Vascular access to the arterial side of the pancreas in the Syrian hamster

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Summary
In order to establish a new approach to the treatment of tumours of the exocrine pancreas of humans, this work was aimed at gaining vascular access to the arterial side of the pancreas in the Syrian hamster. There is to our knowledge no information available in the literature concerning the catheterisation of the arterial side of the pancreas in the Syrian hamster. Preliminary anatomical studies revealed that the coeliac artery could be a possible vascular access to the different lobes of the pancreas in the Syrian hamster.
The lumen of the splenic artery is too small to be catheterised. Injection of Evan´s blue and plastic beads in different sizes into the coelic artery demonstrated distribution to the different lobes of the pancreas as well as to the spleen, the stomach, the duodenum, and the omentum.
This opens up the possibility of a treatment, using biodegradable plastic beads coated with immunomodulators injected on the arterial side of the pancreas, as well as alginate beads harbouring transfected cells, capable of delivering various substances in the area of interest.

Introduction
Human tumours of the exocrine pancreas are often detected too late to be removed surgically and often do not respond significantly to cytostatica drugs or irradiation. Delivery of different therapeutic agents by means of biodegradable beads *(Seljelid et al. 1997)* or microencapsulated producer cells *(Read et al. 2001)* opens up strategies for local delivery in the treatment of different tumours, including tumours of the exocrine pancreas.
However, this demands a better knowledge of the circulation in the pancreas and the distribution of material injected intra arterially. This work demonstrates a technique for intra arterial injection of the pancreas in the Syrian hamster and shows the distribution of the injected particulated substances.
The Syrian hamster is a much used model for pancreatic carcinogenesis. Pancreatic tumours in the Syrian hamster can be induced by a variety of nitrosamines and show remarkable molecular, morphological and clinical similarities with pancreatic tumours in humans *(Hotz et al. 2000)*.

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The hamster pancreas is a well-defined, yellowish-white structure in the central abdomen. It averages 0.46 g. in weight or approximately 0.4 to 0.5% of the total body weight. The general shape of the pancreas is similar to the Greek letter lamda, with the irregularly shaped pancreatic head lying medio-dorsally to the cranial duodenum. The pancreatic head comprises 23% of the weight of the pancreas. It is bordered on the right side by the pyloris, duodenum, and ascending colon. The gastric lobe of the pancreas is attached to the stomach and pyloris by the mesogastric membrane formed from the greater omentum.The gastric lobe comprises 25% of the weight of the pancreas. The splenic lobe follows the greater curvature of the glandular stomach and comprises 40% of the pancreatic weight. It is covered by the greater omentum as it runs cranially to the area of the aorta and caudal vena. The splenic lobe is caudal to the spleen, but not attached to the splenic hilus. Instead, it has a connective tissue attachment to the cranial descending colon. The thin, leaf-shaped duodenal lobe is the smallest, averaging only 12% of the total pancreatic weight. It extends laterally from the duodenal loop and runs caudally enclosed in the mesentery, between the duodenum

and cranial ascending colon. The coelic artery provides blood to the pancreas and branches off the aorta cranial of the mesenteric artery. The first ten millimetres of the coelic artery has a diameter smaller than one millimetre, but is still possible to catheterise. In the area of inteterst, the gastroduodenal artery branches off, and the coelic artery continues after having given off the splenic artery. The splenic artery travels through the splenic lobe of the ancreas and eventually becomes the left gastroepiploic artery, which in turn anastomoses with the right gastroepiploic artery. The right gastroepiploic artery is also supplying the gastric pancreatic lobe. The pancreatic head and duodenal lobes receive arterial blood from the cranial pancreaticoduodenal artery. Venous return from the pancreas is via the splenic, right gastroepiploic, and cranial pancreaticoduodenal veins primarily, with a small portion of the tail of the duodenal lobe draining through the caudal pancreaticoduodenal vein *(Takahasi 1987, Schwarze 1987)*. Since local treatment of tumours of the exocrine pancreas by means of coated plastic beads or encapsulated transfected cells demands a vascular access to the arterial side of the pancreas, we have studied the distribution of plastic beads of various diameters inside the organ after intra arterial injection into the coeliac artery.
Histological examination was used to locate the beads in the different lobes of the pancreas, spleen, duodenum, and omentum.

Materials and Methods

Animals
Twenty-two outbred, adult Syrian hamsters (Charles River, Wiga GmbH , Germany) of both sexes weighing between 140-160 grams were utilized in the experiment. All procedures involving live animals were performed in accordance with institutional guidelines and national legislation.

Health status
The hamsters were found seronegative against Sendai, SV-5, PVM, Reo-3, LCMV, Encephalitozoon cuniculi. The bacteriological/ pathological examinations did not demonstrate the presence of : Campylobacter sp., Campylobacter jejuni, Pseudomonas aeruginosa, Pseudomonas sp., Salmonella sp., Bordetella bronchiseptica, Corynebacterium kutscheri, Klebsiella pneumonie, Klebsiella oxytoca, Mycoplasma pulmonis, Pasteurella multocida, or Pasteurella pneumotropica. The parasitological examinations for ectoparasites were all negative.

Husbandry
Male and female hamsters were housed separately in groups of five or six animals in Macrolon Type 2 cages. They were fed ad libitum with Special Diet Services Rat/Mouse 1 Maintenance (Expanded) feed supplemented by pieces of apple and wheat grain. Processed aspen chips (4 mm x 4 mm x 1 mm) were used as bedding. The room temperature was 21 +/- 2 degrees centigrade with a relative humidity of 55 %+/-10% with 10 air changes per hour.

Plastic beads
The plastic beads used in this experiment were Dynospheres (Dynal, Oslo, Norway). These are solid, mono-sized beads made of styrene with a cross-linking agent *(Seljelid et al. 1985)*. Diameters of 5, 10, and 20 microns were used with one size for each treatment group of animals.The beads are sold commercially in a suspension of water with sodium laurylsulphate as emulgator. Prior to injection,the beads were washed three times in a solution of 0.9 % NaCl and 3.0 % albumin to remove the toxic sodium laurylsulphate and preclude the formation of aggregates. The NaCl acts mainly in removing the toxic emulgator, while the albumin prevents the aggregation. The washed suspension of particles was adjusted to 10 million particles/microliter. As this was a non-recovery study, the particles were not sterilized. If required, this can be achieved by sterile filtration of the fluid and by autoclaving the beads.

Catheter
Portex Polythene Tubing (Portex Limited, Hythe, Kent, UK) with an inner diameter of 0.28 mm and an outer diameter of 0.61 mm was used as catheter material. This was stretched slightly to get an even smaller diameter of the catheter.

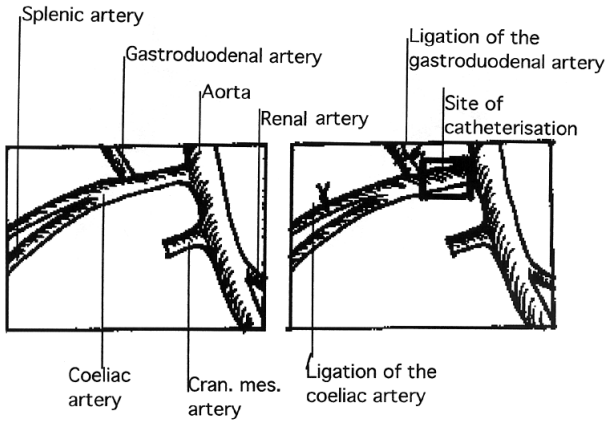
Experimental procedure

Anaesthesia
A combination of one part fentanyl citrate 0.315mg/ml and fluanisone 10 mg/ml with one part

midazolam 5 mg/ml mixed separately in equal volumes of sterile water was used at a dose of 5.6 ml/kg i.p. to give a satisfactory anaesthesia.

Surgical prearation

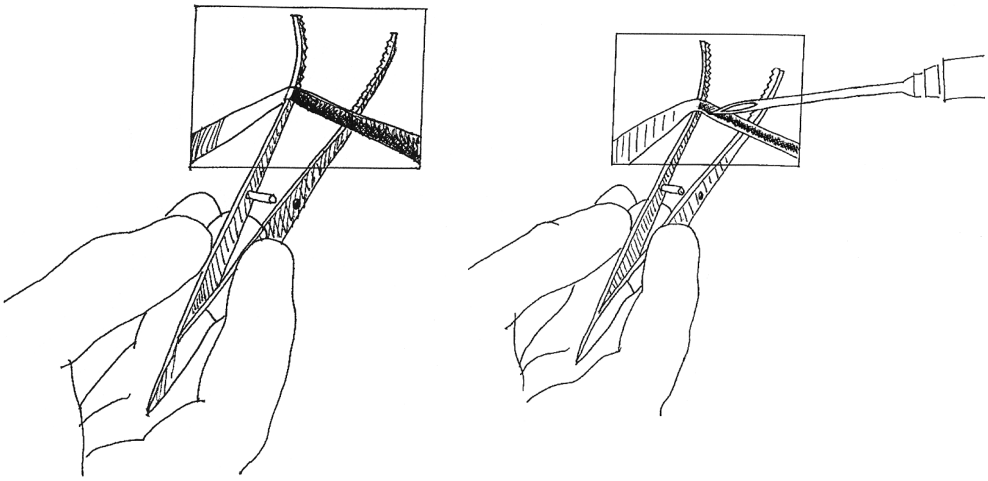
The anaesthetised hamster was laid on its back with its tail towards the investigator. Access to the area of interest was gained through a midline abdominal incision which extended about two-thirds of the length of the abdomen posterior to the xiphoid cartilage. The stomach, spleen, and duodenum were gently pulled out and placed on moistened gauze on the left side of the chest wall. The transverse colon was mobilised gently caudally and also placed on moistened gauze. The right cranio-ventral and the lateral left cranio-ventral parts of the liver were carefully retracted cranially by means of moistened gauze providing a clear view of the left kidney. A small bolster was placed under the thorax to facilitate the exposure of the area of interest. The use of a low power dissecting microscope during much of the operation is recommended. In addition to the normal instruments, some of which should be of a fine calibre, a good supply of cotton-tipped applicators is required. Cotton-tipped applicators were used to push aside the fat between the left kidney and the midline of the dorsal abdominal wall, leaving the aorta, the renal artery and the coeliac artery visible (see Fig. 1 and 2). The branches of the coeliac artery were loosened from adhering tissue by careful dissection using the cotton-tipped applicators and a pair of small, curved forceps. The gastroduodenal artery is ligated and the coeliac artery is ligated distal to the splenic artery (see Fig. 1 and 2).



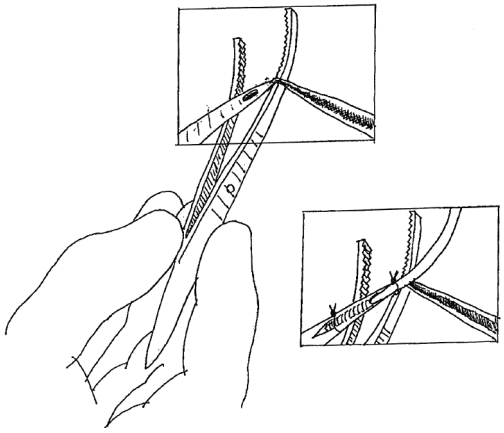
Figures 1 and 2.
Branches of the aorta and the coeliac artery in the area of interest.

Catheterisation procedure

A pair of small, curved forceps was introduced under the coeliac artery between the aorta and the ligation on the coeliac artery. By gently turning the left branch of the forceps upwards, and thereby minimizing the bloodflow, the lumen of the coeliac artery became clearly visible due to the increased blood pressure proximal to the forceps (see Fig. 3). Then the coeliac artery was punctured with a 30 G Microlance 3 cannula. To avoid bleeding after the artery was punctured, the right branch of the forceps was turned upwards, exerting pressure between the aorta and the site of puncture (see Fig. 4). At that point, the catheter was inserted 5-6 mm into the lumen of the artery, the curved forceps were removed, and the vessel was ligated to include the catheter (see Fig. 5).



Figures 3 and 4.
By gently turning the left branch of the forceps upwards, the lumen of the coeliac artery becomes clearly visible and can be punctured.



Figures 5.
To avoid bleeding after the artery has been punctured, the right branch of the forceps is turned upwards, exerting pressure between the aorta and the site of puncture, and the catheter can be inserted.

Injection procedure
Three treatment groups of hamsters, each consisting of two females and two males were prepared in the described manner. The four hamsters in group 1 were injected with 100 microliter of the 5 micron diameter plastic bead suspension (10 million beads/injection).

The four hamsters in group 2 were injected with 100 microliter of the 10 micron diameter plastic bead suspension (10 million beads/injection). The four hamsters in group 3 were injected with 100 microliter of the 20 micron diameter plastic bead suspension (10 million beads/injection). 20 minutes after the injection, the hamsters are euthanised by

an overdose of Thiopentone given into the aorta. One hamster was used as a control and was euthanised at the end of the project.

Collection of tissue samples

Tissue samples from the following organs were taken from all the experimental animals, including one control.

1. Pancreas -Pancreatic head
   -Duodenal lobe
   -Splenic lobe
   -Gastric lobe
2. Spleen
3. Duodenum
4. Stomach (glandular)
5. Omentum

Results

Beads with diameters of respectively 5, 10 and 20 micron were found in all lobes of the pancreas (Fig. 6). Beads with a diameter of 5 and 10micron were also found in the spleen and in the duodenum. No beads was observed in the wall of the stomac or in the omentum.

Table 1 shows the distribution of the plastic beads based on histology after injection into the coeliac artery.

Discussion

Gaining access to an artery or vein is a prerequisite for many physiological, pharmacological, and behavioural studies. Access to the vascular system is required to measure temporal changes in blood-borne humoral substances and to monitor the performance of several organs. Surgical approaches needed to operate on small animals like mice, hamsters, rats, guinea pigs, ferrets, and rabbits with indwelling vascular cannulas are unique. Special strategies are required because of structural or functional constraints imposed by proportional reduction in body mass and blood vessel diameter. In addition, the surgical technique and instrumentation adopted for use with small animals should minimize stress and avoid compromizing the animal´s behaviour. Therefore intravascular studies must be designed to ensure animal comfort and the acquisition of biologically relevant results.

A technique to catheterise the coeliac artery in hamsters is described in this work. The coeliac artery may be used for injections at the arterial side of the pancreas.

After treatment, the beads were found to be distributed in all lobes of the pancreas, and the 5 and 10 micron beads were also found in the spleen and in the duodenum.

Histology provides a qualitative answer concerning the distribution of the beads. The uneven distribution may be due to the differences in size as well as to other factors. The fact that beads with diameters of 5 micron and 10 micron were found in the arteries of the duodenum may indicate that they had slipped through the capillaries of the pancreas and were distributed to other organs as well. It was expected to find beads in the spleen, as blood supply is shared with the splenic lobe of the pancreas.

This study demonstrates that it is possible to get access to the arterial side of the pancreas in the Syrian hamster by catherisation of the coeliac artery. In order to get stuck in the area of interest, an appropriate diameter of the injected beads or microspheres must be chosen. This will enable the beads or microspheres to release the actual substances and eventually influence tumour growth.

Table 1. The table shows the distribution of the plastic beads based on histology after injection into the coeliac artery. 1=Pancreatic head, 2=Duodenal lobe of the pancreas, 3=Gastric lobe of the pancreas, 4= Splenic lobe of the pancreas.

Bead size	Pancreas				Spleen	Duodenum	Stomack	Oment
	1	2	3	4				
5 micon	+	+	+	+	-	-	-	-
10 micon	+	+	+	+	+	+	-	-
20 micon	+	+	+	-	-	-	-	-

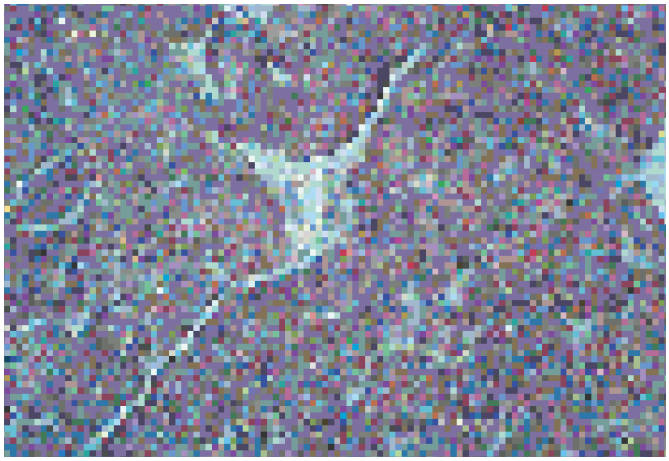


Figure 6. The picture shows a 20 micron plastic bead within the pancreatic tissue side of the pancreas.

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