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The Effect of Combination Therapy with Cyclosporine A and Hydrocortisone on Glucose Metabolism in Diabetic Rats Following Pancreatic Islet Transplantation

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Summary

Glucose tolerance, insulin secretion and *in vitro* insulin action were examined in streptozotocin-induced diabetic rats following pancreatic islet allotransplantation treated with combination of oral cyclosporine A (10 mg/kg) and hydrocortisone (1.5 mg/kg) intramuscularly. 1400 pure islets from multiple donors were implanted either into the portal vein (n=10) or under the renal capsule (n=11). Ten sham-operated non-diabetic animals receiving the same immunosuppressive therapy, 8 healthy animals without any treatment and 10 diabetic animals without immunosuppression following islet transplantation were used as controls. In all transplanted animals blood glucose was normalized by day 3 after transplantation with lower levels in those transplanted intraportally ($p<0.05$). Non-immunosuppressed animals rejected the graft after 6.5 ± 1.2 days after transplantation, Immunosuppressed animals in both groups remained normoglycaemic till the end of the experiment on day 28. Oral glucose tolerance tests and insulin levels on days 10 and 28 improved dramatically. No differences in glucose and insulin levels between intraportal and subcapsular groups were found. Post-load glucose levels in immunosuppressed non-transplanted animals were higher on day 28 than before treatment and were also higher than in the healthy non-treated group ($p<0.05$). *In vitro* insulin action determined by the incorporation of labelled glucose into adipose tissue was impaired only in animals in which islets were transplanted into the liver ($p<0.05$ vs other groups). In conclusion, therapy with cyclosporine A and hydrocortisone prevents allogeneic islet rejection in rats during a short-term experiment. Although glucose tolerance is not completely normalized following transplantation, slight impairment is also demonstrable in healthy animals on the same drug therapy.

Key words

Cyclosporine A – Hydrocortisone – Glucose metabolism – Diabetes – Pancreatic transplantation

Introduction

Pancreatic islet transplantation represents a potential method of treatment of insulin-dependent (type 1) diabetes mellitus. At present several methods have been used to prevent allogeneic islet graft rejection under experimental conditions: immunoisolation, immunomodulation and immunosuppression (Scharp and Lacy 1989). Various immunosuppressive drugs have been able to prevent islet rejection in an allogeneic model for different time periods (Gray and Morris 1987). In clinical transplantation, successful islet survival has been reported from several centres (Federlin *et al.* 1991), always with the use of intensive immunosuppressive treatment which included corticosteroids, cyclosporine A (CsA), azathioprine and lymphocytotoxic antibodies (Ricordi *et al.* 1992, Scharp *et al.* 1990, Warnock *et al.* 1992).

CsA and corticosteroids, the most frequently used immunosuppressive drugs, have been reported to exhibit a diabetogenic effect, the former mainly by impairment of insulin secretion and the latter by inducing insulin resistance (Rizza *et al.* 1982), both of which may impair glucose tolerance following

transplantation of insulin-producing tissue (Saudek *et al.* 1993, Teutscher *et al.* 1994).

Diabetogenic effects of CsA have been reported *in vitro* as well as in experimental animals. Andersson *et al.* (1984) demonstrated decreased DNA and proinsulin production in CsA-treated cultured mice islets; this finding was confirmed by other authors in rodent (Gillison *et al.* 1989, Hahn *et al.* 1986, Helmchen et al 1984), dog (Basadonna *et al.* 1988) and human (Nielsen et al 1986) islets. Impaired glucose tolerance following CsA administration was also found by several authors in experimental animals (Alejandro *et al.* 1989, Dresner *et al.* 1989, Garvin *et al.* 1988, van Schilfgaarde *et al.* 1987, Wahlstrom *et al.* 1990). Furthermore, CsA may also affect early vascular islet engraftment (Rooth *et al.* 1989).

Beside the positive effect of immunosuppressive treatment the potential negative metabolic effects on glucose tolerance should also be taken into account in islet transplantation. The aim of the present study was therefore to examine glucose tolerance, insulin secretion and *in vitro* insulin action in diabetic rats treated by CsA and hydrocortisone following islet allotransplantation into renal subcapsular space or into the liver, and to compare the above parameters with those in healthy animals receiving the same drug therapy.

Methods

Experimental animals. Non-syngeneic Wistar rats (Velaz, Prague) 8–10 weeks old (weighing 200–250 g) were used as islet donors and recipients. The animals were fed a standard pelleted diet with free access to water. The study was approved by the Institute's Local Ethics Committee. Diabetes was induced by i.v. injection of streptozotocin (55 mg/kg 7 days before transplantation) and confirmed by stable non-fasting glycaemia above 24 mmol/l and pathological values of oral glucose tolerance test (OGTT).

Islet isolation. With the animals under flunitrazepam (2 mg/kg i.m.) and ketamine (100 mg/kg i.m.) anaesthesia the pancreas was exposed and pancreatic duct was cannulated through the duodenum using 25 G cannula. The hepatic duct was clamped and 10 mg collagenase (ÚSOL, Prague) dissolved in 10 ml Hanks Balanced Salt Solution (HBSS) and chilled to 4 °C was injected. The distended pancreas was removed and incubated for 20 min at 37 °C. Pancreatic tissue was dispersed and passed through a 0.5 mm plastic mesh. The preparation was washed 3 times in HBSS and centrifuged. The resulting pellet was mixed with 25 % solution of Ficoll 400 DL (Sigma, St. Louis) and centrifuged for 20 min at 4 °C in Ficoll discontinuous gradient (23 %, 18 % and 11 %). The islets were next collected from the upper interface, washed again in HBSS and resuspended in CMRL medium (Sigma, St. Louis) containing 1 % penicillin/streptomycin, 1 % glutamine and 10 % newborn calf serum (all from Sigma, St. Louis). A total of 400–500 islets were obtained from one donor. Islets were handpicked with a micropipette to obtain 1400 pure islets for one recipient.

Islet transplantation. Two hours after isolation, the islets were either injected in 0.5 ml medium into the portal vein using a 25 G cannula or implanted through a 24 G cannula under the renal capsule with the animals under flunitrazepam and ketamine anaesthesia. In control animals only the vehiculum was injected using the same procedure.

Experimental groups. The animals were divided into five groups.

Group I. Ten diabetic rats after intraportal islet transplantation treated with CsA and hydrocortisone.

Group II. Eleven diabetic rats after renal subcapsular islet transplantation treated with CsA and hydrocortisone.

Group III. Ten non-diabetic rats that were sham-operated (subcapsular injection in five rats and intraportal injection in the other five) and treated with CsA and hydrocortisone.

Group IV. Eight control rats without any treatment.

Group V. Ten diabetic rats treated by islet transplantation (five in the liver and five beneath the capsule) without any immunosuppressive treatment. These animals were sacrificed after the loss of graft function.

In groups I, II, and III CsA treatment was started one day before transplantation. The drug was given by a gastric tube once daily in a dose of 10 mg/kg in olive oil. Trough CsA blood levels were measured on days 10 and 28 using a non-specific (for CsA and metabolites) RIA kit (Cyclosporin RIA Kit, Adico, Prague). In groups I, II, and III hydrocortisone was administered intramuscularly (1.5 mg/kg) once daily starting on the day of transplantation.

Non-fasting plasma glucose levels were measured using the glucose oxidase-peroxidase method (Lachema, Brno) before transplantation and on days 0, 1, 2, 3, 5, 7, 9, 12, 14, 21 and 28. Oral glucose tolerance tests (1 g glucose/kg) were performed before starting the study in all animals, after inducing diabetes before transplantation in groups I and II, and on days 10 and 28 in all animals. Serum insulin levels were measured before the OGTT and 60 min after glucose administration by radioimmunoassay (Ria-Sax-Insulin, Sebnitz, Germany) using a rat insulin standard (Novo Nordisk, Denmark).

On day 28, the animals of groups I–IV were sacrificed and a sample of epididymal fat tissue was obtained. The tissue was incubated in the presence of ^{14}C-U-glucose at 4 different insulin concentrations

(0, 50, 250 and 1250 μU/ml). Following incubation, total lipids were extracted, the radioactivity was measured and glucose incorporation calculated (Vrána *et al.* 1969). The results were expressed in ng/mg protein. This investigation was not possible in diabetic rats because there was almost no epididymal adipose tissue present. The livers or kidneys in groups I, II and V were examined histologically.

Statistical analysis. The results are expressed as mean values ± S.E.M. Differences between groups were analyzed using the Kruskall-Wallis analysis of variance.

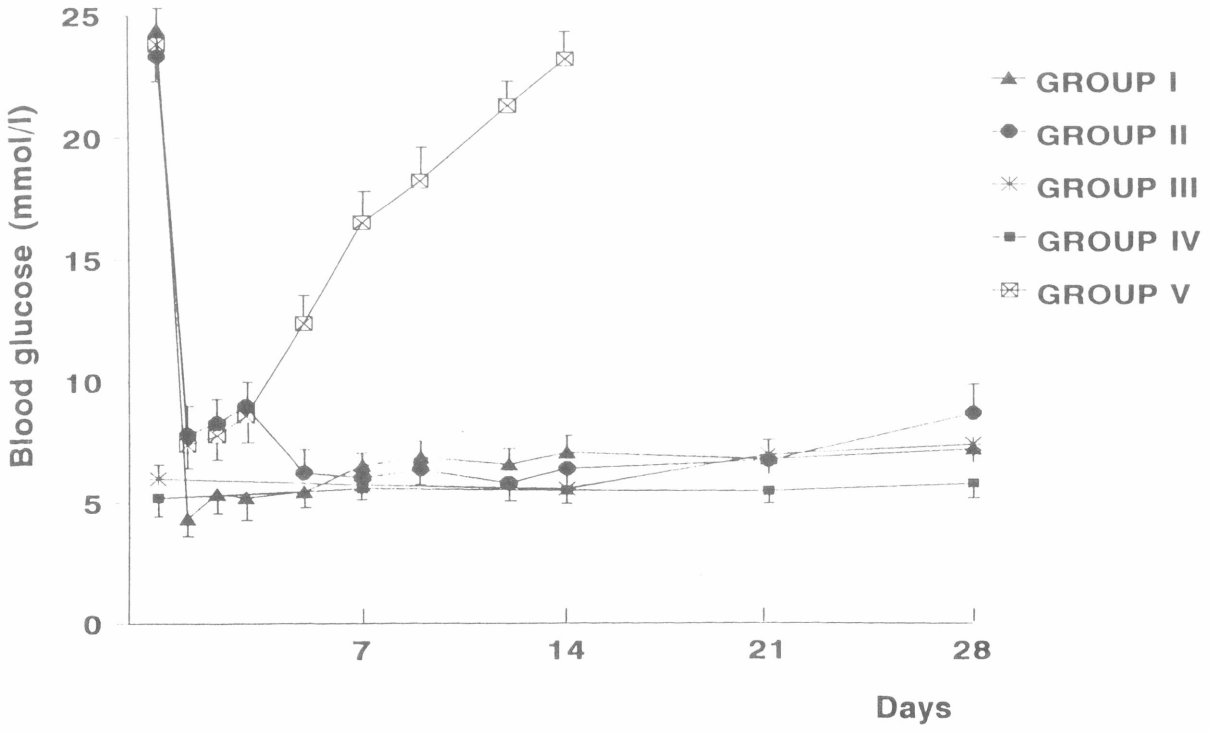

Fig. 1
Non-fasting blood glucose levels during the experiment. Group I: CsA- and hydrocortisone-treated diabetic rats after intraportal islet transplantation. Group II: CsA- and hydrocortisone-treated diabetic rats after renal subcapsular islet transplantation. Group III: non-diabetic CsA- and hydrocortisone-treated rats, Group IV: non-treated non-diabetic rats. Group V: diabetic non-treated rats after islet transplantation. Data are means ± S.E.M.

Results

Induction of diabetes. After streptozotocin administration all animals in groups I, II and V became diabetic as confirmed by non-fasting blood glucose levels above 10 mmol/l, and pathological OGTT values (Fig. 2). Fasting and post-load insulin levels were markedly reduced (Fig. 3). Fig. 1 shows mean non-fasting blood glucose levels in all groups during the experiment.

Fasting and non-fasting blood glucose levels. Following islet transplantation, blood glucose concentrations fell rapidly in groups I, II and V with lower glucose levels in animals with grafts in the portal vein than under the renal capsule (group I vs group II $p<0.05$ on days 1, 2 and 3). No statistical differences between these groups were observed subsequently. On day 28, blood glucose in group IV (healthy control animals) after feeding was lower ($p<0.05$) than in group I (diabetic animals after intraportal transplantation. Diabetic animals treated by islet transplantation without any immunosuppressive treatment again became diabetic 6.5±1.2 days after transplantation on the average.

Oral glucose tolerance tests. Glucose levels during OGTTs are shown in Fig. 2. In immunosuppressed animals (groups I and II), fasting glucose levels became normalized and post-load levels improved considerably following transplantation. After glucose administration, glucose levels were higher in immunosuppressed islet recipients (groups I and II) than in immunosuppressed and non-immunosuppressed control animals (groups III and IV) 30, 60 and 120 min after glucose administration. The differences between groups I and II (after intraportal and subcapsular transplantation) were not significant. In group III (non-transplanted animals treated with CsA and hydrocortisone) the stimulated glucose levels were higher on day 28 than before treatment and were also higher than in group IV (non-treated healthy controls) (both $p<0.05$). Fasting and non-fasting glucose levels remained unaltered.

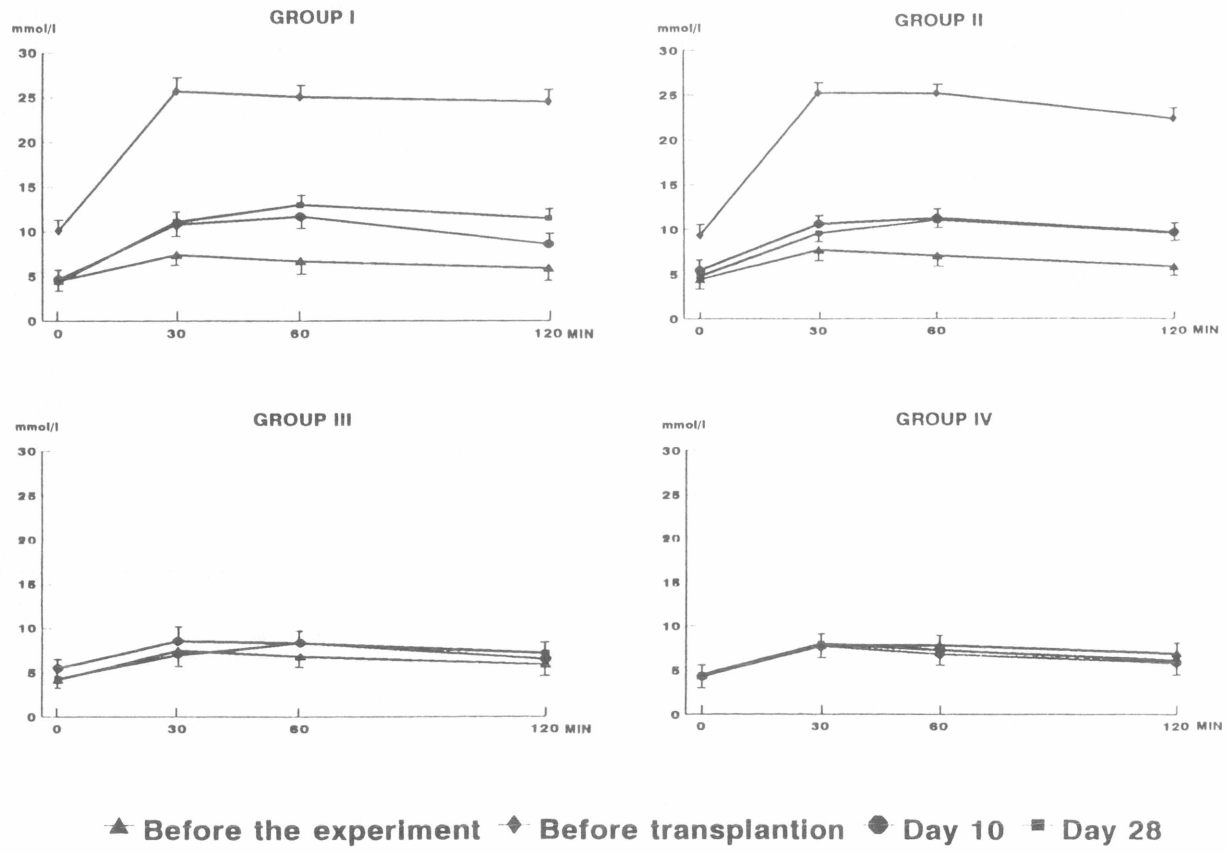

Fig. 2
Blood glucose levels during oral glucose tolerance tests in groups I, II, III and IV. For other description see Fig. 1.

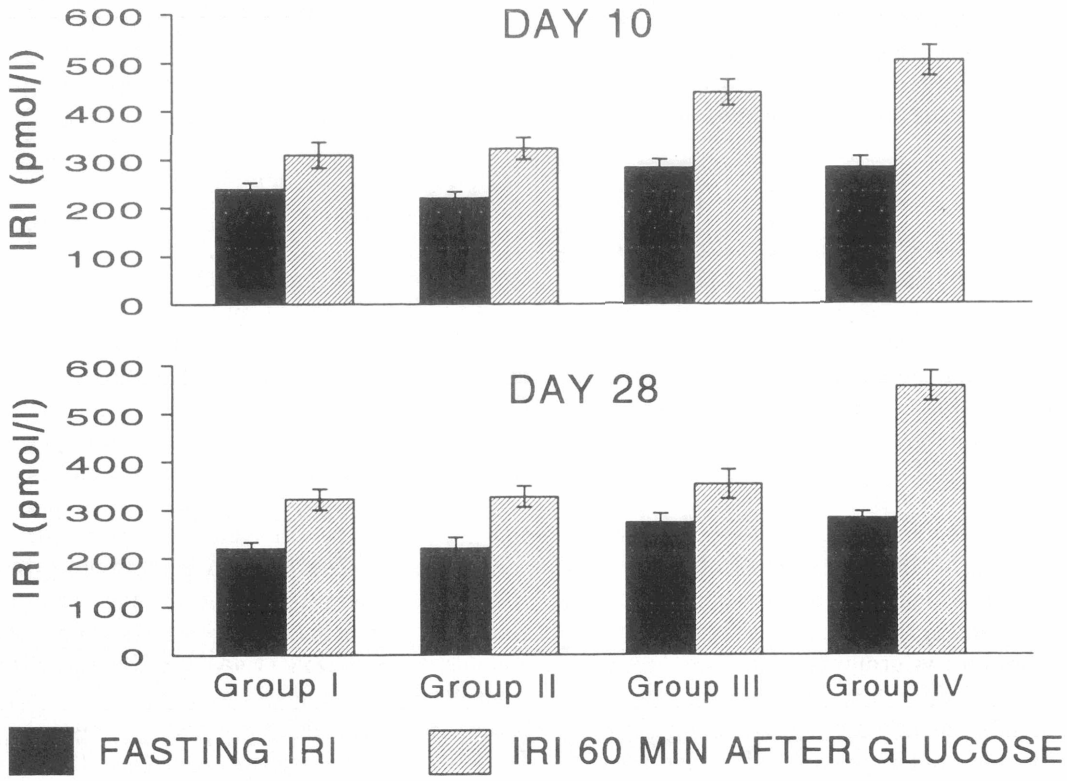

Fig. 3
Plasma insulin levels under fasting conditions and 60 min after administration of glucose (during OGTT) on days 10 (upper panel) and 28 (lower panel) of the study. For other description see Fig. 1.

Fasting insulin levels and insulin levels 60 min after glucose administration are shown in Fig. 3. Both fasting and post-load insulin levels on days 10 and 28 were lower in immunosuppressed islet recipients (groups I and II) compared with groups III and IV ($p<0.05$ for fasting and $p<0.01$ for post-load levels). Insulin levels tended to be lower in group I (intraportal islet placement) than in group II (subcapsular islet placement) following transplantation. The differences were however not statistically significant.

Incorporation of ^{14}C-U-glucose into total lipids of fat tissue as a measure of insulin action is depicted in Fig. 4. The incorporation was significantly reduced in group I (intraportal transplantation with immunosuppression) at insulin concentrations of 50, 250 and 1250 μU/ml as compared with groups II, III and IV ($p<0.01$ vs groups III and IV and $p<0.05$ vs group II). In groups II (immunosuppressed recipients with subcapsular islet placement) and III (immunosuppressed healthy controls), the incorporation was not significantly different from normal values (group IV).

Mean trough blood CsA levels on day 10 in groups I, II and III were 630±78, 610±66 and 215±21 ng/ml whereas on day 28, 710±58, 650±60 and 240±18 ng/ml, respectively. CsA levels were lower in group III (immunosuppressed healthy controls) than in immunosuppressed graft recipients (groups I and II) ($p<0.01$).

Serum creatinine levels on day 10 in groups I, II, III and IV were 39±2.1, 41±1.4, 38±1.5 and 41±1.4 whereas on day 28, 41±2.1, 43±2.2, 44±1.2 and 42±1.9 μmol/l, respectively. The differences between groups or between levels on day 10 and 28 were not significant.

Histological examination revealed morphologically intact pancreatic islets in branches of the portal vein (group I) or in the renal subcapsular space (group II) and positive staining with aldehyde-fuchsin in β-cells. In group V (non-immunosuppressed diabetic islet recipients) only a few islets were found, with lymphocytic infiltration and almost a negative reaction with aldehyde-fuchsin.

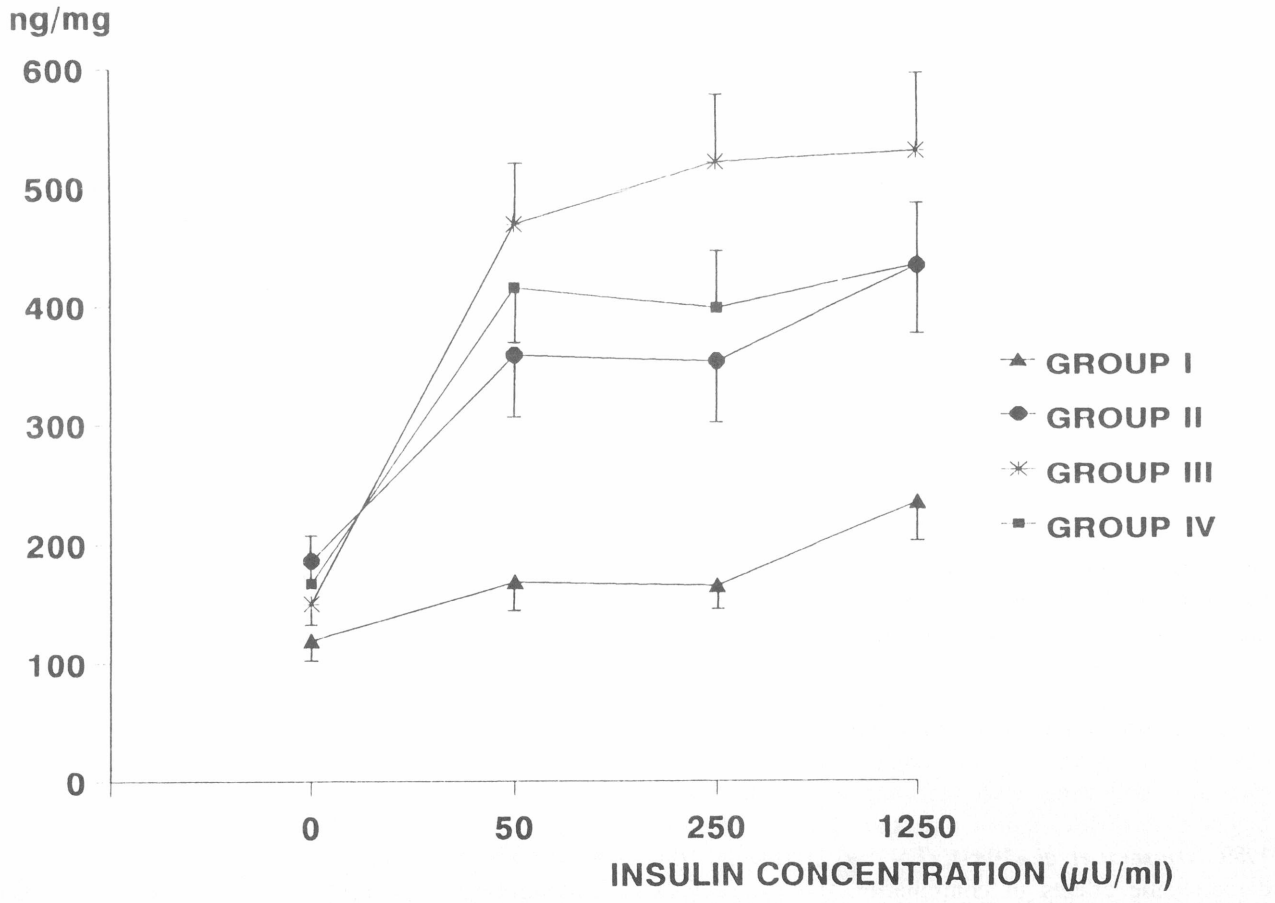

Fig. 4
Incorporation of labelled glucose into adipose epididymal tissue at different insulin concentrations as a measure of *in vitro* insulin sensitivity. For other description see Fig. 1.

Discussion

Allogeneic rat islet grafts in non-immunosuppressed recipients are usually rejected in 5–12 days following the transplant and CsA treatment may significantly prolong their survival (Dibelius *et al.* 1986, Al-Abdullah *et al.* 1991, Gray at al. 1984). In most cases doses of 20 mg/kg per day or higher have been used orally or intramuscularly. In our study we evaluated glucose metabolism in highly immunoresponsive diabetic rats following multiple donor allogeneic islet transplantation treated with a combination of moderate oral doses of CsA (10 mg/kg per day) and intramuscular hydrocortisone (1.5 mg/kg per day). Trough blood levels of CsA were rather low and comparable with those usually achieved in clinical medicine. Non-immunosuppressed control animals rejected the islets within 14 days and became again diabetic 6.5±1.2 days after transplantation on the average. We demonstrated that the combined immunosuppressive therapy was able to prevent rejection and maintain fasting and fed normoglycaemia for 28 days both in animals with islets implanted into the portal vein as well as under the renal capsule.

In diabetic animals treated by islet transplantation, blood glucose dropped below 10 mmol/l by post-transplant day 3 with normalization faster in those transplanted into the portal vein than under the renal capsule (blood glucose on days 1, 2 and 3 in group I vs group II $p<0.05$). While non-fasting blood glucose at the end of the follow-up tended to be lower in group I than in group II, the difference was not significant. Previous studies comparing the survival of intraportal and renal subcapsular islet grafts in rats produced conflicting results. While some authors prefer subcapsular placement (Reece-Smith *et al.* 1982, Yasunami *et al.* 1983), others found better results following intrahepatic placement (Gores *et al.* 1987).

Immunosuppressive treatment itself in healthy animals without islet transplantation did not cause significant impairment of glucose tolerance evaluated according to glycaemic curves and insulin levels. Nevertheless, blood glucose after feeding was slightly higher after 28-day treatment than in healthy non-treated animals and the same was found for blood glucose 120 min after glucose administration. Insulin levels did not differ significantly. Other authors, who observed a more severe impairment of glucose tolerance following CsA administration, had used higher CsA doses than in our study (Alejandro *et al.* 1989, Dresner *et al.* 1989). To our knowledge, the diabetogenic effects of combination therapy of CsA and prednisone in animals has not been studied.

Glycaemic curves did not normalize completely after transplantation but improved considerably. Insulin levels dropped dramatically after diabetes induction and almost no stimulation was demonstrated following a glucose challenge. Both fasting and post-load insulin levels rose after transplantation. However, both levels remained lower than those in healthy non-treated animals as well as in CsA- and hydrocortisone-treated control animals. No differences between group I and group II were found. This is an interesting finding because intraportally secreted insulin exhibits the hepatic first-pass effect before reaching the systemic circulation (Brown *et al.* 1979).

Evaluation of glucose incorporation into the total lipids of epididymal fat tissue as a measure of insulin sensitivity did not reveal significant differences between groups II, III and IV. However, the incorporation was significantly lower in animals with islets transplanted into the portal vein when compared with other groups. This might reflect a certain degree of insulin resistance especially in these animals. What was the cause of this phenomenon, which was only present in this particular group, is not clear but it might be consistent with the slightly worse glucose tolerance in group I than in group II. Systemic insulin levels did not differ in these groups.

Another finding not clearly understood concerns higher CsA levels in transplanted animals compared with CsA levels in control animals treated with the same drugs. Impaired CsA metabolism could be the result of the toxic effect of streptozotocin or of a previous diabetic state. The difference cannot be explained by impaired renal function, as creatinine levels remained stable throughout the whole study in all animals.

In conclusion, we have demonstrated that combination therapy with CsA and hydrocortisone prevents pancreatic islet rejection in highly immunoresponsive allogeneic rats during a short-term experiment. After the transplantation of approximately 1400 islets, glucose tolerance is not completely normalized, but a certain degree of impairment is also seen in healthy animals on the same immunosuppressive regimen. *In vitro* insulin resistance has been demonstrated in intraportally transplanted animals but not in those with islets placed beneath the renal capsule. This finding requires further investigation.

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