

## 122 IMMUNOHISTOCHEMICAL STUDY OF ISOGENEIC FETAL LIVER FRAGMENTS TRANSPLANTS IN THE OMENTUM

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**Objective:** Iso, allo and xeno-fetal liver fragments (FLF) transplantation is an attractive approach for acute and chronic liver failure in animal models. In this study, isogeneic FLF were transplanted into the omentum of rats and their morphological and functional changes were observed by the immunohistochemical study.

**Methods:** FLF were prepared from Wistar rats' fetuses of gestational periods of 19~20 days. They were transplanted into the omentum of isogeneic Wistar rats. One, 3, 6, 7, 10 days after the transplantation, grafted tissues were taken and examined morphologically by routinely H.E. staining and immunohistochemically using polyclonal antibodies, such as anti-albumin, anti-GSH-PO and anti-P450.

**Results and Conclusions:** FLF grafts showed morphological structure resembling adult liver in a weak. Three days after the transplantation, they exhibited strong reactivity with anti-albumin antibody. GSH-PO is produced not in a fetal stage but comes gradually to be produced after birth and reaches at the normal level in about 2 weeks. In the omentum, FLF grafts 3~6 days after the transplantation showed strong reactivity with anti-GSH-PO antibody. Another important enzyme, P-450, which functions for detoxicating metabolizes of harmful drugs was also produced in the 3 day's grafts. These results show that FLF could be converted rapidly to functionally matured status after transplantation into the omentum and one of mechanisms of FLF transplantation could be attributed to this point.

## 123 ENZYME KINETICS OF COMMERCIAL COLLAGENASES AND THEIR INFLUENCE ON PORCINE PANCREATIC ISLET ISOLATION

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A major problem, particular in porcine pancreatic islet isolation, appears to be the quality of commercial collagenase preparations. Crude preparations are contaminated with trypsin-like activity, neutral protease and clostripain. The influence of these enzymes on islet isolation is widely unknown. The aim of our study was to examine various collagenase preparations and the kinetics of enzyme activities during the isolation process. Collagenase activity was assayed by three different biochemical tests, with either collagen, the artificial FALGPA or PZ-Pro-Leu-Gly-Pro-Arg as substrates. Both, trypsin-like and clostripain activity were evaluated using assays with BAEE as substrate, neutral protease activity using the Azocoll test. Islet isolation was basically performed with the semi-automated technique (Ricordi et al., Surgery 107:688-694, 1990). Results: (1) Commercial collagenase preparations showed widely varying patterns of enzyme activities, in particular trypsin-like activity. (2) During the isolation process, trypsin-like and neutral protease activities increased strongly, presumably due to the release of enzymes from the exocrine portion of the disintegrating pancreas, whereas collagenase enzyme activity decreased. (3) Complete inhibition of trypsin-like activity occurred only with one of four enzyme inhibitors. (4) So far, yield and viability of isolated islets was insufficiently dependent on the above characterized enzyme activities. Discussion and conclusions: With different enzymes involved in commercial collagenase preparations, and, a lack of defined enzyme activity patterns, reproducibility of islet yield and islet viability remains a constant concern. Aiming at selected trypsin activity inhibition during the digestion process, it should be possible to overcome this problem.

## PREDICTION OF HUMAN ISLET GRAFT FUNCTION BY A 124 GRAFT-RECIPIENT INDEX (GRI)

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The aim of this study was to examine the relation between in vitro glucose stimulated insulin response and in vivo function of human islets after intraportal transplantation.

The integrated amount of in vitro stimulated insulin production of perfused islets over 80 min was calculated for the transplanted islet equivalents (IEQ/kg body weight of five islet after kidney graft recipients (GRI, range 19.7 - 456.2 mU/kg). The ratio of C-peptide clearance (CPCL) to endogenous creatinine clearance (CRCL) was similar in all patients (median = 18.7%). The coefficients of variation of fractional tubular reabsorption (FTR) of C-peptide (mean = 82.4%, median of CV = 15.6%) were within normal limits throughout the period of observation, so intra-subject corrections for temporal variations in C-peptide elimination were superfluous. In order to proceed to inter-subject comparisons we performed individual multiple regression analyses of effects of FPG, s-Crea, CRCL and daily insulin dosage on FPCP. Only FPG and s-Crea emerged as significant ( $p=0.03$  and  $0.01$ ) independent predictors of FPCP, so the latter was corrected accordingly by adjusting FPCP to a s-Crea of 1.0 mg/dl and a FPG of 100 mg/dl. When corrected FPCP (4th and 5th week post Tx) was plotted against GRI, it was possible to fit an inverse hyperbola of the form:  $FPCP = -constg \cdot GRI^{-1} + FPCPMAX$  ( $R^2=0.784$ ,  $p<0.01$ ). FPCPMAX (1.75 ng/ml per mg/dl s-Crea per 100 mg/dl FPG) represents the maximally attainable C-peptide level after correction for renal function and glycemia. Constg (equivalent to an ED<sub>50</sub>) determines the Graft-Recipient Index value necessary to achieve half-maximal result (FPCPMAX) and was estimated by log-logit analysis as 46.13 mU/kg ( $R^2=0.713$ ,  $p=0.05$ ).

In conclusion, in vivo basal function of human islet allografts is determined by both islet mass (IEQ/kg) and by secretion capacity as measured by perfusion. The proposed Graft-Recipient Index shows saturation properties and will be used in our center to decide whether a given islet preparation merits transplantation into a diabetic patient.

## EFFICIENCY OF HIGHLY PURIFIED ISLETS AUTO- 125 TRANSPLANTED INTO THE PORTAL VEIN AFTER TOTAL PANCREATECTOMY.

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To prevent or ameliorate the diabetic sequelae following pancreatectomy a subtotal pancreatectomy and/or autotransplantation of islet tissue can be performed if pancreatic resection is required for chronic pancreatitis. The efficiency of an islet autograft into the portal vein was studied following total duodenopancreatectomy for non duct dilated chronic pancreatitis and adenomatosis of the duodenum.

### Patient and Methods:

The operation was performed in a 37-year old female. The pancreas was flushed with U.W. solution and stored for 5 hours. Islets were prepared by automated collagenase digestion with subsequent Ficoll gradient purification. A microfluorometric membrane integrity test and glucose perfusion were used to measure islet viability in vitro. Islets were retransplanted into the portal vein under continuous portal vein pressure control. Pre-, intra- and postoperative glucagon challenge with C-peptide levels and daily plasma glucose values was performed.

### Results:

A total of 363 000 (i.e. 7 000/ kg) islets were transplanted corresponding to 643 µl islet volume. Purity was 95 %, viability 90 %. The portal pressure following injection increased from 8 cmH<sub>2</sub>O to a maximum of 13 cmH<sub>2</sub>O after 10 min. and was back to normal after 30 min.. C-peptide (ng/ml) pre/post i.v. glucagon (1 mg) : prepancreatectomy 5.0/12.0 postpancreatectomy 0.4/0.7 and posttrans-plant 3.2/5.8. Fourteen days posttransplant the patient was discharged on a regular diet without exogenous insulintherapy.

### Conclusion:

The autotransplantation of highly purified islet into the portal vein renders normoglycemia to the patient in whom total pancreatectomy is performed without the risk of rising portal vein pressure. The metabolic efficacy however is reduced as compared to the native pancreas which has to be controlled on the long term.