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Magnetic Resonance Imaging Study of Mouse Islet Allotransplantation

J.-H. Juang, C.-R. Shen, J.-J. Wang, C.-H. Kuo, M.-Y. Lin, S.-T. Wu, Z.-T. Tsai, and T.-C. Yen

ABSTRACT

Although only 10% of islet transplant recipients maintain insulin independence, 80% of them are C-peptide positive at 5 years. To better understand the fate of transplanted islets, a magnetic resonance imaging (MRI) technique has been used to detect superparamagnetic iron oxide (SPIO)–labeled transplanted islets. Recently, we successfully used a novel MRI contrast agent, chitosan-coated SPIO (CSPIO) nanoparticles, to monitor mouse islet isografts for 18 weeks after transplantation. In the present study, we tested whether CSPIO could be applied to monitor islet allografts, which are supposedly rejected without immune interventions. Male C57BL/6 and Balb/c mice were used as donors and recipients of islet transplantation, respectively. After overnight incubation with or without CSPIO (10 μg/mL), 300 C57BL/6 islets were transplanted under the left kidney capsule of each Balb/c mouse. Starting from day 10 after transplantation, 3.0-Tesla MRI of the recipients was performed weekly. Four mice were followed for ≥38 days. At 38 and 45 days, 1 islet graft was removed for insulin and Prussian blue staining, respectively. From days 10 to 45 after transplantation, CSPIO-labeled islet grafts were visualized on MRI scans as sustained distinct hypointense spots homogeneously located at the upper pole of left kidney, the site of transplantation. At days 38 and 45, the histology of CSPIO-labeled islet grafts revealed insulin and iron staining colocalized in the same areas. Our results in a mouse allotransplantation model indicated that CSPIO-labeled islets survived as long as 45 days with positive MRI.

Recently, the Edmonton Protocol has markedly improved the success of human islet transplantation.[1] However, ≥2 pancreata are usually required to achieve normoglycemia. Moreover, the long-term function of the transplanted islets has been disappointing.[2,3] Allograft failure may be due to nonimmunologic (eg, insufficient β-cell mass and islet engraftment problems) as well as immunologic (eg, immune rejection, toxicity of immunosuppressants, and autoimmune recurrence) factors.[4] Although only 10% of recipients maintain insulin independence, 80% of them are C-peptide positive at 5 years after islet transplantation.[2] To better understand the fate of transplanted islets, a magnetic resonance imaging (MRI) technique has been used to detect superparamagnetic iron oxide (SPIO)–labeled islets.[5] Recently, we successfully used a novel MRI contrast agent, chitosan-coated SPIO (CSPIO) nanoparticles, to monitor mouse islet isografts as long as 18 weeks after transplantation.[6] In the present study, we tested whether CSPIO could be applied to monitor islet allografts which are supposedly rejected without immune interventions.

From the Division of Endocrinology and Metabolism, Department of Internal Medicine, Chang Gung University and Chang Gung Memorial Hospital, Taoyuan, Taiwan (J.-H.J., M.-Y.L.), Department and Graduate Institute of Medical Biotechnology and Laboratory Science, Chang Gung University, Taoyuan, Taiwan (C.-R.S., S.-T.W.), Department of Medical Imaging and Radiological Sciences (J.-J.W.), Chang Gung University, Taoyuan, Taiwan, Molecular Imaging Center, Chang Gung Memorial Hospital, Taoyuan, Taiwan (C.-R.S., Z.-T.T., T.-C.Y.), and Department of Biological Science and Technology (C.-H.K.), National Chiao Tung University, Hsinchu, Taiwan.

J.-H. Juang and C.-R. Shen had equal contributions on this work.

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Address reprint requests to Dr Jyuhn-Huarng Juang, Division of Endocrinology and Metabolism, Chang Gung Memorial Hospital, 5 Fu-Shin Street, Kweishan, Taoyuan, Taiwan. E-mail: jjuang@adm.cgmh.org.tw

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MATERIALS AND METHODS

Animals

Male C57BL/6 and Balb/c mice of ages 8–12 weeks were used as islet transplant donors and recipients, respectively.[7] The animal experiments were approved by our animal ethics committee.

Islet Isolation

Under anesthesia with sodium amobarbital, pancreatas were distended with 2.5 mL RPMI-1640 medium (Gibco BRL, Grand Island, NY) containing 1.5 mg/mL collagenase (*Clostridium histolyticum,* type XI; Sigma Immunochemicals, St Louis, MO) during incubation in a 37°C water bath. Islets separated by density gradient (Histopaque-1077; Sigma Immunochemicals), were purified by hand picking under a dissecting microscope.[7]

Islet Labeling

Isolated islets were incubated overnight with CSPIO (10 μg/mL) in culture medium. After incubation, they were washed with culture medium before in vitro studies or islet transplantation.[8]

Islet Transplantation

Three hundred C57BL/6 islets cultured with or without CSPIO were transplanted under the left kidney capsule of each Balb/c mouse. The islets were centrifuged in PE-50 tubing (Clay Adams, Parsippany, NJ) connected to a 200-μL pipette tip. With the mouse under amobarbital anesthesia, the left kidney was exposed through a lumbar incision. A capsulotomy was performed in the lower pole of the kidney for the tip of the tubing to be advanced under the capsule of the upper pole, the site of final injection. The capsulotomy was left unsutured.[7]

In Vivo MRI of Transplanted Islets

Starting from day 10 after transplantation, 3.0-Tesla MRI of the recipients was performed weekly. Images were acquired on a 3.0-Tesla MRI scanner (Magnetom Trio with TIM; Siemens, Erlangen, Germany) using a homemade surface coil. A T2*-weighted gradient-recalled echo sequence was acquired for all hosts.[6]

Removal of the Islet Graft

At 38 and 45 days after transplantation, we removed 1 islet graft under amobarbital anesthesia. Via an abdominal incision, the kidney was exposed and under the dissecting microscope, the kidney capsule surrounding the graft was excised and removed with the adherent graft.[7]

Histology and Immunohistochemistry of the Islet Graft

The removed grafts were fixed in formalin for paraffin embedding. Sections were stained for iron with Prussian blue and for the endocrine β-cells with a guinea pig anti-swine insulin antibody (Dako Co, Glostrup, Denmark).[6]

RESULTS

In contrast to the control samples, grafts of CSPIO-labeled islets were visualized by MRI scans from days 10 to 45 after transplantation as sustained distinct hypointense spots homogeneously located at the upper pole of left kidney, the site of transplantation (Fig 1). There was a 52%–63% signal loss throughout the follow-up period. At days 38 and 45, the histology and immunohistochemistry of CSPIO-labeled islet grafts revealed insulin and iron staining colocalized in the same areas (Fig 2).

DISCUSSION

An MRI technique was used to detect SPIO-labeled islets after transplantation. Feridex, a dextran-coated SPIO, is approved by the Food and Drug Administration for human use as a liver imaging contrast agent. Unfortunately, in November 2008, the company ceased manufacturing Feridex. Therefore, several laboratories are searching for new contrast agents for clinical use. Recently, Tsai et al developed a novel MRI contrast agent, CSPIO, by coating SPIO with chitosan, thereby increasing the content of magnetite.[8] Using this novel MRI contrast agent, we have successfully monitored mouse islet isografts for as long as 18 weeks after transplantation.[6] In the present study, we further demonstrated that isolated mouse islets labeled with CSPIO nanoparticles could be visualized by MRI as long as 45 days

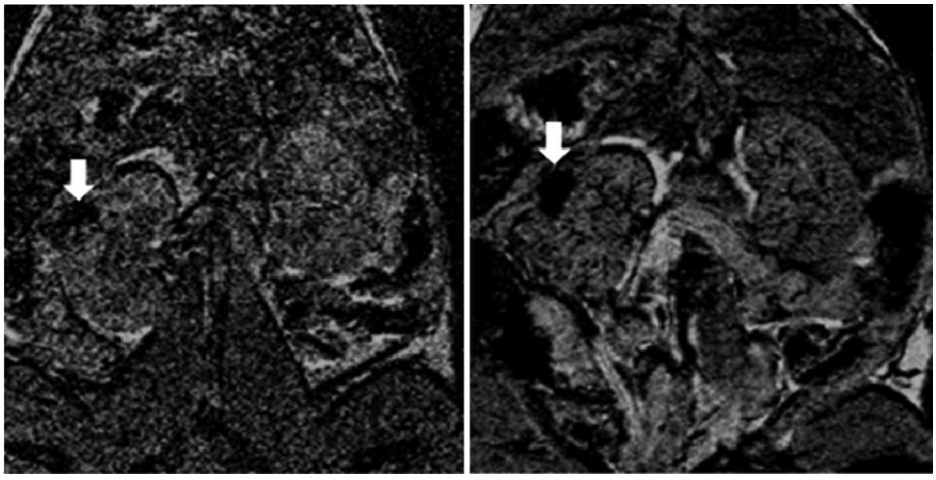

Fig 1. At days 10 (left panel) and 45 (right panel) after transplantation, grafts of CSPIO-labeled islets were visualized on MRI scans as sustained distinct hypointense spots homogeneously located at the upper pole of left kidney (*arrows*).

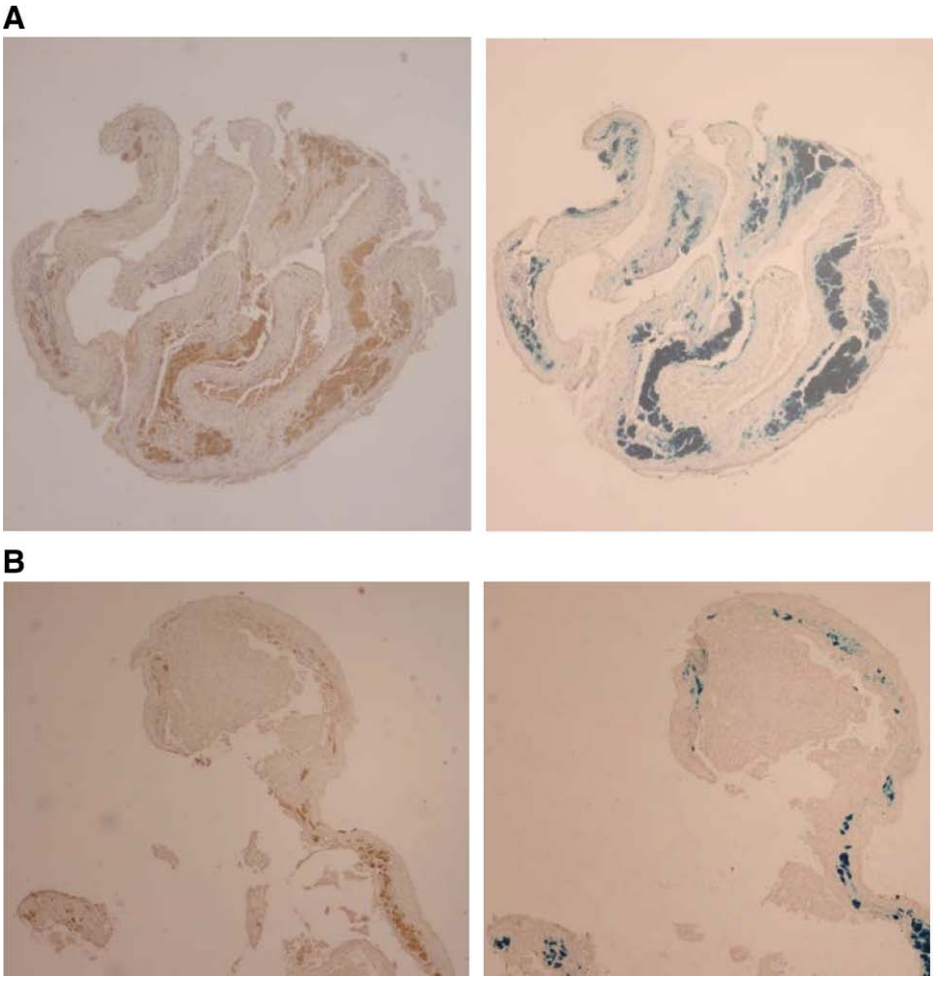

Fig 2. At days 38 **(A)** and 45 **(B),** the histology of CSPIO-labeled islet graft revealed the insulin staining (left panels, brown color) and iron staining (right panels, blue color) colocalized in the same areas.

after allotransplantation. Similarly to our previous syngeneic islet transplantation work, CSPIO-labeled islet allografts were visualized on MRI scans as sustained distinct hypointense spots homogeneously located at the upper pole of left kidney, the site of transplantation. Using the contralateral kidney as a reference, the MRI signal intensity of CSPIO-labeled islet allografts from days 10 to 45 after transplantation was 52%–63%. As we know, the majority of mouse recipients reject their islet allografts within 2 weeks after transplantation.[9] Thus, earlier studies showed that the number of MRI signal voids in the liver declined at 2 weeks[10,11] or 6 weeks[12,13] after intrahepatic islet allotransplantation. In contrast, we observed stationary MRI signal loss of renal subcapsular islet allografts labeled with CSPIO throughout the follow-up period. In addition, the colocalization of insulin and iron staining was confirmed at days 38 and 45, indicating that CSPIO-labeled islets survived as long as 45 days. The longer survival of our CSPIO-labeled islet allografts may be due to a different transplantation site (renal subcapsule vs liver) and/or the use of other SPIOs (dextran- vs chitosan-coated). Although the renal subcapsular site offers better growth conditions for syngeneic islets than the liver,[14] its influence on allogenic islets is unknown. Recently, cytoprotection of chitosan hydrogels was demonstrated in xenogeneic islet transplantation.[15] However, the protective effect of CSPIO needs to be further investigated.

In conclusion, our results in a mouse allotransplantation model indicated that CSPIO-labeled islets survived as long as 45 days with positive MRI.

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