Biochemical and Biophysical Research Communications xxx (2017) 1–7

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Oxygenated thawing and rewarming alleviate rewarming injury of cryopreserved pancreatic islets

Hirotake Komatsu*, Alyssa Barriga, Leonard Medrano, Keiko Omori, Fouad Kandeel, Yoko Mullen

Division of Developmental and Translational Diabetes and Endocrinology Research, Department of Diabetes and Metabolic Researches, Beckman Research Institute of City of Hope, 1500 E. Duarte Rd., Duarte, CA 91010, USA

ARTICLE INFO

Article history:
Received 2 March 2017
Accepted 24 March 2017
Available online xxx

Keywords:
Cryopreservation
Pancreatic islet
Rewarming injury
Oxygenation

ABSTRACT

Background/Aims: Pancreatic islet transplantation is an effective treatment for Type 1 diabetic patients to eliminate insulin injections; however, a shortage of donor organs hinders the widespread use. Although long-term islet storage, such as cryopreservation, is considered one of the key solutions, transplantation of cryopreserved islets is still not practical due to the extensive loss during the cryopreservation-rewarming process. We have previously reported that culturing islets in a hyperoxic environment is an effective treatment to prevent islet death from the hypoxic injury during culture. In this study, we explored the effectiveness of thawing and rewarming cryopreserved islets in a hyperoxic environment.
Methods: Following cryopreservation of isolated human islets, the thawing solution and culture media were prepared with or without pre-equilibration to 50% oxygen. Thawing/rewarming and the pursuant two-day culture were performed with or without oxygenation. Short-term recovery rate, defined as the volume change during cryopreservation and thawing/rewarming, was assessed. Ischemia-associated and inflammation-associated gene expressions were examined using qPCR after the initial rewarming period. Long-term recovery rate, defined as the volume change during the two-day culture after the thawing/rewarming, was also examined. Islet metabolism and function were assessed by basal oxygen consumption rate and glucose stimulated insulin secretion after long-term recovery.
Results: Oxygenated thawing/rewarming did not alter the short-term recovery rate. Inflammation-associated gene expressions were elevated by the conventional thawing/rewarming method and suppressed by the oxygenated thawing/rewarming, whereas ischemia-associated gene expressions did not change between the thawing/rewarming methods. Long-term recovery rate experiments revealed that only the combination therapy of oxygenated thawing/rewarming and oxygenated culture alleviated islet volume loss. These islets showed higher metabolism and better function among the conditions examined.
Conclusion: Oxygenated thawing/rewarming alleviated islet volume loss, with the help of oxygenated culture.

1. Introduction

Type 1 diabetes is a disease where the autoimmunity of the patient destroys insulin-producing beta cells in pancreatic islets. Insulin injections have been the only treatment for controlling hyperglycemia, however, blood glucose often remains difficult to control. An insufficient insulin dose may lead to glucotoxicity induced renal dysfunction, while inversely, overdoses of insulin may lead to lethal hypoglycemia [1]. Recently, pancreatic islet transplantation has been shown to be a safe and effective treatment to eliminate insulin injections and hypoglycemic episodes; however, donor shortages hinder the expansion of the islet transplantation. Isolated islets from cadaveric donors are used for islet transplantation, but they deteriorate easily and are difficult to store. Although not well established, long-term islet storage, or "islet banking", can be a promising solution to solve the donor shortage.

Much advancement has been made in the cryopreservation of single cells, but unlike other cell types, the recovery of

* Corresponding author.
E-mail address: hkomatsu@coh.org (H. Komatsu).

http://dx.doi.org/10.1016/j.bbrc.2017.03.134
0006-291X/

cryopreserved islets remains quite low. The series of procedures required for cryopreservation, thawing and rewarming islets damages them, leading to a significant volume loss, and therefore, cryopreserved islets are not yet clinically applicable. It is known that rewarming injury is deeply related to ATP depletion [2,3], and it is also known that ATP can be efficiently produced by aerobic metabolism with oxygen. Thus, we hypothesized that oxygenation may contribute to alleviating islet damage during thawing and rewarming. We have previously reported that the oxygenated culture of freshly isolated islets effectively prevents islet loss during culture [4]. The protective effect of oxygenated rewarming of cold-stored livers has also been reported [5,6]. In this study, we aimed to maintain islet mass volume using oxygenated thawing and rewarming from cryopreserved islets.

2. Materials and methods

2.1. Isolation of human islets

Human islets were isolated, as described previously, by the Islet Manufacturing Team of the Southern California Islet Cell Resources Center (SC-ICRC) [7]. The use of human tissues in this study was approved by the Institutional Review Board of the Beckman Research Institute of City of Hope.

2.2. Islet area and volume measurement

Islet area was assessed using microphotographs taken under bright field (IX50, OLYMPUS, Tokyo Japan) and measured as described previously [4].

2.3. Cryopreservation of isolated human islets

Isolated human islets were cryopreserved using the islet cryopreservation solution (ICS) shown in Table 1. Five hundred IEQ were placed in a cooled 1.8-mL cryotube (Cryogenic Vial, Nalgene, Rochester, NY, USA) for short-term recovery measurements, and 5000 IEQ for other experiments. Cryopreserved islets were stored in vapor phase of liquid nitrogen for at least three months prior to thawing/rewarming.

2.4. Thawing procedures of cryopreserved islets

Thawing procedures were performed following the protocol described previously [8]. Thawed islets were washed using PIM (R) culture media (PRODO laboratories, Aliso Viejo, CA, USA) including 5% HSA, PIM (G) (PRODO), and Ciprofloxacin hydrochloride (Thermo Fisher Scientific, Waltham, MA, USA) to replace the solution completely. For oxygenated thawing, all solutions were pre-equilibrated under 50% O_2, 45% N_2 and 5% CO_2.

2.5. Rewarming of human islet after thawing

Oxygenated or non-oxygenated PIM(R) media used for washing in the thawing process was also used as islet culture media for rewarming. For oxygenated rewarming, thawed islets were placed in a 24-well plate at 250 IEQ/well on ice with oxygenated culture media, and transferred to a 50% oxygen chamber [4]. This chamber was incubated at 22 °C for 45 min followed by 37 °C for 45 min. Thawing and rewarming procedures took 2 h in total, and these steps were expressed as “Short-term recovery” (Fig. 1A).

Short-term recovery rate was defined as the ratio of post-rewarming islet volume compared to the pre-cryopreservation. Prior to the cryopreservation, approximately 500 IEQ were plated to measure the islet area. These islets were completely retrieved, cryopreserved, then thawed and rewarmed. The area of the rewarmed islets was measured using the same procedure, which gives the short-term recovery rate: Short-term recovery = Islet area_Post-rewarming/Islet area_Pre-cryopreservation. Three independent experiments were performed using islets isolated from the same donor pancreata. Experiments were repeated using islets from four donors.

2.6. Human islet culture after rewarming

After completing the rewarming to 37 °C, islet culture plates were transferred to a 21% O_2 incubator (21% O_2, 74% N_2 and 5% CO_2) or 50% O_2 chamber (50% O_2, 45% N_2 and 5% CO_2) at 37 °C, depending on the experimental conditions, for two days [4]. This step was defined as “Long-term recovery” (Fig. 1A). Long-term recovery rate was defined as the ratio of islet area on day 1 or 2, to the area of post-rewarming islets (day 0). The following four groups were established to determine for long-term recovery rate: 21% O_2 thawing/rewarming followed by 21% O_2 culture (21T21C); 21% O_2 thawing/rewarming followed by 50% O_2 culture (21T50C); 50% O_2 thawing/rewarming followed by 21% O_2 culture (50T21C); 50% O_2 thawing/rewarming followed by 50% O_2 culture (50T50C). Three independent experiments were employed using islets isolated from the same donor pancreata. Experiments were repeated using islets from five different donors.

2.7. Absolute oxygen measurement in oxygenated/non-oxygenated thawing solution and culture media

The absolute oxygen tension in oxygenated/non-oxygenated thawing solution on ice (0 °C), culture media prior to rewarming (4 °C) and culture media at 37 °C on day 2 was measured using an oxygen sensor (Ocean Optics, Dunedin, FL, USA). Measurements were repeated 6 times independently.

Table 1
Composition of islet cryopreservation solution (ICS).

Materials	Molecular Weight	Final Concentration (mM)
L-Cysteine	121.2	1
Adenosine	267.2	10
Alloprinol	136.1	1
D-Mannitol	182.2	30
Glutathione	307.3	6
Potassium hydroxide	56.1	100
Lactobionic acid	358.3	100
Magnesium sulfate anhydrous	120.4	5
Niacinamide	122.1	5
Monobasic potassium phosphate	136.1	15

Please cite this article in press as: H. Komatsu, et al., Oxygenated thawing and rewarming alleviate rewarming injury of cryopreserved pancreatic islets, Biochemical and Biophysical Research Communications (2017), http://dx.doi.org/10.1016/j.bbrc.2017.03.134