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ORIGINAL ARTICLE

Efficiency of co-expression of transcription factors Pdx1, Ngn3, NeuroD and Pax6 with insulin: A statistical approach

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KEYWORDS

Efficiency;
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Abstract *Aim:* The objective of this study was to investigate the time related profile and efficiency of co-expression of the homeodomain proteins Pdx1, NeuroD, Ngn3, Pax6 and caspase3 with insulin, and to establish the time periods post PDL optimum for islets transplantation.
Study design/methods: In this experimental study, immunofluorescent staining procedure was performed on deparaffinized pancreatic duct ligated (PDL) tissues of 78 Sprague–Dawley rats. Quantification of protein coexpression was made using a computerized morphometry. The efficiency of co-expression was arbitrary defined by the value of mean ratio (score without unit) of insulin expression divided by each expression index of the other proteins, occurring within the time interval of 12–24 h post PDL. Statistical tool was used to analyze the efficiency of co-expression of proteins; analysis of variances (one way ANOVA) was used to compare the means of co-expression indexes across the time periods pre- and post PDL. *P*-values less than 0.05 were considered statistically significant; no post hoc test was done.
Results: The curve of insulin expression showed a crossover with that of the co-expression at

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different time periods pre- and post PDL. The optimal or higher efficiency of co-expression was observed for insulin and Ngn3 co-expression, while a good or medium efficiency was noted for the co-expression of insulin with Pdx1, insulin with NeuroD and insulin with Pax6. Low or weak efficiency was observed for the co-expression of insulin with caspase3.

Conclusion: We therefore propose an early islets transplantation using 12–24 h post PDL harvested pancreatic tissues.

1. Introduction

The mammalian pancreas derives from the foregut endoderm. Early differentiation from endodermal cells into endocrine cells forming the pancreatic islets of Langerhans is significantly determined by a cascade of genes activation event [1,2]. During foetal time, the clusters of epithelial cells in the embryonic phase differentiate into the mature endocrine cells of the islets of Langerhans, the duct cells, and the exocrine cells [3,4].

The chronobiology of these differentiated cells is well known: the glucagon-expressing alpha cells are followed by insulin-producing beta cells, somatostatin-producing delta cells and pancreatic polypeptide-producing PP cells [5,6]. Reliable data suggest that protein encoded by some of class B bHLH genes such as neurogenin 3 (Ngn3) and NeuroD are interrelated [7], and expressed in an overlapping and redundant manner [8,9]. Ngn3 is required for endocrine fate determination in the developing mouse pancreas [10]. The proposed position for the transcription factors Pdx1, Ngn3, Pax6 and insulin is based on the timing (chronobiology) of individual protein expression [1,11,12].

As diabetes mellitus (DM) is now a global epidemic, it afflicts around 300 million patients worldwide [13], shifting from developed countries towards developing countries, including sub-Saharan countries [14]. Studies dealing with pancreas development at the molecular level are urgently needed. These studies may be involved in providing cues and potential tools for in vitro generation of functional beta-cells from stem cells after the pancreatic duct ligation (PDL), despite existing controversies in post PDL pancreatic atrophy [15], and remarkable increase in mass of the survived islets [16]. However, the chronobiology of co-expression of Pdx1, Ngn3, NeuroD and Pax6 homeodomain proteins is not known, although there are evident suggestions that transcription factors may be involved in the proliferation of the islet after pancreatic duct ligation [17,18]. The research question was raised as follows: what time before 84 h after PDL might be the best moment to release the duct ligation, or to perform pancreatic transplantation, according to protein co-expression in the rats? Indeed, the autogenous transplantation of PDL tissue in the kidney of diabetic rats performed at 84 h following individual expression of insulin showed a graft failure in 50% of the animal group [19].

Thus, this study aims to investigate the time-related profile and efficiency of the co-expression of homeodomain proteins Pdx1, NeuroD, Ngn3, Pax6, and caspase3 with insulin; and to establish the time periods post PDL optimum for islets transplantation.

2. Materials and methods

2.1. Laboratory animals

Seventy-eight male, randomly selected healthy Sprague–Dawley rats were obtained from the Central Animal Unit of the Faculty of Health Sciences, University of Stellenbosch. The rats were weighed and put into groups of six animals each, corresponding to the time periods post-PDL of 6, 12, 24, 36, 48, 60, 72, 84, 96, 108 and 120 h, and time periods pre-PDL of 0 and 5 h at which animals were killed. While animals in both groups pre-PDL 0 h and 5 h did not undergo duct ligation, animals in group pre-PDL 5 h had the abdomen opened and closed only (sham operation).

2.2. Pancreatic duct ligation

A fully equipped microsurgery laboratory at the Department of Anatomy and Histology was used for the PDL surgical procedure. A night prior to PDL, the rats were housed in clean cages and in a thermally controlled environment with free access to water but no food.

On the day of the surgical procedure, induction of anaesthesia was achieved by 5% halothane vaporized in O_2, such that there was no spontaneous movement and no withdrawal responses to tail or foot pinch. Abdominal hair was shaved using a surgical blade, one centimetre on either side of the linea alba to avoid excessive heat loss. Great care was taken not to abrade or cut the skin. The shaved portion of the abdominal surface was then cleaned with betadine antiseptic solution containing providone-iodine at 10 mg/ml (Adcock Ingram Pharmaceuticals, Industria, Johannesburg, RSA).

The animals were placed on their back (dorsal recumbency); the hind limbs were maintained on the surgical table by means of a paper tape with a minimum tension, to avoid muscle strains. A mid-line laparotomy incision, starting from the tip of the xiphoid process to about one centimetre above the pelvic symphysis, was made to obtain access to the abdominal cavity. The stomach and the duodenum were drawn out and reflected cranially, to expose the pancreas. The topographical position of the pancreas was noted, and cotton buds were used to prise the pancreas away from the surrounding tissue. A Zeiss OPMI-1 operating microscope equipped with a zoom and a focus adjustment (Carl Zeiss, AG, Oberkochen, Germany) aided in identifying the colourless pancreatic duct. A resorbable suture material (5/0 sterile white braided silicone treated polyester, USP, Davis and Geck, Isando, South Africa) soaked in saline solution, was used for a tight single suture occluding duct, made at about 1/3 proximal to the tail end