Comparison of Insulin Secretion between Human Adult and Fetal Pancreatic Islets

Background, objectives: The aim of this study was to compare insulin secreting capacity of human adult and fetal pancreatic islets in order to establish which one is more suitable for transplantation in IDDM patients.

Methods: Both adult and fetal islets were cultured in RPMI 1640 medium for 7 days (short-term cultivation) under conventional static culture. Insulin secreting capacity was expressed as stimulation index after stimulation with low and high glucose concentration (1.6 mM/L and 16.7 mM/L, respectively).

Results: Adult islets had greater stimulation index on day 1 than on day 7 (703.60±2.89% and 72.21±0.58%, respectively), indicating the loss of functional capacity on the 7th day. Fetal islets, on the other hand, showed slightly increased secretory ability on day 7 compared to day one (395.58±0.12% and 371.52±0.107%, respectively), with statistically significant difference (p<0.01). Comparing stimulation indexes of adult and fetal islets on day 1 and day 7, we established that adult islets had greater values on day 1 than fetal islet, p>0.05. Fetal islets had greater stimulation index on day 7 than adult ones and difference is statistically significant (p<0.001).

Conclusions: Based on analyzed results, we concluded that adult islets showed decreased functional ability of insulin secretion in response to glucose stimulation during 7-day cultivation, while fetal ones had slightly increased secretory capacity under the same conditions. These data indicate that fetal tissue cultured in elevated glucose levels is better adapted to culture conditions, while adult tissue loses its functional ability for adequate response on glucose stimulation during 7-day cultivation.

Key words: human, adult, fetal, pancreatic islets, insulin secretion

Vergleich der Insulinsekretion zwischen menschlichen adulten und fütalen Pankreas-Inselzellen

Hintergrund, Ziele: Es soll die Insulinsekretionsfähigkeit zwischen menschlichen adulten und fütalen Pankreas-Inselzellen verglichen werden, um herauszufinden, welche Inselzellen sich für die Transplantation bei IDDM-Patienten besser eignen.

Methoden: Sowohl adulte als auch fütale Inselzellen wurden in RPMI-1640-Medium sieben Tage lang (kurzfristige Kultivierung) unter herkömmlicher statischer Kultur gezüchtet. Die Insulinsekretionsfähigkeit wurde als Stimulationsindex nach Stimulierung mit niedriger und hoher Glukosekonzentration (1,6 mM/l bzw. 16,7 mM/l) ausgedrückt.
Introduction

Despite efficacy of insulin therapy as a treatment for type-1 diabetes mellitus (IDDM) there are numerous clinical complications. Those are the reasons for extensive research of pancreatic tissue transplantation as alternative to insulin therapy during last decade. There are different types of transplantations: whole pancreas, its segments or isolated adult and fetal islets. Xenotransplantation and allotransplantation require obligatory immunosuppression, so they are performed simultaneously or immediately after successful kidney transplantation in IDDM patients with severe end-stage renal insufficiency. Transplantation of isolated human islets has advantages such as simple surgical techniques and possibility of in vitro modulation which allows significant reduction of postoperative immunosuppressive treatment. Human adult islets can be collected by multiple cadaveric donors, but optimal results are obtained by isolating sufficiently large numbers of islets from single pancreas with good tissue compatibility. In this case, there is insulin independence for a 6 years period. Because of their reduced immune response, fetal islet transplantation has advantages over adult islet transplantation, but it is more difficult to achieve insulin independence, because of inadequate islet yields. However, C-peptide levels increased, peaking on day 90 and than decreased up to day 180 (1). Although insulin independence is not noticed in fetal islet transplantation (2), it can be achieved significant reduction in insulin dosage as well as stable glycemic control with maximal survival of avascular graft for one year (3).

Pretransplantation procedures include islet isolation, purification and in vitro cultivation, estimation of graft quality by determining: cytological characteristics (islet number and viability by dithizone staining); histological characteristics (using light and electronic microscopy); degree of islet purification; checking microbiological sterility; establishing functional capacity of islets (perfusion and secretion index) (4). The aim of this study was to analyze functional capacity of human adult and fetal pancreatic islets as adequate insulin response to glucose stimulation (5, 6). This indicates islets ability to adapt and to preserve their main characteristics during isolation and short-term in vitro cultivation.

Islets were cultured in relatively high glucose levels (11mM) which are common in IDDM patients, in order to determine what type of islet (adult or fetal) are more suitable for transplantation and also, what type of islet will be better adapted in vivo after transplantation. We investigated short-term cultivation because it is very important to perform islet transplantation in IDDM patients as soon as possible. Furthermore, in short-term cultivation, there is significant reduction of exocrine tissue after collagenase digestion (7).

Materials and Methods

Human fetal islets were isolated from fetal pancreata (n=10) of gestational age 16-24 weeks. Average weight of fetal pancreatic tissue was 0.476±0.216g. Warm ischemia (time from the beginning of isolation procedure to the moment when cells were placed in the culture vessels) was 52.60±6.75 min, while cold ischemia was 54±13.29 min. Pancreata were obtained after spontaneous or prostaglandin-induced abortion due to medical reasons or after operation (sectio parva). All procedures were performed in Obstetrician Department, Clinical-Hospital Centre Zemun and Institute for Gynecology and Obstetric, Clinical Centre of Serbia. Human adult pancreata were obtained after pancreatectomy in patients with chronic pancreatitis or benign tumors. Operative procedures were performed...
in Institute for gastrointestinal diseases, Clinical Centre of Serbia. In case of tumors, we exclusively took healthy tissue by line of resection. For this study islets from eleven pancreata were employed (four males, seven females, age 60.84±10.44 years). Cold ischemia was 70.45±26.41 min; warm ischemia was 59.40±4.01 min. Average weight of pancreatic tissue was 4.89±0.49g. Islets were isolated under aseptic conditions by non-automated method using collagenase IX, 5mg/mL (Sigma-Aldrich) (15, 16). After semi digestion for 30 minutes at 37 C, supernatant was decanted and cells were washed in HBSS (Aplichem-GmbH) solution containing 20 mM glucose, 5.6 mM glucose, 0.2%FCS (fetal calf serum), pH 7.4. Islets were not purified to avoid cell loss (<50%) during purification and to preserve tissue important for normal cell function. Islet yield was 2-4000 islets/g pancreas. Islets were re-suspended in 10mL RPMI 1640 culture medium (Sigma-Aldrich), containing Ca(NO3)2x4H2O 0.1 g/L, MgSO 4 (anhyd) 0.048 g/L, KCl 0.4 g/L , NaHCO 3 2g/L, NaCl 6g/L, Na2HPO4 (Anhyd) 0.8 g/L,-Glutamine 0.3g/L, D-Glucose 2g/L (11 mM/L), 25 mM/L HEPES, 10% FCS, 100 U/ml penicillin, and 100 μg/ml streptomycin. Cells are incubated at 37 C in a 5%CO2, 95% humidity atmosphere for 7 days in the plastic culture flasks (Falcon 3013, volume 50cm³).

Functional capacity of isolated islets was established by static glucose stimulation assay. Islets were incubate one hour in low, one hour in basal and one hour in high glucose concentration (1.6 mM, 11mM and 16.7 mM) for all analyzed cultures. Insulin secretion index was calculated for each culture and mean values are illustrated on Figure 2. We noticed that both groups of islets showed increased glucose-induced insulin secretion. Adult islets had statistically greater response to glucose stimulation on day 1 (703.60±2.89%) than on day 7 (72.21±0.58%), p<0.01, which indicates the loss of functional capacity of islets during cultivation. Fetal islets also had increased insulin secretion, but greater on day 7 (395.58±0.12%) than on day 1 (371.52±0.107%), p<0.01, which indicates slight increase of insulin secretion during cultivation. On day 1, adult islets had higher secretion indexes (703.60±2.89%) than fetal islets (371.52±0.107%), but difference was NS (p>0.05). On day 7, fetal islets (395.58±0.12%) showed better glucose-induced insulin secreting capacity than the adult ones (72.21±0.58%) and the difference was statistically significant (p<0.001).

Results

Figure 1 shows mean values of insulin secretion induced by different concentrations of glucose (1.67, 11 and 16.7 mM) for all analyzed cultures. Insulin secretion index was calculated for each culture and mean values are illustrated on Figure 2. We noticed that both groups of islets showed increased glucose-induced insulin secretion. Adult islets had statistically greater response to glucose stimulation on day 1 (703.60±2.89%) than on day 7 (72.21±0.58%), p<0.01, which indicates the loss of functional capacity of islets during cultivation. Fetal islets also had increased insulin secretion, but greater on day 7 (395.58±0.12%) than on day 1 (371.52±0.107%), p<0.01, which indicates slight increase of insulin secretion during cultivation. On day 1, adult islets had higher secretion indexes (703.60±2.89%) than fetal islets (371.52±0.107%), but difference was NS (p>0.05). On day 7, fetal islets (395.58±0.12%) showed better glucose-induced insulin secreting capacity than the adult ones (72.21±0.58%) and the difference was statistically significant (p<0.001).
Discussion

Analyzing results (insulin secretion indexes) showed in fig. 2 it can be noticed that on day 1, adult islets had greater response to low and high glucose stimulation than fetal ones. Adult islets showed significant decrease in insulin secretion on day 7, which can be explained by free insulin released by beta-cells due to damage during isolation or cell death. Additional cell loss arises from the increased number of apoptotic events observed in static culture (10,11) and also as a consequence of elevated levels of nitric oxide produced in vitro (12,13). It is, therefore, evident that the islet, once removed from its natural surroundings within the pancreas and placed within the alien environment of the culture flask, becomes deprived of normal physiological organization and exposed to a number of hostile factors that cause its premature demise (14).

Although glucose stimulation assay is a relatively short procedure (3 hour in total), there are data showing that basal insulin secretion of fetal islets has the greatest values on second day of cultivation. This confirms that a significant amount of detected insulin is liberated by 5% of damaged cells (9). Incubation during basal secretion measurements lasts much longer (about 12 hours) so it can not be a criterion for insulin secreting capacity.

We can conclude that there is decrease in insulin secreting ability due to the loss of functional ability of adult islets for adequate response to glucose stimulation. This is probably the reason for not adapting to in vitro conditions. The existing protocols for the in vitro maintenance of primary human islets are however limited, allowing only short-term (less than 72 h) storage of cells under conventional static culture (CSC) conditions (15, 16). Extending the duration of culture beyond this period usually results in a significant loss of structural integrity within the islets and a concomitant decline in glucose-stimulated insulin release (GSIR) (17, 18). Fetal islets showed slightly increased insulin secreting capacity on day 7 compared to day 1. Other studies (19) show decreased secretory capacity of fetal islet in first days of cultivation and increase on day 4-8. It can be explained by quick recovery of cell culture followed by increased basal insulin secretion, as well as, formation of a thin layer of cluster cells (20, 21). This ability does not change significantly up to day 14 of cultivation, but decreases until day 21 of long-term cultivation.

Fetal islets show significant preservation of functional capacity during in vitro cultivation as a result of their better adaptation to culture conditions. Isolation and cultivation of fetal and adult islets took place under identical conditions which are more suitable for physiological and morphological characteristics of fetal islets. Experimental transplantation of human fetal and adult islets in nude mice confirmed that transplanted fetal tissue gave rise to grafts that had significantly higher levels of insulin than those from the same number of adult islets (22). Fetal cells are superior to adult ones because of their ability not only to grow, but also to mature at the implantation site. In fetal tissue, pancreatic ductal cells have potential to proliferate and differentiate into insulin-producing β-cells (23). Study of development of insulin secretion mechanism in fetal, neonatal and adult rats showed that no effect of glucose was observed on the pancreas from 19-day-old fetuses, while islets from adult rats had the greatest response to low and high glucose stimulation (24).

Tissue quantity and islets number do not affect the secretion index analyses, so methods affecting on efficacy of islet isolation do not have great influence. But type of sera added to the medium has an influence during islet cultivation. Optimal conditions for fetal islets cultivation are achieved using fetal calf serum (FCS) (20, 21). Some studies showed the toxicity of FCS on adult islets. In FCS-free media adult islets retain their low and high glucose induced response up to 21st day of cultivation compared to 7th day of cultivation in media containing FCS (25). Presence of FCS in media can reduce insulin biosynthesis up to 50% comparing to control culture in FCS-free medium. In summary, on day 1, fetal islets showed lower insulin secreting capacity than adult islets, but on day 7, fetal islets had greater response to low and high glucose stimulation than the adult ones. During 7 days cultivation, insulin secretion of fetal islets increased, but of adult islets decreased. In conclusion, although fetal islets had smaller insulin-secreting capacity than adult ones on day 1, they showed increased insulin-release capacity during cultivation. On the other hand, functional ability of adult islet decreased during cultivation. Fetal islets are more suitable for transplantation in IDDM patients, because they are better adapted to high glucose conditions.

References


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