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.

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).
The technique used for total pancreatectomy is based on the tech-
cricatic head.
Loupes are used to obtain magnification during resection of the pan-
rified and this is done by tearing them off as far away from the ar-
sed. All the small branches between the splenic vessels
vascularized until most of the total pancreatectomy was completed;
the dorsal surface of the pancreas. The right colonic flexure is com-
mobilization of the left lateral segment of the liver, the aorta is ident-
ized using both long- and short-acting insulin (Actrapid). A fasting

Induction of diabetes using streptozotocin
STZ was a gift from the Upjohn Co, Sweden and The Nether-
s to 4°C and protected from light. The mon-
ture 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 tai-
loring insulin was given (orally and i.m.) on days 0, 1, 2 and 3, and fentanyl was given i. m. when
required for pain relief.

During the first postoperative week, the control group was admin-
istered 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.

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 continuous infusion of lactated Ringers solution. A midline lapa-
rotomy incision is made from the xiphoid process down to the pubic
bone. The incision is extended at the umbilicus with a transverse inci-
sion 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
ortic wash-out. The caval vein is prepared in the same fashion. After
mobilization of the left lateral segment of the liver, the aorta is iden-
tified at the diaphragmatic hiatus and dissected free for a distance of
2 cm. The aorta is most easily entered from the left through the dia-
aphragm. A Kocher’s manoeuvre is performed to enable exposure of
the dorsal surface of the pancreas. The right colonic flexure is com-
pletely mobilized until the inferior mesenteric vein is exposed. This
vein can then be ligated and divided. The hepatic artery is identified

Induction of diabetes using total pancreatectomy
The technique used for total pancreatectomy is based on the tech-
nique 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 bran-
ches 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
cocherization is extended by opening the retroperitoneum up to the
common bile duct and extending along it. The portion of the peri-
toneum 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 super-
ficially by pancreatic tissue. However, these vessels must be
preserved. Only branches that truly support the pancreas can be sac-
rificed and this is done by tearing them off as far away from the ar-
cade as possible. The two pancreatic ducts are identified, ligated and
divided. The portion of the pancreas that is connected to the duode-
num is now easily separated from the duodenum by blunt dissection.
With the vascular plane divided, the head of the pancreas can then be
removed in one or more pieces, preserving the vessels to the duode-
num (Fig. 2).

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