Back to the Reinnervation of the Pancreas After Transplantation? (Experimental Study on Dogs, Cats, and Rats)

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ABSTRACT

Background. Significant functional decrease and sclerosis of the pancreas graft in late delays cannot only be related to chronic rejection. Any transplantation leads to graft denervation, which may be an important cause of dysfunction. Studies concerning graft reinnervation were controversial.

Purpose of the Study. The purpose of this study was to investigate the feasibility and pertinence of a surgically directed reinnervation (SDR) of denervated/neuro-reflex isolated (NRI) or autotransplanted (aTx) pancreas.

Basic Procedures. Anatomy of the nerves penetrating into the pancreas was studied in humans, dogs, cats, and rats. Surgery and physiological investigations were performed in dogs, cats, and rats. Nervous conductivity between NRI, NRI+SDR pancreas, and brain was tested. Load tests with glucose, insulin, and adrenalin were performed; amylase and lipase were determined in fasted and not fasted animals to evaluate the influence of NRI and SDR on pancreatic function. Histology was provided. Observation delays were 6 months.

Main Findings. Anatomic feasibility of SDR in humans and animals was proved. Models of pancreatic tail NRI and surgical reconstitution of the interrupted nervous pathways (SDR) were elaborated in animals. The restoration of the pancreas-brain reflex axis after SDR was electro physiologically proved. As blood glucose curves after load test, exocrine amylase and lipase determination have shown that pancreas NRI or aTx leads to an exaggerated reaction to usual stimulations that may cause the observed graft functional exhaustion in late delays. SDR shortened the period of the graft neuro-reflex isolation, contributed to a quick normalization of its function, and prevented its late degradation.

Conclusion. SDR was shown to be a simple surgical technique, easily performed after the graft surgical revascularization. Its functional and morphological efficiency was tested and proved. Thus, SDR may be recommended in human pancreas transplantation as pertinent.

DURING the last years significant improvement of pancreatic transplantation results (organ as a whole) was achieved [1–4].

Nevertheless, some reports about functional status of the pancreatic grafts several years after transplantation have mentioned functional decrease and morphological sclerosis of the graft [5–7]. It was related to a chronic rejection process. But with the progress of immunotherapy [8,9] the rejection episodes are dramatically reduced and can no more be the only cause of the pancreatic graft dystrophy [10,11].

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The pancreas organ transplantation procedure (as with any other organ grafting) includes only revascularization of the graft and reconstruction of the natural excretion ways [12–14]. So it leads to the nervous isolation of the grafted organ. At the same time the autonomic nervous system is known to regulate both endocrine and exocrine secretion of the pancreas, thus impacting glucose metabolism, as well as digestion processes [15–20].

In cases of extrinsic denervation of the pancreas, the basal pancreatic secretion in humans and the exocrine function in humans and dogs were significantly disturbed [15,21,22].

The question of the restoration of nervous connections between whole pancreas graft and recipient was discussed many years ago [23,24], concerned only spontaneous reinnervation, and never applied to clinics. The majority of authors agreed that the spontaneous growth of the recipient nerves into the graft occurs always very slowly.

Some investigations have shown that, in the case of the intestinal transplantation [25–34], spontaneous reinnervation is achieved too late to allow a complete recovery from the secretion, motility, and absorption dysfunctions caused by denervation. A surgical directed reinnervation of the intestinal transplant was proposed and tested; the conclusion was that it is feasible and efficient for fast penetration of the recipient nerves into the graft and it enhances the normalization of the graft function and morphology [26,35,36]. The idea of a surgical reinnervation of grafts was also proposed by other authors, particularly in the case of somatic nerve injury [37–40], but also for colon, kidney, and islet grafts with various results [41–44].

Hence, it seemed interesting to verify whether the denervation and reinnervation have the same influence on the function and morphology of the pancreas graft as was described in intestinal transplantation.

AIM OF THE STUDY

The aim of the study was to prove the feasibility and pertinence of the surgical directed reinnervation (SDR) of denervated or neuro-reflex isolated (NRI) or autotransplanted (aTx) pancreas for the optimization of the pancreas transplant condition.

The objectives were as follows: (1) on the basis of the study of the human and animal anatomy of the local pancreas innervation, to elaborate models of the NRI of the pancreas from the central nervous system (CNS) and spontaneous reinnervation, to elaborate a method of SDR of pancreatic grafts, (2) to evaluate the NRI influence on the endocrine and exocrine pancreatic function and morphology, (3) to evaluate the NRI + SDR influence on the pancreatic graft function and morphology, and (4) to justify the possibility of the surgical reinnervation of the human pancreas graft.

MATERIALS AND METHODS

The anatomy of the nerves penetrating into the pancreas was studied in 22 humans, 8 dogs, 9 cats, and 8 rats with the help of a binocular loupe MBC-3 with grid in millimeters. Taking into account the specific localization of both vascular sutures and possible nervous sutures during aTx in humans and in animals, the diameters of the dissected nervous fibers going to the pancreas were measured at the levels of the celiac trunk (level I) and at the origin of the splenic artery (level II).

Human cadavers were provided by the Department of Human Anatomy and Embryology of the Peoples' Friendship University of Russia (PFUR). Animal cadavers were provided by the PFUR animal house after euthanasia following local agreed protocol.

The surgical and physiological parts of the study were carried out in 27 dogs, 28 cats, and 94 rats, including fasted and nonfasted protocols.

All the animals used were hosted under standard conditions following "Guiding Principles for Research Involving Animals and Human Beings" Helsinki declaration of 1975.

All of the procedures were carried out under analgesia.

For surgery, general anesthesia was applied as follows in the dogs: induction by Aminosin (Chlorpromazine) 0.5 mL/kg and Droperidol 0.1 mg/kg (1–2 mL 0.005%) 15 minutes before the operation, followed by Thiopental Natrium 5% 0.5 mL/kg. After the operation, analgesia was pursued with intramuscular Analginum (Novalgin) 25% 1 mL.

For physiological study in the cats, intravenous Chloralose ((5ξ)-1,2-*O*-[2,2,2-Trichloroethylidene]-α-*xylo*-hexofuranose) 0.75 μg/kg (IV) was used.

In the rats, all invasive experiments were carried out under ether anesthesia.

Surgical Technique

NRI. Animals were placed in the supine position. After scrubbing and sterile draping, median laparotomy was performed.

The segment body-tail of the pancreas was dissected from all surrounding tissues in such a way that it remained attached to the splenic vessels and the pancreatic duct (Fig 1A). Dissection and hemostasis were performed using electro coagulation. The adventitia of the splenic vessels and pancreatic duct was accurately removed. In rats, residuary pancreas tissue attached to the duodenum was destroyed by electrocution, keeping the pancreatic duct in place. Abdominal cavity was closed without drain.

Pancreas aTx. This operation was performed just like NRI but with section and suture of the splenic vessels following the technique described by Roman RR et al [13,14]. The graft vessels were flushed with room temperature saline without heparin before orthotopic or heterotopic aTx. Warm ischemia time was 50 ± 5 minutes.

Exocrine pancreas secretion was drained into the jejunum by end-to-side suture between pancreatic duct and jejunum with running Catgut 4°°. In 4 dogs with segmental pancreas NRI and NRI + SDR, for the exocrine enzymes collection, pancreatic duct was sutured with the jejunum, a small patch of which was exteriorized to the skin as a modification of skin-intestine Thiry-Vella fistula.

SDR (During NRI or aTx Operations After Vascular Procedures). SDR consisted in paraneural and epineural suture with Prolen 10°° of the edges of the cut nervous branches surrounding the pancreas NRI/graft vessels with nerve trunks of the local neural plexus. For orthotopic reinnervation the nerve trunks surrounding either superior mesenteric artery and celiac trunk or splenic vessels were used. In heterotopic reinnervation the prepared nerve trunks of the graft splenic plexus were sutured with fibers of the hypogastric plexus (Fig 1B).

Control. The control groups in all animal models included animals after a laparotomy performed under general anesthesia followed by the pancreas surgical mobilization.

Follow-up. The animal care complied with the Guide for the Care and Use of Laboratory Animals [45].