Islet Transplantation Inhibits Diabetic Retinopathy in the Sucrose-fed Diabetic Cohen Rat

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Purpose. To study the effect of islet transplantation on the development of diabetic retinopathy in the sucrose-fed diabetic Cohen rat, a useful experimental model of accelerated microvascular disease.

Methods. Syngeneic transplantation of collagenase-ficoll isolated islets by intraportal injection was performed either after 6 weeks or after 12 weeks of diabetes, i.e., before or after the first morphologic retinal changes, respectively. Retinal digest preparations were examined using quantitative morphologic parameters.

Results. After 26 weeks of diabetes, characteristic features of background retinopathy such as a 5% increase in capillary endothelial cells, a 27% pericyte dropout, acellular occluded vessels and, occasionally, microaneurysms developed in untreated animals. Islet transplantation performed after 6 weeks of diabetes completely prevented endothelial cell proliferation and diminished pericyte loss (2950 ± 140 vs 2390 ± 40 in diabetic controls, \( P < 0.01 \)). A significant increase in acellular occluded capillaries persisted (31 ± 14 vs 8 ± 2 in NC; \( P < 0.01 \)). Islet transplantation after 12 weeks of diabetes, i.e., after established pericyte loss, only partially restored capillary cell composition and did not prevent retinal vessel occlusion. These findings indicate that the beneficial effect of islet transplantation on diabetic retinopathy is limited to a time very early in the evolution of this process.

Conclusions. These data suggest that irreversible changes induced by antecedent hyperglycemia play a central role in the progressive development of diabetic retinopathy. Invest Ophthalmol Vis Sci 1993;34:2092-2096.

The sucrose-fed diabetic Cohen rat shares some essential features with human type II diabetes such as genetic and environmental disposition, non-insulin-dependent diabetes, an early phase of hyperinsulinemia and insulin resistance, followed by hypoinsulinemia, a decreased number and sensitivity of insulin receptors, a metabolic response to hypoglycemic drugs such as glibornuride, and the development of retinal and renal complications.1 Therefore, it has been proposed as a model of noninsulin-dependent diabetes mellitus.

Retinal pathology has been defined and the beneficial effects of oral antidiabetic agents on the development of retinopathy have been investigated.2 In previous studies in rats and dogs with an insulin-dependent type of chemically induced diabetes mellitus the development of diabetic retinopathy could only be abolished when euglycemia was reconstituted very early after established diabetes either by islet transplantation or by intensified insulin treatment.3,4 The dysfunction of the islet of Langerhans as a critical factor in the pathogenesis of type II diabetes in humans has been reviewed recently.5 Therefore, the current study should evaluate the significance of restored islet function by transplantation on the development of diabetic retinopathy. Furthermore, by transplanting islets at two different times in the course of diabetes, after 6 weeks, i.e., before the suspected onset of the first morphologic changes, and...
after 12 weeks. After this point, we intended to distinguish between primary prevention and secondary intervention of diabetic retinopathy.

MATERIALS AND METHODS

The procedure of diabetes development in the Cohen rat has been described in detail elsewhere. Briefly, two lines of this strain were selected from the parental Sabra albino rat by feeding a copper-deficient (1.2 ppm vs 2.6 ppm in regular starch diet, analyzed by atomic absorption spectrophotometry), sucrose-rich diet (72% of sucrose vs 72% of starch). Offspring with abnormal glucose tolerance (upward selection) were brother-sister mated and developed hyperglycemia, glucosuria, and diabetic complications. The downward selected or so-called diabetes-resistant line remained normal under the sucrose-rich diet.

In the current study, two series of animals were selected after islet transplantation:

1. Eleven animals received islets from the diabetes-resistant line after 6 weeks of overt diabetes ("early" transplanted group = ETx)
2. Five animals received islets from the same donor strain after 12 weeks of diabetes ("late" transplanted group = LTx).

Five untreated rats (diabetic controls, DC) and four rats of the diabetes-resistant line (nondiabetic controls, NC) served as controls. All animals used in this study were kept and treated according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. The following parameters were measured before and 4, 8, 12, and 16 (LTx 14) weeks after transplantation: body weight, postprandial blood glucose, glucosuria, HbA1, and serum insulin.

Islet transplantation was performed using the method of Lacy and Kostianovsky. Briefly, the donor pancreas was digested with 10 ml collagenase (0.02%) via the ductus choledochus separated from the surrounding small gut and incubated for 45 min at 37°C. Before centrifugation (30 min, 900 G) against a Ficoll (Sigma, Heidelberg, Germany) gradient, the di-

![FIGURE 1. Postprandial blood glucose (a), body weight (b), glycated hemoglobin (c) and plasma insulin levels (d) of early vs late treated (ETx, LTx) and control rats (DC) after 6 weeks of diabetes (black bars) and at the end of the study (white bars) compared to age-matched nondiabetic controls (NC). Mean ± SD.](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933399/)
gest was homogenized by whirling on a vortex for 15 sec and washed twice in ice-cold Hanks solution (Gibco, Eckenstein, FRG).

A total of 1000–1200 freshly isolated islets was given intraportally in the deeply anesthetized animals.

**Retinal Digests**

At the end of the study (i.e., after 26 weeks of diabetes in DC), eyes were removed under deep nembutal narcosis and a modified digestion technique was used to obtain the retinal vasculature (one eye per animal).

Briefly, after retinal isolation, the samples were washed extensively in distilled water. A combined digestion, 5% pepsin in 0.2% hydrochloric acid for 1 hr, then 3% trypsin in 0.2 molar Tris for 3.5 hr, was used to isolate the retinal vessel system. The preparations were stained with periodic acid-Schiff (PAS).

Endothelial cells and pericytes were counted in 10 randomly selected fields of each retina using an image analyzing system (CUE 2, Olympus [Opticals Europe, Hamburg, Germany], magnification 600X). Cells were differentiated according to the criteria of Kuwabara and Cogan. Fields were selected to contain only capillaries (no larger vessels) at a relative capillary density of 20–35% (to avoid area underestimation by capillary overlap). The numbers of both cell types were normalized to the relative capillary density, obtained automatically by the image analyzing system (number of cell per mm² of capillary area) and their ratio was calculated.

Acellular capillaries were quantitated by a modification of the method of Kuwabara and Cogan. Using a grid of 100 fields, 10 microscopic fields covering a total area of 6.76 mm² of retinal area were scored for the presence of acellular occluded vessels (integration ocular Olympus/400X magnification). Each field containing acellular capillary segments was recorded as positive, and values were normalized to mm² of retinal area. Retinas were scored for the presence of saccular microaneurysms.

All morphometric evaluations were performed by an experienced observer blinded to the identity of the samples being examined.

**Statistics**

All parameters are given as mean ± SD. The significance of differences between groups was tested using one-way analysis of variance and the Student-Newman-Keuls Test.

**RESULTS**

Islet transplantation improved metabolic parameters, as shown in Figure 1 (a–d). However, all parameters of the transplanted groups were still different from NC. Vascular texture from the NC group showed regular width throughout and only a few acellular capillaries (Figs. 2a and 3). In precapillary arterioles no significant PAS accumulation was visible and microaneurysms were absent. The E/P ratio was 1.03 ± 0.08. In contrast, capillaries DC exhibited severely affected focal areas, including varicose dilatation, acellular vessels (Fig. 4) and arteriolar accumulation of PAS positive material. Microaneurysms were seen in at least one of the five retinas examined.

Cell counts revealed a 5% increase of endothelial cells (P = 0.024 vs NC) and an 18% loss of pericytes (P < 0.0001 vs NC; Fig. 2b) resulting in a significant increase of the E/P ratio (1.49 ± 0.07; P = 0.0055 vs NC).

In the ETx group, a beneficial effect of transplantation was observed (Fig. 5). Capillary integrity, the absence of severely affected capillary areas and a largely preserved E/P ratio (1.10 ± 0.08; P < 0.0001 vs DC) were observed. However, a significantly higher number of acellular capillaries occurred (31 ± 14 vs NC 8 ± 2; P = 0.01; Fig 2a).
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FIGURE 3. Retinal digest preparations from a nondiabetic control rat of the diabetes resistant line. Regular capillary integrity and cell (endothelial cell and pericytes) composition. PAS staining, original magnification X500.

FIGURE 5. Retinal digest preparations from a rat after early transplantation. Almost regular capillary shape and cell distribution; few vessel abnormalities. PAS staining, original magnification X400.

FIGURE 4. Retinal digest preparations from a diabetic control rat. Example of an extended field of vessel occlusion. PAS staining, original magnification X400.

FIGURE 6. Retinal digest preparations from a rat after late transplantation. Capillary irregularities, acellular capillaries and occlusions and pericyte loss. PAS staining, original magnification X400. In LTx animals, extended fields comparable to those shown in Figure 4 have been found explaining the considerable variation of acellular capillaries in the LTx group.

Postponed treatment, as represented by the LTx group, was accompanied by more severely affected microvasculature, compared to specimens from the ETx group. Retinal vessels exhibited major PAS-positive depositions, comparable to those in DC, and acellular capillaries were widely distributed similar to those in the DC group, although with major variation (Fig 6). Microaneurysms were not found.

Endothelial cell numbers were significantly higher compared to NC ($P = 0.011$), while the pericyte loss was partially restored (Fig. 2b), giving an improved E/P ratio ($1.28$, $P = 0.0035$ vs DC).

DISCUSSION

According to the data obtained in this study, islet transplantation is a valid tool for restoration of glucose metabolism as an irrevocable prerequisite toward the inhibition of complications associated with hyperglycemia. Although the mechanisms underlying impaired islet function in the Cohen rat are unknown, recent studies using single islet cell perfusion uncovered a severely defective insulin synthesis.$^{11}$

Retinal lesions including pericyte loss, endothelial cell proliferation, microaneurysms, focal acellularity, and vessel occlusions seen in the diabetic Cohen rat are widely congruent with those found in other rat models such as streptozotocin-diabetic LEWIS rats or spontaneous diabetic BB rats,$^{12,13}$ suggesting hyperglycemia as the primary factor leading to diabetic complications.

The mechanisms by which hyperglycemia could
exert its damaging effects on the microvasculature comprise excessive polyol pathway activity, myoinositol depletion of special cell compartments, protein kinase C activation via de-novo substrate synthesis, and excessive formation of ketoamines.\textsuperscript{14-16} However, limiting the general impact of these alterations as the biochemical basis of diabetic complications is that they are completely reversible by insulin and do not explain results from the current and previous studies that after a prolonged period of hyperglycemia, reconstituted euglycemia was unable to prevent or even retard retinopathy.\textsuperscript{3,4} The formation and accumulation of advanced glycosylation end products (AGE), leading to irreversible changes that persist on long-lived structural macromolecules even after glucose metabolism has been normalized, is widely congruent with essential features of developing complications\textsuperscript{17}. Some of the AGE are detectable by their ability of autofluorescence\textsuperscript{18} and recently, a relation between precapillary arteriolar depositions of PAS-positive material and autofluorescence with identical spectral properties of AGE was found in retinal digest preparations,\textsuperscript{9} precluding the full development of diabetic retinopathy in a model of experimental diabetic retinopathy. Measurements revealed a 2.6-fold increase of AGE in retinas of diabetic rats and a suppression of their accumulation by aminoguanidine, an inhibitor of AGE formation. Islet transplantation and aminoguanidine treatment have in common that they both lower precursors of advanced glycosylation products. In the current study, precapillary arteriolar PAS accumulation was more effectively reduced by early than by late transplantation, underlining its cumulative character. Furthermore, the superiority of treatments introduced before morphologic changes appear in tissues susceptible to complications over therapeutic approaches introduced late in the course of diabetes mellitus is confirmed.

In the model of the diabetic Cohen rat, islet transplantation was able to inhibit diabetic retinopathy. This suggests the important role of the function of the islets of Langerhans in the pathogenesis of the accelerated microvascular disease in this model.

**Key Words**

diabetic retinopathy, Cohen rat, islet transplantation, microangiopathy, morphometric studies

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**References**


