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Diabetes induction and pancreatic transplantation in the cynomolgus monkey: methodological considerations

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Abstract. The aim of this study was to develop a model for pancreatic transplantation in the primate in order to test a new immunosuppressive drug. Initially, streptozotocin was used to induce insulin-dependent diabetes mellitus, but it was found to be ineffective and associated with a high morbidity. Furthermore, streptozotocin-induced insulin-dependent diabetes mellitus did not always persist, thus invalidating the evaluation of pancreatic graft function. Therefore, total pancreatectomy was introduced and combined with the pancreatic allotransplantation as a single procedure. Enteric diversion of the pancreatic juice was chosen since this avoids exocrine pancreatic insufficiency and facilitates the oral administration of the test drug. Intra-arterial monitoring of blood pressure and blood gases during the operation and avoidance of hypothermia in the animal were found to be the most important factors contributing to a successful outcome from the operative procedure.

Key words: Pancreatic transplantation, in the monkey – Diabetes induction, in the monkey – Streptozotocin, in the monkey

Organ transplantation in non-human primates is a logical intermediate model for investigating new immunosuppressive drugs before embarking on clinical trials. The fact that certain organs may be rejected more strongly than others makes it desirable to test a given drug with several organs, such as the kidney, liver, heart and pancreas. Also, possible side effects of the drug may affect the various transplanted organs differently, a fact that might have important clinical implications.

Recently, we set out to investigate the efficacy of a new immunosuppressive drug, FK 506, for pancreatic transplantation in the cynomolgus monkey. It soon became apparent that several aspects of the model had to be worked out before the actual experiments could begin. Thus, an effective and reliable method for the induction of diabetes in this primate model had to be found. In our experiments, streptozotocin (STZ) treatment was unreliable, so we reverted to total pancreatectomy. Pancreaticoduodenal transplantation, with exocrine drainage to the recipient small bowel, was used to provide physiological reconstruction following the pancreatectomy. The protocols used initially for anaesthesia and intraoperative care were found to be unsatisfactory, but following the modification of several details, it was possible to achieve a satisfactory operative survival rate. It resulted in a reliable, effective and repeatable procedure.

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Material and methods

Animals

Cynomolgus monkeys *(Macaca fascicularis)* with an estimated mean age of 5 years and a mean weight of 3.8 kg (range 1.7–9.3 kg) were used. The monkeys were residents of three separately bred monkey colonies and had been involved in either behavioural studies or in programmes for the induction of antisera. Consent was obtained from the ethics committee for animal experimentation of the University of Limburg prior to the experiments. The animals underwent preoperative blood sampling and an intravenous glucose tolerance test (IVGTT) to exclude the presence of diabetes mellitus or other pre-existing abnormalities. Glucose tolerance was assessed after an overnight fast with a single intravenous bolus injection of 0.5 g/kg glucose. The breeding history guaranteed that donors and recipients were not closely related. Before transplantation, a donor-recipient red blood cell cross-match was performed. Transplantation was only performed between cross-match-negative pairs. The heavier animal from a given pair was selected as the graft recipient. Normal feeding consisted of standard primate pellets (Hope Farm, Woerden, The Netherlands) and fresh fruit. All animals had access to water ad libitum. On the day of surgery, the animals were fasted.

The study comprised the following four groups of animals:

1. Seven monkeys which were injected with STZ with the aim of producing insulin-dependent diabetes mellitus (STZ-group).

2. Three monkeys which underwent total pancreatectomy in order to induce insulin-dependent diabetes mellitus (pancreatectomy group).

3. Six monkeys which received pancreaticoduodenal allografts followed by total pancreatectomy (initial transplant group).

4. Eleven monkeys which received pancreaticoduodenal allografts followed by total pancreatectomy, several adjustments having been made in the management protocol (final transplant group).

In addition there were 17 monkeys that served as donors for the recipients in the transplant groups.

Induction of diabetes using streptozotocin

STZ was a gift from the Upjohn Co, Sweden and The Netherlands. STZ powder was stored at 4°C and protected from light. The monkeys were initially treated with intravenous STZ 50 mg/kg dissolved in phosphate buffer (4°C) pH 5.4 immediately prior to injection. All animals vomited shortly after the injection. If the first dose was ineffective, some animals were given additional doses of STZ as described below. Monitoring of blood glucose and assessment of the need for rehydration were performed twice daily. Rehydration was usually performed on each occasion with 50–60 ml Ringers lactate. If the animals became hypoglycaemic due to the release of insulin from the dying islets, a glucose infusion was commenced. A fasting blood glucose of 16 mmol/l (approximately 300 mg/dl) or more after STZ treatment was considered to indicate that insulin-dependent diabetes mellitus (IDDM) had been induced [16, 17], and insulin therapy was instituted. The treatment was commenced with 2 IU/day of long-acting insulin (Monotard) and then individually tailored using both long- and short-acting insulin (Actrapid). A fasting blood glucose of less than 6 mmol/l was regarded as normal.

Induction of diabetes using total pancreatectomy

The technique used for total pancreatectomy is based on the technique used by Leow and Gray in Oxford, UK. A brief summary is given here. First, the greater omentum is resected in order to prevent development of adhesions postoperatively. Then the transverse mesocolon is transected. The peritoneum is incised along the splenic artery and vein. All the small branches between the splenic vessels and the pancreas can then be identified, ligated and divided. The spleen is left in situ. The pancreatic tail is mobilized until the anterior surface of the superior mesenteric vein is seen (Fig. 1). Only branches truly entering the pancreas should be ligated. Kocherization of the duodenum and mobilization of the right colonic flexure expose the dorsal surface of the pancreas from the right side. The duodenum and the head of the pancreas are freed from the colon. Cranially, the kocherization is extended by opening the retroperitoneum up to the common bile duct and continuing along it. The portion of the peritoneum covering the pancreas, between the duodenal branches of the pancreaticoduodenal arcade, is cut close to the duodenum. Using two sharp microvascular forceps, the pancreaticoduodenal arcades on the anterior and posterior sides of the duodenum, together with their duodenal branches, are separated from the pancreatic surface and preserved. Note that the arcades are sometimes covered superficially by pancreatic tissue. However, these vessels must be preserved. Only branches that truly support the pancreas can be sacrificed and this is done by tearing them off as far away from the arcade as possible. The two pancreatic ducts are identified, ligated and divided. The portion of the pancreas that is connected to the duodenum is now easily separated from the duodenum by blunt dissection. With the vasculature divided, the head of the pancreas can then be removed in one or more pieces, preserving the vessels to the duodenum (Fig. 2).

It is most important to use two microvascular forceps in the separation of the pancreatic tissue from the vessels and the duodenum. Loupes are used to obtain magnification during resection of the pancreatic head.

Anaesthesia and pre- and postoperative care

All handling of the animals is performed wearing gloves. The animals are anaesthetized using ketamine hydrochloride for induction, and halothane and nitrous oxide for maintenance. Ketamine hydrochloride is given intramuscularly at a dose of 5–10 mg/kg body weight. This drug allows sufficient time for blood sampling, rehydration (intravenously and/or subcutaneously), weighing and transport from the cage to the operation room. Full anaesthesia is given for the IVGTT and for abdominal surgery. An endotracheal tube is used for continuous delivery of oxygen, nitrous oxide and halothane. A heated mattress is used on the operating table. The rectal temperature is monitored during surgery with an anal probe and the heart rate is monitored with an ECG. Surgery is performed under sterile conditions by masked, gowned and gloved surgeons.

Donor monkeys have a peripheral venous line installed and are given Ringers lactate 15–20 ml/kg/per hour. A Pulmomat (19.1, Drägerwerke, Lübeck, FRG) is used for ventilation. This ventilator is in routine use in the laboratory and was originally intended for large animals.

In the initial transplant group the recipient monkeys were hydrated through a peripheral venous line (usually in the lower limbs), and were ventilated using the Pulmomat ventilator. The operating room temperature was approximately 18°C, and no arterial line was used. For the final transplant group, the following modifications were made: (a) a central venous line was inserted through the left cephalic vein; (b) an arterial line was inserted in the left brachial artery; (c) glucose was added to the Ringers lactate to obtain a 1% solution which was infused as soon as the pancreatic graft was revascularized until most of the total pancreatectomy was completed; (d) an infant ventilator (Loosco Amsterdam Mk 2, Hoek Loos) was used instead of the Pulmomat; and (e) the room temperature was kept at 30°–32°C.

All recipients were given intraoperative and postoperative prophylactic antibiotic cover consisting of 250 mg of ampicillin and 250 mg of cefotaxime i. v. twice daily on days 0, 1 and 2. Rehydration was performed using a total of 30 ml/kg Ringers lactate given i. v. and s. c. in equal volumes on the evening of day 0 and twice daily on days 1 and 2. Oral fluid intake was commenced from day 3 onwards. Oral feeding started on day 4. Heparin was given in two daily doses of 500 IU s. c. on days 0, 1, 2 and 3, and fentanyl was given i. m. when required for pain relief.

During the first postoperative week, the room where the animals were housed in separate cages was heated to 28°C. Subsequently it was maintained at 22°C. Blood glucose was measured daily for animals in the STZ and pancreatectomy groups and three times per week in the transplanted animals. According to the protocol, transplanted animals were sacrificed if the blood glucose exceeded 40 mmol/l on 2 consecutive days.

The donor operation

The animal is placed in a supine position on a heated mattress and the limbs are tied to the four corners. A peripheral i. v. line is placed for continous infusion of lactated Ringers solution. A midline laparotomy incision is made from the xiphoid process down to the pubic bone. The incision is extended at the umbilicus with a transverse incision through the rectus muscles to give further exposure. The four corners of the wound are turned over and fastened with stay sutures. The aorta is then dissected free at its bifurcation for a distance of 4 cm and ties are prepared for securing the cannula used for the aortic wash-out. The caval vein is prepared in the same fashion. After mobilization of the left lateral segment of the liver, the aorta is identified at the diaphragmatic hiatus and dissected free for a distance of 2 cm. The aorta is most easily entered from the left through the diaphragm. A Kocher's manoeuvre is performed to enable exposure of the dorsal surface of the pancreas. The right colonic flexure is completely mobilized until the inferior mesenteric vein is exposed. This vein can then be ligated and divided. The hepatic artery is identified

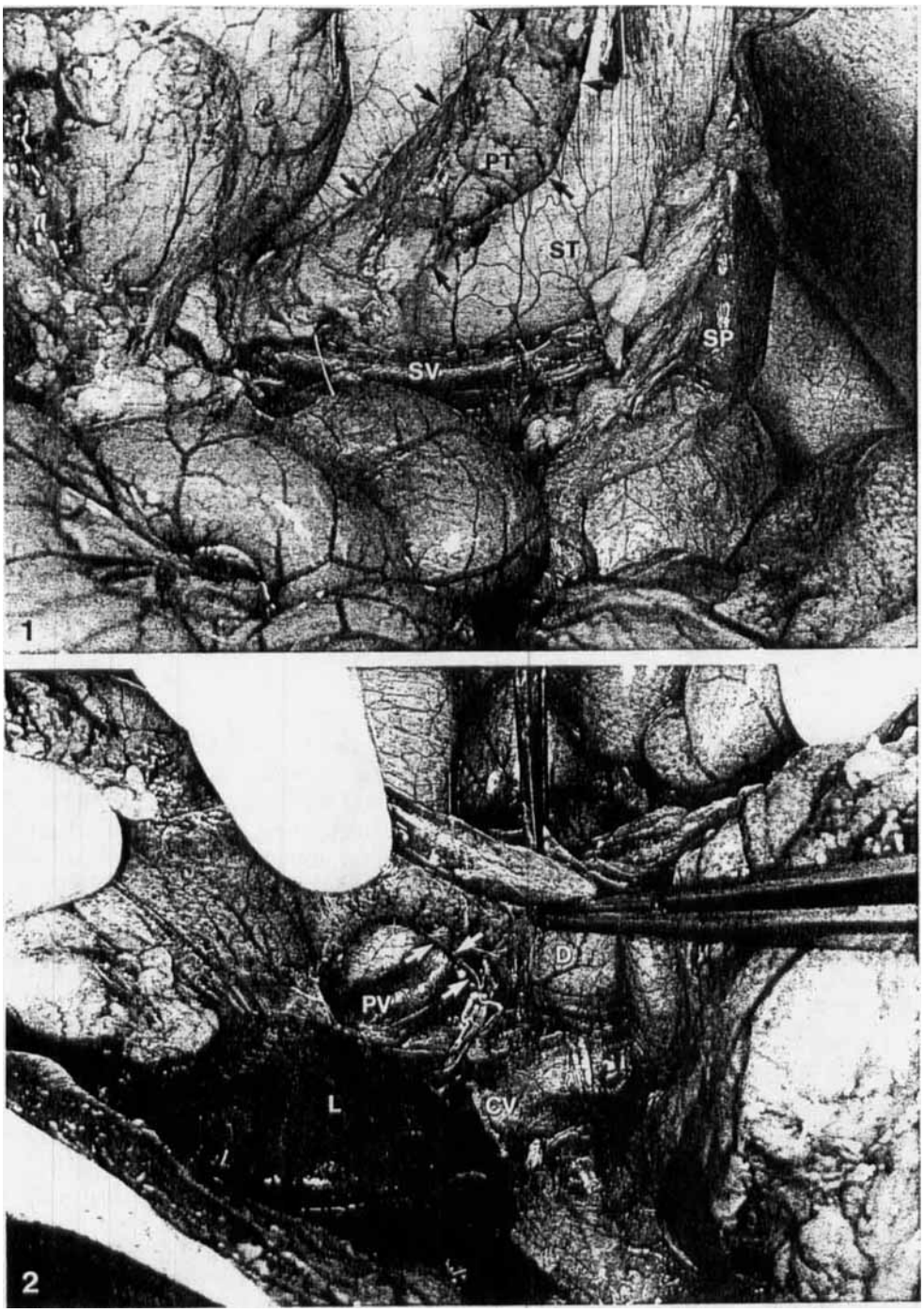

Fig. 1. Total pancreatectomy. The pancreatic tail *(PT)* is mobilized and lifted. The spleen is preserved along with its vasculature. *SV* Splenic vein; *ST* stomach; *SP* spleen

Fig. 2. The total pancreatectomy is completed with the resection of the pancreatic head. The pancreaticoduodenal arcade which is preserved is indicated by the arrows. *L* Liver; *PV* portal vein; *CV* caval vein; *D* duodenum

in the hepatoduodenal ligament and ligated distally to the gastroduodenal artery. The common bile duct is identified, ligated and divided. The portal vein is dissected to its bifurcation, cleaned of connective tissue, and 7-0 prolene placed in the vessel wall to indicate the cranial medial (left) corner of the vein. This tie will be used subsequently to prevent torsion of the portal vein when performing the portocaval anastomosis in the recipient. The lesser omentum is divided after dissecting out the hepatic hilum. Next the greater omentum is opened and the cranial rim of the pancreas is dissected free towards the spleen. The short gastric vessels are ligated and divided. The spleen is mobilized and the splenocolic ligament is divided. Care has to be taken to separate the pancreas from the adrenal gland. The tail of the pancreas and the spleen are mobilized from the left until the coeliac trunk and the superior mesenteric artery (SMA) are seen. Their origins from the aorta are dissected free from the coeliac plexus and the diaphragm. A vessel loop is positioned around the coeliac trunk and the SMA to ensure safe dissection of the connective tissue and coeliac plexus from the right side. The left gastric artery is ligated and divided close to the stomach. The SMA and SMV distal to the pancreas are identified and encircled but not ligated. The duodenum is then transected with a GIA autosuture machine at the fourth part and at the pylorus. The duodenal segment, the whole pancreas and the spleen have then been completely dissected free and are connected to the rest of the animal by only three structures: the aorta via the SMA and the coeliac trunk, the liver via the portal vein, and the small bowel via the superior mesenteric vessels.

Heparin 2000 IU is injected i. v. The aorta is tied off at the level of the diaphragm, and in situ perfusion and cooling are simultaneously commenced with lactated Ringers solution at 4°C via a cannula intro-

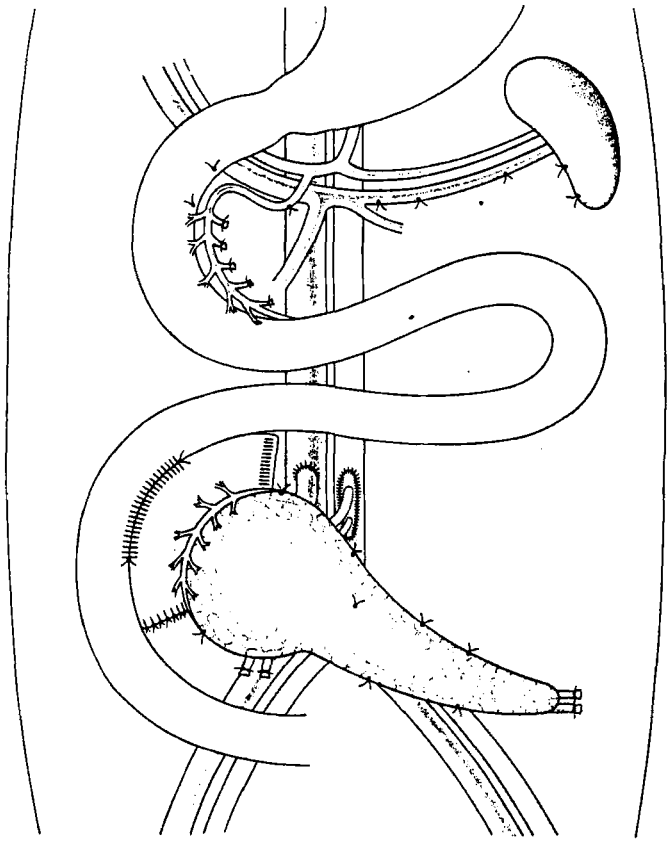

Fig. 3. The aortic patch and the portal vein of the pancreas allograft are anastomosed to the aorta and the inferior vena cava of the recipient. Enteric drainage of the pancreatic juice is via the duodenal segment to the proximal jejunum. The spleen is transplanted along with the pancreas, and initially preserved to increase early portal vein flow. The recipient total pancreatectomy is performed after the transplant procedure. The native spleen is preserved as is the duodenum. The transplanted spleen is removed just before closing the abdomen

duced into the distal aorta. The caval vein is simply transected to allow free drainage of the perfusion fluid. The portal vein is transected obliquely, high in the hilum, to allow the best possible drainage for the graft. By the end of the perfusion, the distal parts of the SMA and SMV are ligated and divided. After approximately 5 min, when 100–200 ml of cold lactated Ringers solution has been infused, the perfusion is stopped. The segment of the aorta containing the coeliac trunk and the SMA is then excised for a distance of approximately 4 cm. The graft is placed in a basin with lactated Ringers solution and sterile ice. The donor animal is sacrificed with an intracardiac injection containing an overdose (2 g) of pentobarbital sodium.

The recipient operation

The anaesthesia and positioning are the same as for the donor animal. The arterial line and the central venous line are positioned and the infusion is commenced. Good exposure is obtained with a midline incision from the xiphoid process to the pubic bone and the placement of a two-blade self-retaining retractor. The distal aorta below the inferior mesenteric artery is dissected free for a distance of 4–5 cm. Branches to the posterior are ligated and divided. The distal caval vein is prepared in the same fashion. The caval vein and the aorta are occluded using vessel loops which encircle the vessels twice. The graft is prepared by opening the aorta on the dorsal aspect and trimming the aortic patch to form an oval shape. The venous anastomosis is made end-to-side between the graft portal vein and the recipient caval vein using 7-0 prolene. Loupes are used to obtain magnification. Care must be taken not to narrow the caval vein. The cross-clamp on the caval vein is released after completing the venous anastomosis and a microvascular clamp is placed on the graft vein. Next, the aorta is cross-clamped and opened longitudinally for approximately 3 cm. The donor aortic patch is sutured to the recipient aorta with 6-0 prolene. The vessels are cleaned with heparinized saline but no heparin is administered systemically at this stage of the procedure. The transplantation proceeds with an antemesenteric enteroenterostomy between the duodenal segment of the graft and the recipient jejunum approximately 20 cm distal to the ligament of Treitz. The anastomosis is performed in two layers using 5-0 Dexon. The distal duodenum, which was previously transected with the autosuture machine, is inverted using a few single sutures. The donor spleen is left in place while total pancreatectomy is performed. Finally, the donor splenectomy is performed. The graft is then positioned so that the arteries and the vein are not twisted, usually with the tail of the pancreas towards the sigmoid colon (Fig. 3). The abdomen is closed in two layers with running sutures PDS 2-0. The ties are inverted below the skin to prevent the animal from untying the knots.

Results

Induction of diabetes

Following the initial injection of STZ, six of the seven animals became hyperglycaemic. Only three of these animals, however, developed IDDM with blood glucose levels > 16 mmol/l. These three monkeys were placed on insulin treatment. One of the animals died on day 8, probably from diabetic coma, and another died after 3 months, possibly from hypoglycaemia.

The third animal underwent pancreatic transplantation 27 days after STZ treatment. Insulin treatment was discontinued after transplantation. Since the animal was to serve as a control, no immunosuppression was given. The fasting blood glucose level remained normal for 6 days and then rose to a maximum of 17.2 mmol/l. Following this it fell to reach normal values on day 15, and then remained normal. The K-values of the IVGTTs declined and then improved (Fig. 4). After 117 days the animal was sacrificed. Histological examination showed graft rejection with complete obliteration of the vessels. Thus, the normoglycaemia could not be ascribed to a functioning pancreatic graft. Rather, the course suggests that the animal had recovered from STZ treatment.

Among the four animals which did not develop IDDM, one died on day 5 and one on day 6. At autopsy, one had signs of severe pneumonia, but the findings in the other were unremarkable. The two remaining monkeys were given several additional doses of STZ; the doses were 55, 60 and 100 mg/kg, and 55 and 60 mg/kg, respectively. The former of these animals developed IDDM with glucose levels over 16 mmol/l after the third injection of STZ, and insulin had to be given (Fig. 5). This animal was sacrificed in good health while still on insulin after 8 months. In the latter animal, the blood glucose remained unaffected although the K-values on the IVGTTs fell (Fig. 6). This animal was sacrificed on day 26 due to diarrhoea and weight loss. Thus, the overall result with STZ was that IDDM could be induced in four of seven animals (57%). How-

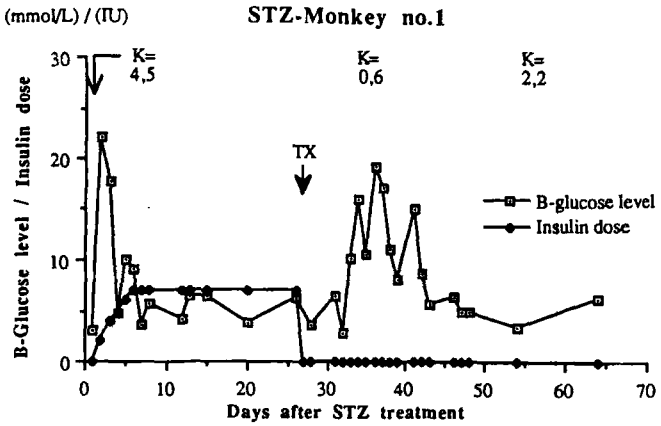

Fig. 4. In this animal STZ (50 mg/kg) induced severe hyperglycaemia, fulfilling the criteria for insulin-dependent diabetes mellitus, and the animal was maintained on insulin until allotransplantation was performed on day 27. From this day onwards no insulin was given. By day 6 post-transplantation, rejection occurred clinically. This was expected since the animal was to serve as a control and no immunosuppression was given. After a period of hyperglycaemia the animal maintained an essentially normal fasting blood glucose level. The K-values of the IVGTTs are given in the figure. The animal was sacrificed 90 days after surgery. Autopsy confirmed severe graft rejection. The course suggested that the animal had recovered from STZ treatment

ever, two of these animals died early, and one presumably recovered from the STZ treatment.

The effect of total pancreatectomy was studied in three animals which were not transplanted. In these animals enzyme substitution was given in the diet (Combizym, Will-Pharma, The Netherlands) but insulin therapy was not instituted. All animals recovered from the operative procedure and all developed hyperglycaemia from the first postoperative day. Two of the three animals died on days 4 and 5. The peak postoperative fasting blood glucose levels in these animals were 17.0 and 16.2 mmol/l, respectively. The third animal was sacrificed when comatose on day 12 after the total pancreatectomy. In this animal the fasting blood glucose exceeded 30 mmol/l. Mean K-value of the IVGTTs fell from 3.3 preoperatively to < 0.2 after the total pancreatectomy.

Total pancreatectomy was also used to induce IDDM in 13 animals subsequently undergoing pancreatic transplantation. Three animals, which were not given immunosuppression and survived more than 24 h, were used to study the efficacy of total pancreatectomy to induce IDDM, since their grafts would be rejected eventually. All developed a fasting blood glucose level of > 16 mmol/l within the first 2 weeks after transplantation, presumably when the grafts were rejecting. One of the animals died after 11 days and one after 29 days. One animal was alive for many weeks with a persistent blood glucose level above 30 mmol/l. This animal was sacrificed on day 64 post-transplantation when the blood glucose level exceeded 40 mmol/l and when weight loss had become severe. There were no surgical complications related to the total pancreatectomy procedure.

Operative survival in the transplant model

In the initial transplant group the perioperative mortality was prohibitive. Out of six transplanted animals, only one recipient survived. This animal was much larger than average with a weight of 9.3 kg. The typical intraoperative findings were a heart rate above 180/min and poor peripheral perfusion in the absence of bleeding. The condition did not respond to i. v. fluid administration. The intraoperative blood glucose was not followed routinely in this group but was found to be extremely low (< 1 mmol/l) in two animals after reperfusion of the graft and on manipulation of the pancreatic tail during total pancreatectomy. Immediately after surgery, all animals had a rectal temperature of 30.5°–33.2°C. One monkey died on the operating table due to a ventricular fibrillation immediately prior to extubation and after the surgery had been completed. The rectal temperature in this monkey was 30.5°C. Four of the five non-survivors were extubated postoperatively. In spite of acceptable spontaneous respiration, only one regained consciousness, and these four monkeys died within 12 h postoperatively.

There were 11 recipients in the final transplant group. A central venous line was used to allow fluid infusion during cross-clamping of the large vessels. Intra-arterial monitoring of the blood pressure made it possible to maintain anaesthesia with the lowest possible concentration of halothane (1%–0.4%). Arterial blood gas analyses were used to guide ventilation rates and volumes, and to indicate when bicarbonate was needed for the correction of

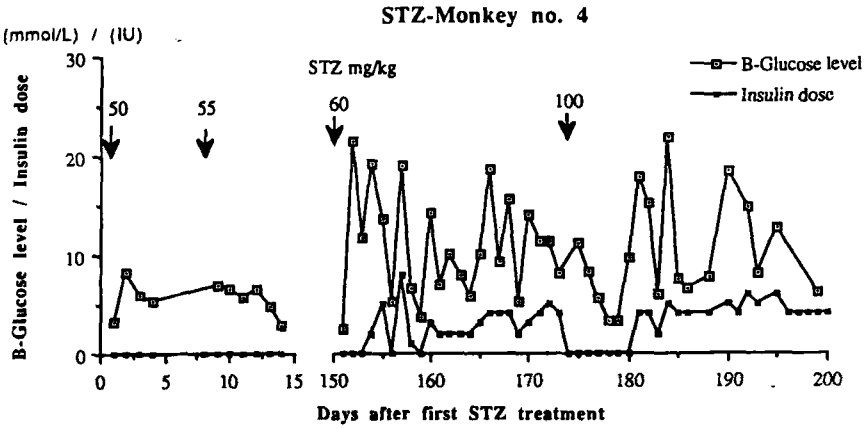

Fig. 5. The first two doses of STZ did not induce IDDM in this animal. After the third dose hyperglycaemia > 16 mmol/l occurred. A fourth dose of STZ was given since the low insulin requirements suggested that true IDDM was not present. However, this did not affect the need for exogenous insulin

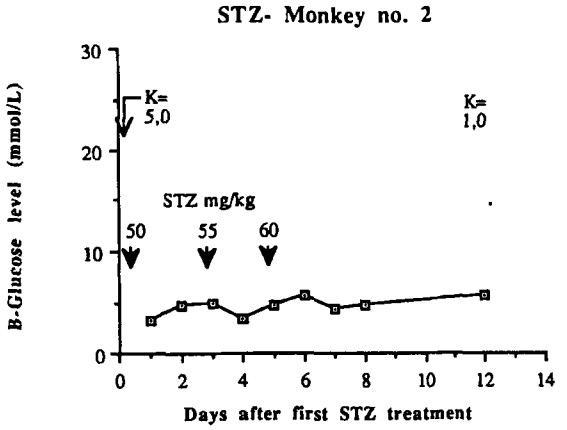

Fig.6. The animal was treated three times with STZ without developing fasting hyperglycaemia. However, the K-values of the IVGTTs fell after STZ treatment

metabolic acidosis, which was usually seen during the latter part of the operation. In this group, intraoperative monitoring of blood glucose was performed every 2 h and solutions containing low amounts of glucose were given from the moment the graft was reperfused until the blood glucose level indicated that this was no longer needed, usually during the latter part of the total pancreatectomy. The room was heated to 30°–32°C. All monkeys were extubated, and nine of the 11 animals regained consciousness immediately after surgery. Two animals died during the first postoperative night. In these animals, the arterial line had failed to function properly, thus hampering adequate intraoperative monitoring. Two animals were lost due to vascular complications in the grafts, and seven animals lived beyond 10 days.

The seven technically successful animals all maintained a normal blood glucose level after transplantation until rejection or drug toxicity occurred, or until they were sacrificed at 90 days. Animals that did not experience rejection or drug toxicity maintained a stable body weight and did not exhibit any signs of exocrine insufficiency such as diarrhoea. Blood transfusions were not given during surgery. No monkeys were lost in either group due to intraoperative blood loss.

Discussion

Cytotoxic drugs such as alloxan [2, 5] and STZ [5, 8–11, 13, 15, 17] have usually been used for the induction of diabetes mellitus in non-human primates. STZ is reported to induce insulin-dependent diabetes mellitus in 14%–55% of treated Rhesus monkeys, while the remainder usually have impaired glucose metabolism of varying degrees [9, 10, 13, 15, 17]. In one report more than 80% of cynomolgus monkeys developed IDDM after a single dose of STZ [16]. We were able to induce hyperglycaemia > 16 mmol/l, thus fulfilling the criteria for IDDM, in four out of seven treated cynomolgus monkeys (57%). In one of these animals, however, true IDDM was not present, or only present temporarily, since it was later possible to discontinue the insulin. In another animal the presence of IDDM may be questioned, since it was possible to reduce the dose to 4 IU/day despite four treatments with STZ (Fig.5). Unfortunately, insulin was not completely tapered off before this animal was sacrificed 232 days after the first treatment with STZ, and thus it is not known whether ketoacidosis would have occurred without insulin treatment. Even if one or both of the early deaths that occurred in the animals without IDDM could have been prevented with more intensive management, and if they had developed IDDM (IDDM was not observed before death occurred), the method appears to be unpredictable, resulting in a number of monkeys which have impaired glucose metabolism but which are not useful for the study of a pancreas transplant model. In contrast to the findings of Takimoto et al. [17] who used rhesus monkeys, we did not find that fasting blood glucose levels above 16 mmol/l within 5 days after STZ treatment always indicated persistent IDDM. This may not be surprising since an apparent reversibility of islet B-cell damage in rats that received lower doses of STZ has been reported [1, 12].

Recovery from STZ-induced hyperglycaemia, with normoglycaemia, has been reported in a non-insulin-dependent rhesus monkey as well [18]. The course in one of our animals is consistent with the occurrence of recovery from STZ-induced IDDM. One explanation for the relatively low incidence of true IDDM in our STZ-treated animals could be that cynomolgus monkeys are more resistant to STZ treatment than rhesus monkeys. However, this is less likely, since more than half of our animals and almost all of the STZ-treated cynomolgus monkeys used by Stegall et al. [16] (who used the same criteria for IDDM) fulfilled the criteria for IDDM. An alternative explanation may be that we used younger animals (mean age in the STZ-group was 6.1 years) than Takimoto et al., who found that their subgroup of younger animals (mean age 6.0 years compared with 8.5 years) were more resistant to STZ-induced B-cell cytotoxicity [17].

Apart from the fact that STZ was not an effective or persistent inducer of true IDDM in our animals, STZ and alloxan have side effects such as nephrotoxicity, hepatotoxicity and (for STZ) carcinogenesis that can make it difficult or even impossible to interpret the side effects of a test drug, such as an immunosuppressive agent [7, 14]. Therefore, we turned to the use of pancreatectomy. The use of pancreatectomy to induce diabetes mellitus in non-human primates was described in the baboon by Gillman et al. in 1958 [6]. The technique we used for total pancreatectomy with preservation of the duodenum in cynomolgus monkeys is the technique used by Leow and Gray in Oxford. With this technique, all animals promptly develop IDDM (personal communication) which was also confirmed in the pancreatectomy group in our study. Preoperative handling is easier, and anaesthesia is presumably safer, than would be the case had STZ treatment been employed 2 to 3 weeks prior to transplantation since no insulin is required. Pancreatectomy, in our hands, proved to be a much more reliable method of inducing IDDM in the cynomolgus monkeys than STZ.

In the experiments by Du Toit et al., baboons were subjected to total pancreatectomy in combination with auto-

transplantation of an intraperitoneal segmental pancreatic graft with open duct drainage to the peritoneal cavity [3]. When allografts were used in their model unusually high doses of oral cyclosporin (up to 85 mg/kg) were needed to ensure prolonged graft survival. This was suspected to be due to poor cyclosporin absorption in the pancreatectomized animal, which frequently had malabsorption despite enzyme supplementation in the diet [4]. Furthermore, free duct drainage into the abdominal cavity frequently resulted in pancreatic ascites, which necessitated paracentesis. In our more physiological model, with a whole organ pancreatic graft and with exocrine drainage to the intestine, the risk for malabsorption due to loss of exocrine function is avoided since the graft produces pancreatic juice which is delivered to the proximal part of the bowel. In accordance with the techniques used in humans, systemic venous drainage of the graft was used despite the fact that portal vein drainage should be the most physiological method of endocrine drainage.

However, the combination of pancreatic transplantation and total pancreatectomy results in a long operative procedure (6–7 h). Therefore, there is no leeway for inappropriate depth of anaesthesia. Fluid and electrolyte substitution need to be carefully monitored since fluid losses from the exposed intestine may be substantial. The combination of a long intra-abdominal procedure with extensive fluid replacement and reperfusion of an ice-cold organ further reduces the animal's ability to maintain an adequate body temperature. The importance of these factors in achieving survival in the tiny primates was underestimated in the first series of transplants. The introduction of an arterial line to monitor the blood pressure was the most important advance in the final series of transplants. It was then possible to maintain adequate anaesthesia as well as improve fluid management throughout the operation. Furthermore, it was immediately obvious if too much tension or pressure was applied to the portal vein during the total pancreatectomy since this would always result in a low systemic blood pressure. This could then be corrected at once. The second important change was to increase the operating room temperature to a minimum of 30°C. The risk of developing ventricular fibrillation is significant, since the body temperature can easily approach 30°C even if a heating mattress is used. Furthermore, an early postoperative extubation in combination with a low body temperature, as in the initial transplant group, may result in an inadequate ventilation leading to death.

An alternative to a single long procedure would be to carry out the two operations separately. In one of their models, Du Toit et al. first performed the transplant procedure and then later performed the total pancreatectomy as a second operation [3]. However, the evaluation of pancreatic graft function with this technique is more complex. Furthermore, the combined risk of two short abdominal procedures may not be less than a single long procedure. With the final protocol in our study, we have shown that these two operations can be performed safely as a single procedure even in small primates.

We believe that the total pancreatectomy, in combination with a pancreatic allotransplantation with enteric exocrine diversion, is a physiological and reliable model for preclinical trials of new immunosuppressive drugs.

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