**TITLE:**

Pancreatic Duct Infusion: An Effective and Selective Method of Drug and Viral Delivery

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**SHORT ABSTRACT:**

Pancreatic duct infusion is an important technique that can allow for lineage tracing, gene introduction, and cell line-specific targeting. A pancreatic duct infusion technique for drug and viral delivery to pancreatic cells is presented here.

**LONG ABSTRACT**

The pancreas is a bifunctional organ with both endocrine and exocrine components. A number of pathologies can afflict the pancreas, including diabetes, pancreatitis, and pancreatic cancer. All three of these diseases mark active areas of study, not only to develop immediate therapy, but also to better understand their pathophysiology. There are few tools to further these areas of study. Pancreatic duct infusion is an important technique that can allow for lineage tracing, gene introduction, and cell line-specific targeting. The technique requires the intricate dissection of the second portion of the duodenum and ampulla, followed by the occlusion of the bile duct and the cannulation of the pancreatic duct. Although the technique is technically challenging at first, the applications are myriad. Ambiguity in the specifics of the procedure between groups highlighted the need for a standard protocol. This work describes the expression of a green fluorescent protein (GFP) within the pancreas after the pancreatic duct infusion of a viral vector expressing GFP versus a sham surgery. The infusion and therefore expression is specific to the pancreas, without expression present in any other tissue type.

**INTRODUCTION:**

The pancreas is a bifunctional organ, with both endocrine and exocrine components. A number of pathologies can afflict the pancreas, including diabetes, pancreatitis, and pancreatic cancer1. All three of these diseases mark active areas of study, not only to develop immediate therapy, but also to better understand their pathophysiology.

A targeted therapeutic delivery method would be beneficial. We have developed a pancreatic duct infusion technique that is an effective and selective method for drug and viral delivery to pancreatic cells. In this model, the pancreatic duct is selectively cannulated, and the common bile duct is occluded, allowing for delivery exclusively to the pancreas.

This technique can allow for the lineage tracing of specific cell types to further elucidate the developmental pathways important to pancreatic development and pathology2,3. Different promoters in viral vectors allow for the specific targeting of cell lines and for the introduction of genes into these cell lines4,5. This work demonstrates the expression of a green fluorescent protein (GFP) within the pancreas after the pancreatic duct infusion of a viral vector expressing GFP versus a sham surgery.

**PROTOCOL:**

All animal experiments were approved by the Animal Research and Care Committee at the Children’s Hospital of Pittsburgh and the University of Pittsburgh Institutional Animal Care and Use Committee.

1. **Preoperative preparation.**
   1. Administer inhaled isoflurane (1-3% for maintenance and up to 5% for induction) via a nose cone to an appropriate anesthetic level. Monitor the adequacy of anesthesia by toe pinch with forceps. The lack of a response is considered adequate, and be sure not to overmedicate, as respiratory depression may occur.
   2. Place the animal on its back on the dissecting microscope stage, with the abdomen centered in view of the objective. Immobilize the animal with surgical tape. Apply ophthalmic ointment to the eyes of the animal to prevent dryness while under anesthesia.
   3. Apply a smooth, thick layer of hair removal cream and leave it in place for 3 min. Check a small area for hair removal. Gently wipe off the cream and hair with 70% ethanol-soaked gauze.
   4. Cleanse and disinfect the abdomen of the animal by wiping it with a 70% ethanol-soaked gauze followed by a betadine-soaked gauze. Maintain sterile conditions throughout the surgical procedure.
2. **Proper exposure.**
   1. Make a midline incision in the skin from the xyphoid process to the umbilicus using a scalpel. Lift the peritoneum with Adson forceps and make a small hole in the midline using scissors.
   2. Extend the peritoneal incision to the length of the skin incision, taking care to remain on the midline (linea alba).

Note: This will produce an upper midline abdominal laparotomy.

* 1. Elevate the liver with blunt retractors to expose the common bile duct. Clamp the common bile duct with a curved bulldog vascular clamp.

Note: This maneuver prevents undesired infusion into the liver.

1. **Identification of the pancreatic papilla and duodenotomy.** 
   1. Identify the duodenum and, with Arruga forceps, retract it caudally to display the papilla.

Note: The papilla is a white spot on the anterior surface of the second portion of the duodenum.

* 1. Retract the duodenum caudally, allowing for the identification of the course of the pancreatic duct. With a 30½-gauge needle, puncture the wall of the duodenum tangentially, directly opposite the papilla.

Note: The needle should follow the same angle as the duct as it enters the duodenum.

1. **Infusion.**
   1. Pass the catheter through the duodenotomy created in step 3.2 until the catheter is visible within the duct. Advance the catheter into the duct no more than 1 cm to prevent the bypass of minor ductal tributaries.
   2. Clamp the catheter in place with a second curved bulldog vascular clamp. Turn on the pump and infuse the selected volume. The volume infused may range from 25-250 μL, with an ideal volume of approximately 150 μL.
   3. Administer an intraperitoneal injection of approximately 1.5 mL of saline to offset losses. Cover the exposed bowel with a moist gauze to prevent desiccation.
   4. At the conclusion of the infusion, remove the bulldog clamp that was holding the catheter in place. Use the bulldog clamp to gently remove the catheter from the pancreatic duct. Release the clamp from the common bile duct.
   5. Close the peritoneum and the skin in one or two layers using a running suture.

Note: There is no need to close the duodenotomy, as it will seal on its own.

* 1. As postoperative analgesia, administer a dose of ketofen at 5 mg/kg during the procedure and one on the following day.
  2. Return the mouse to its cage under a heat lamp until it recovers. Provide the mouse with food and water ad libitum.
  3. Do not leave the animal unattended until it has regained sufficient consciousness to maintain sternal recumbency. Do not return an animal that has undergone surgery to the company of other animals until it has fully recovered.

1. **Sample preparation.**
   1. Place the animal in a carbon dioxide chamber or perform cervical dislocation.
   2. Harvest the pancreas, with the spleen, liver, and duodenum as controls. Fix the tissues in paraformaldehyde overnight at 4 °C. Cryoprotect the sample in 30% sucrose overnight, as previously described6.
   3. Snap-freeze the samples. Section the tissue at a thickness of 6 μm using a microtome. Perform imaging on a microscope with fluorescence imaging, as previously described7.

**REPRESENTATIVE RESULTS:**

With practice and careful surgical technique, the survival rate of these mice should be greater than 95%. One week after infusion, the desired effect should be evident in the pancreas. Mice at 10 to 12 weeks of age were utilized for pancreatic duct infusions. Here we use an adeno-associated virus serotype 8 (AAV8) with a CMV promoter to express green fluorescent protein (GFP), as compared to a sham surgery. Mice pancreases were harvested 7 days after the infusions. Sections of pancreas, spleen, and duodenum were stained with insulin antibody and Hoescht. Figure 1 shows that there is extensive expression of GFP throughout the pancreas in the mice that underwent pancreatic duct infusion with AAV8, as opposed to those that underwent sham surgery. There is no expression in the liver, spleen, or duodenum in these mice, thus documenting the selective nature of the infusion. These data can be confirmed with several other methods, including DNA and RNA expression profiles.

**FIGURE LEGEND:**

**Figure 1. Expression of GFP after pancreatic duct infusion**. (a) Sham surgery as compared to (b) pancreatic duct infusion with AAV8 expressing GFP. The scale bar represents 50 μm.

**DISCUSSION:**

This work describes in detail the methodology behind pancreatic duct infusion, an effective mouse model for the delivery of genes and other molecules specifically and effectively to the pancreas. Ambiguity in the specifics of the procedure between various groups highlighted the need for a standardized protocol8-10.

There are several critical steps of the procedure, beginning with the selection of young, healthy mice. Perforation of the duodenum, however small and controlled, is a traumatic event in the mouse that undoubtedly leads to the recruitment of inflammatory cytokines and other factors that have not been completely characterized. Therefore, a delicate dissection is crucial, as is creating the smallest duodenotomy possible for the introduction of the catheter. The most important step of the entire procedure is the cannulation of the pancreatic duct with the delivery system.

Difficulties can arise with excessive disruption of the pancreatic papilla, leading to an overwhelming systemic inflammatory response. The pancreas is a delicate organ; in the surgical community, it is colloquially described as an organ not to “mess” with. Success rates will vary only slightly with practitioner skill level. As long as bile duct is properly occluded, the infusion will be 100% specific to the pancreas alone.

The learning curve for the procedure is quite steep, and the main limitation is overcome with practice. As previously stated, excessive manipulation of the duodenum and pancreas can lead to an overwhelming inflammatory response that must be avoided.

The procedure has broad applicability across many different disciplines. It can be utilized as a method for targeted gene delivery through the use of specific viral vectors11. The procedure can also allow for the specific infusion of molecules that aid in the elucidation of pancreatic cell function. The specific delivery of certain molecules is an appealing method for modifying the signaling pathways within the islets to produce proliferation12. The system can also obviate the need for transgenic breeding by directly producing a desired genotype and phenotype within the pancreas. The applications are myriad.

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**DISCLOSURES:**

The authors have nothing to disclose.

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