Experimental studies on islet isolation and islet graft function
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In the first part of the introduction (Chapter I) of this thesis an overview of the numerous techniques used in islet isolation procedures is presented. The differing lines of approach for the dissociation of the pancreas which have been applied, and are still being further developed, indicate that a consensus concerning the technical aspects leading to an optimal pancreas dissociation, i.e. dissociation of the exocrine tissue while maintaining the integrity of all islets present in the pancreas, has not yet been reached. These aspects include, amongst others, (i) the composition of the collagenase enzyme mixture, (ii) an identification of the pancreatic structures which have to be dissolved by the enzymes, and (iii) the method of application of the enzymes. Once that a clean cleavage between the islets and the other pancreatic tissue components has been achieved it is reasonable to assume that islet purification procedures will be highly effective, at least in respect of obtaining large numbers of islets without contaminating exocrine tissue, but possibly also without contaminating vascular, ductal and lymphoid tissue.

In the second part of the introduction (Chapter II) an overview of the results of transplantations of islet isografts and autografts is given. Normalisation of basal glucose levels can be readily achieved by isogenic islet transplantation in the rodent model. However, the amount of islet tissue reportedly required to obtain normoglycemia varies considerably, as does the capacity of islet grafts to normalize glucose tolerance. In part this can be attributed to differences in endocrine volume of the islet grafts and to properties of the graft sites, such as route of venous drainage and factors determining the degree of engraftment.

In Chapter II the rationale of the five studies included in this thesis (Chapters III - VII) is given. The rationale of each study is briefly given below in the summary of each chapter.

Since collagen is an important substrate in the enzymatic dissociation of the pancreas for islet isolation, we determined the amount of collagen and its distribution in a comparative study comprising normal pancreata of rat, dog, man, young pig and adult pig (Chapter III). A major factor in islet isolation procedures appears to be the total amount of collagen. In addition, not the amount of collagen in the septa but collagen in the rest of the pancreas, mainly located between the acini, seems to determine the dissociation of the pancreatic tissue. This is illustrated by the higher islet yields which are reportedly obtained from the adult pig pancreas compared to those obtained from the young pig pancreas; the young pig pancreas was found to contain a higher total amount of collagen than the adult pig pancreas but a similar
distribution of collagen in the septa. Conceivably the presence of a capsule containing collagen facilitates the cleavage between endocrine and exocrine tissue. However, the presence of a capsule seems to be of secondary importance in islet isolation procedures, since similar islet yields are obtained from the canine and human pancreas containing a relatively low and high amount of collagen around the islets, respectively, but a similar total collagen content. The general experience that islet isolation procedures are effective in rats can be readily understood, since the rat pancreas contains both a low total amount of collagen and a high amount of collagen around the islets, although the easily obtainable vast amount of experience in these procedures is certainly also an additional positive factor.

Reportedly, higher islet yields are obtained by ductal collagenase administration and the subsequent digestion of the pancreas than by the digestion of chopped pancreatic tissue in a medium containing collagenase. In the study presented in Chapter IV we investigated if the higher yields can be explained by a different distribution of the collagenase enzymes in the pancreatic tissue after ductal application, in particular by a restriction of the enzymes to the exocrine tissue, as compared to during the chopped tissue digestion. We used Indian ink to mimic and visualize the distribution of collagenase in histological sections of pancreases of several species. Ink particles were seen around and even within the islets both after ductal application as well as during chopped tissue collagenase digestion in all species studied. We therefore concluded that collagenase enzymes are not restricted to the exocrine tissue compartment with either technique. This conclusion is substantiated by the similar islet yields obtained in our experiments in rats with either technique, which are also similar to the islet yields obtained by the ductal collagenase method in rats reported in the literature. Nevertheless, the islet yield was only approximately 50% of the endocrine volume of the pancreas, indicating that a substantial loss of islet tissue does occur, most likely due to the peri-insular presence of enzymes in the collagenase preparation during islet isolation procedures.

It is beyond dispute that the success of islet transplantation is determined by the initial amount of endocrine tissue of the grafts. It has recently been argued that a certain degree of 'contamination' of the islet grafts by non-endocrine tissue, i.e. exocrine tissue, lymphoid tissue, vessels and ducts, can be accepted without jeopardizing graft function in clinical islet transplantation since aiming at complete purification of the islet grafts inevitably results in a reduced graft endocrine volume. However, there is experimental evidence that contamination of the grafts by non-
SUMMARY

Transplantation of islets has been successfully performed in rodents, in large laboratory animals and in man. The amount of islet tissue reportedly required to obtain normoglycaemia in the rodent model varies considerably, as does the capacity of islet grafts to normalize glucose tolerance. In part this can be attributed to differences in endocrine volume of the islet grafts in various studies, to the method of assessing graft function, to properties of the graft sites, such as route of venous drainage and to the efficacy of engraftment, or to a combination of these factors. We have analyzed the graft function of rat islet isografts of well-defined endocrine volumes after transplantation to three different sites (kidney, liver and spleen). Graft function was tested in unanesthetized, unstressed rats by the responses to glucose infusion and to a meal. Graft endocrine mass was determined by measuring the total islet volume prior to transplantation. In one study (Chapter VI), grafts contained a similar amount of endocrine tissue as present in the normal adult rat pancreas. All transplanted animals returned to normoglycaemia within one week after transplantation. At one month, basal glucose and insulin levels were similar to controls in rats with grafts to the spleen, but higher in rats with grafts to the kidney or liver. Irrespective of the transplantation site, recipients had higher glucose and lower insulin levels than controls in response to glucose infusion, but in response to a meal these differences from normal were less outspoken. Finally, recipients showed both

endocrine tissue results in an incomplete engraftment of the islets and an enhanced risk graft rejection and therefore should be avoided. Islet graft purification is generally performed by discontinuous density gradient centrifugation, a method which results in the separation of a majority of the islets from the exocrine tissue. However, the capacity of density gradients to also separate lymph nodes, vessels and ducts from the islet grafts is usually not mentioned. We therefore compared seven different density gradient forming materials as to their efficacy for rat islet purification (Chapter V). Continuous density gradients were used in order to determine the buoyant densities of the different pancreatic tissue components. A significant separation of large numbers of islets from the exocrine tissue, was only seen in the albumin, dextran-40 and metrizamide gradients. However, pure islet preparations could not be obtained with any of the gradients studied, since none of the gradients completely separated lymph nodes, vessels and ducts from the islets. As judged in terms of both numbers of islets obtained and islet function tested in vitro, the best results were obtained with gradients composed of metrizamide or of dextran.

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an acute insulin response to glucose infusion as well as a pre-absorptive insulin release after food ingestion, irrespective of the transplantation site. Our findings indicate that the insulin response to glucose infusion and to a meal is quantitatively reduced, but qualitatively intact after transplantation to the kidney, liver or spleen. In the second study (Chapter VIII) grafts comprising either 12.5, 25, 50 or 100% of the endocrine volume in the normal adult rat pancreas were transplanted to the same three sites as in the previous study, i.e. kidney, liver and spleen. All animals with grafts $\geq 25\%$ of the endocrine volume of the rat pancreas returned to normoglycaemia after transplantation. The minimal graft volume for restoring normoglycaemia is probably between 12.5 and 25% since also a small number of grafts of 12.5% were successful. At one month, basal insulin levels were similar to controls in rats with grafts to the spleen, but higher in rats with grafts to the kidney or liver. Irrespective of the transplantation site, recipients had higher glucose and lower insulin levels than controls in response to glucose infusion. In response to a meal, however, only the first phase insulin response was reduced, but the total insulin output during the entire test was similar to controls. Graft performance was found to be graft size dependent. Results of tests performed at two months showed a tendency of increasing responsiveness compared to the results of tests at one month.

In the general discussion (Chapter VIII) we have reviewed the observations of the studies presented in this thesis in the perspective of other studies performed at our laboratory and those performed by other research groups. We argued that on the basis of the morphology of the pancreas it is likely that higher islet yields than currently acquired after enzymatic pancreas dissociation can be obtained by utilizing an appropriate mixture of pure enzymes. However, the exact composition of this enzyme mixture has yet to be determined and, in addition, is also probably species dependent. Therefore, further fundamental research regarding enzymes and their substrates is indicated. We discussed that although islet purification, for instance by density gradient centrifugation, is continuously improved, grafts composed of unaffected and pure islets cannot be obtained since this implies the cleavage of direct endo-exocrine cell-cell contacts. From our islet transplantation experiments we concluded that grafts comprising an endocrine volume of 50% of the normal pancreas show an adequate, short-term, graft function after transplantation when tested under various physiological conditions. An adequate long-term graft function most likely requires portal venous drainage of the grafts which can be obtained by islet transplantation to the spleen.