

Secondary Negative Effects of Isolation Enzyme (s) on Human Islets

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Failure to maintain long-term islet function even after multiple donor islet infusions for one recipient directly proves that there is islet loss after transplantation. Among the possible reasons accounting for islet cell loss, isolation stress plays a major role [1]. The consequences of isolation stress are not limited to disappointing islet, but may also hinder islet functionality after transplantation. In our study, we identified that the most commonly used isolation enzyme, LiberaseTM-HI, negatively influences islet survival and function [2]. We observed that islet insulin secretory capacity is impaired following islet exposure to Liberase. We also found that islets internalize the Liberase and that one or more of its components degrades the insulin molecule *in vitro*. In our previous study, we proposed a novel strategy of isolation that consisted of earlier removal of islets from the isolation milieu, enriched in digestive enzymes. Islet morphology and function were improved versus the standard procedure that caused greater islet damage [3]. The isolation process exposes human pancreatic islets to exogenous isolation enzymes. Exposure to these enzymes, as a result of intraductal injection in the pancreas or simple contact of islets with enzyme components, causes internalization into the islet cells of enzymes and their by-products. Human islets exposed to Liberase-HI exhibit a decreased insulin secretory ability that correlates with the time of exposure. This phenomenon is paralleled by increased expression of adhesion molecules (CD106 and CD62p) and activation of apoptotic pathways (Bax and Bcl-2) in islet cells. Increased functional impairment is also observed after islet transplantation in diabetic

immunodeficient mice. Experimental exposure of islet grafts to exogenous isolation enzymes causes intense inflammation (CD11b positive cells) at the transplant site. The extent of these adverse effects likely deceives the standard qualitative protocols currently in use to assess islet quality *in vitro*. Reducing the secondary effects of exogenous isolation enzymes on isolated human islets may be crucial to enhance the quality of islets as tissue grafts.

1. Response of human islets to isolation stress: role of antioxidative treatment. ***Diabetes*** 2004 Oct; 53(10):2559-68.
2. Harmful delayed effects of exogenous isolation enzymes on isolated human islets: relevance to clinical transplantation. ***American Journal of Transplantation*** 2005 Nov; 5(11):2671-81.
3. Flexible management of enzymatic digestion improves human islet isolation outcome from sub-optimal donor pancreata. ***American Journal of Transplantation*** 2003 Sep; 3(9): 1135-1142.