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ORIGINAL ARTICLE

Interleukin 6 and interleukin 8 play important roles in systemic inflammatory response syndrome of meconium peritonitis

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Abstract
Purpose Meconium peritonitis is caused by an intestinal perforation that may occur in the fetus, followed by severe chemical peritonitis, resulting in high morbidity.
Methods We have experienced six patients with meconium peritonitis. Cystic drainage was performed soon after birth for all patients. We investigated the concentrations of several cytokines and a chemokine (interleukin 8) in the ascites from the six patients with meconium peritonitis. In two patients we also measured the serum cytokines and chemokine level just after birth.
Results Interleukin 6 and interleukin 8 concentrations were very high in the cyst or ascites just after birth. In the serum taken from two patients, the levels of interleukin 6 and interleukin 8 were also high. In five patients who underwent drainage of cysts after birth, systemic inflammation could not be completely suppressed before curative surgery.
Conclusions Interleukin 6 and interleukin 8 play important roles in the inflammatory response syndrome associated with meconium peritonitis, and drainage of cystic fluid did not completely suppress this inflammation. To lessen the high morbidity of meconium peritonitis, efforts should be made to suppress the inflammatory response using new treatment strategies, such as administration of steroids or anti-cytokine therapy to supplement cystic drainage.

Keywords Meconium peritonitis · Systemic inflammatory response syndrome · Fetal inflammatory response syndrome · Interleukin 6 · Interleukin 8

Introduction

Meconium peritonitis is a fetal inflammatory response syndrome (FIRS) [1] that is triggered by meconium leaking into the peritoneal cavity in the fetus. If such chemical inflammation continues after birth, it is called systemic inflammatory response syndrome (SIRS) and makes patient care more difficult. A high morbidity rate of meconium peritonitis is reported even today [2]. However, precisely which cytokines and chemokines are involved in this inflammation remain to be elucidated, and only one experimental report has previously suggested a role for tumor necrosis factor-α [3]. We report herein our investigation of the concentrations of several cytokines and chemokines in the ascites and serum taken from six patients with meconium peritonitis and elucidate their potential roles in the systemic inflammation in these patients.

Materials and methods

We have experienced six patients with meconium peritonitis since 1993. One patient died soon after birth because he was complicated with a diaphragmatic hernia and had severe pulmonary hypoplasia. The other five patients were classified as having giant cystic type meconium peritonitis. In all six patients, ascitic or cystic fluids were drained soon

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after birth and centrifuged at 5,824 g for 10 min; supernatants were kept at −80°C until examination. In two patients, blood samples were also taken just after birth, and these were also kept at −80°C until use. Five patients underwent curative surgery at 5–25 days of age, and they all had ileal atresia and short bowels. In one patient, severe peritonitis caused by methicillin-resistant *Staphylococcus aureus* occurred after curative surgery, and additional peritoneal drainage was needed. Ascites and serum were taken from this patient after surgery and kept at −80°C until they were analyzed.

The following cytokines and a chemokine [interleukin 8 (IL-8)] were measured using commercially available ELISA kits: interleukin 1β (IL-1β), interleukin 2 (IL-2), interleukin 4 (IL-4), interleukin 6 (IL-6), interleukin 8 (IL-8), interleukin 10 (IL-10), tumor necrosis factor-α (TNF-α), and interferon-γ (IFN-γ).

Results

Profiles of the six patients and the degree of inflammation after birth

The patient profiles are shown in Table 1. Four patients were male and two were female. Patient 2 was not diagnosed antenatally, but five patients were diagnosed as having meconium peritonitis while in utero. Patient 3 died soon after birth because he also had a diaphragmatic hernia and severe pulmonary hypoplasia. The gestational age of the six patients at birth ranged from 34 to 40 weeks and 3 days. Their body weight varied between 2,425 and 3,700 g (including the volume of the peritoneal cyst). All six cases except case 3 were giant cystic types; case 3 was an adhesive type with massive had ascites. The cystic fluids or ascites were drained at 0–2 days after birth, and the serum C-reactive protein (CRP) level was monitored as an indicator of systemic inflammation (Fig. 1). Curative surgery was performed at 5–25 days of age. All patients except in case 3 had ileal atresia and had shorter bowels (the small intestine ranged from 70 to 130 cm in length) compared with age-matched normal neonates. The five patients who underwent surgery are all still alive.

Table 2 shows laboratory data immediately after birth indicating the level of systemic inflammation. White blood cell counts were high, from 18,000 to 32,500/mm^3 in all patients. The serum CRP level was high in patients 1, 2, and 4 (17.4, 2.33, and 4.38 mg/dl, respectively) and mildly elevated in patients 3, 5, and 6 (0.49, 0.3, and 0.8 mg/dl, respectively). Erythroblast counts indicated the level of inflammation, which was high in patients 2 and 4 (12% and 75.6%, respectively).

Concentrations of cytokines and a chemokine in cystic or ascitic fluid and serum that were collected after birth (Tables 3, 4)

In the cystic or ascitic fluids, IL-6 and IL-8 were present at very high concentrations (IL-6 level, 393–31,800 pg/ml; IL-8, 35.2–11,000 pg/ml). The levels of these two cytokines did not correlate with the disease term (from gestational age at diagnosis to gestational age at birth) and also were not correlated with the serum whole blood cell (WBC) and CRP levels, as shown in Table 2. IL-1β was mildly elevated, from 8 to 348 pg/ml. TNF-α was at very low levels in most patients. IFN-γ, IL-2, IL-4, and IL-10 were all almost below the level of detection. In patients 2 and 6, IL-10 was mildly elevated, which suggested that an antiinflammatory response had occurred in these patients [4]. In patients 2 and 4, the serum cytokine and chemokine levels were measured. IL-6 and IL-8 were high, whereas IL-1β and TNF-α were mildly elevated.

Increases and decreases of systemic inflammation after cystic drainage

Figure 1 shows CRP changes before and after curative surgery in case 2. The CRP level fluctuated after cystic drainage, but it never came down below the normal value.

Table 1 Profile of six patients with meconium peritonitis

Case	Sex	Fetal diagnosis	Gestation	Body weight (g)	Drainage (days)	Curative surgery (days)	Length of small intestine (cm)	Prognosis
1	M	32 weeks	40 weeks, 3 days	2,920	0–2	25	70	Alive
2	F	–	35 weeks, 0 day	3,700	0.1	5	75	Alive
3[a]	M	33 weeks	35 weeks, 2 days	2,698	0	–	?	Dead
4	M	28 weeks, 2 days	34 weeks, 0 day	2,425	1	16	130	Alive
5	F	28 weeks	36 weeks, 5 days	2,748	0.1	20	110	Alive
6	M	30 weeks	36 weeks, 1 day	2,448	0	5	120	Alive

[a] Case 3 was complicated with diaphragmatic hernia and the patient died at 2 days of age of pulmonary insufficiency and generalized edema

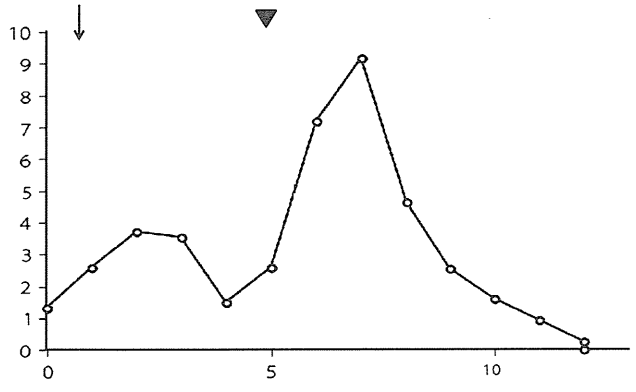

Fig. 1 C-reactive protein (CRP) change in patient 2. Just after birth, the CRP level was 2.33 mg/dl (*y*-axis). After cystic drainage, it fluctuated, but never came down to the normal value. After curative surgery on day 5 after birth (*x*-axis), the CRP level increased as high as 9.0 mg/dl, but decreased thereafter. *Arrow* cystic drainage, *arrowhead* curative surgery

Table 2 Inflammatory states after birth in six patients

Case	Term from diagnosis to birth	WBC[a] (/mm^3)	CRP[a] (mg/dl)	Erythroblasts[a] (%)
1	8 weeks, 3 days	32,500	17.4	0
2	–	29,700	1.33	12
3	2 weeks, 2 days	18,000	0.49	
4	5 weeks, 5 days	15,400	4.38	75.6
5	8 weeks, 5 days	28,800	0.3	
6	6 weeks, 1 day	25,600	0.8	

WBC whole blood count, *CRP* C-reactive protein

[a] Blood samples were taken just at birth

The other four patients (cases 1, 4, 5, and 6) also showed fluctuations in their CRP levels after drainage, and none of them came down below the normal value (data not shown). We concluded that cystic drainage could attain reduction of the local levels of cytokines and the chemokine but could never completely control systemic inflammation caused by meconium peritonitis.

Discussion

In the past few decades, meconium peritonitis has been considered to be a severe, complicated disease with a very high mortality rate [5, 6]. Recently, antenatal diagnosis and progress in treatment have decreased the mortality rate [7, 8], but the morbidity rate is still high because the severe inflammatory response may continue after birth and cause complications [2].

FIRS is a relatively novel concept, but it is now becoming widely accepted by neonatologists and obstetricians [1], and it is closely related to the presence of cytokines such as IL-6 and IL-8 [9, 10]. Meconium peritonitis may be a typical FIRS manifestation at the early stage of the disease in the fetus when one considers its fetal pathophysiology. Meconium is a strong inducer of inflammation via the complement system and the CD14 molecule on macrophages [11]. In the fetus, TNF-α and IL-1β secreted by peritoneal macrophages might play an important role in triggering the inflammatory response in meconium peritonitis [3].

Our present data showed that the inflammatory response continued after birth, and that the patients had varying degrees of inflammatory responses at birth. In other words, the patients with meconium peritonitis are in SIRS just after birth, and suppressing this inflammatory response is very important for successful treatment after birth. Our data also showed that after birth, IL-6 and IL-8 played key roles in inflammation, in contrast to IL-1β and TNF-α, which were suggested to play important roles in experimental meconium peritonitis [3]. We hypothesize that IL-1β and TNF-α work in the fetus to induce an inflammatory reaction in the peritoneal cavity, and that such an inflammatory reaction can result in the formation of a pseudocyst. IL-6 and IL-8 may amplify the inflammation initiated by IL-1 and TNF-α. In some patients, the IL-10 level was increased slightly in the cyst, thus suggesting that these patients may have had antiinflammatory response syndrome (CARS) [4], but even in these

Table 3 Concentration of cytokines and chemokine in cystic fluids

Case	TNF-α (pg/ml) (<5)	IL-1β (pg/ml) (<10)	IL-6 (pg/ml) (<4)	IL-8 (pg/ml) (<2)	IFN-γ (IU/ml) (<0.1)	IL-2 (U/ml) (<0.8)	IL-4 (pg/ml) (<6)	IL-10 (pg/ml) (<5)
1	6	348	1,120	11,000	<0.1	<0.8	–	<2.0
2	<5.0	38	31,800	1,800	<0.1	<0.8	<2.0	9
3	<5.0	16	7,800	591	<0.1	<0.8	<2.0	<2.0
4	23.2	8	393	35.2	0.3	<0.8	–	<2.0
5	<5.0	30	566	8,220	0.2	<0.8	<2.0	<2.0
6	<5.0	2.89	5,200	6,650	0.2	<0.8	–	25

Numbers in parentheses indicate normal value in human blood

TNF-α tumor necrosis factor-α, *IL* interleukin, *IFN-γ* interferon-γ

Table 4 Concentration of cytokines and chemokines in serum after birth

Case	TNF-α (pg/ml)	IL-1β (pg/ml)	IL-6 (pg/ml)	IL-8 (pg/ml)
1	–	–	–	–
2	1.8	2.1	154	66
3	–	–	–	–
4	3	1.13	38.4	104
5	–	–	–	–
6	–	–	–	–

patients, the systemic inflammation still continued, as indicated by the WBC count and CRP level.

The IL-6 and IL-8 concentrations in the cysts did not correlate with the disease term (from first diagnosed gestational age to birth age), or with the serum inflammatory markers such as WBC and CRP in our five patients, although the reasons for this lack of correlation were not clear. We speculate that the inflammatory responses in a fetus may vary according to the process of formation of the pseudocyst wall (this process is likely different in each patient), and once formed, this pseudocyst wall may work as a seawall to prevent the overflow of cytokines into the bloodstream in some patients, whereas in other patients, the wall did not work, leading to severe systemic inflammation. Another important fact was that the IL-6 and IL-8 levels were high in all six patients, but their absolute concentrations varied. Again, the reason for this was not clear, but we speculated that IL-6 and IL-8 were produced by the activated macrophages and neutrophils attracted by the first inflammation evoked by TNF-α and IL-1β. If the adhesive and fibrous pseudocyst wall was promptly formed after first inflammation, it might be difficult to attract macrophages and neutrophils into the cysts, thereby preventing the inflammatory cascade from effectively producing IL-6 and IL-8. In contrast, if the pseudocyst was formed gradually by a necrotic intestinal wall, the wall would have been fragile and easily attracted macrophages and neutrophils into the cyst, allowing the inflammatory cascade to proceed to induce high levels of IL-6 and IL-8. However, these theories are just speculation, and more data are needed to a demonstrate reasonable explanation for these observed differences.

Cystic drainage was reported to be valuable to control the inflammation because it worked to reduce cytokine levels [12], but such treatment alone could not suppress the inflammation completely, as shown by our present data. Therefore, a more effective and stronger anti-cytokine treatment is needed to control SIRS after birth. Steroids are among the promising therapeutic candidates [13]. Other candidates, as apparent from our data, are anti-IL-6 agents [14]. It will be important and valuable to use these medications in the future to control SIRS in meconium peritonitis patients, and this may reduce the morbidity of the disease.

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PEDIATRIC RESEARCH

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Carbohydrate and Energy Metabolism in the Brain of Rats With Thromboxane A_2-Induced Fetal Growth Restriction

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ABSTRACT: Fetal growth restriction (FGR) remains a cause of perinatal brain injury, sometimes leading to neurological and intellectual impairment. Although the mechanisms and pathophysiology of CNS injuries have not been elucidated completely, it is possible carbohydrate and energy metabolism may have an important role in the FGR brain. In this study, FGR was induced in rats by administration of synthetic thromboxane A_2 (STA_2). Pups were delivered by cesarean section. After killing, samples were obtained from the fetuses of both control and FGR rats for evaluation of carbohydrate and energy metabolism in brain tissue. Lactate and pyruvate levels in brain were reduced significantly in the FGR group. Glucose content in brain tissue tended to be increased in the FGR group. In contrast, glycogen content in brain tissue tended to be lower in the FGR group. However, these differences in glucose and glycogen content did not reach statistical significance. Brain high-energy reserves, including ATP, ADP, AMP, and phosphocreatine (P-Cr), were similar in the control and FGR groups. Gluconeogenesis compensated for chronic fetal hypoxia and decreased glycogen storage. Energy metabolism in the FGR brain is likely to be disrupted as a consequence of lower reserves of energy substrates. (***Pediatr Res* 70: 21–24, 2011**)

Fetal growth restriction (FGR) is an important cause of perinatal morbidity and mortality. Surviving small-for-GA (SGA) infants have a higher incidence of neurological impairments including mental retardation and educational and/or behavioral problems (1–3). Several authors have reported that perinatal hypoxic ischemic brain damage may contribute to an increased prevalence of motor, cognitive, and affective disabilities in children born with FGR (4–6). However, the mechanisms underlying these disabilities have not yet been fully elucidated.

Various prenatal factors may affect fetal growth. Pregnancy-induced hypertension (PIH) is an especially important factor in FGR fetuses. In PIH, increasing umbilical vessel resistance causes limitations in uteroplacental blood flow. As a consequence, the supply of oxygen and nutrition to the fetus may decrease (7). FGR fetuses are frequently hypoxic and hypoglycemic (8). During labor, uterine contractions can further compromise placental blood flow and oxygen supply to the FGR fetus, thereby increasing the risk of an intrapartum hypoxic-ischemic event and subsequent brain injury (9). Several authors have also reported that FGR fetuses are not only at great risk of perinatal hypoxic ischemic events but also may be more susceptible to hypoxic-ischemic brain damage, compared with appropriate-for-GA (AGA) fetuses (9,10).

Several studies have investigated the mechanisms of brain injuries in FGR model animals (11–13). Manipulations to induce FGR in animal models included surgical ligation of vessels supplying the uteroplacental unit (12) and maternal starvation (11). However, these models do not necessarily reflect the pathophysiology of FGR. It has been suggested that plasma levels of thromboxane, a potent vasoconstrictor, may exceed prostacycline levels in PIH, whereas in normal gestation, the opposite is true (14–16). This prostacycline-thromboxane imbalance may be of primary importance in the pathophysiology of uteroplacental vascular insufficiency and resultant fetal growth retardation. We have developed an FGR model induced by maternal administration of thromboxane A_2 (17) and consider that this model may more closely resemble the pathophysiology of human FGR compared with other models generated by uterine artery ligation (18) or maternal undernutrition (19). Our FGR model exhibits not only physical growth restriction but also a significant delay in postnatal neurological development (20). The phenotype of our FGR model is therefore very similar to severe human FGR associated with severe PIH.

In general, FGR infants suffer from hypoglycemia. There is evidence that hypoglycemia affects psychomotor development in SGA infants (21). Changes in energy metabolism associated with adaptation of the FGR fetus to chronic hypoxia and hypoglycemia *in utero* may therefore be an important determinant of susceptibility to perinatal hypoxic ischemic brain injury in FGR neonates. Accordingly, it is important to clarify the alterations that occur in carbohydrate and energy metabolism in growth-retarded fetal brains. We hypothesize that the content of substrates in FGR brains is lower than in brains of AGA infants. However, we speculate the energy status in FGR is normal as the majority of FGR infants do not have neurological symptoms. In this study, we examined cerebral carbo-

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Abbreviations: FGR, fetal growth restriction; **P-Cr,** phosphocreatine; **PIH,** pregnancy-induced hypertension; **STA_2,** synthetic thromboxane A_2

hydrate and energy metabolism and used our model of FGR to clarify the mechanism of brain damage in this condition.

MATERIALS AND METHODS

The protocol for this study was approved by the Ethical Committee of the Center for the Promotion of Medical Research and Education, Nagoya University Graduate School of Medicine.

Preparation of FGR rats. Dated pregnant Sprague-Dawley rats were purchased from Chubu Kagaku Shizai, Nagoya, Japan, and osmotic pumps, Model 2M11 from Alza Corporation, Palo Alto, CA. Synthetic thromboxane A_2 (STA_2) analog was kindly gifted by Ono Pharmaceutical Company, Osaka, Japan. All reagents and enzymes used in the study were of the highest commercial grade available. The maternal rats were housed individually and fed *ad libitum* under controlled light cycle (12-h light and 12-h darkness) and temperature conditions. The FGR model rats were produced using our previously reported method (17). In brief, on the 13th day of pregnancy, an osmotic pump filled with STA_2 was implanted into the peritoneal cavity under general anesthesia with pentobarbital. The mean delivery rate of STA_2 was 20 ng/h. On the 20th day of gestation, the fetuses were delivered by cesarean section under ether anesthesia. The fetuses were stabilized for 1 h after birth and then weighed. To avoid hypothermia, the environmental temperature was regulated using a temperature-controlled bath. After decapitation, blood samples were collected for analysis of blood glucose levels. The whole brains were then removed and weighed, and saved for the experiments.

Six pregnant rats were divided into two groups, with three rats being administrated STA_2 to induce FGR, whereas the remaining three animals had no intervention. None of the maternal rats died during pregnancy, and there was no intrauterine/neonatal death of the pups in the study. We were unable to obtain sufficient quantities of tissue from several pups. The lactate and pyruvate content was measured in whole brains of 10 FGR pups from a litter of 13 and also in 14 control pups from a litter of 14. Glucose and glycogen contents in whole brains were evaluated in 7 FGR rats from a litter of 10 and in 13 controls from a litter of 13, whereas energy status in whole brains was evaluated in 9 FGR rats from 1 litter of 12 and in 9 controls from a litter of 13. The total number of pups in the FGR and control groups were 26 and 36, respectively. A proportion of the experimental animals was used to measure plasma glucose levels (10 FGR rats and 13 controls).

Preparation of brain tissue. For determination of lactate and pyruvate contents, the whole brains were removed and homogenized immediately in 2 mL of 0.6% perchloric acid using a Potter-Elvehjem glass homogenizer to achieve optimal preservation of the labile metabolites. The combined procedures of decapitation, brain removal, and homogenization were carried out within 13–15 s. The homogenate was centrifuged at 28,000 × g for 30 min and the resultant supernatant adjusted to pH 6.0 with 3 N potassium carbonate containing 0.5 M triethanolamine, followed by centrifugation at 28,000 × g for 30 min. The final supernatant was stored at −80°C until analyzed. To measure glucose and glycogen contents, the homogenate of the whole brain was added to 50 volumes of 0.03 N HCl and then heated for 10 min at 100°C in sealed tubes. The boiled suspension was used in the assays (22).

Whole brains were homogenized with 10 volumes of ice-cold 6% perchloric acid to evaluate ATP, ADP, AMP, and phosphocreatine (P-Cr) levels. Sampling of the brain tissue was carried out using the same methods as for pyruvate and lactate. After centrifugation of the homogenate at 10,000 × g for 10 min, 0.1 mL of 1 M dibasic potassium phosphate was added to 1 mL of the supernatant. The mixture was then neutralized with 3 N potassium hydroxide and centrifuged at 10,000 × g for 10 min. The supernatant was stored at −80°C until analysis.

Laboratory measurements. Measurement of glucose and pyruvate contents in whole brains were essentially those described by Lowry and Passonneau (23), whereas lactate and glycogen contents were determined by the method of Vannucci and Duffy (22).

The fluorometric methods for ATP, ADP, AMP, and P-Cr were essentially those described by Lowry and Passonneau (23). Determination of adenine nucleotide concentrations allows estimation of the ATP/ADP ratio and adenylate charge ratio, (ATP + 1/2ADP)/(ATP + ADP + AMP). The former approximates energy availability, whereas the latter reflects the equilibrium between high-energy adenine nucleotides mediated by adenylate kinase (24). Plasma glucose was analyzed by the glucose oxidase method using an Ascensia Breeze2 Blood Glucose Meter (Bayer HealthCare, Japan).

Statistical analysis. The results were expressed as median and range. The Mann-Whitney *U* test was used to evaluate the statistical significance of differences between the FGR and control groups. A $p < 0.05$ was considered statistically significant.

RESULTS

Evaluation of fetal growth. The median body weights of pups in the FGR and control groups were 3.64 g (3.35–4.09 g) and 4.18 g (3.74–4.69 g), respectively. Fetuses with FGR were significantly smaller than those in the control group ($p < 0.0001$). The median brain weight in the FGR group was significantly lower than that in the control group [167 mg (144–186 mg) *versus* 180 mg (156–192 mg), $p < 0.0001$].

Evaluation of carbohydrate metabolism. Carbohydrate substrates and metabolites in the whole brains are shown in Fig. 1. The median lactate content in the brains was significantly lower in the FGR group compared with the control group [0.69 μmol/g (0.34–1.67 μmol/g) *versus* 1.19 μmol/g (0.81–1.63 μmol/g), $p = 0.017$]. The median pyruvate content in the brains was also significantly lower in the FGR group than in controls [0.24 μmol/g (0.12–0.43 μmol/g) *versus* 0.31 μmol/g (0.26–0.43 μmol/g), $p = 0.004$]. Total glucose content tended to be higher in FGR rat brains [1.24 μmol/g (0.54–1.96 μmol/g) *versus* 0.58μmol/g (0.37–1.78 μmol/g), $p = 0.081$]. In contrast, total glycogen content in the brains tended to be lower in the FGR pups compared with controls [1.29 μmol/g (1.15–1.66 μmol/g) *versus* 1.53 μmol/g (1.11–1.85 μmol/g), $p = 0.075$]. These differences in glucose and glycogen contents in the whole brains were not statistically significant.

Median plasma glucose was significantly lower in FGR rats compared with controls [24.8 mg/dL (18.0–30.5 mg/dL) *versus* 43.8 mg/dL (39.0–60.5 mg/dL), $p < 0.0001$].

Evaluation of energy status in the brain. The energy state in brain tissue was evaluated by measuring ATP, ADP, AMP, and P-Cr concentrations (Table 1). There were no significant

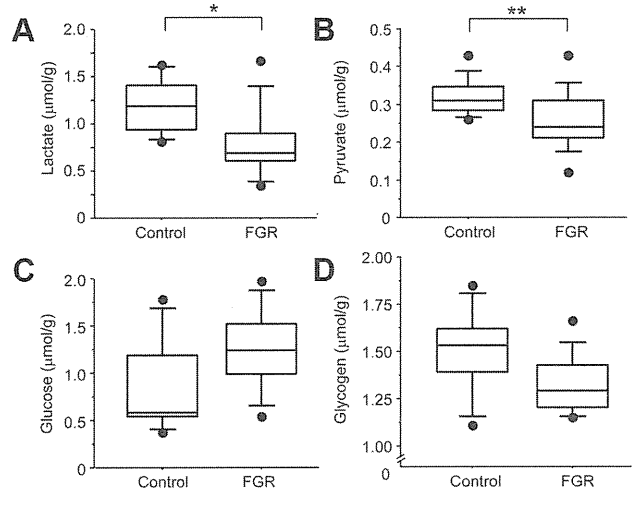

Figure 1. Comparison of carbohydrate substrates in whole brains of the control and FGR groups. (*A*) Lactate contents in control ($n = 10$) and FGR ($n = 14$) pups. (*B*) Pyruvate contents in control ($n = 10$) and FGR ($n = 14$) pups. (*C*) Glucose contents in control ($n = 7$) and FGR ($n = 13$) pups. (*D*) Glycogen contents in control ($n = 7$) and FGR ($n = 13$) pups. The *horizontal bars* within the *boxes* correspond to the median, the *upper* and *lower bars* of the *boxes* to the first and third quartiles, respectively, and the *upper* and *lower whiskers* to the 90th and 10th percentiles, respectively. The observations marked by an *open circle* were considered extreme outliers. $*p < 0.01$, $**p < 0.05$.

Table 1. *High-energy metabolism in the fetal brain*

	Controls (n = 9)	FGR (n = 9)	p
ATP (μmol/g)	1.85 (1.57–3.08)	1.79 (1.45–2.25)	0.392
ADP (μmol/g)	0.33 (0.19–0.41)	0.28 (0.18–0.49)	0.870
AMP (μmol/g)	0.11 (0.09–0.15)	0.10 (0.03–0.20)	0.128
P-Cr (μmol/g)	1.50 (0.70–1.72)	1.17 (0.77–1.50)	0.205
ATP/ADP	6.1 (4.3–12.3)	5.9 (3.7–10.1)	0.627
Charge ratio	0.88 (0.86–0.93)	0.89 (0.82–0.93)	0.773

Charge ratio; (ATP + 1/2ADP)/(ATP + ADP + AMP).

differences in cerebral high-energy reserves, as total ATP, ADP, AMP, and P-Cr contents were similar in the two groups. The ATP/ADP and charge ratio in the FGR brains were also similar to those in the controls.

DISCUSSION

In this study, we evaluated carbohydrate substrates and energy status in the brains of rats with FGR induced by STA_2. Pyruvate and lactate contents in the brain were decreased significantly in FGR rats. Although glucose content tended to be increased in FGR rat brains compared with controls, glycogen content tended to be lower. These differences in glucose and glycogen content in whole brains, however, did not reach statistical significance. There was also no significant difference in cerebral high-energy reserves such as total ATP, ADP, AMP, and P-Cr contents between the two groups. Our results suggest that a normal energy state is maintained in FGR brains even when serum glucose levels are low. However, energy metabolism may be disrupted easily when there is a decreased supply of carbohydrate substrates in the FGR brains. This suggests that FGR brains may be more susceptible to hypoxic-ischemic events.

In view of the known predisposition for human FGR infants to develop neonatal hypoglycemia, glucose metabolism was the subject of early investigations in FGR models. We measured carbohydrate substrates in the brain, and as expected, showed pyruvate and lactate levels were decreased in FGR rats. Glycogen levels tended to be lower in FGR rats compared with controls, although this difference was not statistically significant. In contrast, glucose content in FGR brains tended to be higher when compared with controls. In general, glucose transportation across the placenta and the blood-brain-barrier is facilitated by carrier-mediated diffusion that is dependent on the concentration gradient. Ogata *et al.*(25) showed that growth-retarded fetuses had significantly diminished plasma glucose concentrations 10 and 240 min after uterine artery ligation, which recovered to normal ranges by the 21st gestational day. Our results support the hypothesis that fetal glycogen degradation with resultant hyperglycemia ensures an adequate supply of substrates to vital tissues such as the brain, and provides, at least, some measure of fetal tolerance to interruptions in placental oxygen transport (26).

Brain glycogen is contained predominantly in astrocytes, although its concentration is low compared with that in the liver (27). When brain blood flow is interrupted completely, theoretically energy may become depleted within a few minutes. However, it is possible that glucose utilization is considerably slower in newborn rats (28), and therefore glycogen may be an important substrate in fetal and neonatal brains during periods of asphyxia even when the amount of glycogen stored is small. A reduction in umbilical blood flow results in a decrease in fetal glucose and oxygen uptake. Anaerobic glycolysis is then stimulated by hypoxia to maintain optimal cellular energy balance despite oxygen debt. Glycogenolysis with resulting availability of glucose is an obviously important pathway that contributes to glucose homeostasis during the perinatal period. As glycogen storage in fetal brains is minimal (27), the supply of free glucose *via* glycogenolysis from glycogen storage tissues such as the liver and kidney plays an important role in the survival of the fetus during times of stress. It has been demonstrated that accumulation of fetal hepatic glycogen is decreased in animal models of FGR (25), and in our study we also observed that FGR brains had lower glycogen content. Taken together, these experimental data suggest that FGR brains may have a low tolerance to the effects of undernutrition and adaptive glycogenolysis.

The significance of gluconeogenesis in fetal carbohydrate metabolism remains controversial. Townsend *et al.* (29) showed that gluconeogenic capacity is not expressed *in utero* under unperturbed circumstances, and that the contribution of gluconeogenesis from lactate, pyruvate, or alanine to glucose was quantitatively negligible. The FGR newborn also has depressed activity of enzymes necessary for gluconeogenesis (30). However, another report presented evidence that suggested that three carbon substrates, such as lactate, pyruvate, and alanine are likely to be precursors for hepatic glycogen (31). In experiments on fasted sheep, fetal glucose utilization did not decline to the same degree as the decrease in umbilical uptake, suggesting compensatory glucose production by the fetus (32). Rudolph *et al.* (33) also showed an increase in net glucose production in fetal sheep livers during acute umbilical cord compression.

The substrates in the FGR brain also remain controversial. Kliegman *et al.* (11) examined brain substrates in FGR dogs and showed the levels of lactate, pyruvate, and glycogen were higher in brain tissue of FGR dogs than in controls. However, glucose levels were similar in the FGR dogs and controls. Lin *et al.* (12) also reported high levels of pyruvate and glucose in the brains of FGR rats. By using magnetic resonance spectroscopy, Moxon-Lester *et al.* (13) demonstrated that lactate levels in the brain of FGR piglets and controls were similar. However, in our study, both lactate and pyruvate levels were reduced significantly in the brains of FGR rats. Uteroplacental blood flow reduction induced by STA_2 may stimulate gluconeogenesis at the expense of lactate and pyruvate to adapt to the chronic reduction in delivery of oxygen and substrates. This priority for endogenous fuel provision to vital tissues leads to impaired fetal growth. The reasons for the different results in the analysis of brain substrates between studies are not clearly understood, although we speculate that differences between species and the methodologies used in the FGR animal models may have influenced the results.

In this study, serum glucose levels were significantly lower in FGR rats compared with controls. We did not evaluate glycogen storage in the liver or plasma insulin levels in this

study. However, Ogata *et al.* (25) measured serum glucose and insulin levels and glycogen content in FGR rats and showed that insulin levels were low, despite glucose levels and glycogen storage both being decreased. Therefore, we speculate that the cause of hypoglycemia in FGR rats may be due to reduced storage of glycogen.

Regarding brain energy metabolism, no significant difference was observed in the high-energy reserves, ATP, ADP, AMP, and P-Cr between FGR rats and controls. According to a study by Brown and Vannucci (34) using the Wiggleworth FGR model, the concentrations of various cerebral substrate metabolites including glycogen, glucose, lactate, ATP, and P-Cr were not altered in spite of a significant reduction in brain weight. They ascribed these findings to the absence of hypoxia at the level required to disrupt oxidative metabolism and the energy state of the brain. Kliegman *et al.* (11) demonstrated using a maternal canine starvation model that cerebral charge in FGR neonates was unaffected, whereas their calculated energy reserve was lower than that of controls. Theoretically, depression of oxygen delivery to respiring tissues increases the ADP to ATP ratio (ADP/ATP). This, in turn, acts to stimulate glycolysis (*i.e.* the Pasteur effect), with a subsequent increase in anaerobic ATP production. In this study, the charge ratio in FGR rat brains was similar to that in controls. This result demonstrates that the energy state in FGR brains can be compensated by stimulation of glycolysis under chronic hypoxic conditions, but that FGR fetuses do not revert totally to anaerobic metabolism.

In summary, chronic disturbance in uteroplacental blood flow stimulates fetal glucose production mainly by increasing gluconeogenesis to maintain a normal energy state in the brain. Energy metabolism in the FGR brain will be liable to disruption and stressed further because of decreased storage of carbohydrate substrates.

Acknowledgments. We dedicate this report to Dr. S Mimura who designed the study protocol but died before its the completion.

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V. 資　料

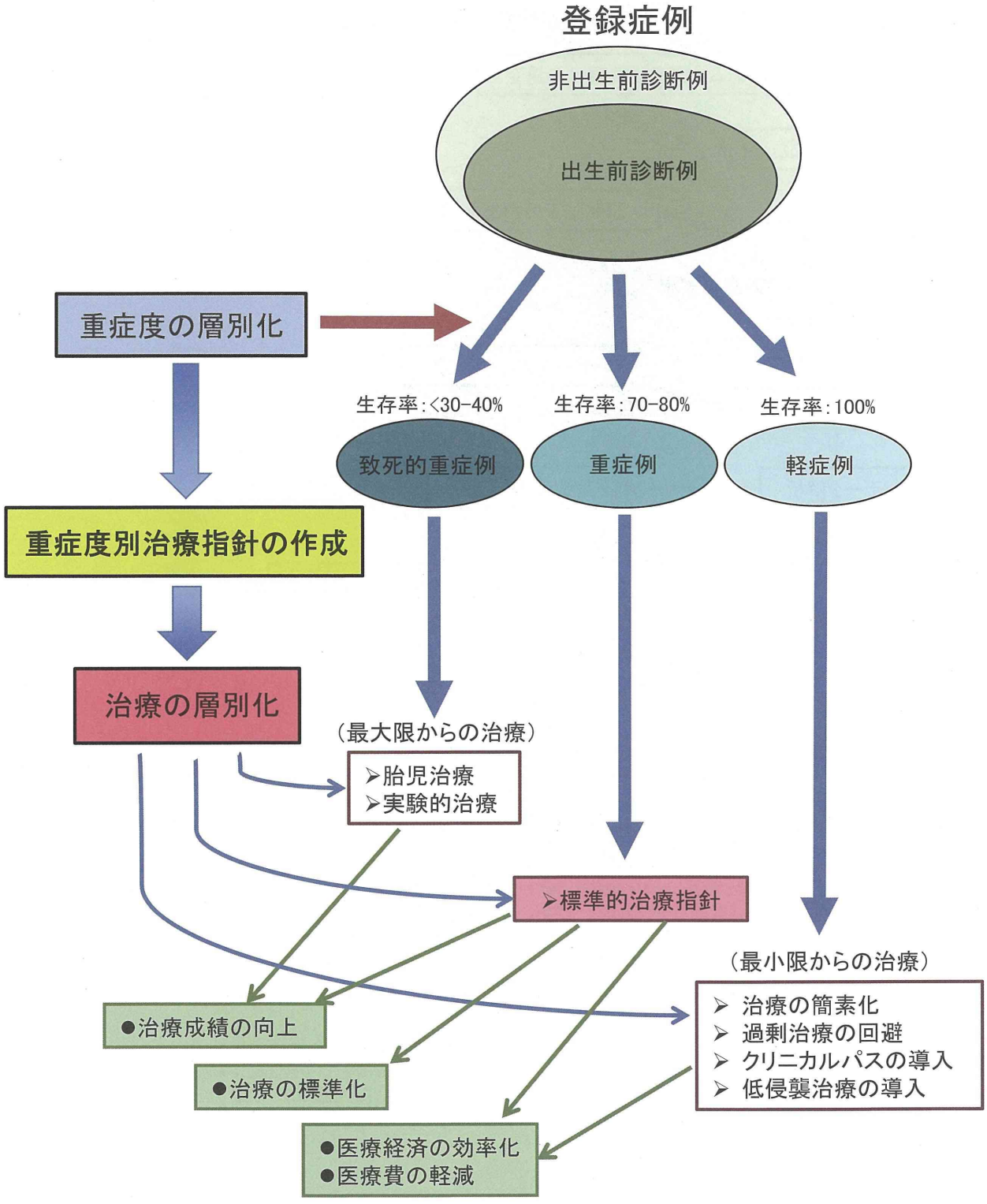
研究奨励分野　研究対象　疾患概要

【疾患名】	新生児横隔膜ヘルニア
【患者数】	推定患者数6,000人（年間発生約200人）
【概要】	先天性横隔膜ヘルニア（以下本症）は、横隔膜の欠損による腹腔内臓器の胸腔内への嵌入による先天性の疾患で、その発生頻度は、出生数2,000人～4,000人あたりに1例である。日本小児外科学会による最新の調査では、本症の年間発症数は約180例程度と報告されている。本邦における先天性横隔膜ヘルニアの予後は、近年改善傾向にあるものの、生存率は未だ約75%に留まっている。また、生存例においても、在宅医療を要する長期後遺障害例が15%程度存在する。
【原因の解明】	本症の疾患の本態は、横隔膜の先天的な解剖学的欠損あるいは形成不全である。しかしながら、横隔膜の欠損とそれに関連した肺低形成および遷延性肺高血圧の病因については、まだ解明されていない。多くの症例は単独で発症し、70%が原因の明らかでない特発性の発症例である。約20%程度は他の原因、例えばビタミンA欠乏症との関連性が実験的に示されている。腹腔内の臓器が横隔膜の欠損孔を通じて嵌入する時期が、肺の発育における非常に重要な時期と一致するため、腸管などの腹腔内臓器による肺の圧迫が肺低形成を生じる主な要因と考えられる。
【主な症状】	横隔膜の欠損孔の大きさと、腹腔内臓器が胸腔内に嵌入する時期が出生後早期に症状を発症するかどうかの重要な因子である。通常、呼吸困難症状を呈した新生児は、酸素投与とマスクを用いた最初の人工呼吸によって症状は一旦改善する。しかし典型的な経過として、この治療により胃や腸がガスで膨らみ、肺をより圧迫し、縦隔偏位がすすむにつれて酸素化の悪化が招来される。最も重症の症例では、生後数時間で死亡する場合もある。しかし、横隔膜の欠損孔の小さい症例では生直後に明らかな症状は出ない。
【主な合併症】	肺低形成と新生児遷延性肺高血圧症が2大合併症である。横隔膜の欠損は時として、染色体異常に伴うことがあり、8%の症例で、13トリソミー, 18トリソミー、ダウン症候群、ベックウィズー・ヴィードマン症候群、ダニー・ドラッシュ症候群などを合併する。しかし家族性の発生はまれである。先天性心奇形を合併する症例も時に認められる。長期的な治療成績は合併奇形の重症度に大きく影響される。肺機能障害、成長発達障害、精神発達遅延、難聴、胃食道逆流症、側弯、漏斗胸などが長期観察中に発症すると言われている。
【主な治療法】	出生前診断された本症の患児にとっては、正確な診断と重症度の評価、それに続く注意深い周産期管理が重要である。近年ではリスクの高い本症の患児に対しては、高炭酸ガス血症容認、低酸素血症容認の考え方のもとに、高頻度振動換気、一酸化窒素吸入療法、人工肺（ECMO）などの積極的な治療が行われている。欧米においては、胎児治療の臨床応用も試みられつつある。一方で低侵襲手術として一部の症例に鏡視下手術が導入されつつある。
【研究班】	新生児横隔膜ヘルニアの重症度別治療指針の作成に関する研究班

Disease Summary

【Name of the disease/symptom】	Congenital Diaphragmatic Hernia (CDH)
【Number of Patients】	6,000 cases (200 cases/year)
【Background】	Congenital diaphragmatic hernia (CDH) is a developmental defect in the diaphragm that allows abdominal viscera to herniate into the chest. CDH occurs in 1 out of 2000 to 4000 births. The Japanese society of pediatric surgeons recently reported that the incidence of CDH was around 180 cases per year. The survival rate of CDH, which has been improved recent years, is still remaining around 80% in Japan. Moreover, 15 % of the survivor of this disease has been suffering from subsequent complication which requires home medical care.
【Cause】	CDH is a simple anatomic congenital defect or ageneis of the diaphragm. However, the pathogenesis of diaphragmatic defect and associated pulmonary hypoplasia and persistent pulmonary hypertension is poorly understood. Most defects develop as isolated defects, ie, sporadically (70%). There are other factors involved in about 20% detected on experiments, such as in relation to medications like vitamin-A deficiency. Because the herniation of abdominal contents through the diaphragmatic defect coincides with a critical period of lung development, lung compression by the herniated viscera and bowel results in pulmonary hypoplasia.
【Major symptoms】	The size of the diaphragmatic defect and the timing of transposition of intraabdominal structures intrathoracically is an important factor in early presentation with symptoms after birth. A newborn in respiratory distress is usually treated with oxygen and ventilation primarily using a mask. Typically, this treatment leads to deterioration of oxygenation by inflating the stomach and the gut, leading to more compression of the lung and to increasing mediastinal shift. Most severe cases will die in a few days after birth. However, some cases with small defects of the diaphragm may not be apparent at birth. In these situations, mediastinal shifts are hardly ever found.
【Major complications】	Pulmonary hypoplasia and persistent pulmonary hypertension are the two major complications. Diaphragmatic defects are seen as well in trisomy 13, 18, or 21 in 8%, such as in Beckwith Wiedemann and Danny Drash syndrome. Familial conditions are rare (2%). Some cases are associated with congenital cardiac abnormalities. Late results are heavily influenced by accompanying congenital morbidity. Reduced lung functions, growth retardation, neurological impairment, healing loss, gastroesophageal reflux. scoliosis and funnel chest are recognized during the long term follow up period.
【Major treatments】	Precise diagnosis and assessment of the severity following a careful perinatal care are important for the patients with prenatal diagnosis of CDH. Recently, aggressive treatments, including the use of high-frequency oscillatory ventilation, inhalation of nitric oxide and extracorporeal membrane oxygenation, under the concept of permissive hypercapnia and permissive hypoxia, have been performed for high risk CDH patients. Clinical trials of fetal intervention have been endeavored in Europe and the United States. Meanwhile, laparoscopic and thoracoscopic surgery are performed in some limited cases.
【Contact information】	Research on the treatmet strategy of congenital diaphragmatic hernia according to the risk-stratified classification.

流れ図


登録症例
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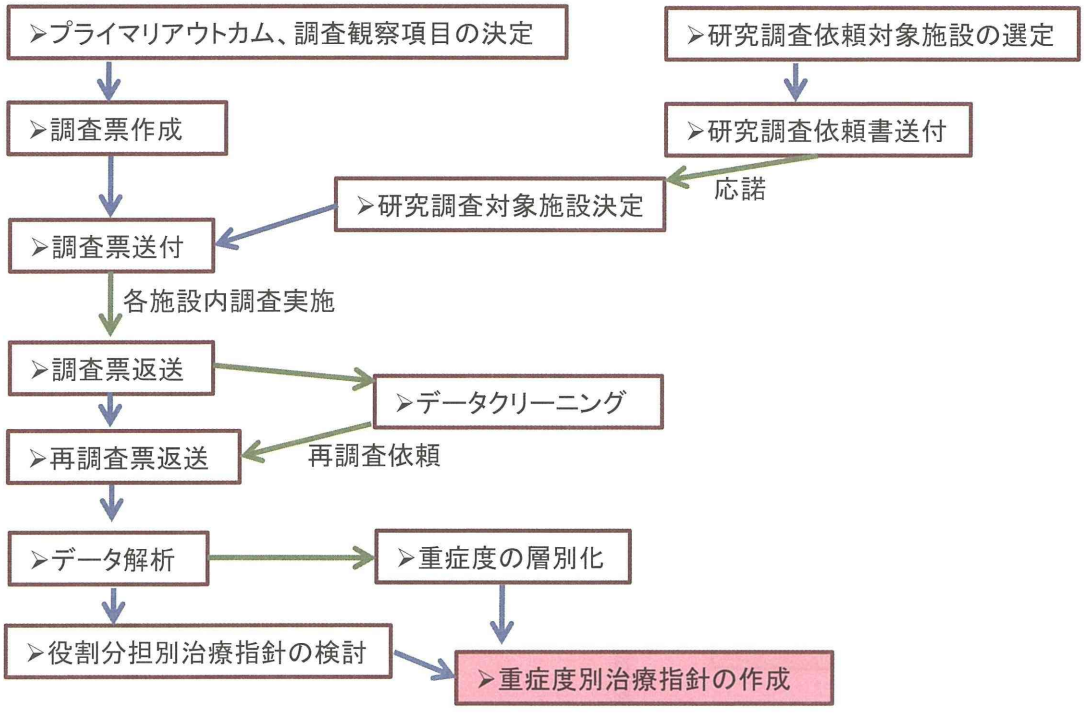
生存率:70-80%
生存率:100%
致死的重症例
重症例
軽症例
重症度別治療指針の作成
治療の層別化
（最大限からの治療）
➢胎児治療
➢実験的治療
➢標準的治療指針
（最小限からの治療）
➢ 治療の簡素化
➢ 過剰治療の回避
➢ クリニカルパスの導入
➢ 低侵襲治療の導入
●治療成績の向上
●治療の標準化
●医療経済の効率化
●医療費の軽減

研究全体のロードマップ

１．重症度別治療指針の作成に関する研究（当該年度）



２．重症度別治療指針の更なる推進に関する研究（次年度採択の場合）

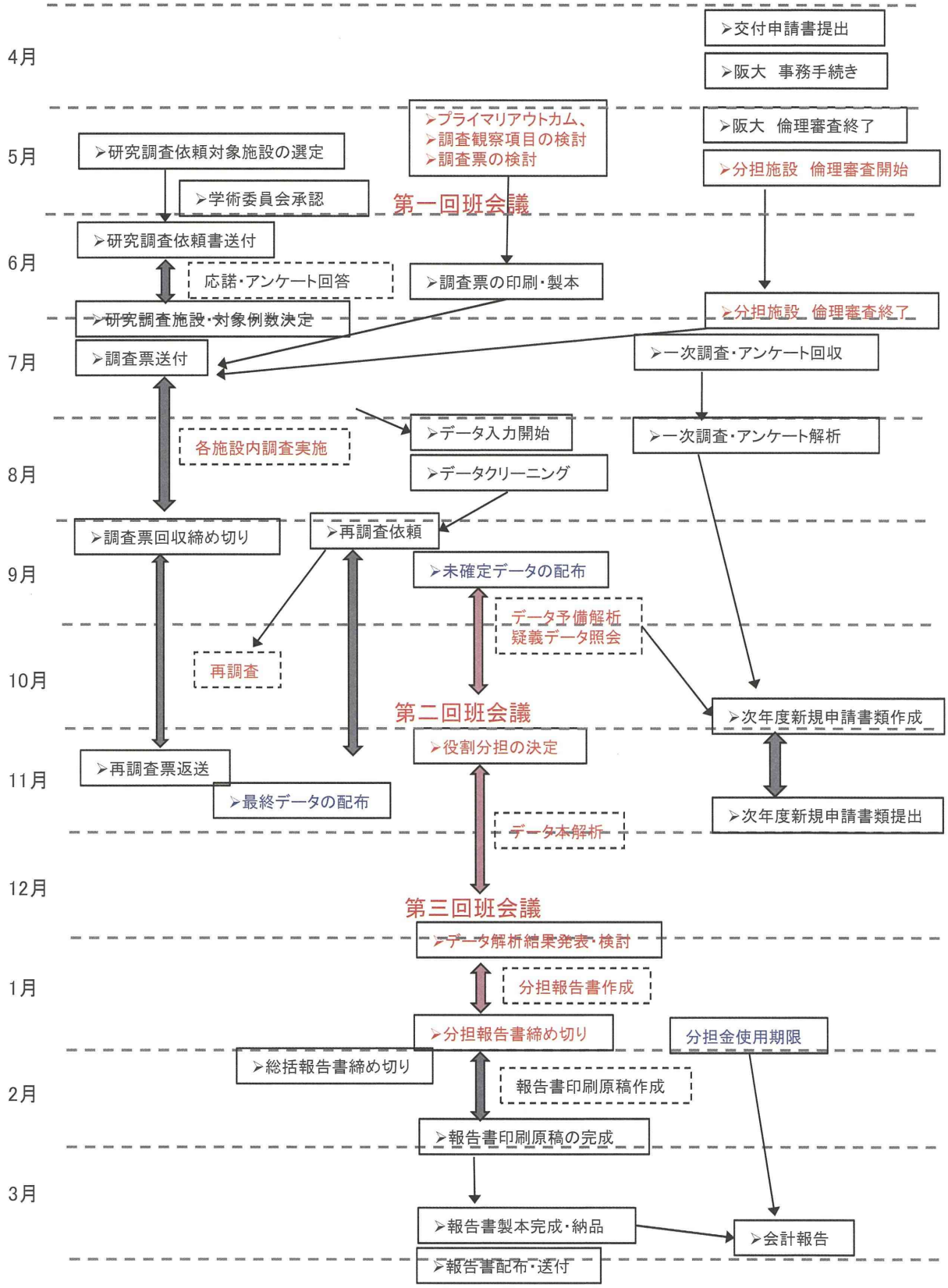
➢重症度別治療指針

①
➢重症度分類に基づいた後ろ向きコホート観察研究
・予め層別化分類を提示して、初年度より規模を拡大して行う

②
➢指針に基づいた治療に関する前向きコホート研究
・事前症例層別化登録による観察研究
・事前症例層別化登録による施設間比較研究

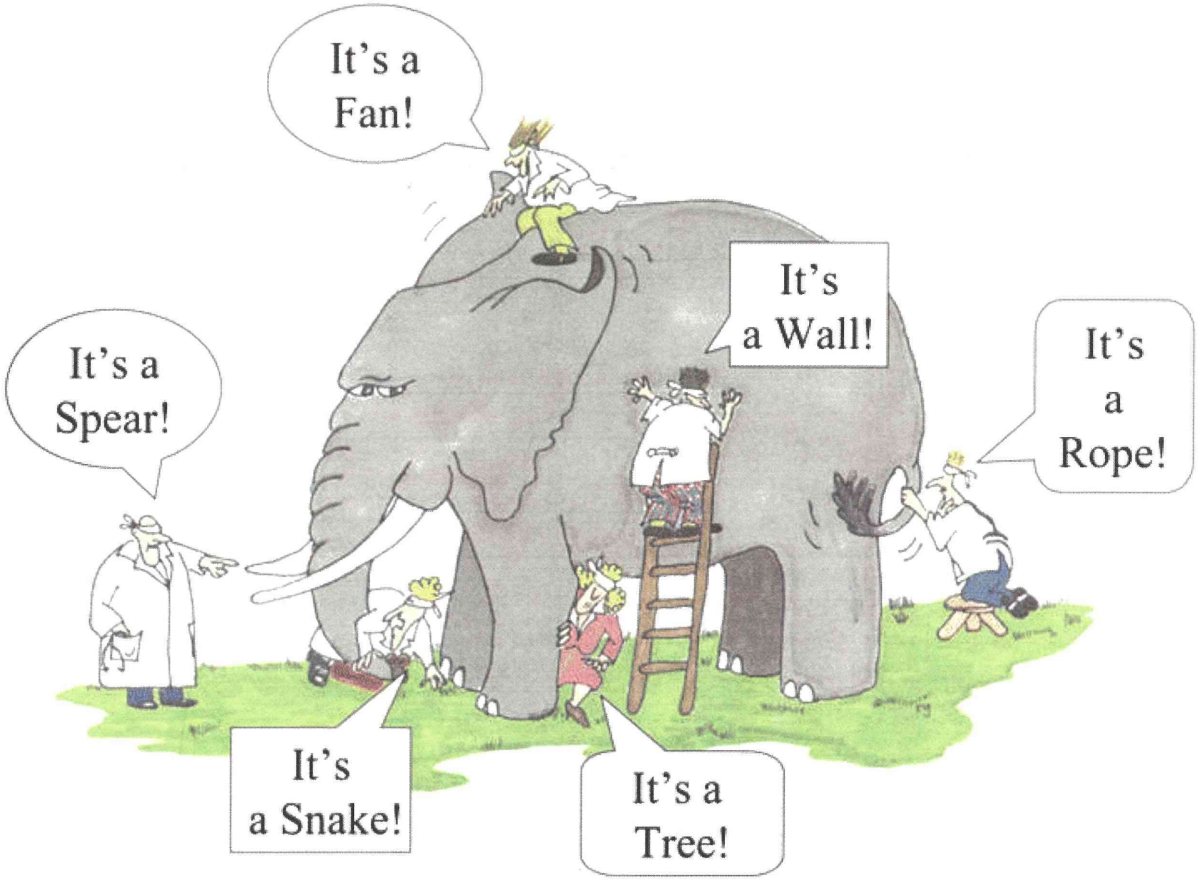
- ①については、調査票を改変して、初年度の規模を拡大して同様の方法で引き続き行う。
- ②については、前向きの登録制度に参加する施設を募り、これまで同様、施設の指針に従って治療を行う施設と、新たに示された治療指針に従って治療した施設間比較研究、および指針使用前後の歴史的比較研究を行う。

CDH研究班　研究日程


4月
5月
6月
7月
8月
9月
10月
11月
12月
1月
2月
3月
➢交付申請書提出
➢阪大　事務手続き
➢プライマリアウトカム、
➢調査観察項目の検討
➢調査票の検討
➢阪大　倫理審査終了
➢研究調査依頼対象施設の選定
➢分担施設　倫理審査開始
➢学術委員会承認
第一回班会議
➢研究調査依頼書送付
応諾・アンケート回答
➢調査票の印刷・製本
➢研究調査施設・対象例数決定
➢分担施設　倫理審査終了
➢調査票送付
➢一次調査・アンケート回収
各施設内調査実施
➢データ入力開始
➢一次調査・アンケート解析
➢データクリーニング
➢調査票回収締め切り
➢再調査依頼
➢未確定データの配布
データ予備解析
疑義データ照会
再調査
第二回班会議
➢次年度新規申請書類作成
➢役割分担の決定
➢再調査票返送
➢最終データの配布
➢次年度新規申請書類提出
データ本解析
第三回班会議
➢データ解析結果発表・検討
分担報告書作成
➢分担報告書締め切り
分担金使用期限
➢総括報告書締め切り
報告書印刷原稿作成
➢報告書印刷原稿の完成
➢報告書製本完成・納品
➢会計報告
➢報告書配布・送付

群盲評象之図


衆瞽
摸象之図


It's a Fan!
It's a Wall!
It's a Spear!
It's a Rope!
It's a Snake!
It's a Tree!

平成23年度　CDH研究班　第1回班会議議事録

日　時　：　平成23年5月28日（土）11：00～15：15
場　所　：　新大阪丸ビル本館（株）ジャパンライフ　3階301会議室
出席者（11名）：稲村先生、臼井、奥山先生、金森先生、神山先生、高橋先生、
田口先生、谷先生、永田先生、服部先生、早川先生、（五十音順）

1）難治性疾患克服研究事業の概要、本研究の今後の日程、本年度の研究予算について
- 研究代表者より説明があり、了承された。
- 一次調査とアンケートについて、日本小児外科学会、学術・先進医療検討委員会に承認依頼中であることが報告された。

2）倫理審査委員会（IRB）への申請について
- 研究代表者施設では、IRBの審査が終了したことが報告された。
- 研究分担者施設では、全施設でIRB申請が開始され、7月までに審査終了の見込みであることが報告された。

3）一次調査の対象施設について
- ある程度の症例数の横隔膜ヘルニアの診療を行っていると推測される施設については、小児外科認定施設・教育関連施設以外の施設にも対象を拡大して、一次アンケートを送付することになった。この点に関連して、研究実施計画書を修正することとした。

4）一次調査の内容およびアンケートの内容について
- 一次調査では、染色体異常や重篤な奇形を合併した出生前診断例の症例数と予後についても、問うこととなった。
- アンケートでは、容認可能な血液ガスデータについて、選択肢形式で問うこととなった。
- アンケートでは、質問1，4，6について、選択肢の文章を若干修正した。
- アンケートでは、質問7，8，9を設けて、人工換気法の選択方針、治療方針決定への心エコーの関与の程度、術前術後管理の主体となる診療科についても、問うこととなった。

6）名古屋大学早川先生、服部先生よりの提案について
- 胎児MRIの3つの所見（不完全肺底部、肝脱出、胃の健側への脱出）をスコアリングすることで、出生前の重症度予測が可能かどうかのstudyを行いたい旨の説明があり、了承された。

7）症例調査票について
- 症例の概要：出生時身長を追加すること、軽症の心奇形の定義、重篤な合併奇形（non-isolated症例）の定義、染色体異常の定義。（これに伴い、研究実施計画書2.2「Isolated CDHの定義」を修正することとした。）
- 出生前所見：胎児MRI所見で、羊水過多の項を削除すること、MRIの所見は初回と最終回を記載してもらうこととした。（これに伴い、研究実施計画書10.4「肺底部不完全描出の定義」を修正し、環状断と訂正して、シェーマを写真に入れ替えること）
- 出生時所見：IUGRの項を削除（代わりに出生時体重、身長からSGAを算出）、胎児麻酔の有無の項を追加、出生直後の鎮静の有無の項を追加する。
- 出生時の血液ガス：24時間以内のベスト（Highest　PaO2とLowest PaCO2）の血液ガスデータと、その時の呼吸条件（FiO2とMAP）を記載してもらうこととした。（OI計算目的）。出生時の

単純レントゲン写真：Shimono らの論文に準じ、肺尖部型・肺門部型の別を問うこととした。（これに伴い、研究実施計画書に定義とシェーマを追加）「術前」の注意書きを追記する。

- 心臓超音波検査：検査時期の同定の項の追加（24 時間以内かどうか）、TR の有無の項の追加、TR の最速流量の追加を行う。「術前」の注意書きを追記する。
- 治療的介入：血液ガスで FiO2 と MAP を記載するのに伴い、呼吸条件の項は削除する。ECMO の適応理由で高度肺低形成を PPHN に変更する。
- その他の薬剤投与：ミリスロールの項を追加する。
- CDH 根治術：横隔膜修復法の項について、「自己筋組織」と「その他」を独立させる。
- 退院までの合併症：CDH 再発、聴力検査異常の項を追加する。

8）経理事務について

- 研究分担金は、研究代表者一括計上とし、研究分担者 1 名あたり暫定的に 15 万円として、今後謝金等で予算に余裕が出れば、再配分する旨の説明があり、了承された。

9）メンバーのメールアドレスについて

- net 上での相談を容易にするため、サーバー上で稼働できるメーリングリストを早急に設定することになった。

以上

平成 23 年度　CDH 研究班　第 2 回班会議議事録

日　時　：　平成 23 年 10 月 27 日（木）18：00～20：00
場　所　：　大阪国際交流センター　3 階 会議室　1
出席者（11 名）：稲村先生、臼井、奥山先生、金森先生、神山先生、塩野先生
高橋先生、谷先生、永田先生、早川先生、藤野先生（五十音順）

1）研究テーマの選定と役割分担について

- 早川先生より、一次調査に関する解析の結果、CDH の治療成績向上のためには、症例の集約化が必要という結論が導き出せること、また、これを論文にすることを予定しているとの報告があった。
- 一次調査の結果については、新生児学会誌または小児外科学会誌などに、アンケート報告として投稿してもよいのではないかという意見があり、来年の新生児学会におけるシンポジウム（二次抄録）との兼ね合いもあるので、今後の動向をみながら投稿も考慮することとなった。
- 高橋先生：右側 CDH についての予後予測因子では 45 例の解析で、出生前因子では MRI の肺底部所見が、出生後因子では highest PaO2、lowest PaCO2 がよい予後予測因子になることが明らかとなった。心疾患合併の CDH については、94 例あり、結論として CDH の重症度よりも合併する心奇形の重症度によって予後が規定されることが明らかとなった。　→　高橋先生には、分担研究として、この 2 つのテーマを中心にご報告いただく。
- 稲村先生：出生前診断の有無についての予後解析の報告があった。また、心臓超音波検査の結果から重症度評価ができるかどうかを検討したところ、生存・死亡については、LVDD、EF、CI、肺動脈径などで有意差が出ることが明らかとなった。　→　稲村先生には、分担報告として、出生後 24 時間以内の心エコー検査からみた予後予測因子についてご報告いただく。
- 奥山先生：Isolated かつ 35 週以上の症例について、手術時期（48 時間以内・以降）と治療成績の関係について解析したところ、Apgar score を 5 以下と 6 以上で重症度をそろえて比較しても、48 時間以内手術の方が、治療期間（人工呼吸、酸素投与、入院期間など）が短縮されることが明らかとなった。　→　奥山先生には、分担研究として手術時期と予後（治療成績）との関係をご報告いただく。
- 早川先生：出生前 MRI 所見によるスコア化について解析を行い、対側肺の肺底部所見や胃の所見、肝の所見で良好な予後予測が可能なことが明らかとなった。スコアに重み付けを行うと、予後予測精度はさらに高めることが可能。　→　早川先生には、分担研究として、出生前 MRI 所見による予後予測についてご報告いただく。
- 手術時の所見に基づく予後予測（重症度予測）については、金森先生に分担研究としてご報告いただく。
- 出生後 24 時間以内の血液ガスデータに基づく予後予測（重症度予測）については、藤野先生に分担研究としてご報告いただく。
- 二次調査についての第一論文（わが国の nationwide survey における contemporary な疫学調査）の報告と、出生後 24 時間以内の所見（Apgar, XP 所見, PDA シャントなど）に基づく予後予測についても、今年度の報告書用の研究テーマとして分担する必要があるが、永田先生に持ち帰っていただき、田口先生と相談してテーマを選択していただくことになった。（後日、九大で第一論文を担当してもらうことをご承諾いただいた。出生後 24 時間以内の所見についての解析は、臼井が担当することとした。）
- それ以外にも、短期合併症や投与薬剤など、まだ解析に使えるデータがあるため、来年度以降に継続して採択された場合に備えて、引き続き研究テーマを検討していくことになった。

2）現在までの進捗状況と今後の日程について

- 事務局より、一次調査が終了し、結果のまとめを日本小児外科学会学術・先進医療検討委員会に提出し、学会ホームページに掲載予定の原稿も審査が終了したことが報告された。
- 早川先生より、一次調査の追加アンケート調査は現在進行中で、現在約 50 の回答が寄せられており、近々もう一度催促するメールを送付する予定であることが報告された。
- 事務局より、二次調査は現在時点で約 560 例のデータ入力を完了し、約 500 例がデータクリーニングも終了したこと、最終的にデータ総数は約 600 例になる予定であることが報告された。
- 11 月末頃を目途に確定データをメール配信し、12 月の班会議で各研究テーマの再確認と、内容が重ならないような相互調整を行ったのち、1 月末必着で分担研究報告書の原稿を書いていただく予定であることが報告された。
- 今年度の分担研究費 15 万円について、12 月末までに使用していただくよう事務局からお願いした。ただし、もし来年から 2 年間の継続課題の研究費が獲得できた場合には、使い残しの分担金を来年分に上積み配分予定であることが報告された。

3）平成 24 年～25 年度研究費補助金申請に際しての新たなテーマについての提案

- 長期フォローについて、追加してデータを集めて、遠隔期の研究を行う。
- 長期フォロー症例について研究するなら、運動能力評価も行う。
- CDH の登録システムを創設してはどうか。
- CDH についての多施設共同の prospective study や CRT などの仲介をしてはどうか。
- 胎児治療の適応決定に繋がる研究を行ってはどうか。

4）平成 24 年～25 年度研究費補助金申請時の分担研究者の推薦について

- もし、胎児治療の適応等についても今後検討するのであれば、産科の先生にもご参加いただいてもよいのではないかという意見があった。

5）その他：

- 今後の学会発表等は、分担テーマを中心に、研究分担者や研究協力者が筆頭となって抄録を提出していただいてよいが、抄録についてはメーリングリスト等で各分担研究者に前もって回覧する。
- 第一論文では、調査票研究に協力していただいた全施設名を credit として、acknowledgement や appendix などに記載する。

6）次回班会議の日程

- 12 月 10 日（土）、新大阪において 11 時頃より 4 時間程度開催の予定。