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New hypothesis on the pathogenesis of ileocecal intussusception

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Abstract

Purpose: Ileocecal 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 a 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 ileocecal valve (ICV) to gain insights into the pathogenesis of intussusception.

Method: Whole-mount preparations of the myenteric plexus from the ileum, ICV, and proximal colon were stained using NADPH diaphorase histochemistry in newborn piglets (n = 3), 4-week-old (n = 3), 12-week-old (n = 3), and adult pigs (n = 3). Using light microscopy, the number of ganglia per square centimeter, the number of cells per ganglion, and the number of ganglion cells per square centimeter 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 the ICV than in the terminal ileum and proximal large bowel in the young animals ($P < .001$).

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

Intussusception is a relatively common surgical emergency in infants and young children with an incidence of 1 to 2 per 1000 births. The peak incidence is between 5 to 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. More than 90% of the cases of ileocolic intussusception are idiopathic, without obvious leading point [3]. The role of the ileocecal valve (ICV) in the pathogenesis of intussusception is not clearly understood, but the invagination usually begins in the distal ileum and progresses through the ICV [1].

Primary intussusception appears in children simultaneously with or after an upper respiratory tract viral infection or enteritis. Patients with intussusception have been reported to shed adenovirus, rotavirus, enterovirus, or herpes simplex

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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 an 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 to gain insights into the pathogenesis of intussusception.

1. Materials and methods

1.1. Tissue sampling and whole-mount preparation

Specimens were taken from the distal ileum, ICV, and proximal colon from newborn piglets (n = 3), 4-week-old (n = 3), 12-week-old (n = 3), and adult pigs (n = 3). The animals were provided by the Institute of Experimental Clinical Research, Skejby Sygehus, University of Aarhus in 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. Ileocecal valves, 3 to 5 cm long segments of terminal ileum, and proximal colon were removed and stored in phosphate buffered saline (PBS) at 4°C until needed.

Whole-mount preparation was carried out using fine pair of forceps and Leica dissecting microscope (Lerca, Wetzlar, Germany). Initially, the bowel segment was opened along the antimesenteric border. Then the connective tissue overlying the serosa was carefully removed. After this the specimen was turned over, and the mucosa together with the submucosa was peeled off the muscular layers. The circular muscle fibers were noted to be quite adherent to the region consisting the myenteric plexus. To avoid damaging the myenteric plexus during dissection, the samples were incubated with the staining solution after removing few of the circular muscle fibers. Once the myenteric plexus was partially visualized after initial NADPH diaphorase (NADPH-d) staining, further dissection was carried out to remove all circular muscle fibers; and the specimen was restained with NADPH-d solution.

1.2. Staining procedure

For NADPH-d histochemistry, the whole-mount preparations were incubated in 1 mg/mL β-NADPH (Sigma, Steinhem, Germany), 0.25 mg/mL nitroblue tetrazolium, and 0.3% Triton-X in 0.05 mol/L Tris-HCL buffer (pH 7.6) at 37°C for 2 hours and then left in staining solution at room temperature. Incubation was performed using free-floating

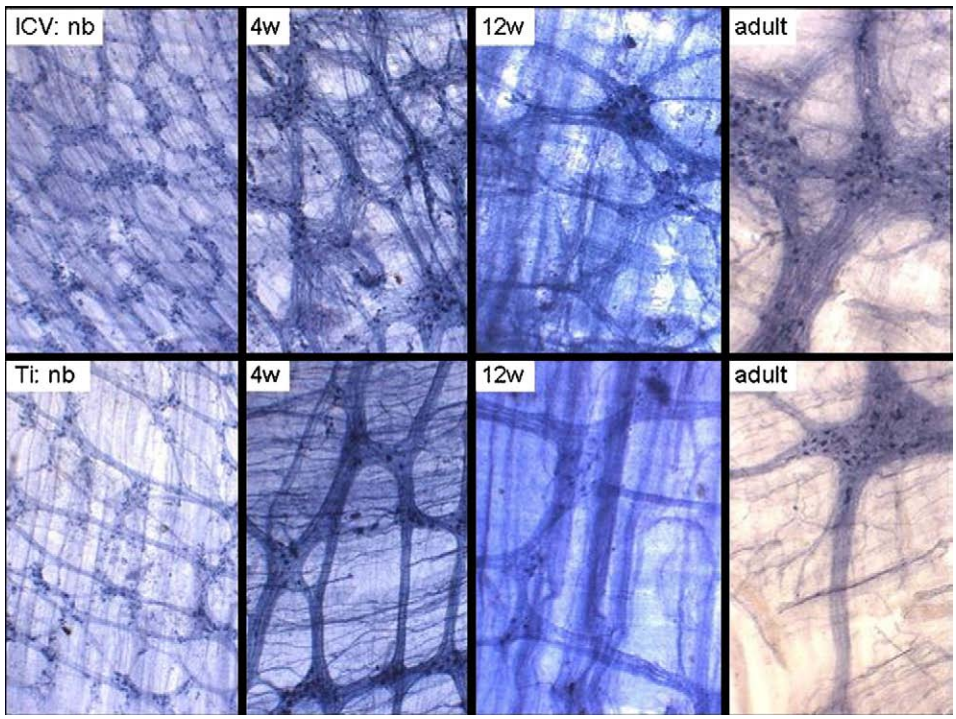

Fig. 1 NADPH diaphorase staining of the myenteric plexus of the ICV and terminal ileum (Ti) in 4 different age groups (original magnification ×40). nb indicates newborn; 4w, 4 weeks old; 12w, 12 weeks old.