Acceleration of Healing of Gastric Ulcers Induced in Rats by Liquid Diet: Importance of Tissue Contraction

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ABSTRACT—We examined the effect of a liquid diet or a combined diet of liquid plus cellulose on the healing of gastric ulcers induced in rats in comparison with that of solid chow. Ulcers were induced in the fundus of the stomach by luminal application of an acetic acid solution. The healing of ulcers could be divided into two phases based on the healing rate: early phase (days 1 to 10) and late phase (days 10 to 20). The liquid diet, but not the combined one, administered for 10 days significantly accelerated ulcer healing in both the early and late phases. The length of the ruptured muscularis mucosa decreased only in the liquid diet group in both phases. Regeneration of the ulcerated mucosa in the chow diet group was observed only in the late phase, it being markedly inhibited in the liquid diet group. The serum gastrin level significantly decreased in the liquid and combined diet groups in contrast to that in the chow group. The liquid and combined diets significantly reduced gastric mucosal DNA synthesis. We conclude that 1) the healing in this gastric ulcer model comprises two phases, and 2) tissue contraction is a major factor for the healing of gastric ulcers in the early phase, while both tissue contraction and regeneration of the ulcerated mucosa are involved in the healing in the late phase.

Keywords: Acetic acid ulcer, Ulcer healing, Serum gastrin, Mucosal proliferation, Gastric distension

It is well known that the healing of gastric ulcers comprises several phases based on the healing rate in humans (1) and experimental animals (2, 3). It was speculated that a different mechanism is involved in each phase. However, the precise mechanism involved in each phase remained unclear. Contraction of the ulcer base and gastric mucosal cell proliferation are believed to play crucial roles in the healing of gastric ulcers (4, 5). Indeed, Ogihara and Okabe (6) recently suggested that the mechanism by which indomethacin delays ulcer healing is partly related to the inhibition of contraction of the ulcer base. We recently reported that unilateral vagotomy accelerates healing of kissing gastric ulcers suggesting the importance of tissue contraction for gastric ulcer healing (7). Therefore, the present study was designed to clarify the mechanism involved in each phase, focusing on tissue contraction and cell proliferation. With this aim, we determined the effect of a liquid diet or a combined diet of liquid plus cellulose on the healing of experimental gastric ulcers induced in rats in comparison with that of a chow diet.

MATERIALS AND METHODS

Animals and nutrients

Male Donryu rats (Charles River Japan, Kanagawa), weighing 250-280 g, were used in all experiments. They were kept in mesh-bottom cages to prevent coprophagy. CE-2 (Nihon Clea, Osaka) and Elental (Ajinomoto, Tokyo) were used for the standard rat chow and liquid diets, respectively. These diets were freely available throughout the experimental period. Elental mainly consisted of amino acids such as L-glutamine, L-serine, L-leucine, lysine-HCl, etc; lipids, minerals and vitamins. This liquid diet was administered to animals by means of a drinking bottle. The diet ingested in one day contained about 60 kcal, which is nearly equal to the value for the chow we used. In the case of the combined diet, the animals were administered liquid diet, from a drinking bottle, and cellulose as the non-nutritive bulk diet together. Alpha-cellulose (Nacalai Tesque, Kyoto) was hardened with agar (Kaneifujimori, Nagano) one day before administration.

Ulcer induction

Gastric ulcers were induced in the fundus by luminal application of an acetic acid solution (7). Under ether anesthesia, the abdomen was incised and the stomach exposed. Both the anterior and posterior walls of the fundus were clamped with forceps with a round ring (ID, 9 mm). A 60% (v/v) acetic acid (0.2 ml) was injected into the clamped lumen by using an injection needle (gauge 21) through the forestomach. Forty-five seconds later, the acid was removed with a needle, and the abdomen was closed. Thereafter, the animals were administered either a standard chow, liquid or combined diet for first 10 days (early phase). In the other study, the animals were fed on chow in the early phase, and subsequently, they were fed on each diet for 10 days. The weight of the animals treated with each diet was determined 10 and 20 days later. The animals were killed with an overdose of ether at 1, 5, 10, 15 or 20 days after acid application, without fasting before sacrifice. At first, blood was collected from the aorta descendens for determination of the serum gastrin level. Then each stomach was removed, opened along its greater curvature and washed with saline. The stomach was extended on a cork board with pins. The area of ulcers was determined under a dissecting microscope (×10; Olympus, Tokyo) with a square grid. The person (S.O.) determining the ulcer size did not know the treatment given to the animals. After determination of the ulcer size, the stomach was fixed with 10% formalin for 24 hr for the histological study. With this intraluminal application method, two ulcers develop on the anterior and posterior walls of the stomach. The reason we selected this ulcer model was to avoid any adhesion of the ulcer base to the surrounding organs, which invariably occurs in the case of conventional acetic acid ulcers. In addition, we had already confirmed that these ulcers heal with time in a similar manner and respond equally to antisecretory agents (7, 8). Therefore, the ulcerated areas on the two walls were summed in the present study.

Histological study

Histological study was performed according to the method previously described by Ogihara and Okabe (6). At autopsy, small pieces of tissue were embedded in paraffin and sectioned at 4 μm. Hematoxylin-eosin staining was performed. The lengths of the ruptured muscularis mucosa and regenerative mucosa at the ulcer edges were measured under a light microscope as parameters of tissue contraction and regeneration of the ulcerated mucosa, respectively.

Determination of serum gastrin levels

A blood sample was centrifuged at 3,500 rpm for 15 min to obtain the serum. Gastrin concentrations were determined by means of a double-antibody, ^{125}I-radioimmunoassay (Sanyou-Kasei, Tokyo). The results were expressed as pg of gastrin per ml of serum.

Determination of proliferative activity

Incorporation of ^{3}H-thymidine into DNA was determined as cell proliferative activity according to the procedure of Johnson and Guthrie (9). Under ether anesthesia, the stomachs of normal animals (without ulcers) were removed, and the fundic mucosa was scraped off with a glass slide. The mucosa was incubated for 30 min at 37℃ in medium 199 (Wako Chemicals, Osaka) containing 2 μCi/ml ^{3}H-thymidine (specific activity: 10 Ci/mmol; American Radiolabeled Chemicals, Inc., St. Louis, MO, USA). The reaction was stopped with perchloric acid, followed by hydrolysis with 0.3 N KOH to remove RNA. DNA was dissolved in 10% perchloric acid and then centrifuged to remove denatured protein. The incorporation of ^{3}H-thymidine into DNA was determined by counting 1.0 ml of DNA-containing supernatant in a scintillation counting system. Using calf thymus DNA as a standard, the DNA contents of samples were determined by the procedure of Burton (10) as modified by Giles and Mayers (11). DNA synthesis is expressed as disintegrations per minute per microgram of DNA.

Determination of gastric distension

Animals with 10-day-old gastric ulcers were deprived of food for 48 hr (water was allowed ad libitum) and then refed on the chow, liquid or combined diet for 2 hr. Subsequently, the animals were killed and their stomachs removed. To fix the outer layer, each stomach was immediately immersed in 10% formalin for 15 min. It was then opened along its greater curvature, and the area of the fundic mucosa was determined with a planimeter (X-PLAN360-i; Ushitaka, Tokyo).

Determination of gastric emptying

Animals with 10-day-old ulcers were fasted for 48 hr and then refed on the chow, liquid or combined diet for 1 hr. They were killed 0, 2 and 4 hr after refeeding, and the gastric contents were collected. These contents were centrifuged at 600 rpm for 5 min and the total volume (ml) determined.

Statistical analyses

All data are presented as means ± S.E.M. Statistical analyses were performed by the two-tailed Dunnett's multiple comparison test (12), values of $P < 0.05$ being regarded as significant.

RESULTS

Changes in body weight

After the operation, the animals fed on the chow gained body weight, the average being 20.7 g from the initial value at 10 days (255.6±4.2 g to 276.3±11.9 g, n=8). The body weights in the liquid and combined diet groups slightly increased for the first 10 days, i.e., 260.6±6.3 g vs 255.0±5.0 g (n=8) and 255.6±6.1 vs 252.5±2.3 g (n=8) at the start, respectively. Similar changes of the body weight were obtained in the late phase.

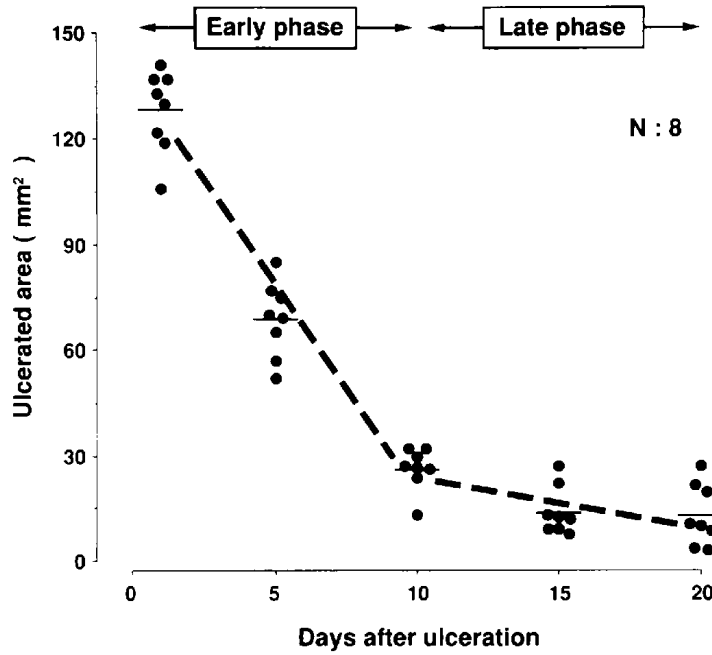

Fig. 1. Healing of gastric ulcers induced in rats fed on chow by intraluminal injection of an acetic acid solution. Note that the spontaneous healing of gastric ulcers can be divided into two phases (early and late) based on the healing rate. ● indicates ulcerated area from individual animals and — indicates the mean of ulcerated areas of these animals.

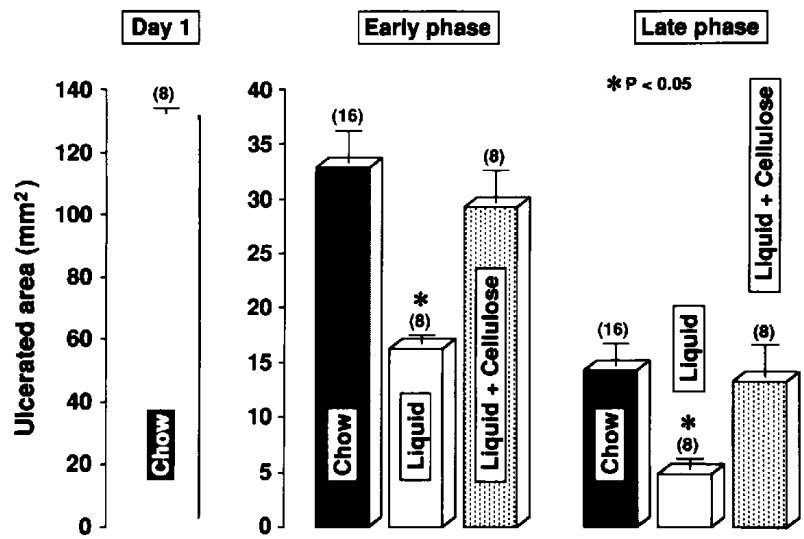

Fig. 2. Effects of chow, liquid and combined diets (liquid plus cellulose) on the healing of gastric ulcers induced in rats. Animals were fed on the chow, liquid or combined diet for 10 days in the early or late phase of ulcer healing. Data are means±1 S.E.M. *Significant difference compared to the chow group, at $P<0.05$.

Effects on ulcer healing

One day after acid application, round and penetrating gastric ulcers consistently developed on both the anterior and posterior walls (128.1 ± 4.1 mm^2, n=8). These ulcers spontaneously healed with time (Fig. 1). Based on the healing rate, the healing can be divided into two phases, i.e., an early phase (the initial 10 days, 10.2 mm^2/day) and a late phase (the following 10 days, 1.3 mm^2/day). Daily administration of the liquid diet for 10 days significantly promoted ulcer healing in the early phase, the ulcerated area being 16.2 ± 1.0 mm^2 vs 32.8 ± 3.0 mm^2 (n=8) in the chow group (Fig. 2). However, the combined diet had little or no effect on ulcer healing in this phase, the ulcerated area being 29.3 ± 2.9 mm^2. In the late phase (chow was administered for the initial 10 days), the liquid diet administered for 10 days also significantly promoted ulcer healing, i.e., 4.8 ± 1.1 mm^2 vs 14.1 ± 1.8 mm^2 (n=8) in the chow group. The combined diet had little or no effect on it (13.1 ± 2.6 mm^2, n=8).

Effects on histological changes

One day after acid application, the damage already extended through the muscularis mucosa into the submucosa and the muscle layer. The length of the ruptured muscularis mucosa was 6.24 ± 0.30 mm (n=8) (Fig. 3). Ten or twenty days after normal ingestion of chow, the length of the ruptured muscularis mucosa had decreased to 4.24 ± 0.21 mm (n=8) or 3.95 ± 0.18 mm (n=8), respectively. Administration of the liquid diet significantly accelerated this decrease in both the early and late phases. In contrast, the combined diet had no effect on the decrease in the length of the ruptured muscularis mucosa in either phase.

Regeneration of the ulcerated mucosa in the chow group was not observed in the early phase, but observed in the late phase (1.13 ± 0.09 mm, n=8). The length of the regenerated mucosa in the liquid diet group was significantly shorter than that observed in the chow group, the value being 0.28 ± 0.11 mm (n=8) (Figs. 3 and 4). However, the combined diet had no effect on the regeneration of the ulcerated mucosa, the value being 1.16 ± 0.16 mm (n=8) in the late phase.

Effects on serum gastrin levels

The serum gastrin levels were 437.5 ± 55.1 pg/ml and 335.4 ± 35.8 pg/ml (n=16) at the end of the early and late phases in the chow group. When animals were administered the liquid or combined diet for 10 days in the early or late phase, the serum gastrin levels significantly decreased compared with those in the chow group, the values being 123.4 ± 13.1 or 138.8 ± 19.8 pg/ml (n=8) and 103.6 ± 10.4 or 140.4 ± 11.3 pg/ml (n=8), respectively (Fig. 5).

Effects on mucosal proliferation

In normal rats fed on the chow, the wet weight, DNA content and DNA synthesis of the gastric fundic mucosa remained unchanged, the values being about 190–200 mg, 180–200 μg and 800–1000 dpm/μg DNA, respectively. However, the wet weight and DNA content were sig-

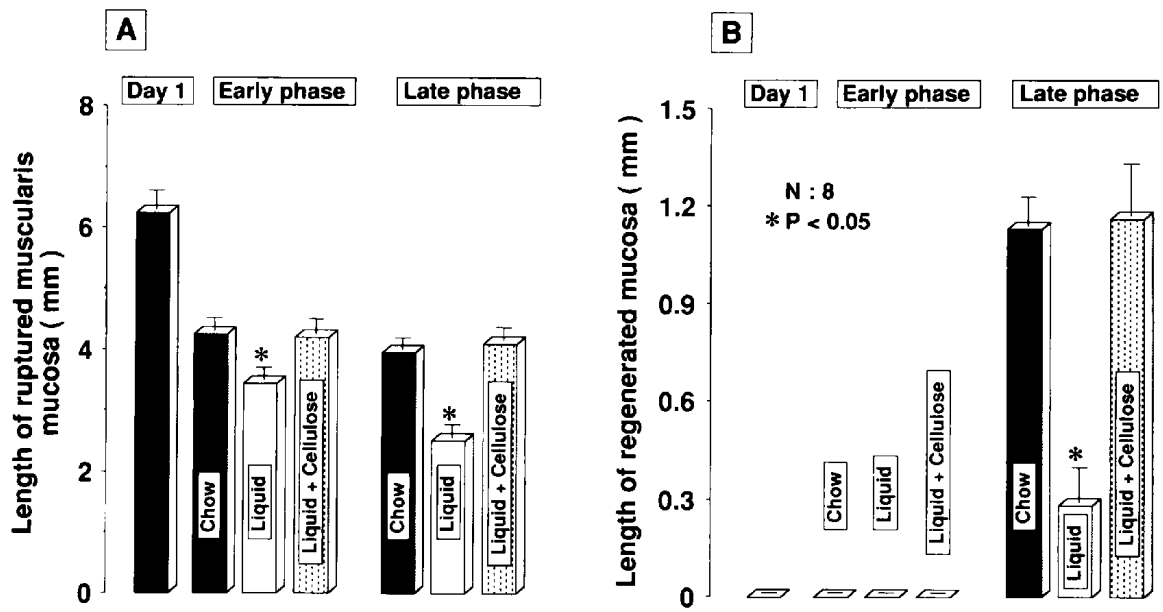

Fig. 3. Effects of chow, liquid and combined diets on the lengths of the ruptured muscularis mucosa and regenerated mucosa in gastric ulcers induced in rats. Data are means ± 1 S.E.M. *Significant difference compared to the chow group, at $P < 0.05$.

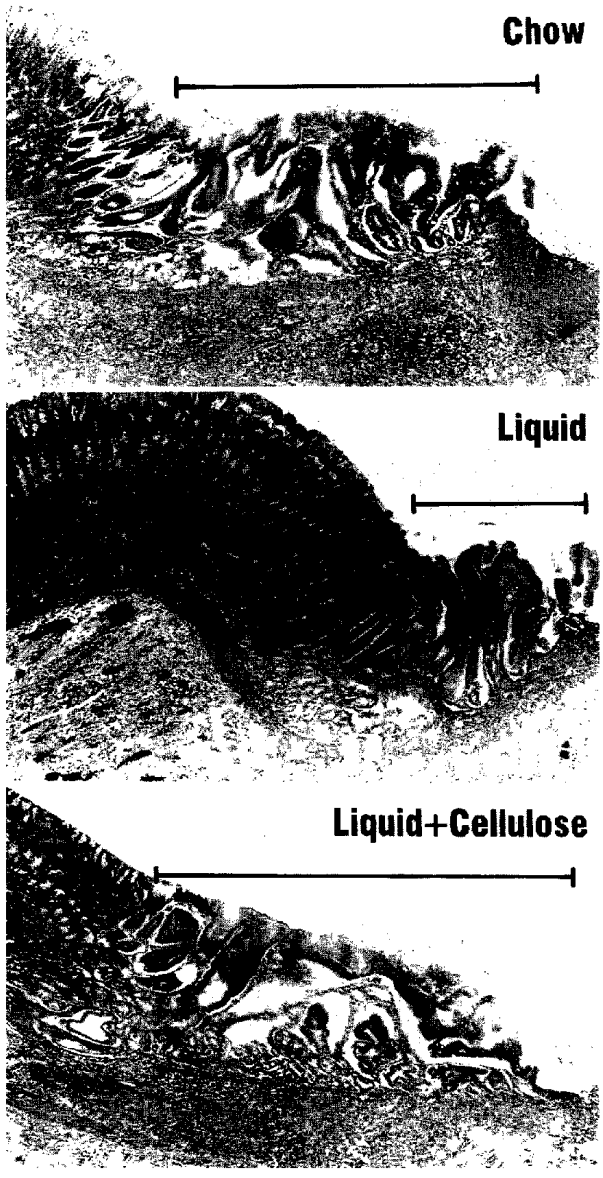

Fig. 4. Microscopic appearance of the margins of gastric ulcers in the late phase. Animals were fed on the chow, liquid or combined diet for 10 days following 10 days administration of the chow (×20). The bars indicate the length of the regenerated mucosa.

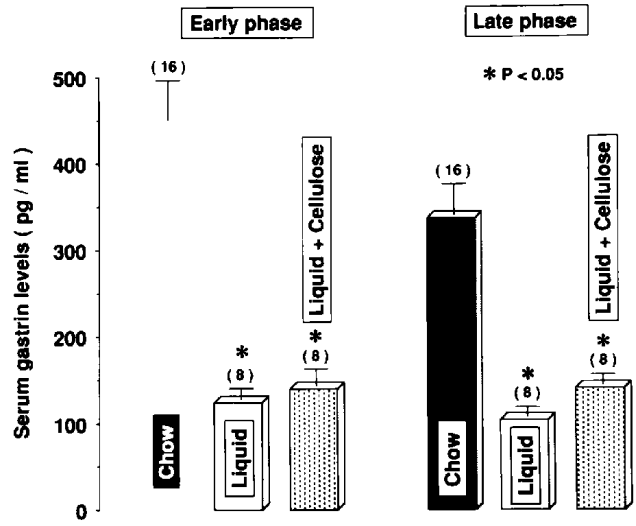

Fig. 5. Effects of chow, liquid and combined diets on the serum gastrin level in rats. Animals were fed on the chow, liquid or combined diet for 10 days in the early or late phase of ulcer healing. Data are means±1 S.E.M. *Significant difference compared to the chow group, at $P<0.05$.

nificantly reduced when the liquid diet was administered for 10 days (Fig. 6). Mucosal DNA synthesis also decreased in these groups, but such a change was observed even after administration for 5 days. On the other hand, the combined diet caused slight reduction of these parameters. The reduction of DNA synthesis after administration for 5 or 10 days was 17% or 44%, respectively.

Effects on gastric distension

After 48-hr fasting, the area of the gastric fundus was 4.5 ± 0.1 cm^2 (n=8). When animals were refed on the chow for 2 hr, the stomach was significantly distended to 8.3 ± 0.3 cm^2 (n=8) (Fig. 7). In the liquid diet group, the gastric distension was significantly inhibited compared with that in the chow group, the value being 5.8 ± 0.3 cm^2 (n=8). The gastric distension caused by the combined diet was quite similar to that observed in the chow group.

Effects on gastric emptying

When fasted animals were refed on the chow, liquid and combined diet for 1 hr, there were no significant differences in the volume of ingested food among the three groups, the values being 3.7 ± 0.5, 3.5 ± 0.6 and 4.6 ± 0.9 ml (n=6), respectively (Fig. 8). The chow or combined diet was gradually emptied from the stomach with time in a similar manner, whereas the liquid diet was emptied much faster than those diets.

DISCUSSION

In this study, we confirmed that the healing of gastric ulcers can be divided into two phases (early and late) based on the healing rate. Halter et al. (2) have shown that the healing rate of traumatic gastric mucosal defects in the pig becomes maximum around the 5th day, with slow initial and late healing velocities. Fitzpatrick et al. (3) also reported that a significant decrease in the ulcer index was observed between 1 and 2 weeks after ulcer induction in rats. The difference in the ulcer model is thought to be the reason why such an initial lag phase was not observed in

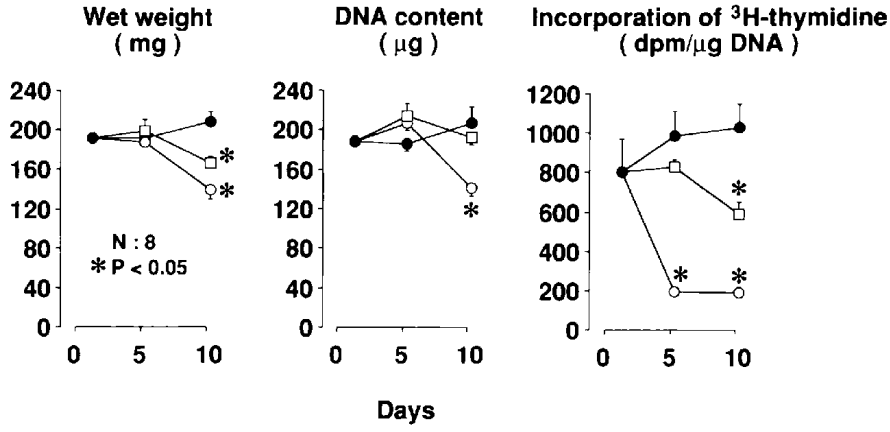

Fig. 6. Effects of chow, liquid and combined diets on proliferative indices in the gastric mucosa in normal rats (without ulcers). Animals were administered the chow (●), liquid (○) or combined diet (□) for 5 or 10 days. Data are means ± 1 S.E.M. *Significant difference compared to the chow group, at $P<0.05$.

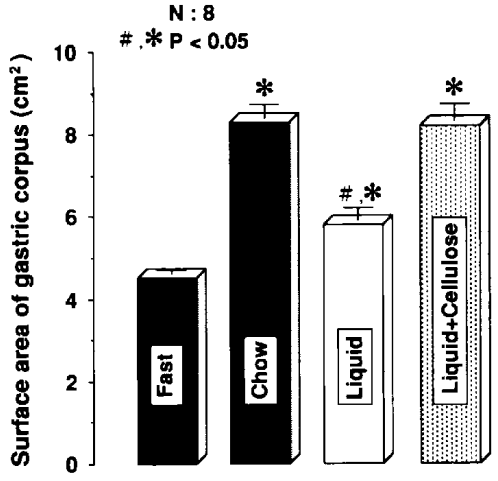

Fig. 7. Gastric distension induced by refeeding the chow, liquid or combined diet in rats with 10-day-old ulcers. Animals were fasted for 48 hr and then refed with each diet for 2 hr. Immediately after refeeding, the stomachs were fixed, and the area of the gastric fundus was determined. Data are means ± 1 S.E.M. *,#Significant differences compared to the fasted group and compared to the chow group, respectively, at $P<0.05$.

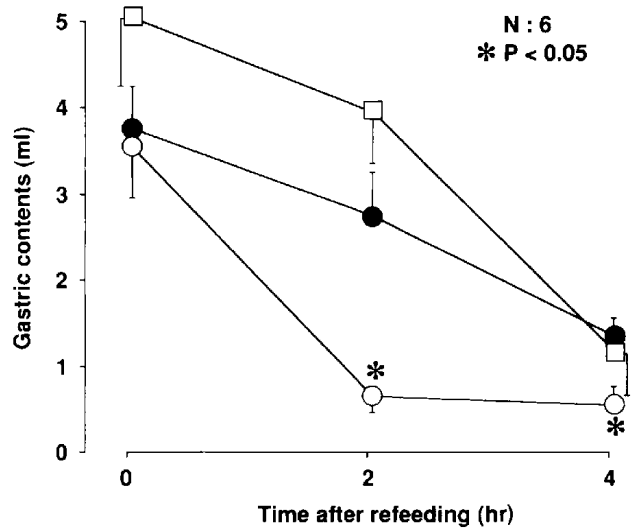

Fig. 8. Gastric emptying of the chow, liquid or combined diet in rats with 10-day-old ulcers. Animals were fasted for 48 hr and then refed with the chow (●), liquid (○) or combined diet (□) for 1 hr. The total gastric contents were determined 0, 2 or 4 hr after refeeding. Data are means ± 1 S.E.M. *Significant difference compared to the chow group, at $P<0.05$.

our gastric ulcer model. They used traumatic gastric mucosal defects and acetic acid-induced gastric ulcers, which are more severe than the gastric ulcers we used.

As expected, the length of the ruptured muscularis mucosa in the animals fed on the chow significantly decreased in the early phase. In the late phase, however, its length was quite similar to that observed at the end of the early phase. On the other hand, regeneration of the ulcerated mucosa in the chow-fed animals was markedly observed only at the end of the late phase. Based on these results, it is likely that the mechanism underlying ulcer healing mainly consists of tissue contraction in the early phase and regeneration of the ulcerated mucosa in the late phase.

In this study, we found that healing of gastric ulcers in both the early and late phases was apparently accelerated when the liquid diet was administered. Previously, we reported that anterior unilateral vagotomy significantly accelerated the healing of kissing gastric ulcers only on the vagal denervated side of the stomach, suggesting involvement of inhibition of the gastric distension caused by unilateral vagotomy (7). At that time, we showed that a liquid diet significantly promoted ulcer healing not only

on the vagal denervated side of the stomach but also on the vagal intact side. In this study, the stomach was fully distended with the refed chow, while it was only slightly distended when the animals were refed on the liquid diet. In addition, we confirmed the well-known fact that liquid is emptied from the stomach faster than solid food (13). These data indicate that the gastric distension was less and shorter in animals fed on the liquid diet compared with those fed on the chow. Of note was that this enhancement of ulcer healing caused by the liquid diet was not observed when it was administered together with cellulose, which distends the stomach to the same degree as the chow. Histological studies also showed that the ruptured muscularis mucosa was shortened after treatment with the liquid diet. It is most likely that a liquid diet is able to accelerate the healing of gastric ulcers through inhibition of gastric distension. The weight gain of the animals fed on the liquid diet was significantly inhibited compared with that of the chow fed group. Although the weight gain in the combined diet group was almost the same as that in the liquid diet group, enhancement of ulcer healing was not observed in that group. Therefore, the possibility that a liquid diet might involve special nutrition that accelerates ulcer healing can be ruled out. Since this beneficial effect of a liquid diet was observed in both the early and late phases, contraction of the ulcerated mucosa appears to influence the ulcer healing in both phases. Ogihara and Okabe (6) provided evidence of the importance of contraction of the ulcer base in ulcer healing. Thus, it is most likely that the ulcer base might contract more easily with inhibited gastric distension.

In general, mucosal cell proliferation is believed to play an important role in ulcer healing (4, 5). Sircar et al. (14, 15) have shown that in rats without ulcers, the administration of a synthetic liquid diet significantly decreased the serum gastrin level and gastric mucosal DNA synthesis. We confirmed their findings in this study. Even in rats with ulcers, we found that regeneration of the ulcerated mucosa was significantly inhibited by liquid diet ingestion. These results suggest that administration of a liquid diet might result in delayed ulcer healing. Indeed, Takeuchi and Johnson (4) reported that healing of gastric ulcers induced in rats was significantly delayed when the animals were fed on an isocaloric liquid diet, which significantly reduced the serum gastrin level and gastric mucosal DNA synthesis. At variance with their findings, however, we found that a liquid diet significantly enhanced ulcer healing. This apparent difference might be explained by the different ulcer models used. They used the acetic acid ulcer model which is pathologically much more severe and involves adhesion of the ulcer base to the liver. On the other hand, our ulcer model is less severe (no damage to the serosal mucosa) and involves no adhesion to the liver. The mechanism by which the liquid diet enhanced the ulcer healing may be attributed to the marked effect of tissue contraction, despite the reduced proliferation of gastric mucosal cells. However, the influence of liquid diet on the action of growth factors, such as angiogenesis or synthesis of extracellular matrix, should be considered as well.

Although the combined diet, like the liquid diet, also decreased the serum gastrin level in both phases, it did not reduce gastric mucosal DNA synthesis to the extent observed with the liquid diet alone. Sircar et al. (15) indicated that the addition of cellulose to a liquid diet did not restore the decreased gastric mucosal DNA synthesis caused by the liquid diet alone. It is hard to explain these different results obtained by two groups. One consideration is that the constituents of the liquid diets used by these two groups may be different, leading to the different results. The question of why gastric mucosal DNA synthesis only slightly decreased with the combined diet can be raised. It is likely that either cellulose stimulated the cell proliferation through physical irritation of the gastric mucosa or extension of the stomach stimulated DNA synthesis. Of note was that regeneration of the ulcerated mucosa was quite similar to that observed with the chow diet. Since the serum gastrin levels were markedly reduced, it is possible that trophic factors other than gastrin, such as EGF or TGF, might be involved in the mechanism underlying the enhanced regeneration (16, 17). Despite the above results, the reason why the combined diet had no effect on ulcer healing seems to be the extensive distension of the stomach caused by the cellulose.

In conclusion, it is suggested that 1) the healing of gastric ulcers is divided into two phases (early and late) and 2) tissue contraction is involved in the ulcer healing in both the early and late phases, while regeneration of the ulcerated mucosa is mainly involved in the late phase.

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REFERENCES

1 Scheurer U, Witzel L, Halter F, Keller HM, Huber R and Galeazzi R: Gastric and duodenal ulcer healing under placebo treatment. Gastroenterology **72**, 838–841 (1977)

2 Halter F, Barbezat GO, Van Hoorn-Hickman R and Van Hoorn WA: Healing dynamics of traumatic gastric mucosal defects in the normal and hyperacid stomach. Dig Dis Sci **25**, 916–920 (1980)

3 Fitzpatrick LR, Jakubouska A, Martin GE, Davis M, Jaye MC and Dionne CA: Acidic fibroblast growth factor accelerates the healing of acetic acid-induced gastric ulcers in rats. Digestion

53, 17–27 (1992)

4 Takeuchi K and Johnson LR: Effect of cell proliferation on healing of gastric and duodenal ulcers in rats. Digestion **33**, 92–100 (1986)

5 Konturek SJ, Brzozowski T, Dembinski A, Warzecha Z, Konturek PK and Yanaihara N: Interaction of growth hormone-releasing factor and somatostatin on healing and mucosal growth in rats; role of gastrin and epidermal growth factor. Digestion **41**, 121–128 (1988)

6 Ogihara Y and Okabe S: Mechanism by which indomethacin delays gastric ulcer healing in the rat: inhibited contraction of the ulcer base. Jpn J Pharmacol **61**, 123–131 (1993)

7 Tsukimi Y and Okabe S: Effect of anterior unilateral vagotomy on healing of kissing gastric ulcers induced in rats. Jpn J Pharmacol **66**, 105–114 (1994)

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

9 Johnson LR and Guthrie PD: Mucosal DNA synthesis: a short-term index of the trophic action of gastrin. Gastroenterology **67**, 453–459 (1974)

10 Burton K: A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochem **62**, 315–323 (1956)

11 Giles KW and Mayers A: An improved diphenylamine method for the estimation of deoxyribonucleic acid. Nature **206**, 93 (1965)

12 Dunnett CW: A multiple comparison procedure for comparing several treatments with a control. J Am Stat Assoc **50**, 1096–1121 (1955)

13 Minami H and McCallum RW: The physiology and pathophysiology of gastric emptying in humans. Gastroenterology **86**, 1592–1610 (1984)

14 Sircar B, Johnson LR and Lichtenberger LM: Effect of chemically defined diets on antral and serum gastrin levels in rats. Am J Physiol **238**, G376–G383 (1980)

15 Sircar B, Johnson LR and Lichtenberger LM: Effect of synthetic diets on gastrointestinal mucosal DNA synthesis in rats. Am J Physiol **244**, G327–G335 (1983)

16 Johnson LR and Guthrie PD: Stimulation of oxyntic mucosal growth by epidermal growth factor. Am J Physiol **238**, G45–G48 (1980)

17 Chen MC, Lee A and Soll AH: Mitogenic response of canine fundic epithelial cells in short-term culture to transforming growth factor alpha and insulin-like growth factor I. J Clin Invest **87**, 1716–1723 (1991)