Dietary zinc supplementation to the donor improves insulin secretion after islet transplantation in chemically induced diabetic rats

Takehiro Mishima[1], MD, PhD, Tamotsu Kuroki[1], MD, PhD, Yoshitsugu Tajima[2], MD, PhD, Tomohiko Adachi[1], MD, PhD, Masataka Hirabaru[1], MD, Takayuki Tanaka[1], MD, Amane Kitasato[1], MD, PhD, Mitsuhisa Takatsuki[1], MD, PhD, and Susumu Eguchi[1], MD, PhD.

[1]Department of Surgery, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan.

[2]Department of Digestive and General Surgery, Shimane University, Shimane, Japan.

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To whom correspondence should be addressed: Takehiro Mishima,
Department of Surgery, Nagasaki University Graduate School of Biomedical Sciences, 1-7-1 Sakamoto, Nagasaki 852-8102, Japan.
TEL 81-95819-7316
FAX 81-95819-7319
E-mail: *tamishima-gi@umin.ac.jp*

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Abstract

Objectives: Zinc (Zn) is related to insulin synthesis, storage and secretion. This study demonstrates the effects of zinc supplementation in donor rats on the outcomes of islet transplantation.

Methods: Donor rats received three different regimens of dietary zinc supplementation for two weeks prior to undergoing pancreas donation: a standard diet containing zinc at 50 ppm (control), 1 ppm (Low-Zn group) or 1,000 ppm (High-Zn group), respectively. Diabetic recipient rats underwent islet transplantation, and the blood glucose levels and insulin secretion were monitored for seven days after transplantation.

Results: The serum and pancreatic zinc levels at the time of donation were significantly lower in the Low-Zn group (48.8±25.5 μg/dl and 11.3±1.9 μg/g) and higher in the High-Zn group (147.3±17.6 μg/dl and 18.7±2.2 μg/g) when compared with those observed in the controls (118.7±7.9 μg/dl and 14.6±2.0 μg/g) ($p<0.05$). The blood glucose levels became re-elevated two days after transplantation in rats receiving islet grafts from the controls and the Low-Zn groups. In contrast, in the rats that received islets from the High-Zn groups,

these were maintained within a normal range ($p<0.01$).

Conclusions: These data indicate that a zinc-rich diet for donor rats improves the function of islet grafts in chemically induced diabetic rats.

Introduction

Type 1 diabetes is characterized by the profound destruction of the insulin-producing β cells of the islets of the pancreas, thus requiring lifetime endogenous insulin replenishment. Despite recent advances in diabetes care, various life-threatening complications, including cardiovascular disease, neuropathy and renal failure, can result in affected patients.

Pancreatic islet transplantation is now a promising treatment for type 1 diabetic patients. However, it still has some issues to overcome. For example, an individual diabetic patient usually requires islets from multiple donors to achieve normoglycemia. Xenotransplantation has thus been used to address the insufficiency in the number of donors (1,2), and many studies have been performed to refine the method for resolving the hypofunction of transplanted grafts (3-11).

It is well known that a deficiency of zinc affects the onset and exacerbation of diabetes (12) because zinc plays important roles in the synthesis, storage and secretion of insulin, as well as β cell death leading to the occurrence of type 1 diabetes. Several studies have demonstrated that increasing the level of dietary zinc can prevent the development of diabetes in animal models (13-15).

Our previous study indicated that a zinc-rich environment is advantageous for the recipient in intraportal islet transplantation (16). Meanwhile, the effects of zinc supplementation on the donor in regard to the graft function following pancreatic islet transplantation have not yet been elucidated.

This study was designed to investigate whether dietary zinc supplementation to the donor can improve the results of islet transplantation in chemically induced diabetic rats.

Materials and Methods

Animals

Male Wistar rats (SLC, Shizuoka, Japan) weighing 250-300 g were used in this study. They were housed one per plastic cage on sawdust bedding and kept at 24±2℃ and 50±20% humidity with a 12-hour light-dark cycle. The rats were fed a CE-2 pelleted diet (Clea Japan, Tokyo, Japan) and provided drinking water *ad libitum*. The animals were checked daily throughout the experiments. All experiments were conducted according to the Guidelines for Animal Experimentation of Nagasaki University.

Zinc supplementation

The donor rats were divided into three groups according to the regimen of dietary zinc supplementation, i.e., a standard pelleted diet containing zinc at 50 ppm (control group, n=10), a zinc-poor diet containing zinc at 1 ppm (Low-Zn group, n=10) or zinc-rich diet containing zinc at 1,000 ppm (High-Zn group, n=10). The animals received the dietary zinc supplementation for two weeks prior to extirpation of the pancreas.

At the time of pancreas donation, blood samples were collected from inferior vena cava for the measurement of the serum zinc levels and serum insulin

values, and pancreatic tissue samples were taken from splenic lobe for the measurement of the pancreatic zinc levels. Pancreatic homogenate was digested in 2N hydrochloric acid for 24 hours at room temperature. The samples were then centrifuged at 7,000 g for 25 minutes, and the supernatant was then used for direct measurements. Serum and pancreatic zinc levels were measured by Atomic Absorption Spectrometry.

Isolation of pancreatic islets

Pancreatic islets were isolated using a modified method of ductal collagenase distention (17) and iodixanol isolation (17,18). Briefly, laparotomy was performed under general anesthesia, and the distal end of the common bile duct was ligated at the entrance of the duodenum with a fine nylon suture. The proximal common bile duct was cannulated with a 24-gauge polyethylene catheter and injected with 10 ml of Hank's balanced salt solution (HBSS) containing 1,200 U/ml collagenase type XI (Sigma Chemical Co., St Louis, MO) to distend the pancreas. The distended pancreas was removed and incubated in a 50-ml conical tube with an additional 5 ml of collagenase solution and then placed in a water bath at 37℃ for 20 minutes. The digestion was stopped by the addition of 40 ml of cold HBSS. The pancreatic tissue was

filtered over 400-μm mesh and then washed twice. Islet purification was performed using a continuous iodixanol (Optiprep (Axis-Shield PoC, Oslo, Norway)) density gradient. The tissue pellets were resuspended in 10 ml of 1.095 g/ml density iodixanol solution. To form a continuous density gradient, 6 ml of 1.087 g/ml density iodixanol solution and 4 ml of Roswell Park Memorial Institute (RPMI) medium were softly added above the tissue layer. Following centrifugation, isolated islets were harvested from the interface between the topmost layers with a pipette. The isolated islets were stained with dithizone (140 mmol/L) and counted. The number of islets was determined using an optical graticule attached to the eyepiece of a dissecting microscope and then was converted to the standard islet equivalent.

Recipient rats

Diabetes was induced in the recipient rats via the intravenous injection of streptozotocin (STZ). Two or three days before islet transplantation, STZ (60 mg/kg) was injected into the superficial dorsal vein of the penis of the recipient rats. After 48 hours, the non-fasting blood glucose levels were measured. Diabetes in rats was defined when the glucose level was greater than 350 mg/dl with severe polyuria, and diabetic rats were prepared for islet

transplantation.

Under general anesthesia, islet transplantation was randomly performed from a single donor to a single recipient with the injection of the harvested islets into the portal vein in which the islets were collected in 0.1 ml of RPMI medium. The amount of transplanted islets was 10 islet equivalents per one gram of body weight of the recipient rat.

Assessment of the islet graft function

The non-fasting blood glucose levels of the recipient rats were measured daily to monitor the function of the islet grafts for seven days after transplantation. The recipient rats were then sacrificed, and the serum insulin values and the serum zinc levels were measured.

Statistics

Unpaired Student's *t*-test was used for the statistical analyses of the differences in the serum and pancreatic zinc levels, the blood glucose levels and the serum insulin levels among the groups. A P value of less than 0.05 was regarded as being statistically significant.

Results

Serum and pancreatic zinc levels, and serum insulin values of the donor rats

The zinc levels in the serum and pancreatic tissues of the donor rats were significantly different among the three groups (Table 1). The serum and pancreatic zinc levels of the donor rats in the Low-Zn group were significantly lower than those observed in the controls ($p<0.05$). Conversely, the serum and pancreatic zinc levels in the High-Zn group were significantly higher than those observed in the controls ($p<0.05$). In addition, significantly lower serum insulin values were observed in the Low-Zn group ($p<0.05$) and higher serum insulin value were observed in the High-Zn group ($p<0.05$) compared to those observed in the control group.

Islet graft function

The blood glucose levels of the recipient rats measured before and after islet transplantation are shown in Figure 1. Elevated blood glucose levels greater than 350 mg/dl in the recipient rats following the STZ treatment decreased to less than 150 mg/dl immediately after islet transplantation. However, the blood glucose levels re-elevated two days after islet transplantation in the rats that received islet grafts from the controls or the Low-Zn diet donors.

Especially in the rats receiving islets from Low-Zn diet donor rats, the blood glucose levels were extremely high, ranging from 350 to 400 mg/dl. In contrast, in the rats receiving islets from High-Zn diet donors, the blood glucose levels were well maintained within a normal range between 150 and 200 mg/dl throughout the experiment. The blood glucose levels of the rats receiving islets from High-Zn donors were maintained at significantly low levels seven days after islet transplantation compared to those observed in the rats receiving islets from Low-Zn donors ($p<0.01$).

The serum insulin levels in the recipient rats seven days after islet transplantation are shown in Table 2. The rats that received islets from High-Zn diet donors demonstrated significantly high levels of serum insulin (3.0±1.5 ng/ml) in contrast to those observed in the rats receiving islet transplantation from the controls (1.7±0.7 ng/ml) or low-Zn diet donors (0.8±0.5 ng/ml) ($p<0.05$). Meanwhile, the serum zinc levels of recipients at the time were approximately 90 μg/g and were not significantly different among three groups.

Discussion

Zinc is known to be important for numerous functions in the pancreas, including insulin synthesis, secretion and signaling, glucagon secretion and pancreatic digestive enzyme secretion and activity (19). It has been reported that islets are the most zinc-rich cells in the body (20). Most intracellular zinc is stored with insulin in the insulin secretory vesicles in pancreatic β-cells as a zinc-insulin complex. The concentration of zinc in these vesicles is quite high at approximately 20 mM (21). Zinc is released together with insulin into the extracellular islet space when insulin is secreted. In addition, zinc is taken up by neighboring cells (22). Zinc forms hexameric crystals with insulin, each of which contains two zinc ions within β-cell insulin granules (23). Zinc-deficient rats are known to exhibit lower insulin secretin and glucose uptake than normal rats (24). It has also been reported that nutritional zinc supplementation improves both fasting insulinemia and glycemia in rodents (15). The mechanism of action of zinc remains unclear, i.e. whether it acts directly on insulin receptors and glucose transporters or indirectly via intracellular pathways of insulin (15).

This study demonstrated the effects of zinc on rat islet transplantation using

dietary zinc supplementation to donor rats for two weeks prior to donation. The serum and pancreatic zinc levels and the serum insulin levels of the donor rats were increased according to the degree of zinc supplementation. Furthermore, the results of the islet function of islet transplantation were also improved. The blood glucose levels were lower in the High-Zn group than those observed in the control and Low-Zn groups, and the serum insulin levels were also higher in the High-Zn group than those observed in the control and Low-Zn groups.

It is also known that islet β cells are vulnerable to oxidative stress (13). It is suspected that excess free radical production may contribute to the death of β cells and lead to the onset of type 1 diabetes (25-28). Pancreatic islets also lack antioxidant protection (29,30), rendering them especially susceptible to damage by free radicals produced during inflammatory and immune processes. In addition, instant blood mediated inflammatory reaction (IBMIR) is a major factor contributing to poor initial engraftment of islets in clinical islet transplantation (3). This reaction is expressed by transplanted pancreatic islets when the islets come in contact with blood in the portal vein (3). IBMIR involves the activation of coagulation and complement systems, which in turn

leads to local ischemia, injury of the endothelium and the upregulation of pro-inflammatory mediators such as ICAM-1 (Intercellular Adhesion Molecule-1) and MCP-1 (Monocyte Chemoattractant Protein-1) (31,32). The mechanisms underlying the effects of zinc supplementation remain unclear, although zinc may be involved in scavenging of free radicals (33,34) or prevention of apoptosis (35,36). Zinc is an essential trace element possessing a wide range of functions and antioxidant properties (37). IBMIR is initiated upon intraportal infusion of islets (38). The present study indicated that the blood glucose levels re-elevate two days after islet transplantation. These findings suggest that IBMIR is a barrier to engraftment in our models of intraportal islet transplantation. Therefore, zinc supplementation is thought to have the potential to improve the results of clinical islet transplantation by reinforcing the islet function and preventing graft loss through IBMIR.

The High-Zn diet donors ate more and grew more compared with the controls. However, food consumption and transitions in body weight did not differ significantly among the groups (data not shown). We thus did not think that any side effects of the High-Zn diet were apparent throughout the experiment. Zinc is considered to be relatively non-toxic to humans (39).

Xenotransplantation has been utilized to resolve donor insufficiency in clinical islet transplantation (1,2). In the setting of xenotransplantation, zinc supplementation may be useful for improving the patient outcomes. In addition, in clinical islet transplantation (allotransplantation), the serum zinc level is a potential indicator of the donor islet function. These data therefore suggest the potential beneficial effects of zinc supplementation in donors for islet transplantation and the applicability of zinc in clinical islet transplantation. Further studies are needed to prove the effects of zinc supplementation on islet transplantation, especially over a much longer period and with respect to aspects of both histology and molecular biology.

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Figure legends

Figure 1. The blood glucose levels of the recipient rats after islet transplantation were consistently high in the Low-Zn group compared with those observed in the control group and were consistently low in the High-Zn group. The levels observed in the High-Zn group were significantly low compared to those observed in the Low-Zn group and were maintained within a normal range throughout the observation.

Table 1

Pancreatic zinc, Serum zinc and Serum insulin level of donor rats

	No. of examined rats	Pancreatic zinc (μg/g)	Serum zinc (μg/dl)	Serum insulin (ng/ml)
Control	10	14.6±2.0*	118.7±7.9*	3.5±1.2*
High-Zn	10	18.7±2.2**	147.3±17.6**	5.2±1.9**
Low-Zn	10	11.3±1.9***	48.8±25.5***	2.4±1.5***

*p<0.05, when compared with control and High-Zn,

**p<0.05, when compared with High-Zn and Low-Zn,

***p<0.05, when compared with Low-Zn and control

Table 2

Serum insulin level of recipient rats

	No. of examined rats	Serum insulin (ng/ml)
Control	10	1.7±0.7*
High-Zn	10	3.0±1.5**
Low-Zn	10	0.8±0.5***

*p<0.05, when compared with control and High-Zn,

**p<0.05, when compared with High-Zn and Low-Zn,

***p<0.05, when compared with Low-Zn and control

Figure 1

Non-fasting blood glucose level after islet transplantation (average)