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Intra-amniotic administration of urinary trypsin inhibitor preserves intestinal contractility in meconium induced intestinal damage in chick embryos with gastroschisis

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Abstract

Background: Intestinal damage causes intestinal dysmotility in gastroschisis. Urinary trypsin inhibitor (UTI) has been shown to prevent intestinal damage in chick embryos with gastroschisis. The effect of intra-amniotic administration of UTI on intestinal motility in gastroschisis has not been investigated.

Methods: Five-day-old fertilized chick embryos were used. Gastroschisis was created through the amniotic cavity without opening the allantoic cavity. There were six groups; control, gastroschisis only, gastroschisis plus meconium and three treatment groups. In the treatment groups, 100 IU/mL, 200 IU/mL and 400 IU/mL UTI were instilled into the amniotic cavity of the gastroschisis plus meconium embryos, respectively. Serosal thickness of the intestines in each group was measured histopathologically. The contractions of the intestines were evaluated by in vitro organ bath technique and the responses were expressed as maximal contraction induced by acetylcholine.

Results: The serosal thickness was significantly increased in the gastroschisis plus meconium, 100 IU/mL, 200 IU/mL UTI groups compared to control and gastroschisis only groups. The serosal thickness of the 400 IU/mL UTI group was similar to control and gastroschisis only groups. Contractility of the intestines was diminished in the gastroschisis plus meconium, 100 IU/mL and 200 IU/mL UTI groups. There was no significant difference regarding contractility among control, gastroschisis only and 400 IU/mL UTI groups.

Conclusion: Intra-amniotic administration of UTI preserves intestinal contractility in chick embryos with gastroschisis. However, preservation of intestinal dysmotility by using UTI in the human gastroschisis cases needs further experimental and clinical trials.

Intestinal damage in gastroschisis has been shown to be dependent to the intra-amniotic meconium concentration [1–4]. Additionally, intestinal damage has been shown to occur when the intra-amniotic meconium concentration exceeds the threshold level [4]. Intestinal damage can be

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prevented by reducing the intra-amniotic meconium concentration below the threshold level [5–11] or by administering intra-amniotic urinary trypsin inhibitor (UTI) [12].

Intestinal damage results in intestinal dysmotility [13,14]. Intestinal dysmotility has been shown to be preserved by reducing intra-amniotic meconium concentration [15]. An experimental study has been planned to investigate the effect of intra-amniotic administration of UTI on intestinal contractility in gastroschisis [16].

1. Materials and methods

Five-day-old fertilized chick embryos (*Gallus domesticus*) were used. Micro-surgical tools and 10× magnifying operation microscope (OPMI-99, Carl-Zeiss, Jena, Germany) were utilized. The chick embryos were divided into six groups. Control group was observed without any intervention. In gastroschisis only group, gastroschisis was created by opening only the amniotic membrane without opening the allantoic membrane on the 5th day of fertilization. The method was described elsewhere [4,12]. Sterile meconium was obtained from a single human newborn. In the gastroschisis plus meconium group, 1/400 meconium suspension was instilled into the amniotic cavity after creation of gastroschisis. In the other three treatment groups, 100 IU/mL, 200 IU/mL and 400 IU/mL UTI (Mochida Pharmaceuticals Co, Yotsuya, Tokyo, Japan) plus 1/400 meconium suspension were instilled into the amniotic cavity of the gastroschisis embryos, respectively. The egg shells were sealed with a sterile film and were placed in the incubator at 37.5 ºC under 80% humidity. On the eighteenth day of fertilization, all embryos were extirpated and protruded intestines were harvested. The study was continued till obtaining 10 live embryos from each group.

Serosal thickness of the intestines was measured histopathologically.

1.1. Preparation of tissues for organ bath studies

The intestinal segments were rapidly removed and placed in Tyrode solution. The solution was kept at 37 ºC and aerated continuously with a 95% O_2 and 5% CO_2 gas mixture at pH 7.35. The tissues were cut into 1 cm strips and mounted in 10 mL organ bath under 0.5 g tension. Tissues were washed with Krebs solution every 15 min during the 60 min resting period. After the equilibration period, cumulative doses of acetylcholine (10^{-9}–10^{-4} molar) were applied. Isometric tensions were recorded with an amplifier system (MP30 Biopac Systems Inc., Santa Barbara, CA, USA) on a computer by using Biopac computer Program.

The data were expressed as millinewton (mN). The differences between the groups were evaluated by analysis of variance (ANOVA) followed by Tukey–Kramer test. The p values lower than 0.01 were accepted as significant.

2. Results

The serosal thickness was significantly increased in the gastroschisis plus meconium, 100 IU/mL, 200 IU/mL UTI groups compared with control and gastroschisis only groups (Table 1). The serosal thickness of the 400 IU/mL UTI group was similar to control and gastroschisis only groups (Table 1).

The contractile response curves of the intestines in the cumulative concentration of acetylcholine are shown in the Fig. 1. Contractility of the intestines was diminished in the gastroschisis plus meconium, 100 IU/mL, 200 IU/mL UTI groups compared with control and gastroschisis only groups (Table 2). The contractility of the 400 IU/mL UTI group was similar to control and gastroschisis only groups (Table 2).

3. Discussion

The contact of the intestines with amniotic fluid in gastroschisis causes edema, shortening, serosal thickening and fibrin accumulation in the intestines [2,4,12,13,17–27]. Intestinal dysmotility due to intestinal damage is the most important prognostic factor in gastroschisis [28,29]. Total parenteral nutrition till recovery of intestinal motility is required. However, expectant treatment including total parenteral nutrition is time consuming, poses morbidity/mortality due to catheter sepsis and metabolic disorders [27,30].

Experimentally the harmful effects of amniotic fluid on the intestines have been shown to be related to the intra-amniotic meconium concentration [3,4,31,32]. When the intra-amniotic meconium concentration exceeds the threshold level, intestinal damage occurs in gastroschisis [3,4]. In addition, increase in the amnio-allantoic fluid ferritin levels has been shown to be a marker for determining intestinal damage in gastroschisis [32].

Histological and functional changes of the intestines in gastroschisis have been shown to be responsible for intestinal dysmotility. Midrio et al. have shown that the maturation of

Table 1 Serosal thickness in experimental groups.

Groups	Serosal thickness [a] (μm)
Control	7.65±0.23
Gastroschisis only	8.65±0.72
Gastroschisis+meconium	29.40±1.79 *
Gastroschisis+meconium+UTI 100 IU/mL	22.70±0.84 *
Gastroschisis+meconium+UTI 200 IU/mL	21.31±1.11 *
Gastroschisis+meconium+UTI 400 IU/mL	9.37±0.33 **

[a] Values are expressed as mean±1 SEM.

* $p<0.01$ compared with control and gastroschisis only groups.

** $p<0.01$ compared with gastroschisis+meconium and 100 IU/mL–200 IU/mL UTI groups.