Development of Canine Models of Type 1 Diabetes With Partial Pancreatectomy and the Administration of Streptozotocin

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We created canine models of type 1 diabetes that were suitable for the assessment of cell therapies, such as islet transplantation and bioartificial pancreas, with low-dose streptozotocin (STZ) injection and partial pancreatectomy. In our model, a 50% pancreatectomy was performed with general anesthesia, followed by systemic injection of 35 mg/kg STZ into a vein of the foreleg. Four weeks after the administration of STZ, the fasting blood glucose level of our model dogs was found to be over 200 mg/dl twice on different days, and we could not detect any canine insulin by the intravenous glucose tolerance test (IVGTT). We therefore diagnosed the dogs to have induced diabetes. Some studies have reported high-dose STZ to be very toxic for both the kidney and liver, and therefore a lower dose is desirable to induce diabetic models without any associated kidney or liver damage. We think that the combination of a partial pancreatectomy can thus make it possible to reduce the dose of STZ, and it is therefore useful for the creation of type 1 diabetes models. We believe that our model is a safe and reliable model for type 1 diabetes in canines to assess the efficacy of pancreas-targeted cell therapies.

Key words: Pancreatectomy; Streptozotocin (STZ); Diabetes; Dogs

INTRODUCTION

Presently, the number of diabetic patients is steadily increasing worldwide. Among these, intensive insulin therapy is essential against type 1 diabetes mellitus (DM) caused by pancreatic islet destruction due to autoimmunity; however, the strict management of blood sugar levels is difficult and the risk of hypoglycemic episodes is high. Moreover, it is also known that microvasculature/peripheral neuropathy cannot be completely prevented. Many strategies, including pancreatic transplantation, pancreatic islet transplantation, and bioartificial pancreas (BAP), have been devised as medical treatments to replace insulin therapy (3,7,10,11). In actual medical practice, seven diabetic patients that underwent allogeneic pancreatic islet transplantation using the Edmonton protocol carried out at the University of Alberta in 2000 successfully withdrew from insulin usage (18). Various studies are still being carried out in the field of pancreatic islet transplantation (12–15), in addition to studies on xenotransplantation of the pancreatic islets of pigs, etc. (2).

However, in transplantation studies that assume xenotransplantation, it is prospected that the transition to clinical application in humans will be very difficult from an ethical standpoint. Accordingly, a large animal diabetes model is essential for appropriate evaluation in vivo. Among large animals, dogs are known to exhibit diabetes symptoms very similar to that of humans and may be said to be the animal that is the most suitable for diabetes studies. Streptozotocin (STZ) is frequently used to induce diabetes in animal models, but high doses may cause additional toxicity of the kidneys and liver. In this study, we developed a safe and reliable diabetes model of a dog by partial pancreatectomy and systemic administration of low doses of STZ. Moreover, a BAP transplanting experiment was carried out using one of these dogs and is being reported together with the study.

MATERIALS AND METHODS

Experimental Animals

Five female beagles (Katayama Chemical, Inc., Osaka, Japan), weighing about 10 kg, were used in this study. The experiments on animals were performed at the Department of Animal Resources in the Okayama University Advanced Science Research Center, and all

procedures were conducted within the guidelines for humane care of laboratory animals and approved by the Okayama University Institutional Animal Care and Use Committee. All dogs used in the study were housed in individual kennels and fed twice daily (morning and evening) on a diet of biscuit fortified with vitamins and minerals.

Diagnosis of Diabetes

We determined the diagnostic criteria of induced diabetes as follows, with reference to articles and various clinical practice guidelines.

1. A fasting blood glucose of 200 mg/dl or more is measured two times on different days.
2. There is no responsiveness toward glucose tolerance testing upon intravenous glucose tolerance test (IVGTT) and dog-type insulin is substantially undetected.

General Anesthesia

Avascularization was lightly carried out by having an assistant fix the forelegs. A route was secured with a 20-gauge indwelling needle (Terumo Corporation, Tokyo, Japan) from the veins appearing on the surface. Diprivan (1%; AstraZeneca, Osaka, Japan) was intravenously injected. A tracheal tube of approximately 6.0 Fr (Japan Medicalnext Co., Ltd., Osaka, Japan) was inserted into the tracheal tube and connected to an artificial respirator. Anesthesia was maintained using Sevofrane (Maruishi Pharmaceutical Co., Ltd., Osaka, Japan) for inhalation. Respiratory care was carried out at a tidal volume of approximately 200 ml and respiratory frequency of approximately 20 times per minute. Diprivan (1%) was injected by 1 ml each time the anesthetic depth became shallow. Muscle relaxants were not used. Atropine sulfate hydrate (0.5 mg; Mitsubishi Tanabe Pharma Corporation, Osaka, Japan) was used to prevent complications such as vomiting, etc., at the time of awakening.

Surgical Procedures

A laparotomy was carried out upon upper ventral incision. It was confirmed that there were no intraperitoneal abnormalities. The omental bursa was opened, and a pink pancreas was confirmed. This was separated from the tail of the pancreas, thrust ligation was carried out where it intersects with the veins of the upper intestinal membrane, and the pancreas tail was subsequently resected. Next, the duodenum was fixed, and the accompanying pancreas uncinate process was resected at a level not damaging the papilla of the vater. The surgical incision was carefully closed upon confirming hemostasis.

Injection of STZ

STZ (Sigma-Aldrich, St. Louis, MO, USA) was initially prepared aseptically as solutions containing 100 mg/ml in trisodium citrate (Sigma-Aldrich) buffer (pH 4.5) and sterilized by filtration through 0.22-μm filters (Millipore Corporation, Billerica, MA, USA). The drug solution was prepared immediately before administration due to the fact that these compounds often display chemical instability in solution form. The solution of 35 mg/kg STZ was systemically injected into a vein in the foreleg or a cervical vein. Next, light sedation with propofol (Sigma-Aldrich) was used, as STZ often causes blood vessel pain.

Postoperative Management

Wound infection is the most common complication in large animal experiments carried out at our institute. From this experience, the wounded area was wrapped with gauze (Hakujuji, Tokyo, Japan) and securely taped, and the wound was managed in order to avoid contact with fecaluria. Since infection from saliva is also common, an Elizabethan collar (Mau, Tokyo, Japan) was used to prevent wound infection due to removal of the gauze and saliva. Cefamezin alfa (Astellas Pharma Inc., Tokyo, Japan) was administered at 0.25 g/day as an antibiotic for 5 days following surgery. The gauze was changed daily for a week as a general rule, and the wound was maintained clean. The stitches were removed on day 14.

Changes in Body Weight

The body weight of the dogs following surgery was measured every 2 weeks.

Biochemical Analysis

Blood was drawn from the dog's foreleg regularly in order to measure the biochemical features of the canine, such as total protein (TP), albumin (ALB), aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), total bilirubin (T-Bil), blood urea nitrogen (BUN), creatinine (CR), amylase (AMY), lipase, Na, K, and Cl. All samples were measured by Idexx Labotatories (Tokyo, Japan).

IVGTT

After partial pancreatectomy and the injection of STZ, the IVGTT was conducted at the point that the fasting blood glucose level of dogs was over 200 mg/dl twice on a different day. The data were compared with those obtained from IVGTT conducted prior to the surgery. For IVGTT, a bolus injection of 50% glucose (Sigma-Aldrich) concentrated solution at 1.0 g/kg of body weight was given. Blood was collected at the time points of 0, 5, 15, 25, 35, 45, 60, and 120 min to measure blood glucose levels and insulin concentrations, using a Medisafe mini GR-102 (Terumo Corporation) and a Canine Insulin ELISA Kit (Mercodia AB, Uppsala, Sweden) according to the provider's protocol, respectively.

Histological Assessment

Autopsy was performed 8 months after surgery and STZ injection. All samples were fixed with 20% formalin (Sigma-Aldrich), embedded in paraffin (Sigma-Aldrich), and processed for staining with hematoxylin and eosin (H&E; Sigma-Aldrich). Insulin staining was also performed for samples taken from the residual pancreas, using anti-insulin antibody (Dako, Carpinteria, CA, USA).

A Case of BAP Transplantation

We are developing a bag-type BAP using a semipermeable membrane, which involves an immunity isolation effect (7). This BAP device was placed inside the abdomen of dog 1 that generated diabetes in this study, and on a later day, a fresh pancreatic islet isolated from a male Lewis rat (12 weeks old; Japan SLC, Inc.. Shizuoka, Japan) weighing approximately 300 mg was infused a total of three times every 7 days (14,200, 9,150, and 10,120 IEQ, respectively). Islet isolation was conducted as described previously (11).

RESULTS

Operation and Postoperative Complication

In order to create a diabetes model of beagle dogs, the pancreas tail was excised where it intersects the veins of the upper intestinal membrane, and subsequently the pancreas uncinate process accompanying the duodenum was extracted at a level not damaging the papilla of the vater. The extracted pancreas weighed an average of 13.7 g, and from the fact that the weight at complete excision of the pancreas carried out on a beagle with the same weight was 28 g, it was found that approximately a 50% pancreatectomy was possible using this method (Table 1).

No acute phase side effects related to surgery such as wound infection, secondary hemorrhage, aspiration pneumonia, etc., were observed. However, a mild tendency for loose stool believed to be due to an impaired exocrine pancreas was observed from a relatively early phase.

Table 1. Detailed Data of the Dogs Actually Used in the Experiment

Dog No.	Body Weight (kg)	Pancreas Weight (kg)	Amount of STZ (mg/kg)	Date of DM After Operation	Survival Days
1	10.5	14.2	35	35	170
2	10.0	12.8	15+35	28	160
3	11.0	15.6	15+35	28	192
4	11.5	12.2	35	23	145
5	11.5	13.8	35	21	125
Average	10.9± 0.65	13.72± 1.32		27.0± 5.43	158.4± 25.3

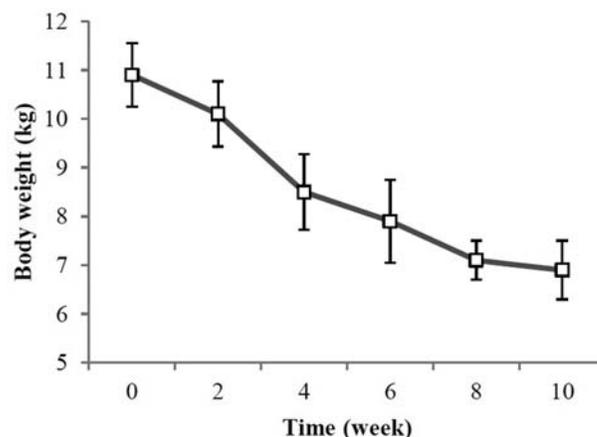STZ, streptozotocin; DM, diabetes mellitus.

Figure 1. Changes in body weight. STZ was intravenously injected after resecting the pancreas tail and pancreas uncinate process of a beagle. The body weight was measured every 2 weeks following surgery.

Changes in Body Weight

STZ (15 or 35 mg/kg) was intravenously injected following pancreatectomy (Table 1). Following pancreatectomy and STZ administration, a decline in body weight was gradually observed, and the average body weight 2 months after operation was approximately 7.1 kg (Fig. 1).

Biochemical Parameters Indicated the Progress of Diabetes

A biochemical test was carried out the fourth week following surgery. No abnormalities were observed in the pancreatic exocrine function upon biochemical tests, and moreover, no other major abnormal findings including liver function/renal function were observed (Table 2).

Table 2. The Biochemical Data of the Dogs Used for the Experiment 4 Weeks Following Surgery

	Average ($n=5$)	Reference Interval
TP (g/dl)	6.44±0.38	5.0–8.0
ALB (g/dl)	3.04±0.26	2.8–4.0
AST (IU/L)	41.8±9.26	10–50
ALT (IU/L)	55.2±18.0	15–70
T-Bil (mg/dl)	0.22±0.13	0.1–0.5
ALP (IU/L)	138.6±31.1	20–150
BUN (mg/dl)	14.2±5.19	10–28
CR (mg/dl)	0.63±0.12	0.5–1.5
AMY (mg/dl)	423.4±63.5	399–1041
Lipase (mg/dl)	660.4±163.8	245–1585
Na (mEq/L)	142.8±2.39	141–152
K (mEq/L)	4.6±0.38	4.4–5.4
Cl (mEq/L)	107.4±2.07	105–115

TP, total protein; ALB, albumin; AST, aspartate transaminase; ALT, alanine transaminase; T-Bil, total bilirubin; ALP, alkaline phosphatase; BUN, blood urea nitrogen; CR, creatinine; AMY, amylase.

Changes in IVGTT Before and After Surgery

An IVGTT test was carried out 4 weeks following surgery. Compared to normal dogs, the blood glucose levels of 4 weeks following surgery stayed about the same with respect to the glucose tolerance test and transited as hyperglycaemia; moreover, dog-type insulin was also not detected (Fig. 2).

Pathological Findings

The residual pancreas was markedly atrophic upon autopsy (Fig. 3). Most parts were replaced by fatty tissues in the pathology specimen as well, and only islet pancreas tissues were observed in some parts (Fig. 4A, B). The vaguely recognized pancreatic islets did not stain at all for insulin, and it was believed that the insulin-secreting ability had disappeared (Fig. 4C). No findings suggesting

complications to other organs such as the lungs, kidneys, liver, etc., were observed.

Changes in Blood Glucose Levels After
BAP Transplantation

We are developing a bag-type BAP using a semi-permeable membrane, which involves an immunity isolation effect. This bag-type device involving an immunity isolation effect was placed inside of the abdomen of dog 1 after generation of diabetes in this study, and starting on day 90, fresh pancreatic islets were isolated from a Lewis rat and infused at three later time points. The blood glucose level declined to 200 mg/dl or less within several days following transplantation with the first and second infusion; however, a tendency was observed for the blood glucose level to subsequently gradually increase. A relatively stable
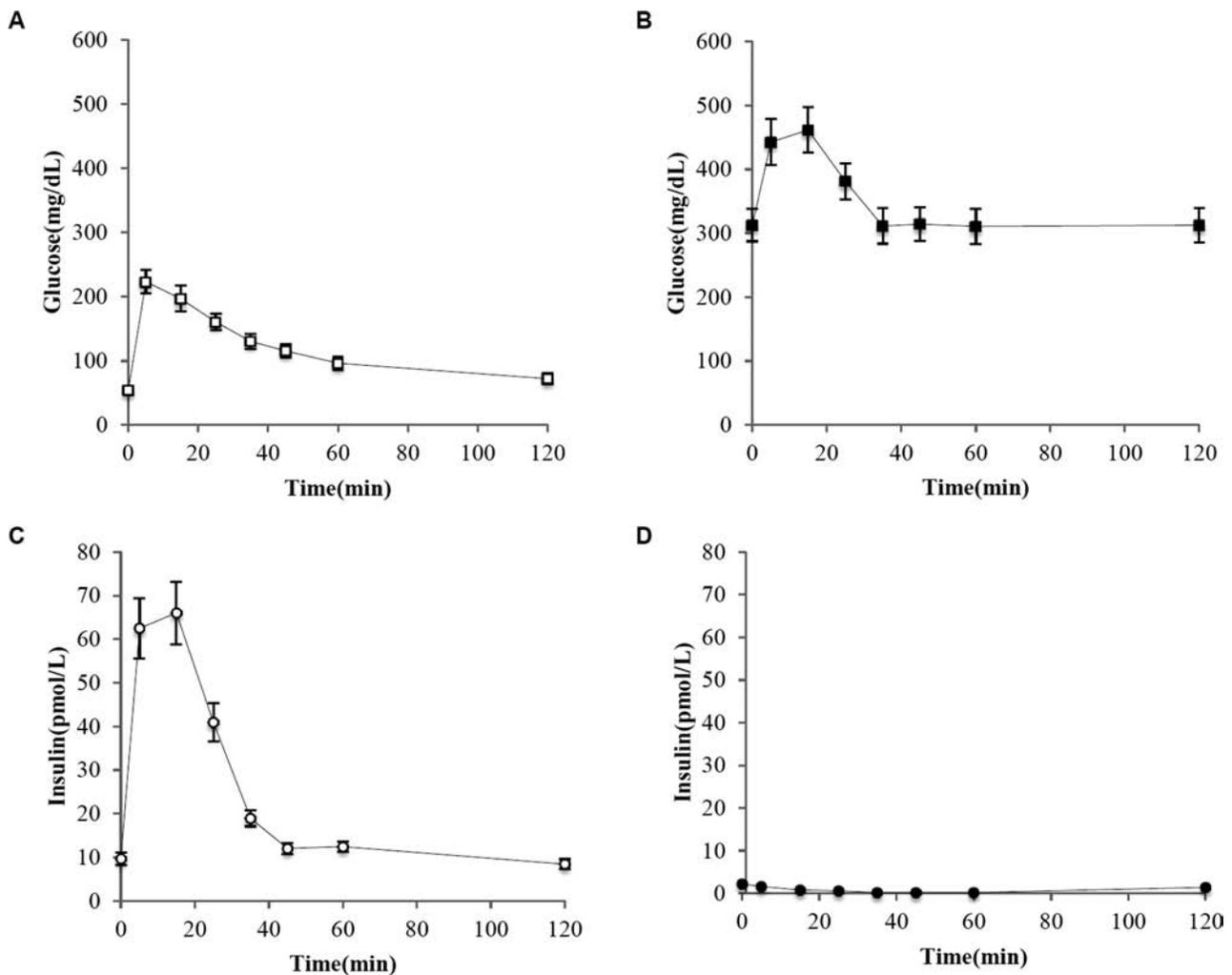

Figure 2. Comparison of the IVGTT in normal dogs and dogs that developed diabetes. (A) The transition of the blood glucose level in normal dogs. (B) The transition of the blood glucose level in diabetic dogs 4 weeks following surgery. (C) The transition of insulin secretion in normal dogs following glucose load. (D) The transition of insulin secretion in diabetic dogs 4 weeks following surgery.
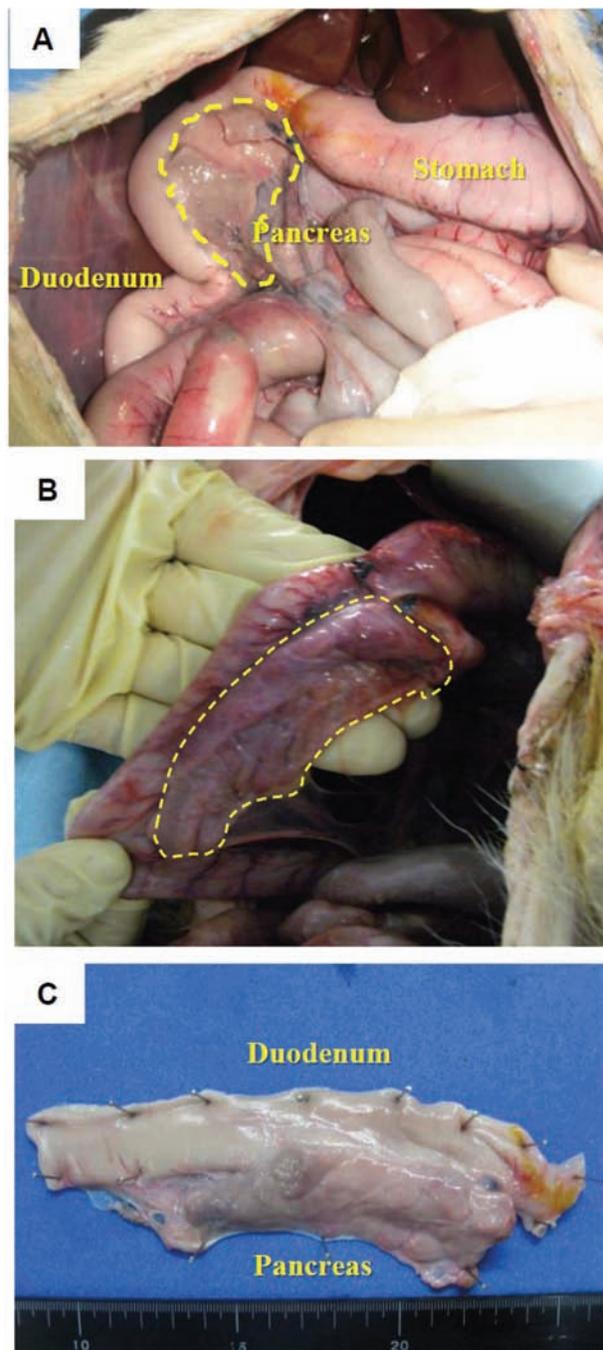
Figure 3. Image at autopsy and image of the resected specimen. (A) Findings in the abdominal cavity upon an autopsy. The remaining pancreas heads (inside the dotted line) are attached to the duodenum. (B) The residual pancreas (inside the dotted line) was thin and observed with prominently atrophic. (C) A clear abnormality was observed in the duodenal mucosa side including the papilla of the vater.

Figure 4. Pathological findings of the excised pancreas (H&E and insulin staining). (A) A low-magnified (100×) H&E-stained image. Most parts are replaced with fatty tissues, with islet pancreas tissues observed in some parts (arrows). (B) A high-magnified (400×) H&E-stained image. These are tissues believed to be barely remaining pancreatic islets. (C) A high-magnified (400×) insulin-stained image. The remaining pancreatic islets did not stain at all from insulin staining.

engrafted in the device and were carrying out good matter/nutrition exchange for at least 2 months. The unexpected death of dog 1 between days 167 and 170 from undetermined causes prevented any follow-up of the BAP.

DISCUSSION

The number of diabetic dogs is increasing following a recent pet boom, with cases of dog owners consulting animal hospitals on the rise (5). Interestingly, dogs are known to exhibit diabetes symptoms similar to that of humans, such as retinopathy, nephropathy, etc. Accordingly, it may be said that dogs are the most suitable research subject in animal experiments related to diabetes. Moreover, they have relatively gentle characteristics

blood glucose level of 200–250 mg/dl was observed for 2 months following the third infusion (Fig. 5). This suggested that the transplanted pancreatic islets may have
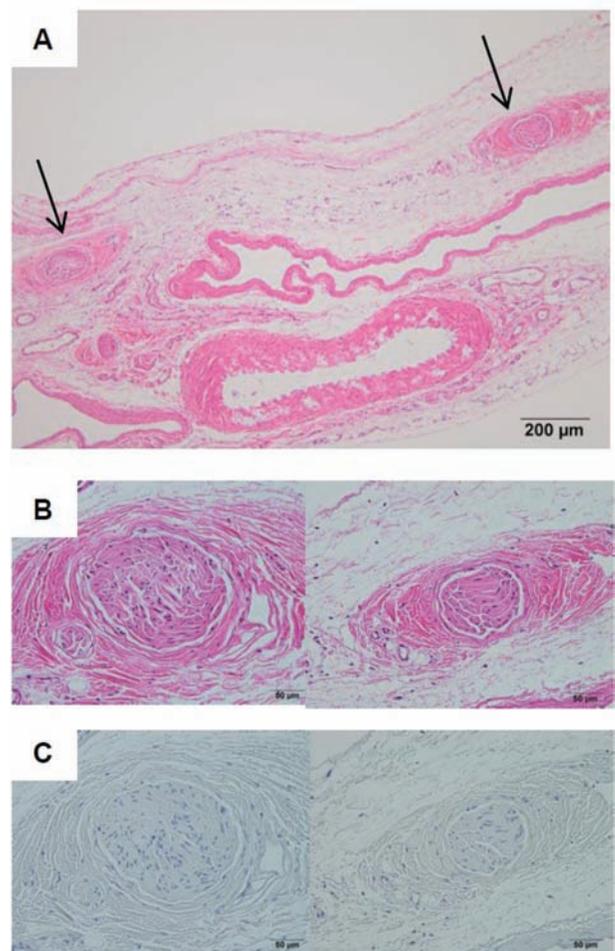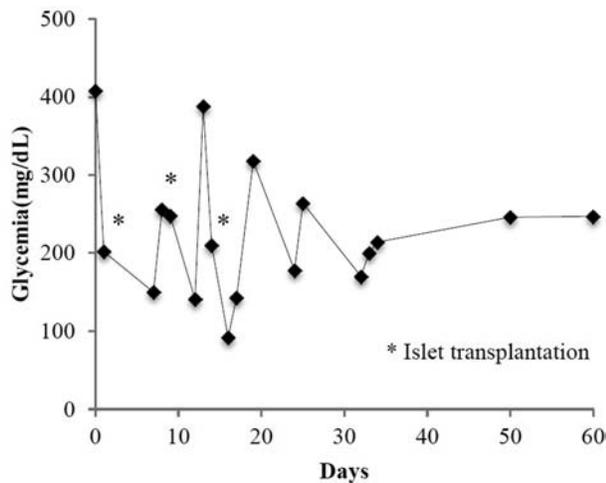
Figure 5. The transition of the blood glucose levels of the dog transplanted with a BAP. The bag-type device comprising an immunity isolation effect with respect to dog 1 is placed, and on a later day, fresh pancreatic islets isolated from a Lewis rat were infused a total of three times every 7 days (14,200, 9,150, and 10,120 IEQ, respectively). Blood glucose levels are shown over a 60-day period. *Days of infusion of pancreatic islets.

among large animals, experimental operations such as anesthesia, blood sampling, etc., are much easier compared to monkeys, pigs, etc., and there is also a merit of simple perioperative management.

There are many reports related to creating a type 1 diabetes model with mammals using STZ (16,19), and reports using large animals such as dogs, pigs, and monkeys can also be found (6,8). The dose of STZ for each animal species differs depending on the article; however, in an example of high-concentration STZ doses of 100 mg/kg or more, complications of liver function and renal function impairment have been reported. In order to obtain a diabetes model with good general condition, diabetes must be induced with the minimum required dose of STZ. Accordingly, we believed that the STZ dose may be reduced by means of carrying out partial pancreatectomy, thereby allowing for a safer and more reliable diabetes model.

In an experiment using the same beagle, Tschoepe et al. (20) systemically administered 28–38.5 mg/kg STZ but were unable to accomplish the creation of a complete diabetes model. Moreover, Anderson et al. (1) administered 40 mg/kg alloxan, which also comprises β cytotoxicity, simultaneously with 35 mg/kg STZ and succeeded in creating a diabetes model; however, necrosis of the liver tissues accompanying wide-ranging fatty infiltration was observed upon autopsy. In our study, 50% pancreatectomy was concomitantly used, and STZ administration at various concentrations was attempted. At first, when 65 mg/kg, which is the standard dose for small animals such as rats, etc., that have not undergone pancreatectomy, was

systemically administered, increased transaminase was observed at an early postoperative phase, and the animal died from liver failure 20 days following surgery (data not shown). In contrast, at 15 mg/kg STZ administration, diabetes was not observed, and an additional administration was required (dogs 2 and 3). Diabetes was generated in all cases administered 35 mg/kg and the liver function/renal function 4 weeks following surgery was good, so this was believed to be the optimal STZ concentration.

In the current studies on creating a diabetes model in large animals, there are many reports mentioning that the pancreas was completely or partially excised. It is known that complications of the digestive system such as diarrhea, etc., are caused regarding cases with complete excision of the pancreas due to the pancreatic exocrine function also being affected. Accordingly, the administration of drugs compensating for the exocrine function becomes necessary. Moreover, reconstruction of the biliary system is required, complicating the surgical procedure and thereby also increasing the risk of complications due to surgery such as bile leakage, secondary hemorrhage, etc. (17).

There are also several reports regarding the creation of diabetic dog models by combining partial pancreatectomy and STZ. Freyse et al. (4) selectively administered 2 mg/kg STZ to the pancreaticoduodenal artery in addition to 77% pancreatectomy and succeeded in creating diabetes models at a ratio of 16:55 dogs. Moreover, although the ratio of excision is not mentioned, Tschoepe et al. administered STZ in different concentrations from the pancreaticoduodenal artery of dogs that underwent excision of the pancreas head as well as excision of the tail and failed in inducing diabetes with the administration of 2 mg/kg STZ but generated diabetes in all dogs at a dose of 25 mg/kg. Morales et al. (9) carried out 90% pancreatectomy and 2 mg/kg STZ administration via the pancreaticoduodenal artery to fox terriers and succeeded in inducing diabetes in all five dogs. The method of selectively directly cannulating and infusing STZ to the pancreaticoduodenal artery is capable of overwhelmingly reducing the dose of STZ and selectively administrating only to the pancreas; therefore, it is theoretically believed to be the most ideal route. However, the method of identifying the artery and directly puncturing requires specialized knowledge, and time is required for acquiring these skills. Moreover, the surgical operation itself becomes complicated, resulting in greater surgical invasion. Consequently, this results in, of course, an increased risk of complications such as pancreatic fistula, secondary hemorrhage, etc.

Our method involves simply resecting the pancreas uncinate process and pancreas tail without touching the biliary system or blood circulatory system; therefore, there is minimal surgical invasion, the surgical time is short, and the risk of complications is minimized. Digestive symptoms such as loose stool, etc., are also mild compared to

complete excision of the pancreas. It is believed that these may be improved by administrating digestive agents compensating for external secretion. Moreover, diabetes is also generated without impairing the liver and renal function by the systemic administration of STZ.

Although simple, the surgical procedure of pancreatectomy and techniques for general anesthesia are required; therefore, regardless of the remaining issues in terms of the universality by which it may be conducted by any researcher, it may be said that the diabetic model that we created in this study is the safest, with good general conditions and the most reliability regarding dogs. We believe that this will become a model useful for evaluating treatments in the frontier of rapidly advancing diabetes treatments such as heterogeneous pancreatic islet transplantation, artificial biopancreas, etc.

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