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The Purification Method Using Iodixanol (Optiprep)-Based Density Gradient Significantly Reduces Cytokine/Chemokine Production from Human Islet Preparations, Leading to Prolong β -Cell Survival During Culture

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Abstract

Improved islet isolation has still been important to obtain adequate islet numbers for islet transplantation. Although Ficoll-based density gradient is widely used for purification in most islet processing centers, OptiPrep-based density gradient is recently used in the limited centers and their clinical outcomes are excellent. Cytokine/chemokine production from islet preparations for transplantation widely varies. Some cytokines/chemokines have been reported to cause apoptosis in human islet preparations after isolation. Reducing cytokine/chemokine production is a key to improve islet numbers after isolation and islet transplantation outcome. The aim of current study is to investigate the variability of pro-inflammatory cytokine/chemokine production from islet preparations purified by different density gradient. After digestion of human pancreata, pre-purification digests were divided into two groups and purified using semi-automated cell processor with Ficoll-based and OptiPrep-based density gradient. Islet preparations were cultured for 2 days and assessed regarding islet cell viability (FDA/PI), fractional β -cell viability and β -cell content. Cytokine/chemokine production from islet preparations was also examined. After purification, purity, post-purification IEQ and islet recovery rate were comparable between two groups. Although FDA/PI and fractional β -cell viability showed no significant differences, β -cell survival during culture significantly increased in OptiPrep-based density gradient group when compared to Ficoll-based density gradient group. TNF- α , IL-1 β , IFN- γ , IL-6 and MIP-1 production from OptiPrep-based density gradient group significantly decreased.

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OptiPrep-based density gradient can reduce cytokine-production when compared to Ficoll-based density gradient, resulting in improvement of quantity of β -cell mass. Cytokine profiling could spot new light on assessment of islet preparations before transplantation.

Improved islet isolation techniques have contributed to the progression of islet transplantation as a treatment for patients with type 1 diabetes mellitus 1, 2. However, many recipients require two or three times islet transplantation to achieve insulin independence. Improved islet isolation has still been important to obtain adequate islet numbers. Although Ficoll-based density gradient has been widely used for human islet purification in most islet processing centers, OptiPrep-based density gradient was recently used in the limited centers. It has been reported that Islet preparations purified using OptiPrep-based density gradient provided better clinical outcomes 3. It is well known that cytokine/chemokine production from islet preparations widely varies. Reducing cytokine/chemokine production from islet preparations may be a key to improve islet transplantation outcomes. The aim of current study is to investigate the variability of pro-inflammatory cytokine/chemokine production from human islet preparations purified using different density gradients.

Materials and Methods

Human islet isolations were performed using automated method 4. After digestion phase, pre-purification digests were divided into two groups and purified using semi-automated cell processor with Ficoll-based or OptiPrep-based density gradient. Human Islet preparations were cultured for 2 days, and assessed regarding islet cell viability (FDA/PI), fractional β -cell viability and β -cell content 5. Cytokine/chemokine production from islet preparations was also examined.

Results

Between Ficoll-based and OptiPrep-based density gradient groups, islet purity (80.0 ± 10.8 % and 74.3 ± 14.2 %, respectively, $p=0.203$), post-purification islet equivalent (IEQ) ($128,504 \pm 28,893$ IEQ and $127,235 \pm 16,970$ IEQ, respectively, $p=0.961$) and islet recovery rate (46.4 ± 4.2 % and 57.3 ± 14.4 %, respectively, $p=0.460$) were comparable. There were no significant differences between these groups by FDA/PI (92.4 ± 2.5 % and 90.6 ± 3.6 %, respectively, $p=0.550$), and fractional β -cell viability in OptiPrep-based density gradient group was comparable when compared to Ficoll-based density gradient group (57.6 ± 8.3 % and 64.5 ± 5.7 %, respectively, $p=0.178$). β -cell survival during culture significantly increased 1.31 ± 0.02 times in OptiPrep-based density gradient group when compared to Ficoll-based density gradient group ($p<0.001$). TNF- α , IL-1 β , IFN- γ , IL-6 and MIP-1 production from OptiPrep-based density gradient group significantly decreased to 22.8 ± 11.8 %, 27.7 ± 14.0 %, 37.3 ± 11.2 %, 45.3 ± 16.2 %, and 38.5 ± 18.6 % of each from Ficoll-based density gradient group ($p<0.05$).

Discussion

Current study clearly demonstrates that use of Ficoll, but not OptiPrep, for purification associates the release of pro-inflammatory cytokine/chemokine and could detrimentally affect islet survival during culture. These findings suggest the reduction of pro-inflammatory cytokine/chemokine production after purification by OptiPrep-based density gradient might indirectly contribute to improved stimulated β -cell survival. We obtained comparable purity, post-purification number of islet and recovery rate using the same density gradient made from either Ficoll or OptiPrep with the same procedure of purification. OptiPrep-based density gradient will be used Clinical Islet Transplant (CIT) trial in U.S.A. Although islets culture offers several advantages such as possible pre-transplant interventions including

immunosuppression 1 3, the number of islet equivalent reduces during culture 2. Our results suggest that the purification method using OptiPrep-based density gradient may be of assistance in improving clinical outcomes, and that Cytokine/chemokine profiling from islet preparations could be helpful to develop better isolation methods.

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