

Interaction of insulin with prokinetic drugs in STZ-induced diabetic mice

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Abstract

AIM: To study the possible interactions of metoclopramide, domperidone and erythromycin in streptozotocin-induced diabetic mice treated with insulin by various parameters.

METHODS: Effects of the individual as well as combined drugs were studied in diabetic mice *via* estimation of the blood glucose and serum insulin levels, small intestinal transit (SIT), gastric emptying (GE), xylose absorption and glucose tolerance tests. Groups were given insulin 2 IU/kg *s.c.*, metoclopramide 20 mg/kg *p.o.*, domperidone 20 mg/kg *p.o.* and erythromycin 6 mg/kg *p.o.* individually and in combination. There were also normal and diabetic control groups. The first set of experiments was carried out to investigate the sub-chronic effect on blood glucose and serum insulin levels in diabetic mice of one week of daily dose administration of the tested drugs individually as well as the combination of insulin with each prokinetic drug. The other five sets of experiments were carried out to investigate

the acute effect of a single dose of each drug individually and in combination on blood glucose and serum insulin levels, SIT, GE, oral xylose absorption and glucose tolerance tests.

RESULTS: The study included the prokinetic drugs metoclopramide (20 mg/kg), domperidone (20 mg/kg) and erythromycin (6 mg/kg), as well as insulin (2 IU/kg), which was individually effective in decreasing SIT, enhancing GE and increasing xylose absorption significantly in diabetic mice. Erythromycin tended to decrease blood glucose level and increase serum insulin level after 1 wk of daily administration in diabetic mice. Erythromycin potentiated the effect of insulin on blood glucose level and serum insulin level whereas other prokinetic agents failed to do so after repeated dose administration in diabetic mice. Metoclopramide or erythromycin in combination with insulin significantly decreased SIT, in diabetic mice, to lower levels than with insulin alone. Administration of prokinetic drugs along with insulin antagonized the action of insulin on xylose absorption. These combinations also increased the rate of glucose absorption from the gut.

CONCLUSION: The present study suggests that prokinetic drugs could potentially improve glycemic control in diabetic gastroparesis by allowing a more predictable absorption of nutrients, matched to the action of exogenous insulin. The use of prokinetics, such as erythromycin, may be interesting in the clinic in decreasing the need for insulin in diabetic patients. The dose of insulin may be safely decreased with erythromycin in chronic treatments.

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Key words: Streptozotocin; Gastrointestinal motility; Insulin; Prokinetic drugs; Intestinal absorption

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INTRODUCTION

Diabetes mellitus is the most common cause of gastroparesis and disturbed gastric and small intestinal intestine motility. Gastroparesis is a syndrome characterized by delayed gastric emptying (GE) in the absence of mechanical obstruction of the stomach. The cardinal symptoms include postprandial fullness (early satiety), nausea, vomiting, bloating, or epigastric pain. Symptoms attributable to gastroparesis are reported by 5% to 12% of patients with diabetes^[1,2]. There is an association between self-reported glycemic control and psychological distress, and the development of gastrointestinal symptoms in diabetics^[3]. Impaired function of the gastrointestinal tract related to diabetes mellitus results from diabetic autonomic neuropathy, impaired sensory innervation and the direct effect of persistent hyperglycemia^[4]. Once established, diabetic gastroparesis tends to persist, despite amelioration of glycemic control. Thus, GE and symptoms are stable over ≥ 12 years follow-up, despite improved glycemic control^[5]. Gastroparesis affects nutritional state and, in diabetics, it also has deleterious effects on glycemic control and secondary effects on organs, leading to increased mortality^[6]. First-line treatment includes restoration of nutrition and medication using prokinetic drugs. Therefore, searching for therapeutic interventions with prokinetic drugs that will improve the specific alterations associated with diabetic gastroparesis represents the most important aim of the present study. Prokinetic drugs have been used for gastroparesis in diabetic patients for a relatively long time and some data about the interactions with insulin in the clinic should be available. It seemed of interest to investigate the possible drug-drug interactions, which may develop from co-administration of insulin and the prokinetic drugs metoclopramide, domperidone and erythromycin. Also, the study aims to highlight the possibility that prokinetics might increase the hypoglycemic effect of insulin.

Prokinetic drugs, commonly used to treat delayed GE, have variable effects on small intestinal motility, and little is known about their effects on glucose absorption. The prokinetic drugs act primarily through neurons since peristalsis is based on neural reflexes. Dopamine antagonists such as metoclopramide and domperidone^[7] are used in this study. Motilides such as erythromycin enhance peristalsis by acting on motilin receptors^[8].

In the present study, streptozotocin (STZ)-induced diabetic mice were treated with insulin and prokinetic agents (metoclopramide, domperidone, erythromycin) individually and in combination. Acute and subchronic studies were carried out to determine whether the prokinetic drugs could improve the blood glucose level and

neuropathy changes in diabetic conditions treated with insulin. This was achieved by measuring some of the biochemical parameters affected by persistent hyperglycemia *via* estimation of blood glucose and serum insulin levels. The acute study were carried out to determine the effect of the test drugs on the gastrointestinal tract motility represented by small intestinal transit (SIT) and GE, in the knowledge that all of the prokinetic drugs used produce acute actions on the gut. The rate of GE is an important determinant of carbohydrate absorption and thus of the blood glucose profile^[9]. Oral xylose absorption and glucose tolerance tests were used as representative indices of carbohydrate absorption changes.

MATERIALS AND METHODS

Drugs and reagents

Insulin (Regular insulin, Novonordisk, Denmark), Metoclopramide (Memphis Pharmaceutical Co., Cairo, Egypt), Domperidone (El Kahira Pharmaceutical Co., Cairo, Egypt) and Erythromycin ethylsuccinate (Abbott Laboratories, Cairo, Egypt) were obtained. Glucose Reagent Kit (Biomerieux, France), Insulin IRMA Kit IM3210 (Immunotech Beckman coulter, Czech Republic), STZ (Sigma Aldrich Chemie, Germany), Phloroglucinol (Sigma chemical Co., United States) and *D*-xylose (Acros Organics, United States) were used. Insulin was diluted with normal saline solution to obtain a suitable strength for injection. Hydroxypropylmethylcellulose (1%) was used as vehicle to administer prokinetic drugs. The other reagents were the highest grade of commercially available products.

Animals

Healthy adult male albino mice weighting between 20-30 g were used in the present study. They were obtained from the animal house of the research department of Kahira pharmaceutical company, Cairo, Egypt. All animals were fed a standard pellet chow and had free access to water. They were maintained under controlled laboratory conditions (temperature, humidity) throughout the study. New groups of mice were recommended for each test carried out. Animals were sacrificed under mild ether anesthesia. Experiments were conducted in accordance with the guidelines set by the animal health research ethics training initiative, Egypt.

Drug treatments

Control groups received equal volumes of vehicle through corresponding routes. Groups were given insulin 2 IU/kg *s.c.*, metoclopramide 20 mg/kg *p.a.*, domperidone 20 mg/kg *p.a.* and erythromycin 6 mg/kg *p.a.* individually and in combination. There were also normal and diabetic control groups. The doses were selected based on the earlier reports, recommended clinical doses and prior pilot experiments^[10-12]. Metoclopramide, domperidone or erythromycin in the dose mentioned above were given alone 15 min before the administration of insulin/ve-

hicle. Insulin was given 50 min before determination of blood glucose and serum insulin levels. Six main sets of experiments were carried out. The first set of experiments was carried out to investigate the subchronic effect on blood glucose and serum insulin level in STZ-induced diabetic male mice of one week of a daily dose of insulin, metoclopramide, domperidone and erythromycin individually as well as the combination of insulin with metoclopramide, domperidone or erythromycin. The other five sets of experiments were carried out to investigate the acute effect of a single dose of insulin, metoclopramide, domperidone and erythromycin individually as well as the combination of insulin with metoclopramide, domperidone or erythromycin on blood glucose and serum insulin level, SIT, GE, oral xylose absorption and glucose tolerance tests.

Experimental induction of diabetes

Diabetes mellitus was induced in overnight fasted mice by a single intraperitoneal injection of freshly prepared solution of STZ (100 mg/kg body weight) in 0.1 mol/L cold citrate pH 4.5^[13,14]. The animals were allowed to drink 5% glucose solution to overcome STZ-induced hypoglycemia^[15]. The control mice were injected with citrate buffer alone. Two weeks after STZ injection, blood samples were collected by tail venopuncture of the mice and used for the estimation of blood glucose levels using an advanced Glucometer ACCU-CHEK (Roche, Germany)^[16,17]. Overnight fasted mice with blood glucose level above 200 mg/dL were selected and used in the present study.

Measurement of blood glucose and serum insulin levels

Blood was collected from the retro-orbital venous plexus according to the method of Cocchetto *et al.*^[18]. Blood was collected into Wassermann tubes using heparinized microhematocrit capillaries. Blood glucose level was measured using advanced a Glucometer ACCU-CHEK. Serum was separated by centrifugation at 11000 *g* for 2 min and serum glucose level was determined immediately using a glucose kit^[19]. There was no significant difference in glucose levels between the two methods. The remaining amount of serum was kept frozen at -20 °C for insulin determination. Serum insulin was estimated by immunoradiometric assay (IRMA)^[20] using an insulin IRMA Kit. This estimation was done 2 min before drug/vehicle administration and 50 min after insulin/vehicle administration.

Small intestinal transit

The passage of a charcoal meal through the gastrointestinal tract of mice was used as parameter for intestinal motility^[10,21]. Overnight fasted mice were treated with test prokinetic drug orally 45 min and/or insulin subcutaneously 30 min before administration of charcoal meal (0.3 mL of a 5% suspension of charcoal in 2% hydroxypropylmethylcellulose solution). After 20 min, animals were killed by cervical dislocation just after mild ether anesthe-

sia. The abdomen was opened, the charcoal marker was identified in the small intestine and tied immediately to avoid movement of marker. The entire intestine was removed by cutting at the pyloric and ileocaecal ends and then washed in water. The distance the meal had traveled through the intestine as indicated by the charcoal was measured and expressed as percent of the total distance from the pylorus to the caecum. SIT = (distance travelled by charcoal/total length of the small intestine) × 100.

Gastric emptying

GE was determined by the phenol red method^[12,22]. The test prokinetic drug was given alone 45 min before administration of phenol red meal. Insulin (*s.c.*) was injected 30 min before the administration of the meal. A solution of 1.5% hydroxypropylmethylcellulose containing 0.05% phenol red as a marker was given intragastrically (0.5 mL/mouse) to overnight fasted mice. Fifteen minutes later, animals were sacrificed by cervical dislocation just after mild ether anesthesia. The abdominal cavity was opened, the cardiac and pyloric ends of the stomach were clamped, and the stomach was then removed and washed with normal saline. The stomach was cut into pieces and homogenized with 25 mL of 0.1 mol/L NaOH. The suspension was allowed to settle for 1 h, 5 mL of the supernatant was then added to 0.5 mL of 20% trichloroacetic acid (w/v) and centrifuged at 3000 *g* for 20 min. 4 mL of 0.5 mol/L NaOH was added to 1 mL of supernatant. The absorbance of this pink colored liquid was measured using a spectrophotometer at 560 nm (Model: Shimadzu 150-20). Phenol red recovered from animals that were sacrificed immediately after administration of the test meal was used as a standard (0% emptying). GE (%) in the 15 min period was calculated according to the following equation: GE (GE)% = 100 - ($x / y \times 100$), x = absorbance of phenol red recovered from the stomach of animals sacrificed 15 min after test meal, y = mean ($n = 5$) absorbance of phenol red recovered from the stomach of control animals (killed at 0 min following test meal).

Oral D-xylose loading test

This test measures intestinal carbohydrate absorption by calculating the plasma concentration of *D*-xylose after ingestion of a known amount of *D*-xylose^[23,24]. The test prokinetic drug was given alone 45 min before administration of xylose. Insulin (*s.c.*) was injected 30 min before the administration of xylose. A 30% solution containing *D*-xylose (0.8 g/kg body weight) was administered by oral gavage to overnight fasted mice. After 60 min of xylose administration, blood samples were drawn from the retro-orbital venous plexus and centrifuged at 11000 *g* for 2 min. Plasma xylose concentrations were measured using a colorimetric assay. The assay involved incubation for 4 min at 100 °C of 20 μL of plasma with 1 mL of colored reagent containing 1 g phloroglucinol in 200 mL glacial acetic acid and 20 mL concentrated HCl, followed by

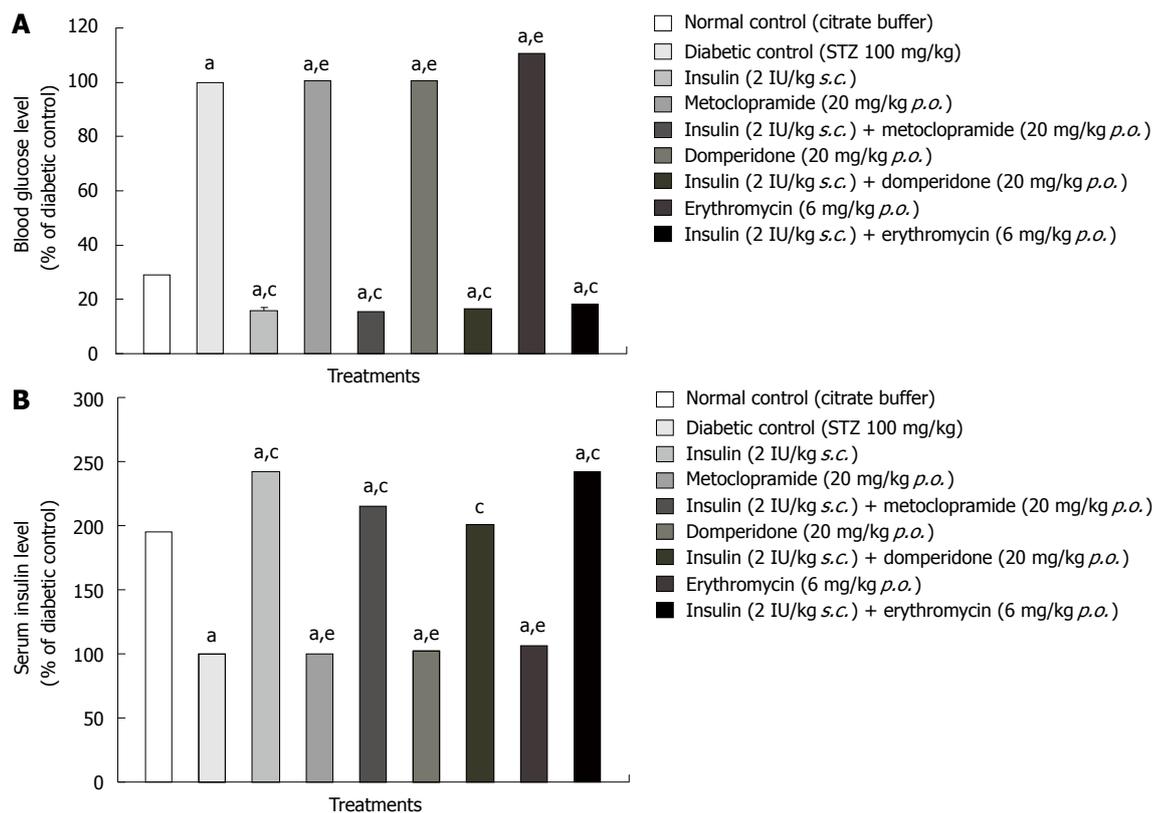


Figure 1 Acute effect in streptozotocin-induced diabetic mice of single doses of insulin, metoclopramide, domperidone and erythromycin individually as well as the combination of insulin with metoclopramide, domperidone or erythromycin. A: Blood glucose level; B: Serum insulin level. Values represent the mean \pm SE of eight mice per group. Significantly different from the normal control value at $^aP < 0.05$; Significantly different from the diabetic control value at $^cP < 0.05$; Significantly different from insulin value at $^eP < 0.05$.

reading of the absorbance at 554 nm using spectrophotometer (Model: Shimadzu 150-20).

Oral glucose tolerance test

The oral glucose tolerance test (OGTT) was used to evaluate intestinal absorption. The test was carried out according to the method of Stümpel *et al.*^[25] and Badole *et al.*^[26]. After the mice were fasted for 12 h, the test compound was administered half an hour before glucose loading. A 50% glucose solution (2.5 g/kg of body weight) was orally administered, and blood was taken from the tail vein at 0, 30, 60 and 120 min afterward. Blood glucose concentrations were determined immediately using an Accu-chek (Roche Diagnostics, Germany). The difference between the value of the diabetic control group and the diabetic treated groups represent the amount of glucose absorption from the intestine affected by the different drugs used in this study in addition to other factors. The extent of absorption of glucose was estimated using the total area under the curve, which represents blood glucose level from t_0 to t_{120} . AUC_{total} is calculated using the trapezoidal rule from t_0 to t_{120} .

Statistical analysis

All data were expressed as the mean \pm SE with 6 to 10 mice per group. Statistical analysis was performed using two way analysis of variance (ANOVA) followed by

Tukey-Kramer multiple comparisons test. For all the statistical tests, the level of significance was fixed at $P < 0.05$.

RESULTS

Effects of insulin and prokinetic drug individually or combined on blood glucose and serum insulin levels in STZ-induced diabetic mice

Figure 1 show the antihyperglycemic effect of insulin against STZ-induced diabetic mice. Acute administration of insulin (2 IU/kg) significantly ($P < 0.05$) decreased blood glucose level to 45.37 ± 4.57 mg/dL and increased serum insulin level to 1.96 ± 0.10 μ IU/kg in diabetic mice close to hypoglycemic value. The acute administration of a single dose of metoclopramide (20 mg/kg), domperidone (20 mg/kg) or erythromycin (6 mg/kg) individually did not affect blood glucose level or serum insulin level in diabetic mice. Acute administration of metoclopramide, domperidone or erythromycin did not affect the action of insulin on blood glucose and serum insulin level (Figure 1). Erythromycin tended to decrease blood glucose level and increase serum insulin level after one week of daily dose administration in diabetic mice. Daily dose administration of insulin (2 IU/kg) for one week significantly ($P < 0.05$) decreased blood glucose level to 45.94 ± 2.60 mg/dL and increased serum insulin level to 2.01 ± 0.02 μ IU/kg in diabetic mice close

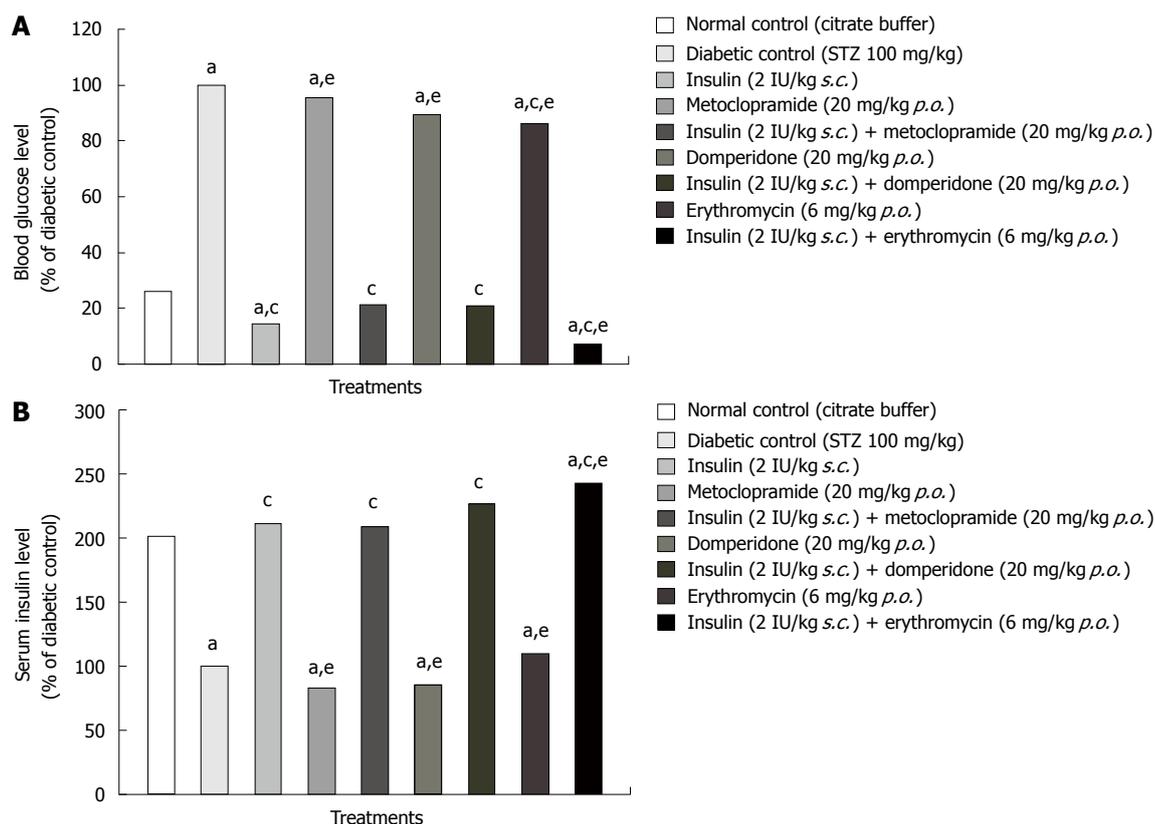


Figure 2 Subchronic effect in streptozotocin-induced diabetic mice of one week of daily administration of insulin, metoclopramide, domperidone and erythromycin individually as well as the combination of insulin with metoclopramide, domperidone or erythromycin. A: Blood glucose level; B: Serum insulin level. Values represent the mean \pm SE of eight mice per group. Significantly different from the normal control value at $^*P < 0.05$; Significantly different from the diabetic control value at $^{\circ}P < 0.05$; Significantly different from insulin value at $^{\#}P < 0.05$.

to hypoglycemic value. There is no interaction between insulin and the test prokinetic, metoclopramide or domperidone, on blood glucose level and serum insulin level after one week of daily administration in diabetic mice. On the other hand, combination of insulin and erythromycin significantly ($P < 0.05$) decreased blood glucose level to 22.9 ± 1.91 mg/dL and increased serum insulin level to 2.18 ± 0.12 μ IU/kg (Figure 2).

Small intestinal transit

The normal control value of the SIT was $56.61\% \pm 2.58\%$ of the total length of the small intestine. Induction of diabetes in mice significantly ($P < 0.05$) increased SIT to $76.90\% \pm 6.12\%$. Insulin (2 IU/kg) significantly ($P < 0.05$) decreased SIT in diabetic mice to $61.05\% \pm 3.85\%$ as compared to diabetic control group. The test prokinetic drugs, metoclopramide (20 mg/kg), domperidone (20 mg/kg) and erythromycin (6 mg/kg), significantly ($P < 0.05$) decreased SIT in the diabetic mice to $50.04\% \pm 2.42\%$, $48.70\% \pm 4.53\%$ and $43.05\% \pm 3.50\%$ respectively. Either metoclopramide or erythromycin in combination with insulin significantly ($P < 0.05$) decreased SIT in diabetic mice and this effect was less than that of insulin alone. Domperidone did not affect the action of insulin on SIT in diabetic mice (Table 1).

Gastric emptying

The normal control value of the GE was $72.50\% \pm 1.68\%$ of the total amount of the phenol red meal given. The high blood glucose level in diabetic control mice delayed GE significantly ($P < 0.05$) to $55.23\% \pm 9.30\%$. Insulin (2 IU/kg), metoclopramide (20 mg/kg), domperidone (20 mg/kg) or erythromycin (6 mg/kg) in the doses employed increased GE significantly ($P < 0.05$) to $95.87\% \pm 2.41\%$, $76.38\% \pm 7.67\%$, $90.92\% \pm 4.92\%$ and $84.77\% \pm 2.11\%$, respectively, compared with diabetic control mice. Administration of prokinetic drugs (metoclopramide, domperidone or erythromycin) along with insulin (2 IU/kg) did not affect the action of insulin on GE (Table 1).

Oral D-xylose absorption test

The normal control value of serum D-xylose concentration was 1.63 ± 0.10 mg/mL after 60 min of D-xylose administration (0.8 g/kg). The amount of xylose absorbed from the GIT significantly ($P < 0.05$) decreased in the diabetic mice, to 0.606 ± 0.030 mg/mL, as compared to normal control group. Insulin (2 IU/kg), metoclopramide (20 mg/kg), domperidone (20 mg/kg) and erythromycin (6 mg/kg) individually increased xylose absorption to 1.64 ± 0.16 mg/mL, 0.989 ± 0.030 mg/mL, 1.162 ± 0.030 mg/mL and 1.469 ± 0.030 mg/mL, respectively. Administration of prokinetic drugs, along with insulin

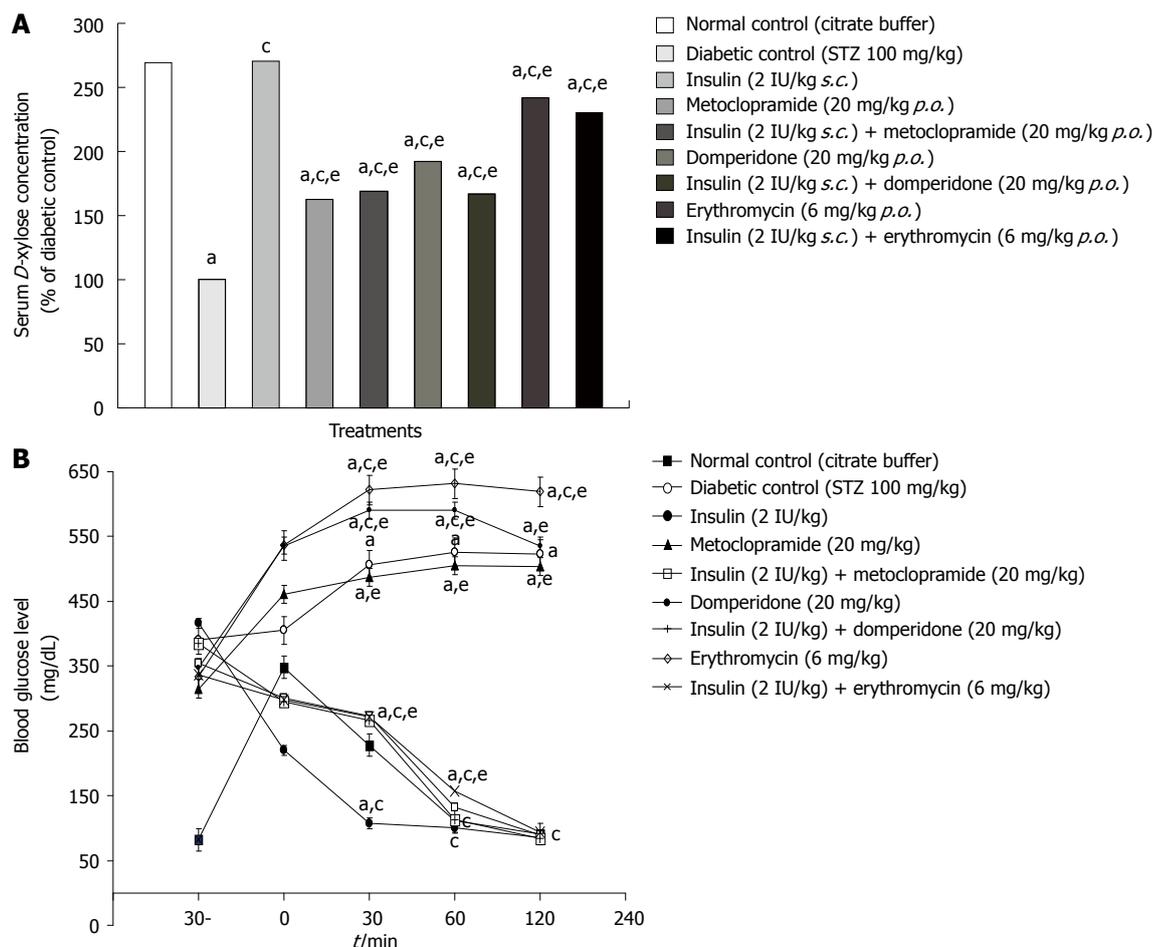


Figure 3 Effect on oral *D*-xylose absorption and tolerance test in streptozotocin-induced diabetic mice of single doses of insulin, metoclopramide, domperidone and erythromycin individually as well as the combination of insulin with metoclopramide, domperidone or erythromycin. A: Oral *D*-xylose absorption test; B: Oral *D*-glucose tolerance test. Values represent the mean \pm SE of eight mice per group. Significantly different from the normal control value at $^aP < 0.05$; Significantly different from the diabetic control value at $^cP < 0.05$; Significantly different from insulin value at $^eP < 0.05$.

antagonized the action of insulin (2 IU/kg) on xylose absorption (Figure 3A) in diabetic mice.

Oral *D*-glucose tolerance test

The OGTT can be used to evaluate blood glucose homeostasis and also indirectly evaluate glucose absorption. As shown in Figure 3B, glucose load (2.5 mg/kg) in normal mice produced rapid increase in blood glucose levels at 30 min and returned to baseline values within 120 min. In contrast, STZ-induced diabetic mice demonstrated basal hyperglycemia (399 ± 14 mg/dL) which remained above 400 mg/dL during all time points determined. The peak increase in serum glucose concentrations in diabetic mice was observed after 60 min of glucose treatment, while that of normal mice observed after glucose loading, indicating delayed glucose homeostasis in diabetic mice. STZ significantly ($P < 0.05$) increased the area under the curve (Figure 4). Insulin (2 IU/kg) significantly ($P < 0.05$) decreased blood glucose level to 107.16 ± 8.51 mg/dL and 100 mg/dL after 30 and 60 min of glucose loading, respectively, and the effects persisted until 120 min (Figure 3B). The area under the curve was significantly reduced to 226.53 ± 12.28 mg/dL after 120 min (Figure 4).

Metoclopramide (20 mg/kg) did not affect blood glucose level where BGL was 487.5 ± 13.6 mg/dL and 505.50 ± 14.55 mg/dL after 30 and 60 min of glucose loading, respectively. Domperidone (20 mg/kg) and erythromycin (6 mg/kg) produced significant ($P < 0.05$) increases in blood glucose level to 590 ± 13 mg/dL, 590.8 ± 17.4 mg/dL and 622.00 ± 23.11 mg/dL, 631.50 ± 21.48 mg/dL after 30 and 60 min of glucose loading, respectively (Figure 3B). In addition, domperidone and erythromycin significantly ($P < 0.05$) increased the area under the curve (Figure 4). Administration of metoclopramide, domperidone or erythromycin along with insulin significantly ($P < 0.05$) increased blood glucose levels as compared to insulin treated values (Figure 3B). Combination of insulin with metoclopramide, domperidone or erythromycin significantly ($P < 0.05$) increased the area under the curve as compared to insulin treated value (Figure 4).

DISCUSSION

Findings of the present investigation revealed that STZ-induced diabetes resulted in a significant increase in SIT and a significant decrease in GE. Abnormalities in GE and small intestinal motor functions were also reported

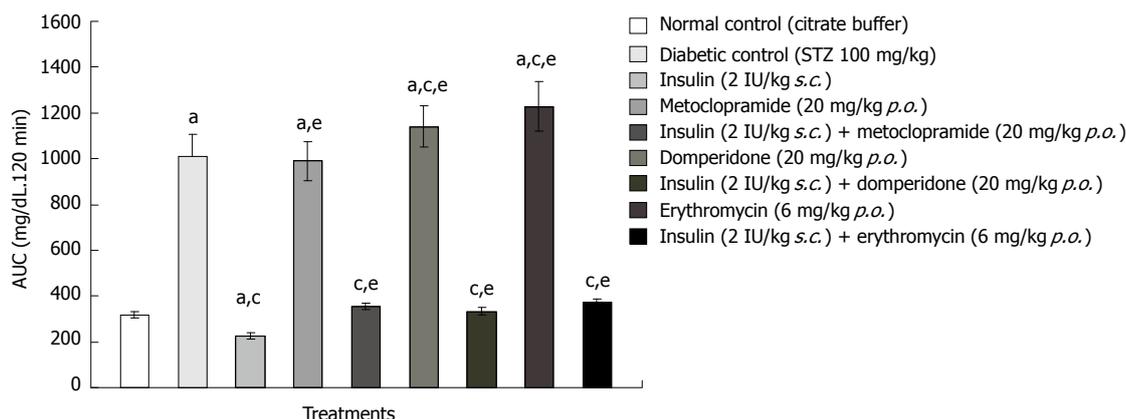


Figure 4 Effect on the area under the curve of blood glucose concentrations during oral glucose absorption test in streptozotocin-induced diabetic mice of single doses of insulin, metoclopramide, domperidone and erythromycin individually as well as the combination of insulin with metoclopramide, domperidone or erythromycin. Values represent the mean \pm SE of eight mice per group. Significantly different from the normal control value at $^aP < 0.05$; Significantly different from the diabetic control value at $^cP < 0.05$; Significantly different from insulin value at $^eP < 0.05$. AUC: Areas under the curve.

in diabetic mice^[27,28]. Increased intestinal transit may be partially due to increased cholinergic and decreased beta-adrenergic receptor activities in diabetic animals^[29]. The delay in GE could be partially attributed to the decrease in the number of myenteric neurons in the stomach as a result of diabetes^[30-32]. Similarly, the increase in intestinal transit could be mediated through the same mechanism. All of the stomach's smooth muscle cells have the ability to produce electric depolarizations "slow waves" from resting potential. These rhythmic contractions are thought to originate in the non-smooth muscle pacer cells in the interstitial cells of Cajal (ICCs)^[33]. GE is delayed because the number of ICCs is markedly diminished in diabetes^[34]. Data of the current study showed that insulin-induced hypoglycemia significantly attenuated SIT and accelerated GE in diabetic mice. These results are partly in agreement with earlier reports^[35-38]. The obtained results might be partially due to the direct effect of insulin and not only due to the antidiabetic effect of insulin in decreasing blood glucose level, thereby leading to decreased SIT. This effect could be due to counter-regulation of hypoglycemia. The actions of insulin on the stomach could be mediated via an insulin stimulant effect on the vagus nerve, as reported by Quigley *et al.*^[39].

Data from the present investigation showed that STZ-induced diabetes resulted in a significant decrease in xylose absorption, in agreement with the finding of Fuessl^[40]. The decrease in xylose absorption could be mediated the decreased rate of GE, which resulted from elevation in BGL, as reported by the present study. This explanation coincides with that given by Rayner *et al.*^[9] and Chapman *et al.*^[41].

Recent studies have shown that modifications of systemic glycemia in OGTT reflect the activity of the intestinal glucose transporter SGLT1^[42]. In the present study, STZ-induced diabetic mice demonstrated basal hyperglycemia, which remained above 400 mg/dL during all time points determined. The capacity of the small intestine to absorb glucose increases in experimentally induced diabetic animals as a consequence of the enhanced

activity and abundance of SGLT1, as shown by Fedorak *et al.*^[43], suggesting SGLT1 as a potential target for glycemic control in diabetic animals. STZ-induced diabetic mice exhibited severe hyperglycemia with increased Na⁺ dependent glucose uptake activity, compared with normal mice^[44]. Acute insulin-induced hypoglycemia increased xylose absorption and glucose absorption from the GIT in the diabetic mice. In the present study, the effects of insulin on the intestinal absorption of sugar did not differentiate between an effect of insulin on the absorption capacity of the mucosa and other factors that may affect total sugar absorption. Some studies have shown that insulin causes increased Na⁺ dependent glucose carrier activity in the small intestine, which leads to increased glucose absorption^[45]. Some studies reported that insulin-induced hypoglycemia accelerates gastric emptying in type 1 diabetes^[37]. The decreased time for movement of sugar from stomach to the small intestine, in addition to its therapeutic effect, decreases the rate of glucose production and increases the rate of glucose utilization by cells^[46].

By studying the prokinetic drugs individually in the current study, domperidone (20 mg/kg *p.o.*) was found to be the most effective agent in the diabetic mice both when compared to diabetic control group and to the other prokinetic drugs. Domperidone, metoclopramide and erythromycin significantly ($P < 0.05$) decreased small intestine transit and accelerated GE in STZ-induced diabetic mice. Similar results have recently been reported from other studies^[47-51]. The inhibitory effect of domperidone on SIT is probably mediated *via* its action on dopamine since it is a dopamine antagonist^[52]. Dopamine has an indirect inhibitory effect through inhibition of cholinergic transmission in the myenteric plexus, which regulates the gastrointestinal tract^[53]. It could be suggested that metoclopramide produces its action through inhibition of presynaptic and postsynaptic D₂ receptors, stimulation of presynaptic excitatory 5-HT₄ receptors and/or antagonism of presynaptic inhibition of muscarinic receptors, in accordance with the conclusions of Valenzuela *et al.*^[54]. The action of erythromycin is prob-

Table 1 Effects of insulin and prokinetic drugs alone and in combination on small intestinal transit and gastric emptying in diabetic mice

Treatments	SIT (%)	GE (%)
Normal control (citrate buffer)	56.61 ± 2.58	72.50 ± 1.68
Diabetic control (streptozotocin 100 mg/kg)	76.90 ± 6.12 ^a	55.23 ± 9.30 ^a
Insulin (2 IU/kg s.c.)	61.05 ± 3.85 ^c	95.87 ± 2.41 ^{ac}
Metoclopramide (20 mg/kg p.o.)	50.04 ± 2.42 ^{ac}	76.38 ± 7.67 ^{ac}
Insulin (2 IU/kg s.c.) + Metoclopramide (20 mg/kg p.o.)	54.30 ± 3.46 ^c	94.91 ± 1.01 ^{ac}
Domperidone (20 mg/kg p.o.)	48.70 ± 4.53 ^{ac}	90.92 ± 4.92 ^{ac}
Insulin (2 IU/kg s.c.) + Domperidone (20 mg/kg p.o.)	62.60 ± 3.07 ^c	92.87 ± 1.14 ^{ac}
Erythromycin (6 mg/kg p.o.)	43.05 ± 3.50 ^{ac}	84.77 ± 2.11 ^c
Insulin (2 IU/kg s.c.) + Erythromycin (6 mg/kg p.o.)	49.14 ± 4.57 ^{ac}	90.86 ± 3.20 ^{ac}

Values represent the mean ± SE of eight mice per group. Significantly different from the normal control value at ^a*P* < 0.05; Significantly different from the diabetic control value at ^c*P* < 0.05; Significantly different from insulin value at ^{ac}*P* < 0.05. SIT: Small intestinal transit; GE: Gastric emptying.

ably mediated *via* its agonistic activity to motilin receptors, which accelerates GE^[8,33].

Metoclopramide significantly increased xylose absorption but did not affect glucose absorption in STZ-induced diabetes. These findings are in agreement with those of Kuo *et al.*^[55]. The action of metoclopramide is mediated through increased plasma concentrations of glucagon like peptide-1 and glucose dependant insulinotropic polypeptide, which are responsible for delay in glucose absorption, although this action does not affect rate of xylose absorption. Domperidone (20 mg/kg) and erythromycin (6 mg/kg *p.o.*) significantly increased xylose absorption and glucose absorption in the diabetic mice as compared to diabetic control group. The effect of erythromycin could be mediated through the action of erythromycin on motilin receptors in the GIT. The action of erythromycin is probably mediated *via* its agonistic activity to motilin receptors, which accelerates GE and increases the rate of sugar absorption.

Combination of domperidone (20 mg/kg), metoclopramide (20 mg/kg) or erythromycin (6 mg/kg) with insulin (2 IU/kg) decreased the amount of xylose absorbed from the GIT when compared to insulin given alone in the diabetic mice. This indicates antagonistic interaction between each two drugs on xylose absorption. It is difficult to explain this action satisfactorily on the basis of the limited information available on the two drugs in this respect. Combination of insulin with metoclopramide, domperidone or erythromycin increased glucose absorption from the intestine when compared to the effect of insulin alone in the diabetic mice. These results suggest that combination of prokinetic drugs with insulin may lead to increased Na⁺ dependent glucose carrier activity in the small intestine. In addition to treating symptoms, prokinetic drugs could potentially improve glycemic control in diabetic gastroparesis by allowing a more predictable absorption of nutrients, matched to the action of

exogenous insulin.

The present study suggested that prokinetics may increase the hypoglycemic effect of insulin. Erythromycin tended to decrease the blood glucose level and increase the serum insulin level after one week of daily administration in STZ-induced diabetic mice. Erythromycin at a dose of 6 mg/kg *p.o.* potentiated the effect of insulin on blood glucose and serum insulin levels after one week of daily administration in diabetic mice where other prokinetic agents failed to do so after repeated administration. Similar results have been reported by Ueno *et al.*^[56]. The action of erythromycin on insulin could be mediated *via* its action as a motilin agonist. It is to be noted that motilin controls cyclic release of insulin through vagal cholinergic muscarinic pathways, as reported by Suzuki *et al.*^[57]. Itoh *et al.*^[58] found that there are no motilin receptors in the pancreas. Therefore, the action of erythromycin on insulin secretion is probably mediated *via* vagal-cholinergic muscarinic pathway stimulation linking to serotonergic receptors. This is a common mechanism in the stimulatory effect of motilin on muscle contraction in the stomach and on pancreatic polypeptide secretion from the endocrine pancreas^[59,60].

The action of erythromycin on gastrointestinal motility in the present study is contradictory to some studies which claim that active motilin receptors do not exist in rodents, and that they only exist as pseudogenes^[61,62]. However, the present results are in agreement with other studies^[63-65]. It is suggested that the action of erythromycin is mediated by binding to central motilin receptors which might be involved in regulation of gastric motility in diabetic rats.

This study dealt with an important issue concerning the use of prokinetic drugs in insulin-treated diabetic individuals. Not all diabetic patients develop gastrointestinal motility disorders or gastroparesis. However, the use of prokinetic drugs might be also relevant in these patients. Erythromycin potentiates the effect of insulin on blood glucose levels and serum insulin levels after repeated administration in diabetic mice. This seems to suggest that the use of prokinetic drugs, such as erythromycin, might be useful in the clinic for decreasing the need of insulin.

In conclusion, combination of insulin with metoclopramide, domperidone or erythromycin increases glucose absorption. This leads to the suggestion that such prokinetic drugs may guard against the risk of severe hypoglycemia associated with diabetic mice treated with insulin. The present study suggests that the use of prokinetic drugs, such as erythromycin, may be useful in the clinic for decreasing the need for insulin in diabetic patients. Use of erythromycin may allow the dose of insulin to be safely decreased in chronic treatments.

COMMENTS

Background

Diabetes mellitus is the most common cause of gastroparesis and disturbed gastric and small intestine motility. Gastroparesis is a syndrome characterized by delayed gastric emptying (GE) in the absence of mechanical obstruction of the stomach. Prokinetic drugs have been used for gastroparesis in diabetic pa-

tients for a relatively long time and some data about the interactions with insulin in the clinic should be available.

Research frontiers

Searching for therapeutic interventions with prokinetic drugs that will improve the specific alterations associated with diabetic gastroparesis was the most important aim of the present study. The research focus was to study the possible effects of metoclopramide, domperidone or erythromycin combined with insulin on different parameters in streptozotocin (STZ)-induced diabetic mice and to highlight the possibility that prokinetic drugs might increase the hypoglycemic effect of insulin.

Innovations and breakthroughs

Diabetic gastroparesis has been managed most successfully with drugs that stimulate GE. Previously prokinetic agents have generally been prescribed in order to relieve symptoms associated with diabetic gastroparesis. The prokinetic drugs metoclopramide, domperidone and erythromycin are all reported to reduce disturbance of gastrointestinal motility. This study deals with an important issue concerning the use of prokinetic agents in insulin-treated diabetic individuals. Not all diabetic patients develop gastrointestinal motility disorders or gastroparesis, but the use of prokinetic drugs might be also useful in these patients. In the present study, erythromycin potentiated the effect of insulin given on blood glucose levels and serum insulin levels after repeated administration in diabetic mice. Combination of insulin with metoclopramide, domperidone or erythromycin increased glucose absorption from the intestine.

Applications

The present study suggests that prokinetic drugs could potentially improve glycemic control in diabetic gastroparesis by allowing a more predictable absorption of nutrients, matched to the action of exogenous insulin. The use of prokinetic drugs, such as erythromycin, may be useful in the clinic for decreasing the need for insulin in diabetic patients. Use of erythromycin may allow the dose of insulin to be safely decreased with in chronic treatments.

Terminology

Gastroparesis is a syndrome characterized by delayed GE in the absence of mechanical obstruction of stomach. Prokinetic drugs enhance gastrointestinal motility by increasing the frequency of contractions in the small intestine or making them stronger, but without disrupting their rhythm. They are used to relieve gastrointestinal symptoms such as abdominal discomfort, bloating, constipation, heart burn, nausea, and vomiting.

Peer review

This manuscript deals with an important issue concerning the use of prokinetic drugs in insulin-treated diabetic individuals. Different prokinetic agents are used alone or in combination with insulin in STZ-induced diabetic mice in order to determine whether there exists any interaction between these drugs in different parameters. The study is interesting and the design is adequate.

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