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EFFECT OF HUMAN ISLET RESCUE GRADIENT PURIFICATION ON ISLET YIELD AND FRACTIONAL BETA CELL VIABILITY

A. Miki^a, C. Ricordi^{a,b,d}, T. Yamamoto^a, A. Mita^a, S. Barker^a, A. Khan^a, R. Alejandro^{a,c}, and H. Ichii^{a,b,d,*}

*a*Cell Transplant Center, Diabetes Research Institute, University of Miami Leonard M. Miller School of Medicine, Miami, FL, USA

*b*DeWitt Family Department of Surgery, University of Miami Leonard M. Miller School of Medicine, Miami, FL, USA

*c*Department of Medicine, University of Miami Leonard M. Miller School of Medicine, Miami, FL, USA

*d*Jackson Memorial Hospital Transplant Institute, University of Miami Leonard M. Miller School of Medicine, Miami, FL, USA

Abstract

It is difficult to consistently obtain sufficient post-purification islet numbers from younger donors, due to the higher proportion of trapped islets after pancreas digestion. Continuous Gradient Purification (CGP), which is currently used in several islet processing centers, sometimes is not efficient in the purification of trapped islets. Rescue gradient purification (RGP) could improve post-purification islet yields, resulting in an increased number of islet cell products that could be transplanted.

68 human islet isolations, in which CGP was followed by RGP were analyzed. The quality of islets from CGP and RGP was assessed by β -cell fractional viability (β FV) and ADP/ATP ratio. Donor age negatively correlated to the proportion of the islets rescued by RGP ($R=-0.52$, $P<0.01$) and to the percentage (%) of trapped islets ($R=-0.46$, $P<0.01$). Age related differences were observed in pancreas weight, digestion time and islet yields from CGP, respectively. Importantly, islets from RGP had an 11% higher β FV compared to islets from CGP. ADP/ATP ratio was also lower in RGP islets vs. CGP islets. RGP improved the efficiency of islet purification from younger pancreata and did not affect islet cell viability, which was actually higher in RGP fractions, indicating that rescued trapped islets from the pellet and lower purity layers are not damaged by the extra purification step and may actually be more viable. RGP could be used to rescue high quality islets from <30% pure islet fractions, which are often discarded in the clinical setting.

It is difficult to consistently obtain sufficient post-purification islet numbers from younger donors due to the higher proportion of trapped islets after pancreas digestion (1). Continuous Gradient Purification (CGP), which is currently used in several islet processing centers, sometimes is not efficient in the purification of trapped islets.

*Address correspondence to: Hirohito Ichii, M.D., Ph.D. Cell Transplant Center, Diabetes Research Institute, University of Miami Leonard M. Miller School of Medicine, 1450 NW 10th Avenue (R-134) Miami, Florida 33136 Phone: (305) 243-3700, Fax: (305) 243-4404, E-mail: hichii@med.miami.edu.

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Recently, rescue gradient purification method (RGP) was reported to be of assistance in maximizing the number of islet preparations successfully used for transplantation protocols (2). It has a potential to purify embedded islets. RGP could improve post-purification islet yield, resulting in an increased number of islet cell products that could be transplanted. The aim of this present study is to investigate RGP efficiency for the purification of islets from young donor pancreata and the viability of islets from RGP.

MATERIALS AND METHODS

Between June 2001 and September 2005, 70 rescue gradient purifications (RGP) in 223 human deceased donor pancreata were processed in our institute. Five pancreata were excluded from this study for long cold ischemic time (CIT, 12>hours). The remaining 65 RGP isolations were analysed by association with donor age in multivariate analysis. The 65 isolations were divided into two groups according to age: Group 1 (age<39) and Group 2 (age>40).

The results of islet isolation and in vitro viability assays such as the beta cell fractional viability assay, ADP/ATP ratio assay, FDP/PI, and static incubation were analyzed for the islet potency test between the two groups.

After the comparison, regression analysis was performed in order to correlate donor age and isolation data.

Islet isolation and purification

Human islets were isolated using the automated method previously reported (2). Islets were purified with the refrigerated computed cell processors (Cobe2991; COBE Laboratories Inc., Lakewood, CO). Continuous gradient purification (CGP) was performed using 1.100 and 1.077 g/ml Ficoll gradients previously reported (3).

After CGP, in cases that islet cell number was sufficient to transplantation and islet cells were remaining in the less pure fraction or COBE bag, RGP was performed additionally to purify the remaining islets from less pure portion. RGP was performed using 1.132, 1.108, 1.096 and 1.037 g/ml Euroficoll gradients (Mediatech, Herndon, VA) as previously reported (3).

Beta cell fractional viability assay and cellular composition assay

Beta cell fractional viability assay and cellular composition assay were performed immediately after islet isolation was accomplished from CGP and RGP as previously reported (4).

Adenosine Diphosphate (ADP)/Adenosine Triphosphate (ATP) ratio assay

To assess the quality of islets from CGP and RGP, ADP/ATP ratio assay was performed as a potency test using the ApoSENSORTM ADP/ATP Ratio Assay Kit (MBL, Nagoya, Japan) according to the manufacture's method.

Statistical analysis

All statistical and regression analyses were performed using the EXCEL software (Microsoft, Redmond, WA) and descriptive statistics are given as means $\pm$ SE. The differences between groups 1 and 2 were considered significant if the P value was <0.05 using an unpaired Student's t test. χ^2 test.

Results

Donor age negatively correlated to the proportion of the islets rescued by RGP (%RGP = RGP IEQ/total IEQ; R= -0.46, P<0.01) and to the % trapped islets (R=-0.52, P<0.01). No significant

differences in BMI, cold ischemia time, gender, pre-purification IEQ RGP IEQ and total IEQ were observed between younger and older donor pancreata (<39 vs. >40). However, a significant age related difference (<39 vs. >40) was observed in pancreas weight (91.6 ± 22.9 vs. 115.0 ± 33.4 g; $P < 0.01$), digestion time (15.1 ± 4.1 vs. 18.2 ± 5.7 min $P < 0.05$) and islet yields from CGP ($214,319 \pm 131,668$ vs. $296,575 \pm 172,509$ IEQ, $P < 0.05$), respectively. Importantly, islets from RGP had an 11% higher β FV compared to islets from CGP. ADP/ATP ratio was also better in RGP islets vs. CGP islets (0.27 vs. 0.83, respectively $P < 0.05$).

Conclusion

RGP improved the efficiency of islet purification from younger pancreases and did not affect islet cell viability, which was actually higher in RGP fractions, indicating that rescued trapped islets from the pellet and lower purity layers are not damaged by the extra purification step and may actually be more viable, RGP could be used to rescue high quality islets from <30% pure islet fractions, which are often discarded in the clinical setting.

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