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Research Article

A Simple and Effective Non-Human Primate Diabetic Model Combining Sub-Total Pancreatectomy and Low-Dose Streptozotocin Injection

Ibrahim Fathi [1], Takehiro Imura [1], Kozue Imura [1], Megumi Goto [1], Yasuhiro Igarashi [1], Akiko Inagaki [1], Makiko Kikkawa [1], Fumiko Ono [2], Hiroo Iwata [3], Yohichi Yasunami [4], Masafumi Goto [1, 5, *]

1. Division of Transplantation and Regenerative Medicine, Tohoku University Graduate School of Medicine, 980-0872, Sendai, Japan; E-Mails: I_Fathy201010@alexmed.edu.eg; takehiro.imura.b1@tohoku.ac.jp; kozue.imura.b7@tohoku.ac.jp; megumi.goto.b1@tohoku.ac.jp; yasuhiro.igarashi.a5@tohoku.ac.jp; akiko.inagaki.b1@tohoku.ac.jp; m.kikkawa@med.tohoku.ac.jp; masafumi.goto.c6@tohoku.ac.jp
2. Department of Veterinary Associated Science, Faculty of Veterinary Medicine, Okayama University of Science, 794-8555, Imabari, Japan; E-Mail: f-ono@vet.ous.ac.jp
3. Compass to Healthy Life Research Complex Program, RIKEN, 650-0047, Kobe, Japan; E-Mail: hiwata00@gmail.com
4. Islet Institute, Fukuoka University, 814-0180, Fukuoka, Japan; E-Mail: yasunami@fukuoka-u.ac.jp
5. Department of Surgery, Tohoku University Graduate School of Medicine, 980-0872, Sendai, Japan

* **Correspondence:** Masafumi Goto; E-Mail: masafumi.goto.c6@tohoku.ac.jp

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Abstract

***Background*:** The existing non-human primate diabetic models, particularly those induced by total pancreatectomy or streptozotocin (STZ) injection, have several disadvantages, including a lengthy surgical procedure, the need for continual supplementation of digestive enzymes,

and delayed oral intake after total pancreatectomy, and severe hypoglycemia and hepatic/renal toxicity following injection of high-dose STZ. This study aimed to develop and optimize a non-human primate diabetic model that would avoid the disadvantages of the current approaches, and compared it with the STZ model.

***Methods*:** Diabetes was induced in male Cynomolgus monkeys (n=4) by performing sub-total pancreatectomy followed by low-dose STZ injection (30 [n=1], 45 [n=2], and 60 [n=1] mg/kg). This model was compared to the STZ injection-only (60 [n=1] and 100 [n=9] mg/kg) model, regarding parameters such as the development of diabetes (measuring serum C-peptide and pancreatic anti-insulin staining), functions of liver (glutamine-ornithine-transaminase and glutamine-pyruvate-transaminase levels) and kidney (BUN and creatinine levels), and the general condition (weight loss, food intake, and activity).

***Results*:** The levels of serum C-peptide after glucose challenge in the present model (0 ng/mL) were comparable to those with the model given 100 mg/kg STZ. The absence of pancreatic insulin staining was observed with the use of 60 mg/kg STZ with sub-total pancreatectomy, as with 100 mg/kg STZ injection only. Animals in the present model did not suffer from hypoglycemic episodes and showed better activity and oral intake than those in the STZ-only model. The levels of liver enzymes were markedly lower in the new model, while those of BUN and creatinine were higher than the STZ-alone model.

***Conclusions*:** Diabetes was successfully induced using the combined technique without the need for a lengthy operation or continual supplementation of digestive enzymes. Furthermore, the reduction in the pancreatic volume allowed for lower utilization of STZ, which led to reduced hepatic toxicity and the absence of hypoglycemic episodes. However, adequate fluid administration may be required to guard against potential renal STZ toxicity.

Keywords

Cynomolgus monkeys; streptozotocin; sub-total pancreatectomy; insulin-dependent diabetes; diabetic model

1. Introduction

Non-human primate (NHP) diabetic models are essential for studies on preclinical pancreatic islet transplantation and development of anti-diabetic medication. Presently, the total pancreatectomy (TP) and streptozotocin (STZ) injection models are the most commonly used for this objective. STZ is a toxin that is largely taken up by pancreatic β cells, as it depends on the glucose transporter-2 (GLUT-2) for internalization [1]. However, due to the presence of GLUT–2 in other cells including hepatic and renal tissues, the use of high doses of STZ, although simple, is associated with hepatic and renal toxicity, and negatively influences the general condition during the post-induction period [2]. Furthermore, the sudden destruction of a large number of β-cells results in hypoglycemic episodes, further complicating the post-induction management.

On the other hand, although the TP model is effective, it is associated with a lengthy procedure which mandates the meticulous dissection and preservation of the duodenal vascular arcade [3]. Therefore, this procedure is associated with a high morbidity rate. Complete removal of the

exocrine pancreatic tissues also requires the continual supplementation of digestive enzymes during the postoperative period [3].

Therefore, we attempted to address the major shortcomings of both models by performing sub-total pancreatectomy (STP) followed by the injection of low-dose STZ (LD-STZ). Hypothetically, the pancreas that remains after a much simpler and less time-consuming procedure than TP should be able to supply the necessary digestive enzymes. Decreasing the amount of pancreatic tissues, and therefore the β-cell mass allows for a reduction in the STZ dose, which in turn can decrease the toxic effects. The destruction of a relatively small mass of β-cells may also help in avoiding hypoglycemic episodes.

In the present study, we compared the STP/LD-STZ model with the STZ injection-only model. To determine the optimum STZ dose, defined as the lowest dose capable of efficiently inducing insulin-dependent diabetes mellitus (IDDM) after STP, we tested several doses of STZ (30, 45, and 60 mg/kg).

2. Methods

2.1 Study Animals

Fourteen male cynomolgus monkeys (*Macaca fascicularis,* aged 4.5 ±0.8 years old, mean weight = 3.88 Kg) were used in this study. The monkeys were supplied by Shin Nippon Biomedical Laboratories, Ltd. (Tokyo, Japan). All the monkeys were tested negative for B virus (cercopithecine herpesvirus 1), Simian Retrovirus, Simian Varicella Virus, and Tuberculin test. The monkeys were housed with matching gender in separate cages with free access to water, and an NHP diet (CLEA old-world-monkey diet "CMK–2"; CLEA Japan, Inc., Tokyo, Japan) was given twice a day.

All the animals that were used in this study were handled according to the Guide for the Care and Use of Laboratory Animals, published by the National Institute of Health [4]. The animal studies were approved by the Animal Care and Use Committee of the Tohoku University (approved protocol ID: 2015 NICHe-Animal–006). All efforts were made to minimize any pain and suffering to the animals.

2.2 Induction of Diabetes

2.2.1 STZ Injection-Only Model

Anesthesia was induced by intramuscular administration of an MMP cocktail containing 0.2 mg/kg Midazolam (Dormicum Astellas Pharma Inc., Tokyo, Japan), 0.03 mg/kg Medetomidine hydrochloride (Domitor, Nippon Zenyaku Kogyo Co., Ltd., Fukushima, Japan), and 0.3 mg/kg Butorphanol tartrate (Vetrophale, Meiji Seika Pharma Co., Ltd., Tokyo, Japan). Inhalational anesthesia (1%–4% isoflurane) was administered through an endotracheal tube (ETT; Entraprime, Intermed Inc., Osaka, Japan) having an internal diameter of 3 mm. An 18- to 20-gauge central venous line (Argyle LCV-UK; Medtronic Co., Ltd., Tokyo, Japan) was passed through the internal jugular vein, through which STZ (Zanosar; Teva Parenteral Medicines, Inc., CA, US) at 60 (n=1) or 100 (n=9) mg/kg, diluted with isotonic saline to a final volume of 10 mL, was administered using a syringe pump (1 mL/min). The animals were continuously hydrated using an electrolyte infusion with 1% glucose; Physio140 (OTSUKA Pharmaceutical Co. Ltd., Tokyo, Japan). Atipamezole

hydrochloride (Antisedan; Nippon Zenyaku Kogyo Co. Ltd.) at 0.1 mg/kg was used to quicken recovery. Intravenous hydration was continued until day 2 using a glucose-electrolyte solution; SOLDEM 3A (TERUMO CO., Tokyo, Japan). On day 0, after 12 h of STZ injection, 50% glucose was administered using a syringe pump (1 mL/h) for another 12 h, with the dose calculated according to the levels of blood glucose after 6 h of infusion. A primate jacket (Lomir biomedical, Quebec, Canada) was used to protect the central venous line during the post-operative period, without restricting the animals' movement.

2.2.2 STP Combined with Low-Dose STZ

Anesthesia was performed using the same medications as for the STZ model, with the use of a face mask instead of ETT. A peripheral line was placed through the lateral or dorsal leg veins and secured in place. The body temperature was monitored using a rectal probe, the core body temperature was maintained between 36–37.5 °C to prevent hypothermia. Before skin incision, 0.5 g Cefotiam (Nipro Pharma Co., Osaka, Japan) was administered. After disinfecting the abdomen using 10% Betadine solution, an upper midline laparotomy was performed. The short gastric vessels were ligated, following which the pancreas was dissected from the splenic side toward the pancreatic neck region. A tunnel was made behind the pancreatic neck and in front of the portal vein. The splenic artery and vein were ligated and divided. After ligation, pancreatic tissue was transected at the level of the portal vein (around 2/3 of the pancreatic tissue was removed) using 2–0 silk (Figure 1). Immediately after resection, the pancreatic duct was cannulated using a 22-gauge cannula, and 1 mL/g ET-K solution (OTSUKA Pharmaceutical Co. Ltd.) was infused to distend the pancreatic tissues. The pancreas was preserved in an ET-K solution and transferred for isolation of islets. After hemostasis was ensured, mass closure of the *linea alba* was performed using 2–0 PDS-II (Ethicon, Tokyo, Japan), followed by skin closure using interrupted sutures. Immediately after pancreatic transection, an STZ dose of 30 (n=1), 45 (n=2), or 60 (n=1) mg/kg was administered (total volume = 10 mL) through the peripheral line using a syringe pump. Reverse anesthesia (0.1 mg/kg atipamezole hydrochloride; Nippon Zenyaku Kogyo Co., Ltd.) was administered and the monkeys were returned to their cages. A dose of 0.05 mL/kg butorphanol (Vetrophale; Meiji Seika, Tokyo, Japan; 5 mg/10 mL) was given as an analgesic on the day of the operation and during the follow-up period, if necessary. A custom-made holder was used to secure the leg containing the peripheral line. Although no glucose solution was administered during the postoperative period, hydration with fluids containing 5–10% glucose (Lactec D or Physiosol No. 3, Otsuka Pharmaceutical Co. Ltd., Tokyo, Japan) was performed on day 0, while SOLDEM 3A (TERUMO Co. Tokyo, Japan) was administered on day 1 and Solita-T4 (Aypharma Co. Tokyo, Japan) was administered on day 2. Oral fluids were allowed on day 2, and normal feeding was gradually resumed from day 6. Prophylactic antimicrobials were administered for three days (Cefotiam 0.5 g; Nipro Pharma Co.).

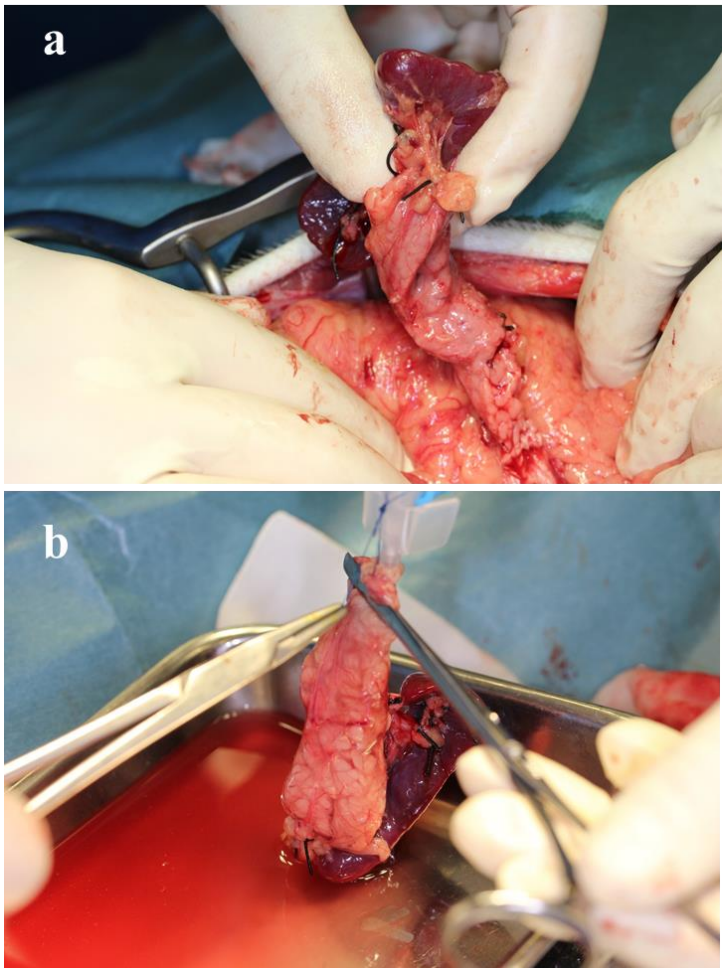

Figure 1 Subtotal pancreatectomy in cynomolgus monkeys. Dissected pancreas and spleen (a); cannulation of the excised pancreas for islet isolation (b).

2.3 Intravenous Glucose Tolerance Test (IVGTT)

Blood sugar levels were measured using a Freestyle Precision Neo portable glucometer (Abbott Diabetes Care Ltd., Oxon, UK) daily before breakfast (around 10 am), and insulin was given accordingly. During parenteral infusion, acting-type insulin (Humulin R, Eli Lilly, Japan) was administered through a syringe pump, followed by the subcutaneous injection of neural protamine Hagedorn insulin (Novolin N FlexPen, Novo Nordisk Pharma Ltd). If the maintenance of blood glucose was desired for a longer, the protocol was modified to incorporate the use of long-acting insulin (TRESIBA FlexTouch, Novo Nordisk Pharma Ltd). Table 1 presents the formula for the calculation of insulin dose. On days 6 to 9, IVGTT was performed after a 17-h fast. Briefly, 1 mL/kg of 50% glucose was injected intravenously, and blood samples at 0 (basal fasting level), 5, 10, 15, 30, and 60 min were collected in BD Microtainer® tubes (ref: 365967; Becton & Dickinson Co., New-Jersey, USA) for determining the levels of C-peptide. Samples were centrifuged at 15,000 *g* for 1.5 min at 4 °C, and the serum was stored at –80 °C. The C-peptide levels were measured using an ultrasensitive human C-peptide ELISA kit (cat#10–1141–01; Mercodia, Uppsala, Sweden), according to the manufacturer's instructions. The absence of fasting and stimulated C-peptide response during IVGTT indicated the development of experimentally-induced IDDM.

Table 1 Protocol for insulin dosage calculation.

Blood glucose level (mg/dL)	Insulin dosage
<70	Administration discontinued
71–100	Previous value × 0.5
101–250	Previous value
251–400	Previous value × 1.2
401–600	Previous value × 1.5
>600	Previous value × 1.8

* Rounded to 0.5 unit increments.

* Initial Insulin dosage is 0.8 U/kg which is initiated when the blood sugar level is 200 mg/dL or more.

* The upper limit of insulin dose is 3 U/kg.

2.4 Follow-Up and Assessment

The general condition, feeding habits, and activities of the monkeys were monitored during glucose measurement and meal administration throughout the study period. A visual score (Table 2) was used for evaluation of the activity. The weights were measured before induction and during post-induction on days 8–15. Since our protocol for the calculation of insulin dose had not been standardized for the first three monkeys in the STZ-only model, it was decided to omit their follow-up data on general condition, activity, and weight loss, from the comparison. Butorphanol (Vetrophale; Meiji Seika, Tokyo, Japan; 0.05 mL/kg) was administered as an analgesic if pain was noted during follow-up.

Table 2 Activity score used in the present study.

Score	Visual finding
1	Lying down and no response to stimulation
2	Difficult to keep posture, reacts to stimulation
3	Maintain sitting but head down
4	Hanging on the front of the cage and runs away with stimulation
5	Actively moving around in the cage as usual

* Evaluation was performed at the same time of the day for all animals by three alternating observers.

* All animals scored 5 at the beginning of the study (before induction).

The levels of hepatic enzymes, including glutamine-ornithine-transaminase (GOT) and glutamine-pyruvate-transaminase (GPT), and parameters of renal function, including blood urea nitrogen (BUN) and creatinine levels, were evaluated before induction (baseline) and on days 6 to 9, using SPOTCHEM EZ sp–4430 (ARKRAY, Inc. Tokyo, Japan). If considerable hepatotoxicity was encountered, hepatoprotective medications including potent neo-minophagen C and glycyrrhizic acid (Griffergen Electrostatic note 20 mL; Nichi-Iko Pharmaceutical Company, Ltd., Toyama, Japan), were administered. Hepatotoxicity and nephrotoxicity were scored with reference to the baseline value (hepatotoxicity score 0: ≤1x; 1: 1–2.5x; 2: 2.5–5x; 3: ≥5x/nephrotoxicity score 0: ≤1x; 1: 1–1.5x; 2: 1.5–3x; 3: ≥3x), similarly as a previously-described scoring system [5].

2.5 Immunohistochemical Assessment

After the completion of the study period (variable depending on the experimentation plan), the pancreata were surgically collected under anesthesia, followed by euthanizing the monkey. The pancreata were fixed in 4% paraformaldehyde for 24 h and embedded in paraffin. Sections of 4-µm thickness were stained using anti-insulin antibodies (#A0564, Polyclonal Guinea Pig Anti-Swine Insulin; DAKO Inc., CA, US). Briefly, the sections were deparaffinized, washed in phosphate-buffered saline (PBS), incubated with 3% H_2O_2/PBS for 3 min for retrieval of antigens, and permeabilized using 0.5% Tween (in PBS) for 5 min (x2). The sections were then incubated with primary antibodies for 30 min at 37 °C, followed by three washes with 0.5% Tween buffer (5 min each) and incubation with secondary antibodies (#K4002, EnVision System, Labeled Polymer Anti-Rabbit; DAKO Inc.) for 30 min at room temperature. This was followed by three washes with 0.5% Tween, incubation with perioxidase solution (for 6 min), washing with PBS and distilled water, hematoxylin staining, dehydration with increasing concentrations of ethanol followed by xylene, and mounting.

3. Results

3.1 Induction of Diabetes and IVGTT

The complete disappearance of fasting C-peptide and complete/nearly complete absence of stimulated C-peptide levels were achieved with a 100-mg/kg dose in the STZ-only model and all the doses tested in the STP/LD-STZ model, confirming the efficient induction of IDDM. Furthermore, although the 60-mg/kg dose in the STZ-only model considerably reduced the stimulated C-peptide response, the levels were still within the detectable range (Table 3).

Table 3 IVGTT results (C-peptide in ng/mL).

Diabetes Induction	STZ (mg/kg)	N	Time (min)					
			0	5	10	15	30	60
STZ	60	1	0.06	0.05	0.06	0.05	0.05	0.03
	100	4	0.00	0.00	0.00	0.00	0.12	0.00
STP + STZ	30	1	0.00	0.00	0.00	0.00	0.00	0.00
	45	2	0.00	0.00	0.00	0.00	0.00	0.01
	60	1	0.00	0.00	0.00	0.00	0.00	0.00

STP, sub-total pancreatectomy; STZ, Streptozotocin

3.2 Histology

Staining for insulin indicated the near-complete absence of insulin-positive cells within pancreatic islets at the following doses: 100 mg/kg in the STZ-only model, and 45 and 60 mg/kg doses in the STP/LD-STZ model (Figure 2). At the lower doses, more areas of insulin staining were observed.

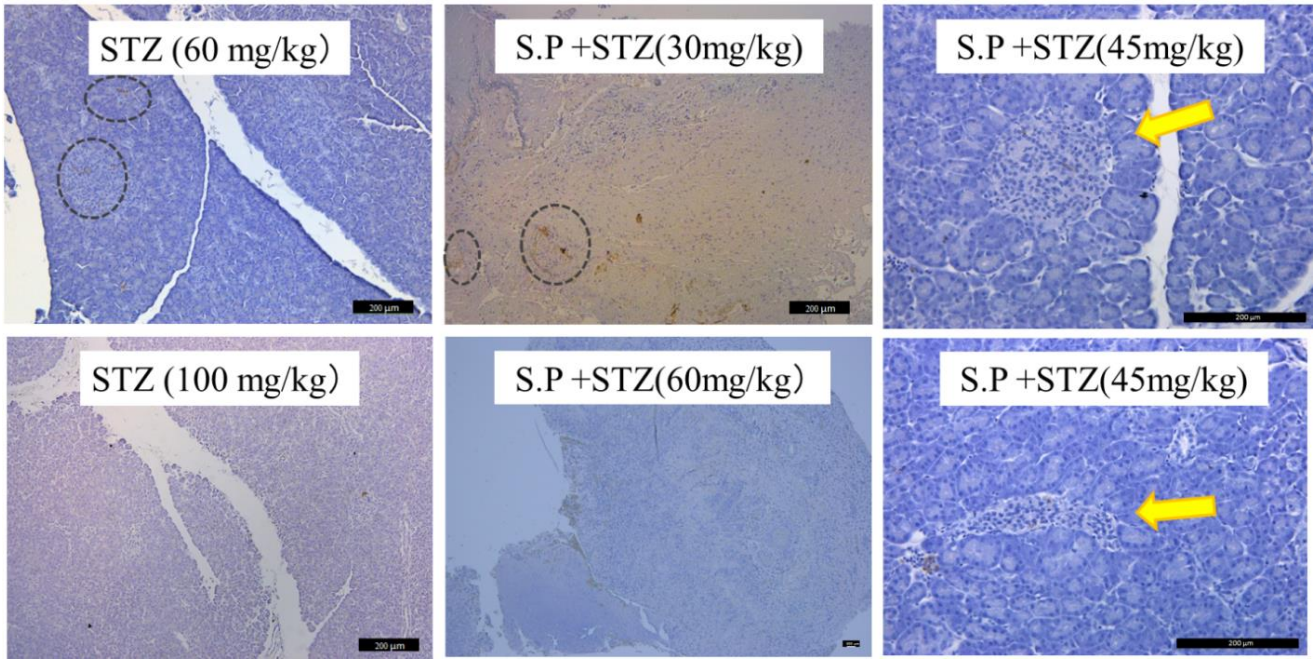

Figure 2 Anti-insulin immunohistochemical staining of the pancreas obtained at euthanasia. Arrows and dashed circles: islet regions. Bar: 200 µm. STP, subtotal pancreatectomy; STZ, Streptozotocin.

3.3 General Condition and Postoperative Management

The extent of weight loss was comparable in both the models (Table 4). The activity scores were relatively higher in the STP/LD-STZ model than in the STZ-only model. Furthermore, one monkey from the STZ group required euthanasia due to weakness. The infusion of 50% glucose was required to prevent the development of hypoglycemic episodes in the STZ-only model but was unnecessary in the STP/LD-STZ model. In the STZ-only model, periocular edema was observed in all the animals with disturbed feeding habits (despite an earlier start to oral feeding).

3.4 Toxicity

The baseline ranges for serum GOT, GPT, BUN, and creatinine in the present cohort were 10–71 IU/dL, 10–69 IU/dL, 13–34 mg/dL, and 0.2–0.8 mg/dL, respectively. After induction of diabetes, evident hepatotoxicity was observed in the STZ-only animals that were given a dose of 100 mg/kg STZ (n=3). Therefore, hepatoprotective medications were administered in the subsequent STZ-only monkeys (Table 4). Thereafter, comparable hepatic functions were observed in the STZ-only and STP/LD-STZ models, although hepatoprotective medications were not used in the latter. In contrast, the renal functions were relatively better in the STZ-only model (Table 4).

Table 4 Biochemical profile, weight changes, and activity score of the study monkeys.

Model type	STZ (mg/kg)	Age (Y)	Hepato-protect. Rx※	Day 6–9 after induction (Baseline between brackets)				Weight loss Kg (%)***	Hepato - toxicity Score	Nephro - toxicity Score	Activity score**
				GOT (IU/L)	GPT (IU/L)	BUN (mg/dL)	Cre (mg/dL)				
STZ only	60	3.7	+	11 (14)	71 (59)	19 (18)	0.4 (0.7)	0.25 (7.2)	1	1	4.0
STZ only*	100	4.4	-	629 (71)	554 (61)	54 (34)	0.8 (0.5)		3	2	
STZ only*	100	5.0	-	223 (62)	289 (69)	25 (15)	0.2 (0.3)		2	2	
STZ only*	100	4.7	-	480 (10)	287 (27)	26 (17)	0.4 (0.4)		3	2	
STZ only	100	4.5	+	36 (23)	121 (32)	25 (15)	0.7 (0.3)	0.44 (13.4)	2	2	4.8
STZ only	100	4.7	+	52 (13)	123 (57)	14 (14)	0.4 (0.4)	0.36 (11.2)	2	0	5.0
STZ only	100	3.6	+	213 (49)	137 (49)	29 (13)	0.6 (0.3)	0.36 (11.3)	2	2	2.2 (E)
STZ only	100	3.2	+	10 (10)	18 (31)	14 (16)	0.3 (0.2)	0.22 (7.3)	0	1	4.1
STZ only	100	3.6	+	12 (10)	67 (33)	18 (15)	0.7 (0.5)	0.52 (14.7)	1	1	4.5
STZ only	100	3.7	+	13 (14)	66 (31)	19 (17)	0.7 (0.5)	0.47 (14.2)	1	1	4.9
STZ + STP	30	6.1	-	172 (10)	93 (10)	59 (28)	0.7 (0.3)	0.35 (7.9)	3	2	4.9
STZ + STP	45	4.9	-	19 (10)	17 (10)	32 (24)	1.3 (0.8)	0.81 (13.2)	1	2	4.0
STZ + STP	45	4.9	-	13 (10)	43 (11)	36 (15)	1.3 (0.7)	1.04 (14.8)	2	2	3.9
STZ + STP	60	5.4	-	25 (10)	93 (18)	44 (23)	0.3 (0.2)	0.15 (3.7)	3	2	5.0

BUN, blood urea nitrogen; GOT, glutamine-ornithine-transaminase; GPT, glutamine-pyruvate-transaminase; STP, subtotal pancreatectomy; STZ, streptozotocin.

(E), Euthanasia on day 8 due to weakness.

※ Hepatoprotective medications: potent neo-minophagen C and glycyrrhizic acid

* Insulin administration protocol was not yet standardized; weight and activity omitted.

** Average activity score from day 1 to day 8.

*** Weight was measured before induction and at day 8–15 post-induction.

4. Discussion

NHP diabetic models are essential for preclinical studies involving pancreatic islet transplantation and anti-diabetic therapeutics. Therefore, the standardization of a simple, reproducible, and efficient model is necessary for experimentation. NHP used for research on diabetes include baboons and macaques. Macaques (rhesus and cynomolgus monkeys) have advantages with regards to size, maintenance, physiology, immunology, and availability over baboons [6, 7].

Diabetes in macaques can either be spontaneous [8, 9] or induced (surgically, chemically, or through gene transfection). Transgenic diabetic models have not yet been developed despite rigorous ongoing research [10]. Therefore, the existing standard methods involve TP and STZ injection. TP is a straight-forward and efficient method for the induction of permanent IDDM but requires meticulous surgery and close monitoring during the intra- and post-operative period. The loss of the exocrine pancreas and all the islet-secreted hormones further complicates management [7]. Nevertheless, the excised pancreas can be used for islet isolation. On the other hand, STZ injection is a less-invasive method that is widely used for the induction of IDDM in NHP [11]. However, the toxin is taken up by all the cells that express GLUT–2, apart from islet β cells. As these cells include hepatocytes and renal tubular cells, the toxin induces hepatotoxicity and nephrotoxicity, especially at higher doses. Although the optimal dose and dose-calculation technique have been debated [12, 13], studies commonly use a higher dose (80–150 mg/kg body weight) and associate it with frequent side-effects (vomiting and severe hypoglycemia), serious complications, and mortality [1, 2, 14, 15]. In order to limit toxicity, several measures have been recommended [5, 7, 16], such as (1) use of clinical-grade STZ (Zanosar©, in the range of 125–150 mg/kg) having higher purity and consistency, (2) continuous fluid administration, (3) intensive and careful monitoring for adverse events, especially if cyclosporin is used as an immunosuppressant [12], and (4) selecting monkeys with lower girth to height ratio. All these measures, except for the last point (neglected for comparative purposes), were applied in the STZ-injection group of the present study. An injection of STZ into the celiac artery after embolization of hepatic and gastric arteries was also reported, which allowed for lowering the dose (50–70 mg/kg) and limited the hepatotoxicity and nephrotoxicity [17].

The purpose of this approach was to overcome the major shortcomings of the existing diabetic primate models, namely the TP and STZ injection models, and to simplify the necessary care during induction and the follow-up period. The surgical procedure for the present model was technically simple, well-tolerated, and unlike TP, was not time-consuming [3] or associated with a high morbidity rate [2]. The high costs associated with obtaining and maintaining primate models render this an important factor to consider. This approach also resolved several issues that could complicate the post-induction period. For example, the remaining portion of the pancreas was sufficient to supply the digestive enzymes in our new model, circumventing the requirement of exogenous supplementation, which was in contrast to the TP model [3]. Furthermore, the absence of hypoglycemic episodes and relatively better general condition compared to the STZ-only model, as a result of reduced STZ dosage, facilitated smooth maintenance during the post-induction period without the need for frequent interventions (e.g., prophylactic glucose infusion and hepatoprotective medications). Although the refinement of the STZ injection protocol in a large cohort revealed that a majority of biochemical hepatic and renal toxicities were mild, 20/53 and

28/53 animals developed ≥ score 2 nephrotoxicity and hepatotoxicity, respectively [5]. Although some of these adverse events were reported to be reversible, [5] management of such events required multiple interventions and sometimes intensive care [5]. Furthermore, although the present study used pharmacological grade STZ and sufficient hydration through a central venous line, it was necessary to administer hepatoprotective medications to prevent hepatotoxicity (Table 4). Another advantage of STP/LD-STZ over the STZ-only model was the availability of the excised pancreas for islet isolation (data not shown) for either autologous or allogeneic transplantation, thus reducing the number of monkeys required.

An STP/LD-STZ model was previously reported in rhesus monkeys [2, 18], and recently in cynomolgus monkeys as well [19]. However, a comparison of different dosages of STZ in cynomolgus monkeys with functional and morphological correlation was essential to minimize the utilized dosage. To the best of our knowledge, this study is the first to perform such a comparison. Jin *et al.* [2] demonstrated that combining STP with LD-STZ (15 mg/kg) was effective in inducing IDDM in rhesus monkeys. This is in contrast to our findings, which indicated that 30 mg/kg STZ was not completely effective in inducing IDDM in cynomolgus monkeys. Furthermore, our model was compared to the refined STZ injection model.

Some of the major concerns regarding the present approach involve the possible development of leakage leading to pancreatic fistula as a result of STP and the probability of regeneration of the remaining pancreas over time. However, we did not encounter leakage in any of the studied animals. On the other hand, regeneration can occur in two scenarios: 1) regeneration of pancreatic islets after STZ injection and 2) pancreatic regeneration after STP. Regarding the first scenario, a study on vervet monkeys showed no evidence of β-cell regeneration after STZ injection [20]. A study on cynomolgus monkeys did show evidence of endogenous C-peptide and insulin-positive cells in 2/11 monkeys after 75 days [21], although this regeneration occurred after islet transplantation that was followed by graft failure. Regarding the second scenario, in our knowledge, relevant long-term data for cynomolgus monkeys are not available. Data from human studies showed no increase in pancreatic volume after STP during a follow-up of 247 ±160 days, as demonstrated by computerized tomography [22]. The study also reported no histological evidence of β-cell proliferation, ruling out the possibility of regeneration in this setting [22]. It should also be noted that if the present model is to be used for tolerance induction studies, performing the procedure without splenectomy is worth considering.

The STP/LD-STZ model showed relatively higher renal toxicity than the STZ-only model, possibly due to inadequate hydration as a result of using a peripheral line in the present model instead of a central venous line, as in the STZ-only model. The objective behind using a peripheral line was to further simplify the procedure for the maintenance of the STP/LD-STZ model. However, the interruption of flow through the peripheral line may have resulted in dehydration and subsequent adverse renal effects in our STP/LD-STZ monkeys. Therefore, we must highlight the importance of proper hydration and patent venous access in the present model, although early oral fluid intake may be considered as a possible alternative.

In conclusion, considering the limited number of animals per group, our findings showed that the STP/LD-STZ model was technically simpler yet effective for the induction of diabetes in NHP models, resulting in a smooth post-induction period and excellent general condition, without the need for repeated interventions or intensive care. At the same time, the present model facilitated the utilization of the excised pancreas for isolation of islets. Both 60- and 45-mg/kg STZ doses

were found to be functionally and morphologically effective, although a lower dose was preferable to minimize toxicity. A larger number of test monkeys and improvement in the hydration approach are required to further standardize this model.

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Author Contributions

Ibrahim Fathi participated in the research design, the performance of the research, and the writing of the paper; Takehiro Imura participated in the performance of the research and the analyses of the experimental data; Kozue Imura participated in the performance of the research; Megumi Goto participated in the performance of the research and the analyses of the experimental data; Yasuhiro Igarashi participated in the performance of the research; Akiko Inagaki participated in the performance of the research; Makiko Kikkawa participated in the performance of the research; Fumiko Ono participated in the performance of the research; Hiroo Iwata participated in the writing of the paper; Yohichi Yasunami participated in the writing of the paper; Masafumi Goto participated in the research design, the performance of the research, and the writing of the paper.

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Competing Interests

The authors declare no conflicts of interest in association with the present study.

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