

Ph.D. THESIS

**EXPERIMENTAL AND CLINICAL DATA
TO THE SURGERY OF THE ILEOCAECAL JUNCTION
IN CHILDHOOD**

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INTRODUCTION

The ileocaecal valve plays a double role in the regulation of the passage of stool. The ileocaecal valve decelerates the stool transport, enhances the ileal absorption and prevents the reflux of the colonic bacteria into the small bowel. It had been thought that it works as a passive valve, but functional integrity of the terminal ileum, ileocaecal valve (or sphincter) and the caecum has been discovered recently.

The ileocaecal junction resembles the gastro-duodenal junction, because in both adjacent organs of the gastrointestinal tract are joining with different function in the digestion. But the coordination of the gastrointestinal motility is different in these two junctions. However the ileal contractions are uncoordinated with colonic contractions in 25-50 % in dogs, 30% in pigs and 70% in humans. The ileocolonic junction is able to behave as a synchronised segment. In contrast the gastro-duodenal motility is completely uncoordinated, i.e. the peristaltic activity of the stomach is not able to spread to the duodenum. The fibrotic isolation between the circular muscle layers and the gap in the myenteric Cajal cell network are considered as anatomical explanations.

The anatomy of the enteric nervous system in the ileocaecal valve has not been well studied. Higher density of neurons has been reported in the ileocaecal valve, but detailed anatomical description with primary reference to the continuity of the enteric nervous system and myenteric Cajal cell network within the ileocaecal valve is not available in the literature. The aim of the first chapter of this study was to explore the detailed neuroanatomy of the ileocaecal junction for proper understanding the motility of the ileocaecal junction.

Intussusception with no leading point is the most common surgical emergency in infants and young children. Motility disturbances of the ileocaecal junction have been considered in the aetiology recently. In the experimental animal model of intussusception, intraperitoneally injected lipopolysaccharides

release large amount of nitric oxide from the myenteric neurons resulting in motility disturbances leading to intussusception. Therefore close link is suspected between the nitregeric innervation of the intestine and the pathogenesis of the intussusception. The second section of the thesis focuses on the role of the nitregeric innervation of the ileocaecal valve and its postnatal changes played in the pathogenesis of the intussusception.

The congenital atresia of the ileocaecal valve with bowel obstruction is extremely rare. So far only three cases have been reported in the literature. The ileocaecal valve was obstructed by a membrane in all of these cases. Complete agenesis of the ileocaecal valve has been reported in an adolescent child who presented with an ileocolic fistula.

The author (first) reports a case of congenital ileocaecal valve agenesis resulting in bowel obstruction in a newborn child, in the third part of the thesis. This section focuses on the importance of differential diagnosis of the membranous atresia and the ileocaecal valve agenesis, and the various treatment options in a newborn.

Acute necrotising enterocolitis or its complications involving the ileocaecal valve, ileocaecal intussusception, caecal volvulus, ileocaecal atresia, and complicated Crohn's disease may necessitate resection of the ileocaecal valve. The loss of the ileocaecal valve results in no more reflux control and the colonic bacteria can colonise the ileum inducing inflammation, increasing the risk of the recurrence of Crohn's disease. This lead to the idea of replacing the ileocaecal valve.

The intussuscepted small bowel nipple valve is the most common technique for ileocaecal valve replacement. These valves work in a passive way, but their effectiveness has been proven in several experiments. With the loss of the ileocaecal valve, there is no control of anterograde stool flow. The accelerated ileal emptying results in a failure of absorption of fatty acids, vitamins and mineraloids. Today with the advances in parenteral nutrition, a

11-cm-long small intestine is sufficient for the long term survival after massive small bowel resection with intact ileocaecal valve. If ileocaecal valve is resected, double the length i.e. approximately 25-cm-long small bowel is required. The replacement of the ileocaecal valve might aim the reduction of the risks associated with parenteral nutrition by shortening its duration of administration.

The studies with intussuscepted nipple valve focussed mainly on their antireflux efficacy, although the feature of the antegrade passage control is also very important in the paediatric surgical practice. There are no data available in the literature regarding the relation of the antegrade and the retrograde hydrostatic parameters of the intussuscepted valve and the hydrostatic parameters of the ileocaecal valve. No study discusses the relation of the hydrostatic parameters and the length of the intussuscepted valve, however these data are very important for the optimal surgical planning of the ileocaecal valve replacement.

The fourth section of the thesis focuses on the coordination of the hydrostatic parameters of the intussuscepted valve and the optimal ileocaecal valve replacement.

The application of the antireflux capacity of the ileocaecal valve is not a new idea in the surgery of other organs. ileocaecal valve has been used for replacement of pharynx and stomach. In the paediatric surgery it has been used for primary biliary reconstruction in extrahepatic biliary atresia.

The Kasai portoenterostomy is still the first choice of the treatment in the extrahepatic biliary atresia. The most common (80%) postoperative complication is the cholangitis. However early studies have been reported the benefits of the application of intussuscepted valves in the Kasai portoenterostomy. Recent clinical trials did not support the effectiveness of these valves in the prevention of postoperative cholangitis. Other experimental studies suggested that the cholestatic liver is especially sensitive for ascending

infections. Therefore a microbiologically highly effective portoenterostomy suppose to reduce the incidence of the postoperative cholangitis. Is it possible that the hydrostatically effective antireflux valves are not effective enough microbiologically, and therefore they were not able to reduce the incidence of postoperative cholangitis? In the only study applying the ileocaecal valve in the portoenterostomy, the incidence of postoperative cholangitis has been reduced by 10 % compared to the original Kasai's method. Is it possible that the ileocaecal valve has better microbiological efficacy despite of its low hydrostatic antireflux activity?

There are no data available in the literature regarding the relation of the hydrostatic and the microbiological efficacy of the antireflux valves. In the fifth chapter of the thesis the microbiological effectiveness of the ileocaecal valve in a portoenetrostomy has been compared to the efficacy of the elongated Roux-Y portoenetrostomy and to the Roux-Y portoenetrostomy fitted with intussuscepted antireflux valve.

AIMS

1. Mapping the microscopic anatomy of the ileocaecal valve by examination of the muscle layers and the enteric nervous system in porcine ileocaecal valves at different age groups.
2. Adaptation of the „Whole-Mount” preparation technique in the study of ileocaecal valve for analysing the spatial patterns of the myenteric plexus of the valve.
3. Analysis of the postnatal changes of the myenteric plexus in ileocaecal valves in pigs of various ages: in newborn, 4-weeks-old, 12-weeks-old and adult pigs.
4. Discussion of the surgical treatment of atresia and agenesis of the ileocaecal valve, based on a case report.
5. Study of ileocaecal valve replacement in mongrel dogs, creation of intussuscepted nipple valves and assessment of its hydrostatic functions for the optimal valve-length selection.
6. Study of the ileocaecal valve used in the surgical treatment of a case of biliary atresia.
7. Comparison of the microbiological efficacy of the ileocaecal bilio-digestive conduit with other bilio-digestive conduits (Roux-Y with elongated limb, Roux-Y fitted with intussuscepted nipple valve).

MATERIAL AND METHODS:

Microscopic anatomy of ileocaecal valve

Experimental animals, histological samples

Specimens were taken from the ileocaecal junctions of pig embryos at 60 day gestation (n=3), and at 12 weeks gestation (n=3). The animals were provided by the Institute of Experimental Clinical Research, Skejby Sygehus, University of Aarhus Denmark. The study was approved by the Danish authority for animal protection with permission number 200601-068. The entire gastrointestinal tract was removed and subsequently fixed using perfusion fixation with 4% paraformaldehyde (PFA). Ileocaecal valves, 3-5-cm-long segments of terminal ileum and proximal colon were removed and stored initially in PBS at 4 °C.

Examination of the muscle layers (Haematoxylin-Eosin staining)

Longitudinal and transverse, 8-10 µm thick slices were made from the paraffin embedded and from the frozen specimens. The paraffin embedded sections were kept at 55 °C for 90 minutes, then deparaffinized in xylene for 2x5 minutes, rehydrated in 100%, 80% and 70% ethanol solutions for 1 minute each, stained in 20% Haematoxylin solution for 10 minutes. After washing in running water for 5 minutes the sections were stained with 1% Eosin solution for 1 minute, dehydrated in 70%, 80% and 100% ethanol solutions for 1 minute each, in xylene for 2x5 minutes and were mounted on slides with „Glycerogel mounting medium”.

Acetylcholinesterase histochemistry:

The staining has been processed by the method of Karnovsky and Roots as modified by Kiernan. The specimens were placed in 10 ml of incubating

solution (65 mM sodium acetate buffer, pH 6.0, 1.7 mM acetylthiocholine iodide, 5 mM sodium citrate, 3 mM cupric sulfate, and 0.5 mM potassium ferricyanide) for 100 minutes at 37 °C. The slides were then rinsed twice in 0.1 M Tris buffer (pH 7.6) and mounted using Glycergel mounting medium.(Dakocytomation).

NADPH-d histochemistry:

For NADPH-diaphorase (NADPH-d) histochemistry, the slides were incubated in 1 mg/ml β -NADPH (Sigma), 0,25 mg/ml nitro blue tetrazolium, and 0.3% Triton-X in 0.05 mol/l Tris-HCL buffer (pH7.6) at 37 °C for 2h, and then and mounted using Glycergel mounting medium (Dakocytomation).

PGP 9.5 fluorescent immunohistochemistry:

The slides were placed in citrate buffer (pH: 6) and microwaved at 650 W for 7 minutes for antigen heat unmasking. Than they were cooled down on ice for 45 minutes and washed in phosphate buffer 2x5 minutes. The samples were blocked with 10 % goat serum (Dakocytomation) for 30 minutes at room temperature. After shaking off the blocking serum, the samples were incubated with rabbit anti-human PGP 9.5 antibody (Dakocytomation), diluted in antibody dilutant (1:500), for overnight at 4 °C. The slides were washed in phosphate buffer 2x5 minutes, than they were incubated with „Texas Red” fluorescent conjugated goat anti-rabbit antibody (Eugene, Oregon USA) diluted in 10% goat serum (1:100), at room temperature for 30 minutes. The slides were mounted with Fluorescein mounting medium by Dakocytomation.

C-kit fluorescent immunhystochemistry:

For C-kit immunhistochemistry similar protocol was followed, only the first antibody was different: rabbit anti-human C-kit (Dakocytomation), diluted in antibody dilutant (1: 50).

Analysis of the postnatal changes of the ileocaecal myenteric plexus

Specimens were taken from distal ileum, ileocaecal valve and proximal colon from newborn piglets (Nb) (n=3), 4 weeks old (4w) (n=3), 12 weeks old (12w) (n=3) and adult pigs (n=3).

Whole-Mount technique

The Whole-mount preparation on the ileum and the caecum was not difficult. It has been carried out using fine pair of forceps and Leica dissecting microscope. Initially the bowel segment was opened along the antimesenteric border. Then the connective tissue overlying the serosa was carefully removed. Following this the specimen was turned over and the mucosa together with submucosa was peeled off the muscular layers. The circular muscle fibres were noted to be quite adherent to the region consisting the myenteric plexus. In order to avoid damaging the myenteric plexus during dissection, the samples were incubated with the staining solution after removing few of the circular muscle fibres. Once the myenteric plexus was partially visualised following initial NADPH-d staining, further dissection was carried out to remove all circular muscle fibres and the specimen was re-stained with NADPH-d solution. The Whole-Mount technique was found to be quite difficult on the ileocaecal valve. At first the ileocaecal valve has to be separated to the ileal and caecal components. It was done relatively easily with the dissecting microscope. After careful removal of the mucosa, the submucosa was peeled off with no fuss. The removal of the circular muscle fibres was much more difficult because of a thin adherent film.

Microscopy and morphometry

Using light microscopy number of ganglia per cm^2 , number of cells per ganglion and number of ganglion cells cm^2 were determined. The counting was

done using eyepiece graticule. Ganglia numbers were counted within an area of 0.5 cm² in the newborns, as the bowel specimen was not large enough to prepare 1 cm² segments. This count was then multiplied by factor of 4 to obtain the numbers per cm². The size of the specimens was not a problem in the larger animals, and counting was performed in a minimum area of 1 cm². Cell numbers per ganglion were counted in a minimum of 25 adjacent ganglia per specimen, and the average was taken. Ganglion cells per cm² were calculated by multiplying the mean number of cells per ganglion by the ganglia numbers per cm². Results were entered into Microsoft Excel, and statistical analysis was performed using one-way Anova test.

Surgery of the ileocaecal valve agenesis

Case report

A male neonate weighing 4200g was born at 41 weeks' gestation by Cesarean section for breech presentation to a nulliparous, 25-year-old-woman with no remarkable family history. The prenatal ultrasound scan showed dilated intestinal loops, but no evidence of polyhydramnios. Abdominal distension with no passage of meconium was reported at 24 hours. An erect plain film of the abdomen showed dilated loops of bowel with fluid levels on the right side and an absent bowel gas pattern in the lower abdomen. A contrast enema showed microcolon up to the mid-transverse colon, with no passage of contrast beyond this point. Based on these findings we made a provisional diagnosis of terminal ileal atresia, and performed emergency laparotomy. Laparotomy revealed that the distended terminal ileum was connected to the transverse microcolon with a 6-cm segment of bowel atrophic bowel. No tenia coli or appendix was identified in this atrophic segment. We also noted a 'V'- shaped defect in the mesentery supplying the ileocecal region. We resected the grossly distended last 8-cm of distal ileum together with the 6-cm segment of atrophic intestine. After

irrigating the lumen of the transverse colon with normal saline to exclude distal obstructions, we performed an end-to-oblique Dennis-Brown anastomosis. Closer examination of the mesentery and small bowel showed evidence of normal rotation. The baby have uneventful postoperative course. He passed meconium on postoperative day 2 and commenced oral feeding on postoperative day 5. When last seen at his 8-month follow-up, he was developing well with height and weight within the 30th percentile.

Histopathological examination of the resected segments revealed complete absence of the ileocecal valve and a 1.5 mm deep blind sac on the tip of the atrophic bowel specimen. Microscopy showed evidence of an atrophic large bowel mucosa and the blind sac showed evidence of atrophic appendix and lack of the tenia coli.

Optimal replacement of the ileocaecal valve

Experimental model

The experiment was approved by the Committee of Animal Research at the University of Debrecen (Permission no: 16/2000.DE MÁB). Mongrel dogs (n=5, age: 2-3-years, weight: 15-20 kg) were subjected to study. The animals were kept under conventional circumstances, in standard cages, were fed with commercially available mixed food, and had free access to water. Anesthesia was performed with a combination of ketamine (10 mg/kg, i.m.) and xilazine (1 mg/kg, i.m.) in multiple doses during the experiment (mean duration 112 minutes in each animal). Heart rate, blood pressure, and body temperature were continuously monitored as well.

Isolation of the intestinal segments

After median laparotomy, a 150-cm length of intestine (jejunum and ileum) was dismembered into five 30-cm-long segments (conduits A, B, C, D

and E). A 15-cm-long segment of the terminal ileum (with the ileocolic valve) and a 15-cm-long colon segment were also isolated (conduit F). Conduit A, without a valve, with propulsive motility intact, was used as control. Conduits B, C, D and E were equipped with stepwise-elongated intussuscepted nipple valves 4, 5, 6 and 7-cm-long, in the middle of the 30-cm-long segment. The caliber and the thickness of the wall of the intestine were the same in all groups of conduits. The mesenteric blood supply and, by means of careful surgery, the peristaltic activity were preserved. 37-°C saline was used to prevent the intestine from becoming cold.

Creation of the intussuscepted valves

Intussuscepted nipple valves were constructed by inserting seromuscular stitches (using Ethibond 4/0 non- absorbable material) around the intestine at equal distances. The intestine was telescoped into itself in the peristaltic direction and the threads were knotted. To determine the minimum and maximum length of the intussuscepted nipple valves used in the experiment we considered the following:

1. When a bowel segment X cm long is telescoped, the bowel wall is folded (doubled) into the lumen over a length of X/2 cm. To build a complete valve, the folded (doubled) bowel should acquire the size of the radius of the intestine tube; otherwise, the center of the valve will remain open. We found the mean diameter of the intestine tube in our canine model to be approximately 4 cm. To build a complete valve an intestine segment at least 4 cm long was needed to telescope.

2. Intussuscepted nipple valves with stepwise elongated length (4, 5, 6, 7, 8, 9, 10 cm,) were created in a preliminary study to observe the changes of the circulation of the intussuscepted bowel wall and to estimate the possible maximum length of intussusception. After 3 hours the stitches were removed

and the intussuscepted segment was examined. Thickening of the bowel wall and marked dark livid discoloration were observed on the invaginated bowel wall longer than 7cm. Therefore no intussuscepted nipple valves were used in the conduits longer than 7 cm.

Record of the hydrostatic parameters

Foley balloon catheters (12 Ch) were fixed at both ends of the conduits. Customary equipment for infusion was connected to the ends of the catheters. The hydrostatic pressures at which the conduits were filled were measured continuously by using the principle of communicating vessels and are given as the differences in fluid levels. A centimeter scale was fixed on the tripod of the infusion equipment to measure the fluid levels. The zero point was at the level of the conduit. The hydrostatic pressures in the conduits were raised by slow elevation (5 cm/min) of the infusion equipment (elevation of the height of the filling fluid column). Continuous X-ray screening of the conduits was performed using image intensifier during the experiment. The moment at which the contrast opened (anterograde) or broke through (retrograde) the valves was observed on the monitor. During the measurement, the conduit was dilated. Gastrographin in 0.9% saline (dilution 1:2) was used as contrast material throughout the experiment. First the anterograde filling was performed in one conduit, and then the contrast fluid was washed out from the conduit by 0.9% saline using the Foley catheters fixed in both end of the conduit. The retrograde filling was subsequently carried out in the same conduit. At the end of the operation, the animals were euthanised with an overdose of the anesthetics.

Statistical analysis

For statistical analyses, SigmaStat 1.0 software (Jandel Scientific Co., Erkrath, Germany) and nonparametric tests were used. One-way RM ANOVA with the Student-Newman-Keuls test and the Mann-Whitney rank sum test were

applied to compare the anterograde and retrograde resistance pressures in the different cases. Data are given as means \pm standard deviations (\pm S.D.). The level of statistical significance was set at $p < 0.05$.

The application of the ileocaecal valve in the reconstruction of the extrahepatic biliary atresia

Experimental model

Only three animals were used in this preliminary experiment (I.-III.) under the previously detailed circumstances.

Creation of the Roux-Y bilioenteric anastomosis

After median laparotomy the common bile duct was ligated and dissected. The first jejunal loop was dissected and pulled up through the mesentery of the transverse colon and a double layer “end-to-end” cholecysto-enterostomy was created with interrupted 4/0 Ethibond nonabsorbable sutures. The continuity of the jejunum was reconstructed with a double layer end-to-side jejuno-jejunosomy. In animal I, the length of the jejunal loop was 60-cm. In animal II, the jejunal loop was only 40-cm long, but it was fitted with a 4-cm-long intussuscepted valve.

Creation of the bilioenteric anastomosis with the ileocaecal junction

In animal III, the bilio-digestive anastomosis was made with ileocaecal junction incorporating the ileocaecal valve. After mobilising the caecum the ileocaecal segment was pulled up through the mesentery of the transverse colon and was anastomosed with the ileal end to the gallbladder. The caecal end was anastomosed end-to-side to the jejunum. The continuity of the ileum was established by end-to-end double layer ileo-colostomy.

Application of the test germs

Seven days after surgery the animals were fed orally with 10^5 human origin *E. faecalis* (ATCC 80171 tribe, Mc Farland > 7.5) germs, which does not occur in the gastrointestinal tract of the dogs. It had been confirmed preoperatively by microbiology test.

Microbiological samples

Twenty four hours after the application of test germs, the animals were anesthetized and laparotomy was performed. Samples were taken for microbiological tests from the gallbladder, oral and aboral end of the efferent conduits and proximal and distal part of the jejunum. The tests were carried out using selective culture media and the results were compared after 72-hrs.

RESULTS AND CONCLUSIONS

Microscopic anatomy of the ileocaecal junction

Haematoxylin-eosin staining:

The transverse and longitudinal sections of the intracaecal part of the ileum, showed 3 distinct muscle layers. The first one, the external circular layer is in continuity with the caecal circular muscle and covered with caecal submucosa and mucosa. Under this external circular muscle, there is a layer of the longitudinal smooth muscle. This homogenous layer arises from the fusion of the ileal and colonic longitudinal muscles and isolates the external circular muscle from the internal circular muscle along the ileocaecal valve except at the very end of the valve.

Acetylcholinesterase (AChE) staining

The AChE staining revealed the AChE positive neurons and fibres on the transverse and the longitudinal sections. The staining showed the specific architecture of the enteric nervous system in the ileocaecal valve: the ileal and coaxial caecal superficial and deep submucosal plexuses and myenteric plexuses.

NADPH-d staining

NADPH-d positive neurons have been found in a great number in the ileocaecal valve. The technique showed the same spatial pattern of the enteric nervous system in the ileocaecal valve than the AChE staining.

PGP 9.5 immunohistochemistry

The PGP 9.5 panneuronal cytoplasmatic marker immunohistochemistry showed the whole size of the ganglions and demonstrated more accurately the

connections between the ganglia and the coaxial structure of the enteric nervous system. On the longitudinal sections the continuity of the plexuses has been found more obvious. There was no gap in the enteric nervous system in the images taken from the free end of the ileocaecal valve in all agegroups.

C-kit immunohistochemistry

The C-kit immunostaining demonstrated the presence of myenteric interstitial cells of Cajal's in the ileal and the caecal plexuses and didn't reveal any break in continuity between the ileal and caecal Cajal cell network.

Spatial pattern of the myenteric plexus

After Whole-Mount preparation and NADPH-d staining we were able to study the meshwork of the myenteric plexus.

The density of the meshwork is different in the terminal ileum, ileal part of the ileocaecal valve and in the caecum. There is no obvious difference between the caecal part of ileocaecal valve and the caecum. Using the dissecting microscope with low magnification we can observe quite a large area compared to that of the light microscope. These pictures show more obviously the similarity between the caecal origin ileocaecal valve plexus and the caecal plexus and the difference between the ileal origin ileocaecal valve plexus and the ileal plexus.

Analysis of the postnatal changes of the ileocaecal myenteric plexus

Changes in the number of the ganglia and neurons:

The number of the ganglia is higher in the plexus of the ileocaecal valve in all agegroups. The largest difference in the number of the ganglia has been found in the newborn group within the ileocaecal junction. But this difference has been reduced with age.

However the number of neurons per ganglion is increased with age, but there was no significant difference within the ileocaecal junction in all agegroups.

The total number of neurons per cm^2 was calculated by multiplying the number of ganglia per cm^2 by the number of neurons per ganglia. According to these data, it is not surprising that the total number of neurons per cm^2 changes similarly to the number of ganglia per cm^2 .

The lipopolosaccharyde model of intussusception pointed out that the intussusception with no leading point develops as a result of a motility disturbance of the intestine and it is related to the high level of NO released from the myenteric neurons.

The vast majority of the intussusceptions happen at the ileocaecal junction. It means that the pathological relaxation of the ileocaecal valve plays major role in the pathogenesis of intussusception.

In my study the highest density of the nitrergic neurons was found in the ileocaecal valve. In my opinion maximal concentration of NO is released in the ileocaecal valve and hence motility disturbance leading to intussusception starts at the ileocaecal valve.

This hypothesis may explain why the primary intussusception develops in the ileocaecal junction.

It is noted in my study that there is a significant decrease in the total number of the nitrergic neurons in the ileum, ileocaecal valve and caecum with age. It means that the danger of excessive NO release is decreasing with age. This may explain why primary intussusception doesn't occur in older agegroups.

Surgical treatment of the ileocaecal valve atresia and ileocaecal agenesis

The case published in the third section of the thesis supports the fact, that there are two type of ileocaecal atresia: ileocaecal valve agenesis and membranous ileocaecal valve atresia. It is important to differentiate these two types as the surgical treatment is different. In a case of membranaceous atresia of the ileocaecal valve, the ileocaecal valve plasty, i.e. the resection of the membrane seems to be more physiological compared to the complete resection of the ileocaecal valve. The complete absence of the ileocaecal valve suggests the need for replacement. If the length of the viable bowel is sufficient, the replacement of the ileocaecal valve is not necessary. Loss of significant bowel length may increase the risk of short bowel syndrome requiring long term parenteral nutrition and therefore ileocaecal valve replacement may be beneficial.

The optimal valve replacement

A check on the hydrostatic properties of the intussuscepted valves revealed a surprisingly high antireflux efficacy, in contrast with the low anterograde resistance. On the other hand, there was a moderate difference between the anterograde and the retrograde hydrostatic resistance of the ileocolic valve. The anterograde resistance of the shortest (4-cm-long) intussuscepted valve did not attain that of the ileocolic valve. This finding could be explained with the different structure of the two valves. The intussuscepted nipple valve has longer wings but no possess sphincter fibres (“high pressure zone”), which should produce contraction in reaction to the increase of intraluminal pressure in the colon/cecum. It seems that its resistance originates rather from passive response. But the resistance of the ileocolic valve appears to come more from its active sphincter contraction, than from its shorter passive

valve wings. We found that an intussuscepted nipple valve length between 6-and 7-cm furnished the anterograde resistance of the ileocolic valve in our model.

We concluded the ileocolic sphincter cannot be replaced perfectly by intussuscepted nipple valve, but by lengthening of the intussuscepted nipple valve higher passive anterograde resistance could be achieved. The shortest possible intussuscepted nipple valve can be used if the main goal in the surgery is to achieve antireflux efficacy, e.g. in clinical conditions such as Crohn disease. In contrast, in short bowel syndrome, where the attainment of appropriately delayed physiological transport is important, the shortest intussuscepted nipple valve will be insufficient. As the anterograde resistance can be enhanced by lengthening the constructed intussuscepted nipple valve, a longer intussuscepted nipple valve should be used to restore the anterograde resistance of the ileocecal valve, but without giving rise to an unsuitable rate of transport. Further investigations are needed to verify the long-term anterograde competence of elongated intussuscepted nipple valves before their clinical application.

The application of the ileocaecal valve in the reconstruction of the extrahepatic biliary atresia

The animals were fed with fluids only on the first postoperative day and then with solid food from the second postoperative day. They were asymptomatic and showed no sign of icterus. The autopsy showed healed anastomoses and there was no evidence of any morphological changes or cholestasis in the conduit.

Results of the microbiological tests

Only the portoenterostomy created with the ileocaecal valve prevented the ascend of the test germs. Neither the elongated Roux-Y limb nor the intussuscepted nipple valve was able to prevent the ascending germ invasion.

Despite its lower hydrostatic antireflux efficacy the ileocaecal valve seemed to be better microbiological barrier compared to the intussuscepted valves.

This result suggests that the hydrostatic efficacy itself doesn't means adequate microbiological efficacy and it can be considered as an explanation, why the intussuscepted valves were ineffective in the prevention of the postoperative cholangitis in the clinical practice.

The microbiological effectiveness of the ileocaecal valve doesn't originate purely from its hydrostatic antireflux efficacy. Other factors such us the active reflexes, Peyer's patches may play an important role as well.

Finally it can be concluded that the ileocaecal valve as a bilio-enteric conduit may be more effective in the prevention of the postoperative cholangitis compared to other portoenterostomies. Therefore it seems to be worth to investigate the possibility of their application.

In the future we would like to increase the number animals included in the experiment and prolong the survival of the animals in the model.

We would like to study the effect of the own intestinal bacteria (instead of foreign test germs) on the expression of inflammatory mediators such us TGF β , monocyte chemoattractant protein 1 (MCP1), interleukin 1 és 6 (IL1, IL 6) in the liver after biliary reconstruction using reverse polymerase chain reaction.

Summary of the most important results and conclusions

1. The thesis first reports the structure of the enteric nervous system of the ileocaecal valve. It reveals that the enteric nervous system of the ileocaecal valve consists of two distinct, ileal and caecal origin coaxial myenteric, superficial and deep submucosus plexuses.

2. The thesis reports the first time application of the Whole-Mount preparation technique in the ileocaecal valve and shows the three dimensional meshwork of the myenteric plexus within the ileocaecal valve.

3. The histological studies revealed the followings:

(a) The two distinct ileal and caecal origin enteric nervous system is in continuity at the free end of the valve.

(b) There is no gap in the myenteric Cajal cell network responsible for the propagation of the “myoelectric” slow wave, as it has been reported between the pyloric and the duodenum.

4. The nitrergic hyperinnervation of the myenteric plexus of the ileocaecal valve may explain why the primary intussusception happens at the ileocaecal junction.

5. The decreasing hyperinnervation of the ileocaecal valve with age may explain the decreasing occurrence of the intussusception in older agegroups.

6. The congenital agenesis and atresia of the ileocaecal valve is rare, but may need surgical intervention in the newborn. Simple resection and anastomosis may be adequate if the length of the bowel is sufficient. Ileocaecal valve-plasty or replacement is suggested if short bowel syndrome is imminent.

7. The ileocaecal valve cannot be replaced with intussuscepted valve perfectly, but by lengthening the intussuscepted valves sufficient antero-grade resistance can be achieved.

9. The shortest possible intussuscepted valve can be used if the main goal in the surgery is to achieve antireflux efficacy, e.g. in clinical conditions such as Crohn's disease, but to achieve proper antero-grade resistance in short bowel syndrome longer intussuscepted valve is recommended.

10. The benefits of the highly effective hydrostatic valves have not been confirmed in our experimental model of Kasai portoenterostomy. However the ileocaecal valve with its lower hydrostatic efficacy has been proved to be better immunological barrier and may reduce the risks of the postoperative cholangitis after biliary reconstruction.

PUBLICATIONS WHICH THIS THESIS IS BASED ON

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