Delivery of Antioxidant Tat-Metallothionein Fusion Protein for Protection of Xenotransplanted Pancreatic Islets from Radical species

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ABSTRACT SUMMARY

Metallothionein (MT) enzyme is known as one of radical scavengers. However, it is rarely expressed in pancreatic islets. When islets are transplanted into patients to cure diabetes mellitus, they are rapidly rejected by host’s immune reactions. Several kinds of radical species produced by host’s immune reactions can strongly affect the viability and functionality of transplanted islets. Here to protect the transplanted islets from radical species, Tat-MT fusion protein was produced and delivered into islets. Collectively, the internalized Tat-MT protein could attenuate radical-mediated islet damage in vitro. In addition, when Tat-MT-delivered islets were xenotransplanted into diabetic mice, they could be protected from the host immune attack. So, Tat-MT delivery can be applied for successful islet transplantation.

INTRODUCTION

Islet transplantation is a promising therapy for treatment of type 1 diabetes mellitus, but outcomes have been disappointing due to host’s immune response which generate oxygen free radicals.

It has been reported that metallothionein (MT), a cysteine-rich and low-molecular-weight protein, provide cellular protection from reactive oxygen species (ROS) such as superoxide, hydrogen peroxide, hydroxyl radical, and nitric oxide by using scavenging property\(^1\),\(^2\),\(^3\). However, MT protein is rarely expressed in pancreatic islets, which means that islets are easily damaged by ROS. So, to overcome this problem, we designed cell penetrating Tat peptide-MT fusion protein (Tat-MT) to enhance MT uptake into islets. The purpose of this study was reducing oxidative stress on islet via delivery of Tat-MT for successful islet transplantation.

EXPERIMENTAL METHODS

Pancreatic islets were isolated from SD rats and digested by collagenase-P. And then it was obtained by Ficoll\(^\text{TM}\) Histopaque density gradient centrifugation. To internalize protein, islets were cultured with 10 \(\mu\)M of Tat-MT fusion protein for 9 hr at 37°C in humidified a 5% \(\text{CO}_2\) air atmosphere. To confirm the Tat-MT internalization into islets, fluorescence-labeled MT and Tat-MT delivered islets were observed by confocal laser scanning microscopy. To determine the response of Tat-MT delivered islets to various concentrations of glucose, untreated or Tat-MT-treated islets (10 \(\mu\)M) were incubated in either a low glucose (2.8 \(\text{mM}\)) or high glucose (20.2 \(\text{mM}\)) solution at 37 \(^\circ\)C for 1hr respectively. The secreted amount of insulin in low and high glucose solution was measured by rat-insulin ELISA kit. And then ROS scavenging function of Tat-MT was assessed by evaluating viability of Tat-MT delivered islets after paraquat (PQ) induced cell death. To evaluate whether Tat-MT can show ROS scavenging effect in vivo model, the untreated or Tat-MT delivered islets were transplanted into kidney subcapsular site of STZ-induced diabetic mice. After 1 day of transplantation, the islet grafts were retrieved and immunohistochemical analysis was performed. To observe the oxidized DNA which is indirect ROS marker, 8-hydroxydeoxyguanosine (8-OHdG) was stained with insulin. And then, to evaluate the efficacy of Tat-MT delivered islets in xenotransplantation model, the untreated or Tat-MT delivered islets were transplanted into kidney subcapsular site of chemical-induced diabetic mice. Islet transplantation rejection was defined when the blood glucose levels were over 200 mg/dL for 2 consecutive days. Islet transplantation survival time was expressed as the median ± standard error (SEM). Other quantitative data were expressed as the mean ± SEM. Statistically analysis was carried out using unpaired t-test or One way ANOVA on Ranks test or Holm-Sidak method by Sigma Plot software (Systat Software Inc., San Jose, CA, USA). P-values < 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Confocal laser scanning microscopy result showed that Fluorescence-labeled Tat-MT was strongly internalized into islets, on the other hand,
fluorescence-labeled MT was rarely delivered into islets (Data was not shown).

For the outcome of transplantation, we performed glucose-stimulated insulin secretion (GSIS) of Tat-MT delivered or untreated islets. Results showed that secreted amount of insulin in Tat-MT delivered islets were not significantly different with that of untreated islets (Data was not shown).

To evaluate Tat-MT can scavenge radical in islets, we check the viability of untreated or Tat-MT delivered islets after treatment of paraquat (PQ) which induce ROS. The viability of untreated islets was 36.0 ± 7.5, whereas the viability of Tat-MT delivered islets was 80.0 ± 4.5 (Fig 1). Results indicated that internalized Tat-MT effectively protected islets, scavenging PQ induced ROS.

Finally, we evaluated Tat-MT delivered islets could restore normoglycemia when they were transplanted into kidney subcapsular site of diabetic immunocompetent BALB/C mice (Fig 3). Results showed that the median survival time of untreated or Tat-MT delivered islets were 10.2 ± 1.9 and 12.2 ± 1.7, respectively. It indicated that Tat-MT could not significantly increase the graft survival of xenotransplanted islets. So, in further study, we will investigate synergistic effect between Tat-MT and other materials which can enhance the islet graft survival in xenotransplantation.

**CONCLUSION**

Tat-MT fusion protein was effectively delivered into islets. Tat-MT strongly protected islets from ROS in vitro and in vivo. In further study, we will investigate the synergism of Tat-MT and other material for enhancing islet graft survival.

**REFERENCES**


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