Intraperitoneal Injection of Oxygenated Perfluorochemical Improves the Outcome of Intraportal Islet Transplantation in a Rat Model

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INTRAPERITONEAL INJECTION OF OXYGENATED PERFLUOROCHEMICAL IMPROVES THE OUTCOME OF INTRAPORTAL ISLET TRANSPLANTATION IN A RAT MODEL

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INTRODUCTION

Islet transplantation has become a clinical option to restore insulin independence to patients with unstable type 1 diabetes mellitus. But one of major obstacles for successful islet transplantation from one donor is early graft loss on islet engraftment. There is a suggestion that as many as 50-75 % of islets fail to engraft following intraportal islet transplantation (IPIT) [1]. Since reconstruction of blood supply with vessel anastomosis cannot be performed in islet transplantation in contrast to pancreas transplantation, hypoxia should contribute to islet graft loss in the immediate post-transplantation.

Perfluorochemical (PFC) has ability as a high oxygen carrier and we published its efficacy to oxygenate the pancreas during the preservation by the two-layer method [2-4]. Here we examined the effect of intraperitoneal oxygenated PFC on the outcome of IPIT.

MATERIALS AND METHODS

Animals

Male Lewis rats weighing 250-300 g were used in this study. Diabetes was induced in recipient rats by intraperitoneal injection of 65mg/kg body weight streptozotocin. Recipients were considered diabetic if their non-fasting blood glucose levels exceeded 350 mg/dl for 3 consecutive days. All animals were maintained in animal care facilities in accordance with the “Guidelines for Animal Experimentation at Kobe University Graduate School of Medicine”.

Islet Isolation and Transplantation

The pancreas was removed under anesthesia with diethyl ether and distended by intraductal injection of 6cc collagenase solution (1mg/cc of Collagenase P (Roche, Indianapolis, IN) in Hanks' balanced salt solution (HBSS; Sigma, St. Louis, MO)), maintaining the injection pressure at 80 mmHg. After digestion, islets were purified with a discontinuous density gradient by using Histopaque 1077 (Sigma, St. Louis, Mo) and HBSS. The crude number of islets in each diameter class was determined by counting after diphenylthioncarbazone staining using an optical graticule. This number was then converted to the standard number of islets equivalents (IEQ; diameter standardizing to 150 μm).

Islets in 0.6cc HBSS were transplanted into the portal vein of a diabetic syngeneic rat anesthetized by pentobarbitalsodium. Before closure of the abdomen, a 5Fr intravenous catheter for cut-down (Atom Medical Company, Tokyo, Japan) was inserted into the abdominal cavity through the subcutaneous tunnel in order to inject the PFC intraperitoneally and remove it if necessary.

Experimental design

First, syngeneic IPIT of 1000, 1500, and 2200 IEQ were performed in order to determine the marginal dose in this study. Daily blood glucose levels for 4 weeks after IPIT were measured. Success of transplantation was defined as maintenance of normoglycemia (<200 mg/dl) for at least 3 consecutive days.

Second, in group 1, oxygenated PFC was intraperitoneally injected on the transplant day and replaced every 6 hours for 2 days following

IPIT. On the other hand, in group 2, PFC with no oxygen, which was saturated by 100% nitrogen, was administered in the same manner. Daily blood glucose levels for 4 weeks after IPIT were measured and intraperitoneal glucose tolerance tests (IPGTT) were examined on the 28^{th} post-transplant day.

Statistical analysis

Results were expressed as mean ± standard deviation and the statistical differences between 2 groups were determined using Fisher's exact test and the Mann-Whitney U test, where appropriate.

RESULTS

Islet yield from one donor rat was 2350 ± 200 IEQ (1070 ± 90 islets) with >90% purity in this study. The success rates of IPIT of 1000, 1500, 2200 IEQ without PFC were 0/6, 1/6, and 6/6, respectively. We used islets of 1500 IEQ for the following transplant experiment.

The success rate of 1500IEQ IPIT was 5/6 in group 1 in contrast with 1/6 in group 2 ($p<0.05$) (Fig 1). The calculated area under the curve of the glucose profile on IPGTT in group 1 was 30500 ± 5500 mg/dl/120 min, which was significantly smaller than that in group 2 (47500 ± 6800 mg/dl/120 min; $p<0.01$) (Fig 2).

DISCUSSION

We show that intraperitoneal oxygenated PFC improves the outcome of IPIT. Hypoxia is thought to be an important factor influenced on the survival of intraportal islets in the immediate post-transplant period [5,6]. From the same viewpoint, Hughes et al. previously reported

that inspiration of hyperoxia could improve the islet engraftment [7]. However, hyperoxia clearly contributes to lung toxicity and recipient's restraint under intubation if the FiO2 is maintained more than 0.5, which are thought to be less clinical relevant. On the other hand, we found in the preliminary data that intraperitoneal PFC could oxygenate the portal vein without adverse events such as ileus and organ dysfunction on blood data. On these points, intraperitoneal injection of oxygenated PFC is thought to be a safer and more feasible method to improve islet engraftment, resulting in successful IPIT with less than marginal dose.

References

1. Contreras JL, Eckstein C, Smyth CA, et al: Activated protein C preserves functional islet mass after intraportal transplantation: a novel link between endothelial cell activation, thrombosis, inflammation, and islet cell death. Diabetes 53:2804, 2004
2. Kuroda Y, Fujino Y, Kawamura T, et al: Mechanism of oxygenation of pancreas during preservation by a two-layer (Euro-Collins' solution/perfluorochemical) cold-storage method. Transplantation 49:694, 1990
3. Matsumoto S, Kuroda Y, Hamano M, et al: Direct evidence of pancreatic tissue oxygenation during preservation by the two-layer method. Transplantation 62:1667, 1996
4. Tsujimura T, Kuroda Y, Kin T, et al: Human islet transplantation from pancreases with prolonged cold ischemia using additional preservation by the two-layer (University of Wisconsin solution/perfluorochemical) cold-storage method. Transplantation 74:1687, 2002
5. Davalli AM, Scaglia L, Zangen DH, et al: Vulnerability of islets in the immediate posttransplantation period: dynamic changes in structure and function. Diabetes 19:1161, 1996
6. Giuliani M, Moritz W, Bodmer E, et al: Central necrosis in isolated hypoxic human pancreatic islets: evidence for postisolation ischemia. Cell Transplant 14:67, 2005
7. Hughes SJ, Davies SE, Powis SH, et al: Hyperoxia improves the survival of intraportally transplanted syngeneic pancreatic islets. Transplantation 12:1954, 2003

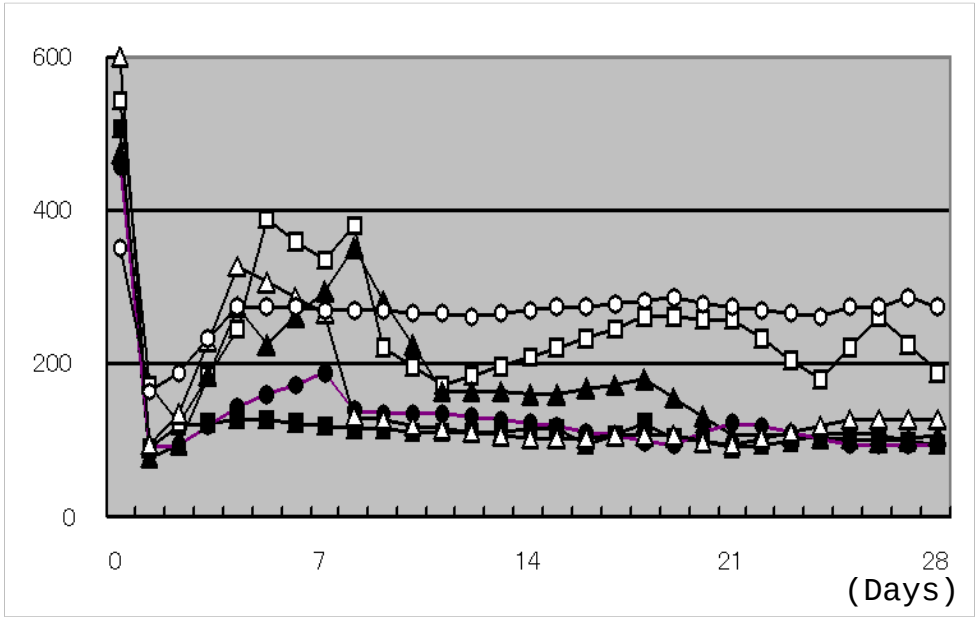
Figure Legends

Fig 1. The daily blood glucose levels after 1500IEQ IPIT. The success rates were 5/6 in group 1 (A) and 1/6 in group 2 (B) ($p<0.05$ vs group 1).

Fig 2. Intraperitoneal oxygenated PFC improved the glucose profile on IPGTTs on the 28^{th} post-transplant day. (A) IPGTTs in group 1. (B) IPGTTs in group 2.

Fig 1.

(A)


Blood sugar level (mg/dl)
600
400
200
0
0
7
14
21
28
(Days)

(B)

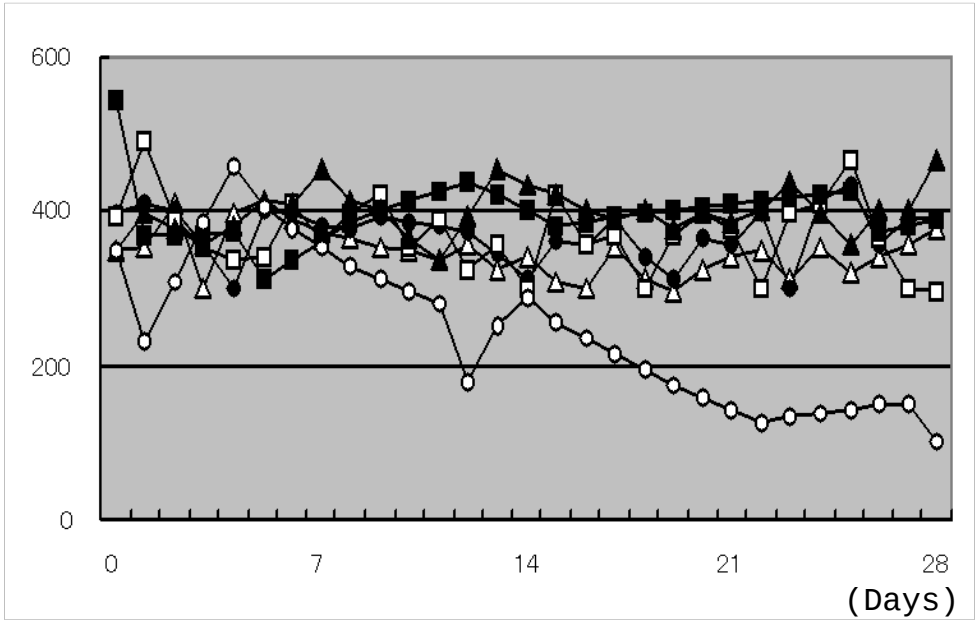
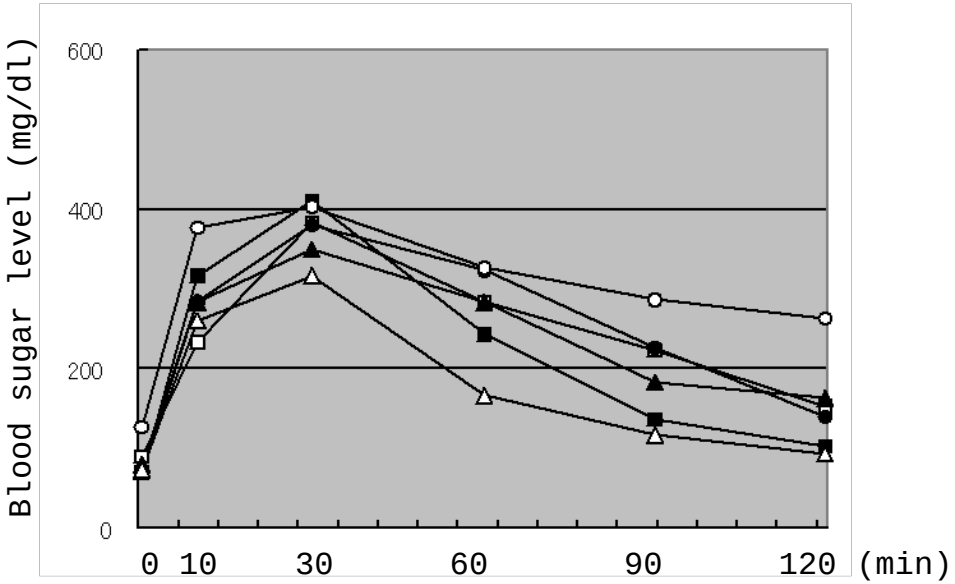
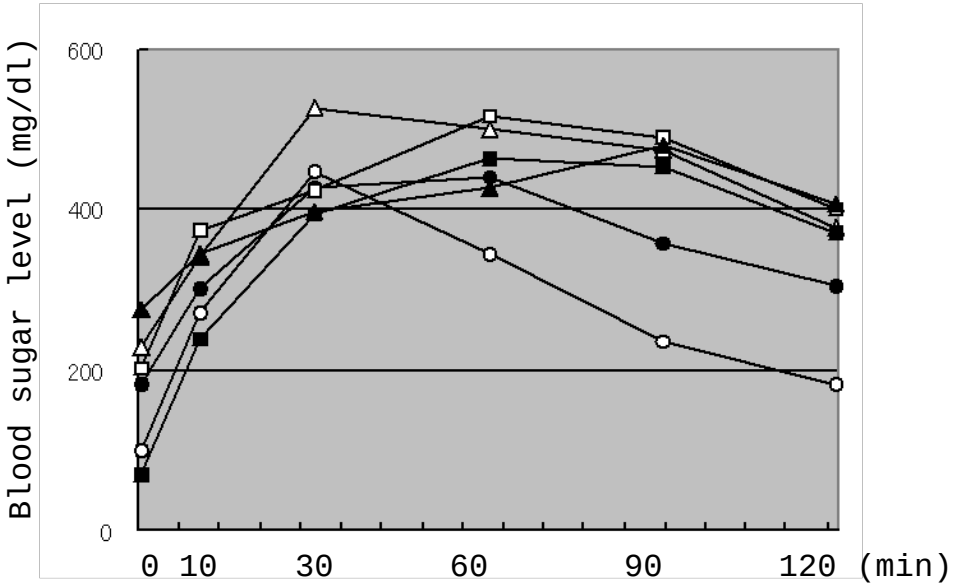

Blood sugar level (mg/dl)
600
400
200
0
0
7
14
21
28
(Days)

Fig 2.

(A)


Blood sugar level (mg/dl)
600
400
200
0
0 10 30 60 90 120 (min)

(B)


Blood sugar level (mg/dl)
600
400
200
0
0 10 30 60 90 120 (min)