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Original Research

The IL-1 Receptor Antagonist Anakinra Enhances Survival and Function of Human Islets during Culture: Implications in Clinical Islet Transplantation

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ABSTRACT

Objectives: Human islet transplantation provides a feasible approach for treatment of type 1 diabetes mellitus but is limited by insufficient pancreatic donors and islet loss during pretransplant culture and posttransplantation. We examined if treatment with Anakinra (Kineret), a clinically approved interleukin-1 (IL-1) receptor antagonist, enhances survival and/or function of human islets during culture as a potential approach to improve the quality and quantity of islets for transplantation.
Methods: Isolated human islets (n=5 donors) were cultured with or without Anakinra. Islet function, β-cell area, apoptosis, β/α-cell ratio and IL-1β release were assessed by glucose stimulated insulin secretion, quantitative immunolabelling and enzyme-linked immunosorbent assay.
Results: β-cell apoptosis increased during islet culture in a time-dependent manner (D0: 1.9 ± 0.4%, D2: 4.4 ± 0.4%, D4: 6.1 ± 0.7%) that was associated with increased islet IL-1β release, decreased islet β-cell area (D0: 72 ± 4.9% vs. D4: 55 ± 6.3%; % islet area) and β/α-cell ratio (D2: 2.3 ± 0.3, D4: 1.7 ± 0.2). Anakinra-treated islets had markedly lower β-cell apoptosis (D2(+An): 2.4 ± 0.4, D4(+An): 3.1 ± 0.6%), higher islet β-cell area (D4(+An): 69 ± 3.8%) and β/α-cell ratio (D4(+An): 2.1 ± 0.15) than nontreated cultured islets ($p<0.05$). Furthermore, Anakinra enhanced β-cell function manifested as increased insulin response to elevated glucose (D2(−An): 2.7 ± 0.2 vs. D2(+An): 3.5 ± 0.4; D4(−An): 2.0 ± 0.3 vs. D4(+An): 3.0 ± 0.2; fold basal) and higher islet insulin content (D4(−An): 60 ± 7.6% vs. D4(+An): 78 ± 7.5%, % D0; $p<0.05$).
Conclusions: Blocking IL-1 receptor with Anakinra protects cultured human islets from IL-1β-mediated β-cell dysfunction and apoptosis. IL-1 receptor blockers may provide a new approach to enhance islet survival and function during pretransplant culture thereby increase the success rate of islet transplantation.

Mots clés:
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au cours du diabète de type 1
culture des îlots
greffe d'îlots

RÉSUMÉ

Objectifs : La greffe d'îlots humains constitue une approche réalisable pour le traitement du diabète sucré de type 1, mais elle est limitée par l'insuffisance de donneurs et la perte d'îlots durant la culture prégreffe et postgreffe. Nous avons examiné si le traitement par l'anakinra (Kineret), un antagoniste du récepteur de l'interleukine 1 (IL-1) cliniquement approuvé, améliore la survie ou le fonctionnement des îlots humains durant la culture à titre d'approche potentielle pour améliorer la qualité et la quantité d'îlots destinés à la greffe.
Méthodes : Les îlots humains isolés (n = 5 donneurs) ont été cultivés avec ou sans l'anakinra. Le fonctionnement des îlots, la zone des cellules β, l'apoptose, le rapport des cellules β/α et la libération de l'IL-1 β ont été évalués par l'insulinosécrétion en réponse au glucose, l'immunoétiquetage quantitatif et le dosage immunoadsorbant lié à l'enzyme.
Résultats : L'apoptose des cellules β a augmenté durant la culture des îlots en fonction du temps (J0 : 1,9 ± 0,4 %, J2 : 4,4 ± 0,4 %, J4 : 6,1 ± 0,7 %) qui était associé à l'augmentation de la libération de l'IL-1β des îlots, à la diminution de la zone des cellules β des îlots (J0 : 72 ± 4,9 % *vs* J4 : 55 ± 6,3 % ; % de la zone des

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îlots) et au rapport des cellules β/α (J2 : 2,3 ± 0,3, J4 : 1,7 ± 0,2). Les îlots traités par l'anakinra ont eu une apoptose des cellules β nettement plus faible (J2(+An) : 2,4 ± 0,4, J4(+An) : 3,1 ± 0,6 %), une zone de cellules β des îlots (J4(+An) : 69 ± 3,8 %) et un rapport des cellules β/α (J4(+An) : 2,1 ± 0,15) plus élevés que les îlots de culture non traités ($p < 0,05$). De plus, l'amélioration du fonctionnement des cellules β traitées par l'anakinra s'est manifestée par l'augmentation de la réponse à l'insuline à un glucose élevé (J2(−An) : 2,7 ± 0,2 *vs* J2(+An) : 3,5 ± 0,4; J4(−An) : 2,0 ± 0,3 *vs* J4(+An) : 3,0 ± 0,2; *fold basal)* et une teneur en insuline des îlots plus élevée (J4(−An) : 60 ± 7,6 % *vs* J4(+An) : 78 ± 7,5 %, % J0; $p < 0,05$). *Conclusions :* Le blocage du récepteur de l'IL-1 par l'anakinra protège les îlots humains de culture du dysfonctionnement et de l'apoptose des cellules β médiées par l'IL-1β. Les antagonistes du récepteur de l'IL-1 peuvent constituer une nouvelle approche à l'amélioration de la survie et du fonctionnement des îlots durant la culture prégreffe et ainsi augmenter le taux de réussite de la greffe d'îlots.

Introduction

Type 1 diabetes mellitus is an autoimmune disease in which islet β-cells are selectively destroyed by the cellular immune system leading to hyperglycemia and life-long insulin dependence (1). In the past decade, human islet transplantation has shown remarkable potential as a means of β-cell replacement in patients with type 1 diabetes (2–6). It is, however, currently limited by the low number of available pancreatic donors and loss of islets during isolation, pretransplant culture, and after transplantation due to both immune and nonimmune factors. A follow-up study from the Edmonton group (4) and subsequent studies (6,7) have shown that approximately 10% of type 1 diabetes recipients of allogeneic islet transplants remained insulin-independent 5 years after transplantation. Thus, finding new approaches to improve viability and function of human islets during pretransplant culture and in islet grafts is of great importance.

Several factors have been suggested to contribute to islet loss during pretransplant culture period (typically 24 to 72 hours) including ischemic time during organ procurement (8,9), mechanical and enzymatic damage during islet isolation process (10), limited access of β-cells in the islet center to oxygen and nutrients (9,11), formation of β-cell toxic islet amyloid (12–14), loss of peri-insular islet extracellular matrix (15) and absence of growth factors, and hormonal and neural inputs that are present in vivo. Previous studies have shown that β-cell death in isolated human islets during culture occurs mainly via apoptosis (12,16). However, the signalling pathways that initiate β-cell apoptosis in cultured human islets are still not well understood.

Interleukin-1β (IL-1β) is one of the key regulators of the body's inflammatory response. It is expressed as a biologically inactive precursor (proIL-1β) that is cleaved intracellularly by the IL-1β converting enzyme to generate the biologically active IL-1β that binds to the IL-1 receptor type 1 (IL-1R1) (17,18). In pancreatic islets, IL-1β expression has been detected in β-cells, resident islet macrophages, ductal cells and vascular endothelial cells (19–22). IL-1β causes β-cell dysfunction and apoptosis in vitro (23–27). Thus, IL-1β signalling pathway has been implicated in the pathogenesis of β-cell death in type 1 diabetes (23,28). Furthermore, exposure to the elevated glucose, fatty acids, leptin or islet amyloid has been shown to increase production and release of IL-1β in islets (14,21,29,30), suggesting that IL-1β and Fas/FasL pathway also contribute to β-cell death in type 2 diabetes independent of an autoimmune reaction, thus providing a link between pathogenesis of type 1 diabetes and type 2 diabetes (31,32). In the present study, we used Anakinra (Kineret), a clinically approved IL-1 receptor antagonist, to examine the potential role of IL-1β in β-cell death during culture under conditions used in the clinical islet transplantation and to test if blocking IL-1 receptor in β-cells can improve the survival and function of human islets during pretransplant culture.

Research Design and Methods

Human islet culture and treatment

Human islets isolated from 5 male and female cadaveric pancreatic donors aged between 46 to 68 years were provided by the Ike Barber Human Islet Transplant Laboratory (Vancouver, BC, Canada) in accordance with approved procedures and guidelines of the Clinical Research Ethics Board of the University of British Columbia. Freshly isolated human islets (hand-picked, purity >90% as assessed by dithizone staining) were cultured (50 islets/well, free floating) in human islet culture medium CMRL (Mediatech, Herndon, VA), supplemented with 5.5 or 11.1 mmol/L glucose, 10% (v/v) fetal bovine serum (Invitrogen Canada, Burlington, ON), 50 U/mL penicillin (Invitrogen), 50 μg/mL streptomycin (Invitrogen) and 50 μg/mL gentamicin (Invitrogen) in humidified 5% CO_2/95% air at 37°C. The culture medium was replaced every 48 hours.

Treatment of human islets with IL-1 receptor antagonist

Human islets were cultured (5.5 mmol/L glucose) in the presence or absence of Anakinra (Kineret; Sobia Pharmaceutics, Denton, MD) for 2 or 4 days. The optimal concentration of Anakinra (10 μg/mL) used in these studies to block the binding of IL-1β to its receptor on islet β-cells was determined by culturing human islets in the presence of recombinant IL-1β (2 ng/mL) with or without different concentrations of Anakinra for 3 days. Paraffin-embedded islet sections were double immunostained for insulin and IL-1β or insulin and terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL) to assess the level of IL-1β bound to IL-1 receptor on β-cells and inhibition of IL-1β-induced β-cell death, respectively.

Measurement of IL-1β and IL-1α release from human islets during culture

At each time point, culture medium was collected and centrifuged (12 000 × *g*, 10 minutes, 4°C) to remove cell debris. The supernatants were frozen immediately at −20°C until assayed. The IL-1β and IL-1α in the culture medium were measured using human specific IL-1β (hypersensitive) and IL-1α ELISA kits according to the manufacturer's instructions (R&D Systems, Minneapolis, MN). The values were reported as total IL-1β (or IL-1α) detected in the culture medium (pg/mL) divided by the number of islets in each well (pg/islet). The culture medium alone (without islets) was used as control for background reading. The sensitivity (minimum detectable concentration) of the ELISA kits used in this study was 0.16 and 1.0 pg/mL for IL-1β and IL-1α, respectively.

Glucose-stimulated insulin secretion test

Human islets from each condition were hand-picked randomly (25 islets, triplicate) and preincubated (1 hour) in 300 μL