Quantitative In Vivo Islet Potency Assay in Normoglycemic Nude Mice Correlates With Primary Graft Function After Clinical Transplantation

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Reliable assays are critically needed to monitor graft potency in islet transplantation (IT). We tested a quantitative in vivo islet potency assay (QIVIPA) based on human C-peptide (hCP) measurement in normoglycemic nude mice after IT under the kidney capsule. QIVIPA was initially tested by transplanting incremental doses of human islets. hCP levels in mice were correlated with the number of several transplanted islet equivalents ($r^2=0.6$, $P<0.01$). We subsequently evaluated QIVIPA in eight islet preparations transplanted in type 1 diabetic patients. Conversely to standard criteria including islet mass, viability, purity, adenosine triphosphate content, or glucose stimulated insulin secretion, hCP in mice receiving 1% of the final islet product was correlated to primary graft function (hCP increase) after IT ($r^2=0.85$, $P<0.01$). QIVIPA appears as a reliable test to monitor islet graft potency, applicable to validate new methods to produce primary islets or other human insulin secreting cells.

Keywords: Islet transplantation, Human, Mouse, Potency assay.

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Despite significant efforts to standardize human islet isolation procedures, primary islet graft function remains variable (1). Reliable islet potency assays are therefore critically needed and numerous methods have been proposed to monitor qualitative or quantitative characteristics of clinical islet preparations (2). None of the currently used tests could however be consistently correlated with primary graft function after clinical islet transplantation (IT). We describe here a simple and reproducible quantitative in vivo islet potency assay (QIVIPA) based on human C-peptide (hCP) measurement in normoglycemic immunodeficient mice after the transplantation under the kidney capsule of a fixed fraction of the final islet product. Initially tested with incremental doses of human islets, the value of this model was confirmed in the clinical setting.

Human islets were isolated at the Lille University Hospital, using a modified version of the automated Ricordi’s method (3). According to French regulations, four islet preparations of adequate quality, but insufficient in number of islets for transplantation, were initially used to develop the QIVIPA model. After 24 hr culture, increasing doses of purified human islets (500, 1000, 2000, 3000 islet equivalent IEQ) were transplanted under the kidney capsule of normoglycemic immunodeficient mice (Swiss nu/nu mice, Charles River Laboratories France, Arbresle, France, n = 25) as previously described (4). Human C-peptide was measured after an overnight fast in mouse tail blood, before transplantation and biweekly for 6 weeks thereafter, using a specific radioimmunoassay (C-peptide IRMA kit, Immunotech, Prague, Czech Republic). With less than 1% of cross-reactivity between human and mouse C-peptides, this assay differentiates the secretion originating from transplanted human islets from the endogenous murine C-peptide secretion that persists in these nondiabetic mice.

As expected, hCP was undetectable (<0.2 ng/mL) in plasma of native nu/nu mice and readily measurable in fasting mice as soon as 2 weeks after transplantation (Fig. 1a). After 6 weeks, immunostaining demonstrated the persistence of insulin positive cells in the abundant and well-demarcated graft in the explanted kidney (Fig. 1b). Undetectable levels of hCP measured in five mice 1 week after nephrectomy further confirmed the absence of endogenous hCP secretion. Noteworthy, increasing numbers of transplanted islets lead to higher hCP levels in a dose dependant manner. As depicted in Figure 1(c), hCP levels measured throughout the study duration (area under the curve) were significantly correlated with the transplanted number of IEQ ($r=0.6$, $P<0.005$). Notewor-
thy, hCP levels measured at day 15 were already predictive of the overall results ($r=0.9, P<0.0001$).

To confirm the clinical pertinence of QIVIPA, we subsequently designed a validation study using eight consecutive islet preparations transplanted in four patients with brittle type 1 diabetes enrolled in a phase 1 to 2 study of IT with the Edmonton Protocol (clinicalTrial.gov NCT00446264). A standardized sample (1%) of each preparation was taken from the final clinical product and transplanted under the kidney capsule of a nude mouse. With only one exception because of a technical failure (capsule rupture), all mice were successfully transplanted and remained available for hCP measurement at day 15. In parallel, each islet preparation was routinely evaluated as previously described (5), using standard in vitro quantitative (islet and beta cell number, islet mass, and purity) and qualitative (viability, adenosine triphosphate [ATP] and insulin content, and glucose stimulated insulin secretion) methods.

By design, all transplanted preparations met product release criteria of at least 250,000 IE (and/or 4000 IE/kg) with a purity above 30% and a viability of at least 90%, and exogenous insulin could be withdrawn within 3 weeks after last infusion in the four patients. The primary function of each preparation, as reflected by the increase in basal C-peptide level (mean±SD: 0.9±0.5 ng/mL) 1 week after the infusion (2), seemed however strikingly variable (Fig. 2). These results suggest that even in experienced centers using "optimal" islet preparations isolated with state of the art techniques from selected donors, islet graft potency may vary considerably.

We also confirmed that all in vitro criteria currently used to qualify islet preparations for transplantation, such as islet mass, viability, purity, ATP content, or glucose stimulated insulin secretion are only poorly correlated with clinical graft outcome (2). This study eventually failed to confirm the recently suggested correlation of primary graft function with the crude number of islets (6) or of beta cells (7). Other recently described methods such as beta-cell viability assessment by laser scanning cytometry (8), ADP/ATP ratio (9), or oxygen consumption rate (10) were not tested. These more sophisticated techniques do not however quantify islet specific function within the final cell product and have not yet been correlated with clinical outcome.

Conversely, we found here that QIVIPA results were significantly correlated with clinical primary graft function (Table 1). As shown in Figure 2, hCP levels measured in non diabetic nude mice, 2 weeks after transplantation of a standardized sample of the final product seemed more reliable than the number of IEQs generally reported for characterizing the potency of a given islet preparation. First described two decades ago (4), in vivo testing of human islet function in

**FIGURE 1.** Outcome of QIVIPA after transplantation of incremental numbers of human islet in nu/nu mice: (a) Biweekly hCP levels in mice after transplantation; (b) Persistence of insulin positive cells under the kidney capsule, 6 weeks after transplantation; (c) Area under the curve of hCP levels during 6 weeks.
immunocompromised mice has been extensively used for experimental studies. Hering et al. (11) also recently used this approach to assess the potency of six clinically transplanted islet preparations. Based on the delay of reversal of STZ-induced diabetes (12), we chose here to use normoglycemic mice as previously proposed by Gaber et al. (13). Initially undetectable in mice, fasting hCP increased within 2 weeks in a dose-dependent manner after transplantation of incremental human islet numbers from the same islet preparations. The hCP levels measured during 6 weeks confirmed the stable function of islets transplanted in this normoglycemic environment, mimicking the period of tight glycemic control obtained by continuous insulin administration in the immediate period after transplantation. When transplanted in a non immune environment, endocrine tissues such as islets (14) and parathyroid glands (15) become fully revascularized within 7 to 14 days. To limit the duration of the test, we therefore chose hCP levels at day 15 as the primary endpoint for the clinical validation of QIVIPA. Sampling 1% of the final product appeared as the best compromise between an islet mass sufficient (i.e., at least 2000 IEQ) to ensure largely detectable fasting hCP levels in the mouse (i.e., above 1 ng/mL), and a limited tissue volume (i.e., under 100 μL) to limit the risk of technical failure. To sensitize in vivo islet testing, Gaber (13) measured stimulated hCP levels after intraperitoneal glucose injection. This model could however be biased by inter individual variations of in vivo glucose absorption and utilization and remained qualitative. As illustrated by our 12% failure rate, IT under the mouse kidney capsule remains a technical challenge and requires dedicated and well trained staff. If necessary, the fraction of the final islet product devoted to evaluation could be reasonably increased to transplant 2 or 3 mice per preparation and further increase the consistency of QIVIPA. Quantitative in vivo islet potency assay intraassay variability could also be reduced without prolonging its duration by taking into account the mean of several daily measures around day 15.

In summary, we described here a new quantitative bioassay in normoglycemic immuno-incompetent mice that seems significantly correlated with the primary graft function after intraportal infusion in type 1 diabetic patients. Despite the retrospective nature of bioassays, QIVIPA could be of significant help to monitor graft potency in IT clinical trials. Using normalized islet numbers from the same islet preparations as illustrated in the first part of this study, this analytical model could also facilitate the preclinical evaluation of new procedures aiming to increase islet potency. Finally, QIVIPA could prove precious to compare human insulin secreting cells derived from alternative sources with human primary islets (16).

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REFERENCES


AUTHOR QUERIES

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