of insulin returned high blood glucose levels to within normal ranges (120–140 mg/dl) and significantly antagonized the delayed healing of these lesions. The delayed healing in diabetic rats was also significantly promoted by recombinant human basic fibroblast growth factor (>300 ng/kg × 2) without any effect on blood glucose level. In normal rats, the mucosal levels of bFGF increased significantly in response to gastric injury at 3 days after HCl treatment. The mucosal bFGF levels in streptozotocin-diabetic rats were significantly lower under basal conditions before HCl treatment and did not increase after injury, yet such dysregulation of bFGF production was partially restored by insulin treatment. rhbFGF even at 1000 ng/kg had no effect on gastric acid secretion in either normal or streptozotocin-diabetic rats. These results suggest that diabetic conditions have deleterious influences on the healing of acute gastric lesions in both an insulin- and bFGF-sensitive manner, and that the administration of exogenous bFGF antagonizes the delayed healing of gastric lesions observed in diabetic animals.

Insulin-dependent diabetes exerts various influences on gastric functions, such as acid secretion and gastric emptying (Feldman et al., 1979; O’Reilly and Long, 1987; Belai et al., 1991). STZ is known to possess diabetogenic properties, causing selective destruction of pancreatic β cells. Recent studies showed the aggravation of gastric mucosal ulcerogenic responses to starvation or stress in STZ-diabetic rats (Piyachatrurawat et al., 1991; Takeuchi et al., 1994a). However, the effect diabetes has on the healing of gastric lesions has not been studied.

Diabetes is characterized by multiple vascular complications including diabetic microangiopathy and is known to impair the events associated with normal wound healing (Enser and Avery, 1984; Goodson and Hunt, 1986). Diabetes results in alterations in mRNA levels of bFGF in various tissues of rats with STZ-induced diabetes, and insulin treatment partially normalized these levels (Karpen et al., 1992). These findings suggest that dysregulation of bFGF may contribute to the development of microangiopathy as well as the impaired wound healing. Indeed, results of one study showed that wound healing is markedly retarded in STZ-diabetic animals and that this retardation is antagonized by treatment with bFGF (Klingbeil et al., 1991). This angiogenic polypeptide is a potent endothelial cell mitogen that also affects the proliferation of other cell types, including fibroblasts, smooth muscle cells and epithelial cells (Folkman and Klagsbrun, 1987). Thus, many of the cell types that are required for tissue replacement at the ulcer site are modulated by bFGF. In fact, several studies have shown using an acid-stable rhbFGF, that bFGF exerts a healing promoting effect on experimentally induced gastric and duodenal ulcers in rats (Folkman et al., 1991; Satoh et al., 1991; Szabo et al., 1994), while another study demonstrated a similar effect on reepithelialization after superficial injury of isolated bullfrog fundic gastric mucosa (Paimela et al., 1993).

The purpose of our study was to examine the influence of diabetes on the healing of acute gastric lesions and the healing promoting effect of bFGF on these lesions under diabetic conditions.

ABBREVIATIONS: bFGF, basic fibroblast growth factor; rhbFGF, recombinant human basic fibroblast growth factor; STZ, streptozotocin; EGF, epidermal growth factor; TGF-α, transforming growth factor-α; MAb, monoclonal antibodies; PBS, phosphate-buffered saline.

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conditions induced in rats by STZ. We also measured the expression of naturally occurring bFGF in the damaged mucosa of normal and STZ-diabetic animals.

Materials and Methods

Male Sprague-Dawley rats (230–250 g, Charles River, Shizuoka, Japan) were used. The animals were fed standard rat food and tap water ad libitum. One week after purchase, they were given STZ (70 mg/kg) i.p. and fed normally thereafter. The control animals received an equal volume of saline.

General procedure. The animals were used in the experiments from 1 wk after i.p. injection of STZ. Blood was sampled from the tail vein and BGL were determined by Glucostar-Glucostix (Miles-Sankyo Co., Ltd., Tokyo, Japan). Animals with BGL of less than 300 mg/dl under nonfasting conditions were excluded from study.

Development and healing of gastric lesions. Two weeks after the injection of STZ, the animals were placed in individual cages with raised mesh bottoms and deprived of food but allowed free access to tap water for 18 hr before the experiment. They were given 1 ml of 0.6 N HCl orally. Some of the animals were killed 1 hr after HCl treatment; the rest were fed normally from 1 hr later and killed on various days (1, 3, 5 and 7 days) after HCl treatment. In addition, the effects of the repeated administration of insulin and basic fibroblast growth factor (rhbFGF; acid resistant mutein, CS-23) (Seno et al., 1988) on the healing of these lesions were investigated. Insulin (4 U/rat) was given s.c. once daily for 5 days to ensure better systemic action, while rhbFGF (30–1000 ng/kg) was given orally twice daily for 5 days for expecting the local action. In all cases, the animals were killed under deep ether anesthesia; 24 hr after the final injection of insulin or 18 hr after the final administration of bFGF. To examine the effects of the above treatment on BGL, blood samples were taken immediately before HCl treatment and at the autopsy. The stomachs were removed, inflated by injecting 8 ml of 2% formalin for 10 min to fix the gastric tissue wall, opened along the greater curvature and examined for lesions under light microscope with a dissecting microscope with a square grid (3 m × 3 m and stained with H&E. Histological injury was assessed using the following criteria according to a previous paper (Takeuchi et al., 1994b); 1, no damage; 2, shallow damage not exceeding 25% of the mucosal depth; 3, moderate damage reaching beyond 25% of the mucosal depth but not exceeding 75% and 4, deep damage reaching beyond 75% of the mucosal depth. The length of sections with type 2, 3 or 4 damage was expressed as a percentage of the total section length and used as a histological index.

Determination of acid secretion. Acid secretion was measured in pylorus-ligated stomachs of both normal and STZ-diabetic rats on various days after HCl treatment. The animals were fasted for 18 hr before the experiment. The abdomen was incised under light ether anesthesia and the pullers ligated. Four hours after the ligation, the animals were killed under deep ether anesthesia and the gastric contents collected. After centrifugation for 15 min at 3000 rpm, each sample was measured for volume and titrated to pH 7.0 against 0.1 N NaOH using an automatic titrator (Hiranuma Comtite-8, Hiranuma, Tokyo, Japan). In some cases, the effects of insulin (4 U/rat) and rhbFGF (1000 ng/kg) on acid secretion was examined in normal and 2 wk-STZ-diabetic rats. Insulin or rhbFGF was given s.c. or i.d., respectively, immediately after the pylorus ligation. Control animals received the vehicle alone.

Determination of bFGF in gastric mucosa. The amount of bFGF in the gastric mucosa was determined in both control and STZ-diabetic rats on various days after HCl treatment. The corpus mucosa (10 mm²) was punched out using a cork borer, rinsed with cold saline and weighed. The tissue was minced in a test tube containing modified 50 mM Tris-HCl buffer (pH 7.6) in a volume 19 times the wet weight of the tissue. The Tris-HCl buffer contained 1.63 M NaCl, 10 mM ethylene-diaminetetraacetic acid (Sigma Chemical Co., St. Louis, MO), 1 mM phenylmethylsulfonyl fluoride (Wako, Osaka, Japan), 1 mM P-aminophenyl methanesulfonyl fluoride HCl (Wako) and 0.05 mM N-ethyl maleimide (Sigma). The tissue was then homogenized with a polytron (T-8; Ika, Yokohama, Japan) on ice, and centrifuged for 2 min at 10,000 × g. The supernatant was stored at −80°C until the assay. The bFGF content of each sample was measured by a sandwich enzyme immunoassay according to the method of Watanabe et al. (1991), using three MAb (Mab 52, Mab 98 and Mab 3H3; Wako) for human bFGF. In brief, Mab 52 or Mab 98 was dissolved in 0.1 M carbonate buffer (pH 9.6) at a concentration of 10 μg/ml. Of a mixture of two solutions 100 μl were added to each well of a 96-well microtiter plate. After overnight incubation at 4°C, the plate was washed with PBS (0.02 M phosphate buffer, pH 7.2, containing 0.15 M NaCl) and each well incubated with 300 μl of buffer A (PBS containing 25% Blocking Ace; Snow Brand Milk Products Co., Tokyo, Japan) overnight at 4°C. After the plate was washed with PBS, 100 μl of each sample or standard rhbFGF diluted in buffer A were added to each well. After a 24-hr incubation at 4°C, the plate was washed with PBS and 100 μl of Mab 3H3 solution in buffer A was added to each well. After a 2-hr incubation at 25°C, the plate was washed and the bound peroxidase activity was measured with O-phenylenediamine. In some cases, the mucosal amount of bFGF was determined in the stomachs of STZ-diabetic rats treated with insulin. In this case, insulin (4 U/rat) was given s.c. once daily for 3 or 5 days after HCl treatment.

Preparation of drugs. Drugs used were streptozotocin (Wako), insulin (Novo, Tokyo, Japan), and rhbFGF (acid-resistant mutein: CS-23, Takeda Ltd., Osaka, Japan). rhbFGF was dissolved in 1% NaHCO₃ buffer (pH 8.5), although other drugs were dissolved in saline. Each agent was prepared immediately before use and given p.o., i.p. or s.c. in a volume of 0.5 ml/100 g of body weight.

Statistics. Data are presented as means ± S.E. from 4 to 14 rats per group. Statistical analyses were performed using a two-tailed Dunnett’s multiple comparison test (Dunnett, 1955); P < .05 were considered significant.

Results

Body Weight and Blood Glucose Concentrations

The initial body weight of animals receiving injections with saline increased over the 21-day experimental period. In contrast, diabetic animals injected with STZ lost 11.9% of body weight within 3 wk (fig. 1). After injection of STZ, BGL under nonfasting conditions reached significantly high levels (331.3 ± 19.4 mg/dl) at 1 wk as compared with basal values (147.1 ± 7.4 mg/dl) and remained significantly elevated for 2 wk thereafter. Control rats showed stable BGL without significant changes (124.7–148.3 mg/dl) during the 3-wk test period.

Development and Healing of HCl-Induced Gastric Lesions

Intragastric administration of 0.6 N HCl (1 ml) caused severe hemorrhagic lesions in the glandular part of the stomach, mostly in the corpus mucosa, in both control and 2-wk-STZ-diabetic rats. The area of the lesions did not significantly differ between these two groups; the lesion score obtained 1 hr after administration of HCl was 132.6 ± 19.7 mm² in control rats and 102.4 ± 8.8 mm² in STZ-diabetic rats (fig. 2). In the control group, the lesions healed rapidly within

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the lesions; the lesion scores on day 3, 5 and 7 after HCl treatment were significantly greater in STZ-diabetic rats than in control rats. Moderate and deep histological injury was observed more frequently in the stomachs of STZ-diabetic rats than in control animals (table 1); in some diabetic rats, the damage on day 5 was still deep into the mucosa without reepithelialization.

**Acid Secretory Changes After HCl Treatment**

Acid secretion in the pylorus-ligated rats was not significantly different between control and STZ-diabetic rats before HCl treatment. A significant reduction in the acid output was observed in control rats for 3 days after HCl treatment; the reduction in acid output was 63.5 and 32.7% on day 1 and day 3, respectively (fig. 3). Similar changes in acid secretion were observed after induction of gastric lesions in STZ-diabetic animals. The acid output was significantly reduced (50.4%) on day 1 but reverted to normal levels on day 3 after HCl treatment. On day 5 in STZ-diabetic rats, the acidity still showed significantly lower values than that observed before HCl treatment, but the volume of gastric juice was significantly increased, resulting in no change in acid output. Although the acidity on day 3 in STZ-diabetic rats was significantly higher than that observed on day 3 in normal rats, there was no significant difference in the acidity on day 5 between these two groups.

**Effects of Insulin and rhbFGF on Healing of Gastric Lesions**

*Healing of lesions.* Because a marked increase of BGL was observed in STZ-treated rats during the experimental period, we examined the effects of insulin on the delayed healing of HCl-induced gastric lesions in STZ-diabetic rats. Injection of saline s.c. once daily for 5 days after HCl treatment did not significantly modify the healing of gastric lesions in STZ-diabetic rats; the lesion score and BGL on day 5 were 59.8 ± 7.4 mm² and 353.4 ± 10.2 mg/dl, respectively (fig. 4). Daily injection of insulin (4 U/rat/day) to the diabetic

**Table 1: Histology of HCl-induced gastric mucosal lesions in rats**

<table>
<thead>
<tr>
<th>Group</th>
<th>Shallow (%)</th>
<th>Moderate (%)</th>
<th>Deep (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 day</td>
<td>11.0 ± 1.9</td>
<td>10.8 ± 2.9</td>
<td>4.0 ± 2.3</td>
</tr>
<tr>
<td>3 days</td>
<td>8.0 ± 1.8</td>
<td>3.0 ± 1.3*</td>
<td>2.7 ± 0.8</td>
</tr>
<tr>
<td>5 days</td>
<td>4.0 ± 1.3*</td>
<td>1.8 ± 0.7*</td>
<td>0.8 ± 0.8*</td>
</tr>
<tr>
<td>STZ-diabetic rats (2 wk)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 day</td>
<td>10.2 ± 1.8</td>
<td>10.4 ± 1.5*</td>
<td>6.2 ± 1.8</td>
</tr>
<tr>
<td>3 days</td>
<td>6.8 ± 1.7</td>
<td>7.9 ± 1.5†</td>
<td>6.9 ± 2.3†</td>
</tr>
<tr>
<td>5 days</td>
<td>4.8 ± 1.4†</td>
<td>5.2 ± 1.2†‡</td>
<td>4.8 ± 1.5†‡</td>
</tr>
<tr>
<td>+ Insulin (4 U/rat)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 days</td>
<td>5.8 ± 1.2</td>
<td>2.3 ± 0.8§</td>
<td>1.4 ± 0.6§</td>
</tr>
<tr>
<td>+ rhbFGF (300 ng/kg × 2)</td>
<td>6.2 ± 1.4</td>
<td>1.6 ± 0.8§</td>
<td>1.0 ± 0.6§</td>
</tr>
</tbody>
</table>

The animals were given 1 ml of 0.6 N HCl orally and were fed normally from 1 hr later. Histological injury was scored as described in "Materials and Methods," and histological damage with shallow (not exceeding 25% of the mucosal depth), moderate (reaching more than 25% of the mucosal depth but not exceeding 75% of the mucosal depth) and deep (reaching more than 75% of the mucosal depth) injury was expressed as a percentage of the total section length. All values are presented as means ± S.E. from 8 to 14 experiments in each group. Statistically significant difference at P < .05; * from 1 day in normal group; † from 1 day in STZ group; ‡ from the corresponding values in normal group; § from the values on day 5 in STZ group.
rats for 5 days after HCl treatment decreased BGL significantly from 346.6 ± 10.2 mg/dl to within normal ranges (121.3 ± 14.1 mg/dl) and significantly enhanced the delayed healing of gastric lesions observed in STZ-diabetic rats, the lesion score being 25.4 ± 6.1 mm². Moderate and deep histological injury was observed less frequently in the stomachs of these rats than in the diabetic rats without insulin treatment (table 1). Body weight loss observed in STZ rats was not significantly affected by the 5-day-insulin treatment, the values being 241.3 ± 6.8g and 244.8 ± 4.3g, respectively, in STZ- and STZ plus insulin-treated rats.

Daily administration of rhbFGF (30–1000 ng/kg ³ 2) did not affect the high BGL in STZ-diabetic rats at any dose levels, and the values remained elevated at more than 300 mg/dl even after 5-day treatment with rhbFGF. However, the deleterious influence of diabetic conditions on the healing of gastric lesions was dose-dependently antagonized by rhbFGF treatment. The healing of HCl-induced gastric lesions in STZ-diabetic rats was dose-dependently accelerated by rhbFGF and a significant effect was observed at 300 and 1000 ng/kg, the lesion score at day 5 being 16.1 ± 5.7 and 18.2 ± 4.0 mm², respectively (fig. 5). As was the case with insulin treatment, moderate and deep histological injury was less frequently observed in the stomachs of rhbFGF-treated rats than in diabetic rats without any treatment, and the severity of the histological injury was not significantly different when compared to normal rats (table 1). Administration of rhbFGF for 5 days, similar to insulin treatment, did not significantly affect the changes in body weight observed in STZ-diabetic rats (not shown).

Acid secretion. Because the healing of gastric lesions is modified by luminal acidity, we examined the effects of insulin and rhbFGF on gastric acid secretion in normal and 2-wk-STZ-diabetic rats. A single injection of insulin slightly increased both the volume (17.9%) and acid output (30.3%) in normal rats when compared to controls, but neither of these changes was significant (table 2). Although STZ-diabetic rats secreted slightly less acid when compared to normal rats, similar effects were observed after administration of insulin in the diabetic animals; the volume and acid output increased 25.4 and 18.1%, respectively. However, rhbFGF given at the highest dose (1000 ng/kg) did not have any effect on gastric acid secretion in the pylorus-ligated stomachs of either normal or STZ-diabetic rats.

Mucosal Levels of bFGF During Healing of Gastric Lesions

In control rats, the amount of bFGF expressed in the stomach was about 1000 ng/g tissue. These values were significantly increased on day 3 after induction of damage by 0.6 N HCl but returned to basal levels on day 5 (fig. 6). The mucosal levels of bFGF in STZ-diabetic rats were significantly lower under basal conditions and did not significantly respond to damage by HCl; the values remained within similar ranges (600–700 ng/g tissue) before and after HCl treatment, and were significantly lower on days 3 and 5 as compared to normal groups. Daily injection of insulin restored the lowered expression of bFGF in the injured mucosa of STZ-diabetic rats and significantly increased the levels of bFGF in the gastric mucosa on day 3, the values being 1203 ± 76 ng/g tissue. The levels of bFGF (826 ± 98 ng/g tissue) on day 5 in these rats tended to increase after insulin treatment but did...
not reach significant levels (638 ± 58 ng/g tissue; P > .05) as compared with those observed in rats treated with STZ alone.

**Discussion**

Clinical conditions such as diabetes, obesity, glucocorticoid treatment and chemotherapy, are known to impair the events associated with normal wound healing. In particular, the influence of diabetes on the healing of dermal wound has been studied extensively (Klingbeil et al., 1991). Our study showed for the first time the deleterious influence of diabetes on the healing of gastric mucosal lesions in STZ-diabetic rats and further demonstrated that rhbFGF exerts healing promoting activity in the face of distinct physiological impairments observed in diabetic rats that reduce the rates of healing of acute gastric lesions.

Gastric lesions induced by 0.6 N HCl healed rapidly and were partially reepithelialized within 5 days with granulation at the injury site. We have reported that the healing of these lesions was significantly impaired by functional ablation of capsaicin-sensitive sensory nerves or the repeated administration of nitric oxide synthase inhibitors (Takeuchi et al., 1994b; 1997). As expected, diabetic conditions also had a deleterious influence on the healing of such lesions and significantly delayed the reconstruction process of the injured mucosa when examined both macroscopically and histologically. In this study, all STZ-treated animals developed a persistent hyperglycemia, which was observed 1 wk after STZ injection. The development of gastric lesions induced by HCl was not modified during the 2 wk of diabetic conditions, because no difference was observed in the severity of initial damage in response to HCl between normal and diabetic rats. However, the healing of such lesions was apparently impaired by diabetes and the mucosal defect without reepithelialization was persistently observed even 5 to 7 days after HCl treatment. Because daily injections of insulin to normalize BGL significantly antagonized the impaired healing of gastric lesions in STZ-treated rats, it is assumed that the deleterious influence of diabetes may be insulin-dependent and not caused by nonspecific action of STZ. It remains unknown, however, whether the insulin effect appears through amelioration of hyperglycemic conditions or through some other specific actions of this peptide. Because loss of body weight was similarly observed in STZ-diabetic rats with

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**TABLE 2**

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>No. of Rats</th>
<th>Volume (ml/4 hr)</th>
<th>P</th>
<th>Acid Output (μEq/hr)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal rats</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin</td>
<td>4 U/rat</td>
<td></td>
<td>7.8 ± 0.7</td>
<td>&gt;.1</td>
<td>198.2 ± 31.8</td>
<td>&gt;.1</td>
</tr>
<tr>
<td>NaHCO₃</td>
<td></td>
<td></td>
<td>9.2 ± 0.9</td>
<td>&gt;.1</td>
<td>258.3 ± 26.1</td>
<td>&gt;.1</td>
</tr>
<tr>
<td>rhbFGF</td>
<td>1000 ng/kg</td>
<td></td>
<td>7.7 ± 0.6</td>
<td>&gt;.2</td>
<td>201.8 ± 24.3</td>
<td>&gt;.2</td>
</tr>
<tr>
<td>STZ-diabetic rats</td>
<td></td>
<td></td>
<td>7.9 ± 0.5</td>
<td>&gt;.2</td>
<td>210.6 ± 28.4</td>
<td>&gt;.2</td>
</tr>
<tr>
<td>Saline</td>
<td></td>
<td></td>
<td></td>
<td>&gt;.1</td>
<td>173.7 ± 16.2</td>
<td>&gt;.1</td>
</tr>
<tr>
<td>Insulin</td>
<td>4 U/rat</td>
<td></td>
<td>8.4 ± 0.9</td>
<td>&gt;.2</td>
<td>205.1 ± 21.5</td>
<td>&gt;.2</td>
</tr>
<tr>
<td>NaHCO₃</td>
<td></td>
<td></td>
<td>6.5 ± 1.2</td>
<td>&gt;.2</td>
<td>168.9 ± 23.7</td>
<td>&gt;.2</td>
</tr>
<tr>
<td>rhbFGF</td>
<td>1000 ng/kg</td>
<td></td>
<td>6.8 ± 0.7</td>
<td>&gt;.2</td>
<td>176.3 ± 18.1</td>
<td>&gt;.2</td>
</tr>
</tbody>
</table>

All values are presented as the means ± S.E. from five to eight rats per group. Acid secretion was measured by pylorus ligation in normal and 2 wk-STZ diabetic rats (4 hr). Insulin (4 U/rats) or rhbFGF (CS-23: 1000 ng/kg) was given s.c. or i.d., immediately after the pylorus ligation. None of the treatments gave results that were significantly different at P < .05 from the corresponding values in the saline group.
in control diabetic rats most of the histological injury was observed with the full-thickness wounds in diabetic mice. Previous studies showed using genetically diabetic mice, that the top-
ical factors including luminal acid (Halter et al., 1980; Ka-
mada et al., 1983; Takeuchi and Johnson, 1986). Because acid secretion was decreased in the damaged stomach after ad-
ministration of 0.6 N HCl and because these changes in acid secretion were hardly affected by diabetes, it seems unlikely that healing was delayed by modulation of luminal acidity. In fact, neither insulin nor rhbFGF significantly affected the acid secretory ability of the stomach in diabetic rats, although both agents significantly promoted the delayed heal-
ing in STZ-diabetic rats. However, our study showed that STZ-diabetic rats displayed rapid recovery of acidity (P < .05) on day 3 after injury compared to normal rats. Because rhbFGF did not have any effect on gastric acid secretion in either normal or diabetic rats, it is unlikely that the in-
creased expression of tissue bFGF inhibited parietal cell activ-
ity, similar to EGF and TGF-α (Goldenring et al., 1993; Tsunoda et al., 1993; Wang et al., 1993). Certainly, if the expression of EGF and TGF-α are increased in response to injury, similar to bFGF (Polk et al., 1992), then one may assume that the recovery rate of acidity in the injured mu-
cosa is influenced by local production of these substances.

In general, wound healing undergoes multiple phases, such as synthesis of extracellular matrix materials, angiogenic response and epithelial migration over newly granu-
lated tissue, ultimately resulting in tissue rebuilding (Tar-
nawski and Halter, 1995). bFGF is a locally acting growth factor and a potent angiogenic polypeptide that stimulates the growth of other cells such as fibroblasts and smooth muscle cells as well as epithelial cells (Folkman and Klags-
brun, 1987). The beneficial effect of rhbFGF on the impaired healing of gastric lesions may be explained by earlier obser-
vations that rhbFGF indeed enhances a cascade of cellular events that are part of the mitogenic response produced by this polypeptide (Satoh et al., 1991; Paimela et al., 1993; Szabo et al., 1994). Certainly, because the angiogenic re-
ponse is closely associated with the neovascularization of the injured tissue, there is a possibility that the delay in the healing under diabetic conditions is partly due to mucosal blood flow insufficiency and the beneficial effect of rhbFGF is attributable to the amelioration of mucosal blood flow re-
ponse.

It has been reported that bFGF mRNA levels are altered by diabetes in a tissue-specific fashion and that the altered production of bFGF may contribute to the pathophysiological states of various diseases associated with diabetic conditions (Karpen et al., 1992). bFGF mRNA levels increased several fold in eye, heart and brain, but decreased in skeletal muscle. In contrast, insulin-like growth factor mRNA levels were significantly decreased in all tissues except brain (Fagin et al., 1989). In our study, we measured gastric mucosal levels of bFGF before and after damage by HCl and found that bFGF levels were significantly increased on day 3 after HCl treatment. In STZ-diabetic rats, not only was there less ex-
pression of bFGF in normal stomachs, but also, bFGF levels in the injured gastric mucosa did not increase after HCl

or without insulin treatment, it is unlikely that malnutrition plays an important role in the impaired healing of gastric lesions observed in such diabetic animals.

Genetically diabetic and obese mice are both hyperglyce-
mic and obese, conditions that have been correlated with impaired healing of the dermal wound in mice and implicated with healing problems in humans (Énser and Avery, 1984; Klinbeil et al., 1991). Significant impairment of healing was observed with the full-thickness wounds in diabetic mice when compared to normal heterozygous controls. Previous studies showed using genetically diabetic mice, that the top-
ical application of bFGF promoted the formation of granula-
tissue, the healing of full-thickness incisional wounds and the epithelialization of partial wounds in pigs (McGee et al., 1988; Klingbeil et al., 1991). Similarly, we found that acid stable bFGF (rhbFGF) significantly enhanced the delayed healing of HCl-induced gastric lesions in STZ-diabetic ani-
mals. In the diabetic rats treated with rhbFGF, the histolog-
ical injury on day 5 was restricted to the upper part of the mucosa and not distributed deeper in the mucosa, although in control diabetic rats most of the histological injury was

still deep, reaching more than 75% of the mucosal depth. These results are in good agreement with the findings by others who showed, using an acid-stable rhbFGF, that bFGF exerts a healing promoting effect on gastric and duodenal ulcers in rats (Folkman et al., 1991; Satoh et al., 1991; Szabo et al., 1994).

The tissue reconstructing processes are influenced by se-
veral factors including luminal acid (Halter et al., 1980; Ka-
mada et al., 1983; Takeuchi and Johnson, 1986). Because acid secretion was decreased in the damaged stomach after ad-
ministration of 0.6 N HCl and because these changes in acid secretion were hardly affected by diabetes, it seems unlikely that healing was delayed by modulation of luminal acidity. In fact, neither insulin nor rhbFGF significantly affected the acid secretory ability of the stomach in diabetic rats, although both agents significantly promoted the delayed heal-
ing in STZ-diabetic rats. However, our study showed that STZ-diabetic rats displayed rapid recovery of acidity (P < .05) on day 3 after injury compared to normal rats. Because rhbFGF did not have any effect on gastric acid secretion in either normal or diabetic rats, it is unlikely that the in-
creased expression of tissue bFGF inhibited parietal cell activ-
ity, similar to EGF and TGF-α (Goldenring et al., 1993; Tsunoda et al., 1993; Wang et al., 1993). Certainly, if the expression of EGF and TGF-α are increased in response to injury, similar to bFGF (Polk et al., 1992), then one may assume that the recovery rate of acidity in the injured mu-
cosa is influenced by local production of these substances.

In general, wound healing undergoes multiple phases, such as synthesis of extracellular matrix materials, angiogenic response and epithelial migration over newly granu-
lated tissue, ultimately resulting in tissue rebuilding (Tar-
nawski and Halter, 1995). bFGF is a locally acting growth factor and a potent angiogenic polypeptide that stimulates the growth of other cells such as fibroblasts and smooth muscle cells as well as epithelial cells (Folkman and Klags-
brun, 1987). The beneficial effect of rhbFGF on the impaired healing of gastric lesions may be explained by earlier obser-
vations that rhbFGF indeed enhances a cascade of cellular events that are part of the mitogenic response produced by this polypeptide (Satoh et al., 1991; Paimela et al., 1993; Szabo et al., 1994). Certainly, because the angiogenic re-
ponse is closely associated with the neovascularization of the injured tissue, there is a possibility that the delay in the healing under diabetic conditions is partly due to mucosal blood flow insufficiency and the beneficial effect of rhbFGF is attributable to the amelioration of mucosal blood flow re-
ponse.

It has been reported that bFGF mRNA levels are altered by diabetes in a tissue-specific fashion and that the altered production of bFGF may contribute to the pathophysiological states of various diseases associated with diabetic conditions (Karpen et al., 1992). bFGF mRNA levels increased several fold in eye, heart and brain, but decreased in skeletal muscle. In contrast, insulin-like growth factor mRNA levels were significantly decreased in all tissues except brain (Fagin et al., 1989). In our study, we measured gastric mucosal levels of bFGF before and after damage by HCl and found that bFGF levels were significantly increased on day 3 after HCl treatment. In STZ-diabetic rats, not only was there less ex-
pression of bFGF in normal stomachs, but also, bFGF levels in the injured gastric mucosa did not increase after HCl

Fig. 6. A, Mucosal expression of bFGF in control and STZ-diabetic rats before and at various days after the administration of 0.6 N HCl. Data are presented as the means ± S.E. from four to six rats per group. Statistically significant difference at P < .05; * from the values observed in the intact stomach (before HCl treatment) of normal group; #from the corresponding values in normal group. B, Effect of insulin on the mucosal bFGF levels in STZ-diabetic rats after HCl treatment. Insulin (4 U/rat) was given s.c. once daily for 5 days after HCl treatment. Data are presented as the means ± S.E. from four to six rats per group. Statistically significant difference at P < .05; * from the corresponding values in normal group; #from STZ alone.
treatment. This suppression of bFGF levels in STZ-diabetic rats was significantly antagonized by daily injection of insulin, suggesting that dysregulation of bFGF production may contribute to the delayed healing of gastric lesions observed under diabetic conditions. It has been shown that bFGF is widely distributed in various cells, especially in vascular endothelial cells, fibroblasts, smooth muscle cells and macrophages (Cordon-Cardo et al., 1990), and even is generated to some extent in the epithelium (Folkman et al., 1991). Therefore, it is possible to assume that bFGF levels in the mucosa are lower in STZ-diabetic rats because of the deeper gastric injury in their stomachs (less epithelium). However, the levels of bFGF were already low in intact stomachs of STZ-diabetic rats without any injury. In addition, the mucosal bFGF levels in normal rats were higher on day 3 after injury than on day 5, although the severity of the gastric lesions was much less on the latter day. On the basis of these findings, it is unlikely that the changes in bFGF levels observed in this study are attributable to the amount of intact epithelium of the stomach. At present, it remains unclear which types of cells are responsible for the increased expression of bFGF in the injured tissue, but they may include both the injured cell itself and inflammatory cells such as macrophages.

In conclusion, our results taken together suggest that diabetic conditions have deleterious influences on the healing of acute gastric lesions in both insulin- and bFGF-sensitive manner, and that exogenously administered rhbFGF may be effective in enhancing the delayed healing of gastric lesions in diabetic rats, through an acid-independent mechanism (table 3). Further studies with other models of gastric lesions or with other models of diabetes are required to confirm the universality of our findings in diabetic rats and to elucidate the mechanism of delayed healing of gastric lesions as well as the relationship between insulin deficiency and dysregulation of bFGF production under diabetic conditions.

References


TABLE 3

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Normal Rats</th>
<th>STZ-Rats</th>
<th>STZ-Rats + Insulin</th>
<th>STZ-Rats + rhbFGF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healing Acid secretion</td>
<td>Normal</td>
<td>Impaired</td>
<td>Recovered</td>
<td>Recovered</td>
</tr>
<tr>
<td>bFGF contents</td>
<td>Decreased</td>
<td>Decreased</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>BGL</td>
<td>Increased</td>
<td>Decreased</td>
<td>Increased</td>
<td>NT</td>
</tr>
<tr>
<td>Body weight</td>
<td>Normal</td>
<td>Decreased</td>
<td>Decreased</td>
<td>Increased</td>
</tr>
<tr>
<td>Acid secretion†</td>
<td>Normal</td>
<td>Slightly decreased</td>
<td>Slightly recovered</td>
<td>Slightly decreased</td>
</tr>
</tbody>
</table>

Normal rats that have normal BGL, body weight gain and acid secretory activity showed spontaneous healing of HCl-induced gastric lesions with concomitant increase of bFGF in the mucosa. In STZ-diabetic rats that show increased BGL and decreased body weight, the healing of gastric lesions was impaired in association with decreased expression of bFGF. Insulin treatment recovered these changes observed in STZ-diabetic rats, except the decrease of body weight, although rhbFGF recovered the impaired healing response in STZ-diabetic rats, without any effect on the increased BGL, body weight loss or acid secretion. Acid secretion† was determined in the stomach after HCl injury, although acid secretion† was measured in intact stomachs without HCl injury, NT, Not tested.


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