Pancreatology xxx (2017) 1–9

Contents lists available at ScienceDirect

Pancreatology

journal homepage: www.elsevier.com/locate/pan

Kinetics of pancreatic tissue proliferation in a polymeric platform in mice

Luciana Xavier Pereira [a], Celso Tarso Rodrigues Viana [a], Laura Alejandra Ariza Orellano [a], Simone Aparecida de Almeida [a], Marcela Guimarães Takahashi de Lazari [a], Letícia Chinait Couto [a], Anilton Cesar Vasconcelos [a], Silvia Passos Andrade [b], Paula Peixoto Campos [a, *]

[a] Department of General Pathology, Institute of Biological Sciences, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil
[b] Department of Physiology and Biophysics, Institute of Biological Sciences, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil

ARTICLE INFO

Article history:
Received 7 July 2017
Received in revised form
19 December 2017
Accepted 20 December 2017
Available online xxx

Keywords:
Pancreas
Animal model
Platform
Proliferation

ABSTRACT

Background/Objectives: Pancreas regenerative capacity after injury is not always sufficient to comply with the body's requirement of digestive enzymes and hormones. We present an alternative system to induce pancreas parenchyma proliferation (exocrine and endocrine components), rather than regeneration or remodeling in normoglycemic mice.
Methods: Porous discs of polyether-polyurethane were surgically placed adjacent to the native pancreas and removed at days 15, 30 and 45 after implantation. No exogenous growth factors or extracellular matrix components were added to the platform. The synthetic matrix provided a platform that was filled with parenchymal and non-parenchymal pancreas tissue as detected by histological analysis. Immunohistochemistry analysis were performed to identify insulin positive cells in the newly formed tissue. In addition, angiogenic, inflammatory and metabolic parameters were carried out in those mice.
Results: At day 15, the pores of the platform were filled with inflammatory cells, spindled-shaped like fibroblasts, extracellular matrix components, blood vessels and clusters of pancreatic parenchyma (acini, ducts and islet-like structures). At days 30 and 45 the pancreas features remained well organized; its organization resembled that of a native pancreas. Interestingly, besides islet-like structures that showed positive cells to insulin, some ductal cells were also positive for insulin immunostaining. No significant differences in serum glucose and c-peptide concentrations during the experimental period were detected.
Conclusions: The plain synthetic porous platform (without addition of exogenous molecules) placed adjacent to the native organ exhibits potential to restore and/or expand exocrine (acini, ducts) and endocrine (β-cell mass) components in pancreatic injuries and in high metabolic demand.

Introduction

The pancreas is a complex organ composed of endocrine and exocrine compartments. Injury to the exocrine compartment causes severe clinical complications. In acute pancreatitis, high levels of amylase and lipase are present in the circulation but normally, this condition resolves without incident within days or weeks [1–3]. In contrast, chronic pancreatitis, involve advanced inflammatory and fibrotic alterations [3–5]. Lesion to β-cells in the endocrine compartment causes diabetes, which is characterized by hyperglycemia and is associated to micro and macrovascular alterations, being considered a global epidemic disease [6].

Several animal models have been reported which selectively injury the exocrine component (caerulein administration, duct ligation) or the endocrine compartment (streptozotocin administration, subtotal pancreatectomy), providing valuable insights into mechanisms of pancreas regeneration [1]. Studies with these models showed that acinar cells could regenerate from self-proliferation [7,8]. Whereas, beta cells can have multiple origins as from other endocrine cells [9], duct cells [10], acinar cells [11],

* Corresponding author. Federal University of Minas Gerais, Av. Antônio Carlos, 6627 – Pampulha, CEP 31270-901, Belo Horizonte, Minas Gerais, Brazil.
E-mail address: paulapc@icb.ufmg.br (P.P. Campos).

https://doi.org/10.1016/j.pan.2017.12.011
1424-3903/

self-duplication [12–15] or progenitor cells [16,17]. However, the different repair responses of adult pancreas depends on the nature of damage [18] and it regenerative capacity is not always sufficient to comply with the body's requirement of digestive enzymes and hormones, taking to chronic injury and aggravation of diabetes [9,13,19]. There is, thus a need for the development of strategies to improve pancreas regenerative capacity and/or replacement.

We have used synthetic polyether-polyurethane as an implanted platform to analyze the interaction between the host and the foreign material in the subcutaneous or peritoneal cavity of rodents. In previous publications, we showed that in the absence of exogenous addition of growth factors or extracellular matrix components, the implants become infiltrated by a proliferative fibrovascular tissue containing parenchymal cells of closer organs 8 days after implantation [20,21]. More recently, we reported that this polymeric platform adherent to the native mice pancreas filled with human adipose stem cells (hASCs) was able to induce growth of pancreatic parenchyma decreasing glucose levels in diabetic animals [22]. Thus, in contrast with other in vivo models used to investigate regenerative capacity, our model requires no incision to the animal's internal organs and no addition of exogenous growth factors and/or extracellular matrix components to the platform. As the model does not involve reduction of organ mass or ligation of ducts, it presents an alternative system to induce internal organ proliferation, rather than regeneration or remodeling. We hypothesized that this experimental model could be further exploited to investigate whether the platform would be colonized by exocrine and endocrine pancreatic cells. In order to achieve that we analyzed the kinetics of the cell population inside the platform at different time points post-implantation. Understanding the cellular processes involved in this response is central to the development of tissue engineering strategies using the newly formed pancreatic tissue.

Material and methods

Animals

The Ethics Committee of Animal Use (CEUA) of the Federal University of Minas Gerais, (protocol number 43/2015) approved all animal procedures. We used 8-week-old male C57BL/6 mice (n = 10/group) provided by Centro de Bioterismo (CEBIO) of the Federal University of Minas Gerais (UFMG). All mice were provided with standard chow pellets (NUVILAB CR-1 Brazil) and water *ad libitum* in our pathogen-free facility, and maintained under a 12-h light/dark cycle.

Experimental design

Synthetic polyether-polyurethane matrices in disc format, 5 mm thick x 8 mm in diameter (Vitafoam Ltd, Manchester, U.K.), were soaked overnight in 70% ethanol and sterilized by boiling in distilled water for 30 min prior to implantation [23]. All animals were anesthetized with a mixture of ketamine and xylazine (60 mg/kg and 10 mg/kg, respectively). The abdominal hair was shaved and the skin wiped with 70% ethanol. An incision (<1 cm long) was made in the upper left quadrant of the animals' abdomen. The implant discs were placed directly on the pancreas to create an interface between the synthetic matrix and the biological tissue. The incisions were closed with silk braided absorbable suture. The animals were kept isolated (one animal/cage) and monitored post-surgery for any signs of infection or discomfort. Post-operatory analgesia was not administrated for mouse because it could interfere with the interpretation of experimental results, since the main drugs used in experimental research with animals have effects in inflammatory process, such as opioids [24], among them the Buprenorphine [25] and nonsteroidal anti-inflammatory drugs (NSAIDs) such as Acetaminophen [26].

After 15, 30 and 45 days, the animals were anesthetized with ketamine/xylazine and euthanized. The implant and pancreas were carefully dissected from the adherent tissue, removed, and weighted. In addition, animal blood samples were collected. They were then processed as described below for various assays.

Animals glycemic metabolism analysis

The blood glucose levels were measured with On Call® Plus Blood Glucose Meter (ACON Laboratories, Inc. San Diego CA, USA). The animals' blood glucose levels were measured weekly throughout the experiment. The blood samples collected during euthanize were centrifuged at 4 °C for 10 min at 3000 rpm to obtain serum to measure c-peptide level. C-peptide levels were measured using the C-peptide ELISA Kit (DRG Instruments GmbH, Germany) according to manufacturer's protocol.

Histological and immunohistochemical (IHC) analysis

The implants and pancreas of the animals were removed, and fixed in 10% formol for 24 h. After paraffin inclusion, sections with 5-μm thickness were stained with hematoxylin and eosin (H&E), while other sections were processed for immunohistochemical analysis. A total of 30 fields/slide were captured with a digital camera JVC TK-1270/JCB connected to a microscope with a magnification of x400 to analyze the number of pancreatic islet, acini, ducts and blood vessels in the native pancreas and intra platform. Additionally, the percentage of pancreatic area infiltrating the platform was determined. The morphometric parameters were analyzed by Image Pro Plus 7.0 software.

Immunohistochemistry analysis of insulin producing-cells and cell proliferation were performed in deparaffinized sections of the implants. The sections were hydrated, and submitted to subsequent blocking steps: endogenous peroxidase block with 30% H2O2: methanol (1:1) for 15 min and 5% BSA for 1 h at ambient temperature. The slides were then incubated overnight at room temperature with the primary antibody: guinea pig antibody anti-insulin (1:500 - Dako) to insulin producing-cells and other slides were incubated with mouse monoclonal antibody anti CDC-47 (1:300 - Neomarkers) to cell proliferation; followed by incubation with Dako kit LINK and HRP–streptavidin (1 h each). DAB chromagen was used to visualize the peroxidase activity. Sections were then counterstained with hematoxylin. The negative control was performed by omitting the primary antibody and carried out simultaneously.

The presence of cells in apoptosis was examined intra implant by TUNEL (TdT mediated dUTP nick end labeling) in sections (5 mm thickness) using a commercial kit (TdTFragEL DNA Fragmentation Detection Kit, Cat QIA33; Calbiochem, San Diego, CA, USA). This allows identification of cells in apoptosis in marked terminal fragments of DNA (portion 39-OH), associated with the characteristically fragmented nuclear DNA. The method was applied according to the manufacturer's instructions. The morphometric parameters described above were analyzed by Image Pro Plus 7.0 software.

Measurement of cytokine levels produced intra implants

The cytokines TNF-α and CCL2 in the implants were determined by Immunoassay Kits (R and D Systems, USA) according to manufacturer's protocol. The implants were homogenized in PBS pH 7.4 containing 0.05% Tween and centrifuged at 10,000 × g for 30 min. Following that procedure, 100 μl of the supernatant were used to

Please cite this article in press as: Pereira LX, et al., Kinetics of pancreatic tissue proliferation in a polymeric platform in mice, Pancreatology (2017), https://doi.org/10.1016/j.pan.2017.12.011