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Increased secretion of insulin and proliferation of islet β-cells in rats with mesenteric lymph duct ligation

Ko Nagino [a,1], Junji Yokozawa [a,1], Yu Sasaki [a], Akiko Matsuda [a], Hiroaki Takeda [a], Sumio Kawata [a,b,*]

[a] Department of Gastroenterology, Faculty of Medicine, Yamagata University, Yamagata 990-9585, Japan
[b] Hyogo Prefectural Nishinomiya Hospital, 13-9 Rokutanji-cho, Nishinomiya 662-0918, Japan

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ABSTRACT

Background & aims: It has been suggested that intestinal lymph flow plays an important role in insulin secretion and glucose metabolism after meals. In this study, we investigated the influence of ligation of the mesenteric lymph duct on glucose metabolism and islet β-cells in rats.

Methods: Male Sprague–Dawley rats (10 weeks old) were divided into two groups: one underwent ligation of the mesenteric lymph duct above the cistern (ligation group), and the other underwent a sham operation (sham group). After 1 and 2 weeks, fasting plasma concentrations of glucose, insulin, triglyceride, glucose-dependent insulinotropic polypeptide (GIP), and the active form of glucagon-like peptide-1 (GLP-1) were measured. At 2 weeks after the operation, the oral glucose tolerance test (OGTT) and intravenous glucose tolerance test (IVGTT) were performed. After the rats had been sacrificed, the insulin content of the pancreas was measured and the proliferation of β-cells was assessed immunohistochemically using antibodies against insulin and Ki-67.

Results: During the OGTT, the ligation group showed a significant decrease in the plasma glucose concentration at 120 min ($p < 0.05$) and a significant increase in the plasma insulin concentration by more than 2-fold at 15 min ($p < 0.01$). On the other hand, the plasma GIP concentration was significantly decreased at 60 min ($p < 0.01$) in the ligated group, while the active form of GLP-1 showed a significantly higher level at 90 min (1.7-fold; $p < 0.05$) and 120 min (2.5-fold; $p < 0.01$). During the IVGTT, the plasma insulin concentration in the ligation group was significantly higher at 2 min (more than 1.4-fold; $p < 0.05$). Immunohistochemistry showed that the ratios of β-cell area/acinar cell area and β-cell area/islet area, and also β-cell proliferation, were significantly higher in the ligation group than in the sham group ($p < 0.05$, $p < 0.01$ and $p < 0.01$, respectively). The insulin content per unit wet weight of pancreas was also significantly increased in the ligation group ($p < 0.05$).

Conclusions: In rats with ligation of the mesenteric lymph duct, insulin secretion during the OGTT or IVGTT was higher, and the insulin content and β-cell proliferation in the pancreas were also increased. Our data show that mesenteric lymph duct flow has a role in glucose metabolism.

1. Introduction

Lymphatic vessels not only play a physiological role in maintenance of homeostasis and the immune response together with blood vessels, but also participate in the development of pathological states such as inflammation and cancer metastasis [1]. Mesenteric lymphatic vessels have a specific function, including absorption of long-chain fatty acids from the small intestine. Ligation of the mesenteric lymph duct is reported to prevent acute pulmonary dysfunction during ischemia/reperfusion and trauma/hemorrhage shock-induced cardiac contractile dysfunction [2,3]. Recently, it has been reported that impairment of lymphangiogenesis promoted by Prox1 haploinsufficiency leads to adult-onset obesity [4].

Incretins, glucose-dependent insulinotropic polypeptide (GIP), and glucagon-like peptide-1 (GLP-1) are produced in enteroendocrine K cells and L cells, respectively in the intestine in response to ingestion of carbohydrate and fat [5–7]. Both GIP and GLP-1 stimulate insulin secretion from pancreatic β-cells, accounting for ~50–70% of postprandial insulin secretion from the pancreas [8,9]. Recently, it has been shown that intestinal lymph is enriched with GIP [10] and GLP-1 [11], and studies using the lymph fistula rat model have demonstrated that the carbohydrate and lipid contents of food affect the secretion of GIP and GLP-1 into intestinal

Abbreviations: GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1; OGTT, oral glucose tolerance test.

* Corresponding author. Address: Department of Gastroenterology, Faculty of Medicine, Yamagata University, 2-2-2 Iida-Nishi, Yamagata 990-9585, Japan. Fax: +81 23 628 5311.
E-mail address: Sumio_Kawata@pref.hyogo.lg.jp (S. Kawata).

[1] These authors contributed equally to this work.

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Table 1
Comparison of biochemical data between the lymph duct ligation and sham groups at 1 and 2 weeks after the procedure.

Characteristic	1 week after ligation		2 weeks after ligation	
	Ligation	Sham	Ligation	Sham
Body weight (g)	354 ± 19.4	370 ± 10.2	380 ± 25.3	394 ± 15.1
Epididymal fat/body weight (%)	ND	ND	1.3 ± 0.04	1.4 ± 0.08
Glucose (mg/dL)	112 ± 6.3	124 ± 4.5	115 ± 4.6	127 ± 4.8
Triglyceride (mg/dL)	66 ± 5.4	65 ± 5.1	53 ± 3.7	54 ± 4.0
Total cholesterol (mg/dL)	73 ± 3.6	76 ± 3.9	72 ± 3.3	75 ± 2.5
Insulin (ng/mL)	0.29 ± 0.03	0.42 ± 0.08	0.36 ± 0.06	0.45 ± 0.09
C-peptide (pg/mL)	534 ± 108	738 ± 270	388 ± 49.7	572 ± 113
GLP-1 (pmol/L)	5.4 ± 1.3	5.7 ± 2.8	4.3 ± 0.7	4.6 ± 1.8
GIP (pg/mL)	14.6 ± 2.8	14.8 ± 2.8	14.0 ± 2.2	15.0 ± 2.3
Adiponectin (μg/mL)	4.8 ± 0.3	4.8 ± 0.3	7.4 ± 0.4	6.4 ± 0.4

Data are presented as mean ± standard error.

lymph vessels [12,13]. The incretin concentrations in lymph are significantly higher than in portal or systemic blood [10,11]. These findings suggest that intestinal lymph flow plays an important role in insulin secretion and glucose metabolism after meals. However, the consequences of interference with intestinal lymph flow on glucose metabolism and insulin secretion have not been investigated.

In this study, we examined changes in glucose metabolism and insulin secretion resulting from blockage of mesenteric lymph flow by ligation of the thoracic duct in rats.

2. Materials and methods

2.1. Construction of an animal model

Seven-week-old male Sprague–Dawley rats were purchased from CLEA Japan, Inc. (Tokyo, Japan) and fed a normal diet after acclimation to the laboratory environment. At 10 weeks, the rats were subjected to lymph duct ligation above the cisterna chyli in the peritoneal cavity. To visualize lymph ducts, 5 ml/kg olive oil was orally administered to the rats 2 h prior to surgery [14]. Pentobarbital (40 mg/kg) was then injected into the peritoneal cavity to anesthetize the rats, and laparotomy was performed to expose the thoracic duct in the peritoneal cavity. The rats were divided into two groups: those subjected to lymph duct ligation (ligation group) and those subjected to a sham procedure, i.e. laparotomy only (sham group).

Preliminary experimental tests revealed that the tension of the cisterna chyli was lost by 3 weeks after surgery. Therefore, the entire study was conducted using rats no later than 2 weeks after surgery. This study was conducted after receiving approval from the Animal Center Ethical Committee of Yamagata University Medical School.

2.2. Methods for collection of blood samples and removal of pancreas tissue

A total of 16 rats, 8 from each of the ligation and sham groups, were used for the study. Blood samples were collected from the tail vein of rats before surgery (at 10 week of age) and at 1 week (11 weeks of age) and 2 weeks (12 weeks of age) after surgery. All samples were collected following an overnight (12 h) fast. Blood samples were placed into tubes containing ethylenediaminetetraacetic acid (EDTA), aprotinin, and dipeptidyl peptidase-IV (DPP-IV) inhibitor (Millipore, Billerica, USA) so that the final concentrations after addition of the sample became 1.25 mg/ml, 500 KIU/ml, and 50 μM, respectively. Plasma samples were stored at −80 °C.

At 2 weeks after ligation, a portion of tissue samples from the pancreatic body region was fixed in 4% paraformaldehyde for 24 h at room temperature and embedded in paraffin according to the conventional method. Unused pancreatic tissues were instantaneously frozen in liquid nitrogen and stored at −80 °C.

2.3. Blood biochemical tests

Plasma levels of glucose, triglyceride, total cholesterol, insulin, C-peptide, total GIP, activated GLP-1, and adiponectin were measured using glucose CII test Wako, triglyceride E-test Wako, Cholesterol E-test Wako (Wako, Osaka, Japan), Rebis Insulin-rat T, Rebis C-Peptide-rat U-type (Shibayagi, Shibukawa, Japan), and a Rat/Mouse GIP (Total) ELISA kit, and levels of activated GLP-1 using a GLP-1 (Active) ELISA kit (Millipore, Billerica, USA), and an Adiponectin (rat) ELISA kit (AdipoGen, Seoul, Korea), respectively.

2.4. Oral glucose tolerance test (OGTT)

OGTT was carried out on the ligation ($n = 8$) and sham ($n = 8$) groups with the animals under anesthesia. Blood samples were collected from the two groups via a tail vein at 2 weeks after surgery prior to glucose oral administration (0 min) and at 15, 30, 60, 90, and 120 min after administration of 2.0 g/kg glucose (gastric administration) using a gavage tube [15].

The insulinogenic index, an index of insulin secretion, was also calculated as described previously [16].

2.5. Intravenous glucose tolerance test (IVGTT)

IVGTT was carried out on each group with the animals ($n = 8$, respectively) under anesthesia. Blood samples were collected from the rats via a tail vein at 2 weeks after surgery prior to glucose administration (0 min) and at 2, 5, 7.5, 10, 15, 30, 45, 60, and 90 min after bolus injection of 1.0 g/kg glucose into the femoral vein.

2.6. Immunohistochemistry of pancreatic β-cells

Pancreatic tissue was collected 2 weeks after surgery and embedded in paraffin for immunohistochemical studies from a total of 16 rats: 8 from the ligation group and 8 from the sham group. The paraffin block containing pancreatic tissues was sliced into 3-μm-thick sections using a microtome (EMS-100L; ERMA Inc., Tokyo, Japan). Immunostaining of the thin-sectioned tissues was then performed as described previously [17]. For measurement of β-cell proliferation, double immunostaining for insulin and Ki-67 (a cell proliferation-associated nuclear antigen) was performed as described previously [18]. As the primary antibody, anti-rat Ki-67 mouse antibody (1:25, Dako, Glostrup, Denmark) was used. Nuclei were stained using hematoxylin.

AxioVision AutoMeasure (Carl Zeiss, Oberkochen, Germany) was used to analyze the images of the stained pancreatic tissue