Formation of Cholangiogenic Cysts Following Intrahepatic Islet Transplantation in Streptozotocin Diabetic Rats

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ABSTRACT

Permanent hyperinsulinemia and the resulting overstimulation of the insulin receptor signaling pathway is suspected as a trigger of cancer genesis in the livers of type 2 diabetic patients. Liver tissue (LT) surrounding transplanted pancreatic islets (PI) can be permanently exposed to insulin in even higher concentrations than in type 2 diabetic patients. Therefore, this study examines the effect of PI transplantation (Tx) on LT in animals with streptozotocin (STZ)-induced diabetes mellitus. The suboptimal mass (400 or 1000) of isogeneic PI was transplanted into either the portal vein or under the kidney capsule of diabetic Brown Norway (BN) rats. Healthy BN rats treated with 400 isogeneic PI transplanted in the portal vein served as a control group. During the first 6 months after PI Tx, small and infrequent cystic lesions developed in animals with STZ diabetes, irrespective of the Tx site. In 10 months, frequent and complex cystic lesions appeared in these animals. In the control group, several small lesions were detected but not until 10 months after the PI Tx. In summary, STZ is the likely main inductor of hepatic cystic lesions, but the contribution of PI was not confirmed.

THE TRANSPLANTATION of isolated pancreatic islets (PI) into the hepatic portal vein is currently considered a relevant alternative to pancreas organ transplantation [1]. It is much safer but less efficient in restoring insulin independence in type 1 diabetic patients [2]. Typically, one recipient of isolated PI needs to undergo transplantation sequentially from more than one donor [2]. After the first transplantation of the suboptimal number of islets, the graft cannot fully normalize glucose control and, therefore, beta cells are maximally and permanently stimulated to secrete insulin. The Markman and Brendel groups reported on the combined effect of stimulated insulin secretion, mild hyperglycemia, and steroid-free immunosuppressive protocol (preserving insulin sensitivity of the recipient) on liver tissue, detecting multiple steatotic lesions in recipient livers several months after islet transplantation [3,4].

In addition to its metabolic effects, insulin is an efficient growth factor. In type 2 diabetic patients who are exposed to permanent hyperinsulinemia, the overstimulated "mitogenic arm" of the insulin receptor (IR) signaling cascade is considered a conceivable mechanism in the pathogenesis of hepatocellular carcinoma, which is 2.4 times more frequent in comparison with healthy populations [5]. This effect can be even more significant in the case of focal "hyperinsulinemia" after islet transplantation into the liver.

Pancreatic islets represent only 1%–2% of whole organ tissue in total. However, islet perfusion extracts even more than 20% of all blood entering the pancreas [6]. The rich perfusion of PI allows prompt and efficient insulin wash-out toward the portal vein. Despite this, cells surrounding islets are exposed to insulin in high concentrations and acinar cells adjacent to islets have been reported to be morphologically different to their more distant cells (larger, polynuclear, and containing more granules) [7]. In addition to that, blood flow entering the islets engrafted within branches of the portal

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vein represents less than 20% of the native blood flow in the pancreas [8]. Therefore, the dilution and washing away of insulin is slow and a locally high concentration of insulin persists longer (perhaps permanently) in the proximity of transplanted islets. Under such conditions, the stimulatory effect of insulin on liver cells can be more pronounced than has been described in type 2 diabetic patients.

Almost 40 years ago several authors have reported typical cystic liver lesions in animals with diabetes induced by streptozotocin (STZ) injection and subsequent transplantation of PI [9–11]. They concluded that STZ is a triggering factor in causing the development of cystic lesions. Contrary to these reports, several recent articles by Dombrowski et al have referred to hepatocellular adenomas, hepatocellular carcinomas, and cholangiomas in animals with spontaneous autoimmune diabetes treated with transplantation of subtherapeutic islet mass [12,13]. They propose that the combined effect of islets, maximally stimulated to secrete insulin, with mild hyperglycemia is a carcinogenesis triggering mechanism independent of other factors [12,13].

Based on these results, we hypothesize that the reported effects should manifest themselves faster and more severely in high-insulin–sensitive animals. Therefore, the Brown Norway (BN) rats known as insulin high-sensitive model were used in our experiments [14]. The main goal of our study was to test the effect of potentially carcinogenic STZ, insulin released from the graft, and hypeglycemia in chosen rat strain. Herein we report results of the first step of our study focused on the development of cystic changes after islets transplantation into STZ diabetic rats. The effect of the islets themselves was tested in healthy animals by transplanting 400 isogeneic islets into the liver portal vein. The combined effect of STZ and islet transplantation was tested in animals with severe STZ-induced diabetes treated using intraportal transplantation of 400 isogeneic islets. Testing the effect of STZ without the local influence of islets, we used a control group consisting of animals with severe STZ-induced diabetes treated by transplantation of 1000 isogeneic islets under the left kidney capsule.

METHODS

All animals were held according to the European Convention on Animal Care with free access to food pellets and water. The Animal Care Committees of the Institute for Clinical and Experimental Medicine and the Ministry of Health of the Czech Republic approved protocols related to this study. BN rats served as PI donors and recipients (250–320 g, respectively). PI were isolated using collagenase digestion (Sevapharma, Prague, Czech Republic) and subsequently purified in discontinuous Ficoll density gradient (Sigma-Aldrich, St. Louis, MO, United States; 1.108 g/mL, 1.096 g/mL, 1.069 g/mL, and 1.037 g/mL). Isolated islets were cultured in basic media (CMRL-1066, Fetal Calf Serum 10%, HEPES 5%, Penicillin/Streptomycin/Glutamine 1%) overnight at 37°C and 5% CO_2 atmosphere [15]. The next day, islets were harvested from tissue cultures, counted, and transplanted into recipients. The transplantation was performed under general anesthesia induced by intramuscular injection with a mixture of ketamine (100 mg/mL; 2.4 mL) + medetomidin (1 mg/mL; 0.6 mL) in a dose of 0.065 mL/100 g [16].

Table 1. Macroscopic Observation in Recipient Livers of Individual Groups 6 and 10 Months After Transplantation of PI

	6 mo				10 mo			
	Grade 1	Grade 2	Grade 3	Grade 4	Grade 1	Grade 2	Grade 3	Grade 4
Group A	5				5			
Group B		4	1				3	2
Group C		4	1				4	

Islet recipients were divided into 3 groups according to treatment protocol. Group A consisted of healthy animals (n = 5) that had undergone transplantation of 400 PI by injection into the ileocaecal vein (peripheral branch of the portal vein) [17]. Bleeding was prevented with Avitene (Davol Inc., Warwick, RI, United States), which was attached to the puncture, as described previously [18]. Group B included animals (n = 5) with severe diabetes (glycemia > 20 mmol/L) induced by an intraperitoneal injection of STZ (50 mg/kg, after 8 hours of fasting), which was followed 1 week later by transplantation of 400 isogeneic islets into the ileocaecal vein. Severely hyperglycemic animals of Group C (n = 5) underwent transplantation of 1000 isogeneic islets under the left kidney capsule 1 week after the STZ injection as a model mimicking the systemic insulin administration. Blood glucose levels (AccuChek Performa glucose sensor, Roche, Switzerland) and weights of all diabetic animals were monitored daily during the first week, weekly during the first month after transplantation, and then monthly. A macroscopic examination of liver morphology was performed 6 and 10 months after islet transplantation in all groups. The observation was graded as follows: Grade 1 to 4 simple cystic lesions <3 mm in diameter; Grade 2 many simple cystic lesions combined with up to 3 complex cystic adenomas; Grade 3 multiple complex cystic lesions; and Grade 4 general transformation of liver tissue to cystic lesions. Liver tissue obtained from all animals at the end of the study was fixed in 10% formalin and processed for a routine histological assessment.

RESULTS

The injection of STZ induced severe chemical diabetes (blood glucose levels >20 mmol/L) in 90% of treated animals; 10% of animals remained normoglycemic and were not included in the study. Islet transplantation in all animals from Groups B and C corrected fasting blood glucose levels to the physiological range (4.5–6.2 mmol/L), but blood glucose levels in fed animals ranged between 5.0 and 15.0 mmol/L. In Group A, blood glucose levels remained normal, irrespective of islet transplantation. At the time of transplantation surgery, all recipient livers were macroscopically normal with no visible cysts. Six months after transplantation (Table 1), several simple cystic lesions up to 3 mm were detected in all animals of Groups B (Fig 1) and C (Fig 2). Ten months after islet transplantation, a second macroscopic examination was performed and large complex cystic lesions occupying a substantial part of the liver were detected in all animals of Groups B and C (Table 1). In 2 animals with islets transplanted into portal vein, most of the liver tissue was cystically transformed. A histological examination of the liver revealed the proliferation of