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Immunohistochemical detection of meconium in the fetal membrane, placenta and umbilical cord☆

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ABSTRACT

Objective: To develop the immunohistochemistry specific for meconium in the placenta, fetal membrane and umbilical cord.
Study design: We previously reported the specific presence of zinc coproporphyrin I (ZnCP-I) in human meconium and demonstrated the possible diagnostic use of an elevation in maternal plasma ZnCP-I levels in cases of amniotic fluid embolism. In this study, we developed a new specific monoclonal antibody for ZnCP-I and applied it to the immunostaining of meconium in the placenta, fetal membrane, and umbilical cord.
Results: Immunoreactivity of ZnCP-I clearly and specifically identified meconium in the placenta, fetal membrane, and umbilical cord. It was especially useful in cases of severe chorioamnionitis to detect meconium in the macrophages surrounded by numerous neutrophils. In more than half of the cases, meconium was detected in clear amniotic fluid at delivery, suggesting previous exposure.
Conclusions: Immunohistochemical detection of ZnCP-I is a highly sensitive histological diagnosis of meconium.

1. Introduction

Meconium is a bile-stained material present in the small bowel of fetuses long before mid-gestation, and which moves in the intestinal lumen with contractions of the intestinal wall, but is usually eliminated after birth [1]. Some investigators have reported the presence of meconium staining to be associated with an increased incidence of adverse neonatal outcome [2,3]. Others have demonstrated no association between the presence of meconium stain and neonatal levels of arterial pH, carbon dioxide pressure, or base excess [4–6]. Nathan et al. examined a large number of samples and found that the impact of meconium on neonatal morbidity and mortality is rather small and primarily related to meconium aspiration syndrome [2], especially for thick meconium [7]. However, the role of meconium as the primary factor contributing to meconium aspiration syndrome is controversial [8], because autopsy studies have suggested prenatal origins of intrauterine infection and/or chronic hypoxia [8,9]. Thus, the pathophysiological involvement of meconium in neonatal outcome is still contentious. We speculate that the unavailability of universal diagnostic criteria for meconium staining is one of the reasons for the confusion. Indeed, it is difficult to distinguish thick from thin meconium-stained amniotic fluid by gross appearance [10] and the histological detection of meconium is not always reliable, especially using the hematoxylin–eosin (HE) stain [1,11].

The aim of the present study was to develop immunohistochemistry specific for meconium. We previously reported the presence of zinc coproporphyrin I (ZnCP-I) in human meconium [12] and demonstrated the possible diagnostic use of an elevation in maternal plasma ZnCP-I levels in cases of amniotic fluid embolism in Japan [13]. In the present study, we newly developed a specific monoclonal antibody against ZnCP-I and applied it to the

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Table 1
Clinical features of subjects. Values are expressed as the mean ± standard deviation. The gross appearance of amniotic fluid at delivery was classified as clear (−), thin (+), and thick (++). PIH; Pregnancy induced hypertension. FGR; Fetal growth restriction. APS; Apgar score.

Meconium staining	Meconium in amniotic fluid at delivery by gross appearance	
	Clear (−)	Thin (+) or thick (++)
Age	33 ± 5.3	32 ± 5.4
Gestational week	37 ± 0.8	39 ± 1.2
Placental weight (g)*	540 ± 98	574 ± 124
Birth weight (g)	2815 ± 336	3085 ± 463
Intrauterine infection	0	3
PIH	0	3
FGR	0	1
Non-reassuring fetal status	1	9
APS (5 min <7)	0	0

immunostaining of meconium in the placenta, fetal membrane, and umbilical cord, comparing the findings with results of standard screening by HE, Prussian-blue staining, and the gross appearance of amniotic fluid at delivery.

2. Materials and methods

2.1. Subjects

After delivery, all specimens of umbilical cord, fetal membrane and placenta were stored at 4 °C in a refrigerator at Hamamatsu University Hospital between June 2009 and April 2011. The tissues were kept in the dark for avoiding exposure to light [14]. The tissues were then dropped in 10% formaldehyde (0.1 M sodium cacodylate buffer, pH 7.4) at room temperature until used. Two researchers (N.F. and C.Y.) retrospectively selected a total of 78 cases of placentas according to the data on the gross appearance of meconium in amniotic fluid, i.e. clear (−), thin (+), and thick (++) meconium, which was assessed by midwifes at delivery. 50 cases were selected as thin (+) or thick (++) meconium and 28 cases as clear amniotic fluid. Table 1 indicates clinical features of 78 pregnant women, whose placentas were analyzed.

The intestine was obtained at autopsy of a neonate who died one day after birth at 35 weeks of gestation.

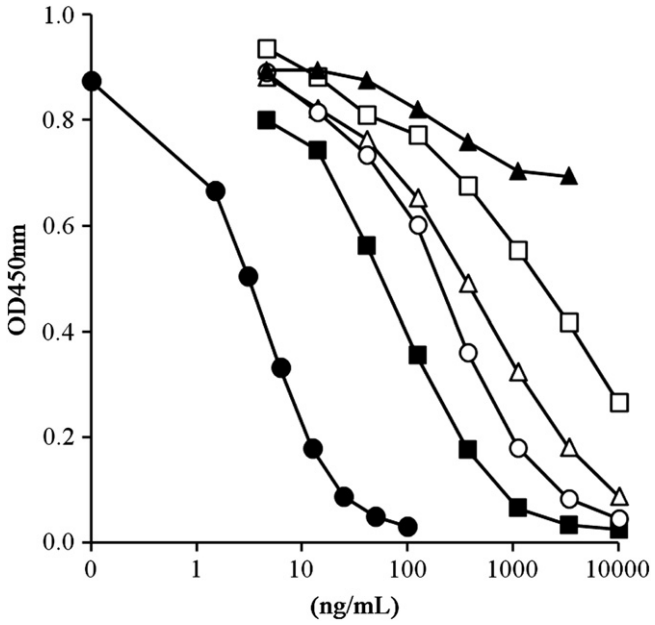

Fig. 1. Cross-reactivity of the monoclonal antibody raised against zinc coproporphyrin I (ZnCP-I) with major porphyrins. Closed circles indicate the reactivity to ZnCP-I. Open and closed triangles indicate coproporphyrin I and protoporphyrin IX, respectively. Open and closed squares indicate coproporphyrin III and uroporphyrin I, respectively. Open circles indicate uroporphyrin III.

Meconium was obtained from a neonate delivered at 38 weeks of gestation, which was expelled approximately 2 min after birth.

2.2. Measurement of ZnCP-I level in the meconium

ZnCP-I level in the meconium was measured as previously described [12]. In brief, 100 mg wet weight meconium was dissolved 1.6 mL distilled water and after

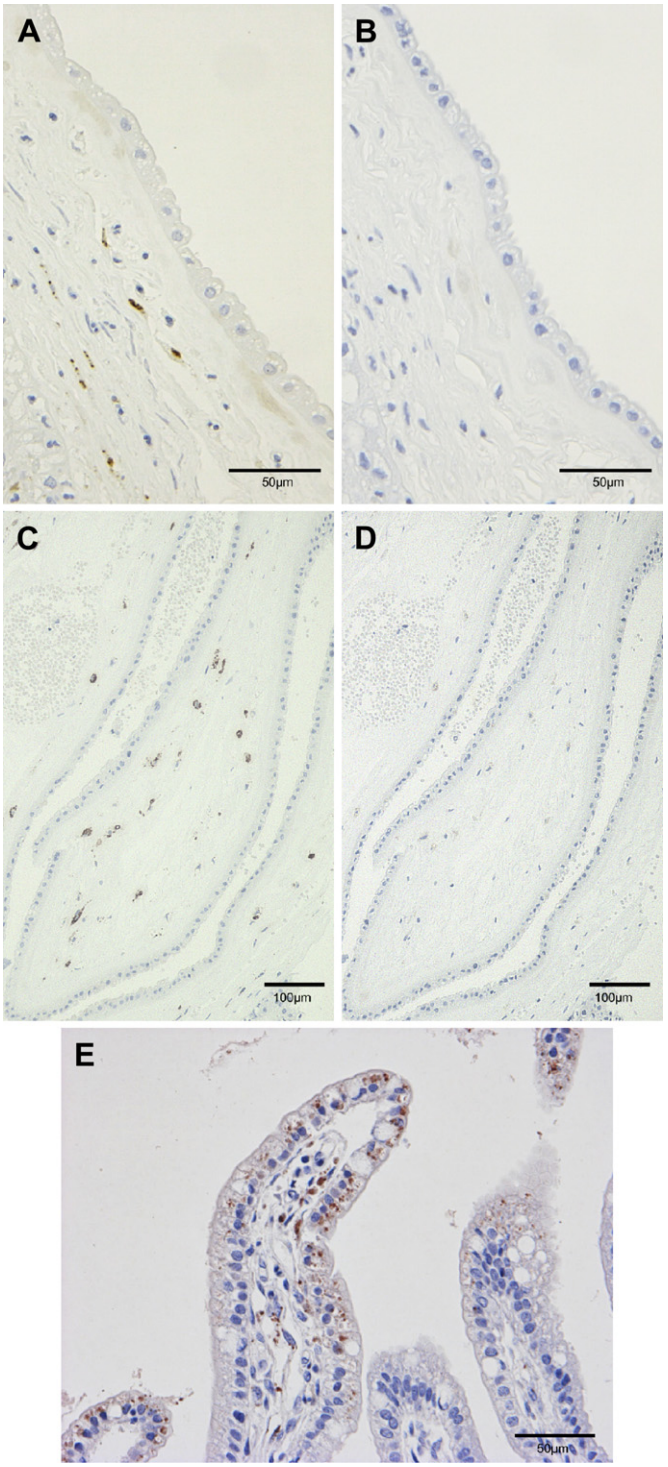

Fig. 2. Immunohistochemical detection of ZnCP-I in term placenta (A, B), term fetal membrane (C, D) and neonatal intestine (E). Brown cytoplasmic granules in macrophages indicate ZnCP-I immunoreactivity (A, C and E), which completely disappeared with the pre-absorption of 0.1 μM ZnCP-I (B) or 15.7 mg wet weight/L meconium containing 0.1 μM ZnCP-I (D). Black bars indicate 50 μm (A, B and E) and 100 μm (C, D).