Surgical techniques for quantitative nutrient digestion and absorption studies in the pig☆

Henry Jørgensen⁎, Anja Serena, Peter K. Theil, Ricarda M. Engberg

University of Aarhus, Faculty of Agricultural Sciences, Department of Animal Health and Bioscience, P.O. Box 50, DK-8830, Denmark

ARTICLE INFO

Keywords:
Catheterised pigs
Flow probe
Absorption
Total T-cannula

ABSTRACT

Surgical techniques allow quantitative measurement of nutrient digestion and absorption in pigs. The present paper presents our updated techniques for anaesthesia and surgery. The surgery technique of catheterization of the portal vein, mesenteric vein and mesenteric artery, as well as the fitting of a flow probe for continuous portal blood flow measurements in sows is described. Further, the cannulation of the terminal ileum with a dirigible bi-directional T-cannula for the total collection of ileal digesta is described.

© 2010 Elsevier B.V. All rights reserved.

1. Introduction

The use of various surgical techniques is a prerequisite for quantitative measurements of nutrient digestion and absorption in pigs (Jørgensen et al., 1992; Jørgensen et al., 1997; Pluske et al., 1995; Sauer et al., 1983). These techniques involve surgical access to the digestive tract facilitating the quantitative collection of digesta, and to the associated blood vessels, e.g. liver vein, portal vein, mesenteric vein and mesenteric artery to allow studies on the fate of nutrients in metabolism. During the last 5 years, a total of 260 pigs with a body weight in the range of 40–60 kg (grower pigs) and 150–250 kg (sows) underwent surgery in our laboratory. The present paper presents our updated techniques for anaesthesia and surgery.

2. Management of pigs around surgery and anaesthesia

The pigs are housed individually in smooth sided pens with a concrete floor (area of 3 m²) providing floor heating. The temperature is maintained between 18 and 20 °C. All animals are fed individually with free access to fresh drinking water. During an adaptation period of 10 days before surgery, the animals get used to the staff, the new environment, and standard routines like feeding, pen cleaning and weighing.

Prior to anaesthesia the pigs are deprived of feed for a period of 16 h. Anaesthesia is induced by i.m. injection (mg/kg BW) of 1.25 mg tiletaminchdrochlorid and 1.25 mg zolazepamhydrochlorid (Zoletil 50 Vet., Virbac S.A., Cedex, France), 0.25 mg butorphanoltartrat (Torbugesic® Vet, Scan-Vet Animal Health A/S, Fredensborg, Denmark), 1.25 mg ketaminhydroclorid (Ketaminol® Vet., Intervet Danmark A/S, Skovlunde, Denmark), 1.25 mg xylazin (Rompun® Vet., Bayer A/S, Lyngby, Denmark). The pigs are intubated and access to an ear vein is established. The surgery area is shaved and cleaned with soap and water followed by disinfection with chlorhexidine solution (0.5% in 80% alcohol). Anaesthesia is extended by continuous i.v. infusion of 10 mg propofol (Propofol, Frensenius Kabi AB, Uppsala, Sweden) and 25 μg fentanyl (Haldid™, Janssen Pharmaceutica, Beerse, Belgium) per kg BW per h. The pigs are ventilated with a mixture of O₂: atm air (1:1, v/v) and i.v. fluid treatment is provided (0.9% NaCl, 10 mL/kg/h). The currently used anaesthesia procedure provides a quiet and fast recovery.

After surgery, the pigs are returned to their smooth-walled holding pens to minimise trauma caused by catching the cannula or exteriorized catheters on the sides of the pen. The pigs are given 10–14 days to recover and regain full appetite. Antibiotic treatment (Streptocillin®) and postoperative pain reliever are provided ((Flunixin 2.2 mg/kg BW, Finadyne® vet., Schering-Plough Animal Health, Farum,
3. Arterial–venous difference across intestinal tissue and blood flow to the liver

A modified procedure that is routinely used in our laboratory to measure continuously portal blood flow and to quantify the absorption of nutrients in growing pigs and sows by arterial–venous difference across intestinal tissue is described in the following (Fig. 1).

3.1. Preparation of catheters

The catheters for the mesenteric artery (MA) (Tygon®, polyvinyl chloride, Cole Parmer® Instrument Co., IL, USA; ID.040, OD.070) and the portal vein (PV) (Tygon®, ID.050, OD.090) with a length of 175 cm are prepared before surgery. Catheters are straightened out by heating in a water bath at 37 °C and then hanging up for a minimum of one week. The tips of the catheters are cut with a scalpel diagonally with an angle of 45° to enlarge the surface opening. The catheter tip is further cut at an angle of 90° and smoothened by a heated scalpel to prevent irritation of the vessel and blood coagulation. The catheter is checked, and heparin (100 IU/ml) is deposited. The function of the catheter is confirmed by arterial bleeding (branch of ductus choledochus) by blunt dissection. An ultrasonic blood flow probe (28A probe, 28 mm for sows and 20A probe 20 mm for growing pigs; Transonic System Inc., USA) is placed around the portal vein and closed. A flowmeter (Transonic® T206 flowmeter with P-option, Transonic System Inc., USA) is used to monitor the blood flow and to ensure that the flow probe is working correctly. The catheterization of the portal vein can be performed either through the liver or through the mesentery vein (Vena mesenterica cranialis).

3.2. Insertion of flow probe

The pigs are placed in dorsal recumbence and a midline incision is made. At the liver hilus the portal vein is isolated from the lymphoid tissue, connective tissue and the ductus choledochus by blunt dissection. An ultrasonic blood flow probe (28A probe, 28 mm for sows and 20A probe 20 mm for growing pigs; Transonic System Inc., USA) is placed around the portal vein and closed. A flowmeter (Transonic® T206 flowmeter with P-option, Transonic System Inc., USA) is used to monitor the blood flow and to ensure that the flow probe is working correctly. The catheterization of the portal vein can be performed either through the liver or through the mesentery vein (Vena mesenterica cranialis).

3.3. Insertion of PV catheter through the liver and through the mesentery vein

To get access to the PV through the liver, the liver tissue is cut off transversally approximately 3–4 cm from the top of a liver lobe. On the surface of the hepatic parenchyma, a portal vessel is identified at the Glissons’ triades, which are recognised by arterial bleeding (branch of Arteria hepatica). The PV catheter is filled with heparin (100 IU/ml) and a guide wire (TSF-28-180, Cook Denmark) is inserted until 2 cm of the guide wire becomes visible at the catheter tip. The guide wire is threaded into the portal vein branch exhibiting the biggest vessel diameter compared to the artery and the bile duct. The catheter is inserted and the guide wire redrawn approximately 3 cm. The catheter is inserted further until it appears in the PV as verified by palpation. In order to avoid disturbance of the flow probe by the inserted catheter, the final position of the catheter is about 2 cm cranial to the flow probe. The guide wire is removed; the function of the catheter is checked, and heparin (1000 IU/ml) is deposited. The catheter is fixed to the liver tissue by a 0.7 cm long cuff of silicone tubing, which is glued to a piece of polyvinyl net. The cuff is placed around the PV catheter and fixed by suture. The polyvinyl net is anchored in the hepatic tissue by suture. Alternatively the PV can be catheterised from the mesentery vein following the same procedure as for the mesentery artery, which is described below.

3.4. Catheterisation of the mesenteric artery

A branch of the mesenteric artery is identified in the mesenterium and isolated 3–4 cm from connective tissue by blunt dissection. Two ligatures are placed around the vessel in a distance of about 2 cm to control haemorrhage during introduction of the catheter. With a pair of micro-scissors, a transverse incision through one third of the vessel is made. The catheter is filled with heparin (100 IU/ml) and a guide wire (TSF-25-180, Cook Denmark) is inserted as described
previously. The catheter is inserted 10–15 cm in the artery and the guide wire is withdrawn. The catheter functionality is checked and a heparin solution (1000 IU/ml) is deposited in the catheter. The catheter is further fixed by anchoring the silicone cuffs in the mesenterium (Dexon 2, Davis and Geck, Cyanamid, Hampshire, UK). In our experience, the longevity of the mesentery arterial catheter is limited and the catheterization of another branch of the mesenteric artery is recommended.

The cable of the flow probe is exteriorized through the right flank and blood catheters are tunneled subcutaneously up to the same location. Placing the catheters in this position allows blood sampling even when the pigs are lying down. Catheters and flow probe cable are placed together in a protecting fabric bag. After surgery the wound is covered by plus ulcus wound plaster (Coloplast A/S, Denmark) and the surrounding skin is then covered with “non sticky barrier film” (3 M-Cavilon, Germany) to prevent possible skin allergy induced by the elastic adhesive bandage (Tensoplast, Smith & Nephew Medical Limited, Denmark) used to fix the bag on the skin. Finally, the bandage is covered by Stülpa-fix Gr. 6 (Hartman, Denmark) to keep it in proper position. The catheters are rinsed with 0.9% saline solution, filled with heparin (1000 IU/ml), closed and placed in the fabric bag. The proper function of the catheters is checked two times a week and heparin is always deposited before closing the catheter. The bandages are changed when appropriate.

In growing pigs, our measurements of portal blood flow are in the range of 1.2 to 1.8 l/min, which is in accordance with Hooda et al. (2009) and Yen and Killefer (1987), who use the flow probe method and the indicator dilution method, respectively. In sows, a portal blood flow of approximately 4 l/min is measured (Serena et al., 2009). In accordance with Hooda et al. (2009), we consider the direct blood flow measurement as a reliable tool in long-term studies of kinetics and quantification of nutrient absorption.

4. Total collection of ileal content — growing pigs and sows

The necessity to obtain separate estimations of the digestive events in the main intestinal segments has been acknowledged. Different methodologies have been developed and various methods to collect ileal digesta (re-entrant cannulae, T-cannulae, SICV-cannulae) have been reviewed (Danfær and Fernández, 1999). A modification of the simple T-cannulae allowing total collection (total T-cannulae) has been used by Bach Knudsen and Jørgensen (2007). This T-cannula (Fig. 2(A)) is made from rigid polyvinyl chloride (PVC). When inserted into the terminal ileum, the tube fits tightly to the mucosal side of the small intestine, only permitting the digesta to pass through the tube. To prevent digesta outflow or digesta accumulation and undesirable microbial fermentation between collections, a PVC cylindrical stopper (5-cm length and 2.1-cm o.d., Fig. 2(a)) is introduced into the lumen of the barrel. To prevent digesta flow between cannula and ileal mucosa, a double velour patch fabric (USCI® Debakey®, Massachusetts, USA, 11 × 2 cm) is cut and formed around the cannula and intestine and sutured together with a silk suture (Fig. 2(B)).

5. Conclusion

In conclusion, the present surgery methods are a reliable tool to obtain quantitative results in nutrient digestion and absorption studies.

Conflict of interest

There is no conflict of interests.

References