SUBCUTANEOUS PANCREAS TRANSPLANTATION

AN EXPERIMENTAL STUDY IN RATS AND DOGS

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SUBCUTANEOUS PANCREAS TRANSPLANTATION

AN EXPERIMENTAL STUDY IN RATS AND DOGS

SUBCUTANE PANCREAS TRANSPLANTATIE

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PROEFSCHRIFT

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Aan Karin en Reinier
Aan mijn ouders

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CHAPTER 1
25 YEARS OF PANCREAS TRANSPLANTATION

1.1. INTRODUCTION

Glucose metabolism is regulated by the islets of Langerhans, the endocrine part of the pancreas. In insulin–dependent (type I) diabetes mellitus (IDDM) the insulin–producing beta–cells of the islets are destroyed, which results in major metabolic imbalances. IDDM usually starts in childhood, and reaches its peak incidence around 13 years of age. The estimated incidence in the Netherlands is 11 per 100,000 for 0–19 year–old children and is still increasing. The prevalence is about 4500 for this age–group [1].

In spite of more insight into the etiology of IDDM, the real pathogenesis is still unclear [2][3][4]. It is generally assumed to be evoked by an autoimmune reaction against pancreatic beta–cells, possibly triggered by one or more environmental factors, maybe a virus. Hereditary susceptibility appears to play an important role, as the majority of type–I diabetics express either the major histocompatibility alleles DR3 or DR4, or both. In a patient's family one or more relatives often suffer from diabetes too. Humoral as well as cellular immunological abnormalities have been found at the onset of diabetes mellitus. Immunosuppressive therapy such as Cyclosporine A (CsA) can delay but not prevent the onset of hyperglycemia in newly diagnosed diabetics [5]. As long as the real pathogenesis is not understood, no prophylactic or causal therapies can be developed. This means that only non–causal treatments are available: exogeneous insulin therapy or transplantation of endocrine pancreatic tissue.

Insulin–therapy

Since the isolation of insulin by Banting (1922) [6] insulin–therapy has been highly developed. Although injection is still the main form of administration, new techniques in insulin–delivery devices, imitating the biological system more closely, are being refined; they include continuous subcutaneous infusion and the "artificial pancreas" with a feedback mechanism by continuous glucose monitoring [7][8][9]. The results of latter technique, however, are still unsatisfactory yet due to insulin crystallization and failure of the glucose sensor in course of time [10]. Tight control of glucose metabolism is not only necessary to avoid hypoglycemia and hyperglycemia, but also to avoid or to delay secondary diabetic complications: macrovascular and microvascular lesions, leading to nephropathy,

retinopathy and neuropathy [11][12]. The dilemma of insulin-therapy is not only how to maintain good metabolic control, but also to find a compromise between this tight control and sociopsychological factors affecting the patient. A compromise implies that optimal but not perfect normoglycemia can be achieved, still leading to secondary diabetic complications. In spite of insulin therapy Deckert et al. estimated the mortality rate of type I diabetics to be 2-6 times that of the non-diabetic population, indicating that about 50% die before 35 years suffering from diabetes, with nephropathy and cardiac diseases as the main causes of deaths [13]. Others estimate one-third less life expectancy for the diabetic patient compared to the general population; diabetics would be 25 times more prone to blindness, 17 times more prone to kidney disease, would have five times more gangrene and twice more often heart diseases than non-diabetics [14]. These disturbing figures made physicians aware of the deficiencies of insulin as long-term therapy and made them focus on the most natural and precise form of glucose regulation by a pancreatic tissue transplant.

Pancreatic tissue transplantation

Although the first experiments with non-vascularized pancreas tissue transplantation were described at the end of the last century [15][16], it was not until 1966 that the first human vascularized pancreatic allotransplantation was performed, by Kelly et al. [17]. Although kidney transplantation has shown an increasing success rate [18], transplantation of the pancreas encountered many complications that led to poor graft survival and high mortality, and this subdued the initial enthousiasm about clinical pancreas transplantation [19]. The most experienced group, from the University of Minnesota, reported 14 whole pancreas transplantations between 1966 and 1973, resulting in only four living patients and one functioning graft after one year [20]. Until 1977 64 pancreas transplantations had been performed worldwide, but only two grafts still functioned after one year [21]. In those days much research was done in the laboratories to improve the techniques, but attention was also paid to transplantation of endocrine pancreatic tissue only, aiming at bypassing the exocrine part, which appeared to be the main cause of the problems in vascularized pancreatic transplantation [22].

1.2. TRANSPLANTATION OF ISLETS OF LANGERHANS

In the early 1970s, after a long history of experimental work begun in the 19th century, Ballinger and Lacy were the first to achieve long-term amelioration of diabetes with syngeneicly transplanted isolated islets in rats [23]. Until then the development of islet transplantation had been hindered by the inability to isolate the islets from the exocrine tissue, which affects islet transplantation in several ways. Not only the exocrine enzymes may damage the endocrine tissue [24], but also contamination with lymphoid, ductular, or acinar tissue results in more rapid rejection because of their higher immunogenicity [25].

Technical progress

Microdissection of the pancreas was replaced by digestion using the enzyme complex collagenase [26][27]. Separation of endocrine and exocrine tissue using density gradients, mostly of Ficoll [28], led to the first promising results in islet transplantation.

An insufficient yield of purified islets from one donor, especially in larger animal models and humans, and early rejection of the allotransplants, still remained the major obstacles in this form of pancreas transplantation. Islets were transplanted to different sites such as the spleen by arterial injection or injection in the splenic pulp or the liver by intraportal administration [29]. To bypass the rejection problem more immuno-privileged sites were used, for example the testes, the anterior chamber of the eye, the cerebral ventricles, the thymus, under the renal capsule, or in millipore chambers [30][31][32][33][34]. In general, transplantation via the portal route led to the best functional but not immunological results. With increasing experience isolation techniques improved resulting in a better yield of purified islets [35]. With the further purification of the islets, portal hypertension could be avoided and long-term normoglycemia could be achieved in several experimental models, but the sensitivity to rejection remained an unsolved problem.

Immunomodulation

For many investigators the pancreatic islet tissue has been an attractive target for their immunomodulating experiments. The MHC-class II positive dentritic cell, scattered throughout the tissue, is supposed to be one of the instigators in the rejection process [36]. Therefore, to eliminate these cells several modulation techniques such as low temperature culture or culture at high oxygen concentration, pretreatment with monoclonal antibodies, and gamma or UV-light irradiation, were applied with some

success in rodents but quite less in larger animals [37].

Human islet transplantation

Until the early 1980s some progress in this field of pancreatic tissue transplantation was made in experimental models, but not in humans [38][39][40]. Although islet transplantation did not cause complications, no patients became insulin–independent. These disappointing results urged the investigators and clinicians to continue the vascularized pancreas transplantation program, awaiting better results in islets transplantation. Recently some groups reported success with human pancreatic islet transplants: they observed (temporary) insulin–independence in a few patients [41][42]. Perfection of islet isolation, determination of the correct islet dosage, duration of islet culture, duration of peritransplant insulin therapy, and improvement of immunosuppression contributed to this progress and may further improve the islet transplantation results in humans.

1.3. VASCULARIZED PANCREAS TRANSPLANTATION

Vascularized pancreas transplantation was revived in the 1970s. Much experimental work was done with improving results. Several groups (re)started their clinical programs. Some problems have been solved, but many questions remained and new questions were evoked as listed in Table 1.1. and discussed below.

1.3.1. Procedures for handling the exocrine pancreas

Apart from graft rejection, the exocrine pancreas evokes the major problems in pancreas transplantation, leading to results that compare unfavourably with those of other organs. Complications to the secretory function of the exocrine part may develop pancreatitis, infection, fistulas and graft thrombosis [19][43]. To overcome these complicating factors many methods dealing with the exocrine pancreas have been developed. Generally these techniques can be distinguished into draining and non–draining procedures (Table 1.2.).

Table 1.1. Considerations in vascularized pancreas transplantation

– Procedure concerning the exocrine pancreas
– Whole versus segmental pancreas transplantation
– Vascularization and prevention of thrombosis
– Systemic versus portal venous drainage
– Graft ischemia and preservation
– Immunosuppression
– Monitoring of rejection
– Pancreas with or without kidney transplantation
– Metabolic control after pancreas transplantation and influence on secondary diabetic complications
– Recurrence of the diabetic disease process in the pancreas graft

Table 1.2. Procedures for handling the exocrine pancreas

CLOSED PANCREATIC DUCT:
– duct–ligation
– duct–obliteration
OPEN PANCREATIC DUCT:
– free intraperitoneal drainage
– gastrointestinal drainage
– urinary drainage

1.3.1.1. Duct–ligation

Except for the very first clinical (duct–ligated) pancreas transplant by Kelly et al. [17], the first transplantations by the Minnesota group consisted of pancreaticoduodenal transplants (4 cutaneous duodenostomies and 8 Roux–en–Y duodeno–jejunostomies) [20]. As pointed out before, the results were very disappointing, because of leakage of pancreatic secretions through the duodenal anastomoses and high susceptibility to rejection of the duodenum [19]. Duct–ligation was (re)introduced, having the theoretical advantage of inducing exocrine tissue atrophy while leaving endocrine tissue intact. Enteric anastomoses were not needed and transplantation of the pancreatic tail segment only would be possible. However, acute and chronic inflammatory reactions induced by duct ligation might involve the entire pancreas, including the endocrine part, resulting in recurrence of diabetes [44][45].

Results

Initial experiments with duct–ligation were often complicated by severe autolysis of the graft and peripancreatitis caused by leakage of pancreatic enzymes through the dissected lymph vessels. This occurred in 77% of the transplanted dogs as reported by Bartos et al. [43]. Also Papachristou et al. encountered many complications with in situ duct–ligated canine pancreatic segments and only had 25% maintenance of normoglycemia by pancreatic autografts in spite of normal function at the first postoperative day. They noticed fistulas, edematous grafts with pseudocysts, dilated ducts and fibrosis [46]. More groups had this bad experience in dogs [47][48] and in pigs [49], but some were able to improve the early post–operative results by in situ duct–ligation before transplantation [47] or by postoperative administration of drugs [49]. Good postoperative results after duct–ligated pancreas transplantation in dogs with minimal postoperative complications and good function have also been reported [50][51].

Duct–ligated pancreas transplantation appeared to be a feasible technique in rats; in experienced hands the complications are negligible and a success rate of 90% can be obtained [52].

Long–term function

Long–term endocrine function after duct–ligation has been found to be more or less successful. Early postoperative diminution of endocrine capacity within two months [50][53] and late deterioration after 6 months [44][45], supposedly due to profound fibrosis, have been observed. In contrast, de Gruyl et al. found stable endocrine function (75% of normal values) up to 3 years after in situ duct–ligation in dogs; they also described a good endocrine function 5 years after pancreatic autotransplantation [48][54].

The long–term results in rats are conflicting as well. Some groups observed normal graft function up to 2 years after duct–ligated syngeneic transplantation [52][55], whereas others noticed impaired function after 6 months follow–up and even complete graft failure after 1 year in 5 out of 12 grafts [56]. Bodziony et al. described a remarkable increase of pancreas insulin (per gram tissue) by in situ duct–ligation in rats. In combination with morphological findings, demonstrating regeneration of islet tissue, this may indicate that the rat pancreas is capable to compensate for the damage induced by duct–ligation [57].

Duct–ligation of human grafts

In humans about 10 duct–ligated pancreas grafts have been transplanted so far. Severe complications were ascribed to be related to the surgical technique, however, most transplants failed due to reasons other than damage by duct–ligation [20][58] [59]. Graft survival for more than one year has not been observed [60][61].

The duct–ligation technique was abandoned because of its poor overall results, but also because more promising techniques became available.

1.3.1.2. Duct–obliteration

Complications inherent to exocrine draining procedures forced research groups to innovative experiments. In 1977 Dubernard et al. were the first to introduce the duct–obliteration technique, in which the exocrine duct is occluded with Neoprene, a synthetic latex polymer that hardens under the influence of alkaline pH [62]. As with duct–ligation the procedure has the advantage of being safe, but in contrast to duct–ligation it causes a more chronic, less damaging, inflammation, that might lead to a better maintenance of islet–tissue. Other occluding polymers have also been used such as cyanoacrylate tissue adhesive [63], prolamine [64], polyisoprene [65], and silicone rubber [66].

Results

Using the duct obliteration technique many groups had fewer complications, experimentally as well clinically. Compared to duct–ligation less early postoperative failures were seen [46]. In comparison to the enteric drainage procedures especially infections were less common [67][68]. Duct–obstruction was continued to be associated with problems such as fistulas, peritonitis, abscesses, and acute failures. Many of these, however, were evoked by the location of the graft (subcutaneous or extraperitoneal) or by inexperience with the occlusion technique [51][69][70] [71]. Various adaptations of the operation technique (omentoplasty, intraperitoneal location) and the postoperative care (peritoneal lavage) improved the success rate [72][73][74].

Some groups advocate the "delayed duct–obliteration" technique diverting the exocrine secretions percutaneously for some weeks before occluding the duct. Thus, fistulas occur less frequently. Furthermore, in the period during which the postoperative pancreatitis extinguishes, the exocrine secretions can be used for rejection monitoring [75][76].

Long-term function

The carbohydrate metabolism after duct-obliteration is subject of much discussion. Long-term normoglycemia can generally be achieved, but stimulation tests often reveal impaired function. Although in rats it is possible to have normal glucose tolerance tests for more than one year after duct-obliterated pancreas transplantation [77][78], there are conflicting findings in dogs. Reports about good long-term function [79][80] are outnumbered by those describing impaired graft function or even graft failure within a few months after duct-obliteration [51][70][81]. Studies dealing with this subject have been done by Gooszen [82]. He found a quantative as well as a qualitative loss of islet function in duct-obliterated pancreas grafts in dogs. After 18-24 months he observed a reduction of 50% of islet cells and 70% of insulin secretion of the duct-obliterated left pancreatic lobe as compared to the non-obliterated left lobe. Abnormal K-values and insulin release patterns during intravenous glucose tolerance tests (IVGTT) were already seen after one month and stabilized up to two years postoperatively. Pancreatic polypeptide (PP) secretion after bombesin stimulation was abolished by duct-obliteration of the left lobe, suggesting that the histological changes led to an intrinsic denervation of the islets, whereas PP release still could be activated after enteric stimulation, probably via the hormonal enteropancreatic axis [83]. After disruption of the islets new "pseudo-islets" were observed twelve months after duct-obliteration [82]. These findings suggest that early damage of the graft with distortion of the islet structure and the advancing fibrosis after duct-obliteration reduce the endocrine capacity.

Obliterated grafts show impaired function also in humans. Normal glycosylated hemoglobin A1 (HbA1c) levels are generally found, but 30-45% of the patients show abnormal glucose metabolism during oral glucose tolerance tests [73][84][85], although immunosuppressive therapy may partially be responsible for these results. Other groups reported long-term deteriorating endocrine function in both allografts [86] and autografts [87]. Thus, rejection episodes may further contribute to the degrading process in duct-obliterated grafts, but are not responsible alone.

Because of its safety, duct-obliteration is one of the techniques most widely applied. In the period 1986-1990, 16% of the human grafts were duct-obliterated. According to the International Pancreas Transplant Registry the 1-year graft survival was comparable with that found with the intestinal drainage technique (56% and 52%, respectively), but worse compared to grafts drained to the bladder (65%, $P<0.001$) [88].

1.3.1.3. Free intraperitoneal drainage

Another option, avoiding congestion of the exocrine secretions in the duct, was simply leaving the duct open, draining freely into the peritoneal cavity. Especially in experimental models the peritoneum proved to have a good capacity to resorb pancreatic juice, resulting in a low complication rate [89][90]. The ducts however, quite often sealed off spontaneously after some time, inducing fibrosis of the graft [51][91][92]. Furthermore, in man this technique appeared to be associated with a high technical complication rate, including intractable ascites [93]. These factors contributed to success rates inferior to those obtained in other techniques. Open duct drainage was therefore abandoned.

1.3.1.4. Gastrointestinal drainage

This procedure, which is most physiological, involves diversion of the exocrine secretion to the gastrointestinal tract. In case of a segmental graft this may be done by an end-to-end pancreaticoenteric anastomosis, using a Roux-en-Y loop or by an end-to-side anastomosis, which can be made either to a Roux loop or simply to a bowel loop. If the entire pancreatic graft is used, the anastomosis can be made with a duodenal patch or segment [20][94].

In the first clinical pancreaticoduodenal transplantations the duodenal component was responsible for a high incidence of lethal complications, mainly because intestinal rejection was difficult to control with the immunosuppressives of those days [19]. When cyclosporine became available and surgical experience increased, pancreatic grafts with reduced duodenal segments appeared to be a good alternative again [95][96].

Opening of the intestines has often been complicated by leakage of the anastomoses and by infections [97]. Improvement of the techniques, for example by temporary postoperative percutaneous diversion of the pancreatic secretions, and ample experience reduced the nonimmunological graft failures dramatically [98]. However, using this technique one of the most experienced groups still had 21% graft losses in their most recent series resulting in 38 relaparotomies in 42 patients after combined kidney and pancreas transplantation [99].

Gastric drainage

To avoid enteric bacterial contamination gastric drainage has also been employed, either by telescoping the graft into the stomach [97] or by performing a ductal anastomosis [100]. The additional assumption was that activation of digestive

enzymes might be prevented due to the low pH of the gastric juices. The Cambridge group only has been using this technique routinely, also because they preferred a paratopic position of the pancreas graft with a portal venous drainage [101].

Functional results

Experimental and clinical reports of long-term endocrine function of enteric drained grafts show good results. Glucose tolerance tests were found to be normal or near-normal [102][103] and appeared to be better than with duct-ligated grafts [53] or duct-obliterated grafts; the latter was investigated in a prospective study by the Lyon/Milan group [67].

1.3.1.5. Urinary drainage

Experimental and clinical observations suggest that the acinar tissue is more sensitive to rejection than endocrine cells. Lymphocyte infiltrates in the exocrine pancreas precede those in the endocrine part [58][104][105]. However, exocrine serum parameters do not correlate with the rejection process, and rise in plasma glucose levels has been demonstrated to be a late indicator of pancreas allograft rejection. By the time immunosuppressive treatment is adapted, irreversible damage to the endocrine tissue has been inflicted, resulting in only 30% reversal of rejection [20][106]. Urinary diversion of the graft exocrine secretions has the advantage of allowing exocrine pancreatic function to be assessed directly by measurement of pancreatic enzymes in the urine. This can be performed as long as the pancreas is functioning and not only in the early postoperative weeks as is the case with delayed duct-obliteration or temporary diversion in the enteric drainage technique [106][107].

Technical facts

Urinary drainage was introduced by Gliedman et al. performing a duct-ureter anastomosis [108]. When it appeared that urine amylase might be of importance for early monitoring of rejection, this technique was taken up by others. The pancreatic duct can be anastomosed to the recipient's ureter after nephrectomy [109], but drainage into the bladder as introduced by Sollinger et al. is the technique most frequently used because it has better results, and the kidneys do not have to be sacrificed [110][111][112]. Different techniques have been used: duct-to-bladder anastomosis, mainly in segmental pancreas transplantation [113], and pancreaticoduodenocystostomy in case of a whole pancreas, only leaving a duodenal button around the papilla of Vater [114] or by a side-to-side anastomosis

duodenal segment and the bladder [96][115].

Severe complications may occur, but morbidity is comparable to other techniques [110][116]. Comparison of technical complications between the duodenal button and duodenal segment technique appears to be in favour of the latter.

Drainage to the bladder may give rise to the additional problem of metabolic acidosis due to chronic loss of bicarbonate from the pancreas and duodenal segment [115]. In combination with impaired renal function this may be quite dangerous and necessitate lifelong suppletion of bicarbonate or even conversion of bladder to enteric drainage [111][117][118]. Nevertheless, the advantage of earlier recognition of an ongoing rejection process by determination of urinary amylase levels results in a better graft survival than with other techniques, while glucose metabolism is comparable to grafts with enteric drainage [88][106].

1.3.2. Whole versus segmental pancreas transplantation

Whether a segmental or whole pancreas graft is transplanted is determined by the harvesting procedure in the donor. The procedure in cadaver donors is less complicated if only the tail segment with the splenic vessels is removed, especially when also the liver is donated. It is also possible, however, to procure a whole pancreas and liver from the same donor with some modifications of the vasculature when transplanted [119]. In living-related donation obviously, only the tail segment can be harvested [120]. However, transplantation of a reduced endocrine mass results in limited insulin production [121][122][123], similarly as is observed when islet mass is reduced in other surgical procedures or in living-related pancreas graft donors [124][125]. Although normoglycemia can be achieved by a reduced pancreatic mass, glucose stimulation tests may be disturbed, dependent on the residual endocrine mass. Systemic venous drainage, which is the situation after transplantation in most cases, compensates the impaired serum insulin level to normoinsulinemia or even hyperinsulinemia by bypassing the liver; but generally it does not effect the glucose values during stimulation tests [83][126]. La Rocca et al. found higher insulin levels and a less abnormal OGTT response rate in recipients of whole pancreas grafts than in those with segmental grafts [127]. Enteric drainage in the former group versus duct-obliteration in the latter group may have contributed to the better results. Other groups could not demonstrate any difference in endocrine function between segmental and whole pancreatic grafts, all with visceral drainage [126][128].

It seems important that enough residual endocrine tissue is available to intercept loss of islet tissue by ischemia, fibrosis, or rejection episodes [129]. In rat allograft

experiments survival was prolonged by optimizing the conditions and extension of endocrine tissue mass [130]. Differences in one-year graft survival by increased pancreas volume in humans have not been observed [131].

1.3.3. Vascularization and prevention of thrombosis

More than other transplants the pancreas graft is highly prone to thrombosis. In the early series (1974-1983) thrombosis of the pancreatic vessels occurred in about 15-25% of the transplants, i.e. it accounted for half of the technical failures [21] [132][133]. Undoubtedly this is due to the fact that the pancreas is a low-flow organ. In dogs it was estimated that the pancreas takes only about 10% of the celiac arterial blood flow [134]. After splenectomy the pancreatic blood flow through the splenic vein averaged only 4.8 ml/min [135]. In addition, peri-operative damage to the graft by manipulation, ischemia and duct-obstruction leads to increased tissue resistance by edema resulting in a further decrease of blood flow [136].

Several methods have been introduced to prevent or reduce the occurrence of thrombosis. End-to-end anastomosis of the splenic vein of the graft to the recipient's vein was abandoned when disastrous results were obtained in dogs [137]. End-to-side anastomosis is the clinical technique of choice. How to deal with the arterial part was examined in detail by Florack et al. in dogs [138]. They had the best results with the end-to-side technique, whereas others preferred the so-called jump technique implying end-to-side anastomoses of both proximal and distal splenic ends [139][140]. Calne et al. introduced the arteriovenous fistula in the distal splenic vessels in order to increase the blood flow in both vessels [134]. After initial enthousiasm this technique was employed by only a few groups; most groups preferred the simple end-to-side technique without a fistula [141].

To imitate the physiological situation some groups transplanted the pancreas together with the spleen [113][142]. Thrombosis seemed to be reduced, but signs of graft-versus-host disease (GVHD), in one case fatal, the risk of laceration of the spleen, and the necessity of ABO-compatibility between the donor and recipient were complicating factors, making this technique not very popular [95][96][107]. Irradiation of the spleen before transplantation may abrogate the risk of GVHD [142][143].

Most groups have introduced an agressive postoperative anticoagulation program, consisting of dextran, aspirin, heparin, coumarin-type agents, or a combination of these. With regard to thrombosis there may be an improvement, but bleeding and hematoma may occur, resulting in an increased frequency of reinterventions. Despite

many efforts to suppress thrombosis, it still remains one of the most important causes of early graft failure (8–16% in the last series) [84][144][145].

1.3.4. Systemic versus portal venous drainage

Usually the pancreas graft is placed heterotopically with vascular anastomoses to the inferior abdominal vessels, i.e. with a systemic venous drainage. This unphysiological state has consequences for the metabolic profile. Peripheral hyperinsulinemia with portal hypoinsulinemia is one of the consequences when the liver is bypassed [146][147]. About 50% of the insulin, when secreted from the pancreas into the portal system, is retained in the liver during its first passage [8]. A second result of transplantation, viz. denervation of the graft, might play an additional role in the origin of hyperinsulinemia. Bewick et al. described that in dogs only the combination of denervation and portal–to–caval venous transposition led to hyperinsulinemia, as did heterotopic transplantation, but not denervation or venous transposition alone. Insulin levels were two to three times higher than in unmodified controls, although this had no effect on basal or stimulated blood glucose levels [148]. These additional effects of denervation and systemic drainage were found earlier by Sells et al. in pigs. In this model venous transposition without denervation already caused hyperinsulinemia with impaired rise of glucose levels after glucose stimulation [149]. Some groups could not confirm these results [121], whereas others found hyperinsulinemia in varying degrees [50][70][122]. The results seem to depend on the experimental model and the precise technique used.

Variance of venous drainage in clinical transplantation

In clinical pancreas transplantation it is difficult to determine the exact role of systemic venous diversion on carbohydrate metabolism, because it may be influenced by many other technical and therapeutical factors. After heterotopic pancreas transplantation in humans, serum insulin levels may range from normal or slightly elevated to remarkably increased, although normoglycemia was found by most groups [73][126][150]. A twofold increase of serum insulin with normoglycemia was found by Pozza et al. and they observed an unexpected and unexplained hyperketonemia. The latter may indicate that the metabolism in the liver is impaired as a result of reduced portal venous insulin levels [150].

Apart from these metabolic consequences, hyperinsulinemia is suggested to be associated with atherosclerosis. There is evidence that insulin has biologic activity on the arterial wall that may be relevant to the development of atheromatous lesions

[151].

Another disadvantage of systemic venous drainage could be the observation in dogs that it may lead to reduced insulin response to glucose and even to recurrence of diabetes mellitus in the long run. This may be due to exhaustion caused by continuous overstimulation of the endocrine pancreas [152].

More physiological hormone blood levels can be achieved by insulin delivery through the portal route [153]. Calne et al. put this into practice by their paratopical pancreas transplantation technique, using the recipient's splenic vessels [154]. This procedure has been adopted by others in a small number of patients [97][155]. Peripheral hyperinsulinemia was indeed avoided and normal serum glucose levels were obtained [156]. Sutherland et al. used the recipients' mesenteric vessels in patients with unsuitable iliac blood–vessels due to severe atherosclerosis. In this group they found normoinsulinemia with mean plasma glucose concentrations during OGTT that were lower than in patients with systemic venous drainage [157].

Up to now immunological benefits of grafts having a portal venous drainage, observed in some rat models, have not been confirmed in larger animals or humans [158].

Although the differences between the two venous drainage systems are not distinct, it is still unclear which metabolic deviations can be accepted or have to be avoided in order to prevent or reverse secondary diabetic complications. The portal venous anastomosis technique is not employed on a large scale because of its technical and practical aspects.

1.3.5. Graft ischemia and preservation

Cold and warm ischemia times should be limited to avoid damage to the graft, which may lead to edema, pancreatitis, thrombosis, or primary non–function of the graft. For logistic reasons, however, prolongation of the cold–ischemia is desirable.

Two methods of experimental graft preservation have been used: the simple cold storage technique and the continuous hypothermic perfusion technique [159]. They were equally successful, but because the latter is more complex, most pancreas grafts are currently stored by immersion at $4^{o}C$ [160]. While in dogs normal graft function can be obtained after warm– and cold–ischemia times up to one hour and 48–72 hours respectively [161][162][163], detrimental in porcine pancreatic transplants effects have been observed after 4 hours of cold storage [164]. In dogs, long–term graft function and histology were not affected by 24–hour cold preservation [165]. Much depends on the storage solution used. Crystalloid

Collin's solution has been used by many groups experimentally as well as clinically. More sophisticated hyperosmolar solutions, such as silica gel filtered plasma (SGFP) and particularly University of Wisconsin (UW) solution are evident to be more suitable for prolonged cold-ischemia time [166][167].

In humans, pancreas grafts seem to be more prone to damage by ischemia than in dogs. Warm-ischemia time should preferably be kept as short as possible, not exceeding half an hour [20][59]. Using the crystalloid preservation solutions most groups preferred to limit cold-ischemia time to 6 or maximally 12 hours [71][98] [168], With the development of hyperosmolar solutions, however, extension up to 30 hours seems to be justified, thus making pancreas transplantation not an emergency procedure [169]. Data of the International Pancreas Transplantation Registry did not reveal differences in one year graft survival rates between grafts with different preservation times up to 24 hours. A one centre study, only using hyperosmolar solutions in 130 combined kidney-pancreas grafts, shows similar technical failure rates and endocrine function studies between 2 and 6 weeks postoperatively in groups with different cold ischemia times (<6, 6-12, 12-24, and >24 hours, respectively) [169]. However, long-term results of prolonged ischemia times on endocrine function have not yet been reported.

1.3.6. Rejection and immunosuppression

In experimental models the pancreas has proven more susceptible to rejection than other vascularized allografts [170][171]. Enhancement procedures, treatment with either conventional immunosuppressive drugs or with CsA, and combinations of these therapies are less effective in pancreatic transplantation [172][173] [174][175]. This is the main reason why until recently 30% of clinical pancreatic allografts were lost by rejection in the first year. Improved immunosuppressive regimens and progress in monitoring of rejection has reduced this figure to 10-20% in the past few years [110][145].

Matching for tissue antigens

The value of MHC-matching in pancreas grafting has been demonstrated both in dogs and in humans. The effect in humans is mainly on account of matching for DR, not A or B-locus antigens [176][177]. Klempnauer et al. performed whole rat pancreas and islet transplantations in many donor-host combinations across several major or minor histocompatibility disparities. While heart and kidney allografts survived permanently if transplanted in only non-MHC incompatible rats, pancreas and

islet allografts were acutely rejected [178][179]. This suggest a more important role of non-MHC histocompatibility antigens in the rejection process of pancreatic grafts. Until recently HLA-matching in humans could hardly be done aiming at prevention of prolonged ischemia time. This is the reason why most recipients of cadaver grafts are completely mismatched or only partially matched for HLA-antigens [96][110][156]. This situation, which might lead to grafts more prone to acute and also to chronic rejection, had to be compensated by more agressive immunosuppressive treatment [73]. With the improvement of graft preservation, enabling graft cold ischemia times of more than 24 hours, tissue matching can be paid more attention to.

Immunosuppression

Conventional therapy with azathioprine and prednisone led to moderate immunosuppression. CsA monotherapy or CsA in combination with prednisone were not sufficient either [20][180]. In experimental pancreas transplantation a combination of CsA and azathioprine was found to act synergistically [171][175]. However, the immunosuppressive effect of all mentioned combinations in pancreas transplantation appeared to be inferior to the effect observed in transplantation of other grafts, so that other combinations were introduced. In humans, triple-therapy (CsA-azathioprine-prednisone) was more successful and has now been applied by several groups [73][96][181][182]. Most patients are now being treated with quadruple immunosuppression: triple-therapy plus temporary antilymphocyte or antithymocyte globulin (ALG/ATG) or anti-T-cell monoclonal antibodies like OKT3, in order to avoid acute rejection in the first two weeks [98][106][110][183]. Rejection crises are mostly suppressed by high doses of prednisone or by ALG, ATG, or OKT3 therapy [145] [184]. Toxicity and the inadequacy of the current drugs have stimulated a continuing search for new anti-rejection agents, of which FK 506 seems to have the highest potency. The results of the first clinical trials, which have been started recently, are promising [185].

Side-effects

The major disadvantage of multiple drug immunosuppression is the increased risk of side effects. Apart from complications induced by each drug on its own, combination therapy may lead to synergism of some of these effects. In pancreas transplantation the diabetogenic effects of corticosteroids and CsA, but also of FK 506, are of special importance. Although the insulin resistance due to steroids seems to be a minor effect, it might become more important when superimposed on the diabetogenic effect of CsA

[186]. Gunnarsson et al. found a (reversible) impairment of glucose metabolism after conversion from azathioprin–prednisolone to CsA–prednisolone therapy [187]. They suggested a peripheral insulin resistance due to CsA, because they found higher C–peptide serum concentrations when CsA was given, concentrations which were not related to impaired renal function [188]. They were not able to demonstrate a reduction of prednisolone clearance by CsA, as decribed by others [189][190]. Several groups observed a reversible decrease of insulin release from CsA treated islet–cells [191][192]. Although low serum concentrations of CsA only seem to result in impaired insulin release [193][194], high levels damage the beta–cells directly, leading to degenerative changes [191][195]. Acute and sub–acute functional and morphological alterations by CsA were found to be reversible, but long–term effects are not yet clear [195].

1.3.7. Monitoring of rejection

The pancreas is not only prone to rejection but also has a limited capacity to recover from damage as evoked by rejection crises [20]. Therefore rejection episodes have to be suppressed at an early stage to preserve sufficient islet tissue to guarantee optimal metabolic control. Early monitoring of rejection requires reliable parameters with a high specificity and sensitivity and which have to be simple to make day–to–day monitoring possible.

Endocrine functional changes such as fasting hyperglycemia or glucosuria occur late in the rejection process and mostly represent irreversible islet destruction [106]. Measurements of beta–cell hormone release (peripheral C–peptide) show too much variability and are influenced by too many factors such as immunosuppressive drugs and kidney function [196]. The 24–hour glucosuria/urinary C–peptide ratio, corrected for renal function, has been employed to bypass some of these variabilities, but it also appeared to be not specific enough, and not very practical [196].

Urinary parameters and cytology

The bladder–drainage technique was developed with the aim to develop a transplantation procedure with few complications; it appeared to have the additional advantage of rejection monitoring by urinary amylase measurements. Hyperglycemia appeared to be preceeded for some days by a drop in urinary amylase, bicarbonate, protein, and pH [110][197][198], especially when the concentrations were based upon 24–hour determinations [111]. In contrast, changes in serum amylase were found to be of no predictable value [198]. The parameters used in the urinary drainage

technique can be applied in the technique of temporary percutaneous exteriorization of the exocrine duct [75][199]. If these parameters are combined with pancreatic juice cytology, rejection episodes can be detected in the first few postoperative weeks. Klima et al. reported a sensitivity of 87% and a specificity of 97% [200][201].

Histology

Histologic examination of biopsies is most reliable, but in all transplantation techniques except the bladder-drainage technique, a reexploration was supposed to be needed and therefore it has only been done if prompted by extreme changes of other parameters [202]. More recently it was shown that percutaneous needle core biopsies as used in kidney and liver grafts, can be a save diagnostic procedure in pancreas transplantation without the formation of fistulas [203]. Fine-needle aspiration biopsy may be a safer tool, although it is difficult to distinguish between the different features of pancreatitis, rejection and viral infection [204]. Bladder drained grafts are well accessible for histologic examination by the transurethral route: transduodenal pancreas biopsies can be taken, but also biopsies from only the duodenal segment or only the button may reflect the condition of the pancreas graft [205].

Miscellaneous

Noninvasive methods such as Technetium-scanning or Indium-labeled-platelet-scanning may be helpful in addition to other parameters, but are not practical for regular monitoring [206][207].

The level of urinary neopterin, a metabolite produced by activated T-cells, was found to be elevated early in the rejection proces; this metabolite, however, it is not organ-specific and is also influenced by immunosuppressive drugs and viral infections [208]. Urinary radioimmunoreactive insulin and urinary prostaglandin were found to be early markers of rejection in dogs, but their exact value has not yet been evaluated clinically [209][210]. More recently serum anodal trypsinogen and pancreatic secretory trypsin-inhibitor were suggested to be reliable diagnostic indicators of acute pancreas rejection after human transplantation [211].

Simultaneous kidney/pancreas transplantation

Simultaneous kidney and pancreas transplantation from the same donor to uremic recipients has the advantage of allowing the pancreas to be indirectly monitored for possible rejection by assessing kidney function through serum creatinine levels. Serum creatinine levels appear to increase some days before serum glucose when both

transplants are threatened by rejection [72]. Not only chemical (serum creatinine versus glucose) but also histological findings demonstrate that the renal graft shows earlier signs of rejection than the endocrine part of the pancreas. When serum creatinine level is elevated as a sign of renal rejection, usually serum glucose is still within its normal range. Simultaneously there is a diffuse cellular infiltrate in the kidney and the exocrine pancreas but often not in the endocrine part [104]. Several reports indicate that there may be more renal than pancreatic rejection episodes in simultaneous transplantation; and even complete rejection of the kidney while the pancreas remains preserved [72][96][168][212].

1.3.8. Pancreas transplantation alone or combined with kidney transplantation

In the first years of clinical pancreas transplantation only patients with end-stage nephropathy receiving a kidney transplant were selected for pancreas transplantation [120]. Due to the initial poor results a pancreas was only transplanted in patients with far advanced diabetic complications. In the absence of good early parameters for rejection of the pancreas graft rejection episodes could be detected by determination of kidney function. Obviously, this advantage does not hold good when the grafts are obtained from different donors. Owing to the progress in rejection monitoring, predominantly with regard to the bladder-drainage technique, more single pancreas transplantations are currently being performed in non-uremic patients [213].

Graft interaction

There is much discussion about the possible interaction of the two organ transplants concerning graft survival. In the first period of combined transplantation, kidney graft survival seemed to be highly impaired if the kidney was transplanted in combination with a pancreas graft [214][215]. The results, however, were influenced by factors such as high patient mortality due to technical failures with the pancreas graft, a poor HLA-match, and high doses of immunosuppressive drugs leading to nephro-toxicity. The results improved by modified immunosuppressive regimens: by decrement of the CsA dosage and by addition of ATG/ALG or OKT3 to the triple drug protocol. The most recent figures on kidney graft survival show hardly any difference between solitary kidney transplants and kidneys transplanted with pancreas grafts [216].

If transplanted with a kidney, pancreas grafts do not seem to be harmed, but even seem to benefit from the presence of the kidney, as demonstrated in experimental models [217][218][219]. Also clinically the simultaneous transplantation of a kidney seems to provide a protective effect on the pancreas. In comparison to pancreas

transplants alone, the incidence of reversible rejection episodes and graft loss from rejection were lower in simultaneous pancreas/kidney transplants. Possible explanations are (1) renal rejection episodes may function as early indicators of pancreas rejection, (2) preoperative uremia might still have an immunosuppressive effect early in the postoperative period, and (3) a lymphocyte entrapment phenomenon takes place in the transplanted kidney [220].

1.3.9. Metabolic control by insulin therapy versus pancreas transplantation and their influence on secondary diabetic complications

Endocrine function after pancreas transplantation is determined by several factors: reduced islet mass (as a result of segmental grafting, rejection episodes, fibrosis, or ischemic injury), hormone delivery into the peripheral versus the portal circulation, denervation, relatively impaired renal function, and immunosuppressive treatment. Despite these complicating factors the pancreas transplant corrects the metabolic abnormalities of diabetes rather precisely. Technically successful transplantation results in normoglycemia with normal 24–hours profiles and normal HbA1c levels in the majority of patients. Only stimulation tests of the endocrine system often reveal sub–normal or abnormal values (30–40% of the patients) [221]. As discussed before the question is whether this level of metabolic control is adequate to prevent or reverse diabetic complications and whether the morbidity and mortality attending transplantation would outweigh the disadvantages of exogenous insulin therapy.

1.3.9.1. Metabolic control with insulin therapy

Intensified insulin therapy by advanced techniques, home blood glucose monitoring, and education, has led to improved metabolic control but in general does not reach the level obtained by pancreas transplantation, especially in brittle diabetics [12][222]. In a majority of patients with pump therapy HbA1c levels are still outside the normal range [223]. Aiming at normoglycemia the risk of hypoglycemia is serious and potentially dangerous [224][225].

Conversion from conventional to intensified insulin therapy may slow down or arrest established microvascular complications, although reversal can hardly be achieved. Several groups have reported that, surprisingly, retinopathy is more frequently deteriorated shortly after conversion to continuous subcutaneous insulin infusion therapy (CSII), than in conventionally treated patients in which a continuous progression was found [226][227][228][229]. However, after a follow–up of two years,

no progression and even a marginal improvement was found in the CSII–groups, contrasting the conventional groups [230][231][232][233]. A similar trend was not observed with intensified conventional insulin therapy during 5 years in spite of long–term improvement of metabolic control [234]. While acute or early diabetic neuropathy may be brought to arrest or may slightly improve after long–term treatment with CSII, the influence on long–established and advanced lesions is doubtful [232][233]. The same applies to nephropathy: slightly elevated albumine excretion in urine can be reversed after CSII, but progression of clinically manifest nephropathy is unaffected by intensified control [231][235][236][237]. Furthermore, in patients with renal allografts diabetic nephropathic lesions may develop again within 2 years after kidney transplantation [238].

1.3.9.2. Metabolic control with pancreas transplantation

Extensive experiments in rats have shown that pancreas transplants provide protection against neuropathy and all major lesions of nephropathy for more than two years [239][240]. Other groups were also able to reverse mesangial changes, characterizing diabetic nephropathy, by endocrine tissue transplants [241][242].

In recent years clinical data about long–term effects of pancreas transplantation on diabetic microvascular changes have become available. A comparison with the results obtained with insulin therapy is difficult because of differences in degree of advancement of diabetic complications between both groups. It should be noted that up to now most pancreas transplantations have been performed in uremic patients and patients with advanced diabetic complications in whom a point of no–return might have been passed.

Nephropathy

One of the most important observations after combined renal and pancreatic transplantation is that the pancreas graft protects the kidney against nephropathy for at least 4 years [243][244]. In nonuremic recipients of a pancreas graft Sutherland et al. found a decline in renal function in the first year but a stabilization in the second year after transplantation [213]. In non–uremic diabetics, histological examination of renal biopsies revealed a reduction in glomerular mesangial volume two years after pancreas transplantation [245].

Neuropathy

Although autonomic nerve dysfunction does not seem to improve, several groups described that combined kidney–pancreas transplantation had beneficial effects on polyneuropathy [246][247][248]. A considerable part of these effects, however, should be attributed to the elimination of uremia, not only the tight normoglycemia [246]. Secchi et al. found equal improvement of nerve conduction velocity in the first year after combined pancreaticorenal transplantation in comparison to kidney–alone transplantation. After the second postoperative year, however, the results were further improved in the combined group to the detriment of the kidney–alone group. This suggests that the effect on neuropathy by metabolic control by the pancreas graft become evident at the long–term in contrast to the effect induced by the kidney graft [249]. Besides, also after pancreas transplantation in non–uremic patients slight improvement of peripheral nerve functional parameters has been observed [213].

Retinopathy

The Minnesota group found that rapid conversion to normoglycemia by a pancreas graft may deteriorate retinopathy in the first postoperative year, a phenomenon also observed with insulin therapy [250]. In nonuremic recipients, retinopathy has been found to progress in 41% of the eyes and to remain stable in 59%; at two years, visual acuity was unchanged in 83% [213]. In a follow–up study by the Munich group in patients with combined kidney and pancreatic allografts, visual acuity remained stable in 32% of the patients, improved in 56% and deteriorated in 12% after a mean follow–up of 21 months. Patients with grafts surviving for more than 12 months had no further deterioration of visual acuity [248]. It should be mentioned that there were no control groups in the latter two studies. As with other diabetic complications, the course of diabetic retinopathy after pancreas transplantation seems to depend on the grade of progression before transplantation [251].

Microangiopathy

The Munich group used transcutaneous oxygen tension measurement and telethermography as a method to measure changes in microcirculation. In patients with a mean follow–up period of 18 months after combined renal and pancreatic transplantation, they observed a significant improvement of these parameters compared to renal graft recipients receiving insulin [248]. Because these changes already were observed early posttransplant, functional rather than structural changes were probably responsible for the better vascular reactivity to the physiological stimuli applied. However, in spite of this observation and the finding of an improved peripheral

neuropathy, 5 of 20 patients with successful pancreas grafts developed a diabetic foot resulting in toe amputation [73].

1.3.10. Recurrence of the diabetic disease process

Although the exact pathogenesis is still unknown there is considerable evidence supporting the hypothesis that type I diabetes mellitus is a chronic autoimmune process that selectively destroys the insulin-producing beta-cells of the pancreatic islets [2]. The theoretical risk of recurrence of the autoimmune process in the pancreatic graft was confirmed as reality by Sibley et al., who described four cases of recurrent diabetes mellitus in recipients of a HLA-identical pancreatic graft, not receiving immunosuppressive therapy [252]. In a later report on 100 histopathologic examinations obtained from biopsies and obduction, they found an isletitis in 25% of the grafts, in both fully-matched and mismatched grafts. In mismatched grafts it was not possible to differentiate isletitis caused by rejection and inflammation and isletitis caused by disease recurrence. Recipients of fully matched grafts (from identical twins or identical siblings) were not treated at all or only treated with minimal immunosuppression. Isletitis occured in 50% (11/22) of these grafts, associated with a selective loss of beta-cells in 82% (9/11), suggesting disease recurrence, leading to graft loss in all but one patient [253].

In the BB-rat diabetes model, which is very closely related to the human type I diabetic disease process, an autoimmune reaction in islet transplants resulted in graft failure. Such an autoimmune reaction was not observed in other endocrine tissues transplanted in the same model [254][255][256].

Although isletitis may occur in matched and mismatched grafts, selective loss of beta-cells is only seen in HLA-identical grafts [252]. Two explanations can be adduced for this. Firstly, the autoimmune process only attacks islet-cells having identical HLA-antigens, which is consistent with the hypothesis that recurrence of the disease is a MHC-restricted phenomenon [257]. Secondly, in fully immuno-suppressed mismatched recipients the anti-rejection therapy is also suppressing the autoimmune process. Observing the occurrence of islet cell antibodies after HLA-mismatched pancreas transplantation in 7 out of 23 recipients, Bosi et al. suggested a possible reenhancement of islet cell autoimmunity in type I diabetic patients, also after HLA-mismatched pancreas transplantation [258]. Because immunosuppressive drugs may delay the onset of hyperglycemia in newly diagnosed diabetics [5][259], they might be able to prevent the recurrence of diabetes in the pancreatic graft too. Indeed, Sibley et al. were able to delay the occurrence of

autoimmune isletitis in HLA–identical pancreatic grafts by giving immunosuppressive therapy [252].

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CHAPTER 2
AIM OF THE STUDY

2.1. INTRODUCTION

Type I diabetes mellitus is not only physically a disabling disease, but also psychologically and socially. Most patients will develop late diabetic complications which result in blindness, renal failure, myocardial infarction, cerebral vascular accidents and amputation of the extremities due to gangrene. Due to this morbidity the average survival time of patients with insulin–dependent diabetes diagnosed before the age of 31, is 40 years. Only 25–30% of the patients will not develop late diabetic complications [1]. Conventional insulin–therapy has proven to be insufficient to protect against these complications.

Furthermore, patients, mostly being children at the onset of type I diabetes, are put under restraint, because of their diet, a forced regular life pattern, and insulin–therapy with multiple daily blood–sugar controls and injections. Insulin–therapy and monitoring of its effect on metabolism have improved in the last decades. In spite of this the diabetic complications are still an unsolved problem, and so, long–term quality of life is poor.

Transplantation of islets of Langerhans may be the alternative. This form of transplantation is still in an experimental stage, due to logistical problems. Lack of simple islet isolation techniques with sufficient yield of purified cells and lack of adequate treatment of rejection hinder clinical exploitation on a large scale. Although some success in clinical islet transplantation has been obtained recently, long–term results in humans are not available. Therefore it is not known whether this type of transplantion can prevent or reverse diabetic complications [2][3].

Vascularized pancreas transplantation has been studied more extensively in man and can restore normal glucose homeostasis. There is evidence that, dependent on its timing in the course of the disease, it can halt or even reverse microvascular complications. However, most transplantations are performed in persons with far advanced diabetic complications. It is not possible to select those patients who are at high risk for developing diabetic complications outweighing the complications of transplantation and the side effects of the antirejection drugs. Consequently, determination of the most optimal timing of transplantation is hardly possible, neither in general nor for the individual patient.

In the last decade much progress has been made with pancreas transplantation

resulting in better graft and patient survival (Table 2.1.).

Good results are mainly obtained in experienced transplantation centers. These groups increasingly perform pancreas transplantation in non-uremic patients, but in most clinics pancreas transplantation is still combined with kidney transplantation in patients with end-stage diabetic nephropathy. Non-uremic patients are mostly considered for transplantation if other diabetic complications are far advanced [4].

Table 2.1. One-year pancreas graft and patient survival from 1966 to 1990 according to the Pancreas Registry Report [5][6].

		1-year graft survival	1-year patient survival
1966-1977	n=64	3 %	41 %
1978-1982	n=201	21 %	71 %
1983-1985	n=519	39 %	78 %
1986-1987	n=672	53 %	86 %
1988-1990	n=1183	70 %	91 %

2.2. SUBCUTANEOUS PANCREAS TRANSPLANTATION

The aim of pancreas transplantation is to prevent or delay late diabetic complications and to improve quality of life. Therefore pancreas transplantation should preferably be performed before diabetic complications develop, at least before they have reached a point of no return, in other words in young patients. In view of their age and life expectation the following conditions should be fulfilled:

1. the transplantation technique should have a minimum of complications, i.e. minimal morbidity and mortality,
2. the pancreas transplant should have long-term function, preventing diabetic complications,
3. side effects of (life-long) immunosuppression should be minimal, at least should not exceed the side-effects and difficulties of insulin-therapy,
4. parameters have to be available to discriminate patients at risk from patients at low risk to develop serious diabetic complications, in order to determine the indication for transplantation.

Intraperitoneal pancreas transplantation has proven to be a high-risk operation with serious complications such as fistulas, infection, formation of pseudocysts and abscesses, ascites and abdominal pain. This morbidity has led to a high mortality although the results have been improved. The alternative may be an extra-abdominal location of the graft, e.g. subcutaneous transplantation. Subcutaneous transplantation is technically simpler and permits daily examination of the graft by physical examination. Identification of pancreatic fistulas and other complications are more easily detectable and so is early graft destruction. The graft is more accessible for diagnostic biopsies and, if needed, it can be removed easily. Subcutaneous pancreas transplantation has been performed with success in many experimental models, such as mice [7], rats [8], sheep [9], pigs [10] and dogs [11]. Ducts were ligated or obliterated mostly [12][13], but also free percutaneous draining procedures [14] or more complicated anastomoses to the parotid duct [15] or esophagus [16] have been carried out. Good postoperative as well as long-term results were reported [9][10][11][17][18], but as with most techniques, also early postoperative complications, failures and impairment of long-term graft function have been obtained [19][20].

Human subcutaneous autotransplantation has been performed in a few patients after chronic pancreatitis [21][22]. Duct-obliterated grafts were transplanted to the femoral vessels. Fistulas were the main postoperative complication, which closed spontaneously in all patients. Patients were mostly insulin-independent after transplantation, although the endocrine graft function hardly can be determined because often only a subtotal pancreatectomy was performed. Cases of human subcutaneous allotransplantations have thus far not been reported.

2.3. AIM OF THIS STUDY

The aim of this study was to investigate whether subcutaneous pancreas transplantation is a feasible technique which results in long-term insulin-independence, which can be used in early pancreas transplantation, before late diabetic complications will develop. In this surgical model also the third condition for early pancreas transplantation was tested: immunosuppressive protocols with non- or low-toxicity. Especially biological procedures (blood transfusions and anti-class-II donor pretreatment) were used in combination with low-dose immunosuppressive drugs. The studies were performed in an experimental rat model and a preclinical dog model.

The next specific questions were studied:

* can long-term pancreas-graft function be achieved if placed in a subcutaneous position?
* has this position immunological consequences?
* can effective immunosuppressive protocols be developed with no or minimal side-effects, resulting in long-term graft function?

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CHAPTER 3
MATERIALS AND METHODS

3.1. EXPERIMENTS IN RATS

Animals

Rats of the inbred WAG and BN strains were used. The WAG strain is homozygous for the RT-1^u haplotype, the BN strain for the RT-1^n haplotype, resulting in a incompatibility for class I and class II antigens of the major histocompatibility complex. Syngeneic skin transplantation was performed regularly to test the genetic homogeneity of the strains. The animals, obtained from the Central Institute for Laboratory Animals-TNO, Zeist, The Netherlands, had been bred under specific pathogen-free conditions, were 12-16 weeks old and weighed 200-320 g.

Induction of diabetes

Recipients were rendered diabetic with 65 mg/kg of streptozotocin (Zanosar^R, a gift from Upjohn, Ede, The Netherlands) administered intravenously at least two weeks before transplantation. Rats showing two consecutive blood glucose values above 17 mmol/l were considered diabetic.

Anaesthesia

Intravenous injection of streptozotocin, transfusion of blood, blood sampling and operations were all carried out under ether anaesthesia. IVGT-tests were performed under Nembutal^R anaesthesia, 1 ml/kg intraperitoneally containing 60 mg/ml pentobarbital (Abbott BV, Amstelveen, The Netherlands).

Pancreas transplantation: donor procedure

Pancreatectomy was carried out under nonsterile conditions as described by Lee et al. [1] with some modifications. After the abdomen was opened through a midline incision the stomach was clamped and lifted over the thoracic wall. The ligament of Treitz was divided. The transverse colon was bluntly dissected from the duodenum and the mesenteric vessels, which were divided between ligatures at the caudal border of the pancreas. A splenectomy was carried out. The left gastric artery and vein were identified beneath the esophagus and divided between ligatures. Then, the hepatic artery was carefully separated from the portal vein and ligated and cut together with the bile duct at the liver hilus. The pancreas with the choledochal duct was ligated

close to the duodenum and dissected, leaving the duodenum in situ. After blunt dissection of the suprarenal aorta, the superior mesenteric artery was ligated. After injection of 1 ml of 1% heparin (Thromboliquine^R, Organon, The Netherlands) the aorta was clamped above the celiac artery. The portal vein was cut at the liver hilus and an aorta patch with the celiac artery was dissected. The pancreas was flushed gently with 2 ml of chilled (4°C) Hanks' balanced salt solution (HBSS) to remove residual donor blood and placed in HBSS while preparing the recipient.

Pancreas transplantation: recipient procedure

Cervical subcutaneous transplantation: a paramedian cervical incision was made up to the muscle layer. The common carotid artery and jugular vein were exposed by careful dissection and clamped. First the aorta patch (about 2 mm in diameter) was anastomosed end–to–side to the carotid artery using a Mirafil 9–0 suture (Braun–Melsungen AG, W.–Germany). After two corner stitches, continuous sutures were made using about five steps. Then the end–to–side anastomosis was made between the portal and jugular vein, also using two corner stitches and a five step continuous Mirafil 9–0 suture, stitching the hindwall from the inside of the lumen. The graft was placed in a bluntly widened subcutaneous pouch on top of the blood–vessels, without traction. The skin was closed using staples.

Intraperitoneal transplantation: the abdomen was opened through a midline incision. The intestines were wrapped in moistened gauze and moved laterally after which the aorta and caval vein were exposed and clamped. The donor aorta patch with the celiac artery was anastomosed end–to–side to the recipient's infrarenal aorta and the donor portal vein end–to–side to the recipient's inferior vena cava, using Mirafil 8–0 as a continuous suture. The grafts were placed intraperitoneally.

Peroperative and postoperative treatment

Rats were allowed to take complete rat food (Hope Farms BV, Woerden, The Netherlands) and water ad libitum before and after grafting. No antibiotic treatment was given.

Follow–up

Graft function was monitored by regular nonfasting glucose measurements in blood. Transplantations were considered to be successful, if the concentrations of glucose in the blood normalized below 10 mmol/l within 24 hours postoperatively. Unsuccessful transplantations were not included in the results. The day of graft failure was defined as the first day of two consecutive measurements of hyperglycemia above 14 mmol/l.

After follow–up, the transplants of normoglycemic rats were removed, whereafter hyperglycemia had to return. If not, the rat's own pancreas function was supposed to have recovered and the animal was excluded from the experiment.

In syngeneicly transplanted rats IVGT–tests were performed. One over–night fasting rats were injected with 0.5 g/kg glucose, and serum glucose was measured before and 5, 10, 20, 30, 45, and 60 minutes after bolus injection. K–values (%/min decline of serum glucose during IVGTT) were calculated as described before [2].

3.2. EXPERIMENTS IN DOGS

Animals

Male and female beagles, obtained from the Central Institute of Laboratory Animals–TNO, Zeist, The Netherlands, were used as donors and recipients. Female dogs had never been pregnant. The dogs weighed 10–20 kg and their ages ranged from 1–3 years. Mongrel dogs from the central animal facilities of the Erasmus University were used as blood donors. Beagles were typed for the conventional DLA–A, B and C locus antigens as described earlier [3]. Donor–host selection was further based on the outcome of mixed lymphocyte cultures (MLC) as reported earlier [4]. Allogeneic pancreas transplantation was performed by exchange of grafts between two mismatched (MLC–positive), nonlittermate beagles.

Induction of diabetes

In dogs diabetes was induced by surgical resection of pancreas tissue in the same session as transplantation. Technical details are described below. After transplantation stools were tested twice on the exocrine proteolytic enzyme α–chymotrypsin, in order to demonstrate surgical sufficiency as described before [5]. Both values had to be less than 2 U/g to be sure that pancreatectomy was complete.

Anaesthesia

Premedication of atropin (0.5 mg) and 2 ml thalamonal^{R} (0.05 mg fentanyl and 2.5 mg droperidol per ml) was administered one hour before operation. Anaesthesia during operation consisted of 200 mg thiopental (Nesdonal^{R}, Rhône–Poulenc Pharma, Amstelveen, The Netherlands) and 0.1 mg fentanyl i.v. and endotracheal enflurane (Ethrane^{R}, Abbott BV, Amstelveen, The Netherlands) supplemented with nitrous oxide and oxygen. Thalamonal^{R} and fentanyl were obtained from Janssen Pharmaceutica BV, Tilburg, The Netherlands.

Pancreas transplantation

Operations were performed under sterile conditions. The left pancreatic segment, comparable with the pancreatic tail in man, was used as transplant. In the allogeneic groups, grafts were exchanged between two beagle dogs, which were operated simultaneously. Autotransplantations were performed one at a time.

Donor procedure

The abdomen was opened through a midline incision. The left pancreatic segment was freed from surrounding tissue. The splenic vein was dissected close to the portal vein. The left gastric vessels were divided between ligatures, whereafter the celiac trunk was dissected. Unless mentioned otherwise, a splenectomy was carried out, leaving about 2 cm of the splenic vessels distally to the pancreas graft in situ. The left pancreatic segment was ligated and divided, close to the uncinate process. In general the vascular supply of the segment consisted of the splenic vessels; in a few cases an aberrant mesenteric artery branch was included. The transplant was removed and flushed with heparinized physiological saline at 4°C.

The remaining right pancreas segment was removed carefully according to Markowitz [6] with maintenance of the duodenal vascularization.

Recipient procedure

Transplantation was performed to the neck of the recipient. Through a paramedian cervical incision the common carotid artery and the external jugular vein were dissected over a 7 cm stretch and clamped. Vascular anastomoses, using the double-bridge technique as described by Agnes et al. [7], were made between the splenic artery and the common carotic artery and the splenic vein and the external jugular vein with 6-0 prolene (Ethicon GmbH, Norderstedt, W.-Germany) continous sutures. The double bridge technique implies that the two ends of each splenic vessel were anastomosed end-to-side to carotic artery and jugular vein, respectively. In case of an aberrant mesenteric branch, a mesenteric patch was anastomosed end-to-side to the common carotic artery. The graft was placed in a subcutaneous pouch. Two wound drains were left behind.

Peroperative and postoperative treatment

Peroperative infusion consisted of physiological saline, glucose 5% and at the end of the ischemia of the graft 250 ml of glucose 10% with 0.5 g KCl to avoid metabolic disorders. Dextran 10% (Rheomacrodex^{R}, NPBI BV, Amsterdam, The Netherlands) was administered to inhibit intravascular coagulation: 75 ml during operation, 50 ml

6 hours after operation and 100 ml on the first day after operation. Postoperative fluids consisted of mixed glucose/saline infusion. Dogs received antibiotics (2 ml Depomycine^R, Gist Brocades, Delft, The Netherlands) intravenously during the first five postoperative days. Drains were removed if they were no more productive (about the third day). After one day on fluid food the animals were fed with standard dog food twice a day (8.30 and 17.00 h.). Exocrine pancreatic enzymes were supplemented by Pankreon^R tablets (Kali–Chemi Pharma GmbH, Hannover, W.–Germany), 8 every meal.

Follow–up

Graft function was monitored by regular nonfasting glucose, amylase and lipase measurements in blood. The criteria about graft function and failure in rats applied to pancreas grafts in dogs as well: transplantations were considered to be successful, if blood glucose values normalized below 10 mmol/l within 24 hours postoperatively. Unsuccessful transplantations were not included in the results. The day of graft failure was defined as the first day of two consecutive measurements of hyperglycemia above 14 mmol/l. IVGT–tests were performed in one over–night fasting dogs by injection 1.0 g/kg glucose. Serum glucose values were measured before and 5, 10, 20, 30, 45 and 60 minutes after bolus injection, from which K–values were calculated.

3.3. GENERAL MATERIALS AND METHODS

Laboratory determinations

Blood samples were taken from rats by bleeding from the tail. Blood from dogs was sampled by peripheral venous puncture.

Serum glucose was determined by the Gluco–quant^R glucose test (Boehringer GmbH, Mannheim, W.–Germany), a hexokinase method in hemolysed blood with an extinction measurement at 340 nm UV–light. Screaning measurements of serum glucose were done by Glucostix^R teststrips in a Glucometer II^R photoreflexionmeter (Ames Division, Miles Laboratories Ltd, Slough, Great Britain).

Serum lipase was determined by an enzymatic spectophotometric method at 340 nm UV wavelength, amylase by an enzymatic colorimetric test at 405 nm UV–light (Boehringer GmbH, Mannheim, W.–Germany).

αChymotrypsine in feces was determined with N–acetyl–L–tyrosine–ethylester (ATEE) as substrate as described before [8]

Insulin–values were measured in the experiments using SMS 201–995 (chapter 9) by

radioimmunoassay (Ins-RIA-100^{R}, Medgenix, Brussels, Belgium).

Blood transfusions

In rats donor blood was obtained by punture of the abdominal aorta or by bleeding from the tail. One milliliter of fresh heparinized blood was transfused at weekly intervals via the penile vein of the recipient.

In dogs donor blood was sampled from mongrels by intravenous puncture. At 4, 3 and 2 weeks before transplantation 100 ml of heparinized whole blood from three different mismatched donors was transfused to the recipient.

Immunosuppressive drugs

If mentioned, rats were treated with Cyclosporin A (Sandimmune^{R}, Sandoz, Switzerland) by one or more intramuscular injections in the hind leg at a dosage of 15 mg/kg. Cyclosporin A (CsA) was dissolved in olive oil.

All allografted dogs were treated daily with 2 mg/kg azathioprine (Imuran^{R}, a gift from Wellcome, The Netherlands) and 1 mg/kg prednisolone (Di-Adreson-F^{R}, Organon NV, Oss, The Netherlands) intravenously. Autotransplanted animals were not treated. If the leukocyte count dropped below $3x10^{6}$/l, azathioprine was withdrawn temporarily.

Irradiation

In the study of donor pretreatment, donor rats were treated 5 days before transplantation, with 10 Gy whole body irradiation, generated by a ^{137}CS-gamma-source (Atomic Energy of Canada, Ltd.).

Monoclonal antibodies

In the donor pretreatment experiments, the monoclonal antibody F 17.23.2 was used. F 17.23.2 is a mouse monoclonal antibody. The tissue culture supernatant used had a concentration of 15 μg/ml. It is directed against class II determinants of rats having the a, l and n haplotypes but not of c or u haplotypes [9][10]. Consequently, the monoclonal antibody is reacting with the class II antigens of the BN rat but not of the WAG rat. In the staining procedures two monoclonal antibodies were used: F 17.23.2 and OX 6. OX 6 is a commercially available anti class II monoclonal antibody, but MHC haplotype non-specific (Flow Laboratories, UK).

SMS 201-995

SMS 201-995 is a somatostatin analogue octapeptide (Sandostatin^R, Sandoz, Basel, Switserland). Rats were treated three times a day with 2 μg/kg dissolved in 0.2 ml physiological saline subcutaneously.

In dogs SMS 201-995 was administered s.c. in three daily doses of 25 μg/animal (about 2 μg/kg) dissolved in 2.5 ml 0.9% NaCl.

Histology

After sacrifice the grafts were routinely processed for histology, and stained with hematoxylin/eosin.

The effect of the class II antigen reducing procedures was checked by immunohistology using a peroxidase-antiperoxidase (PAP) staining technique. Cryostat sections were incubated with F 17.23.2 during 45 minutes, but not if the transplant was pretreated with monoclonal antibodies. After washing, a second step using swine antimouse antibody (SwAM/7S; Nordic, Tilburg, The Netherlands) was applied for 30 minutes. Then, after washing, sections were covered with mouse PAP-complex (M/PAP; Nordic, Tilburg, The Netherlands) during 30 minutes. Finally, after washing, slides were incubated with 3,3'-diaminobenzidine-tetrahydrochloride and embedded.

Statistic analysis

The Student's-t test, Wilcoxon's rank sum test and the chi-square test were used to test statistical significance. The limit of significance was set to $p<0.05$.

3.4. REFERENCES

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CHAPTER 4
TECHNICAL DETAILS OF SUBCUTANEOUS PANCREAS TRANSPLANTATION IN RATS AND DOGS

4.1. INTRODUCTION

In chapter 3 the surgical details of the pancreas transplantations in rats and dogs are described. The purpose of performing subcutaneous transplantation is to develop a safe technique with a minimum of complications. As pancreas transplantation is not primarily a life saving procedure, mortality due to this operation should not be accepted. The initial postoperative results of the techniques in rats and dogs are discussed in this chapter.

4.2. EXPERIMENTAL PROTOCOL

The technical results of subcutaneous pancreas transplantation in rats are discussed on the basis of 238 – syngeneically and allogeneically – transplanted grafts during the whole study. In dogs a total of 49 autologous and allogeneic grafts were transplanted in subcutaneous position, the results of which are described below.

4.3. RESULTS

4.3.1. Rats

The described technique of subcutaneous pancreas transplantation in rats appeared to be a feasible technique. Total operation time ranged between 60–90 minutes and ischemia time never exceded 60 minutes (mean 47.1 ± 5.8 minutes). The animals tolerated the operation very well. In rats 90% of the transplantations was technically successful: from a total of 238 grafts performed during the whole study, 23 were primarily non-functional. Failures were mainly caused by insufficient vascular anastomoses (bleeding and thrombosis) and hemorrhagic autolysing pancreatitis. Postoperative enlargement of the graft was about three times the normal size. The most important complications were percutaneous fistulas originating from pseudocysts and abscesses, with an occurrence rate of about 20% . They were easy to deal with, seldom resulting in death (<2%).

4.3.2. Dogs

During the study, including autologous as well as allogeneic transplantations, 49 dogs were grafted. The technical details are shown in Table 4.1.. In the beginning we started with transplantation of the pancreas to the groin using the femoral vessels. However, this location resulted in technical failures due to kinking of the blood vessels, which results in thrombosis, and traumatic disruption. Therefore, a switch was made to the cervical region.

In dogs the mean ischemia time was 67.4 ± 13.2 minutes. Inspite of the cervical location and use of the double bridge vascular anastomosis technique, 31% of the grafts failed to function, thrombosis being the main cause (Table 4.2. and 4.3.). 67% of the grafts were functioning well with normoglycemia within 24 hours post-operatively. Three grafts (6%) had reduced blood glucose levels after transplantation, but the values exceeded the 10 mmol/l limit, reported as "dysfunctioning" grafts. Again 18% of the dogs were lost for follow-up due to death or graft failure during the first postoperative weeks (Table 4.3.), resulting in only 49% (24/49) successful grafts for follow-up. Apart from thrombosis, ischemia was one of the main causes of graft loss. Twelve left pancreatic segments had proved aberrant blood vessels (24.5%): 11 grafts with an artery originating from the superior mesenteric artery and one with a combination of an aberrant artery from the mesenteric artery with an aberrant vein to the portal vein. These blood vessels were cut accidentally or reanastomosed, which resulted in seven graft failures due to ischemia or thrombosis. One dog was lost from intestinal ischemia after mesenteric reanastomosis. Pancreatitis only two times led to early graft failure. Subcutaneous abscesses and fistulas were seldom seen. There were no dogs lost due to the recipient procedure itself.

4.4. DISCUSSION

Our surgical procedure of subcutaneous duct-ligated pancreas transplantation in rats was found to have a success rate of 90% which is in agreement with, or even better than found by other groups [1][2][3]. Using the "no-touch technique" the incidence of graft pancreatitis, which may result in graft failure, was minimal. In dogs the results were quite different. Only 49% of all transplants could be used for follow-up. Thrombosis (25%) and ischemia mostly due to problems with aberrant blood vessels (12%), were the main causes of failure during the first postoperative week. 24.5% (12/49) of the left pancreatic segments had aberrant blood vessels, particularly an arterial vessel originating from the superior mesenteric artery. Also other groups

described many vascular variations, often resulting in ischemia and thrombosis. Florack et al. [4] found 10% of the arterial blood supply originating from the superior mesenteric artery and another 12% aberrant veins in mongrel dogs. Van Schilfgaarde et al. described 20% aberrant arteries in Beagle dogs [5].

In man thrombosis of the graft is estimated at 10–15% [6][7], in dogs 20% or more [4][5][8][9]. In an attempt to overcome the problem of vascular thrombosis after pancreatic transplantation, various modifications of the arterial and venous anastomosis techniques have been described, each with varying degrees of success. We used the double bridge technique according to Agnes et al. [10], who had no vascular complications with this technique (0/6). However, in our study also this technique led to a high failure rate due to thrombosis, to a similar degree as in other techniques. Furthermore, disadvantages using this procedure are prolonged operation and ischemia times.

In conclusion, the technical results of subcutaneous transplantation were in agreement with the results of other groups. The rate of thrombosis in dogs was comparable to results described in other reports (± 25%). In our hands the double bridge technique did not seem to limit the rate of thrombosis. In both experimental models grafts were seldom lost due to local (non–vascular) complications, although in rats fistulas, pseudocysts, and abscesses were complications which occurred frequently.

Table 4.1. Technical details of 49 autologous and allogeneic subcutaneous pancreas transplants in dogs

dog		function	failure
1	auto/g	funct.	fibrosis (w 59)
2	auto/g	dysf.	aberant SMA branch cut perop.; disrupted blood vessels (d 12)
3	auto/g	dysf.	ischemia + thrombosis (d 7); part. dyscirculation perop.
4	auto/c	funct.	fibrosis (w 48)
5	auto/c	funct.	sacrifice
6	auto/c	funct.	death during narcosis (d 23)
7	auto/c	n.f.	aberant SMA branch cut perop.; venous thrombosis
8	auto/c	n.f.	ischemia; aberant SMA branch reanastomosed
9	auto/c	funct.	pancreatitis; increasing serum glucose (d 8)
10	auto/c	funct.	16 hours p.o. sudden death e.c.i.
11	auto/c	funct.	fibrosis (w 66)
12	auto/c	funct.	sacrifice
13	auto/c	funct.	sacrifice
14	allo/c	funct.	rejection (d 14)
15	allo/c	funct.	rejection (d 23)
16	allo/c	n.f.	ischemia; aberant SMA branch cut perop.
17	allo/c	n.f.	arterial + venous thrombosis; aberant SMA branch cut perop.
18	allo/c	funct.	ischemic necrosis (d 12)
19	allo/c	funct.	rejection (d 43)
20	allo/c	funct.	rejection (d 12)
21	allo/c	funct.	necrotizing pancreatitis (d 14)
22	allo/c	funct.	rejection (d 64)
23	allo/c	funct.	rejection (d 15)
24	allo/c	n.f.	venous thrombosis
25	allo/c	n.f.	arterial thrombosis
26	allo/c	dysf.	ischemia (d 2); aberant SMA branch cut perop.; aberant vein reanastomosed

dog		function	failure
27	allo/c	n.f.	ischemia e.c.i.
28	allo/c	funct.	arterial thrombosis (d 2)
29	allo/c	funct.	arterial thrombosis (d 7); aberant SMA branch reanastomosed
30	allo/c	funct.	intestinal ischemia after reanastomosis SMA (d 1)
31	allo/c	funct.	rejection (d 17)
32	allo/c	funct.	rejection (d 9)
33	allo/c	funct.	rejection (d 14)
34	allo/c	funct.	rejection (d 15); aberant SMA branch reanastomosed
35	allo/c	n.f.	arterial thrombosis
36	allo/c	funct.	rejection (d 19)
37	allo/c	n.f.	arterial thrombosis
38	allo/c	n.f.	arterial thrombosis; aberant SMA branch reanastomosed
39	allo/c	funct.	arterial thrombosis (d 5)
40	allo/c	funct.	rejection (d 10); aberant SMA branch reanastomosed
41	allo/c	unknown	p.o. abdominal bleeding
42	allo/c	funct.	rejection (d 23)
43	allo/c	funct.	rejection (d 40)
44	allo/c	funct.	rejection (d 17)
45	allo/c	n.f.	arterial thrombosis
46	allo/c	n.f.	thrombosis of pancreatic artery and of SMA after reanastomosis
47	allo/c	funct.	rejection (d 14); aberant SMA branch reanastomosed
48	allo/c	funct.	rejection (d 22)
49	allo/c	funct.	rejection (d 10); aberant SMA branch reanastomosed

auto= autograft; allo= allograft; g= transplanted to the groin; c= cervical transplantation; funct.= postoperative function; dysf.= per- and postoperative dysfunction; n.f.= primary non-function; SMA= superior mesenteric artery; d= postoperative day; w= postoperative week.

Table 4.2. Primary postoperative function of 49 autologous and allogeneic subcutaneous pancreas transplants in dogs

	n	%
good function	33	67%
dysfunction	3	6%
non-function	12	25%
unknown function	1	2%
	49	100%

Table 4.3. Causes of failure of 49 autologous and allogeneic subcutaneous pancreas transplants in dogs

	n	%
lost for follow-up:		
thrombosis	12	25%
ischemia	6	12%
traumatic disruption of blood vessels	1	2%
pancreatitis	2	4%
intestinal ischemia	1	2%
abdominal bleeding	1	2%
sudden death e.c.i.	1	2%
narcosis	1	2%
useful for follow-up:		
fibrosis	3	6%
rejection	18	37%
sacrifice	3	6%
	49	100%

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CHAPTER 5
EFFECT OF PREOPERATIVE BLOOD TRANSFUSIONS ON ALLOGRAFT SURVIVAL

5.1. INTRODUCTION

In the last decade pancreas graft survival has improved considerably, mainly as consequence of a better transplantation technique [1]. Rejection is still a major problem which is manifested in a steep decline in graft survival percentage in the first year after grafting [2]. There is reluctance in performing pancreas transplantation in early stages of type I diabetes with the aim to prevent secondary diabetic lesions. Beside a low risk transplantation technique, a second requirement for such an endeavour is obvious but difficult to attain: an effective immunosuppressive treatment of low toxicity, which can be safely given for a long period. In this chapter the results with preoperative blood transfusions as a form of immunosuppressive treatment with no or little toxicity are reported.

Preoperative treatment of the recipient with blood transfusions has proven to be a good adjunct to immunosuppression with drugs in other organ transplantations [3][4][5]. However, little is known about the effect of blood transfusions on pancreas graft survival. In our experiments blood transfusions were given alone or combined with low-dose immunosuppressive drugs; in rats with Cyclosporin A (CsA) and in dogs with azathioprine and prednisolone. In previously reported kidney transplantation experiments in the same dog model as we used, the effect of preoperative blood transfusions on kidney graft survival has been demonstrated, provided it was combined with postoperative treatment with azathioprine and prednisolone and not with low-dose CsA [6]. This was the reason to combine the transfusions in dogs with conventional azathioprine/prednisolone treatment in order to demonstrate whether they might have any effect.

5.2. EXPERIMENTAL PROTOCOL

5.2.1. Rats

CsA and pancreas graft survival

The effect of different doses of CsA on pancreas allograft survival was investigated, in order to determine the most useful dose in further investigations. Group I (n=16):

untreated controls. Groups IIa,b and c were treated with different numbers of intramuscular shots of 15 mg/kg CsA: group IIa (n=9): one shot of CsA post-operatively (day 0); group IIb (n=6): two shots on day 0 and day 3 postoperatively; group IIc (n=4): 3 shots (days 0, 3 and 6).

Blood transfusions with CsA and pancreas graft survival

Group I (n=16) consisted of untreated controls. In group IIa (n=9) rats were treated with 15 mg/kg CsA on day 0. Recipients were given three syngeneic blood transfusions in group III (n=7), one donor-specific blood transfusion (DST) in group IV (n=12) and three DST's in group Va (n=12). Group VII (n=8) received one preoperative DST and CsA at day 0. Blood transfusions were given at weekly intervals before transplantation, the first one week after induction of diabetes with streptozotocin. Transplantation was performed one week after the last transfusion.

Timing of blood transfusions in relation to induction of diabetes and transplantation

To exclude any effect of the diabetic state of the recipient during the preoperative blood transfusions on the outcome of graft survival, blood transfusions were given after and before injection of streptozotocin. Group Va (n=12): streptozotocin on week −4, DST's on weeks −3, −2 and −1 before transplantation. Group Vb (n=6): DST's on weeks −5, −4 and −3, streptozotocin on week −2 before transplantation. Group Vc (n=6): streptozotocin on week −6, DST's on weeks −5, −4 and −3 before transplantation.

Diabetes and allograft survival

To investigate whether diabetes or diminished glucose homeostasis might have any effect on graft survival, allogeneic heart grafts were transplanted heterotopically in rats as described before [7], with different pretreatment protocols. Experimental groups: VII (n=6): controls without pretreatment. Groups VIII and IX: induction of diabetes in recipients by streptozotocin, 3 weeks before allogeneic heart transplantation. Group VIII (n=5): isogeneic pancreas transplantation one week before heart transplantation, to restore normoglycemia. Group IX (n=8): sham operation, by small bowl resection one week before heart transplantation. Rejection of heart grafts was determined by absence of palpable abdominal heart contractions.

5.2.2. Dogs

There were three experimental groups: group I (n=7): non-transfused, splenectomized, Azathioprine/Prednisolone (Aza/Pred) treatment; group II (n=7): transfused, spenectomized, Aza/Pred treatment; group III (n=4): transfused, non-splenectomized, Aza/Pred treatment.
In all but four dogs splenectomy was performed after transsection of the spenic vessels. In four dogs (group III) the spleen was kept in place, leaving the short gastrosplenic vessels for vascularisation. This was done to investigate whether the spleen plays a predominant role in the transfusion phenomenon as was demonstrated in previous experiments in dogs [8].

5.3. RESULTS

5.3.1. Rats

CsA and pancreas graft survival

The results of CsA treatment are shown in Table 5.1. and Figure 5.1.. There was a significant prolongation of graft survival after treatment with only one dose of 15 mg/kg CsA (17 ± 4 vs 12 ± 2 in controls, $p<0.01$) and two or three shots were able to further extend graft survival (26 ± 12 and 44 ± 20 days respectively).

Blood transfusions with CsA and pancreas graft survival

The effects of blood transfusion pretreatment on allogeneic pancreas transplantation with or without CsA postoperatively are shown in Table 5.2.. Subcutaneous pancreas grafts in animals pretreated with three syngeneic blood transfusions (group III) survived as long as in untreated animals (group I): 12 ± 1 and 12 ± 2 days respectively. Allogeneic donor-specific blood transfusions prolonged graft survival significantly to more than 23 ± 15 days using one DST and more than 29 ± 15 with three DST's ($p<0.01$ in both groups compared to groups I and III). However, donor-specific blood transfusions resulted only once in "permanent" (>60 days) graft survival, while three blood transfusions gave no better result than one. Pancreas grafts did benefit from one postoperative dose of CsA as shown before (group IIa). Blood transfusion combined with low-dose CsA seemed to work additionally, but the result (>34 ± 20 days in group VI) was not significantly different from the control groups with monotherapy (group IIa and IV). However, significantly more grafts survived longer than 23 days (the longest survival time in the CsA-treated group), compared to groups IIa and IV ($p<0.05$; chi-square test).

Timing of blood transfusions in relation to induction of diabetes and transplantation

As described before, three preoperative donor specific blood transfusions to diabetic recipients significantly prolonged pancreas graft survival. If the transfusions were administered before the induction of diabetes, the effect was nearly completely abrogated (group Vb vs Va: 16 ± 2 and >29 ± 15 days respectively, $p<0.02$, Table 5.3.). However, if the recipients were treated with blood transfusions after induction of diabetes, but the pancreas was transplanted 3 weeks after the last transfusion as in group Vb, also the transfusion effect was abrogated (group Vc vs Va: 13 ± 2 and >29 ± 15 days, respectively, $p<0.01$).

Diabetes and allograft survival

Heterotopic heart grafts in plain recipients survived 8–9 days (group VII, Table 5.4.). If they were transplanted to recipients, three weeks after induction of diabetes with streptozotocin, but with a succeeded subcutaneous isogeneic pancreas graft, heart grafts survived 9.6 ± 2.9 days (group VIII, range 7–14), which is not different to group VII. One heart graft had a prolonged survival, but it was in a recipient with a fulminant pancreatitis during follow–up. In group IX the heart grafts were transplanted in diabetic rats, not receiving a pancreas isograft, but undergoing a sham operation (small bowl resection) one week before transplantation. The diabetic state had no influence on heart graft survival (8.5 ± 0.5 days).

Table 5.1. The effect of Cyclosporin A on subcutaneous pancreas allograft survival in rats

group	n	Cyclosporin A	graft survival (days)	MST ± SD
I	16	——	9,9,10,10,10,10,11,11, 11,12,12,12,13,14,15,16	12 ± 2
IIa	9	day 0	10,14,15,18,18,18,19, 20,23	17 ± 4
IIb	6	day 0 + 3	12,20,24,24,30,46	26 ± 12
IIc	4	day 0 + 3 + 6	16,45,54,60	44 ± 20

Cyclosporin A doses: 15 mg/kg; MST ± SD = mean graft survival time ± standard deviation in days.

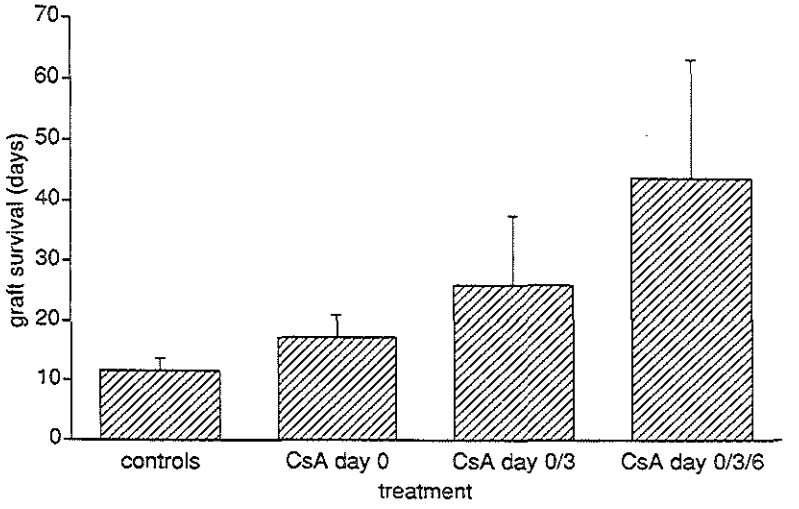

Figure 5.1. The effect of Cyclosporin A (15 mg/kg/gift) on subcutaneous pancreas allograft survival in rats.

Table 5.2. The effect of donor specific blood transfusions and Cyclosporin A on pancreas graft survival in rats

group	n	transfusion	CsA	graft survival (days)	MST ± SD
I	16	–	–	9,9,10,10,10,10,11,11, 11,12,12,12,13,14,15,16	12 ± 2
IIa	9	–	+	10,14,15,18,18,18,19, 20,23	17 ± 4
III	7	3x syn. tr.	–	10,11,11,12,12,12,13	12 ± 1
IV	12	1x DST	–	13,14,14,15,16,17,18,18, 19,22,46,>60	>23 ± 15
Va	12	3x DST	–	15,16,17,18,20,23,24,27, 31,46,49,>60	>29 ± 15
VI	8	1x DST	+	14,14,16,26,33,51,>60,>60	>34 ± 20

3x syn. tr.= syngeneic blood transfusions (WAG) at days –21,–14,–7; 1x and 3x DST= donor specific blood transfusions (BN) at days –7 and –21, –14, –7 respectively; CsA= 15mg/kg Sandimmune^{R} i.m. at day 0. MST ± SD = mean graft survival time ± standard deviation in days.
Wilcoxon's rank sum test: group IIa vs I $p<0.01$; IV vs I $p<0.01$; Va vs I $p<0.01$; Va vs IV: N.S.; VI vs I $p<0.01$; VI vs IIa and IV: N.S.

Table 5.3. The effect of timing of preoperative blood transfusions on pancreas allograft survival

group	n	treatment	graft survival (days)	MST ± SD
I	16	----	9,9,10,10,10,10,11,11, 11,12,12,12,13,14,15,16	12 ± 2
Va	12	diab --> 3x DST --> Pax	15,16,17,18,20,23,24, 27,31,46,49,>60	>29 ± 15
Vb	6	3x DST --> diab --> Pax	13,15,15,17,17,18	16 ± 2
Vc	6	diab --> 3x DST --> 3 weeks --> Pax	10,11,13,13,13,15	13 ± 2

diab = induction of diabetes with streptozotocin; 3x DST = donor specific blood transfusions at weekly intervals; Pax = allogeneic pancreas transplantation; 3 weeks = 3 weeks interval between last transfusion and transplantation; MST ± SD = mean graft survival time ± standard deviation in days. Wilcoxon's rank sum test: Vb vs Va: $p<0.02$; Vc vs Va: $p<0.01$.

Table 5.4. Effect of streptozotocin induced diabetes on heterotopic allogeneic heart transplantation

group	treatment	graft survival (days)	MST ± SD
VII	Hax	8,8,8,9,9,9	8.5 ± 0.5
VIII	diab --> Psx --> Hax	7,8,8,11,14*	9.6 ± 2.9
IX	diab --> sham --> Hax	8,8,8,8,9,9,9,9	8.5 ± 0.5

Hax = allogeneic heart transplantation; diab = induction of diabetes with streptozotocin (week -3); Psx = syngeneic pancreas transplantation (week -1); sham = laparotomy and small bowl resection (week -1); MST ± SD = mean heart graft survival time ± standard deviation in days; * = rat with severe graft pancreatitis.

5.3.2. Dogs

The results of the effects of blood transfusions on pancreas graft survival in dogs are summarized in Table 5.5.. Dogs treated postoperatively with azathioprine and prednisolone rejected their grafts after 17 ± 5 days. When they were pretreated with three third-party blood transfusions the mean graft survival was 29 ± 19 days, which is not significantly different from the control group. However, 3 out of 7 grafts were rejected beyond the normal range of the control group (40, 43 and 64 days after operation), and this occurred 3–7 days after azathioprine had to be withdrawn because of leukopenia. In group III the spleen had been preserved to exclude a negative effect of splenectomy on the blood transfusion effect. The results were not significantly different from those in group II (14 ± 3 days), but there were no prolonged survivors at all.

Table 5.5. Graft survival of subcutaneous pancreas transplantation in dogs. The effect of preoperative third party blood transfusions and peroperative splenectomy

group	n	transfusions	splenectomy	graft survival	MST + SD
I	7	-	+	10, 14, 14, 17, 19, 22, 23	17 ± 5
II	7	+	+	10, 12, 15, 23, 40*, 43*, 64*	29 ± 19
III	4	+	-	9, 14, 15, 17	14 ± 3

MST ± SD = mean graft survival time ± standard deviation in days; *= azathioprine discontinued.

5.4. DISCUSSION

One of the necessities of pancreas transplantation, especially in young diabetics is long-term mild, i.e. non-toxic immunosuppression. Preoperative blood transfusions in combination with immunosuppressive drugs have been shown to be of great benefit for kidney and heart allografts. Doubt has recently risen about the role of blood transfusions in kidney transplantation, because of the dominating role of Cyclosporin

A, which results in excellent graft survival [9][10]. In pancreas transplantation good, long-term results are lacking, if strong multiple immunosuppressive drug schedules are omitted, so that there is need for additional immunosuppression, possibly by blood transfusions. However, the effect of blood transfusions in pancreas transplantation, mostly in rats, has been the subject of only a few investigations.

Sutherland mentioned that the administration of blood transfusions in humans led to a better graft survival, however, without reporting the exact data [11]. In rats Perloff et al. did not find any blood transfusion effect on pancreas or skin allografts, whereas such an effect was observed for heart grafts [12]. Islet allografts may benefit from donor specific, but not from third party blood transfusions in rats [13]. Other groups had to deplete the blood from Ia-bearing cells by U.V.-irradiation [14] or by anti Ia-serum [15] to have any immunosuppressive effect. Selawry et al. demonstrated a DST effect on islet allografts in rats only in combination with ALS [16].

In the experiments presented in this chapter the effect of blood transfusions on pancreas allograft survival was rather disappointing. While one or more DST's resulted in 100% permanent heart and 60% permanent kidney allograft survival in previous experiments performed in the same donor-host rat combination [17][18], one or three DST's improved pancreas graft survival, but only led to permanent long-term graft survivors in 1 out of 12 cases in each group. CsA monotherapy prolonged graft survival significantly in different doses, but the mean graft survival after one DST was not further prolonged significantly by addition of low-dose CsA compared to both monotherapies. However, significantly more grafts survived beyond the range of CsA-treated recipients (>23 days). Cooke et al. also found a disparity between heart and pancreas grafts with a comparable protocol. They were able to prolong pancreas graft survival slightly significantly with short-term CsA, but not with preoperative DST's. However, there was no additional role of CsA to preoperative blood transfusion treatment, in contrast to heart transplantation, in which both therapies were additive [19].

In the preclinical dog-model the results were comparable: the blood transfusion protocol, proven to be effective in kidney grafts with 60% surviving grafts beyond 60 days [6], appeared to be less effective to pancreas grafts. However, it should be noted, that three out of seven dogs might have become longer survivors if not the immunosuppression had to be withdrawn partially due to azathioprine induced leukopenia. We have no explanation for the high rate of leukopenia, especially not because it occurred in one of the two groups in which the spleen had been removed, whereas splenectomy is supposed to diminish the likelihood of development of

azathioprine–induced leukopenia [20]. Recently, our protocol in dogs was also used by others [21]. They came to the same conclusions as we did: no significant improvement of graft survival by preoperative blood transfusions with Aza/Pred treatment. But they were able to prolong their pancreas graft survival, if they combined their treatment with antirejection therapy with prednisolon bolus injections when serum glucose rose. This treatment of rejection crises was effective in 71% of the cases in the blood transfusion pretreatment group, but only in 25% of the crises in the non–pretreated group, resulting in a significant prolongation of graft survival. So, blood transfusions might protect the graft by less severe rejection crises.

Perioperative splenectomy has been found to abrogate the blood transfusion effect in kidney graft recipients in previous experiments performed by our group [8]. Also in mice the spleen appeared to be a necessity to induce the blood transfusion effect [22], although other groups could not demonstrate an abrogation of the transfusion effect by splenectomy [23][24]. To exclude this phenomenon as the possible cause of the disappointing results of blood transfusions in the pancreas experiments we preserved the spleen in four dogs. Surprisingly, in this group pancreas grafts survived as long as in non–transfused controls. However, the timing of splenectomy may play a crucial role. Marquet et al. performed the splenectomy one week after the last transfusion and one week before kidney transplantation [8], while in the present experiment the spleen was dissected during transplantation of the pancreas. The immunological system, among others the suppressor–cell activity, is affected in diabetic patients, not only due to the autoimmune disease, but also dependent on the degree of metabolic control [25]. Therefore, induction of enhancement by blood transfusions might be impaired in diabetic animals. However, if blood transfusions were administered before the induction of diabetes in rats, the transfusion effect was almost completely abrogated. The conversion of sequence of streptozotocin and blood transfusions led to a delay of time between the last transfusion and transplantation of 3 weeks. But also when blood transfusions were given to diabetic animals, followed by a 3 week interval, the transfusion effect was abrogated. So, the interval between the last blood transfusion and transplantation plays a predominant role on the effect of blood transfusions on pancreas allograft survival in this rat model. The effect of the diabetic state during transfusion seemed to be of rather unimportance, but could not be excluded. In dogs the transfusions were administered to non–diabetic animals, so, neither in this model the moderate transfusion effect could be ascribed to diabetes. To exclude whether a diabetic state of the recipient was influencing the outcome of allograft survival in rats, heart grafts were transplanted in diabetic animals or animals, normoglycemic with a successful pancreas isograft. In these groups there was no

difference in graft survival compared to untreated controls. Remarkable was heart graft survival of 14 days in one of the rats bearing a pancreas isograft with a necrotizing pancreatitis, but which not yet resulted in hyperglycemia. The severe inflammation might be the cause of the prolonged survival in this rat.

Conclusion

The blood transfusion effect on pancreas graft survival is quite moderate in comparison to other vascularized grafts. This difference in reactivity to the detriment of the pancreas graft has also been demonstrated using other enhancing procedures [26][27]. Therefore, it should be considered whether this moderate effect will out-weigh the negative effects of blood transfusions, such as the risks of sensitisation and transfusion reactions.

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CHAPTER 6
ATTEMPT TO REDUCE CLASS II ANTIGENS IN THE PANCREAS GRAFT AND ITS EFFECT ON THE SURVIVAL OF ALLOGRAFTS

6.1. INTRODUCTION

The major histocompatibility complex (MHC) encodes for antigens, that play important roles in the immune response which results in rejection. Particularly the class II MHC antigens are major stimulatory antigens in the rejection process. Matching for these antigens results in prolongation of survival of several types of grafts [1][2][3]. Mixed lymphocyte reactions (MLR), which are in vitro models of transplantation immune reactions, are stimulated by class II–positive cells, which can be blocked by anti–class–II antibodies [4][5]. The bone–marrow derived class II–positive antigen presenting cell – the dendritic cell – plays an important role in triggering the rejection cascade. Blocking or eradication of these cells in the graft can delay the rejection process [6].

Davies and Alkins were the first to demonstrate that the survival of MHC–incompatible rat hearts could be prolonged by injecting recipients with antibodies directed against the graft class II antigens [7]. Also others have shown that elimination of class II–positive antigen presenting cells – more specifically, dendritic cells – from organ grafts reduces the graft's immunogenicity, which results in prolonged or even indefinitive graft survival in various animal models. Several methods of donor pretreatment have been used, for example culture of allogeneic tissue at low temperature [8] or in 95% oxygen [9]; gamma or u.v. irradiation [10][11]; pretreatment of allografts with monoclonal antibodies directed against class II–positive cells [12][13], or more specifically, dendritic cells [14], and donor pretreatment with blood [15] or immunosuppressive drugs [16][17].

In experimental models the pancreas has proven to be more susceptible to rejection than other vascularized allografts [18][19]. Enhancement procedures, treatment with conventional immunosuppressives or with cyclosporine A (CsA) and combinations of these therapies are less effective in pancreatic grafts [20][21][22][23]. The aim of the study described in this chapter was to investigate whether donor pretreatment of the vascularized pancreas can diminish the immunogenicity of the graft and so can decrease the necessity of high doses of immunosuppressive drugs.

6.2. MATERIALS AND METHODS

Only the rat model was used in this study. Grafts were pretreated, using two procedures:
1. whole body irradiation of the BN donors with 10 Gy, 5 days before transplantation.
2. perfusion and incubation of BN pancreas grafts with monoclonal antibody F 17.23.2 (reacting with class II of the donor, not of the recipient). After isolation of the graft and removal of residual blood with HBSS, the graft was gently perfused and incubated with undiluted F 17.23.2 tissue culture supernatant for 30 minutes in vitro at 4°C. After incubation the graft was washed again by HBSS perfusion and used for immuno-histology or transplantation.

Immunohistology

The effect of F 17.23.2 pretreatment or total body irradiation on the expression of class II antigens was assessed by immunohistology using the peroxidase-antiperoxidase (PAP) staining technique as described in chapter 3. The effect of irradiation on the content of dendritic cells in the pancreas was compared to the effect in heart grafts using PAP staining with the anti-class II monoclonal antibodies F 17.23.2 and OX 6. The number of class II positive cells was counted per mm2 in irradiated (n=3) and non-irradiated (n=3) grafts. Grafts pretreated with F 17.23.2 (n=3) were stained according to the PAP technique, but with the omission of the monoclonal antibody, in order to confirm a binding of the antibody to the dendritics by the perfusion procedure.

Transplantation

Experimental groups: Group I (n=8) consisted of non-treated controls, whereas the rats in group II (n=9) were treated with CsA. Grafts of group III (n=4) were pretreated with F 17.23.2. Donors were irradiated 5 days before transplantation in group IV and V; recipients were not treated postoperatively (group IV, n=5) or treated with CsA (group V, n=8). CsA treatment consisted of single dose of 15 mg/kg on the day of transplantation.

6.3. RESULTS

The number of class II positive cells before and 5 days after irradiation in the pancreas and the heart is shown in Figure 6.1.. There was a reduction of cells in the pancreas of 80-85% (49 ± 6 to 11 ± 2 cells/mm^2 with F 17.23.2 staining, and

34 ± 2 to 5 ± 1 cells/mm^2 with OX 6 staining before and after irradiation, respectively). In heart grafts the number of class II positive cells was reduced with about 90% (24 ± 4 to 2.5 ± 0.5 cells/mm^2 with F 17.23.2 staining, and 22 ± 1 to 2 ± 1 with OX 6 staining before and after irradiation, respectively).

When the antibody step in the PAP-staining procedure on F 17.23.2 perfused grafts was omitted, there were no class II positive cells visualized at all. This was in contrast to slides in which the PAP-staining was completed with the monoclonal antibody, indicating an absence of efficacy of the perfusion. The results concerning graft survival are summarized in Table 6.1.. Untreated pancreas grafts survived 10 ± 1 days (group I). Pretreatment with monoclonal antibodies (group III), as expected, did no result in prolonged graft survival. Although there was a reduction of class II positive cells in irradiated pancreas grafts, it did not prolong graft survival in non-CsA-treated recipients (11 ± 1 and 10 ± 1 days, respectively). Low-dose CsA treatment (group II) led to a slight but significant prolongation of graft survival (14 ± 2 days, $p<0.01$ compared to group I). Surprisingly, pretreatment of the donor with 10 Gy led to annihilation of the immunosuppression induced by CsA (12 ± 1 days, $p<0.05$, compared with group II).

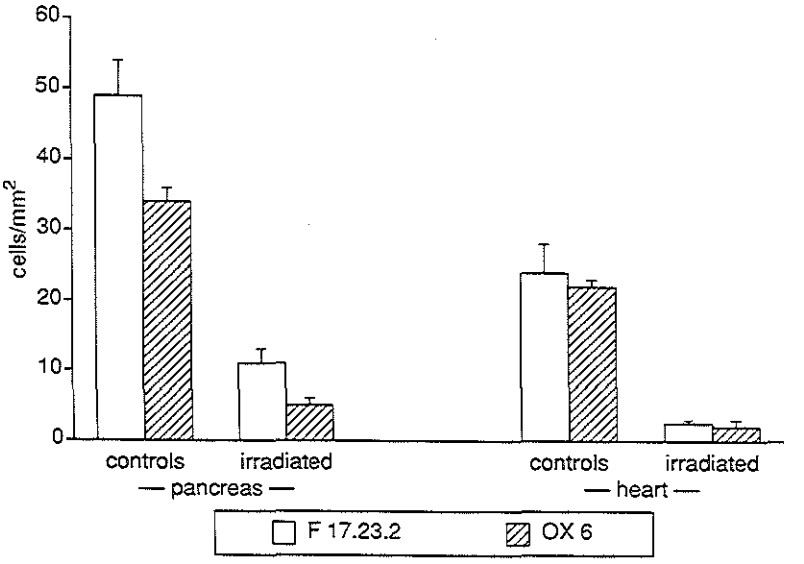

Figure 6.1. Effect of irradiation of BN rats on the content of class II positive dendritic cells in pancreas and heart. "Controls": non-irradiated rats. "Irradiated": irradiated rats five days before counting.

Table 6.1. The effect of donor pretreatment with anti-class II monoclonal antibodies and irradiation on pancreas graft survival in rats

group	n	donor pre-treatment	recipient treatment	graft survival (days)	MST ± SD
I	8	--	--	9,9,10,10,10,10,11,12	10 ± 1
II	9	--	CsA	11,13,13,13,14,14,14, 16,16	14 ± 2
III	4	F17.23.2	--	10,10,11,12	11 ± 1
IV	5	10 Gy	--	10,10,10,11,11	10 ± 1
V	8	10 Gy	CsA	11,11,11,12,12,12,14,14	12 ± 1 *

F17.23.2 = anti class II antibody perfusion on the day of transplantation; 10 Gy = 10 Gy gamma irradiation of the donor on day -5; CsA = 15 mg/kg dose of CsA on day of transplantation; MST ± SD = mean graft survival time ± standard deviation; * $p < 0.05$ compared to group II.

6.4. DISCUSSION

Many investigations have been performed dealing with the role of class II positive dendritic cells in the rejection process. In several experimental models it has been shown that elimination of the dendritic cells can prolong allograft survival, whereas restoration with these cells induces rejection [6][14][24].

In our transplantation model we were not able to impair immunogenicity of the graft by reduction of class II positive cells by irradiation. Perfusion of the graft with the F 17.23.2 monoclonal antibody was not effective, although the technique used was the same as found to be effective by others using kidney and heart allografts [12][13][25]. Most probably, the monoclonal antibody that we used was not able to diffuse through the wall of the bloodvessels into the interstitium. Both methods, irradiation and perfusion, have been used in many experimental models, but there is much variability in effectiveness. The results are dependent on the type of graft and the animal model used. Of course, the main condition is that enough class II antigens of the dendritic cells are occupied by monoclonal antibodies or are destructed by irradiation to annihilate their function in the rejection process. With regard to the irradiation experiments, the technique is widely accepted to produce almost complete destruction of lymphoid tissue, with total suppression of immunological capacities

[26]. Gamma-irradiation with 10 Gy has been shown to induce more than 95% elimination of dendritic cells, resulting in prolonged graft survival, and to work synergistically with low-dose CsA in some islet transplantation models [27][28]. However, in the rat model that we used, still 15-20% of the target cells in the pancreas and 10% in the heart were left, apparently enough to initiate rejection. It has been reported that at least 90-95% of the class II positive cells have to be eliminated to impair graft immunogenicity [10][27]. Lloyd et al. were also unable to prolong pancreas graft survival after irradiation. They used a pretreatment schedule of cyclophosphamide with 10 Gy gamma irradiation, resulting in 10-15% of the dendritic cells still present in the graft at the time of transplantation [29].

Other arguments for failure of anti-class II graft pretreatment in pancreas transplantation are that class I antigens may have a initiating role in the rejection process. If the preferential way by class II antigens is blocked or that the donor dendritic cell function is taken over by those of the recipient [30][31][32] [33]. Also minor histocompatibility antigens are supposed to be able to initiate the rejection in pancreas transplantation. Although in the rat grafts such as kidney and cardiac allografts are only rejected if transplanted across a major histocompatibility barrier, the pancreas still can be rejected if transplanted across an incompatibility for only minor antigens [2]. This would mean that the rejection process can follow other pathways, bypassing the dendritic cells.

Elimination of the immunosuppressive effect of CsA by donor irradiation has never been described before. However, in our study the mean graft survival of the combined therapy was slightly diminished compared to the CsA group ($p=0.045$), which is an observation we can not explain.

Conclusion

We were not able to suppress graft immunogenicity by alteration or depletion of antigen presenting cells in the vascularized pancreas graft by the use of anti-class II monoclonal antibodies or by irradiation. Perfusion of the graft with monoclonal antibodies was technically not successful, and irradiation only caused a 80-85% reduction of class II positive cells in pancreas grafts, not resulting in prolonged graft survival.

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CHAPTER 7
A COMPARISON BETWEEN SUBCUTANEOUS AND INTRAPERITONEAL PANCREAS TRANSPLANTATION IN RATS

7.1. INTRODUCTION

The subcutaneous location of the pancreas graft might have adverse, site-specific immunological and functional consequences. Little is known about the immunological aspects of subcutaneously transplanted allografts. In rats a detrimental effect has been described if heart grafts were transplanted subcutaneously instead of intraperitoneally [1]. We made a comparison between subcutaneous and intraperitoneal pancreas grafts, based on allograft survival and histology.

7.2. EXPERIMENTAL PROTOCOL

7.2.1. Immunological study

An immunological comparison was made between subcutaneous and intraperitoneal pancreas transplantation by determination of graft survival in the BN to WAG rat model. Recipients of subcutaneous grafts in group I (n=16) and intraperitoneal grafts in group II (n=14) were non-treated. Groups III and IV were treated preoperatively with one donor specific blood transfusion (DST) and groups V and VI with three DST's. Groups III (n=12) and V (n=12) received subcutaneous grafts, IV (n=11) and VI (n=12) intraperitoneal grafts. Groups VII (subcutaneous grafts, n=6) and VIII (intraperitoneal grafts, n=7) were treated postoperatively with 15 mg/kg CsA at day 0 and 3.

7.2.2. Histological study

To determine the morphological differences between subcutaneous and intraperitoneal pancreatic transplants 18 rats received a pancreas transplant: 6 rats a subcutaneous syngeneic transplant, 6 a subcutaneous allograft and 6 an intraperitoneal allograft. Histological examination was performed after sacrifice at days 2, 4, 6, 8 ,10 and 12 after transplantation.

7.3. RESULTS

7.3.1. Immunological study

The comparison of graft survival between subcutaneously and intraperitoneally transplanted allografts in rats is presented in Table 7.1.. Untreated rats had a mean graft survival of 12 days (groups I and II), independent of the site of the transplant. After pretreatment with one donor specific blood transfusion, which resulted in prolonged graft survival (groups III and IV, 23 ± 15 and 15 ± 3 days respectively, $p<0.01$ compared to the respective controls), there was no significant difference in mean graft survival in relation to graft location. However, subcutaneous pancreas grafts survived longer than intraperitoneal grafts after three preoperative donor specific blood transfusions: >29 ± 15 vs 17 ± 7 days respectively ($p<0.01$). In both groups, treated with one and three DST's, there were more survivors beyond 16 days (the maximum in the control groups) of subcutaneous than intraperitoneal grafts; 7/12 versus 2/11 in the one DST group and 10/12 versus 2/12 in the three DST's group (X^2–test $p<0.02$). Finally, treatment with two doses of CsA resulted in prolonged graft survival (groups VII and VIII, 26 ± 11 and 20 ± 4 days respectively, $p<0.01$ compared to their controls), although graft location did not influence the outcome.

7.3.2. Histological study

In subcutaneous syngeneic as well as allogeneic pancreas grafts, there was more necrosis during the first week and subsequently more fibrosis than in intraperitoneal allografts. The necrosis and fibrosis was most abundant in the subcapsular part of the subcutaneous graft and the peripancreatic tissue. In more than 50% of the subcutaneous grafts (syngeneic as well as allogeneic) abscesses, especially subcapsular, were found, in contrast to the intraperitoneal grafts, in which no abscesses were seen. Compared to intraperitoneal allografts, subcutaneous allografts showed earlier deterioration of the exocrine and endocrine tissue. Comparison of subcutaneous syngeneic versus allogeneic grafts showed longer preservation of exocrine and endocrine tissue in the syngeneic grafts.

Table 7.1. Comparison of subcutaneous versus intraperitoneal pancreas graft survival

group	n	treatment	site	graft survival	MST ± SD
I	16	----	s.c.	9,9,10,10,10,10,11,11, 11,12,12,12,13,14,15,16	12 ± 2
II	14	----	i.p.	10,10,10,10,11,12,12,12, 12,12,13,13,14,14	12 ± 1
III	12	1 DST	s.c.	13,14,14,15,16,17,18,18, 19,22,46,>60	23 ± 15
IV	11	1 DST	i.p.	12,12,14,14,14,15,15,16, 16,17,22	15 ± 3
V	12	3 DST	s.c.	15,16,17,18,20,23,24,27, 31,46,49,>60	>29 ± 15
VI	12	3 DST	i.p.	10,14,14,14,14,15,15,15, 15,16,25,38	17 ± 7
VII	6	CsA	s.c.	12,20,24,24,30,46	26 ± 11
VIII	7	CsA	i.p.	15,19,19,19,19,20,29	20 ± 4

DST= donor specific blood transfusion; CsA= 15 mg/kg bwt Cyclosporin A day 0 + 3; s.c.= subcutaneous; i.p.= intraperitoneal; MST ± SD = mean graft survival time ± standard deviation. Wilcoxon's rank-sum test: III and VII vs I resp. IV and VIII vs II: $p<0.01$; I vs II, III vs IV and VII vs VIII: N.S.; V vs VI: $p<0.01$.

7.4. DISCUSSION

Recipients without postoperative treatment or with low-dose CsA therapy rejected subcutaneous or intraperitoneal pancreas grafts equally, independent of transplantation site. Previously, Marquet et al. [1] observed a shorter graft survival of subcutaneously transplanted rat heart grafts than intraperitoneally transplanted grafts. Whether this difference was due to an immunological or a mechanical cause, was beyond the scope of the investigation. In our study there seemed to be an opposite difference, if the recipients were pretreated with donor specific blood transfusions. Although the mean graft survival was not significantly different between the groups treated with one DST, graft survival after three DST's was more prolonged in the subcutaneous group than in the intraperitoneally transplanted group. In both pretreatment protocols (1 and 3 DST's) significantly more subcutaneous grafts had a prolonged survival (beyond 16

days), than intraperitoneal grafts. It is difficult to find good arguments to explain these differences. In studies on the blood transfusion effect using several experimental models, blood injected subcutaneously is found to have a different immunological, mainly sensitizing effect, whereas blood injected intravenously may result in immunosuppession [2]. This suggests that subcutaneous release of antigen induces an other immunological cascade. It might be that for grafts in the s.c. position not only the intravascular expression of tissue antigens, but also the subcutaneous way of antigen presentation plays a role, particularly after profuse release of antigen due to tissue autolysis. In the histological studies we found a more fulminant postoperative pancreatitis in the subcutaneous than in the intraperitoneal grafts. This non-specific inflammation might give rise to a different antigen expression or a different rejection pattern, resulting in the observed difference in graft survival.

During the first twelve postoperative days we observed more abscesses and necrosis in the subcutaneous grafts and faster deteriorating exocrine and endocrine tissue than in intraperitoneal grafts. This is in accordance with the observations of most other groups, in which the tissue damage mostly, but not always resulted in more primary graft failures [3][4][5]. The histological features of necrosis, abscesses and fibrosis, are also assumed to impair the endocrine reserve capacity, which results in rather poor long-term function of the subcutaneous grafts [6]. In intraperitoneal pancreas transplantation the peritoneum is able to resorb exocrine fluids leaking from minute ductal disruptions and cut lymphatics, which is not possible in the subcutaneous technique [3][4][6][7].

Whether the technique of duct-ligation in itself plays an additional role in the morphological and functional results is not quite sure. As described by Reemtsma et al., complications of post-transplant necrosis and thrombosis were overcome, if the duct was ligated 6 weeks before transplantation, resulting in complete fibrosis of the exocrine part of the graft [8]. Some groups found better results with duct-obliteration than with duct-ligation [4], but procedures with subcutaneous pancreas transplantation with open-duct drainage [5][6] or with enteral drainage [9] were described to have a comparable frequence of complications and failures.

Conclusion

In our rat model we were not able to find immunological differences to the detriment of subcutaneous pancreas transplantation, if compared to the intraperitoneal location. In contrast, after donor specific blood transfusions, graft survival was more often prolonged when the pancreas was in a subcutaneous than in an intraperitoneal site. Histologically the subcutaneous location seems to be a disadvantageous place, with

more abscesses and fibrosis than in the intraperitoneal grafts, which might protect the graft from rejection but ultimately may have a detrimental effect on graft fuction.

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CHAPTER 8
LONG-TERM FUNCTIONAL RESULTS OF SUBCUTANEOUS PANCREAS TRANSPLANTATION IN RATS AND DOGS

8.1. INTRODUCTION

Pancreas transplantation is not primarily, as in heart and liver transplantation, a live saving operation. It is an elective intervention, in order to improve quality of life, to reduce the development of diabetic complications and finally to improve patient survival. To avoid or reduce diabetic complications, a high level of glucose homeostasis should be achieved [1][2]. Therefore, the transplantation procedure not only must be safe, but also the transplant should have a long-term function at a high quality level.

In this chapter long-term functional results of syngeneic grafts in rats, and autologous pancreas grafts in dogs are discussed.

8.2. EXPERIMENTAL PROTOCOL

8.2.1. Rats

The following groups, including non-transplanted controls, were compared. Group I (n=9): non-transplanted non-diabetic contols; II (n=4): non-transplanted diabetic controls. Twenty-five WAG rats received a syngeneic subcutaneous pancreas transplant to perform IVGT-tests at the first (group III, n=6), second (IV, n=7), fifth (V, n=6) and ninth (VI, n=6) postoperative month. To exclude the influence of age, rats were also tested more than 6 months after a comparable operation, i.e. heart transplantation: group VII (n=4). Heterotopic intraperitoneal heart transplantation was carried out in non-diabetic rats as described before [3]. Functional studies on syngeneically transplanted grafts were performed using intravenous glucose tolerance tests (IVGTT), from which K-values were calculated. Pancreas grafts were sent for histological examination after sacrifice.

8.2.2. Dogs

Six dogs, which underwent autologous subcutaneous pancreas transplantation, were followed for more than one year. Transplantation was performed to the neck of the recipient, except for one dog, in which the pancreas was transplanted to the groin,

using the femoral vessels. Serum glucose levels were determined and IVGT–tests were carried out preoperatively and at regular intervals postoperatively (1, 4, 9, 21, 34, 52, 65 and 78 weeks). Grafts were sent for histological examination after sacrifice.

8.3. RESULTS

8.3.1. Rats

Functional results

Non–fasting serum glucose levels in syngeneically transplanted rats were within normal limits during the nine months observation time (Figure 8.1.). Streptozotocin induced diabetic rats had preoperative glucose values of 21.1 ± 1.3 mmol/l, which normalized to 6.8 ± 1.5 mmol/l the first day after transplantation and were stable up to nine months. After transplantectomy rats became diabetic again (18.6 ± 3.5 mmol/l).

However, if expressed in IVGTT–derived K–values, graft function deteriorated remarkably (Table 8.1./ Figure 8.2.). Preoperative diabetic K–values (0.61 ± 0.08 %/min) returned to normal non–diabetic values postoperatively. One and two months after transplantation K–values (2.47 ± 0.58 and 2.13 ± 0.53 %/min resp.) were not significantly different from normal values (2.31 ± 0.39 %/min). However, at 5 and 9 months after transplantation mean K–values had significantly deteriorated (1.32 ± 0.26 and 1.04 ± 0.26 %/min resp.) compared to the preoperative and 1–month values ($p<0.001$). To exclude the influence of age of the rats, IVGT–tests were done in rats more than 6 months after heart transplantation. Also compared to this group (2.23 ± 0.26 %/min) K–values had significantly deteriorated ($p<0.001$).

Histology

On histological examination most tissue was occupied by necrosis and abscesses, leaving only small parts of fibrotic pancreatic tissue. Microscopically a polymorphonuclear infiltrate, reflecting post–transplant pancreatitis, was observed up to one month, whereafter the inflammation extinguished. At the same time the maximum of fibroblastic activity was overcome, although the degree of fibrosis still increased up to nine months, resulting in total disappearance of the exocrine acinar tissue. Islets of Langerhans could still be observed at nine months, although in course of time the configuration of islet cells became less coherent.

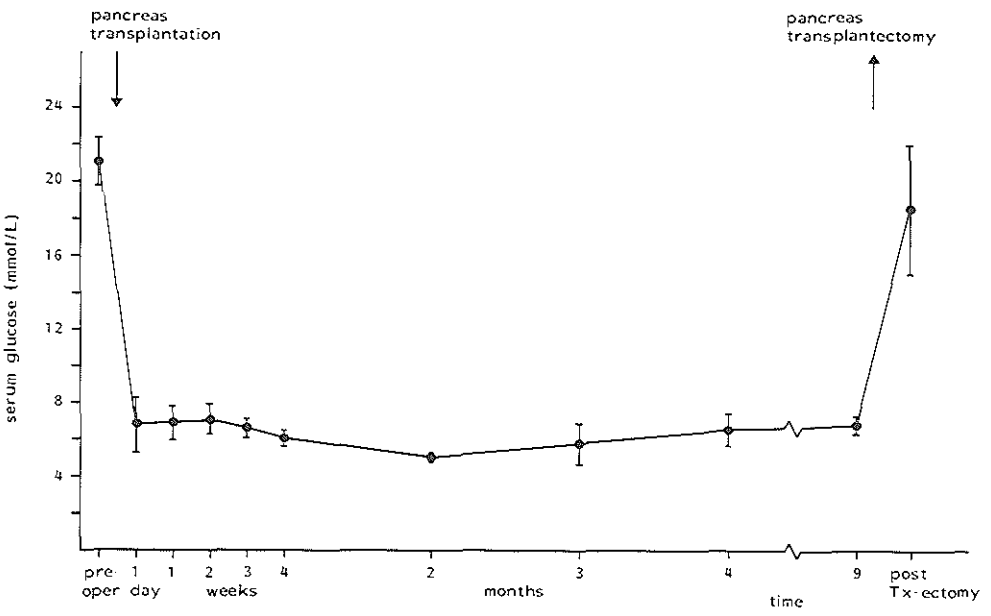

Figure 8.1. Serum glucose levels ± standard deviations before and after subcutaneous pancreas transplantation and after transplantectomy in streptozotocin induced diabetic WAG rats receiving syngeneic pancreas transplants (n=5).

Table 8.1. Mean K-values before and after syngeneic subcutaneous pancreas transplantation in rats

group	n		mean K-value ± SD (%/min)	Student's-t-test
I	9	normal rats	2.31 ± 0.39	
II	4	diabetic rats	0.61 ± 0.08	II vs I $p<0.001$
III	6	1 month p.Px	2.47 ± 0.58	
IV	7	2 months p.Px	2.13 ± 0.53	
V	6	5 months p.Px	1.32 ± 0.26	V vs III $p=0.001$
VI	6	9 months p.Px	1.04 ± 0.26	VI vs III $p<0.001$
VII	4	>6 months p.Hx	2.23 ± 0.26	

p.Px/p.Hx = post pancreas resp. heart transplantation; SD = standard deviation.

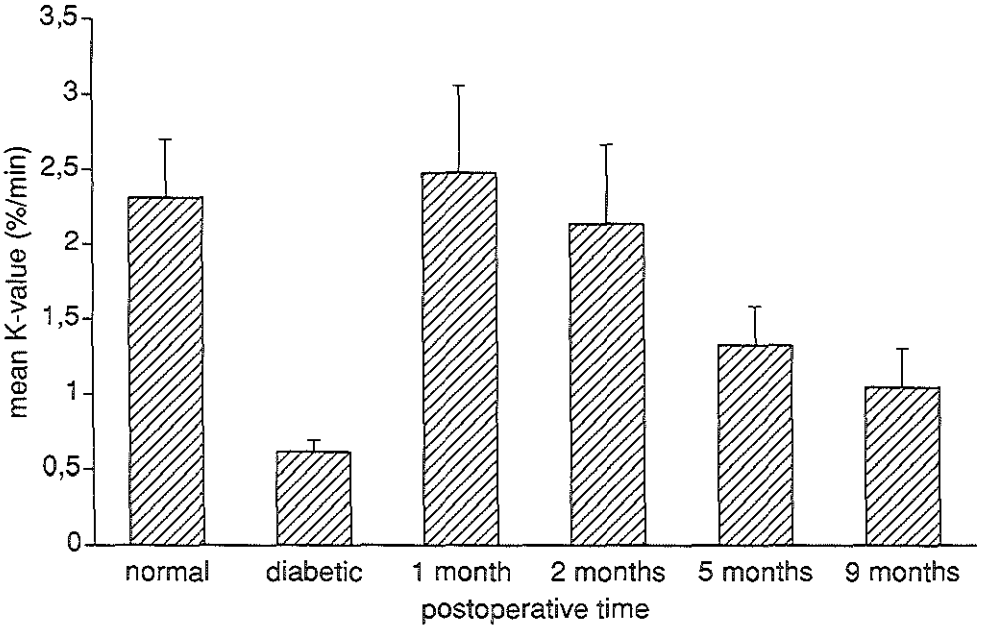

Figure 8.2. Mean K–values derived from IVGT–tests ± standard deviations before and after syngeneic subcutaneous pancreas transplantation in WAG rats.

8.3.2. Dogs

Functional results

Glucose homeostasis was reduced directly after transplantation. Serum glucose of dogs with functioning grafts, although slightly increased, were within normal limits (Figure 8.3.). However, K–values decreased from a mean preoperative value of 4.59 ± 0.88 to a mean of 1.69 ± 0.58 %/min ($p<0.001$) in the first postoperative week. Graft function recovered slightly in the first postoperative months, with mean K–values of about 50% of the preoperative values (2.41 ± 0.59 and 2.44 ± 0.81 %/min at 4 and 9 weeks respectively). However, during the next months graft function further deteriorated, which resulted in three graft failures around one year (48, 59 and 66 weeks respectively) and to further reduction of mean K–values of the remaining 3 grafts to 1.51 ± 0.38 %/min at 78 weeks (Figures 8.3. and 8.4.).

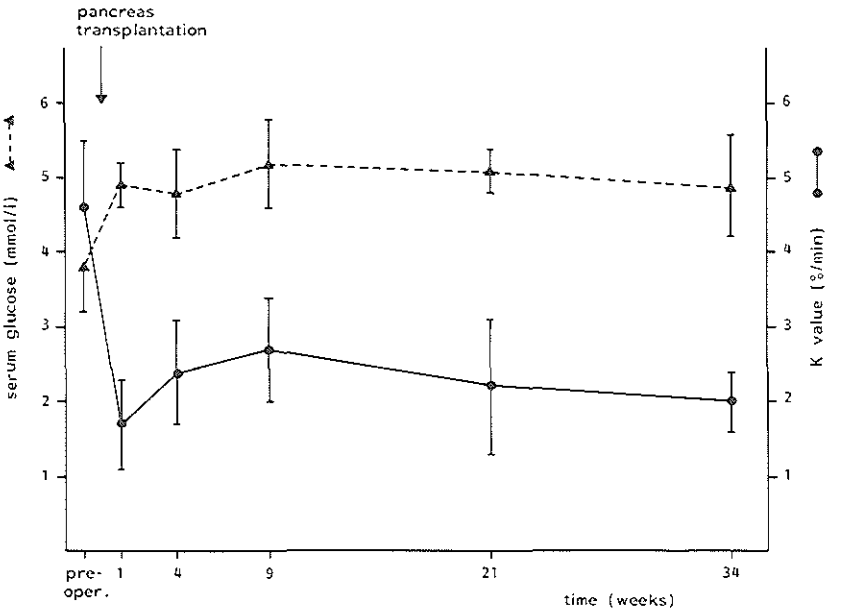

Figure 8.3. Serum glucose levels and IVGTT derived K-values ± standard deviations before and after segmental duct-ligated subcutaneous cervical pancreas autotransplantation in dogs (n=5).

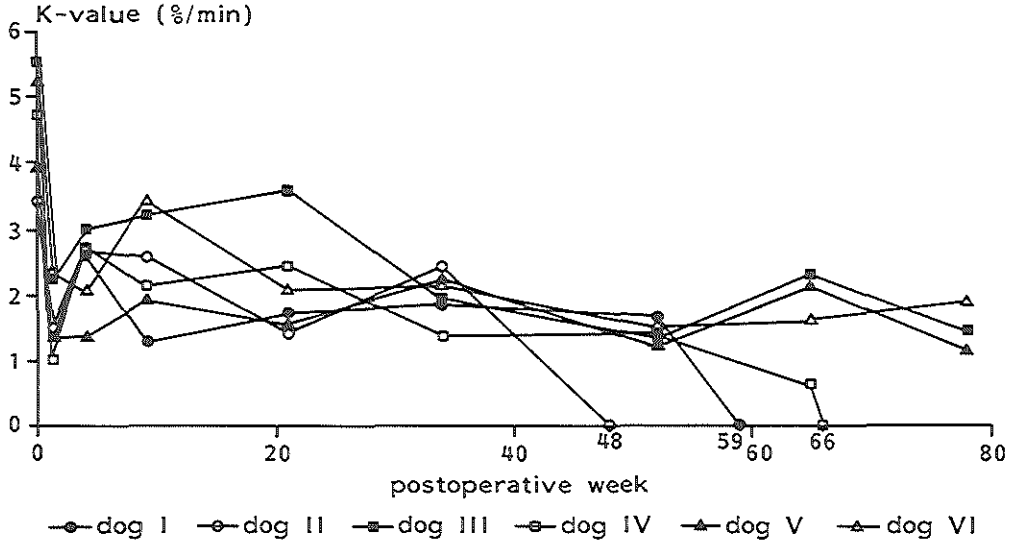

Figure 8.4. Pancreas graft function (IVGTT derived K-values) after subcutaneous autotransplantation in dogs.

Histology

The histological picture of the grafts after sacrifice was in agreement with the functional results. In the three failing grafts only a few clumps of endocrine cells could be found between the totally fibrosed exocrine part, in contrast to the functioning grafts, in which still clusters of apparently normal islets of Langerhans were identified. In the failing grafts vacuolization was seen in the endocrine cells, being absent in functional grafts. In all grafts remarkable neuromatous hyperplasia could be observed.

8.4. DISCUSSION

Although serum glucose values were within normal limits after transplantation in rats as well in dogs, the subcutaneous duct-ligated pancreas transplants were not able to achieve long-term normal glucose homeostasis in response to intravenous glucose injection. During the first postoperative months IVGT-tests in rats were normal, but deteriorated around the fifth month. Spontaneous hyperglycemia, however, was not observed until the ninth month. In dogs IVGT-tests were impaired immediately after transplantation. After a minimal function during the first week, in which pancreatitis was most abundant, mean graft function was stable during the first six months on a level of 50% of preoperative values. After six months graft function again deteriorated resulting in spontaneous hyperglycemia in three out of six dogs around one year postoperatively. Other groups have also described long-term results with deterioration and even failure of duct-ligated subcutaneous grafts [4][5]. Baumgartner et al. found K-values of about 50% of the preoperative values after segmental transplantation, although their results were independent of duct management (open/ligated/ obliterated) or graft location (intraperitoneal/ subcutaneous) and did not further deteriorate after one year [6]. Ekberg et al. described a long-term follow-up of 10 dogs for more than 18 months with segmental duct-obliterated subcutaneous grafts in the groin, which resulted in three failures at respectively 21, 27 and 60 months postoperatively [7]. They had no failures before or around the first postoperative year as we had, which might be explained by the difference of duct-obliteration versus duct-ligation.

Duct-ligation of intraperitoneal pancreas grafts is mostly reported to be associated with endocrine insufficiency in dogs [8][9], although negligible effects have also been described [10][11][12]. Duct-obliteration with synthetic rubber might give less tissue damage. Papachristou et al. had better technical as well as long-term functional results after duct-obliteration in comparison to duct-ligation, with only one

diabetic IVGTT curve out of eleven technically successful subcutaneous grafts after one year [13][14]. However, also this technique does not prevent destruction of islet architecture and deterioration of endocrine graft function [15].

Our finding, especially in rats, that fibroblastic activity is extinguished during the first two postoperative months, while the density of fibrosis still progresses in long-term functioning grafts, is in agreement with others, although they found no further deterioration of the glucose tolerance curves in experiments in dogs, after it had stabilized after the first postoperative month [7][15]. However, Gooszen et al. observed a progressive quantitative and qualitative deterioration of insulin secretion up to 24 months after duct-obliterated segmental pancreas transplantation, due to disruption of normal islet cell architecture.

In intraperitoneal pancreas transplantation the peritoneum is supposed to resorb exocrine fluids, which is not possible using the subcutaneous technique. In combination with duct-ligation, it results in formation of pseudocysts, abscesses and local tissue destruction, including the graft itself. These histological changes, including the fibrotic component as mentioned before, in combination with transplantation of only a segment of the pancreas, further impairs endocrine reserve capacity, which must be assumed to be the main reasons of the rather poor results of long-term function of subcutaneous grafts.

Conclusion

Subcutaneous transplantation of pancreas grafts in rats and dogs results in a progressive decline of endocrine function. More necrosis and abscesses are found with subcutaneous transplantation of a duct-ligated pancreas graft, than in intraperitoneal grafts. In combination with fibrosis and transplantation of only a segmental graft, it resulted in deteriorating glucose homeostasis in rats and even graft failure in dogs. Long-term function of the graft must be one of the priorities, if one decides to perform pancreas transplantation in young diabetic patients. In view of this aspect, subcutaneous transplantation has no advantage, in comparison to intraperitoneal transplantation.

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CHAPTER 9
EFFECT OF THE SOMATOSTATIN ANALOGUE SMS 201-995 ON POST-TRANSPLANT PANCREATITIS AND GRAFT FUNCTION IN RATS AND DOGS

9.1. INTRODUCTION

There are several methods for drainage of the exocrine duct of the pancreas after transplantation. The techniques mostly used are duct obliteration during [1] or some weeks after [2] transplantation and drainage into a visceral organ (jejunum [3], stomach [4] or bladder [5]). The bladder drainage technique seems to be superior to the other techniques, whereas intestinal drainage or polymer duct injection give better results than drainage to the stomach [6][7][8]. Each procedure has its own early postoperative complications, but acute pancreatitis remains a main postoperative event after all procedures [9][10]. It may not only result in local complications, but also to a decay of tissue due to necrosis and fibrosis, which reduces the reserve capacity of the graft [11][12]. In the early or late postoperative course this may give dysfunction of the graft and/or recurrence of the diabetic state.

Due to its capacity to inhibit various endocrine secretory systems investigators and clinicians became interested in somatostatin, a growth-hormone-release-inhibiting-factor (SRIF). After its first isolation in 1973 [13] and its synthesis one year later by Brazeau [14], this tetradecapeptide appeared not only to inhibit the secretion of growth hormone, but also of thyrotropin, insulin, glucagon, gastric hormones and of pancreatic exocrine hormones in normal individuals [15]. However, a therapeutic role was limited due to its short half life time (3 minutes) and the appearance of rebound secretion after discontinuation of the treatment [16].

Some years ago a somatostatin-analogue octapeptide was synthesized (SMS 201-995), which was about 45 times more active in comparison to native somatostatin in rhesus monkeys [17], had a much longer half-life time (113 minutes) [18] and caused no rebound hormone hypersecretion [19]. The development of this analogue gave new impetus to the therapeutic application of somatostatin. Both somatostatin and somatostatin-analogues appear to have inhibitory effects on pancreatic endo- and exocrine secretion [20][21], on pancreatic blood flow [22] and seem to have therapeutic effects on experimentally induced pancreatitis [23][24] and clinical cases of pancreatitis-derived high-output fistulas [25][26].

The aim of the present study was to investigate in rats and dogs whether SMS was

able to limit post-transplant pancreatitis, and to what extent this might have beneficial effects on endocrine pancreas function.

9.2. MATERIALS AND METHODS

Three experimental models were used:
1. syngeneic pancreas transplantation in rats.
2. allogeneic pancreas transplantation in rats.
3. an in situ pancreatitis experiment in dogs.

9.2.1. Rats

Experimental groups and postoperative treatment

Group I (n=10) WAG to WAG syngeneic pancreas transplantation and SMS 201-995 treatment; II (n=10) WAG to WAG syngeneic controls; III (n=7) BN to WAG allogeneic pancreas transplantation and SMS 201-995 treatment; IV (n=8) BN to WAG allogeneic controls.

SMS 201-995 was administered at the beginning of the recipient operation (i.e. about 45 minutes before revascularisation of the graft) and during the first seven postoperative days in three daily doses (08.30, 12.30 and 17.00 hrs.) of 2 µg/kg dissolved in 0.2 ml physiological saline. Controls received physiological saline.

Postoperative follow-up

Groups I and II: on postoperative days 2, 4, 6 and 8 one ml of blood was collected at 08.00 h. for serum glucose, amylase and lipase determinations and replaced by one ml of syngeneic whole blood from a normal rat to keep the hematocrit within the normal range. After one night fasting an intravenous glucose tolerance test was carried out at the ninth postoperative day and K-values were calculated. Afterwards the transplant was removed and sent for histological examination.

Group III and IV: the day of graft rejection was determined by non-fasting serum glucose measurements (>14 mmol/l).

9.2.2. Dogs

Many factors are involved in post-transplant pancreatitis such as ischemia time and flushing of the graft. In order to reduce these variables, we decided to use a model in which pancreatitis was induced the in situ pancreas tail.

Induction of pancreatitis

The tail of the pancreas was denervated and dissected from surrounding tissues: dissection of all nervous tissue from all feeding and draining blood vessels of the pancreatic tail over about 3 centimeters (the gastrosplenic vessels, the splenic vessels distally from the pancreas and, if existing, the aberrant vessels); ligation and transsection of the left gastric artery and vein and transsection of the visceral peritoneum surrounding the pancreatic tail. The tail was ligated at the isthmus and divided from the right lobe of the pancreas which results in a free-floating pancreatic tail except for its arterial and venous supply. To mimic the ischemia time in pancreas transplantation, clamps were placed during 15 minutes on the arterial vessels proximally and distally from the pancreas tail after an intravenous injection of 2500 IU of heparin. After removal of the clamps 1 ml of protamin (10 mg, KabiVitrum BV, Amsterdam, The Netherlands) was administered intravenously. Pancreatectomy of the right lobe was performed sparing the duodenal vascularisation.

Experimental groups and postoperative treatment

Group I (n=5) treatment with SMS-201-995; group II (n=5) controls (physiological saline).

SMS 201-995 was first administered at the beginning of the operation procedure and afterwards in three daily doses of 25 μg/dose dissolved in 2.5 ml 0.9% NaCl subcutaneously (on 8.30, 12.30 and 17.00 hrs.). Controls received equal volumes of physiological saline. Treatment was stopped 4 weeks postoperatively. Additional postoperative treatment was similar as in the transplantation experiments.

Postoperative follow-up

Regular determinations of serum glucose, amylase, and lipase were performed before and after operation. IVGT-tests were performed before, 10 days after, and 4 and 9 weeks after operation. Insulin secretion (expressed as Area Under the Curve = AUC-values) was measured and K-values, derived from serum glucose measurements, were calculated. Blood sampling and the IVGTT's took place before the morning SMS/saline treatment. A relaparotomy to take a biopsy from the pancreas was carried out seven days postoperatively. After 9 weeks the dogs were sacrified and pancreas tissue was sent for microscopic examination.

9.3. RESULTS

9.3.1. Rats

In the syngeneically transplanted control group (II) two out of ten rats died, one due to bleeding from a hemorrhagic pancreatitis and one due to a necrotizing pancreatitis in combination with pneumonia. In the SMS-treated group (I) all rats survived. Between the two surviving groups there was no significant difference in pancreatitis reflected by mean serum lipase and amylase in the first postoperative week, when the post-transplant pancreatitis is most abundant (Table 9.1.): 53 ± 29 U/l and 8108 ± 1444 U/l (group I), and 186 ± 388 U/l and 10,888 ± 9876 U/l (group II), respectively. The peak-values in the first postoperative week ranged from 40 - 300 (group I) and 40 - 1750 U/l (group II) for serum lipase (normal values 0 - 20 U/l), and 7600 - 19,000 (group I) and 6000 - 42,800 U/l (group II) for serum amylase (normal values 5800 - 8600 U/l), respectively.

Glucose homeostasis in rats was similar in the untreated and SMS-treated groups (Table 9.1.): mean serum glucose in the first postoperative week was 7.1 ± 0.7 (group I) and 6.8 ± 0.5 mmol/L (group II). IVGT-tests at day 9 resulted in K-values of 1.82 ± 0.47 %/min in group I and 1.56 ± 0.39 %/min in group II.

In the allogeneically transplanted rats there were no graft failures due to post-transplant pancreatitis. As shown in Table 9.2. SMS had no effect on allograft survival. In groups III and IV graft survival amounted to 10 ± 1 days.

9.3.2. Dogs

In the pilot study in dogs one animal in each group was lost for technical reasons (one intraabdominal bleeding and one sudden death without apparent cause in the first 24 hours postoperatively). In the SMS-group one extra dog was sacrificed four weeks postoperatively because of cachexia. As in rats, neither in dogs the mean serum lipase and amylase, measured as reflection of pancreatitis, was different in the two remaining groups during the first week: 1321 ± 1838 vs 1780 ± 1038 U/l for serum lipase and 4914 ± 2797 vs 4673 ± 1648 U/l for serum amylase in group I and II, respectively (Table 9.3.). Three IVGT-tests were performed at day 10 and weeks 4 and 9. K-values and Insulin-AUC-values expressed absolutely as well as percentage of the preoperative values did not show significant differences one and four weeks postoperatively. However, the K-values at 9 weeks were significant in favour of the SMS-group ($p<0.01$), while the insulin-AUC-values showed the same tendency, but were not significantly different (Table 9.3.).

9.3.3. Histological results

Histology did not reveal differences between experimental groups and controls. In rats diffuse mixed–cellular infiltrate and severe fibrosing activity could be observed at day 9. In general the islets were well preserved. The exocrine tissue showed various degrees of atrophic reaction, not related to treatment. In the acute phase after sham transplantation in dogs, the degree of cellular infiltration, edema and congestion of ductuli was similar in both groups. After 9 weeks acinar tissue could not be observed, being replaced by severe fibrosis, whereas islets were still well preserved without any difference between both groups.

Table 9.1. Effect of SMS 201–995 on pancreas graft function in rats during the first nine postoperative days

	SMS–group (I)	Controls (II)
N	10	10
Deaths	0	2
Serum glucose (mmol/l) *		
mean ± SD	7.1 ± 0.7	6.8 ± 0.5
Serum lipase (U/l) *		
mean ± SD	53 ± 29	186 ± 388
range peak values	40 – 300	40 – 1750
median peak values	75	95
Serum amylase (U/l) *		
mean ± SD	8108 ± 1444	10888 ± 9876
range peak values	7600 – 19000	6000 – 42800
median peak values	9950	11400
Mean K–value ± SD (%/min) +	1.82 ± 0.47	1.56 ± 0.39

SMS 201–995 treatment: 3 daily doses of 2 µg/kg s.c., days 1 to 7; * serum levels were determined on days 2, 4, 6 and 8; SD = standard deviation; + K–values were derived from IVGTT's on day 9.

Table 9.2. Effect of SMS 201-995 on pancreas allograft survival in rats

Group		n	graft survival	MST ± SD
III	SMS 201-995	7	9,9,9,10,10, 11,11	10 ± 1
IV	controls	8	9,9,10,10,10, 10,11,11	10 ± 1

SMS 201-995 treatment: 3 daily doses of 2 µg/kg s.c., days 1 to 7.
MST ± SD = mean graft survival ± standard deviation; graft survival and MST in days.

Table 9.3. Effect of SMS 201-995 on pancreas function after sham-transplantation in dogs

	SMS-group (I)	Controls (II)
N	5	5
Deaths	1	1
Serum glucose (mmol/l) *		
mean ± SD	7.2 ± 0.6	7.1 ± 0.7
Serum lipase (U/l) *		
mean ± SD	1321 ± 1838	1780 ± 1038
range peak values	300 - 5800	680 - 6000
Serum amylase (U/l) *		
mean ± SD	4914 ± 2797	4673 ± 1648
range peak values	2500 - 10400	2700 - 9000
Mean K-value ± SD (%/min) +		
preoperative	2.92 ± 0.86	2.32 ± 0.21
10th day	1.58 ± 0.35	1.48 ± 0.24
4th week	1.67 ± 0.36	1.63 ± 0.47
9th week	2.17 ± 0.43	1.16 ± 0.15 ≠
mean insulin-AUC ± SD (U.min/l) +		
preoperative	3348 ± 1170	3022 ± 705
10th day	2422 ± 1198	2604 ± 393
4th week	2080 ± 405	2003 ± 421
9th week	2335 ± 1073	1333 ± 667

SMS 201-995 treatment: 3 daily doses of 2 µg/kg s.c., week 1 to 4; * serum levels were determined every day during the first postoperative week; + K-values and insulin-AUC were derived from IVGTT's; SD = standard deviation; ≠ $p<0.01$ (Student's-t test).

9.4. DISCUSSION

Pancreatitis is one of the main complications after transplantation of the pancreas. It may give rise to exocrine pancreatic leakage and to formation of pseudocysts, abscesses and fistulas. The chance of thrombosis of the vessels of the graft is increased by the changed pancreatic blood flow [27]. Long-term endocrine graft function can be reduced by tissue damage and fibrosis of the graft due to the severe pancreatitis [11][12].

Improvement of surgical techniques, graft preservation, anticoagulation therapy and immunosuppressive protocols have resulted in graft and patient survival moving towards the transplantation results of other vascularized organs [3][28]. Non-surgical treatment directed against the pancreatitis have not yet been very successful. Results of radiation of the graft, treatment with Trasylol^{R}, corticosteroids or glucagon are controversial [27][29]. Somatostatin, particularly the long-acting analogue SMS 201-995, may be able to suppress the exocrine secretion of the pancreas, resulting in amelioration of the pancreatitis.

Experiments in normal animals and man have demonstrated an inhibiting effect of somatostatin on exocrine pancreatic secretion, apart from influences on several other hormonal mechanisms [20][21]. There are many theories about the mechanism of action of somatostatin on the exocrine pancreas. The pancreas may be suppressed via inhibition of pancreas stimulating hormones, for example CCK, secretin and motilin or via a direct action on somatostatin receptors on exocrine cells [21][30][31]. Furthermore, others have demonstrated an inhibiting effect of somatostatin on splanchnic, particularly pancreatic blood flow, possibly influencing the pancreatic endocrine and exocrine function [22][32][33]. Also a non-hormonal cytoprotective effect of somatostatin by stabilisation of cell membranes has been suggested [34].

In several rat models of severe pancreatitis induced by intraductal injection of agressive agents, resulting in 100% mortality within 24 hours, somatostatin (SRIF) and SMS 201-995 in the same dosage as we used, were able to diminish pancreatitis. It evoked a significant reduction of serum lipase and amylase and less severe histological changes and consequently reduced mortality [23][35]. In our experiments in rats we also found mortality in the syngeneic control group: 2 rats died due to pancreatitis versus 0 in the SMS group. However, there was no significant reduction of postoperative amylase and lipase values. The same was found in the study in dogs. In experiments with bile-induced pancreatitis in dogs also others did not find a significant reduction of serum parameters after somatostatin (SRIF) treatment, but it resulted in better clinical and histological features [24]. These latter parameters were not different

in our experiments, neither in rats, nor in dogs.

After transplantation of the pancreas, Liu et al. could not demonstrate a beneficial effect of another somatostatin analogue (L363,586) on posttransplant pancreatitis or pancreas function during continuous somatostatin infusion in the first postoperative week [36]. They found, however, a slight but significant beneficial effect in the somatostatin group on serum amylase levels and IVGTT-K-values in the second week (when somatostatin infusion was stopped), which was nullified again in the third postoperative week. This rebound effect by the original somatostatin analogues, does not occur with the SMS 201-995 analogue.

Two groups, using SMS 201-995 in much higher dosages as we did, demonstrated only minor effects on pancreatic grafts. Nicholson et al. used three daily dosages of 5 μg/kg in pigs and only found a significant effect on bloodflow, but not on the occurence of thrombosis or on the release of amylase or trysine [33]. Garvin et al. demonstrated a significant effect of one intravenous bolus injection of about 8 μg/kg SMS in dogs on meal induced amylase and bicarbonate release. However, this effect was demonstrable for only two hours after injection. Basal values were not influenced [31].

During treatment with SMS there was no difference in glucose homeostasis in rats, nor in dogs as expressed in serum glucose and IVGTT-derived insulin and K-values in comparison to controls. But, after discontinuation of SMS, K-values were in favour of the SMS groups, although they were only significant in dogs (ninth postoperative week). This suggests that the endocrine reserve capacity is better preserved in the SMS groups than in controls. This is not reflected in prolongation of allogeneic graft survival in rats, although it should be noted that no immunosuppressive therapy was given in this study. Otherwise, differences might have been more subtile, displaying a possible effect of SMS. Furthermore, the experimental model of pancreas duct-ligation induce fulminant post-transplant pancreatitis, which might be too intense, to enable any measurable beneficial effect of SMS. Long-term functional studies should be performed to investigate whether the differences measured after discontinuation of SMS will indeed result in long-term better graft function.

Conclusion

We were not able to demonstrate a pancreatitis-reducing effect of SMS 201-995 after pancreas transplantation in rats or dogs. Allograft function was also not prolonged in rats. Islet cell function, however, was better preserved by SMS 201-995 in dogs after (sham) transplantation.

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GENERAL DISCUSSION

Insulin–dependent diabetes is a disabling chronic illness in which the patient has to cope with daily restrictions to regulate glucose metabolism and with symptoms of secondary diabetic complications on the long term. Although conservative anti–diabetic therapy has been improved, it cannot obviate the daily discomfort nor can it prevent the long–term disability. Both have substantial impact on the quality of life of the patient and also have important socio–economic consequences.

Vascularized pancreas transplantation has the ability to improve quality of life, abandoning the tyranny of injections, dietary restrictions, blood glucose monitoring, hypoglycemia, and a rigid life style [1][2]. It may also decelerate or even stop progression of some, probably not all, secondary diabetic complications [3]. At least subjectively, secondary complications are improved [4]. There are indications that life can be prolonged by pancreas transplantation [2][5].

Pancreas transplantation including strong immunosuppressive therapy, however, is associated with complications and mortality that are (partially) counteracting the advantages of transplantation. These latter facts are of decisive importance to restrict pancreas transplantation to diabetic patients with advanced secondary complications. In addition, it is not yet possible to select those patients who are at high risk for developing diabetic complications that are more morbid than the side effects of immunosuppression.

Progress has been made in the field of islet transplantation. Still it is in an experimental (clinical) stage [6]. Isolation techniques have been improved and it has been made possible to store islets with crypreservation techniques [7][8]. This can make possible the use of multiple preparations to prevent rejection. In addition it permits the ability to bank islets. The application of the microencapsulation technology is currently limited by the need for materials that do not stimulate fibroblastic response in the recipient. Macroencapsulation intravascular devices require nonthrombogenic surfaces that are resistant to fibrin deposition while they maintain an effective interface with blood and/or tissue fluids [9]. Besides, unlike to vascularized transplantation, the use of isolated islet has never been proven to reverse or prevent secondary diabetic complications [10][11].

As mentioned in chapter 2, pancreas transplantation should preferably be performed before diabetic complications develop, at least before they have reached a point of no return, in other words in young patients. The subcutaneous location of the pancreas graft was studied to bypass major technical complications such as abdominal sepsis and

its concomitant symptoms. Apart from technical and functional studies, this model was used to investigate the value of non-pharmacological immunosuppressive protocols in order to reduce the immuneresponse, which in its turn enables a reduction of (toxic) immunosuppressive drugs. Both technical and immunological aspects are of primary importance in order to shift the moment of transplantation to an earlier period.

Technical results in these studies in rats and dogs were not different from the results of intraperitoneal transplantation reported in the literature [12][13]. Especially thrombosis was a major reason of early graft failure in dogs, similarly as described by others [13][14]. Post-transplant pancreatitis only led to temporary, minor local complications. More obvious was the deterioration of graft function on the long term, and in dogs even graft failure in 50% of the cases about one year after transplantation. The unavoidable combination of subcutaneous transplantation with duct obstruction (ligation or obliteration) must have been the main reason for the deteriorated graft function. The process of inflammation and fibrosis appeared to be more pronounced in subcutaneous than in abdominal grafts, as was confirmed by histology. More abscesses and fibrosis were seen in subcutaneous grafts, undoubtedly due to more local destruction by pancreatic exocrine secretions compared to intraperitoneally grafted recipients, in which the peritoneum has a resorbing capacity. Duct obliteration will, if anything, only partially limit the destruction of endocrine tissue compared to ligation [13][15][16]. A possible option is temporary exocrine drainage, having a twofold advantage: it reduces the inflammatory reaction and facilitates monitoring of rejection from the exocrine fluid collections.

Maybe it is not completely surprising that treatment with a pharmacological agent was not effective in reducing the full-blown pancreatitis after transplantation of a duct-ligated graft. Endocrine function in dogs, however, seemed to be better preserved after SMS 201-995 treatment, although final conclusions can only be drawn after long-term follow-up in larger groups of animals. If any, only treatment of pancreatic fistulas seems to be an indication for the use of somatostatin in transplantation surgery, as confirmed in the literature [17]. Furthermore, if rejection monitoring via amylase production in the postoperative period is envisaged (in urine or other exocrine samples), somatostatin would interfere with these measurements.

The results of pancreas transplantation world-wide are steadily improving, with one-year patient and graft survival rates approaching the outcome of kidney transplantation [18]. The number of technical failures, until recently the major problem, is now smaller than that of failures due to rejection, however, the severity of complications still shows that the pancreas is a difficult organ to handle. While the technical failure rate in well-established centers was used to be up to 30% until recently, it is now less

with a mortality rate of 5–10% [19][20]. However, in spite of agressive antirejection therapy (triple or quadrupple therapy in most centers) about 15% of the grafts are still lost due to rejection in the first year [21].

In our experiments in rats and dogs it was evident that the pancreas graft is prone to rejection. It has been shown that CsA therapy or pretreatment with blood transfusions in the BN–WAG rat model enabled permanent survival for kidney or heart grafts [22][23]; however, in this study the pancreas graft only sporadically survived for more than 60 days using the same protocols. Also in dogs a pretreatment protocol used formerly to induce long–term kidney graft survival only led to a slight but not statistically significant prolongation of pancreas graft survival [24]. These findings, in combination with those of others, strongly suggest that the pancreas may evoke a different immune response or may be more vulnerable to rejection than either the heart or kidney [25][26]. The nature of this difference is yet unclear, but it may be related to differences in the content of passenger leukocytes, the expression of MHC and organ specific antigens, or the importance of minor histocompatibility antigens.

A negative role of the position of the graft in the outcome of survival was excluded by comparing subcutaneous with intraperitoneal grafts. Grafts in subcutaneous position did not have a worse survival if untreated or treated with CsA. Even after blood transfusions, subcutaneous grafts did better than intraperitoneal grafts. We were not able to give a satisfactory explanation of this effect.

We attempted to pretreat the graft in order to decrease its immunogenicity, ultimately enabling a reduction of immunosuppressive drugs. Donor irradiation only led to a partial reduction of class II positive cells (80–85%), not resulting in prolongation of graft survival. Perfusion of the graft with monoclonal antibodies was likewise not successful. It remaines doubtful, whether the technique of donor pretreatment, so successful in experimental islet–cell transplantation, will ever provide a meaningful solution in vascularized pancreas transplantation [27][28]. Not only the proportion of inactivated cells should be near 100% to annihilate the dendritic pathway of rejection, but also other pathways which appear to be responsible for rejection of the pancreas graft should be blocked efficiently.

The future

An increasing number of institutions are currently switching to the bladder–drainage technique [18][29]. As technical problems appear to decrease, the early treatment of rejection episodes becomes (relatively) more important. The endocrine part of the graft has a limited reserve capacity to sustain repeated attacks of rejection. Therefore, early detection of rejection, which can be attained from urine samples after exocrine

drainage to the bladder, is essential [29][30].

New clinical antirejection protocols and better monitoring of rejection have led to better graft survival than 5–10 years ago, and the results will further improve with the development of new immunosuppressive agents [31][32]. Since it appears to be possible to accept preservation times of more than 24 hours, HLA matching can play an important role in further improvement of graft survival rates [33].

Further insight in immunological systems and improvements in applied immunology, for instance the production of specific monoclonal antibodies, might provide a more important role for specific biological antirejection therapies which might improve graft survival.

Islet transplantation has gradually been developed, and recent success with this technique in humans gives hope for the future [6]. With improving immunosuppression and experience in immuno–biology further progress will definitely be made. However, as mentioned before, still it is not yet clear, whether isolated islet transplants will have any ameliorating effect on secondary diabetic complications.

Medical and biological investigators are not only making progress in the field of transplantation immunology, but also in the field of diabetes immunology, genetics, and etiology. It may be expected that they will provide the ultimate tools for prevention or early treatment of type I diabetes, relegating pancreas transplantation to the past.

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SUMMARY

In the past 25 years pancreas transplantation has developed into a valuable treatment for insulin–dependent diabetic patients with advanced secondary diabetic complications. The best results in terms of pancreas graft survival have been achieved with combined pancreas–kidney transplantation. However, apart from nephropathy patients undergoing combined pancreas–kidney transplantation may have secondary diabetic complications, such as retinopathy and neuropathy, which may have reached a point of no return, so that the quality of life can only improve partially after transplantation. In the course of years transplantation has become more successful in terms of decreased morbidity and mortality, allowing pancreas transplantation in non–uremic patients. However, the operative and immunosuppressive drug–induced side effects still restrict the indication of pancreas transplantation to patients with advancing diabetic complications.

Insulin dependent–diabetes mellitus (IDDM) usually starts in childhood, and restrains the young patients in their life pattern, because of the insulin therapy, and diet involved. A compromise between a tight control for optimal glucose regulation and an acceptable social and psychological freedom of the patient is therefor essential. In view of the socio–psychological limitations for the young patient and the aim to prevent secondary diabetic complications, we tried to find ways to shift the moment of transplantation to an earlier period.

An overview of 25 years of pancreas transplantation is given in chapter 1. Technical and immunological aspects are reviewed, and the effects of transplantation on the diabetic disease process are discussed.

The aim of the study is elaborated in chapter 2. Early pancreas transplantation needs an operative technique without or with only a few complications and immuno–suppression with low toxicity. The combination of early and long–term side effects of transplantation should not outweigh the long–term side effects of diabetes mellitus treated with insulin. A subcutaneous position of the duct–ligated graft was chosen to bypass the intraperitoneal location and its attendant life–threatening complications. The functional and immunological consequences of this position of the graft were investigated. Besides, in this model several immunosuppressive modalities were tested. The operation techniques and general material and methods in the rat and dog models are described in chapter 3.

The feasibility of the transplantation techniques in rats and dogs are presented in chapter 4. Transplantation of the duct–ligated pancreas to the cervical region had a success rate of 90% in rats, while 2/3 of the transplantations in dogs was technically

successful. Thrombosis, partially due to anatomically vascular variance, was the main cause of primary graft dysfunction in dogs (25%).

The immunosuppressive effect of preoperative blood transfusions to the recipient, as described for other transplants, was tested for the pancreas (chapter 5). To reduce the immunogenicity of the graft, it was attempted to eradicate MHC–class II antigens in the pancreas graft (chapter 6). If effective, both pretreatment modalities could be of value to reduce the dose of conventional immunosuppressive drugs, a main condition for long–term administration after performing early pancreas transplantation.

In both animal models, rat and dog, blood transfusions were found to be less effective than in other (heart and kidney) allografts (chapter 5). Variation in timing of blood transfusions in relation to transplantation interfered with the outcome, but appeared not to be responsible for the worse results compared to other organs. The diabetic state of the recipient could not be found to influence the results of graft survival.

To reduce the number of MHC–class II antigens of the graft, donor rats were pretreated with gamma–irradiation, or grafts were perfused with anti–class II monoclonal antibodies (chapter 6). Irradiation reduced the number of class II positive cells to 15–20% of the normal number of cells, but did not improve graft survival. In vitro perfusion of pancreas grafts with monoclonal antibodies was not effective at all: immunohistology of the perfused grafts did not reveal any adherence of antibodies to the class II positive cells, nor was graft survival prolonged.

Of special importance is that the subcutaneous position of the graft should not have negative effects on graft survival. In chapter 7 a comparison is made between subcutaneous and intraperitoneal transplantation in rats. The outcome of subcutaneous grafts in non–immunosuppressed or CsA treated rats was equal compared to intra–peritoneal grafts, even, survival in blood transfusion pretreated recipients was better. Histological comparison revealed a more fulminant pancreatitis with formation of abscesses in subcutaneous grafts. This might give rise to a worse endocrine function of the graft, which was investigated and described in chapter 8.

The main goal of pancreas transplantation is a better metabolic control than that possible with insulin therapy, not only temporary, but long–term. Graft function was evaluated after syngeneic transplantation in rats and after autologous transplantation in dogs. In both models graft function deteriorated; in rats after a few months, whereas in dogs diabetes even recurred after about one year postoperatively in 50% of the cases. The combination of duct–ligation and the subcutaneous position of the graft, resulting in pancreatitis and fibrosis, is supposed to be responsible for these results.

To reduce the pancreatitis after transplantation, the effect of the somatostatin analogue SMS 201–995 was investigated (chapter 9). It was not possible to

demonstrate a pancreatitis-reducing effect with SMS 201-995 in rats or dogs. However, islet function was better preserved in SMS-treated dogs two months after sham-transplantation.

At the end the whole study is generally discussed. It is concluded that the subcutaneous position of the graft will not contribute to better transplantation results, neither functionally nor immunologically. The immunogenicity of the graft requires adequate immunosuppression and so, together with the technical morbidity, stands in the way of early transplantation before diabetic complications become manifest. Finally some words are devoted to the future of pancreas transplantation.

SAMENVATTING

In 25 jaar is pancreas transplantatie uitgegroeid tot een volwaardige behandeling voor patiënten met insuline afhankelijke diabetes mellitus met vergevorderde secundaire diabetische complicaties. De beste resultaten in termen van pancreas transplantaat overleving worden behaald na een gecombineerde pancreas–nier transplantatie. Echter, naast de bestaande nefropathie zijn in deze gevallen andere secundaire diabetische complicaties, zoals retinopathie en neuropathie, veelal irreversibel, zodat de kwaliteit van het leven slechts gedeeltelijk kan worden verbeterd door transplantatie. In de loop der jaren zijn de transplantatie resultaten verbeterd met een verlaging van de morbiditeit en mortaliteit, zodat transplantatie in niet–uremische patiënten tot de mogelijkheden is gaan behoren. Echter, als gevolg van de operatieve en de door immunosuppressiva geïnduceerde bijwerkingen is de indicatie voor pancreas transplantatie nog immer beperkt tot de groep patiënten met vergevorderde diabetische complicaties.

Insuline afhankelijke diabetes mellitus begint doorgaans op jeugdige leeftijd. De ziekte beperkt de jonge patiënt in zijn/haar vrijheid, als gevolg van de insuline therapie, het dieet en zo het regelmatige leefpatroon. Er moet gezocht worden naar een compromis tussen een strakke controle voor optimale bloedsuikerregulatie en een acceptabele sociale en psychologische vrijheid van patiënt. Gezien deze beperkingen voor de jonge patiënt en met het doel secundaire diabetische complicaties te voorkomen, trachtten wij te komen tot verbeteringen om zo het moment van transplantatie naar een vroeger tijdstip te kunnen verschuiven.

In hoofdstuk 1 wordt een overzicht gegeven van vijfentwintig jaar pancreas transplantatie. Technische en immunologische aspecten worden besproken en de effecten van transplantatie op het diabetische ziekteproces worden bediscussieerd.

Het doel van het onderzoek wordt weergegeven in hoofdstuk 2. Vroege pancreas transplantatie vereist een operatie techniek met slechts een beperkt aantal complicaties en een weinig toxische immunosuppressieve therapie. De combinatie van vroege bijwerkingen en bijwerkingen op lange termijn als gevolg van transplantatie behoren de bijwerkingen van langdurige insuline behandelde diabetes mellitus niet te over treffen.

Een subcutane ligging van het duct–onderbonden transplantaat werd gekozen om zo de intraperitoniale ligging met zijn levensbedreigende complicaties te voorkomen. De functionele en immunologische gevolgen van deze ligging van het transplantaat werden onderzocht. Daarnaast werden in dit model verschillende immunosuppressieve tech–

nieken getest. De operatietechnieken en algemene materialen en methoden in het ratten en honden model worden beschreven in hoofdstuk 3.

De haalbaarheid van de transplantatietechnieken in ratten en honden worden gepresenteerd in hoofdstuk 4. Transplantatie van de duct–onderbonden pancreas naar de halsregio in ratten had een succespercentage van 90%, terwijl tweederde van de transplantaties in honden technisch succesvol was. Trombose, deels als gevolg van variaties in de vaatvoorziening, was de belangrijkste oorzaak van primaire dysfunctie in honden (25%).

Het immunosuppressieve effect van preoperatieve bloedtransfusies aan de ontvanger, zoals beschreven voor andere transplantaten, werd onderzocht voor het pancreas (hoofdstuk 5). Teneinde de immunogeniciteit van het transplantaat te verminderen werd getracht het aantal MHC–klasse II antigenen in het pancreas transplantaat te reduceren (hoofdstuk 6). In geval van effectiviteit, zouden beide voorbehandelingstechnieken van waarde kunnen zijn teneinde de dosis van conventionele immunosuppressiva te kunnen reduceren, één van de belangrijkste voorwaarden voor langdurige toediening na vroege pancreas transplantatie.

In beide diermodellen, rat en hond, bleken bloedtransfusies minder effectief dan in andere transplantatiemodellen, zoals bij hart en nier transplantatie (hoofdstuk 5). Variatie in het moment van toediening van bloedtransfusies ten opzichte van transplantatie interfereerde met de resultaten, echter dit kon niet verantwoordelijk gesteld worden voor de slechtere resultaten ten opzichte van andere organen. Het al dan niet aanwezig zijn van diabetes mellitus beïnvloedde de resultaten van transplantaat–overleving niet.

Om het aantal MHC–klasse II antigenen in het transplantaat te reduceren, werden donor ratten voorbehandeld met gamma–bestraling, of werden transplantaten geperfundeerd met anti–klasse II monoclonale antilichamen (hoofdstuk 6). Bestraling beperkte het aantal klasse II positieve cellen tot 15–20% van het normale aantal cellen, hetgeen echter niet effectief bleek om transplantaat overleving te verbeteren. In–vitro perfusie van pancreas transplantaten met monoclonale antilichamen was in het geheel niet effectief: immunohistologie van de geperfundeerde transplantaten liet geen binding zien tussen antilichamen en klasse II positieve cellen, noch werd de transplantaat overleving verlengd.

Van speciaal belang is dat de subcutane ligging geen negatieve gevolgen heeft voor de transplantaat overleving. In hoofdstuk 7 wordt een vergelijking gemaakt tussen subcutane en intraperitoniale transplantatie in ratten. De overleving van subcutane transplantaten in onbehandelde of met CsA behandelde ratten was niet slechter, zelfs was de overleving in bloedtransfusie voorbehandelde ontvanger beter in vergelijking

met intraperitoniale transplantaten. Histologische vergelijking liet een heftiger pancreatitis zien met vorming van abcessen in de subcutane transplantaten. Dit zou aanleiding kunnen geven tot slechtere endocrine functie van de transplantaten, zoals onderzocht en weergegeven in hoofdstuk 8.

Het belangrijkste doel van pancreas transplantatie is een betere bloedsuikerregulatie dan met insuline therapie, niet alleen tijdelijk maar ook op de lange termijn. Transplantaat functie werd vervolgd na syngene transplantatie in ratten en autologe transplantatie in honden. In beide modellen verslechterde de transplantaat functie, in ratten na een aantal maanden terwijl in honden in 50% van de gevallen de diabetes zelfs terugkeerde na gemiddeld één jaar. De combinatie van duct–onderbinding en de subcutane ligging van het transplantaat, leidend tot pancreatitis en fibrose, wordt geacht voor deze resultaten verantwoordelijk te zijn.

Teneinde de pancreatitis na transplantatie te beperken werd het effect van het somatostatine analogen SMS 201–995 onderzocht (hoofdstuk 9). Het was niet mogelijk een pancreatitis reducerend effect van SMS 201–995 in ratten en honden aan te tonen. Wel was de eilandjes functie van met SMS behandelde honden twee maanden na sham–transplantatie beter behouden gebleven.

Aan het eind wordt de hele studie in het algemeen bediscussieerd. Hierin wordt aangegeven dat de subcutane ligging van het transplantaat niet bijdraagt tot betere transplantatie resultaten, noch functioneel noch immunologisch. De immunogeniciteit van het transplantaat vereist adequate immunosuppressie. Dit samengevoegd met de technische morbiditeit staat het zo een vroege transplantatie, voordat diabetische complicaties manifest worden, in de weg. Tenslotte worden enkele woorden gewijd aan de toekomst van pancreas transplantatie.

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