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SHORT COMMUNICATIONS

Glucose Turnover and Insulin Secretion in Dogs with Pancreatic Allografts

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Summary. The endocrine function of pancreaticoduodenal allografts was studied in six dogs and compared with that of normal animals. The grafts were able to prevent the diabetic ketosis that was observed in a control group after total pancreatectomy without following transplantation. — Systemic hyperglycaemia enhanced the insulin release from the transplanted pancreas, as measured by increased IRI levels after intravenous glucose administration. In contrast, the stimulation of insulin secretion by oral glucose loading was less than in normal dogs, while the glucose assimilation was also increased in transplanted animals. — It was speculated that the duodenum might be more susceptible to immunological damage than the pancreas, and that consequently an impairment of the resorption of glucose and of the production of intestinal factors controlling the secretion of insulin may result. — In the near future, pancreatico-duodenal transplantation does not appears to become a therapeutical alternative to the conventional treatment of diabetes mellitus in man.

Introduction

The endocrine function of the transplanted pancreas has been repeatedly studied since 1892 when Minkowski used the technique of pedicle transplantation of a pancreatic lobe, by which he demonstrated the relationship between the pancreas and diabetes mellitus [2, 4, 6, 9, 17, 19, 20, 21]. An orthotopic pancreatico-duodenal transplantation permitting a normal exocrine as well as endocrine function of the graft was used for the first time by Largiadèr *et al.* [12, 13, 14]. The transplantation en bloc of the functionally-connected pancreatico-duodenal complex is a technique that may eventually advance to a therapeutical procedure in the surgical treatment of chronic pancreatitis, carcinoma of the pancreas and maybe also diabetes mellitus. In view of this last possibility we were prompted to study the endocrine function of these grafts. Since the dogs survived the operation for approximately three weeks, the experiments were not carried out in the immediate postoperative period when the animals did not yet feed normally and when carbohydrate metabolism was still disturbed.

The response of the graft to systemic hyperglycaemia was tested by intravenous glucose administration, and the function of the glucose-responsive "enteroinsular axis" [18] was investigated by oral glucose tolerance tests. Some of the results have previously been published in preliminary form [7].

Materials and Methods

Adult mongrel dogs of either sex weighing between 19 and 26 kg were used. The surgical procedure for pancreatectomy and pancreatico-duodenal allotransplantation is schematically shown in Fig. 1. The method, the surgical results and the exocrine function of the allograft have been described in detail elsewhere [12, 13, 14]. Immunosuppressive therapy consisted of daily administrations of Azathioprine (4—8 mg per kg body weight). All recipient dogs had this treatment. After the immediate postoperative period of three to four days the dogs were again fed normally. No intravenous or oral glucose tolerance tests were performed prior to the 10th postoperative day. The animals were fasted 12—16 h before the tests.

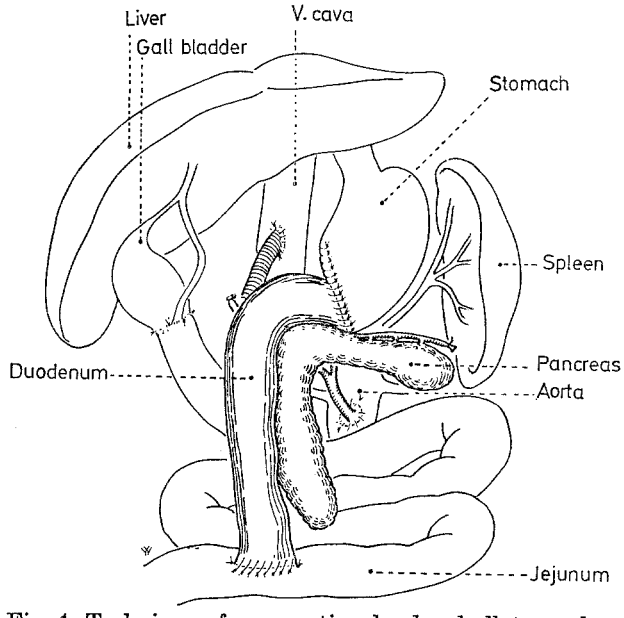

Fig. 1. Technique of pancreatico-duodenal allotransplantation in the dog. (From 14)

Intravenous and oral glucose tolerance tests were performed by the method of Conard [5] and Arnould *et al.* [1] in unanaesthetized animals. Glucose was given intravenously (0.5 g/kg) or orally by gastric tube (2.0 g/kg, in a 25% solution containing 4.25 g of NaCl).

Blood samples were obtained by venipuncture before glucose administration and every 5 to 10 min

thereafter for 50 to 75 min. The kinetics of glucose utilization were studied as described by Arnould [1], using the rate of disappearance of blood glucose-^{14}C activity after intravenous injection of 40 μCi of glucose-U-^{14}C. T½ of blood glucose and radioactivity was determined graphically on semilogarithmic paper.

Blood glucose was measured with glucose oxidase [3]. Plasma free fatty acids were extracted and titrated according to Gordon [8]. ^{14}C-labelled glucose was obtained from the Radiochemical Centre, Amersham, England. It was isolated from blood in the form of osazones and counted in a low background flow counter. The values obtained were corrected for self-absorption. Serum immunoreactive insulin was determined by a double antibody immunoassay[1].

Results

1. *Pancreatectomy and prevention of diabetic ketosis by pancreas transplantation*

In four dogs total duodeno-pancreatectomy with choledocho-duodenostomy and gastro-jejunostomy was performed, and their blood sugar, plasma free fatty acids and acetone were determined. The daily infusion of 1 l of slightly hypotonic and alkaline solution (equal parts of 0.95% NaCl, 1.6 M sodium bicarbonate and distilled water) corrected fluid losses and acidosis to some extent. The survival time of the dogs after pancreatectomy ranged between 7 and 31 days. All animals developed severe diabetes mellitus and ketosis, and finally died. The mean levels of fasting blood sugar and plasma free fatty acids between the 7th and 10th day postoperatively were 337.2±32.0 mg% and 1.33±0.05 mval/l respectively (mean ± SEM). In contrast, the fasting blood sugar of 3 dogs was the same before and 10 days after pancreatectomy followed by pancreas transplantation and Azathioprine therapy (78.0±3.9 mg%, 83.0±11.8 mg%). The administration of Azathioprine to control animals which were not operated upon did not influence the fasting blood sugar.

2. *Kinetics of glucose utilization and insulin secretion in response to glucose administration. Influence of transplantation and/or Azathioprine therapy*

a) Oral glucose tolerance tests. Oral glucose administration to healthy dogs normally elicits only a small rise of blood sugar, and T½ of the ^{14}C-activity of blood glucose varies appreciably from one animal to another. When the same animals were tested before and after transplantation an increase in T½ of ^{14}C-activity of blood glucose was apparent at each instant after glucose loading (Table 1). Oral glucose elicited an increase in the disappearance rate of ^{14}C-glucose in each dog in all groups. Transplantation and Azathioprine, as well as Azathioprine alone, seemed to reduce this response to glucose to some extent (Fig. 2).

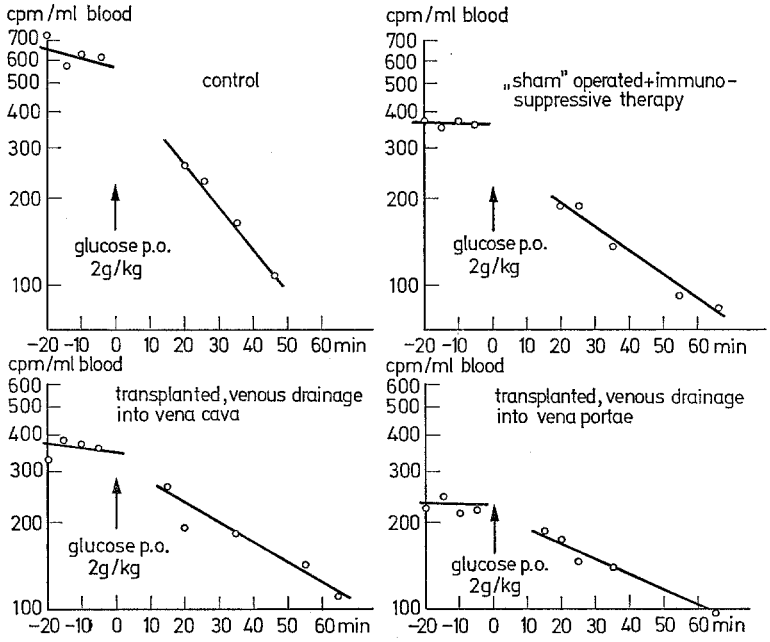

Fig. 2. Glucose assimilation before and after oral glucose in dogs with pancreatico-duodenal transplants. After intravenous injection of 40 μCi of glucose-U-^{14}C the disappearance of blood glucose-^{14}C-activity was measured before and after intragastric administration of unlabelled glucose (2 g/kg). The glucose load was given at 0 min. One animal was tested in every group. For technical details see section on methods

1 We are grateful to Prof. E. Flückiger and Dr. W. Hammer, Sandoz AG, Basel, for these determinations.

Serum immunoreactive insulin was about doubled 30 min after glucose in the control group, whereas it was only insignificantly influenced in recipients of allografts (Table 1).

b) Intravenous glucose tolerance tests. Intravenous injection of glucose resulted in a sharp increase of the blood sugar after 10 min. 30 min after the injection a clear-cut reduction of the blood sugar level was apparent in all animals (Table 2). T½ of blood glucose-^{14}C-activity again seemed to be somewhat prolonged after transplantation, but also after treatment with Azathioprine alone.

Table 1. *Results of oral glucose tolerance tests*
Blood sugar and IRI values were measured before and 35 min after oral glucose (2 g/kg). 60 min prior to the administration of oral glucose a trace amount (40 μCi) of glucose-U-^{14}C was given intravenously, and the biological half life of blood glucose (T ½) was graphically determined on semilogarithmic paper by plotting the fall in radioactive glucose per ml of blood after oral glucose administration

Group	Dog No.	T½ min	Blood glucose mg/100 ml	IRI μU/ml
	40	24	94	13
			86	41
Control	211	26	86	13
			154	26
	259	38	80	13
			92	23
Immunosuppressive	83	34	81	13
therapy			120	21
Transplanted	45	43	69	11
venous drainage			88	18
into inferior	211	39	106	19
vena cava			115	21
Transplanted	259	77	46	11
venous drainage			85	12
into portal vein				

Serum IRI was about doubled 10 min after intravenous glucose in the control group, and the insulin release from the transplanted pancreas was of the same order of magnitude. Azathioprine alone appeared to have no effect on IRI levels.

Discussion

The endocrine function of the transplanted pancreas has previously been studied by the measurement of postoperative blood sugar levels [2, 19, 20, 21], as well as by intravenous glucose tolerance tests and the determination of IRI-levels [9, 19]. Our data obtained by the same methods indicate that a transplanted dog pancreas is indeed able to enhance insulin release in response to systemic hyperglycaemia and confirms the findings of Ota *et al.* [9, 19].

The duodeno-pancreatic complex not only represents an anatomical and surgical unit, but also a physiological one. It is well known that the duodenum plays an important role in the humoral control of exocrine as well as endocrine pancreatic function. The technique used for duodeno-pancreatic transplantation in our experiments was developped by Largiadèr and coworkers [14] with the intention to preserve both the endocrine and the exocrine function of the graft. As a further advantage of this method, no pancreatic regeneration is possible after duodeno-pancreatectomy as had been reported when other techniques were used [2]. The investigation of the function of the glucose-responsive "entero-insular axis" [18] by oral glucose administration revealed that although the glucose assimilation rate was somewhat increased in response to the insulino-secretory stimulus, IRI-levels remained essentially unchanged.

Table 2. *Results of intravenous glucose tolerance tests*
Blood sugar and IRI values were measured before, 10 and 30 min after intravenous glucose administration (0.5 g/kg). Again, a trace amount (40 μCi) of glucose-U-^{14}C was given intravenously 60 min before the tolerance tests, and T½ of blood glucose was determined as explained in the legend to Table 1

Group	Dog No.	T½ min	Blood glucose mg/100 ml	IRI μU/ml
			64	13
	3	25	162	36
			77	11
			74	14
	123	30	192	28
			139	28
Control			48	13
	305	42	105	38
			70	22
			72	11
	279	17	180	27
			83	10
			84	9
	86	37	183	21
			118	24
			70	9
Immunosuppressive	3	39	189	27
therapy			135	9
			80	12
	123	40	172	56
			123	22
			63	20
	173	41	148	—
			106	26
Transplanted			66	9
venous drainage into	x	36	162	32
inferior vena cava			104	19
			65	9
	86	75	147	22
			121	19

An impairment of glucose resorption cannot be the only cause for the deficient insulin response, since blood sugar values after glucose loads were not higher in the control group than in the transplanted dogs, and yet IRI values were twice as high. Thus, our results are in agreement with the conclusion of Arnould [1] that the pancreatic response to oral glucose in dogs is influenced by intestinal factors as well as by systemic hyperglycaemia. In dogs with pancreatico-duodenal

transplantation this regulatory intestinal function seems to be impaired. It has previously been observed by post mortem examination that the immunological reaction of the pancreas is less pronounced than that of the duodenum. In our study, four out of ten dogs died after transplantation because of severe ulcerating inflammation of the duodenum [13].

One may conclude, therefore, that the pancreatico-duodenal grafts are unable to respond in a normal manner to oral glucose because of the signs of immunological rejection of the duodenum, whereas the more resistant B-cells may be stimulated by systemic hyperglycaemia.

By the surgical technique routinely used in this study, the vena portae of the graft was implanted into the vena cava of the recipient animal, so that the pancreatico-duodenal venous blood bypassed the liver. The effect of the porto-caval shunt on glucose metabolism has repeatedly been studied [9, 15, 16, 22]. No clear cut effects of porto-caval transposition on the fasting blood sugar values and on the glucose assimilation rate were observed by Lorenz *et al.* [15].

In our series one porto-portal anastomosis was performed. We failed to observe any difference in the carbohydrate metabolism in this animal and the metabolism in the other transplanted dogs. Thus, our results confirm the observation of Jdezuki *et al.* [9].

Pancreatico-duodenal transplantation has already been therapeutically used for the treatment of "severe human diabetes mellitus and its complications" [10, 11]. The patients required no insulin treatment for 31 to 134 days postoperatively. Since immunological complications affecting the duodenum seem to be present long before failure of the B-cells becomes apparent, we still have a long way to go before pancreatico-duodenal transplantation might become a feasible therapeutic tool in the treatment of diabetics. However, transplantation of the pancreas continues to be an interesting experimental means for the study of the pathophysiology of diabetes mellitus, as it was already in Minkowski's times.

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