Cryopreservation of Hamster Pancreatic Islets Using a Rapid Cooling Rate

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ABSTRACT: To clarify the possibility of developing a rapid cooling rate for islet cryopreservation, we used a cooling rate of 25°C/min for hamster pancreatic islet cryopreservation using 15 per cent dimethylsulfoxide as a cryoprotectant. After preservation, these islets were examined for their morphology and function by assaying the insulin release after glucose stimulation and the contents of the insulin and DNA in 10 islets. In addition, islet cell replicatory activity was investigated by an autoradiographic technique. The effects of transplantation of the islets upon isogeneic and xenogeneic transplantation were also examined. Freezing using a rapid cooling rate of 25°C/min was found to be as effective as a slow cooling rate of 1°C/min for hamster islet cryopreservation. Morphologically, the cryopreserved islets appeared to be similar to the non-frozen cultured islets. The glucose-stimulated insulin release and cell replicatory activities in vitro also remained unchanged, whereas the number of cells per islet decreased slightly after cryopreservation. The grafting of cryopreserved islets normalized streptozotocin induced hyperglycemia following isogeneic transplantation. On the other hand, no prolongation of graft survivals in the case of the xenogeneic transplantation of hamster islets to rats was observed. The isogeneically transplanted islets exhibited the same cell replicatory activities in vivo, which was even higher compared that of normal hamster pancreatic islets in situ. In conclusion, the present findings indicate that hamster pancreatic islets can be successfully cryopreserved using a rapid cooling rate, however, it does not appear that this treatment reduces islet vulnerability to xenogeneic graft rejection.

KEY WORDS: cryopreservation, hamster pancreatic islets, rapid cooling rate, transplantation

INTRODUCTION

Pancreatic islet storage is one of the major problems for successful islet transplantation.

There are many preservation methods, such as culture, low temperature preservation, and cryopreservation, but cryopreservation may be the only potentially long lasting preservation method. Concerning the use of cryopreservation for pancreatic islet storage, there are several factors which have to be considered in order to obtain the best preservation of islet cells. The most important one is the cooling rate. Previously, relatively slow cooling rates (0.3–1.0°C/min) had been used.
in experiments on the cryopreservation of isolated pancreatic islet of Langerhans.\textsuperscript{1--3}

Such slow rates had been utilized since it was thought that freezing damage to the pancreatic islets could thus be reduced, mainly by avoiding intracellular ice crystal formation in the freezing process. Some authors have nevertheless demonstrated that the function of pancreatic islet cells preserved at more rapid cooling rates can still be retained after freezing and thawing.\textsuperscript{4--7} The freezing process takes about 2 hours at a cooling rate of 1.0°C/min. In this study, we applied a more rapid cooling rate to shorten the time of exposure for the non-frozen islets to various deleterious solute effects before freezing and also in order to simplify the cryopreservation method. Furthermore, Taylor et al.\textsuperscript{8} recently reported that rapid cooling rates destroyed lymphoid cells, so in pancreatic islet cryopreservation, "passenger" leukocytes\textsuperscript{9} might be killed with such a rapid cooling rate which might produce an immunological advantageous tissue for grafting. Finally, the effects of transplantation with rapidly frozen islets not only for isogeneic, but also for xenogeneic transplantation were examined.

\textbf{Materials and Methods}

\textit{Animals}

Rats and mice have previously been used as normal experimental animals, but we used 5--8 week old hybrid male golden hamsters for the \textit{in vitro} studies of morphology and function. For the \textit{in vivo} experiments with isogeneic transplantation, 5--8 week old male inbred GN descent golden hamsters from the Nippon Institute for Biological Science, Tokyo were used as donors and recipients while for xenogeneic transplantation, the same donors were used along with 4--5 week old male inbred Fisher descent rats from the Nippon Institute for Biological Science which were used as recipients.

\textit{Islet isolation and culture}

Islets of Langerhans were isolated by the method described by Lacy and Kostianovsky\textsuperscript{10} with some modifications. After mincing, pancreatic tissue was digested with 4 mg/ml Sigma type V collagenase dissolved in 5 ml Hank's balanced salt solution, and agitated firmly in a 37°C water bath. The digested pancreatic tissue was then rinsed with Hank's solution 3 times, and only islets of Langerhans were collected by hand.

After isolation, islets were cultured in a free floating fashion in sterile plastic dishes with a culture medium RPMI-1640 (Research Institute for Microbial Disease, Osaka University, Osaka) supplemented with 10 per cent calf serum for 3 days prior to freezing and for 3 days after thawing, to allow the islets to recover from the damage due to isolation, freezing and thawing, as reported previously.\textsuperscript{11,12}

\textit{Freezing-thawing procedure}

After culturing, the islets were frozen using a programmable temperature controller (Profreeze, Nihon Freezer, Tokyo). About 100 islets were put into sterilized small aluminum bottles containing 0.5 ml Hank's solution supplemented with 10 per cent calf serum, antibiotics and 5--20 per cent dimethylsulfoxide (Me$_2$SO, Sigma Chemical, St Louis) as a cryoprotectant. The islets were cooled at a rate of 8°C/min and kept for 30 minutes at 0°C to allow for Me$_2$SO equilibration. Then the islets were cooled at the rate of 25°C/min to −70°C, and then subsequently plunged into liquid nitrogen for 2 weeks. After storage, the islets were thawed in a water bath at 37°C. When the ice melted, the contents of the 4 bottles were rinsed with 40 ml Hank's solution in order to remove the Me$_2$SO, and centrifuged at 1500 r.p.m. for 2 minutes. Then islets were cultured in dishes with 5 ml culture medium for 3 days. Control non-frozen culture islets were isolated by the same method, and cultured only for 3 days. Other experiments comprised islets cryopreserved at a cooling rate of 1°C/min, but otherwise was treated as above.