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Effects of restoring normoglycemia in type 1 diabetes on inflammatory profile and renal extracellular matrix structure after simultaneous pancreas and kidney transplantation

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ABSTRACT

Aims: Patients with type 1 diabetes and end-stage renal disease with simultaneous pancreas and kidney (SPK) or kidney transplants alone (KA) were recruited 9–12 years post transplantation. We investigated differences between these groups with regard to inflammatory parameters and long-term structural changes in kidneys.

Methods: Blood samples were analyzed by ELISA and multiplex for chemokines, cytokines, growth factors, cell adhesion molecules and matrix metalloproteinases. Kidney graft biopsies were analyzed by electron microscopy for glomerular basement membrane thickness. Heparan- and chondroitin sulfate disaccharide structures were determined by size exclusion chromatography mass–spectrometry.

Results: The SPK and the KA group had average glycated hemoglobin A1c (HbA1c) of 5.8% (40 mmol/mol) and 8.6% (70 mmol/mol) respectively. SPK recipients also had 16.2% lower body mass index (BMI) and 46.4% lower triglyceride levels compared with KA recipients, compatible with an improved metabolic profile in the SPK group. Plasminogen activator inhibitor (PAI-1), C-reactive protein (CRP) and vascular endothelial growth factor (VEGF) were lower in the SPK group. In kidney graft biopsies of the KA-patients an 81.2% increase in average glomerular basement membrane thickness was observed, accompanied by alterations in heparan sulfate proteoglycan structure. In addition to a decrease in 6-*O*-sulfated disaccharides, an increase in non-*N*-sulfated disaccharides with a corresponding slight decrease in *N*-sulfation was found in kidney biopsies from hyperglycemic patients.

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Conclusions: Patients with end stage renal disease subjected to KA transplantation showed impaired inflammatory profile, increased thickness of basement membranes and distinct changes in heparan sulfate structures compared with SPK recipients.

1. Background

Patients with type 1 diabetes have increased risk of macrovascular complications such as atherosclerotic cardiovascular diseases including myocardial dysfunction and stroke [1,2], as well as microvascular complications including neuropathy, retinopathy and nephropathy [3]. Diabetic nephropathy is characterized by hypertension, albuminuria, reduced glomerular filtration rate (GFR) and kidney morphological changes [4]. These overt effects are the long-term consequences of changes in several important signaling systems affected by hyperglycemia [5], by changes in the renin-angiotensin system, increased transforming growth factor β (TGF-β)-activity, promoting fibrotic processes and the activation of immune cells in the circulation and changes in inflammatory pathways, all contributing to systemic endothelial dysfunction [6].

Long-term complications in the kidneys of patients with type 1 diabetes result from a series of molecular changes involving changes in glucose metabolism leading to generation of advanced glycation end products (AGEs), changes in O-glycosylation of cytosolic and nuclear proteins, activation of protein kinase C and increased expression of reactive oxygen species and TGF-β [7]. All these changes contribute to the development of diabetic nephropathy, which in turn can lead to end-stage renal disease (ESRD), where dialysis and kidney transplantation are treatment options. Kidney transplantation improves patient survival compared with long-term dialysis [8,9].

Further treatment options for patients with diabetic ESRD also include pancreatic transplantation [10], and these patients can receive simultaneous pancreas and kidney (SPK) or kidney transplants alone (KA). Several studies demonstrate improved survival and reduced cardiovascular events in SPK transplans compared with KA recipients [11–13], but long-term follow-up studies are needed to demonstrate possible differences in effects of SPK transplantation versus kidney transplantation alone [14]. Recurrence of diabetic allograft nephropathy might also be prevented (References listed in [15]. Furthermore, improved glycemic control as a result of pancreatic transplantation may lead to lowered risk of cardiovascular disease, both in patients with type 1 and type 2 diabetes [16,17].

The importance of strict blood glucose control to decrease the risk of long-term complications has been well documented in large clinical trials, both for patients with type 1 and type 2 diabetes [3,16,18]. The importance of inflammation in development of complications has been addressed [6,19] as has also the long-term effects of endothelial dysfunction [20]. Furthermore, the importance of changes in the extracellular matrix (ECM) in the kidney glomeruli, mesangial matrix and interstitium has also been studied in relation to changes in kidney function in diabetes [4].

Proteoglycans (PGs) are essential structural and functional components of the ECM. PGs are proteins substituted with long sugar chains – glycosaminoglycans (GAGs) – able to interact with a range of partner molecules, and being essential for many of the functions of the PGs. The extent and functional consequences of such interactions are affected by the structural composition and degree of post-translational modifications of the sugar entities, including sulfation. One particular ECM component, heparan sulfate (HS) PGs, has been the focus of several studies. Chondroitin sulfate (CS)/dermatan sulfate (DS) PGs however, have been studied only to a limited extent [4]. A series of studies have supported the notion that changes in the amount and structures of HSPGs may lead to lower charge density in the basement membranes of the kidneys, thereby contributing to proteinuria [21]. However, some recent studies have provided conflicting data and challenged this hypothesis. This issue has been reviewed and the complexity of the regulation of kidney filtration suggest that HSPGs may be one of several factors involved in the development of albuminuria in diabetes [7,22].

The purpose of this study was to investigate the long term effects of hyperglycemia on inflammatory parameters and on ECM and PG structures important for renal filtration. Our results suggest improved inflammatory and metabolic profiles in the normoglycemic SPK recipients. In kidneys, differences in basement membrane thickness and distinct HSPG structures were observed.

2. Methods

2.1. Ethics statement

Experiments and tests were approved by the regional ethics committee for Southern Norway, and followed the guidelines of the Helsinki declaration. All participants gave written consent for their participation.

2.2. Patients and study design

Twenty-seven patients with type 1 diabetes and former ESRD leading to kidney transplantation (Table 1) were included at Oslo University Hospital, Rikshospitalet, Department of Transplant Medicine, Section of Nephrology. This is a subgroup of a larger study, used for a targeted investigation of inflammatory and ECM parameters. Data, blood samples and kidney biopsies were collected 9 or more years after transplantation, from patients with functional grafts only.

This study is a cross-sectional single-center study compromising follow-up data on patients attending the only hospital performing transplantations in Norway. Surgical