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Low Susceptibility of Stress Ulcer in Diabetic Rats: Role of Cholinergic Gastric Motility

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Abstract

The induction of gastric hemorrhage ulcer by cold restraint stress (CRS) and the changes of cholinergic gastric motilities were studied in streptozotocin (STZ)-induced diabetic (DM) rats to compare with the age-matched normoglycemic rats. After receiving cold restraint stress for 4 h, a lower of both gastric motility and hemorrhagic ulcer was found in the STZ-induced diabetic rats in a time-dependent way from 0-16 week (s) parallel with the induction of diabetes. The mucosal ulceration induced by CRS was correlated with the amplitude ($r = 0.8928$, $p < 0.001$) and frequency ($r = 0.8674$, $p < 0.001$) of gastric motility in diabetic rats. A significant ($p < 0.01$) lowering of mucosal glutathione levels and mucus production also was observed in stressed diabetic rats. Under stress condition, the cholinergic gastric motility and mucosal lesion were markedly reduced by an intraperitoneal injection (i.p.) of atropine (1.0 mg/kg, i.p.) or bilateral vagotomy in 8-week STZ-DM rats. Otherwise, bethanechol (2.0 mg/kg, i.p.), 2-deoxy-D-glucose (135 mg/kg, i.p.) and metoclopramide (20 mg/kg, i.p.) stimulated gastric motility and aggravated mucosal lesions significantly ($p < 0.05$). It is indicated that cholinergic gastric motility was lowered in DM rats in a manner can decrease mucosal lesion induced by CRS.

Key Words: diabetes, stress ulcer, gastric motility, glutathione, rat

Introduction

Gastric motility and acid secretion may be greatly associated with mucosal damage. Increase in gastric motility may concern with gastric ulceration induced by indomethacin (30) or stress (31). Further, high concentration of intraluminal acid may cause severe gastric mucosal ulceration in either aspirin-treated dogs (16) or diabetic rats (15) via gastric acid-back-diffusion.

Vagal neuropathy is one of the important manifestations in patients (10) or animals (5, 18) with diabetes mellitus (DM). It may be involved in the pathogenesis of metabolic syndromes, including hypoglycemia, unawareness and defective glucose counter-regulation insulin-dependent diabetes mellitus patients (10). In the gastrointestinal system, vagal neuropathy may result in hyposecretion of gastric acid and the delay of gastric motility (2, 11). Since the reduction of gastric acid secretion may be beneficial to the prevention of peptic ulcer formation, it was suggested that gastric acid autoinhibition resulted from vagal neuropathy may contribute to the lower incidences of peptic ulcers in DM patients (14). Moreover, DM-produced vagal neuropathy may cause impairment of gastric motility (6, 11). Whether this dysfunction of gastric motility caused by neuropathy may attenuate CRS-ulcer in rats, however is unknown. The aim of the present study is to demonstrate the truth that gastric motility was lowered in DM rats to eliminate the CRS-induced stomach ulcer formation. Changes of mucosal glutathione (GSH) and mucus production also were evaluated due to previous reports that gastric GSH and mucus possess cytoprotective effects on the gastric mucosa (16, 33, 17, 19).

Materials and Methods

Animals

Male Wistar rats, weighing 200-250 g, were

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obtained from and housed in The Laboratory Animal Center, National Cheng-Kung University, Tainan, Taiwan, Republic of China. The rats were deprived of food but allowed free access to tap water overnight. Under light diethylether-anesthesia, streptozotocin (STZ 65 mg/kg) dissolved in citric saline (pH 4.0), was immediately injected into rat femoral vein for inducing diabetes (DM). Age-matched control rats were received the same volume of citric saline. Both DM and control rats were housed individually in a room with 12-h dark-light cycle and with central air conditioning (25 °C temperature, 70% humidity). They were allowed free access to water and pellet diets (the Richmond standard, PMI Feeds, Inc. St. Louis, MO, U.S.A.). The animal care and experimental protocols were in accord with the guidelines of The National Science of Concil of Taiwan, Republic of China (NSC 1994). The blood glucose concentrations were determined on the next day after administration of STZ and the time rats were killed. The concentrations of blood glucose were determined on a chemistry analyzer (Technicon, ames; RA-50, Elkhart, IN. USA), and blood glucose levels over 250 mg/dl were considered to be DM. The manifestations of DM, such as polyuria, polydipsia and body weight loss were recorded every day.

Influences of DM on CRS-Induced Gastric Ulcer

In this experiment, rats were divided into four groups. Group 1 and 2 contained 8-week STZ-DM and age-matched non-DM rats without CRS, respectively. Group 3 and 4 were 8-week STZ-DM rats and age-matched non-DM rats received 4 h CRS, respectively. Each group contained 8 animals. Gastric motility of rats was recorded during the experiment. At the end of the experiment, rats were killed under ether anesthesia. The arterial blood samples were collected for determining glucose levels, and their stomachs were dissected. The mucosal injuries were evaluated by morphological and histological study. In addition, the concentrations of mucosal GSH and mucus were measured.

Time-Course Effects of DM Progression on CRS-Induced Gastric Mucosal Lesions

The experiment is performed to clarify the lowering of CRS-induced mucosal lesions is associated with DM-development. After 0 (the experiment was started right after STZ was given to the animals), 1, 2, 4, 8 and 16 week (s) of DM induction, rats were received 4-h CRS. Age-matched non-DM rats were served as control. Gastric motility was recorded 30 min before CRS and during CRS. After CRS was achieved, rats were killed and the concentrations of mucosal GSH, mucus and injuries were determined.

Effects of Vagotomy or Cholinergic Drugs on CRS-Induced Gastric Lesions in DM Rats

Gastric vagotomy was performed in 8-week STZ-DM rats as demonstrated by Shay (27). Sham vagotomy was used as control. In brief, rat stomachs were exposed under diethylether-anesthesia. The subdiaphgramatic bilateral truncal vagi were severed. Care was taken to avoid injury of blood vessels supplied on the lower esophagus. The vagotomy was performed 30 min after the basal motility was stabilized. Anticholinergic drug, atropine and several cholinergic contractile agents, such as bethanechol (BCH), 2-deoxy-D-glucose (2-DG) and metoclopramide (MTCP), were given to rats intraperitoneally just before CRS. Control rats were challenged with the same volume of saline. The dose of atropine (1.0 mg/kg) used in the study has been shown to completely inhibit colonic motor activity induced by corticotropin releasing factor (CRF) in rats (20). The doses of BCH, 2-DG and MTCP were 2, 20 and 135 mg/kg, respectively. All of these doses caused optimal stimulation of gastric motility in dose-response curves of each agent in normoglycemic rats of without CRS in our pilot study.

Measurement of Gastric Motility

Gastric motility was determined by using a balloon method as previously described (29). Briefly, the rat abdomen was opened under diethylether-anesthesia. A small incision was made on the forestomach from which gastric contents were gently expelled out. Then, a balloon with a catheter connected to a 3-way stopcock was inserted in the stomach through the same incision. The incision of forestomach and abdominal wound of the animals were secured with suture. The balloon in the stomach was then instilled with tap water through the 3-way stopcock. The intrapressure of the balloon was calibrated by using a manometer, and was kept at 10 mmHg before recording. The balloon catheter was then connected to a pressure transducer (Doppler Master, Model DM-1, Hopkinton, MA. USA) together with a polygraph (Lectromed Multitrace 4, Letchworth Garden, Great Britain). With this device, gastric motility can be recorded continuously as intraballoon pressure changed. After gastric motility was stabilized, the first 30-min of the motility was recorded and was taken as basal motility. Then, both DM and non-DM rats were restrained and removed into the cold refrigerator. Gastric motility was recorded again for 4 h thereafter. The motility was quantified by counting

the number of contractions (frequency of the motility) with amplitudes over 10 mmHg and by measuring the height of amplitude of each contraction (clear spike) during the test period. The mean of these values in each rat was estimated. The CRS-induced changes (%) in amplitude was calculated as follow: mean amplitude during CRS / mean basal amplitude x 100% in each rat. The change in frequency was calculated in the same manner. The means of changes in amplitude or frequency of gastric motility from 8 rats were determined.

Measurement of Gastric Mucus

Gastric mucus was assessed by the method described previously (8). Namely, the rat stomach was excised and washed with tap water. The sample was immersed in 10 ml of a solution containing alcian blue (1.0 mg/l), sucrose (0.16 mol/l) and sodium acetate (0.05 mol/l) for 2 h. To remove the unbound dye, the sample was washed for 15 and then 45 min in a 0.25 mol/l sucrose solution. The mucus-bound dye was eluted by immersing gastric mucosa in 10 ml of 0.5 mol/l $MgCl_2$ solution for 2 h. The obtained solution was mixed with 10 ml of diethylether. The absorbance of the color aqua solution was measured on a spectrophotometer (Hitachi, U3210, Tokyo, Japan) at 605 nm. The amount of alcian blue extracted from the tissue was analyzed against a standard curve which was obtained from known graded concentrations (5-50 mg/l) of alcian blue solution. All samples were measured in duplicate. The results were expressed as μg alcian blue/g wet tissue.

Gastric Lesions Induced by CRS

The method of inducing CRS-lesion was similar to that used by William (32). This method can produce consistent and rapid CRS-lesions in rats. After recovery from diethylether-anesthesia, the rat was restraint in the cold environment that was provided by a ventilatory refrigerator maintained at 4.0±0.1°C. After received CRS for 4 h, rats were killed under diethylether-anesthesia, and the abdomen was opened. The arterial blood was collected from the abdominal aorta near the branches of kidneys. The blood glucose was immediately measured. The stomach was then dissected and filled with 10 ml of 1% formalin for 10 min. Gastric mucosa was exposed by opening the stomach along the greater curvature and put it on a glass plate. The length (mm) and the width (mm) of ulcer on the gastric mucosa were measured with a planimeter (1×1 mm) under a dissecting microscope (x 0.7–x 3.0; American Optical Scientific Instrument 569, Buffalo, NY, USA). The ulcer areas were determined as previously described (17); ulcer area = length x width x $\pi/4$. The total ulcer area (mm^2) of each stomach was recorded. The mucosal lesions were examined by a person unaware of experimental procedures. Histological studies of the stomach also were conducted by methods previously demonstrated (17). Briefly, after gross examining, the specimens taken from corpus were blocked and immersed into 10 % neutral formalin for two days. Blocks were then dehydrated in series of alcohol, cleared in xylene and embeded in paraffin. Sections (7-mm thickness) were cut and stained with hematoxylene and eosin as routine histological procedures. Each section was examined under a microscope (Nikon HF, X-IIA, Tokyo, Japan), and the extent of tissue damage was quantified. The section was scored with an index of 0-5 in which 0 indicated normal appearance of mucosal cells; 1, mild injury in the epithelial cells; 2, mild injury in the upper part of mucosal cells; 3, hemorrhage or edema in the mid or lower part of mucosal cells; 4, degranulation or necrosis of the epithelial cells and 5, serious cell disruption of lower part of the mucosa. The index of each section was evaluated on a cumulated basis to give a maximal score of 15.

Assay of Mucosal GSH

The quantitation of gastric mucosal GSH was performed by a method as previously described [17]. In brief, after the final sample was collected, the rat stomach was dissected. The corpus mucosa was scraped using two glass slides on ice, weighed and homogenized immediately in 2 ml of phosphate buffer (0.1 M NaH_2PO_4 plus 0.25 M sucrose, pH 7.4). Acivicin (250 μM), an irreversible inhibitor of γ-glutamyltransferase, was added to the homogenate to inhibit the catabolism of GSH. The samples were then centrifuged at 4000 r.p.m. for 15 min at 4°C. To determine the recovery of reduced thiol, the supernatant was added with or without GSH (200 μmol reduced GSH contained in phosphate buffer solution, pH 7.0). Subsequently, 0.5 ml of 0.25 M trichloroacetic acid was added to 1.0 ml of the supernatant of each sample, and kept at 4°C for 30 min. After centrifuged the sample at 3000 r.p.m. for 15 min, the supernatant was used to determine GSH using 2,2-dinitro-5,5-dithio-dibenzoic acid. The optical density was measured at 412 nm on a Hitachi spectrophotometer (model U-3210, Tokyo, Japan). All samples were measured in duplicate. Recovery of added internal standard was greater than 90% in all experiments. Absorbances of the samples were measured against a standard curve contrasted with freshly prepared GSH solutions (0.05-0.5 mM) which were treated in the same manner as the tissue samples.

Table 1. Effects of CRS on Gastric Musosal GSH, Mucus Production, Motility Changes and Arterial Blood Glucose Concentrations in DM and non-DM rats

	non CRS		CRS	
	non-DM	DM	non-DM	DM
GSH (μmol/g wet tissue)	2.5±0.2[a]	2.0±0.2[b]	1.8±0.1[b]	1.4±0.2[c]
mucus (mg/g wet tissue)	284.2±21.8[b]	325.8±20.2[a]	245.4±11.7[b]	199.5±23.1[c]
motility (%)				
spike number	100.5±4.5[b]	93.0±4.0[b]	236.0±13.1[a]	96.0±9.0[b]
amplitude	104.5±14.5[b]	81.5±18.5[b]	166.7±8.8[a]	99.0±8.8[b]
ulcer area (mm^2)	0.0±0.0[d]	2.0±0.1[c]	116.8±8.0[a]	10.3±3.6[b]
Blood glucose (mg%)	115.5±14.3[d]	468.7±24.5[b]	144.6±11.5[c]	532.6±21.2[a]

The experiment was conducted in 8-week STZ-DM and age-matched non-DM rats. CRS was carried out for 4h. Each test contained 8 rats. Data are expressed by means±SEM. Values in a row with different superscripts are significantly different. ($p<0.05$). a>b>c>d. Abbreviations: CRS=cold restraint stress, GSH=glutathione, DM=diabetes mellitus, STZ=streptozotocin.

The results obtained from tissue samples were expressed as micromol per gram wet tissue.

Measurement of Gastric Mucosal Acetylcholine Contents

Gastric corpus mucosal acetylcholine (ACh) contents in 8-week STZ-DM and aged matched non-DM rats were measured using HPLC-ED with an immobilized-enzyme reactor (12). The tissues were homogenized in 500 μl of 0.2 M perchloric acid containing 0.01% EDTA-2Na and 20 nM ethylhomocholine. The homogenates were centrifuged at 10000 g for 20 min at 4°C. Then, a 25 μl of the supernatant was injected directly into a HPLC-ED system which consisted of a M45 pump (Waters Assoc., Milford, Mass., U.S.A.), refrigerated microsampler (Carnegic, Medicine), chromatographic columns , an immobilized column (Bio Analytical System, Tokyo, Japan), a column heater (LC-23B, BAS, U.S.A.) and an electrolyte detector (LC-4B, BAS) installed with a platinum working electrode (TL-10 A, BAS). The mobile phase consisted with 50 mM sodium phosphate buffer (pH 8.3), 1 mM tetramethylammonium chloride and 70 mM sodium 1-octanesulphonic acid, at a flow rate of 0.8 ml/min. The chromatographic and immobilized enzyme columns were maintained at 35°C with a column heater. The applied potential was + 500 mV versus Ag/AgCl.

Chemicals Used

The following chemicals in reagent grade were used. Acivicin, alcian blue, EDTA-2Na, ethylhomocholine, rat hemoglobin, reduced glutathione, sodium 1-octanesulphonic acid, streptozotocin, tetramethylammonium chloride, trichloroacetic acid, acivicin, bethanechol, metoclopramide, 2-deoxy-D-glucose, n-butanol, perchloric acid, pyridine, sodium laurylsulfate, 1,1,3,3-tetramethoxypropane and 2-thiobarbiturate were purchased from Sigma, St. Louis, Mo. U.S.A. or Wako, Tokyo, Japan or Fluka, Buchs, Switzerland. The purity of all drugs was over 98%. All chemical solutions were freshly prepared before use.

Statistical Analysis

The data obtained from the experiments were expressed as means±SEM. Significant differences in the data of experiments for single measurement traits were analyzed statistically by using ANOVA. In Fig. 5 and Table 1, data were analyzed by Tukey honestly significant difference test for pairwise comparison after ANOVA (21). A p-value of 0.05 or less was considered statistical significant. A simple regression analysis was used to determine the correlation between two different variances.

Results

Comparison of Ulcer Formation and Blood Glucose Levels in DM and Non-DM Rats under either Normal or CRS Condition

It was indicated that in the DM rat, a greater gastric mucus production and a slightly lowered mucosal GSH levels was found in the normal condition. A smaller incidence of mucosal injury also was observed in these animals. The arterial blood glucose in the DM rats increased to 4 folds of that in

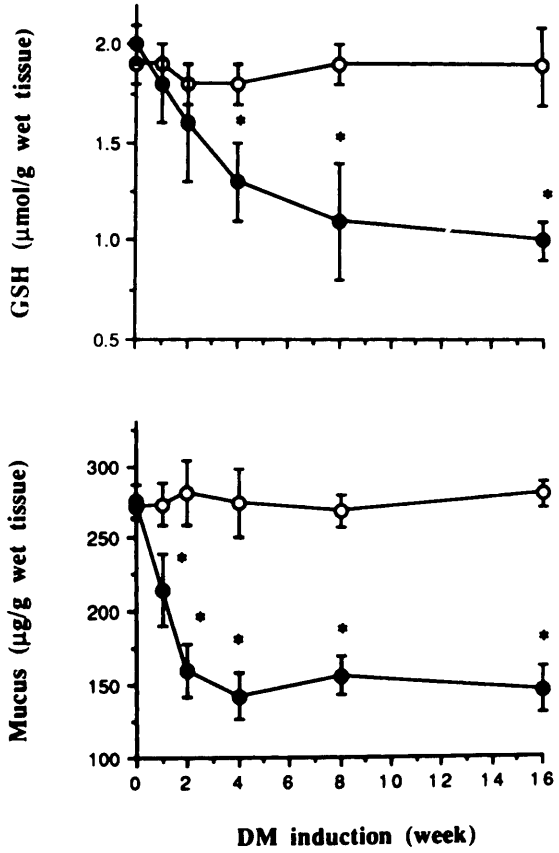


Fig. 1. Changes in gastric mucus and mucosal GSH production in 0-16 week STZ-DM (●–●) and age-matched non-DM (O–O) rats after CRS
Streptozotocin (65 mg/kg in 1.0 ml citric saline) was challenged intravenously to rats 8 weeks before experiments. Age-matched non-DM rats received citric saline only. Each test contained 8-10 rats. * p< 0.05.

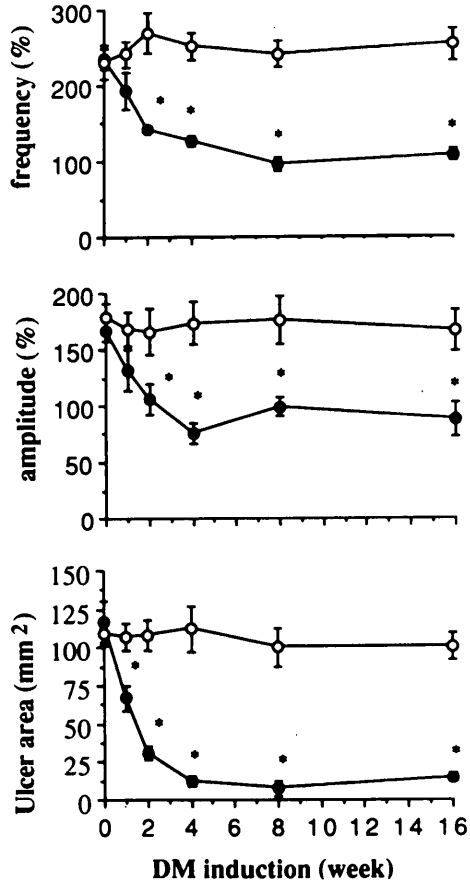


Fig. 2. Changes in amplitude and frequency and gastric mucosal lesions in 0-16 week STZ DM (●–●) and non-DM (O–O) rats under CRS
Intravenous STZ (65 mg/kg in 1.0 ml citric saline) was challenged to rats 8 weeks before experiments. Age-matched non-DM rats received citric saline only. Each test contained 8 rats. *p< 0.05.

non-DM rats (Table I). Gastric corpus mucosal ACh contents in 8-week STZ-DM rats (7.6±0.3 ng/g wet tissue, n= 7) were significantly lower (p< 0.001) than those in age-matched non-DM rats (11.3±0.5 ng/g wet tissue, n= 7).

After received CRS, both DM (8-week STZ-induction) and non-DM rats showed a significant (p< 0.05) reduction in GSH levels and mucus productions in the gastric mucosa. A marked enhancement in gastric motility by increase in amplitude and frequency of contractions was achieved in non-DM rats. However, such elevation in gastric motility caused by CRS was not found in DM rats with 3.7-fold of higher blood glucose levels than non-DM rats. Although CRS did not significantly change either amplitude or frequency in DM rats, it caused a significant (p< 0.05) increase in lesion formation.

Under the same CRS condition, DM rats produced a lower mucosal GSH contents and mucus production than that in non-DM rats. A marked decrease in gastric motility, including amplitude and frequency, and lesion formation was observed.

Time Course of DM-Induced Lesion Formation

In order to ascertain that the lowering of CRS-lesion is associated with DM formation, the influences of DM-progression on gastric mucosal GSH, mucus production and lesion formation in the CRS condition was investigated. As shown in Fig. 1, a gradual decrease in mucosal GSH contents and mucus production was obtained in 0-16- week STZ-DM rats. The maximal reductions in mucosal GSH and in mucus contents were achieved at 2 weeks after STZ was challenged. Thereafter, they maintained almost the same levels 16-week after STZ was given. These mucosal GSH contents and mucus pro- duction in DM rats were significant lower than that in non-DM rats with the same experimental schedules. In Fig. 2, either the amplitudes or the frequencies of

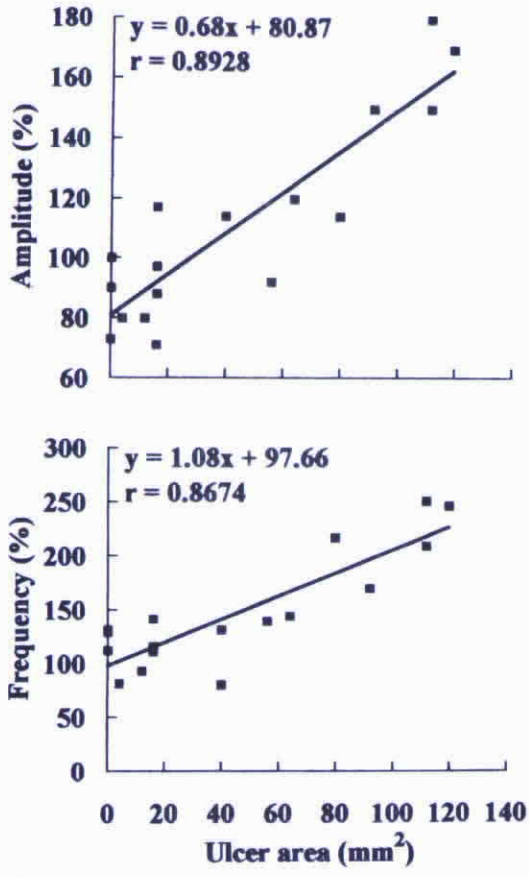
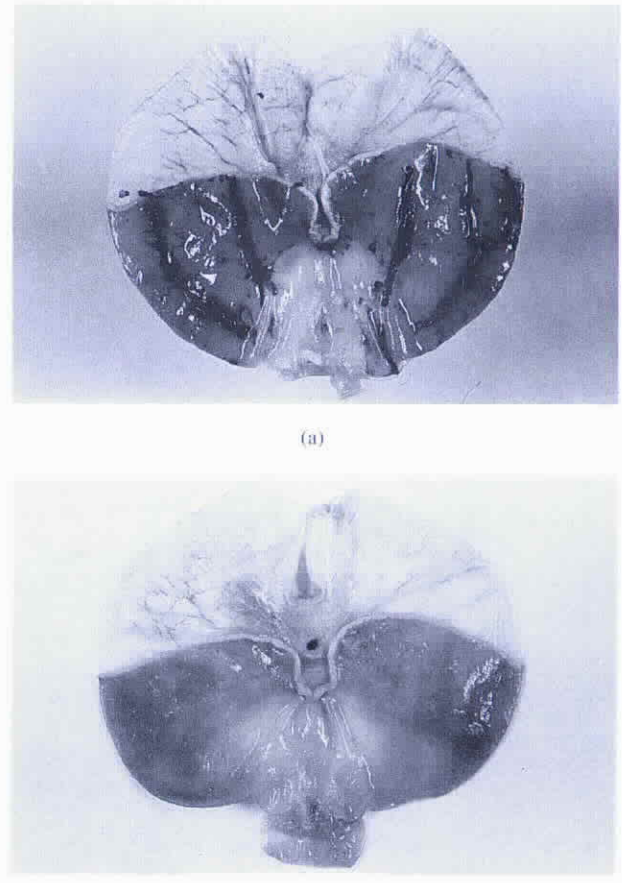


Fig. 3. Correlations between amplitude and mucosal lesions as well as between frequency and mucosal lesions in 8-week STZ-DM rats exposed to CRS



(a)

(b)

Fig. 4. Morphological studies of gastric mucosa in either non-DM or DM rats exposed to CRS
Note that the mucosa of non-DM rats produces severe band-stripe hemorrhage lesions that are observed mainly on the corpus of gastric mucosa (A). Only mild damage is observed on the gastric mucosa of 8-week STZ-DM rats (B).

gastric motilities also were reduced along with the DM development. Gastric lesion formation in these DM rats also was in a DM development-dependent manner.

The decrease in gastric lesion formation was closely correlated with either the amplitude ($r = 0.8928$, $p < 0.05$) or frequency ($r = 0.8674$, $p < 0.05$) of gastric motility in DM rats received CRS (Fig. 3). Apparently, gastric motility was lower in the DM rats, and this was contributed to the lowering of CRS-induced mucosal lesion formation.

Morphological and Histological Investigation of Gastric Mucosa

As shown in Fig. 4, after received CRS, the mucosa of non-DM rats produced intensive stripe-band lesions accompanied with severe hemorrhage which were mainly produced on the corpus but seldom on the antrum or the forestomach. Fig. 5 is the quantification of gastric mucosal cell damage in either DM or non-DM rats exposed to CRS. Greater damage of epigastric and midpart of mucosal cells accompanied with severe gastric edema were produced in non-DM rats exposed to CRS. Under the same condition of CRS, the gastric mucosa of 8-week STZ-DM rats produced only slight injuries. The damages of these DM rat gastric mucosal cells were far less intensive than that in non-DM rats histologically.

Effects of Vagotomy and Cholinergic Drugs

The effects of gastric vagotomy, atropine, BCH, MTCP and 2-DG on gastric motility and mucosal lesions in 8-week STZ-DM rats exposed to CRS were shown in Table II. After bilateral vagotomy at lower esophagus, the amplitude of gastric motility in DM rats under CRS was markedly reduced. Intraperitoneal atropine at the dose of 1.0 mg/kg also was effective in inhibition. Either atropine or vagotomy abolished the lesion formations in these CRS-DM rats. Intraperitoneal BCH (2.0 mg/kg), on the contrary,

Table 2. Effects of BCh, MTCP and 2DG, Atropine and Vagotomy on Gastric Motility Changes and Hemorrhagic Lesions in 8-week STZ-DM Rats Under CRS

	Control	BCh	MTCP	2-DG	Atropine	Vagotomy
	---	2.0 mg/kg	20.0 mg/kg	135.0 mg/kg	1.0 mg/kg	---
Motility						
spike number	96.0±9.0	179.0±30.3*	179.0±10.5*	164.0±1.0*	88.0±12.1	91.0+18.3
amplitude	99.0±8.8	172.7±34.0*	126.7±12.0*	146.0±21.0*	17.5±2.1*	41.0±5.2*
Ulcer area (mm^2)	9.2±3.6	161.0±22.8*	18.8±4.4*	28.0±4.0*	0.0±0.0*	0.0±0.0*

All drugs were given by i.p. (1.0 ml) at the beginning of CRS. Vagotomy was conducted during gastric surgery. Control rats received sham vagotomy and saline. Each test contained 6 rats. *p<0.05. Abbreviations: BCH=bethanechol, MTCP=metoclopramide, 2-DG=2-deoxy-D-glucose, STZ=streptozotocin, DM=diabetes mellitus.

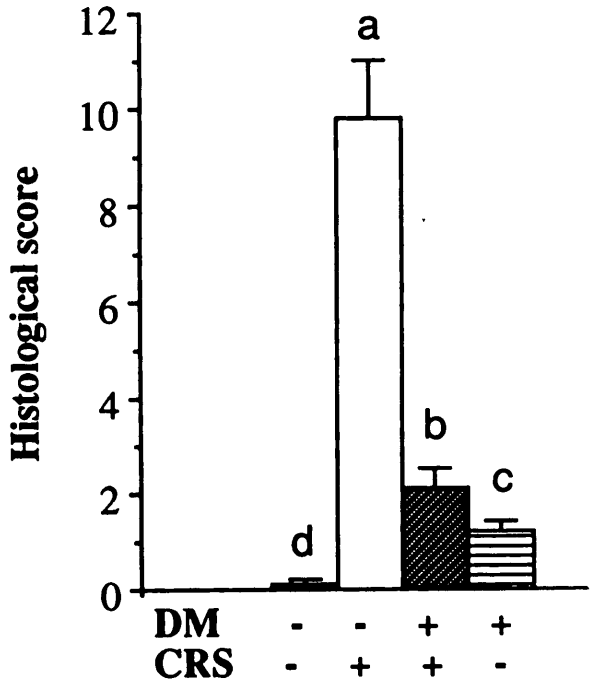


Fig. 5. Histological scores of DM and non-DM rats exposed to CRS Eight-week STZ-DM and age-matched non-DM rats were used. The scores of mucosal cell damages are estimated as described in the section of materials and methods. Each test contained 6 rats. Values with different superscripts are significantly different (P< 0.05). a>b>c>d.

produced enhancements of both amplitude and frequency of gastric motility, and promoted lesion formations. Particularly, the ulcers were aggravated 22.3 folds over control (saline-treated) group. When MTCP and 2-DG were challenged to DM rats, the amplitude and frequency increased to 1.3 and 1.5 folds over control group, respectively. Whereas the frequencies were elevated to 1.9 and 1.7 folds over the control, respectively. Gastric mucosal lesions in these DM rats also were significantly aggravated by MTCP and 2-DG.

Discussion

In the present study, when DM rats were exposed to CRS, a reduction of mucus production was found. Gastric mucus is important in the healing of mucosal hemorrhage ulcers induced by stress (19) or by ischemia/reperfusion (26). The increase in mucus secretion is involved in the mucosal protective effects of prostaglandin E_2(3, 16). However, a reduction of mucus production in DM rats receiving CRS despite that the mucosal lesion was greatly inhibited. This indicated that mucus might not play a critical role in the lowering of CRS-induced lesions in DM rats.

Glutathione, the γ-glutamyl-cysteinyl-glycine tripeptide, is known to play pivotal role in the cellular defense system. It acts to prevent lipid peroxidation produced by oxyradicals and/or oxygen derived species. Document indicated that free radical is associated with stress-induced gastric mucosal ulceration in feeding restricted (22) or DM animals (24). Glutathione also may affect gastric motility and permeability in addition to mucosal cytoprotection (28, 29). In the other document, a lower gastric mucosal GSH in patients with peptic ulcer was proposed (13). This decrease in gastric mucosal GSH contents may be one of etiologic factors in CRS-induced lesion formations. The present study also showed that gastric mucosal GSH concentrations in CRS-DM rats were decreased in a DM-progressing-dependent manner. This may imply that in rat gastric mucosa, the biosynthesis of mucosal GSH may be reduced during the development of DM. However, the generation of oxyradicals was increased in both CRS (25) and DM (23, 34). Thus, the consumption of GSH to scavenge free radicals for maintaining homeostasis in the CRS-DM rat can not be ruled out.

Under CRS, the present study also demonstrated that gastric motility and mucosal damage were decreased in a DM-progressing manner. Moreover, high correlations between mucosal lesions and changed amplitude (r= 0.8928) as well as mucosal

lesions and altered frequency (r=0.8674) were observed in the CRS-DM rats. The decrease in mucosal damage induced by CRS in DM rats related to the elimination of gastric motility seems possible.

It is widely recognized that vagus nerves and cholinergic agonists intensively regulate gastrointestinal motility that can be diminished by vagotomy and/or vagoneuropathy. In the present study, a decreased gastric corpus mucosal ACh content was found in 8-week STZ-DM rats. The results implied that vagal neuropathy actually occurred in DM rats used in the present research. Other document also reports that neuropathy can occur in 2-month STZ-DM rats (18). The inhibition of gastric motility and ulcer formation by vagotomy or atropine indicated that cholinergic nerves played an important function in the CRS-induced ulcers. Pharmacologically, 2-DG can stimulate vagal activity by competition with glucose in the central vagal nucleus. An excitation of vagus nerves can cause acetylcholine release to increase gastric motility. Bethanechol, an agonist of muscarinic (M1) cholinoceptor, shows a marked stimulation in smooth muscle. Under the condition of CRS, both gastric motility and mucosal lesions were greatly enhanced by BCH. Due to the potent stimulation of gastrointestinal motility, MTCP is widely used in clinics for the treatment of gastroparesis in DM patients (24). We found that MTCP enhanced gastric motility in parallel to the formation of mucosal lesion induced by CRS in DM rats. These results indicated that the decrease of gastric motility in the DM rat seems important for the lowering of CRS-provoked mucosal damage.

Mechanisms for the low susceptibility to CRS-induced gastric damage in DM rats may be multiple. Plasma glucose may modulate gastric motility (1), and insulin can increase gastrointestinal motility (4, 7). The parameters, such as an alteration of glucose or insulin levels as well as the changes in sympathetic functions (9) or gastric secretion or change in CRF (20) may also associate with low stress ulcer in DM rats. They need to be identified in advance.

In conclusion, decrease in gastric motility resulted from cholinergic neuropathy may at least be responsible for the low susceptibility of stress ulcer formation in STZ-DM rats.

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