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Effects of intrauterine treatment on interstitial cells of Cajal in gastroschisis☆

Rahsan Vargun[a], Tanju Aktug[a,*], Aylin Heper[b], Meltem Bingol-kologlu[a]

[a]*Department of Pediatric Surgery, Ankara University School of Medicine, Ankara 06100, Turkey*
[b]*Department of Pathology, Ankara University School of Medicine, Ankara 06100, Turkey*

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Abstract

Aim: An experimental study was performed to investigate the effects of amnio-allantoic fluid exchange and intrauterine bicarbonate treatment on intestinal damage and interstitial cells of Cajal (ICC) in gastroschisis.

Materials and Methods: Thirteen-day-old fertilized chick eggs were randomly allocated into 4 groups as control, gastroschisis, gastroschisis + amnio-allantoic fluid exchange, and gastroschisis + bicarbonate treatment groups. In the treatment groups, amnio-allantoic exchange and bicarbonate treatments were performed for 3 days, after creating gastroschisis. Specimens were processed for hematoxylin-eosin and c-kit immunohistochemistry on the 18th day of incubation, after macroscopic examination. The intestines were evaluated with light microscopy for the presence of mucosal congestion and muscular and serosal edema. Mean muscular thickness and density of ICC were measured.

Results: Mean muscular thickness significantly increased in the gastroschisis group when compared with control and treatment groups. Labeling intensity, morphology, and localization of the ICC were similar in all groups. Mean ICC density significantly decreased in the gastroschisis group when compared with the control group ($P < .01$), and it significantly increased after amnio-allantoic fluid exchange treatment ($P < .01$).

Conclusions: The decrease in ICC density encountered in damaged intestinal loops in gastroschisis was prevented with intrauterine treatment. The beneficial effects of amniotic exchange on intestinal motility may depend on both prevention of intestinal damage and preservation of ICC density and function. The density of ICC might be a reliable numeric parameter both to predict intestinal motility disorders in gastroschisis and to compare the effectiveness of intrauterine treatment methods.

Although prognosis has improved during the advent of parenteral nutrition, gastroschisis remains a major malformation with potential long-term complication owing to prolonged intestinal dysfunction [1]. It is well known that exposure to gastrointestinal waste products in amniotic fluid causes intestinal damage, which can be prevented by amniotic fluid exchange [2-9].

Despite the etiology of intestinal damage being understood, its relationship to intestinal dysfunction remains unclear. Smooth muscle thickening, decreased smooth

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* Corresponding author. Ankara Universitesi Tip Fakultesi, Cocuk Cerrahisi Anabilim Dali, Dikimevi, 06100 Ankara, Turkey. Tel.: +90 312 362 3030/6278; fax: +90 312 362 6400.

E-mail address: tanju.aktug@medicine.ankara.edu.tr (T. Aktug).

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muscle contractility, and decreased acetylcholinesterase and acetylesterase activity in myenteric ganglions are suggested to be responsible for motility dysfunction in gastroschisis [10-12].

Interstitial cells of Cajal (ICC), first described by Cajal, are regarded as the pacemaker cells of the gut because they generate the spontaneous slow waves of smooth muscle layers [13-15]. Distribution of ICC was found to be abnormal in several diseases in which intestinal peristalsis is impaired, such as Hirschsprung disease, hypoganglionosis, neuronal intestinal dysplasia, and infantile pyloric stenosis [16-20]. Recently, delayed maturation of ICC and smooth muscle cells has been shown in a rat model of gastroschisis [21]. These data suggest the possibility of motility dysfunction secondary to pacemaker deficiency in gastroschisis.

Amniotic or amnio-allantoic fluid exchange is one of the most studied intrauterine treatment methods, which has been shown to prevent intestinal damage and motility dysfunction in both experimental and clinical studies of gastroschisis. As an alternative treatment, buffering of amniotic fluid by bicarbonate solution has been suggested as a less complicated method to eliminate harmful effects of intestinal waste products by decreasing their enzymatic activity [22]. However, the effects of both amniotic exchange and bicarbonate treatment on the pacemaker system of the intestines have not been evaluated [4,7,8,22]. Therefore, an experimental study was performed to investigate the effects of amnio-allantoic fluid exchange and intrauterine bicarbonate treatment on intestinal damage and ICC in gastroschisis.

1. Materials and methods

Ten-day-old fertilized chick eggs were incubated at 37°C in 80% humidity. The chicken embryo has an allantoic cavity totally separate from the amniotic cavity. The urine and gastrointestinal waste products are excreted into the allantoic cavity.

The eggs were randomly divided into 4 groups: control (group 1), gastroschisis only (group 2), gastroschisis + amnio-allantoic fluid exchange (group 3), and gastroschisis + bicarbonate treatment groups (group 4). In group 1, the amnio-allantoic membrane was opened through an eggshell window to create a common cavity. In group 2, a defect in the abdominal wall was introduced to create gastroschisis. In group 3, gastroschisis was created, and amnio-allantoic fluid exchange was performed. In group 4, gastroschisis was created, and bicarbonate treatment was performed.

Operative procedures for all groups were performed on the 13th day of incubation, as in previously published studies [4-6]. Both allantoic and amniotic membranes were opened during creation of the gastroschisis model, and thus, an amnio-allantoic fluid mixture, which resembles human amniotic fluid, was created. A defect in the umbilical stalk near the abdominal wall was made, and intestinal loops were exteriorized from the abdomen. In groups 3 and 4, a catheter was placed into the amnio-allantoic cavity after creating gastroschisis. In group 3, amnio-allantoic fluid exchange was performed as described by Aktuğ et al [4-6]. For the exchange, a 0.075% saline solution containing 128 mEq/L Na^+, 148 mEq/L Cl^-, and 20 mEq/L K+ was used. This is approximately isoosmolar to the amnio-allantoic fluid of chick embryos before the 15th incubation day. Knowing that the volume of amnio-allantoic fluid is 9 mL on the 14th incubation day and is reduced to 3 mL on the 18th day of incubation, we performed amnio-allantoic fluid exchange once per 24 hours with 1 mL on the 15th and 16th days and with 0.5 mL on the 17th day. In group 4, bicarbonate treatment was performed by instilling an 8.4% $NaHCO_3$ solution into the amnio-allantoic cavity on the 15th, 16th, and 17th days of incubation, as described by Kanmaz et al [22].

The eggs were sealed with sterile plastic dressing and incubated as stated previously, after surgical interventions. By daily inspection, the survival of the embryos was checked and the dead embryos were discharged.

On the 18th day of incubation, the embryos were exteriorized and weighted, and macroscopic intestinal changes were recorded. The intestines of the embryos were fixed with 10% formalin and embedded in paraffin blocks. The histopathologic changes were analyzed on hematoxylin-eosin–stained sections, and the density of the ICC was evaluated blindly on immunohistochemically c-kit (CD117)–stained sections by 2 pathologists. Mucosal congestion, muscular or serosal edema, and muscular thickness were the analyzed histopathologic features. Muscular thickness was measured by Olympus BX40 microscope with an ocular grid attachment (Olympus Optical Co, Japan). Mean muscular thickness was detected by evaluating both the thinnest and thickest portions of the muscularis propria. The immunohistochemical staining was performed by using the streptavidin-biotin-peroxidase method and primary antibody CD117 (Novocastra, 1/50; Vision BioSystems, United Kingdom) with Benchmark

Table 1 Mean muscular thickness and ICC density according to groups

Groups	Number	Muscular thickness (μm; mean ± SD)	ICC density (mean ± SD)
Control	10	102.5 ± 3.9	11.6 ± 0.6
Gastroschisis	8	126.2 ± 4.1*	9.0 ± 0.2**
Amnio-allantoic fluid exchange	9	108.8 ± 2.4	10.7 ± 0.2
Bicarbonate treatment	8	101.2 ± 5.0	10.1 ± 0.5

* $P < .05$, mean muscular thickness significantly decreased in the gastroschisis group when compared with the control and amnio-allantoic fluid exchange groups.

** $P < .01$, mean ICC density significantly decreased in the gastroschisis group when compared with the control and amnio-allantoic fluid exchange groups.