NEW HYPOTHESIS ON THE PATHOGENESIS OF ILEOCAECAL INTUSSUSCEPTION

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ABSTRACT

Purpose: Ileocaecal intussusception is a relatively common surgical emergency in infants and young children. The etiology of intussusception is not clearly understood. Nitric Oxide (NO) is a major inhibitory neurotransmitter in the enteric nervous system, which causes relaxation of the smooth muscles. In the Lipopolysaccharide induced experimental model of intussusception, altered intestinal motility is shown to be the result of increased NO released from various inflammatory mediators, which in turn leads to increased incidence of intussusception. The aim of this study was to examine the age related changes in the nitrergic innervation of the ileocaecal valve (ICV) in order to gain insights into the pathogenesis of intussusception.

Method: Whole-mount preparations of the myenteric plexus from ileum, ileocaecal valve and proximal colon were stained using NADPH diaphorase histochemistry in Newborn piglets (Nb) (n=3), 4 weeks old (4w) (n=3), 12 weeks old (12w) (n=3) and at adult pigs (n=3). Using light microscopy neuronal cells per ganglia, number of ganglia per cm^2 and number of ganglion cells per cm^2 were determined.

Results:

There were striking regional and age-related differences in nitrergic innervation of myenteric plexus. Density of nitrergic neurons was significantly higher in ICV compared to terminal ileum and proximal large bowel in the young animals ($p<0.001$).

Conclusion:

These findings suggest that the inflammatory reactions which usually precede intussusception may cause overproduction of NO by the nitrergically hyperinnervated ICV causing relaxation of the ICV and thereby facilitating ileocecal intussusception.

INDEX WORDS:

Intussusception**,** nitrergic innervation, ileocaecal valve

INTRODUCTION

Intussusception is a relatively common surgical emergency in infants and young children with an incidence of 1-2 /1000 births. The peak incidence is between 5-10 months of age and it has been reported in young animals as well [1,2]. The etiology of intussusception in most infants remains unclear. Over 90% of the cases of ileocolic intussusception are idiopathic, without obvious leading point [3]. The role of the ileocaecal valve (ICV) in the pathogenesis of intussusception is not clearly understood, but the invagination usually begins in the distal ileum and progress through the ICV [1].

Primary intussusception appears in children simultaneously or following an upper respiratory tract viral infection or enteritis. Patients with intussusception have been reported to shed adenovirus, rotavirus, enterovirus or herpes simplex in their stools [4]. There is also evidence that intussusception may be associated with bacterial infection [5]. Intraperitoneal injection of bacterial lipopolysaccharides (LPS) has shown to induce intussusception in mice [6]. In the LPS induced experimental model of intussusception, altered intestinal motility is shown to be the result of increased release of Nitric Oxide (NO), which in turn leads to increased incidence of intussusception [7]. Regional difference in the density of nitrergic neurons in the myenteric plexus has been reported along the length and the circumference of the intestine [8]. There is no information available about the distribution of nitrergic neurons in the ICV. The aim of this study was to examine the age related changes in the nitrergic innervation of the ICV in order to gain insights into the pathogenesis of intussusception.

MATERIAL AND METHOD

Tissue sampling and whole-mount preparation

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). The animals were provided by the Institute of Experimental Clinical Research, Skejby Sygehus, University of Aarhus Denmark. The study was approved by the Danish authorities of animal protection, 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 in PBS at 4 ºC until needed.

Whole-mount preparation was 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 quiet 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.

Staining procedure

For NADPH-diaphorase (NADPH-d) histochemistry, the whole mount preparations 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 ºC for 2h, and then left in staining solution at room temperature. Incubation was performed using free-floating technique on a 12-well cell culture dish. After the desired staining intensity was achieved, specimens were washed in PBS for 15 min and then mounted on Polysine slides (BDH) using Glycergel mounting medium (Dakocytomation).

Microscopy and morphometry

Ganglia numbers per cm^2, and neuronal cells per ganglia were counted using conventional light microscopy. The counting was done using eyepiece graticule. Ganglia numbers were counted within an area of 0.5 cm^2 in the newborns, as the bowel specimen was not large enough to prepare 1 cm^2 segments. This count was then multiplied by factor of 4 to obtain the numbers per cm^2. The size of the specimens was not a problem in the larger animals, and counting was performed in a minimum area of 1 cm^2. Cell numbers per ganglia were counted in a minimum of 25 adjacent ganglia per specimen, and the average was taken. Neuronal cells per cm^2 were calculated by multiplying the mean number of cells per ganglia by the ganglia numbers per cm^2. Results were entered into Microsoft Excel, and statistical analysis was performed using one-way Anova test.

RESULTS

The overall morphology of the myenteric plexus changed from a wide-open network within the terminal ileum to a more condensed network within the ICV in all age groups [Figure 1]. The ICV contained significantly more nitrergic ganglia (2925±196) and neurons (44290±1489) per cm^2 than the terminal ileum (1332±269 and 24379±3026) in the newborn piglets. Similar differences were noted in all age groups but the differences were more marked within the newborn and 4 weeks old groups [table, figures 2 and 4]. There was no statistically significant differences noted in the number of ganglion cells per ganglion between the different regions of the bowel examined (15±7 in ICV and 18±11 in terminal ileum in the newborn group) [Figure 3].

Within each part of the bowel examined, the total number of nitrergic neurons per cm^2 decreased with advancing age (44290±1489 in the ICV at newborn age to 6443±549 at adult age). The rate of fall was highest between 4 and 12 weeks of age in all parts (37973±3583 at 4 weeks to 13882±2065 at 12 weeks in the ICV) [Figure 4]. After 12 weeks of age, the rate of decrease in cell numbers per cm^2 became less marked, but continued into adulthood.

DISCUSSION

The etiology of intussusception in the majority of infants remains unclear. The greater disproportion between the size of ileum and the ileocaecal valve in infants compared to that in older children has been suggested as a contributing factor for intussusception [9]. The popular theory at present suggests that there is a primary lymphoid hyperplasia in the distal small intestine secondary to infective agents, and that some of these enlarged lymphoid aggregates or hypertrophied Peyer's patches become entrapped in the intestine, serving as lead points for the intussusception [10]. However, in the Lipopolysaccharide induced animal model of intussusception none of the animals showed prominent mucosal lymphoid follicles, hypertrophied Peyer's patches or other pathologic process at the apex of the intussusception [6]. Grant et al studied 60 children with idiopathic intussusception and also found no pathological leading point in these children [11].

The role of increased intestinal peristalsis in intussusception has been observed in various studies. Gastrin injection enhanced peristalsis in animal model, and serum gastrin level in patients with intussusception was found higher than in controls [12]. Oral administration of Acetylcholinesterase inhibitor pyridostigmin caused increased peristalsis and diarrhoea in dogs, and lead to an increased incidence of intussusception [13].

Motility enhancing effect of NO has been observed in several studies in the past. For instance, the laxative magnesium sulphate is known to cause its effects through NO [14]. On the other hand, decreased neuronal NO function can result in aperistalsis and obstructive sphincters [15]. Increased NO level was measured in LPS model of intussusception. Both, local (colonic tissue) and systemic (plasma) NO levels were found to be high in rats following intraperitoneal LPS administration.

Selective block of nitric oxide sythase (NOS) by N-nitro-L-arginine methyl ester (L-NAME) prevented intussusception in this model [16,17], confirming that intussusception was secondary to the raised NO levels.

There are three isoforms of NOS: endothelial NOS (eNOS) and neuronal NOS (nNOS) normal constituents of cells and are Ca2+ dependent for activity; the third isoform inducible NOS (iNOS) is Ca2+ independent and is present in various cells including vascular endothelial cells and macrophages[18]. Inflammatory mediators (i.e. products of arachidonic acid cascade) excite the neurons of the myenteric plexus, the major local source of NO in the intestine. This excitement has been shown to increase NO production, by stimulating iNOS, locally and systemically [19,20].

Previous studies have shown that the number of myenteric nitrergic neurons in a given area of bowel increases in the caudal to rostral direction in the small intestine [21]. The markedly increased amount of myenteric neurons in the terminal ileum and the ICV identified in our study may explain why the intussusception develops mainly at the terminal ileum and progresses to involve the ileocaecal valve.

We hypothesise that the large number of myenteric neurons within the terminal ileum cause release of large quantity of NO in response to bacterial or viral infection, which in turn leads to initiation of intussusception within the terminal ileum due to impaired peristalsis. Progression of this intussusceptum may be facilitated by the significant relaxation of the ICV, caused by the release of large amount of NO by myenteric neurons.

Enteric neurons change their chemical coding and their number and density during development. Most quantitative studies examining the age-associated changes in the number of neurons in the myenteric plexus revealed significant decrease in neuronal density with advancing age [22-25]. This observation is consistent across

species and appears to be region specific in the GI tract**.** Gabella (1987) studied developmental changes with whole mount preparations of small intestine of various animal models consisting of longitudinal muscle and myenteric plexus stained with nitro blue tetrazolium (NBT) for β-NADH reductase and determined that the population of neurons decreased by approximately one-half between the ages of 3 and 30 months [22]**.** In guinea pigs the spatial density as well as the total number of myenteric neurons was decreased in the small intestine, a process that does not seem to depend solely on increased intestinal length and diameter throughout the adult life span [23]. Recently cuprolinic blue has been used to stain the myenteric plexus in rats at five time points between 3 and 27 months. This comprehensive study showed decreases of 20-30% in the duodenum, 15-20% in the jejunoileum, and 30-40% in the colon and rectum [24]. This study further illustrated that the neurodegeneration continues in an approximately linear fashion throughout the remainder of the animal's life. Wester et al has shown that this neurodegeneration is seen in the myenteric plexus during the first years of life in humans as well [25].

We have also found an age-related loss of nitrergic neurons within the myenteric plexus in both the terminal ileum and the ICV in this study. It is well documented that the incidence of intussusception is at its peak within the age group of 5-10 months in children, and within the very young animals [1]. This could be predicted by our above hypothesis, as the maximum numbers of nitrergic neurons are seen within the terminal ileum and ileocaecal valve between the newborn and 4 week old animals.

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FIGURE LEGENDS

Table: The * shows the significant p value between the values of ileum and ICV

Figure 1: NADPH-d staining of the myenteric plexus of ICV and terminal ileum in four different age groups (40 x magnification)

Figure 2: Number of ganglia/cm^2

Figure 3: Number of cells /Ganglia

Figure 4: Number of neurons/cm^2

Table

	Age	Terminal Ileum	ICV	Proximal large bowel
Ganglia/cm^2	Nb	1332 ± 269	2925 ± 196 *	1712 ± 51
	4w	813 ± 257	1491 ± 294 *	896 ± 51
	12w	337 ± 149	654 ± 159 *	143 ± 20
	Adult	54 ± 28	103 ± 24 *	75 ± 8
Cells /Ganglia	Nb	18,3 ± 11,25	15,14 ± 7,6 *	21 ± 7
	4w	23,46 ± 11,78	25,46 ± 12,15 *	36 ± 10
	12w	24,74 ± 11,41	21,22 ± 12,99 *	34 ± 15
	Adult	37 ± 14,9	62,32 ± 22,88 *	34 ± 15
Cells/cm^2	Nb	24379 ± 3026	44290 ±1489 *	35952 ± 357
	4w	19084 ± 3027	37973 ± 3583 *	32256 ± 510
	12w	8342 ± 1701	13882 ± 2065*	4862 ± 300
	Adult	1998 ± 418	6443 ± 549 *	2250 ± 120

*P<0.001

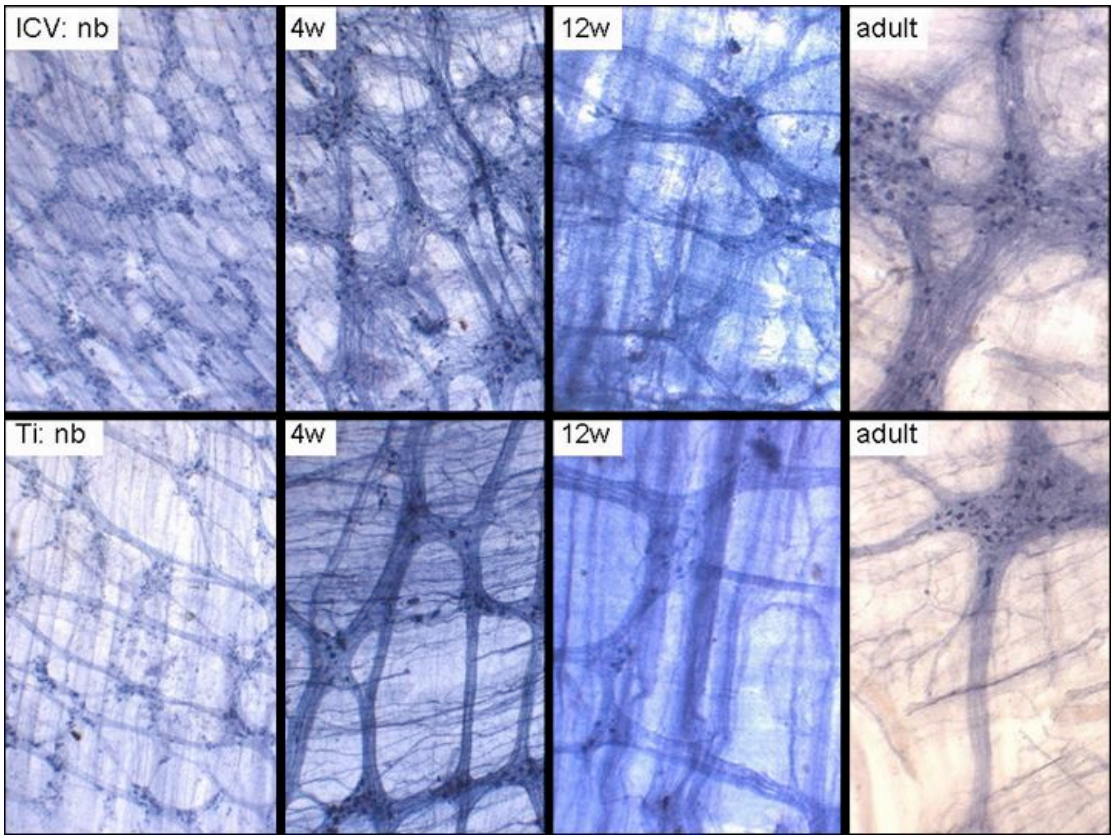

Figure 1: NADPH-d staining of the myenteric plexus of ICV and terminal ileum in four different age groups (40 x magnification)

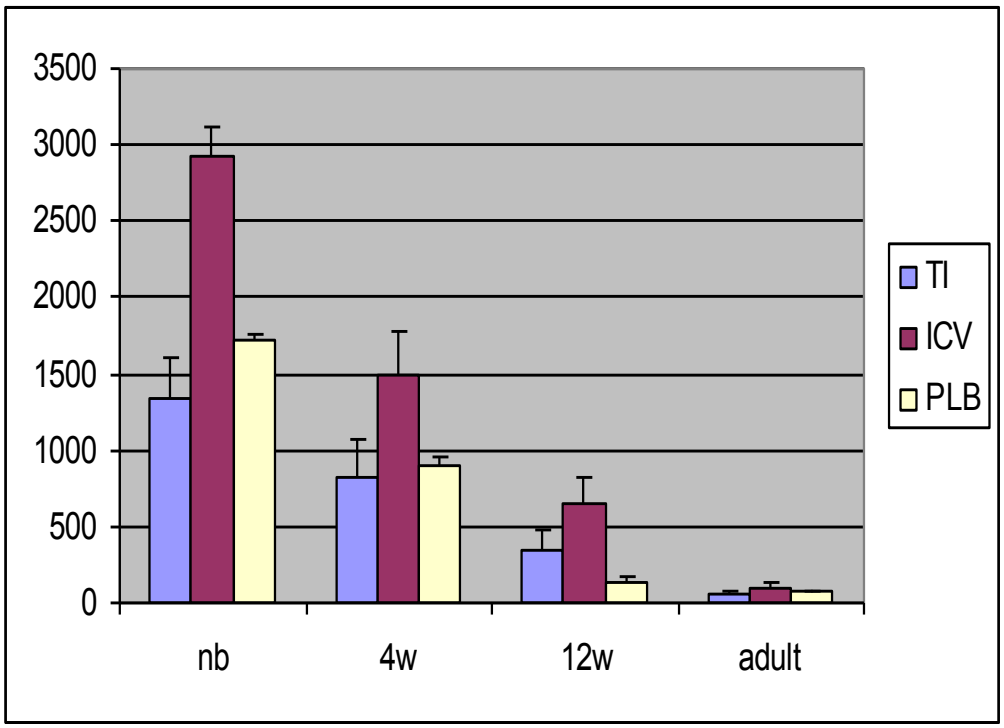

Figure 2: Number of ganglia/cm^2

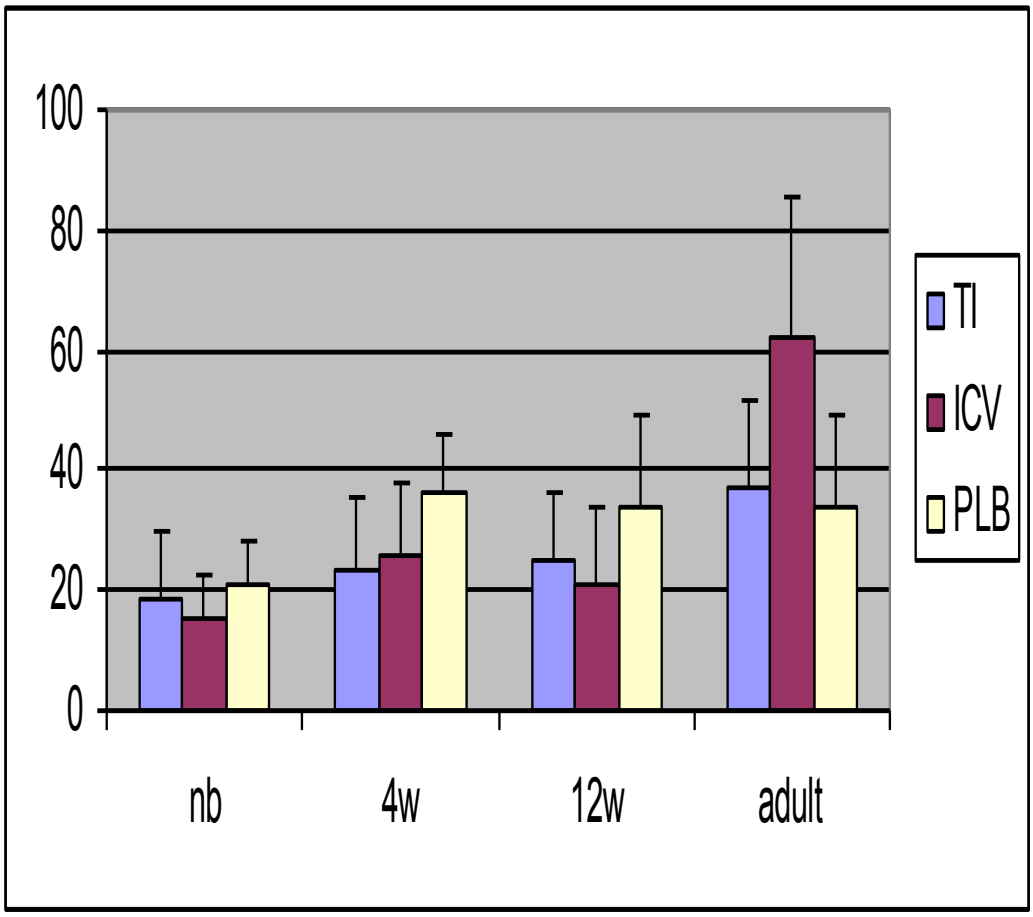

Figure 3: Number of cells /Ganglia

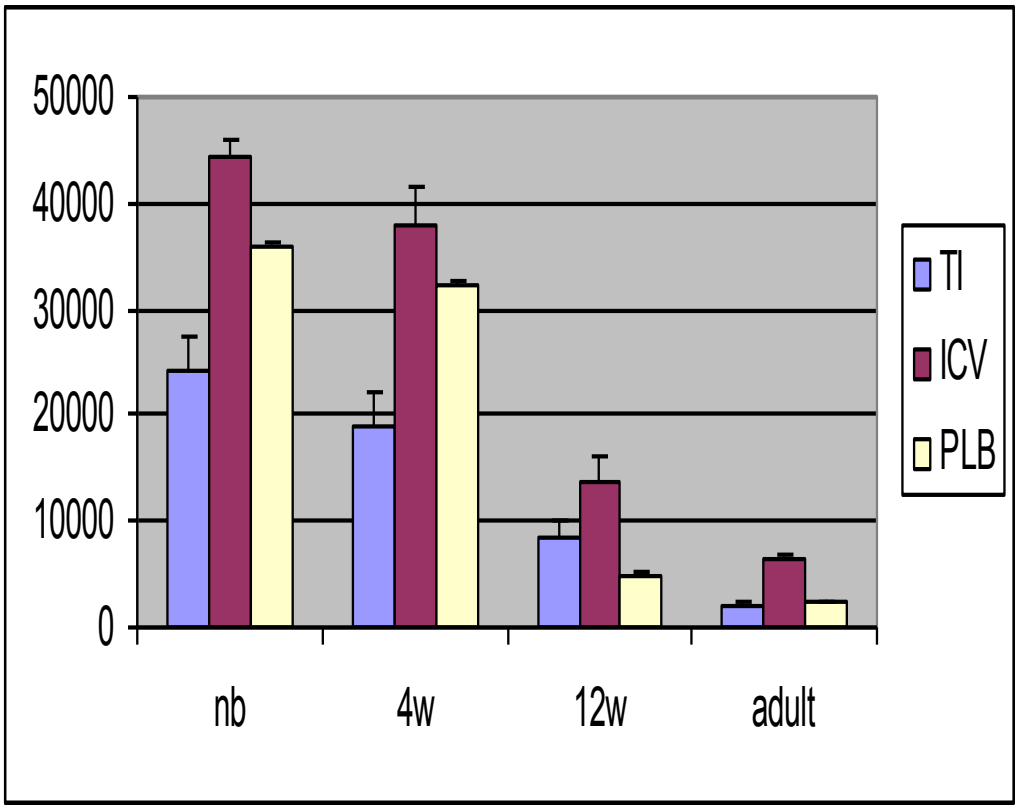

Figure 4: Number of neurons/cm^2