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Original Research Article

Monitoring changes in plasma levels of pancreatic and intestinal enzymes in a model of pancreatic exocrine insufficiency – induced by pancreatic duct-ligation – in young pigs

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ABSTRACT

Purpose: Plasma levels of pancreatic and intestinal enzymes were measured after pancreatic duct ligation (PDL) to monitor pancreatic exocrine insufficiency (PEI) in a model using young pigs.
Material/methods: Five, 6 week-old pigs (10.9 ± 0.2 kg), underwent PDL while age-matched, un-operated pigs were used as controls. Plasma levels of immunoreactive cationic trypsinogen (IRCT), amylase, lipase, and diamine oxidase (DAO) activities were analyzed for 48 days after PDL, including 1 week of oral pancreatic enzyme supplementation (PES) with Creon®.
Results: PDL resulted in an arrested body growth and a rapid surge of pancreatic enzymes (IRCT, amylase and lipase) into the plasma. Nine days after PDL, the plasma levels of these pancreatic enzymes had decreased. IRCT then remained below the level in un-operated pigs while amylase only fell below control at 25 days. The intestinally derived marker DAO and plasma protein levels were unaffected by PDL but DAO decreased slightly with time in PEI pigs. One-week of oral PES restored body growth, but had little effect on pancreatic enzyme plasma levels, except for a tendency towards increased DAO.
Conclusions: The study showed that PEI developed within 1–2 weeks after PDL and that only IRCT is a reliable plasma enzyme marker for this. The reduced plasma DAO indicated that PEI also affected the intestines, while PES therapy restored growth of the PDL pigs and slightly increased plasma DAO, suggesting an improved intestinal function.

1. Introduction

An appropriate pancreatic exocrine function is essential for the digestion and absorption of food while pancreatic exocrine insufficiency (PEI) with a lack of pancreatic enzymes in the intestines results in maldigestion, malnutrition, poor growth or weight loss and an increased mortality [1,2]. PEI is a complication due to pancreatitis, cystic fibrosis or pancreatic duct obstruction and is also fairly common in insulin-dependent diabetes.

Different PEI animal models including mice, rats, cats and dogs have been used to mimic human PEI and to study the aetiology and pathology of PEI [3]. However, a more suitable animal model can be obtained in the pig since the pig is omnivorous and the porcine digestive system is similar to man. Moreover, PEI can be achieved relatively easily in the pig by ligation of the accessory pancreatic duct, which during organogenesis becomes the functioning, main pancreatic duct, without affecting the separate bile duct and the bile flow [4]. After PDL, the young pigs develop steatorrhoea and show impaired growth, but apart from this, appear to behave normally [5]. PEI in pigs dramatically decreases the levels of digestive enzymes in the small intestine, resulting in a substantial decrease in nutrient digestibility [6]. In addition, PEI appears to affect the gut barrier function with an increased intestinal permeability compared to normal pigs [7].

Blood plasma levels of different pancreatic enzymes have been used as markers to diagnose acute and chronic pancreatitis and to monitor pancreatic exocrine function. For that reason, plasma amylase and lipase activities have often been assessed [8]. In addition, immunoreactive cationic trypsinogen (IRCT) has been shown to be a valuable parameter for assessing PEI in man [9] and

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development of pancreatic function in young pigs [10]. Measuring plasma DAO activity has been suggested to be a non-invasive method for evaluating the integrity of the small intestinal mucosa, since DAO is a crucial enzyme involved in polyamine metabolism, playing an essential role in the regulation of intestinal mucosal growth and differentiation [11].

In the present study, we aimed to follow the development of PEI by monitoring the blood plasma levels of pancreatic enzymes, such as trypsin, amylase, and lipase, following PDL in a young, growing, pig model. In addition, plasma DAO activity was measured to assess the intestinal mucosa function after PDL. These plasma enzyme parameters as well as the body growth were monitored during the development of PEI after PDL surgery as well as during and after oral pancreatic enzyme substitution with a porcine enzyme preparation.

2. Material and methods

2.1. Animals

The study was approved by the Local Ethics Review Committee on Animal Experiments at Lund/Malmö, Sweden. The experiments were performed on crossbred ((Yorkshire × Swedish Landrace) × Hampshire) pigs, obtained from the Odarslöv research farm, belonging to the Swedish University of Agricultural Sciences (Alnarp, Sweden). At 6 weeks of age, 5 weaned pigs (10.9 ± 0.2 kg) were selected for PDL surgery. For their welfare, three weeks post-surgery PDL pigs were kept two per cage and subsequently were housed individually in pens in the same stable at 20 ± 2 °C and with lights on from 7.00 to 19.00. In addition, three groups of 5–6 un-operated pigs, 6, 9 and 12 weeks-old, from the research farm were used as controls for normal development. All the pigs had free access to water via a drinking nipple and were offered a commercial pelleted grower feed (Växtill 320, Lantmännen, Sweden) at 2% of their body weight, twice per day (at 08.00 and 16.00, i.e., totally 4% per kg and day). The pelleted feed had a composition of water 12%; crude protein 17.5%; crude fibre 4.7%; crude fat 4%; ashes 5.9%; calcium 0.9%; phosphorus 0.7%; nitrogen 2.8%; potassium 0.6%; sodium 1.5 g/kg; lysine 11.0 g/kg; methionine 3.8 g/kg; cysteine + methionine 6.9 g/kg; threonine 6.6 g/kg.

2.2. Surgery

After an overnight fast and premedication with azaperone (Stresnil®, Janssen Pharmaceutica, Belgium, 2.2 mg/kg i.m.), the pigs were anaesthetized with 0.5–1.5% of Isoflurane (Baxter Healthcare Corporation, USA) in an O_2/air mixture via an endotracheal tube, using a close-circuit respiratory flow system (Komesaroff Medical Developments, Melbourne, Australia). All surgeries were performed under aseptic conditions. For PDL, a 14–18 cm long incision was made posterior to the sternum along the *linea alba*. The accessory pancreatic duct (the main duct in pigs) was isolated and ligated at 2 and 3 cm distance from the duodenal papilla with double silk sutures and then cut between the ligatures. The abdomen was then closed with 3 layers of sutures. Post-operative pain was prevented by administration of buprenorphine (Temgesic®, Schering-Plough AB, 0.01 mg/kg i.m.) for 2 days post-surgery.

At 20 days after the PDL, a catheter was placed in the anterior *vena cava* via the external jugular vein, to enable repeated blood collections during the experiments.

2.3. Experiments

The progress of PEI in the pigs was monitored for 29 days following the PDL surgery, and then, during and after PES (see Fig. 1). On day 29 after PDL, the PEI pigs were administered a single dose of enteric-coated porcine pancreatic enzymes (4 capsules of Creon® 10,000, Abbott Healthcare Products Ltd, Southampton, United Kingdom) together with the morning meal. Then for a one-week period, from day 31 to day 38, the pigs were given PES, i.e., the pigs received 4 enzyme capsules per meal, i.e., a total of 8 capsules per day. One capsule of Creon® contains 10,000 units of lipase, 8000 units of amylase and 600 units of protease (units according to the European Pharmacopoeia).

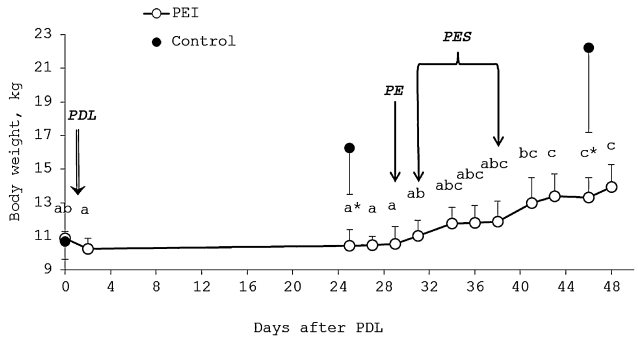

Fig. 1. Body weight of pigs (mean ± SD) during the 48 days following pancreatic duct ligation (PDL). On day 29 the pigs were given a single dose of pancreatic enzymes, Creon® (PE) together with their feed. During day 31–day 38 the PEI pigs were treated with PES. Body weights of age-matched control pigs from the same herd are shown as reference. Different letters (a,b,c) – indicate significant differences ($p \leq 0.05$) between values at different time-points while * indicates significant differences vs corresponding control pigs.

To monitor body weight changes, the pigs were weighed before the morning feeding. Blood samples were also taken in the morning before feeding either by direct puncture of the cranial *vena cava* (from the un-operated control pigs and from the PEI pigs from day 2 to day 16 post-PDL) or via the jugular vein catheter (for the PEI pigs from day 25 to day 48 following PDL). Blood samples of 5 ml were collected into syringes containing EDTA (0.20 mg) and a protease inhibitor (1000 kIU, Trasylol®, Bayer, Leverkusen, Germany), as described previously [12]. The blood samples were immediately cooled on ice and then centrifuged at 3000 × *g* for 15 min at 4 °C. Plasma was separated and stored at −70 °C until analyses.

2.4. Analysis

The plasma levels of IRCT were measured by a 2-step competitive ELISA using a rabbit antiserum against porcine cationic trypsin as the primary antibody (Dept. of Animal Physiology, Lund University, Sweden) and an alkaline phosphatase-conjugated goat anti-rabbit IgG as the secondary antibody (Sigma–Aldrich, St. Louis, MO, USA) as described previously [13]. After adding the substrate, p-NPP, the reaction was followed at a wavelength of 405 nm and a standard curve was generated by using purified porcine cationic trypsin (Novo Nordisk, Bagsvaerd, Denmark). Samples with a resulting absorbance outside the standard test range were re-diluted and re-measured.

Plasma amylase activity was analyzed using ethylidene-pNP-G7 (4,6-ethylidene-p-nitrophenyl-alpha, D-maltoheptaoside) as the substrate, according to the manufacturer's instructions (Infinity Amylase Liquid Stable Reagent; Thermo Electron, Victoria, Australia), modified for microplate usage. Once the substrate has been cleaved by α-amylase, the smaller fragments produced can be targeted by α-glucosidase, which causes the final release of the chromophore, pNP. The rate of formation of pNP, as measured by the increase in absorbance at 405 nm, is proportional to the α-amylase activity present in the plasma sample.