Extended xenograft survival of islets transplanted intratesticularly reinforces the testis as an immune-privileged site

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Abstract
The testis has been shown to be a privileged site for transplantation of allogenic islets in rodents. Pancreatic islets transplantation is a potential attractive treatment for type 1 diabetes. The aim of the precent study was to assess whether the testis of Balb/c mice would have an immune privilege nature as a host for islets transplantation. The isolated guinea pigs (*Cavia cobaya*) pancreatic cells (500 cells/20 g of recipient body weight) were transplanted into the testis or peritoneum of alloxan-induced diabetic Balb/c mice. The results showed that intratesticular xeno-transplanted islets were able to survive up to 14 days significantly ($P<0.05$) longer compared to those of islets transplanted intra peritoneally. The data suggest that testis of Balb/c mice can be used as a model for immune privilege- and transplantation studies.

Key words: *xeno-transplantation, testis, pancreatic islets, alloxan diabetes*

Introduction

The immuno-privileged status of the testis has been appreciated for a variety of transplanted tissues including pancreatic islets[1]. Pancreatic islets transplantation is a potential attractive treatment for type 1 diabetes.[1,2,3] Studies on the use of testis and their cell components such as Sertoli cells as co-transplanted cells to improve prolonged survival of pancreatic transplantation have been conducted extensively in the last decade. Similar studies as well as information regarding immuno-privileged status of the testis, especially in transplantation studies are still limited in Indonesia. The aim of the present study was to assess whether the testis of Balb/c mice available in Indonesia would have an immune privilege nature as a host for islets transplantation.

Materials and Methods

The local male or female guinea pigs (*Cavia cobaya*) weighing between 500 and 600 g were used as pancreatic islet cell donors. The local male Balb/c mice weighing between 25 and 35 g were made diabetic by intravenous injection of 200 mg/kg body weight alloxan monohydrate[4] and were used as the recipients of islet xenografts.

Islet isolation

Islets were isolated as suggested by2 with slight modification and purified by means of Ficoll. gradient centrifugation. The purity of the cells was tested using dithizone (DTZ) stain and was examined under inverted microscope. The islet preparations were then cultured at 37° C in a humidified atmosphere of 5% CO_2 for 2-4 days before transplantation.

Islet transplantation

Two to four days after alloxan injection, only those of male diabetic mice with blood glucose concentration in excess of 200 mg/dl (tested using GlucoDr Strip test) were used as recipients. Sites of xenotransplantation were intra testicular (IT) and intra peritoneum (IP). No transplantation was made on the diabetic mice as controls (CTR). Transplantations were carried out according to[2] and[5] with slight modification. A total of 500 isolated guinea pig islets (>90% purity) per 20 g of recipient body weight were injected into IT or IP regions in 0.1 ml Krebs Ringer buffer (KRB) using a small tuberculin syringe. The control group was injected with the same volume of KRB alone.

Blood glucose monitoring

Blood glucose levels were monitored from the tail vein on days 0, 2, 4, 6, and 14 following transplantation using GlucoDr Strip test according to the manufacturer's procedure.

Statistical analysis

The analysis of xenograft survival data was performed by the expressions of blood glucose change index (BGCI) for each treatment or control of during the time relapse after transplantation. Comparison of means between treatments and controls, four replications each, was carried out using ANOVA by the aid of SPSS 13.0 for Windows. BGCI was

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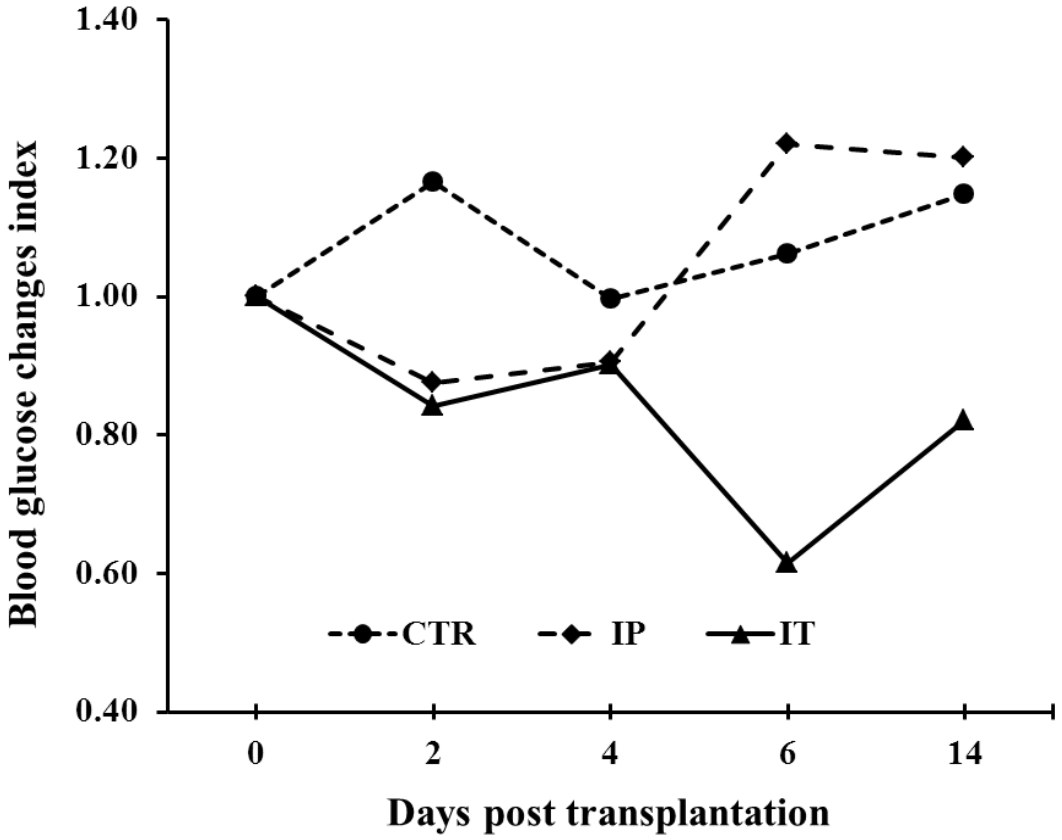

Figure 1: Prolonged xenograft survival of guinea pig (*C. cobaya*) pancreatic islets transplanted intra testicularly (IT) or intraperitoneally (IP) into alloxan-induced diabetic Balb/c mice, expressed as blood glucose changes index (BGCI). Islets transplanted IT survived longer ($P<0.05$) compared to that of IP or CTR (n=4).

calculated as follows: $[1 – (BG_0\text{-}BGn/BGn)]$, where BG_0 was the mice blood glucose at day 0 while BGn was the mice blood glucose at day n post transplantation either treated or control animals.

Results

The patterns of blood glucose changes index (BGCI) throughout experiments is presented in Figure 1. It can be seen clearly that BGCI of alloxan-induced diabetic mice decreased significantly ($p < 0.05$) up to 14 days when guinea pig islets were transplanted into mice intratesticularly (IT) compared to the control (CTR) group.

Alloxan-induced diabetic mice with either IT or IP grafted islets showed decreasing in BGCI on day 2 and 4 post islets transplantation. Beginning on day 6^{th} up to 14^{th}, especially on day 6^{th} the BGCI of IT was the lowest then increased on day 14^{th} but it was still lower than that of IP or CTR group.

Discussion

The present study shows that the blood glucose of alloxan-induced diabetic mice was able to be reverted via islets transplantation. It has been reported elsewhere that islets graft survival is profoundly affected by the organ site selected for the placement of the islets.[6] In the present study, extended survival of islets xenograft up to day 14 clearly occurred when the islets were grafted intra- testicularly. A similar study on islet xeno-transplantation[7] showed that rat islets transplanted into the testis of streptozotocin-induced diabetic mice were able to maintain normoglycaemia of the recipients for 12.4 days, which are comparable to this study.

Testicular Sertoli cells (SCs) have been reported as cells that play a major role in protection of allogeneic and xenogeneic grafts from host immune destruction.[8] This was reinforced by the results of research conducted by Han et al.[1] that when islet cells were co-transplanted with Sertoli cells (Sertoli-islet cell aggregates, SICA) under the kidney capsule of diabetic rats, 90% (9/10) of diabetic rats remained normoglycaemic at 60 days posttransplantation. In contrast with Han, Wang et al.[9] who had transplanted neonatal porcine islets alone (naked islets) or cocultured islets with Sertoli cells (islet/Sertoli cells) into an omental site and other locations of nonimmunosuppressed rodents, had revealed that co-transplantation only last about 2-4 days. It appears that in addition to Sertoli cells, other factors also exist, one

of which is lyso-glycerophosphocholines as endogenous immunosuppressive agents in the bovine and rat gonadal fluids.[10]

Regardless of how the mechanisms of xenografts can be accepted by the testis and what factors are involved in it, which still continuously needs to be investigated, the present study reinforces the results of earlier studies that the testis of Balb/c mice is a potential site to be used as a model for immune-privileged and transplantation studies.

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