PROGRESSION OF NEPHROPATHY AFTER ISLET OF LANGERHANS TRANSPLANTATION IN ALLOXAN-INDUCED DIABETIC RATS[1]

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SUMMARY: We studied the effects of islet of Langerhans transplantation (IT) on the kidney lesions of rats with alloxan-induced diabetes. Forty-five inbred male Lewis rats were randomly assigned to 3 experimental groups: group GI included 15 non-diabetic control rats (NC), group GII included 15 alloxan-induced diabetic rats (DC), and group III included 15 alloxan-induced diabetic rats that received pancreatic islet transplantation prepared by nonenzymatic method from normal donor Lewis rats and injected into the portal vein (IT). Each group was further divided into 3 subgroups of 5 rats which were sacrificed at 1, 3, and 6 months of follow-up, respectively. Clinical and laboratorial parameters were recorded in the mentioned periods in the 3 experimental groups. For histology, the kidneys of all rats of each subgroup were studied and 50 glomeruli and 50 tubules of each kidney were analyzed using light microscopy by two different investigators in a double blind study. The results showed progressive glomerular basement membrane thickening (GBMT), mesangial enlargement (ME), and Bowman's capsule thickening (BCT) in the 3 experimental groups throughout the follow-up. These alterations were significantly more severe in DC rats at 6 months when compared to NC rats ($p < 0.01$). However, the degree of GBMT, ME, and BCT observed in DC rats was not statistically different from IT rats at 1, 3, and 6 months. In addition, Armanni-Ebstein lesions of the tubules (AE) and tubular lumen protein (PRO) observed in DC rats were also observed in IT rats all over the study. These lesions were never present in NC rats. We conclude that IT did not prevent progression of kidney lesions in alloxan-induced diabetic rats within 6 months after transplantation.

SUBJECT HEADINGS: Islet transplantation. Diabetic nephropathy. Alloxan-induced-diabetes.

INTRODUCTION

Diabetes mellitus is a world-wide health problem with a high prevalence of secondary complications[18]. However, there is no a suitable treatment for controlling these complications.

Insulin therapy does not prevent progression of chronic lesions of disease on vessels, kidneys, eyes and nerves of diabetic patient[4,5,40]. Exogenous administration of insulin does not maintain physiologic glucose levels during a 24 hours period; as consequence the dysmetabolism of diabetes cannot be eliminated.

Insulin pumps[1] and continuous implantable insulin systems[13] which were programmed to release insulin by demand and to maintain plasma glucose at a constant levels have not also been capable to control the chronic complications of diabetes[19,38]. Although these systems may improve the metabolic control of diabetes[39], they have not solucioned the instability of the disease, the glucagon-somatostatin interaction, and the counter-regulatory response of organism to the hypoglycemia[25], which probably should also be involved in its genesis.

Now a days, pancreas transplantation is routinely used in type I diabetic patients with advanced

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disease[36]. This procedure corrects the metabolic abnormalities of diabetes[28] and has been able to prevent, stabilize and/or reverse the chronic lesions of diabetes[35]. However, pancreas transplant implies in a major surgery associated to several complications and its potential risks for the patients[10].

Evidences have suggested that metabolic disorders caused by functional damage of the pancreatic beta-cells are the causes of diabetic complications[30,40]. Therefore, islet of Langerhans transplantation (IT) is the most rational method for treatment of diabetes. This method is capable to reestablish the normal endocrine function in man[37] and animals[2]. In addition, it is also associated to low index of morbity and mortality without the problems caused by a major surgery. However, the real effects of IT on chronic lesions of diabetes have been under investigation. The study protocols are multiple and results are variable and conflictants[27]. In present work we addresse the kidney lesions of alloxan-diabetic rats and the use of IT as a means of prevention of these lesions. We hope to contribute for better understanding of diabetes and its advanced therapeutic propositions.

METHOD

Animals

Forty-five inbred male Lewis rats, weighing approximately 250 g were randomly assigned to 3 experimental groups: group GI consisted of 15 non-diabetic control rats (NC), group II included 15 alloxan-induced diabetic control rats (DC), and group GIII included 15 alloxan-induced diabetic rats that received pancreatic islet transplantation from normal donor Lewis rats injected into the portal vein (IT). Each group was further divided into 3 subgroups of 5 rats which were sacrificed at 1, 3, and 6 months of follow-up, respectively. Rats that died throughout the follow-up were replaced.

Diabetes was induced by intravenous administration of alloxan (J.T. Baker Chemical Co., Phillipsburg, New Jersey) in a single dose of 42 mg/kg of body weight. Only rats with glucose levels above 200 mg/dL, urine glucose levels above 3,000 mg/dL, and severe clinical alterations as polydipsia, polyuria and polyphagia were included in the experiment.

Clinical and laboratorial analyzes

Seven days pre-islet transplantation and 4 days, and 1, 3, and 6 months post-islet transplantation, rats were housed in metabolic cages for 24 hours. During this time, their body weight, food and water intake, urine output, blood and urine glucose levels, and plasma insulin levels were recorded. These same parameters were also recorded for NC and DC rats. Blood and urine glucose levels were determined by enzymatic method (Celm. Equi. Lab., São Paulo, Brazil), and plasma insulin levels determined by radioimmunoassay (DPC Co., Los Angeles, CA, USA).

Islet preparation

Islets of Langerhans were prepared by nonenzymatic method according to the original procedure discribed by Hinshaw et al[11]. Briefly, the pancreas from 4 rats were excised simultaneously and immediately immersed in Hanks solution at 4°C. After excess fat has been trimmed, the pancreas was minced with scissors into fine 3 to 5 mm fragments. They were then gently hand-pressed through a stainless steel sieve of 0.25 mm pore size and repeatedly rinsed with cold culture medium (RPMI - 1640, Sigma Chemical Company, St. Louis, USA) at 4°C. The obtained pancreatic tissue was transferred to a 50 ml centrifuge tube which was placed in an ice bath for 3 minutes. The tube was then centrifuged in a refrigerated centrifuge (4°C) during 3 sequences. After the first centrifugation (500 revolutions/min) for 4 minutes, the supernatant was decanted into another tube and the pellet containing exocrine material was discarded. The tube with the supernatant was then centrifuged at 2,000 revolutions/min in a second centrifugation for 5 minutes. The obtained pellet containing the islet tissue was diluted with RPMI and it was processed again in a third centrifugation (2,000 revolutions/min; 5 minutes). The supernatant was discarded and the pellet was diluted in 2 ml of Hanks solution (4°C). A sample of this prepared suspension was taken and tissue viability determined by 1% trypan blue exclusion. Two milliliters of islet tissue suspension were injected into the portal vein in IT rats 14 days after alloxan-diabetes induction.

Histology

Tissue fragments of the kidneys of all rats in each subgroup were fixed in 10% formalin, embedded in paraffin and stained with Hematoxilin-Eosin. Fifty glomeruli and 50 tubules from each kidney were analyzed under light microscopy by two different observers in a double blind study. A total of 250 glomeruli and 250 tubules were examined for each subgroup by each of two investigators. Glomerular basement membrane thickening (GBMT), mesangial enlargement (ME), Bowman's capsule thickening (BCT), Armanni-Ebstein lesions of the tubules (AE), and tubular lumen protein (PRO) were studied. Both glomerular and tubular lesions were scored on a scale of 0 (absent), 1 (minimum), 2 (medium), and 3 (severe). The discrepancy between the two observers was 5% or less.

Statistical analysis

Laboratory and clinical data were analyzed according to Morrison's Profile Analysis[26] and histology by variance and Student-test; $p < 0.01$.

RESULTS

Clinical and laboratory

NC rats showed no evidence of clinical or biochemical alterations throughout the follow-up. All rats of this group had a progressive gain of body weight with water and food intake, urine output, blood and urine glucose levels, and plasma insulin within normal values. In contrast, DC rats presented progressive loss of body weight with significant increase of blood and urine glucose levels, significant decrease of plasma insulin and severe clinical alterations as polyuria, polyphagia and polydipsia (Table I). Four diabetic rats (26,6%) died over the course of study due to metabolic abnormalities and/or pneumonia associated to cachexy in the subgroups of 1 month (1 rat), 3 months (1 rat), and 6 months (2 rats). These rats were further replaced.

Table I - Metabolic parameters in non-diabetic control and alloxan-induced diabetic rats 14 days after induction.

Parameters	Non-diabetic control rats (n = 15)	Alloxan-diabetic rats (n = 30)
Water intake (ml/24h ± SD)	45.6 ± 12.3	100.1 ± 29,4*
Food intake (g/12h ± SD)	17.9 ± 6.8	38.4 ± 7.0*
Urine output (ml/24h ± SD)	8.3 ± 1.4	50.0 ± 14.3*
Blood sugar (mg/dL ± SD)	128.7 ± 15.4	298.4 ± 19.4*
Insulin (µU/ml ± SD)	43.4 ± 5.1	26.4 ± 2.1*

*p < 0.01 when compared to non-diabetic control rats.

Of the islet transplanted rats, 1 rat died in the immediate postoperative period due to acute portal hypertension and it was replaced. Beginning at the 4th postoperative day, 11 transplanted rats (73,3%) showed complete resolution of hyperglycemia, glucosuria, polyuria, polyphagia, polydipsia, and complete restoration of plasma insulin levels observed in pre-transplant period (Table II). Four rats from the subgroups 1 month (1 rat), 3 months (2 rats) and 6 month (1 rat) respectively (26,6%) presented a partial improving of theses abnormalities. In these animals we observed a significant decrease in water and food intake, urine output, blood and urine glucose levels, and a significant increase of plasma insulin levels when compared to DC rats, mainly at the first month after transplantation. However, the means of the values of these parameters were progressively deteriorating throughout the study. Beginning at the 2th month after transplantation the values in these rats were better than DC rats but, they were never similar to NC rats (Fig. 1).

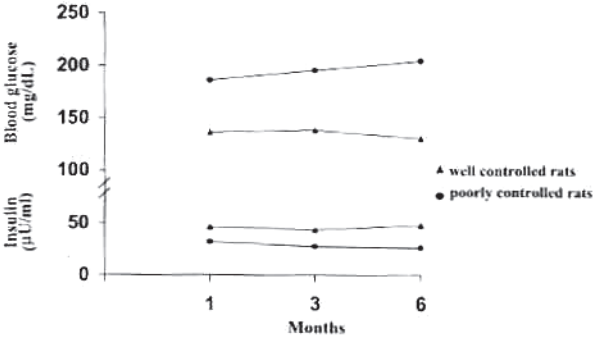

Fig. 1 - Means of blood sugar and plasma insulin in islet transplanted rats with and without good metabolic control.

Histology

Histologic analysis of the kidneys of the 3 experimental groups showed progressive GBMT, ME, and BCT throughout the follow-up. These alterations were significantly more severe in DC rats when compared to NC and IT rats at 6 months ($p < 0.01$). However, the degree of GBMT, ME, and BCT was not statistically different in the 3 experimental groups at 1 and 3 months. DC rats also showed AE, characterized by moderate to severe vacuolization of the tubules and PRO, characterized by the presence of protein in the lumen of the tubules, already observed after 1 month. NC rats showed no evidence of AE or PRO.

Table II - Clinical and laboratorial alterations in alloxan-induced diabetic rats before and after islet transplantation[a].

Parameters	Pretransplant (day 14) (n = 15)	Posttransplant (months) 1 (n = 5)	3 (n = 5)	6 (n = 5)
Water intake (ml/24h ± SD)	96.7 ± 36.0	60.4 ± 15.2*	65.6 ± 18.7*	70.3 ± 12.4*
Food intake (g/12h ± SD)	35.3 ± 6.1	20.8 ± 5.6*	24.8 ± 5.3*	19.6 ± 6.0*
Urine output (ml/24h ± SD)	56.4 ± 12.3	24.5 ± 16.2*	30.6 ± 15.8*	32.0 ± 12.8*
Blood Sugar (mg/dL ± SD)	286.4 ± 22.1	161.4 ± 26.8*	166.7 ± 30.0*	167.4 ± 36.5*
Insulin (µU/ml ± SD)	24.5 ± 1.8	39.1 ± 2.0*	35.3 ± 1.8*	37.2 ± 1.4*

[a] including 4 rats without good metabolic control

*p < 0.01 when compared to pretransplant values

In the IT group we observed a decrease of the degree of GBMT, ME, and BCT when compared to DC rats at 1, 3, and 6 months. However, the differences were not statistically significant. IT rats also showed minimum or moderate AE and PRO, mainly at the 6th month of follow-up. The degree of glomerular and tubular alterations observed in

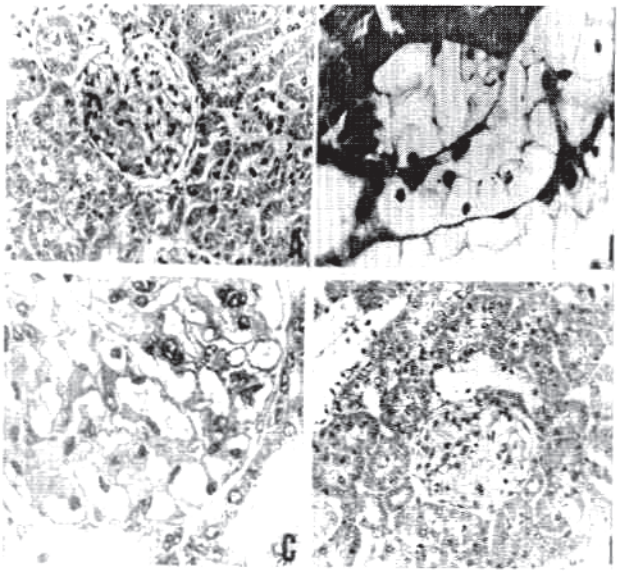

Fig. 2 - Renal glomeruli and tubules of rats sacrificed at 6 months in the 3 experimental groups. A - glomerulus an tubules of non-diabetic control rat showing normal structures (H & E, 400x); B - Armanni-Ebstein lesions with renal tubules showing severe glycogenic vacuolization (clear cells) in alloxan-diabetic rat (H & E, 1000x); C - detail of glomerulus of alloxan-diabetic rat showing intense thickening and duplication of basement membrane and increase of mesangial matrix (H & E, 1000x); D - glomerulus and tubules of islet transplanted rat showing moderate thickening of glomerular basement membrane and vacuolization of the tubules (clear cells - H & E, 400x).

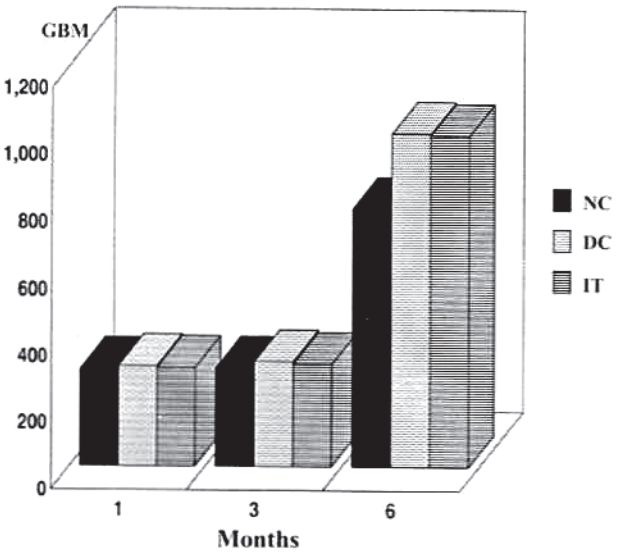

Fig. 3 - Total scores of glomerular basement membrane (GBM) in each subgroup, into the 3 experimental groups, obtained from two different observers (NC: non-diabetic control; DC: diabetic control and IT: islet transplanted rats).

Table III - Means ± SD of glomerular basement membrane thickening (GBMT), mesangial enlargement (ME), and Bowman's capsule thickening (BCT) obtained by total scores from two different investigators for the three experimental groups.

Groups and Parameters	Months		
	1[a]	3[a]	6
Non-diabetic control			
GBMT	0.58 ± 0.30	0.59 ± 0.40	1.54 ± 0.30
ME	0.73 ± 0.50	0.79 ± 0.40	1.56 ± 0.47
BCT	0.28 ± 0.40	0.63 ± 0.52	1.36 ± 0.35
Diabetic control			
GBMT	0.60 ± 0.52	0.63 ± 0.55	1.99 ± 0.31*
ME	0.75 ± 0.52	0.81 ± 0.45	2.00 ± 0.33*
BCT	0.31 ± 0.48	0.65 ± 0.45	1.88 ± 0.27*
Islet transplantation			
GBMT	0.59 ± 0.40	0.60 ± 0.42	1.94 ± 0.37[b]
ME	0.75 ± 0.54	0.83 ± 0.46	1.98 ± 0.41[b]
BCT	0.27 ± 0.36	0.63 ± 0.41	1.85 ± 0.40[b]

* $p < 0.01$ when compared to non-diabetic control rats
[a] non-significant for the three experimental groups
[b] non-significant when compared to diabetic control rats

transplanted rats with sustained normoglycemia was not also statistically different from rats with observed glycosuria and/or hyperglycemia over the course of experiment. The renal glomeruli and tubules of rats in the 3 experimental groups at 6 months are displayed in Fig. 2. Total scores of GBMT and means ± SD of scores of GBMT, ME, and BCT are showed in Fig. 3 and Table III, respectively.

DISCUSSION

Evidences have suggested that metabolic abnormalities caused by functional damage of the pancreatic beta-cells leading to hypoinsulinemia and hyperglycemia are the causes of diabetic nephropathy[21,22]. Therefore, the good control of the glucose metabolism has been considered of utmost importance in the prevention of this complication.

Unfortunately, conventional treatment of type I diabetes mellitus with insulin does not prevent progression of nephropathy and other chronic complications of diabetes[4,15,17].

Several experimental studies have reported that islet transplantation (IT) is capable to reestablish the endocrine dysbalance of diabetes[14] and controlling the chronic complications of disease[9,12,42]. However, these findings are still controversial[27] and the successful results usually observed with IT in animals have not been extrapolled for man[29,37].

Experimental diabetes in the rat induces morphological and functional changes on the kidney similar to the changes observed in human[33]. At present is open to discussion as to whether the control of glucose metabolism achieved by IT is sufficient for the prevention or reversal of these diabetic lesions.

Mauer et al[24]. reported that IT was capable to decrease mesangial matrix and progression of glomerular lesions in streptozotocin-diabetic rats 3 months following transplantation with sustained normoglycemia. However, in subsequent study Mauer et al.[23] showed that the index of mesangial thickening remains slightly increased in transplanted rats six to nine weeks after IT.

Weber et al[41] while studying the effects of IT on renal function and morphology of short-and long-term diabetic rats reported decrease of glomerular basement membrane thickening (GBMT) and normalization of the urine protein excretion in streptozotocin-diabetic rats within 3 months after transplantation. These findings were further confirmed by others[3,7,31]. However, conflictants results have been usually found in this field.

Steffes et al[34] reported that successful IT and correction of hyperglycemia failed to reverse GBMT despite documentation that this treatment reverses other manifestations of diabetic glomerulopathy in the rat including the increased urinary albumin[20] and mesangial thickening[24]. These authors suggested that GBMT, *per se*, is not a significant factor causing albuminuria. In addition, the rate of GBMT turnover may be very slow in rats with long-standing diabetes, suggesting that there is an early period of reversibility of the kidney lesions by IT that probably should not be exceeded.

Gtzsche et al[8] related that IT was uncapable to revert the material glomerular basement membrane accumulation despite reversibility of renal hypertrophy in diabetic rats within four weeks of normoglycemia. They suggested that probably one month of normoglycemia has been quite insufficient to remove the accumulation.

However, Orloff et al[27] in a long-term study showed that pancreatic islet transplantation failed to maintain strict metabolic control of diabetes and preventing nephropathy in alloxan-diabetic rats that received islet tissue prepared with collagenase and injected into the portal vein.

Previous study in our laboratory demonstrated that pancreatic islet tissue obtained from inbred rats using nonenzimatic method was as efficient as pancreatic islet tissue obtained by collagenase digestion to maintain metabolic control of alloxan-diabetic rats[32].

At present issue we demonstrated that IT failed to prevent the kidney lesions in diabetic rats despite good metabolic control in large number of rats. On the other hand, we did not observe statistically significant difference in degree of the glomerular and tubular alterations of the "well controlled rats" with sustained normoglycemia after IT when compared to the "poorly compensated rats" with glycosuria and/or hiperglycemia. Paradoxly, Mauer et al[23] reported improvement in glomerular morphology in islet transplanted rats despite persistent hyperglycemia; however, normal levels of plasma insulin were achieved.

These remaining doubts show that is still unclear whether the normalization of blood glucose or insulin or both is necessary for the prevention renal lesions. Federlin et al[6] have shown that some animals with reversed kidney lesions by IT still had glycosuria which expresses at least temporarily hyperglycemia. It might suggest be more important for the reduction of renal lesions to normalize insulin levels than those of blood glucose. In our study, however, rats with elevated glucose levels also had subnormal insulin levels in the correspondent moments of evaluation over the course of experiment. Köesters et al[16] have shown that islet transplant normalizes the phagocytic function of the mesangial cells. Therefore, it is also possible that improvement of the renal lesions in diabetic rats after IT might be primarily consequence of better phagocytic activity of the mesangial cells than strict control of glucose metabolism.

CONCLUSION

Islet of Langerhans transplantation (IT) is a method with enormous potential for treatment of insulin-dependent diabetes mellittus. However, is open to discussion whether it is capable to control the metabolic abnormalities of diabetes and its chronic complications.

At present work we concluded that IT failed to prevent renal changes in alloxan-induced diabetic rats despite good control of hyperglycemia. It suggests that many other factors, not addressing in this study, as the glucagon-somatostatin interaction and action of the insulin-like hormones, might be participating in their genesis. Therefore, in order to confirm the real effectiveness of IT on chronic lesions of diabetes, new investigations will still be necessary to elucidate the questions reported herein.

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