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Exocrine pancreas-brain axis – studies on pig models

Oś zewnątrzwydzielnicza trzustka-mózg – badania na modelu świni domowej

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Summary

Biomedical research has proven that both diet and life style practices, including food compositiion, food quality, eating behavior, and physical activity profoundly affect the overall health status of the world's population. New treatment approaches, including the use of functional food compounds can ameliorate the effects of the malnutrition which usually arises as a result of poor eating behaviours and poor food quality. Maldigestion, which is the main component of the malnutrition observed in modern society, is mainly a consequence of the lack of pancreatic enzymes. The absence or low level of pancreatic enzymes is commonly described as exocrine pancreas insufficiency (EPI). The EPI which occurs in newborns is an accepted physiologiocal state, while in the elderly it occurs as a result of age-related impairment of the exocrine pancreas. In both cases however, enzyme replacement therapy with pancreatic or pancreatic-like enzymes of microbial origin is applied to the patients. Pancreatic insufficiency (lack of active pancreatic enzymes in the gut) is often associated with marked neurological alterations related to cognitive function. However, studies dedicated to the investigation of brain function and morphology under conditions of malnutrition caused by EPI and the subsequent effects of dietary supplementation with pancreatic enzymes , are lacking – both in human and animal models.

The main aim of the present review was to describe the effects of the presence of active pancreatic or pancreatic-like enzymes within the gut, on brain morphology and function in a pig model.

Streszczenie

Badania biomedyczne dowiodły, że zarówno przyzwyczajenia dietetyczne, styl życia (włączając w to skład i jakość diety), jak i aktywność fizyczna mają ogromny wpływ na ogólny stan zdrowia ludności na świecie. Nowe zalecenia dietetyczne, w tym wykorzystanie żywności funkcjonalnej, mogą zmniejszyć skutki niedożywienia, powstałego zazwyczaj w wyniku złych nawyków jedzeniowych i złej jakości żywności. Jednakże czynnik wewnętrzny – niestrawność – jest główną przyczyną niedożywienia obserwowanego we współczesnym starzejącym sie społeczeństwie. Jest on przede wszystkim wynikiem zaniechania produkcji enzymów trzustkowych w wieku starszym. Brak lub niski poziom enzymów trzustkowych jest powszechnie określony jako niewydolność zewnątrzwydzielnicza trzustki (NZT). NZT dzielimy na fizjologiczną – występującą u noworodków, oraz spontaniczną – pojawiającą się w podeszłym wieku. W obu przypadkach pozytywne efekty przynosi enzymatyczna terapia zastępcza preperatami trzustkowymi, np. Creon 10 000, lub enzymami trzustko-podobnymi pochodzenia mikrobiologicznego. Ostra niewydolność trzustki jest często związana ze zmianami neurologicznych, takimi jak obniżenie funkcji poznawczych i świadomości. Jednakże brakuje badań poświęconych funkcji mózgu i jego morfologii w warunkach NZT oraz skutków suplementacji diety enzymami trzustkowymi – zarówno w modelach ludzkich, jak i zwierzęcych.

Celem niniejszego przeglądu badań własnych jest pokazanie wpływu obecności aktywnych enzymów trzustkowych lub trzustko-podobnych w jelicie na morfologię i funkcje mózgu w modelu świńskim.

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Abbreviations: CCK – cholecystokinin; CFA – coefficient of fat absorption; EPI – exocrine pancreatic insufficiency; IgG – immunoglobulin G; LCPUFA – long chain polyunsaturated fatty acids; NCAM – neural cellular adhesion molecule; NEFA – non-esterified fatty acids; PLEM – pancreatic-like enzymes of microbial origin; TG – triacylglycerides

INTRODUCTION

Exocrine pancreatic insufficiency (EPI) is a major consequence of diseases that lead to the loss of pancreatic parenchyma (pancreatitis, cystic fibrosis or obstruction of the main pancreatic duct; decreased pancreatic stimulation, celiac disease) and/or the acid-mediated inactivation of pancreatic enzymes (Zollinger-Ellison syndrome). In addition, gastrointestinal and pancreatic surgical resections (e.g. gastrectomy, duodenopancreatectomy, gastric by-pass surgery) are frequent causes of EPI (1). Low levels of pancreatic enzyme secretion are also observed in piglets as well as in both pre-term and full term human babies (2-4) and elderly people (5, 6). A deficiency in pancreatic digestive enzymes may result in the maldigestion and malabsorption of essential nutrients, which can in turn lead to malnutrition and weight loss in adults and to impaired growth and development in young individuals, if left untreated (7). Conventional treatment of EPI involves replacement of pancreatic enzymes with a pancreatic enzyme preparation from pigs. But despite high doses of pancreatic enzymes used during therapy, normalisation of digestion does not often occur and only partial corrections of the malnutrition have been reported (8-10).

Acute and chronic pancreatic insufficiency is often associated with marked neurological alterations related to cognitive function (11). Many patients with chronic pancreatitis report symptoms that are associated with a decrease in cognitive function, such as depressive symptoms (12-14), sleep disturbances (15) and the use of opioid medication (16).

The potential application of pig models, which mimick EPI conditions, in the exploration of brain development and function in infants and individuals with chronic malfunction of the exocrine pancreas, such as patients with cystic fibrosis, patients following oncology surgery and the elderly (6, 17, 18) was investigated. We have proved that the EPI pig model is a sensitive tool which allows us to test the effects of the presence of active enzymes within the gut on the neurological status of the animal. Thus, the EPI pig model could serve as a promising, sensitive tool for the investigation of the mechanisms responsible for pancreatitis-related neurological alterations and their correction.

In elderly humans pancreatic function is reduced. Previous studies (5) have confirmed that the function of the pancreas in the elderly is impaired. Thus, one can postulate that low levels of pancreatic enzyme secretion are characteristic of both neonates and the aged. In both cases, the pancreas responds poorly to exogenous stimuli, such as the gut hormones of the cholecystokinin (CCK) family and secretin, which play an important role in the regulation of exocrine pancreas secretion.

A number of different animal models which mimick the lack of active pancreatic enzymes within the gut have been developed. The most common models used for this purpose are rodents (rats, mice) and pigs (both minipigs and regular pigs) (19). The porcine models are of importance for applied physiology and medicine, since at the functional and developmental level, humans and pigs share many similarities with regards to the gastrointestinal tract, genitourinary structures and the development of the brain and pancreas (20-22).

In the studies reviewed, it was of high priority to reveal the coherent possibilities of pig EPI models to serve as experimental tools mimicking conditions in human individuals with chronic malfunction of the exocrine pancreas/lack of active pancreatic enzymes in th gut. A coherent animal model which would allow us to investigate the neurological status of such patients, as decribed above, could serve as a powerful tool in understanding the mechanisms responsible for pancreatitis-related neurological alterations and the correction of such alterations.

PHYSIOLOGICAL EPI IN NEWBORN UNGULATES ENSURES PROPER BRAIN DEVELOPMENT

Immature gut function is comparable in all newborn mammals, including humans, and coincides with very low levels of exocrine pancreatic enzyme secretion (3, 23). Moreover, even existing enzymes activity in newborn pigs (ungulates) is blocked during first hours of life by specific pancreatic and colostrum trypsin inhibitors. The activation of trypsinogen to trypsin (international classification number – 3.4.21.4) initiates the activation of other pancreatic enzymes by trypsin, as well as the autocatalytic activation of trypsinogen to trypsin. Trypsin inhibitors, from the pancreas itself or from the colostrum fed to the piglets, during the first few hours of life block the function of other active pancreatic enzymes. These circumstances allow for the absorption of IgG from the gut into the bloodstream, before gut closure takes place, usually between 24-36 hours after birth. Following the first 24 hours after birth, colostrum production is converted to milk production. Thus, the concentrations of Casal – a colostrum trypsin inhibitor, and that of Bowman – a pancreatic trypsin inhibitor, are reduced. The pancreatic enzymes – even though they are secreted in very small amounts at this stage, can begin to function resulting in digestion of the milk ingested by the newborn. One should keep in mind that milk of an ideal composition requires very low amounts of pancreatic enzymes to be digested into the simple components required for appropriate absorption from the gut (4). Pancreatic function begins to improve after weaning, when the pigs start to consume dry food (24). Pancreatic enzyme activity reaches optimal levels approximately 2-3 weeks after weaning.

Moreover, in ungulates, immunoglobulins from the pregnant sow, are not able to cross the placenta and pass into the circulation of the foetus (25). Neonatal pigs are therefore "agammaglobulinemic" at birth and have no form of immune protection at systemic and mucosal sites (26). Their survival depends directly on the acquisition of maternal immunity from the colostrum. The ability of the neonatal intestinal cells to absorb whole macromolecules and transport them intact across the epithelium into circulation is unique to ungulates e.g., a unique characteristic of the pig intestine during its development (27). During the short time after birth, the porcine gut is completely "open" to macromolecule absorption, while intestinal "closure" begins approximately 6-12 hours after beginning colostrum ingestion and is complete by between 24-36 hours. Such transfer of macromolecules ensures the uptake of immunoglobulins and other growth factors necessary for proper neonatal development. So, we have chosen the newborn, colostrum-deprived piglet as a model of the physiological EPI of neonates. Data obtained from a previous study (28) show that infant exocrine pancreatic insufficiency is required during the early stages of postnatal development. Inactive pancreatic enzymes provide the possibility for macromolecule absorption, including that of immunoglobulins, which can effect the maturation of the central nervous system, thus ensuring appropriate microgliogenesis and neuronal migration, as well as the appropriate level of neural cell adhesion molecules. This concept is of importance for both human infants and newborn pigs. It is possible that preterm neonatal human babies are unable to obtain all the necessary IgG, intrauterinally, and as such their brain development may be deteriorated, similarly to that observed in newborn, colostrum-deprived pigs. In both cases one can expect consequences for brain development. Moreover, maldigestion of macromolecules in adults, which can appear as a result of EPI condition, can affect the immunological status of people, due to the pervasion of whole proteins or their fragments in blood vessels.

PATHOLOGICAL EPI DETERIORATES BRAIN STATUS

In order to artificially mimick the pathological EPI condition, an attempt was made to adapt the surgical pig model of EPI for investigation of the digestion and absorption of nutrients in infants (18). The young pigs were adapted to nursing with a liquid milk formula, conventionally used to feed human infants, with essentially different amounts and proportions of nutrients to that of regular pig feed (i.e. regular pig feed for that age group of pigs usually contains only 2-3% of fat, whereas the milk formula used in the study contained approximately 30% fat). No adverse clinical signs associated with the adaptation of pigs to the liquid milk formula diet or pathological post-mortem macroscopic or microscopic findings (along the gut or in the liver), were observed following the seven days of enzyme treatment, as well as after the moderately long-lasting period (3 weeks) of milk formula feeding. All pigs adjusted well to the liquid diet and parameters of lipid absorption (coefficient of fat absorption (CFA), triglycerides level (TG), non-esterified fatty acids level (NEFA), lipaemic index (LI)) significantly differed between experimental and control groups of pigs. So, it was shown that the pig model of surgically induced EPI provides complete absence of pancreatic enzymes in the gut lumen and is highly reactive to the exogenous and endogenous stimuli. Thus, the model could serve as a sensitive tool, used to test the supplementation of food with exogenous enzymes and for testing functional food ingredients. The proposed EPI pig model reflects both physiological (infant EPI, age-related EPI) and pathological EPI and could be used to elucidate important questions regarding the influence of maldigestion and malabsorption for growth, maturation and development of the organism during different postnatal developmental stages. Moreover, since pig models are validated as the most similar to the human organism, results obtained in the pig studies can be used as the last stages of pre-clinical research.

As has been previously demonstrated, the physiological EPI condition in infants can influence the condition of the brain. Thus, an attempt was made to elucidate the effects of surgically induced experimental EPI on brain morphological structure and function (29, 30). The data obtained demonstrated that EPI resulted in a reduction in the number of pyramidal neurons in the pigs' hippocampus, a reduction in the level of NCAM and development of fewer glial cells, which in turn leads to a pathological increase in the behavioural activity of the pigs. It is possible that these negative effects can be significantly prevented by functional food enriched with pancreatic-like enzymes of microbial origin (PLEM). The positive effects of functional food enriched with PLEM, in terms of brain function observed in the study, might be relevant with respect to the clinical use of a PLEM-enriched diet which can be used for the treatment of EPI. Results obtained from the pig model, are in complete agreement with human studies which show a defecit in cognitive function, as well as the presence of pathological activity in humans suffering from chronic and acute pancreatitis. It is worth noticing, that in our studies the changes in activity were dependent on the diet consumed by the experimental animals. The enrichment of the standard (3%) pig diet with PLEM led to increased activity of the piglets, but the supplementation of the high-fat (18%) diet with PLEM gave the opposite effect – experimental animals decreased their activity and the behaviour values were close to those measured in healthy, control animals. The results obtained highlight the extremely important role of the presence of active pancreatic lipase within the gut, in order to be able to utilise LCPUFA from the diet.

CONCLUSIONS

In summary, we dare to suggest that the pig model of EPI, both physiological and pathological EPI,

can be used to elucidate the mechanisms responsible for pancreatitis-related neurological alterations, and for the development and testing of new functional food components and therapeutical strategies for infants and elderly people with chronic malfunction of the exocrine pancreas, in order to protect them from brain retardation related to lack of the pancreatic enzymes. Moreover, one can speculate that future food composition should consider the existence of a certain essential amount of fat. Enrichment of the standard pig diet with PLEM and fat, up to 18%, gave the opposite effect compared to when the using the normal diet, with 3% fat. Experimental animals fed the diet containing 18% fat, decreased their EPI-induced, pathological activity levels to values close to that observed in healthy, control animals. The results obtained also highlight the extremely important role of fat in the diet for brain development and function. However, the most intriguing question is if "silent" – bariatric surgery related functional exocrine pancreatic insufficiency (lack of active pancreatic enzymes in the gut) can affect brain function and structure. The latter should also be considered in the elderly when pancreatic insuficiency is obvious.

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