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The intestinal damage induced by lipid soluble meconium subfraction is profound compared to the intestinal damage induced by water soluble meconium subfraction

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ABSTRACT

Background: It is unclear which substances in meconium are responsible for intestinal damage in gastroschisis. An experimental study was designed to investigate the effects of the lipid or water soluble subfractions of meconium on the intestines of gastroschisis in a chick model.
Methods: Meconium was pooled, homogenated, rota-evaporated dry and diluted. Meconium subfractions were obtained from water soluble and lipid soluble extracts of the meconium. Five days old fertilized chick embryos were used and divided into 5 groups: control, sham, water soluble meconium subfraction, lipid soluble meconium subfraction and whole meconium. All embryos were extirpated on the 18 days and the intestines were harvested for histopathological examination. Serosal thickness was measured under light microscopy.
Results: Serosal thickness of the meconium (36.36 ± 2.8 μm), the water soluble meconium (14.15 ± 0.93 μm) and the lipid soluble meconium (23.88 ± 1.69 μm) subfractions groups were significantly increased compared with the control (7.47 ± 0.68 μm) and the sham (7.48 ± 0.71 μm) groups ($p < 0.001$). Serosal thickness of the lipid soluble meconium subfraction group was significantly increased compared with the water soluble meconium subfraction group ($p < 0.001$). Serosal thickness of the meconium group was significantly increased compared to both the water and the lipid soluble meconium subfraction groups ($p < 0.001$).
Conclusion: Lipid soluble meconium subfraction induces more intestinal damage compared to water soluble meconium subfraction.

Intraamniotic meconium has been shown to be responsible for the intestinal damage (ID) in gastroschisis [1–8]. It is unclear which substances in meconium are responsible for ID in gastroschisis. Experimental research regarding meconium aspiration syndrome (MAS) showed that meconium reduces surfactant function in vitro [9]. The lipid soluble meconium subfraction has been in vitro shown to exert stronger inhibitory effect on surfactant function than the water soluble meconium subfraction [9,10]. The lipid soluble meconium subfraction has been shown to exert more detrimental effects on pulmonary dysfunction compared with water soluble meconium subfraction [11].

The effects of the lipid and the water soluble subfractions of meconium on ID in gastroschisis have not been investigated. An experimental study has been conducted to investigate the effects of the lipid or the water soluble subfractions of meconium on the intestines of gastroschisis in a chick model.

1. Materials and methods

1.1. Meconium preparation

Sterile meconium was obtained from 15 newborns less than 24 h of postgestational age by applying a sterile urine collecting bag to the perianal area after cleansing with 1% chlorhexidine solution. Meconium was pooled, homogenated, rota-evaporated dry and diluted with sterile saline up to a final concentration of 110 mg/ml (dry weight).

1.2. Water soluble extract of meconium

Water extract from the meconium void of lipids was obtained with chloroform:methanol 2:1. Meconium, 1 g dry weight, was added to 5 ml sterile water and mixed with 20 ml chloroform:methanol 2:1. The mixture was allowed to separate into two phases and centrifuged at 15,000 relative centrifugal force (RCF) for 15 min. The top water phase of the medium was reextracted once with 10 ml chloroform:methanol 2:1. The water–methanol phase was dried under a stream of nitrogen, and redissolved in sterile saline.

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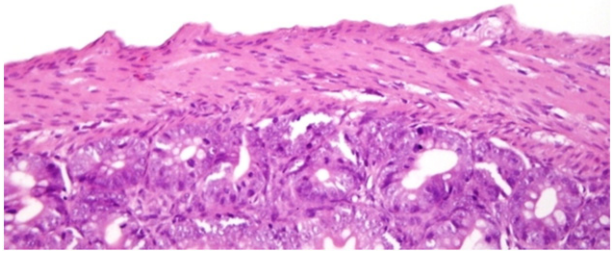

Fig. 1. Photomicrograph of the intestinal wall from the control group (H&E, original magnification ×100).

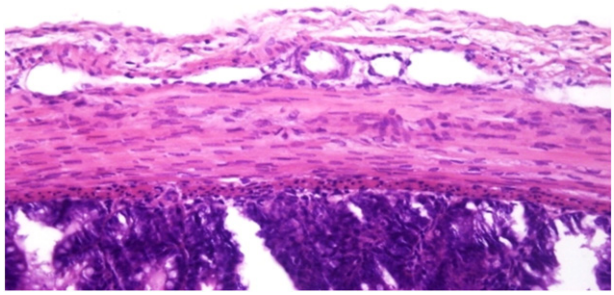

Fig. 3. Photomicrograph of the intestinal wall from the water soluble meconium subfraction group (H&E, original magnification ×100).

This water extract mixed with distilled water and 1/400 concentration meconium suspension was prepared [11].

1.3. Lipid soluble extract of meconium

Lipid extract free from water-soluble components was obtained by the succeeding procedure: 2.5 g dry-weight meconium was added to 37.5 ml sterile water, mixed at 4 °C overnight, centrifuged at 15,000 RCF for 15 min, and the water phase was removed. The precipitate was washed with sterile water and again centrifuged at 15,000 RCF for 15 min. The water phase was removed for the second time. The remaining water insoluble fraction was collected and redispersed in sterile saline at 38 °C. This lipid extract mixed with distilled water and 1/400 concentration meconium suspension was prepared [11].

The subfraction extracts of meconium and the solutions were stored in 10 ml volumes at −20 °C, when necessary they were slowly dissolved up to 30 °C and mixed homogenously before administration.

The study protocols were approved by the Institutional Animal Care and Use Committee (85/2012). Five days old fertilized chick embryos (Gallus Domesticus) were used. The operative procedure for all gastroschisis groups were performed as described previously [2,12].

Briefly, under 10× magnification of the operating microscope (OPMI-99, Carl Zeiss, Jena, Germany), through an eggshell window, to make the vessels visible, allantoic membrane was wetted by 0.75% NaCl solution. Allantoic cavity was retracted laterally and the amniotic cavity was opened. Approximately 0.5 ml amniotic fluid (AF) was aspirated into a syringe and saved for reinstillation. An abdominal wall defect was created with iris scissors and micro forceps, in a diameter of approximately 2.5 mm on the right side of the umbilical stalk near the abdominal wall. After reinstillation of the AF, eggs were sealed with a plastic dressing (Tegaderm, 3M Health Care, St. Paul, USA) and incubated at 37.5 °C and 80% humidity. The eggs were rotated manually, 1–2 times a day. By daily inspection, the viability of the embryos was checked and the dead embryos were discarded. The experiments were continued until 10 surviving embryos were obtained for each group.

The chick embryos were divided into 5 groups:

Group 1 (Control) (n: 10): The eggs were not opened and embryos were observed without any intervention.

Group 2 (Sham) (n: 10): Gastroschisis was created only.

Group 3 (Water soluble meconium subfraction) (n: 10): After creation of gastroschisis, the water soluble meconium subfraction was instilled into the amniotic cavity.

Group 4 (Lipid soluble meconium subfraction) (n: 10): After creation of gastroschisis, the lipid soluble meconium subfraction was instilled into the amniotic cavity.

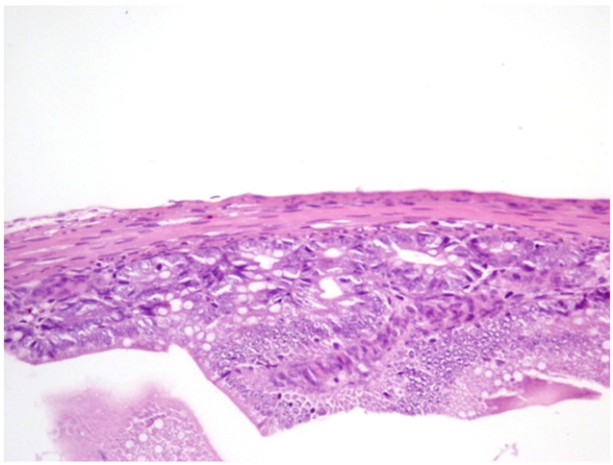

Fig. 2. Photomicrograph of the intestinal wall from the sham group (H&E, original magnification ×100).

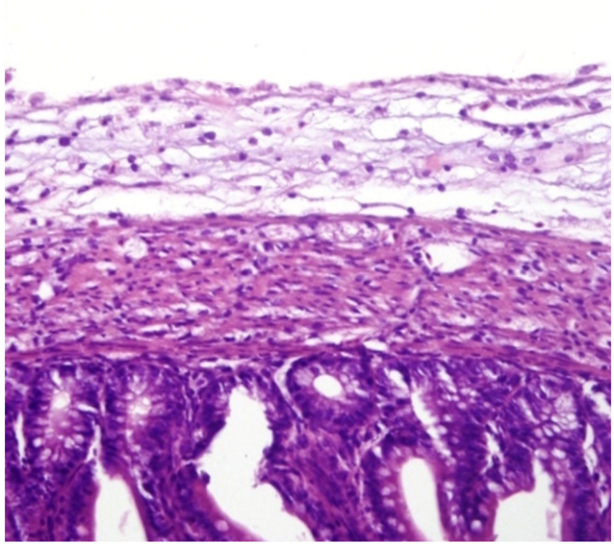

Fig. 4. Photomicrograph of the intestinal wall from the lipid soluble meconium subfraction group (H&E, original magnification ×100).