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Original Article

Impaired Glucose Tolerance Secondary to Partial Intestinal Ischemia is Reversed by Sitagliptin

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Abstract

While diabetes is a predisposing factor for intestinal ischemia, mesenteric ischemia is associated with impaired glucose tolerance. We evaluated the effect of partial intestinal ischemia on glucose tolerance, GLP-1 and insulin level in forty non-diabetic SD rats. Rats were randomly assigned into 4 groups; SHAM; SHAM + sitagliptin (30 mg/kg/day); intestinal ischemia; and intestinal ischemia + sitagliptin (30 mg/kg/day). The superior mesenteric artery was partially occluded. At the 11th day, glucose tolerance, plasma GLP-1 and insulin levels were measured along with histological changes in the pancreas and insulin-secreting beta cells. Intestinal ischemia resulted in significant impairment of glucose tolerance with reduction of plasma levels of GLP-1 and insulin. Treatment with sitagliptin partially ameliorated these changes. Beta cells in the pancreas were not affected by ischemia. These results suggest that impaired glucose tolerance with intestinal ischemia might be secondary to functional impairment of beta cells secondary to decreased basal GLP-1 secretion.

Introduction

Small intestine is a major source of glucagon-like peptide-1 (GLP-1) (1). GLP-1 has several physiological actions which are considered beneficial in diabetic patients. It is a potent secretagogue for insulin (2). GLP-1 potentiates insulin action and increases insulin sensitivity (3). Moreover, it has been shown that GLP-1 has hemodynamic and cardioprotective capacities (4). The half -life of GLP-1 is only 1-2 minutes due to its rapid metabolism (5) by an enzyme called dipeptidyl peptidase-4 (DPP-4) (6).

The interaction between GLP-1 and diabetes is complex. GLP-1 secretion in response to a

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standardized meal was found to be significantly decreased in patients with type II diabetes in comparison to glucose tolerant subjects (7). Whether impaired GLP-1 secretion is a primary phenomenon in the pathogenesis of diabetes or a consequence of diabetes itself is yet to be revealed. First-degree relatives of patients with type II diabetes have normal GLP-1 secretion in response to oral glucose challenge; suggesting that the GLP-1 secretion abnormality seen in diabetes may be acquired (8).

As GLP-1 is secreted from the intestine, it would be interesting to know if any changes in the intestinal hemodynamics could affect the secretion or metabolism of this hormone. The aim of the present study was to evaluate the effect of partial intestinal ischemia on glucose tolerance, insulin and GLP-1 levels in non-diabetic rats and whether a DPP-4 inhibitor, namely sitagliptin, could modify these effects.

Methods

The experiments were conducted in accordance with regulations specified by the local ethical committee, Faculty of Medicine Menoufiya University, Egypt.

Animals

Forty adult pathogen-free Sprague Dawley rats (weighing 200-250 g) under similar housing conditions.

Reagents

ELISA kits were used for the assay of Glucagon like peptide-1 (Phoenix Pharmaceuticals, INC., USA) and insulin (Phoenix Pharmaceuticals, INC., USA). Monoclonal antibodies against insulin (Dako, Glostrup, Denmark) were used for immunohistochemical study of beta-cell mass in the tail of pancreases. The anesthetic, ketamine, was provided by Amoun (Egypt) as 10 ml vials (Ketamar) with a concentration of 50 mg ketamine/ml. Sitagliptin was obtained from Merck Sharp & Dohme Corp.

Experimental protocol

The 40 rats were randomly divided into the following groups (n = 10 in each group):

Group (I): SHAM: Animals in this group were subjected to all steps in the surgical induction of intestinal ischemia except narrowing of the superior mesenteric artery (SMA).

Group (II): SHAM and sitagliptin: As group (I) but starting from day 5 after the operation, rats were treated with sitagliptin (30 mg/kg/day) orally for 7 days.

Group (III): Intestinal ischemia: As group (I) but rats were subjected to the partial narrowing of the superior mesenteric artery (SMA).

Group (IV): Intestinal ischemia and sitagliptin: As group (III) but starting from day 5 after the operation, rats were treated with sitagliptin (30 mg/kg/day) orally for 7 days.

Induction of intestinal ischemia

Rats were anesthetized by ketamine (50-100 mg/kg, i.p.) (9). A midline laparotomy was performed and the SMA was partially constricted below the origin of the middle colic artery to avoid affecting the blood supply of the inferior pancreatic-duodenal artery that supplies the pancreas. The SMA was partially occluded by placing a single ligature of 3-0 silk around both the SMA and blunt-tipped needle (using a 22-gauge needle), placed alongside the SMA. The needle was then removed from ligature, creating a callipered constriction of SMA. The procedure resulted in about 30-40% reduction of SMA blood flow as verified by a Doppler flow meter. After that, sterile saline (1 ml) was injected into the peritoneal cavity for rehydration, and the abdomen was closed in layers.

The animals had 4 days for postoperative recovery. At the 11th day, rats were anaesthetized and subjected to intravenous glucose tolerance test (IVGTT) over 50 minutes. By the end of the test,

blood samples from the femoral vein were used to measure plasma levels of GLP-1 and insulin. Animal were then sacrificed and SMA partial narrowing with the absence of gangrenous intestinal loops was then verified. Pancreatic tissue was dissected for histological examination.

Intravenous glucose tolerance test.

Rats were fasting for 16-18 hours (overnight) prior to the glucose tolerance test. The animals had free access to drinking water during the fasting period. Prior to performing the glucose tolerance test, baseline glucose level was recorded for each rat (blood samples were taken from the tail). D-glucose (10 g/dl) was injected in the femoral vein over 1 minute in a dose of 1 mg glucose/gram body weight (2.5 ml in 250-g rat). At 2, 5, 20, and 50 minutes after injection, blood samples were collected from the tail vein (10). Measurement of blood glucose was done by using the blood glucose checker [GLUCOTREND®2 (Roche Group, UK)] and (Accu-Chek® Active) strips (Roche Group, UK).

Histological and immuno-histochemical study of beta-cells

Tissue samples (tail of the pancreas) were dissected, and fixed in 10% formalin for 5 days, then processed as usual to make paraffin blocks. Sections of 5 microns were stained with haematoxylin and eosin (H and E) for routine histological study. Identification of insulin-secreting beta-cells of pancreas was performed by using immuno-stain with anti-insulin monoclonal antibodies as described by the manufacturer.

Statistical analysis

Data is expressed as Mean±SEM. Data was analyzed using SPSS version 16. Variables were tested for normality distribution using Kolmogorov-Smirnov test. Comparison between groups was done by analysis of variance (ANOVA) followed by Tukey test. A *p* value of less than 0.05 was considered statistically significant.

Results

1. IVGTT:

After injection of glucose, the blood glucose level at the 2nd minute showed a significant increase and remained significantly higher at 5th and 20th minutes in comparison to the basal level. With exception of the ischemia only group, the 50th minute measure was not significantly different from the basal level indicating that 50 minutes were enough for the homeostatic mechanisms to readjust the blood glucose level (Table I). Regarding the comparison between groups, partial intestinal ischemia led to significant elevation of basal level of blood glucose as well as all values in the different time points of IVGTT. Treatment with sitagliptin partially ameliorated the changes induced by partial intestinal ischemia (Table I).

2. Insulin and GLP-1 levels:

In comparison to SHAM group, the ischemic group had a significant decrease in both insulin and GLP-1 levels. Again, treatment with sitagliptin partially ameliorated the changes induced by partial intestinal ischemia. Of note, treatment with sitagliptin resulted in elevation of insulin and GLP-1 levels in group II in comparison to SHAM group (Table II).

TABLE I: Effect of superior mesenteric artery ischemia and sitagliptin on intravenous glucose tolerance test [mean±SEM] (n=10).

Group	*Basal blood glucose mg/dl*	*2 min mg/dl*	*5 min mg/dl*	*20 min mg/dl*	*50 min mg/dl*
I (SHAM)	114±1.98	270±2.02*	224±2.33*	142±1.65*	112±1.87
II (SHAM-Sitagliptin)	91±1.89	257±1.56*	210±2.26*	100±1.90*%	94±1.64
III (ischemia)	143±2.38%f&	405±2.81*%f&	331±2.33*%f&	270±1.87*%f&	205±1.83*%f&
IV (ischemia-Sitagliptin)	121±2.05f&†	322±2.37*%f&†	267±1.85*%f&†	131±2.20%f&†	127±1.73%f&†

* = significant difference from basal blood glucose within the same group; % = significant difference from group I; f& = significant difference from group II; † = significant difference from group III (P<0.05).

TABLE II : Effects of intestinal ischemia and sitagliptin on GLP-1 plasma level (pmol/l) and insulin (µ/ml) in different rat groups (mean±SEM).

GLP-1	*Insulin*	*Groups*
I Sham	9±0.6	23.3±1.13
II Sham + sitagliptin	13.22±0.7*	29±1.26*
III Intestinal ischemia	6.33±0.3*%	18.35±0.42*%
IV Intestinal ischemia + sitagliptin	8.76±0.3%f&	25.1±0.77%f&

*Significant difference from group I; % = significant difference from group II; f& = significant difference from group III (P<0.05).

3. Histological changes in the pancrease:

For both SHAM and ischemic groups; the islands of Langerhans were embedded within the acini forming the endocrine part of pancreas**.** The islets contain several cell types and were highly vascularized. They appeared as non-capsulated pale stained area inside the pancreatic lobules (Fig. 1). Insulin secreting beta-cells could be demonstrated in immune-histochemically stained sections with anti- insulin antibodies. They composed the major cell population of the islets that occupied mainly the central zone. The anti-insulin antibody reaction was seen as brown granules occupying the cytoplasm of the beta-cells. Positively stained beta-cells were seen among pancreatic acini. A positively stained immune reaction for insulin was also seen in the endothelial cells lining blood capillaries (Fig. 2). No differences were detected between different groups indicating that SMA partial ischemia did not affect insulin-secreting cells.

Discussion

The relationship between GLP-1 and Type II diabetes is somewhat complex. In general, meal-stimulated GLP-1 secretion in diabetic patients is impaired (7). Studies on first-degree relatives of patients with type II diabetes suggested that the GLP-1 secretion abnormality seen in diabetes may be acquired (8). A yet to be answered question is whether impairment of GLP-1 secretion is a contributing factor in the pathogenesis of diabetes or is just a consequence of diabetes itself.

The most important cause of chronic intestinal ischemia is atherosclerosis (11, 12). An important predisposing factor to such condition is diabetes mellitus (12). As the terminal ileum is an important source of GLP-1 (1), the present study investigated

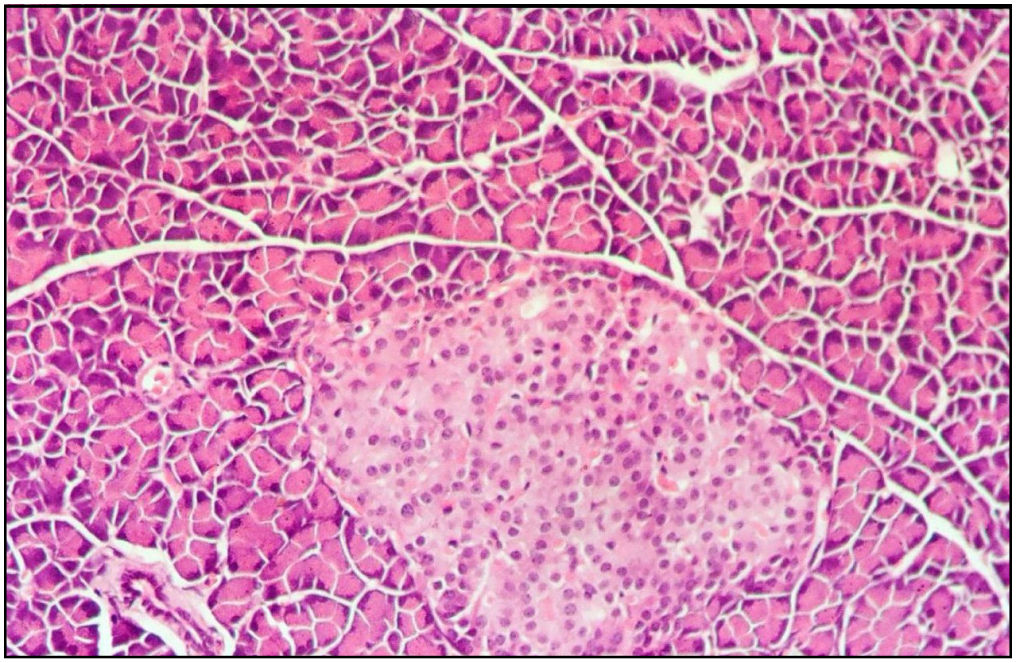

Fig. 1 : A photomicrograph of a section of pancreas from rats subjected to partial occlusion of the superior mesenteric artery (H & E). Lobules packed with acini and an islands of Langerhans situated in the center. Blood vessels and exocrine duct were seen among exocrine acini indicating that ischemia did not influence the pancreas. Magnification: x 200.

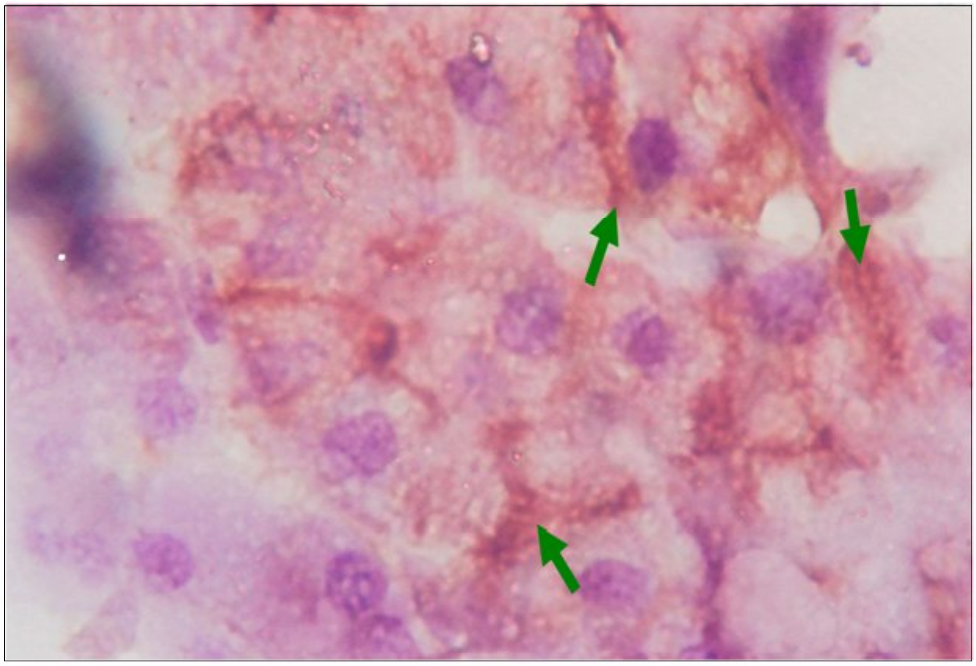

Fig. 2 : A photomicrograph of a section of pancreas from rats subjected to partial occlusion of the superior mesenteric artery stained with anti-insulin antibodies. It indicates that ischemia did not influence beta-cells. Magnification: x 400.

whether intestinal ischemia, by turn, could affect glucose homeostasis. We demonstrated that after 10 days of partial intestinal ischemia, IVGTT was negatively affected.

Abnormal glucose tolerance in case of mesenteric ischemia was reported only once by Sedlack and Bean (13). In a parallel way, resection of 5 cm of the small intestine 5 cm distal to the ligament of Treitz in adult rats resulted in reduced insulin release after IVGTT; an effect unrelated to corticosterone nor IL-6 changes (14). A simple explanation to the report of Sedlack and Bean (13) is that mesenteric ischemia affected the blood flow to the pancreas. To rule this out, we have intentionally constricted SMA below the origin of the middle colic artery to avoid affecting the blood supply of the inferior pancreatic-duodenal artery that supplies the pancreas. In addition, histological and immunohistochemical evaluation of pancreatic tissue of the rats subjected to intestinal ischemia showed no significant differences from the SHAM-operated rats. The present study indicated that beta-cells might be affected at the molecular or functional level rather than being directly injured by mesenteric ischemia. We demonstrated that reduced insulin levels in the ischemic group was secondary to reduced GLP-1 levels and that using a DPP-4 inhibitor partially restored GLP-1 and insulin levels and improved IVGTT. It was reported that, in diabetic patients, defects in the secretion of the incretin hormones are responsible for the reduced incretin effect and hence the inadequate insulin secretion but not responsible for development of diabetes (15).

Of note, it is well-known that GLP-1 is secreted mainly in response meals but not secondary to intravenous glucose (16). Intravenous glucose tolerance test rather than oral glucose tolerance test was done, as absorption of oral glucose from the intestine would be greatly affected by the intestinal ischemia. Nevertheless, it was found that there is a continuous secretion of GLP-1 from the intestine that contributes to measurable basal level of the hormone (5). Although no studies investigated the role of basal GLP-1 in insulin secretion, indirect evidence suggested that the basal hormone levels might be important. Using the GLP-1 analogue, exenatide, in diabetic patients was found to increase the already impaired first-phase insulin secretion (17); an event that occurs too early before the meal stimulates GLP-1 secretion. Such finding may suggest a role of basal GLP-1 in early insulin secretion. In another direction,

GLP-1 might have a direct protective mechanism against islet injury. A DPP-4 inhibitor was reported to suppress STZ-induced islets injury in monkeys; an effect which was dependent on activation of the IGFR/Akt/mTOR signalling pathways by GLP-1 (18). Although the histopathological evaluation did not reveal beta-cell injury that could be attributed to ischemia in this model, affection at the molecular level cannot be excluded. A third possibility that could explain the results of the present study is away from islet affection theory. It was found that GLP-1, through an islet-independent mechanism, can inhibit hepatic glucose production (19, 20). We believe that intestinal ischemia with subsequent impairment of portal drainage in this model might reduce the hepatic action of GLP-1. So, the amelioration of derangement in glucose homeostasis after using sitagliptin might be partially attributed to restoration of GLP-1 in the liver.

Of note, during early development of the model we used acute complete occlusion of the SMA. However, we could not exclude the effect of stress and surgical trauma from affecting glucose tolerance (data not published). Stress stimulates the hypothalamo-pituitary-adrenal axis activating both pituitary adrenocortical and the sympatho-adreno-medullary systems (21). By turn, catecholamines elevate the blood glucose due to stimulation of glycogenolysis and inhibition of insulin secretion (22). Partial ischemia was then used to avoid the effect of stress and as it is also more similar to atherosclerosis; where the blood flow is impaired rather than complete deprivation. One limitation in the present study that should be considered is that part of the L-cells that secret GLP-1 is localized in the rectum (23) and few are localized in the duodenum (1); both are not supplied by the SMA and could escape the effect of ischemia in this model.

Conclusion

In conclusion, chronic intestinal ischemia in rats resulted in significant elevation of basal blood glucose level and impaired IVGTT due to a decrease in basal GLP-1 secretion. Whether such effect of ischemia is a primary contributing factor in the pathophysiology of type II diabetes or just an add-on factor that worsen the disease is a point yet to be investigated.

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References

1. Mortensen K, Christensen L, Holst J, Orskov C. GLP-1 and GIP are colocalized in a subset of endocrine cells in the small intestine. *Regul Pept* 2003; 114: 189–196.
2. Kreymann B, Ghatei M, Williams G, Bloom S. Glucagon-like peptide-1 (7-36): a physiological incretin in man. *Lancet* 1987; 2: 1300–1304.
3. Zander M, Madsbad S, Madsen J, Holst J. Effect of 6-week course of glucagon-like peptide 1 on glycaemic control, insulin sensitivity, and beta-cell function in type 2 diabetes: a parallel-group study. *Lancet* 2002; 359: 824–830.
4. Goodwill A, Mather K, Conteh A, Sassoon D, Noblet J, Tune J. Cardiovascular and hemodynamic effects of glucagon-like peptide-1. *Rev Endocr Metab Disord* 2014; 15: 209–217.
5. Meier J, Nauck M, Kranz D, Holst J, Deacon C, Gaeckler D, Schmidt W, Gallwitz B. Secretion, degradation, and elimination of glucagon-like peptide 1 and gastric inhibitory polypeptide in patients with chronic renal insufficiency and healthy control subjects. *Diabetes* 2004; 53: 654–662.
6. Mentlein R, Gallwitz B, Schmidt W. Dipeptidyl-peptidase IV hydrolyses gastric inhibitory polypeptide, glucagon-like peptide-1(7–36) amide, peptide histidine methionine and is responsible for their degradation in human serum. *Eur J Biochem* 1993; 214: 829–835.
7. Toft-Nielsen M, Damholt M, Madsbad S, Hilsted L, Hughes T, Michelsen B, Holst J. Determinants of the impaired secretion of glucagon-like peptide-1 in type 2 diabetic patients. *J Clin Endocrinol Metab* 2001; 86: 3717–3723.
8. Nyholm B, Walker M, Gravholt C, Shearing P, Sturis J, Alberti K, Holst J, Schmitz O. Twenty-four-hour insulin secretion rates, circulating concentrations of fuel substrates and gut incretin hormones in healthy offspring of type II (non-insulindependent) diabetic parents: evidence of several aberrations. *Diabetologia* 1999; 42: 1314–1323.
9. Wixson S, Smiler K. Anesthesia and analgesia in rodents. In: Kohn DF, Wixson S, White W., eds. Anesthesia and Analgesia in Laboratory Animals. San Diego, Academic Press 1997 pp. 165–203.
10. Pacini G, Thomaseth K, Ahrén B. Contribution to glucose

tolerance of insulin-independent vs. insulin-dependent mechanisms in mice. *Am J Physiol Endocrinol Metab* 2001; 281: E693–E703.

11. Hohenwalter E. Chronic mesenteric ischemia: diagnosis and treatment. *Semin Intervent Radiol* 2009; 26: 345–351.
12. Zeller T, Rastan A, Sixt S. Chronic atherosclerotic mesenteric ischemia (CMI). *Vascular Med* 2010; 15: 333–338.
13. Sedlacek R, Bean W. Abdominal “angina”: The syndrome of intermittent ischemia of mesenteric arteries. *Annu Intern Med* 1957; 46: 148–152.
14. Strommer L, Wickbom M, Wang F, Herrington M, Ostenson C, Arnelo U, Permert J. Early impairment of insulin secretion in rats after surgical trauma. *Eur J Endocrinol* 2002; 147: 825–833.
15. Freeman J. Role of the incretin pathway in the pathogenesis of type 2 diabetes mellitus. *Cleve Clin J Med* 2009; 76: 12–19.
16. Herrmann C, Goke R, Richter G, Fehmann H, Arnold R, Goke B. Glucagon-like peptide-1 and glucose-dependent insulin-releasing polypeptide plasma levels in response to nutrients. *Digestion* 1995; 56: 117–126.
17. Fehse F, Trautmann M, Holst J, Halseth A, Nanayakkara N, Nielsen L, Fineman M, Kim D, Nauck M. Exenatide augments first and second phase insulin secretion in response to intravenous glucose in subjects with type 2 diabetes. *J Clin Endocrinol Metabol* 2005; 90: 5991–5997.
18. Zhang Y, Chen Y, Lu Y, Tian B. DPP IV inhibitor suppresses STZ-induced islets injury dependent on activation of the IGFR/Akt/mTOR signaling pathways by GLP-1 in monkeys. *Biochem Biophys Res Commun* 2014; 63: 2196–2202.
19. Ahrén B. Hepato-Incretin Function of GLP-1: Novel Concept and Target in Type 1 Diabetes. *Diabetes* 2015; 64: 715–717.
20. Jun L, Millican R, Hawkins E, Konkol D, Showalter A, Christe M, Michael M, Sloop K. Absence of glucagon and insulin action reveals a role for the GLP-1 receptor in endogenous glucose production. *Diabetes* 2015; 64: 819–827.
21. Desborough J. The stress response to trauma and surgery. *Br J Anaesth* 2000; 85: 109–117.
22. Nonogaki K. New insights into sympathetic regulation of glucose and fat metabolism. *Diabetologia* 2000; 43: 533–549.

23 Eissele R, Goke R, Willemer S, Harthus H, Vermeer H, Arnold R, Göke B. Glucagon-like peptide-1 cells in the gastrointestinal tract and pancreas of rat, pig and man. *Eur J Clin Invest* 1992; 22: 283–291.