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Regenerative capacity of the enteric nervous system: is immaturity defining the point of no return?

Christina Oetzmann von Sochaczewski, MD,[a,*,1] *Katharina Wenke, MD,*[a] *Andrew Grieve, MD,*[b] *Chris Westgarth-Taylor, MD,*[b] *Jerome A. Loveland, MD,*[b] *Roman Metzger, MD,*[c] *and Dietrich Kluth, MD, PhD*[d]

[a] *Department of Pediatric Surgery, University Hospital of Hamburg, Hamburg, Germany*
[b] *Department of Pediatric Surgery, Chris Hani Baragwanath Academic Hospital, Johannesburg, South Africa*
[c] *Department of Pediatric and Adolescent Surgery, Paracelsus Medical University, Salzburg, Austria*
[d] *Department of Pediatric Surgery, University of Leipzig, Leipzig, Germany*

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ABSTRACT

Background: Intestinal obstruction in newborns is associated with intestinal motility disorders after surgery. Alterations in the enteric nervous system (ENS) might cause abnormal peristalsis, which may then result in intestinal motility disorders. We aimed to quantify alterations in the myenteric plexus after a ligation and to test if these alterations were reversible.

Methods: Small intestines of chicken embryos were ligated in ovo at embryonic day (ED) 11 for either 4 d (ED 11-15) or 8 d (ED 11-19). Both treated groups and control group were sacrificed and intestinal segments examined by means of both light and electron microscopy.

Results: The number of proximal myenteric ganglia increased (ED 19, 30.7 ± 3.16 *versus* 23.1 ± 2.03; $P < 0.001$) in the 8-d ligature group but had values similar to the control group in the 4-d ligature group. The size distribution was skewed toward small ganglia in the 8-d ligature group (ED 19, 83.71 ± 11.60% *versus* 3.88 ± 4.74% in the control group; $P < 0.001$) but comparable with the control group in the 4-d ligature group. Subcellular alterations in the 4-d ligature group were reversible.

Conclusions: The pathologic alterations in the ENS were fully reversible in the 4-d ligature group. This reversibility might be linked to the degree of immaturity of the ENS.

* *Corresponding author*. Department of Pediatrics, University Medicine Mainz, Langenbeckstraße 1, D-51331 Mainz, Germany. Tel.: +49 173 802 8001; fax: +49 613 117 6608.
E-mail address: c.oetzmann@gmail.com (C.O. von Sochaczewski).
[1] Present address: Department of Pediatrics, University Medicine Mainz, Langenbeckstraße 1, D-55131 Mainz, Germany.
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Introduction

Congenital small bowel obstruction is often caused by intestinal atresia and needs to be surgically corrected soon after birth. A fetal vascular event, secondary to mesenteric ischemia, volvulus, strangulation, or intussusception remains the main pathophysiological explanation.[1,2] The availability of adequate surgical treatment and sophisticated neonatal intensive care has substantially decreased mortality rates. Nevertheless, prolonged intestinal motility disorders (IMDs) are a common postoperative problem and impair the quality of life in one third of affected infants.[3]

The enteric nervous system (ENS) and the interstitial cells of Cajal (ICC) both regulate intestinal motility. Apart from a subpopulation of ICCs acting as pacemakers and generating slow-wave contractions, which are crucial for the propulsion of gastrointestinal contents, the ENS comprising neurons, glial cells, and another subpopulation of ICCs involved in neurotransmission integrate information from different sources to control the gastrointestinal motility patterns with a large degree of independency from the central nervous system.[4,5]

Alterations in the morphology and distribution of enteric neurons and ICCs because of intestinal atresia have been hypothesized to cause persistent IMDs after atresia repair.[6,7] These changes in the architecture of the ENS, although described extensively, have only fragmentarily been documented in a quantitative manner. Two studies reported a hypoplastic myenteric plexus (MP) in the intestinal segment proximal to the atresia, whereas alterations in the gut distal to the atretic segment were absent.[6,8] An avian model of experimental intestinal atresia further revealed a higher neuron density the earlier vascular events were caused.[9]

The chicken embryo is a valid model for the human development because of the high similarity in morphology, development, and gene homology.[10,11] Hence, several groundbreaking studies of ENS development were conducted using the chicken embryo.[10,11] Because of these advantages, the chicken embryo has been used as a model for prenatal intestinal obstruction before.[9,12]

A postmortem study, in which the emergence of intestinal atresia and meconium ileus during gestation was compared, revealed that meconium ileus is a disease of the second trimester of pregnancy, whereas intestinal atresia predominantly occurs in the third trimester.[13] The immature ENS, which is typically found in children with meconium ileus without cystic fibrosis, is mainly characterized by small-sized myenteric ganglia.[14,15] The immature state of the ENS disappears after surgical correction of meconium ileus, after which IMDs are no longer present.[14,15]

We aimed to quantify the alterations in the ENS and determine the number and density of the myenteric ganglia in the intestinal sections of chicken embryos. In addition, we analyzed whether the ligation procedure affected the cellular features of these ganglia and the recovery potential in the function of the length of the in-ovo ligation period. Furthermore, we examined whether the ENS showed signs of immaturity and whether the pathologic changes in the ENS could be reversed.

Materials and methods

Fertilized eggs (White Leghorn (Gallus gallus domesticus), Co Lohmann, Cuxhaven, Germany) were incubated in a Bios-Midi incubator (Heraeus, Hanau, Germany) at 37.5°C, with a relative air humidity of 80% under standard hatching conditions.

Animals were divided into three groups: 8-d ligature, 4-d ligature, and control.

Eight-day ligature group: intestinal ligature was placed at embryonic day (ED) 11. Ten vital embryos per ED were harvested for analysis from ED 12 to 19.

Four-day ligature group: animals were treated in the same way as the 8-d ligature group. The ligature was removed at ED 15. After the procedure, the eggs were reincubated, five vital embryos were harvested per ED and analyzed from ED 16 onward.

Control group: five embryos without any surgical intervention were sampled per ED. Another five embryos per ED had a sham operation: the eggshell and the chorioallantoic membrane were opened, but neither the intestine nor the vasculature was manipulated. The results of the subgroups were identical; therefore, they were combined to one control group.

The microsurgical techniques and tissue preparation were described previously.[16] In brief, the eggshell and the chorioallantoic membrane were opened at ED 11 and the postumbilical bowel was ligated at 8 ± 1 cm distal to the omphalomesenteric artery. In the 4-d ligature group, the ligature was released with microscissors at ED 15. Embryos were decapitated; full circumference specimens were harvested proximally and distally to the ligature. Specimens for transmission electron microscopy were in 5-10 mm distance to the ligature, specimens for acetylcholine esterase staining were of 10-15 mm distance to the ligature, silver impregnation specimens had a distance of 15-20 mm to the ligature. Equivalent specimens were sampled from the control group. All experiments were carried out in accordance with the institutional standards and regulations on animal welfare. A permit by the local committee for the protection of animal law was not necessary as in-ovo experiments were neither subject to the national regulation of animals nor they subject to the directive 2010/63/EU as the animals did not hatch, thus did not fulfill the definition of Article 2 no. 4 of the directive 2010/63/EU.

Acetylcholine esterase staining

Samples were embedded in a Peel-A-Way T8 container (Polysciences Inc, Warrington, PA) in Tissue-Tek Compound (Weckert Labortechnik, Kitzingen, Germany), snap frozen in liquid nitrogen (Linde Air AG, Hamburg, Germany), and stored at −80°C. Frozen samples were sliced at 8 μm with a Leica CM3000 cryostat (Leica Instruments GmbH, Nussloch, Germany), air-dried at room temperature for 12 to 24 h, and then either processed directly or stored at −30°C. Tissue was stained with hematoxylin and eosin and tested for acetylcholine esterase according to the standard protocols.[17,18]