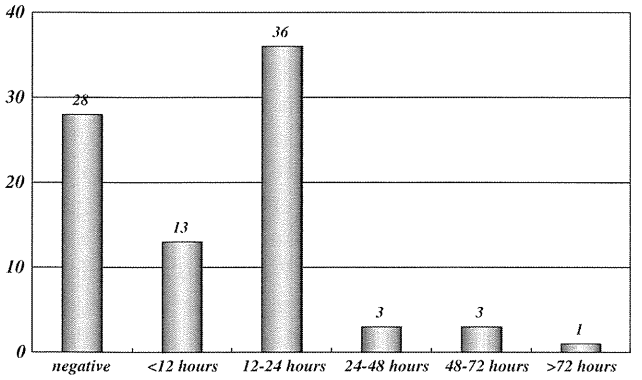

Fig. 1 Time to positivity of blood cultures

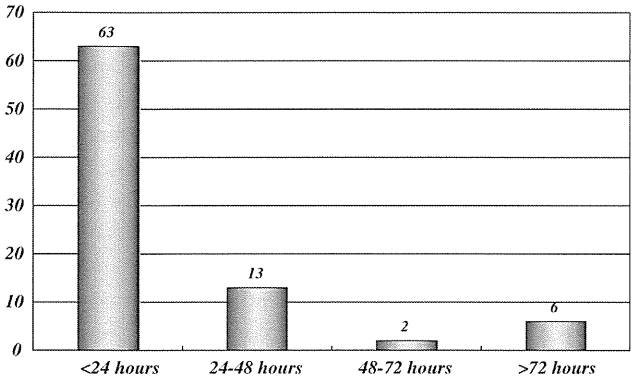

Fig. 2 Time to fever defervescence

event was found to be enterocolitis, whereas three events were recognized as CRBSI. For 28 events with negative culture, it was determined that the febrile event is not caused by CRBSI. Of the 28 events, in 3 events in which the fever persisted over 48 h, another obvious origin was determined. These results are summarized in (Table 2).

Of the 84 laboratory examinations, the 56 exams with positive blood culture were termed the positive group. The other 28 exams were termed the negative group. The count of white blood cell (WBC), the proportion of the neutrophil (%neu) and C-reactive protein (CRP) of each group were investigated as indicators of bacterial infection. The median and interquartile range of each examination is described in (Table 3). No statistical difference was observed in the three examinations (WBC; $p = 0.15$, %neu; $p = 0.11$ and CRP; $p = 0.64$).

During the trial period, no CVC was removed due to failure to control the infection. Replacement of the CVC was performed three times because of breakage or obstruction of the CVC. On all three occasions, we were able to replace the CVC in the same vein.

Discussion

When patients depend on PN, the existence of a venous access site for the CVC must be ensured. Once the catheter has been removed, the vein will be occluded. When the removed CVC reveals a negative culture, the vein may be wastefully occluded. In fact, it is reported that half of the CVCs removed for suspected CRBSI result in a negative culture [3]. When the venous access site for CVC insertion is no longer available, it may be fatal for patients who depend on PN. Therefore, we should strive to treat patients with CRBSI without removing the CVC when they depend on PN.

However, CRBSI is a devastating complication for patients with CVC. The best treatment is to remove the infected CVC [2] when CRBSI occurs. Furthermore, it is reported that the most sensitive and specific technique for diagnosis of CRBSI is the culture of the catheter tip [4]. But removal of CVC may not be the best choice for the patients who depend on PN.

We must consider CRBSI when patients with CVC develop high-grade fever. However, the difficulty of the exact diagnosis of CRBSI lies in the lack of specific

Table 2 Summary of the clinical course of febrile events in our patients

		Blood culture			Total
		Positive		Negative	
		Within 48 h	After 48 h		
Fever resolves within 48 h	Number of events	48	3	25	76
	Diagnosis	CRBSI	Contamination	Viral infection	
Fever persists over 48 h	Number of events	4	1	3	8
	Diagnosis	1 (enterocolitis), 3 (CRBSI)	Infection of EB virus	1 (enterocolitis), 1 (otitis media), 1 (side effect of vaccination)	
Total		52	4	28	84

Table 3 Laboratory test results

	Blood culture		
	Positive	Negative	*p*
WBC	7,305 ± 5,002	8,350 ± 5,565	0.15
%neu	70.3 ± 19.5	65.05 ± 21.4	0.11
CRP	0.41 ± 1.27	0.47 ± 0.92	0.64

Values are expressed in (median ± interquartile range)

symptoms and signs suggestive of CRBSI [5]. It is essential to treat it as soon as possible in a proper way. For prompt initiation of treatment, prompt diagnosis of febrile origin is required.

Blood culture is the required examination to diagnose CRBSI. For diagnosis of CRBSI, quantitative blood cultures and differential time to positivity have a high-degree of accuracy [6]. However, these examinations cannot always be performed. First, it is difficult to collect a suitable sample for blood culture from peripheral vein with suitable procedures because the vessels of the children who depend on PN tend to be exhausted. Furthermore, these procedures cannot be performed in all hospitals. In our hospital, only blood culture obtained through CVC is carried out. Even if the results of this procedure are an overestimation, it is useful as it incorporates necessary conditions of the diagnostic criteria for positive quantitative blood cultures or differential time to positivity. As a result, 52 (93%) specimens were revealed to be positive 48 h after being collected. We regard them as CRBSI, whereas the remaining four specimens were considered false positives with contamination. Shah et al. [7] mentioned similar indication; blood cultures detected 48 h after specimen collection did not involve CRBSI.

Specification of febrile origin is an arduous task. At least, it should be assessed whether the fever was caused by bacterial infection before the antibiotics were administered. In this study, we examined differences between two groups that were divided based on the results of blood culture (positive or negative group). We found no difference between the two groups in terms of the indications for bacterial infection such as WBC, %neu and CRP. This result can be explained by the fact that we collected the blood samples quickly after we had noticed the febrile episode. But it is incongruous to postpone treatment initiation until the laboratory data are observed.

Gram-negative bacilli such as *Escherichia coli* or *Klebsiella* were detected frequently from blood cultures in our study. In many other reports, MRCNS is the species of bacteria detected most frequently [3, 8, 9]. This discrepancy can be explained by the difference of the composition of the underlying disease. In our study, most of the patients suffered from motility disorders of the alimentary tract. On the other hand, in other reports, CVC was applied in most cases for short bowel syndrome. When we analyzed the three patients with short bowel syndrome, a similar trend was observed; we examined the blood culture nine times and revealed positive culture five times. Of the five tests, gram-positive cocci were detected in four tests.

Another important issue is the length of time antibiotics are administered. In the guidelines published by Infectious Disease Society of America, it is recommended that

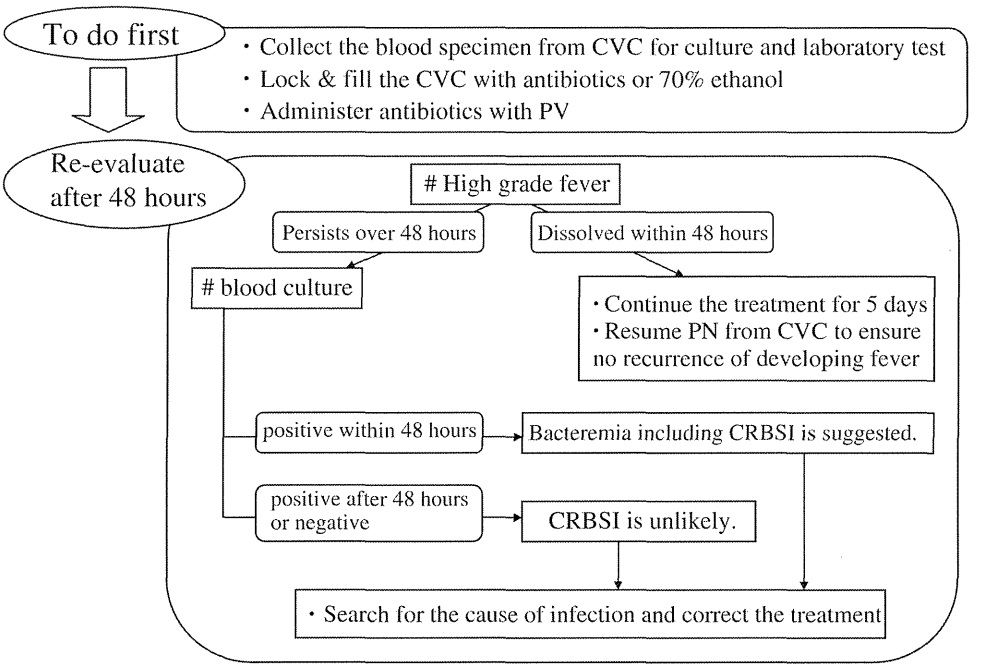

Fig. 3 Our strategy against suspected CRBSI in PN patients

antibiotics should be administered for 10–14 days [10]. In our hospital, infected CVC was locked and filled with antibiotics or 70% ethanol and the antibiotics were administered from the peripheral vein for 5 days. This treatment reduced the patients' fevers within 48 h in 76 (90%) events. When the CVC is locked, the supply of micro-organisms is cut off. Furthermore, the antibiotics or ethanol used in the CVC will destroy the microorganisms.

In conclusion, we recommend that patients with CVC who develop a high-grade fever suggestive of CRBSI begin treatment immediately. Treatment for CRBSI should be started before exact identification of the cause of infection. After 48 h, we can obtain the result of blood culture whether the fever is resolved or not. The treatment can be corrected after the re-evaluation according to the information (Fig. 3). It is noteworthy that using our strategy, no CVC was removed because of failure to control infection.

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ORIGINAL ARTICLE

Effect of an omega-3 lipid emulsion in reducing oxidative stress in a rat model of intestinal ischemia–reperfusion injury

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Abstract
Objectives The usefulness of omega-3 lipid emulsions has been extensively studied. The objectives of the present study were to examine the effect of an omega-3 lipid emulsion in reducing oxidative stress in a rat model of intestinal ischemia–reperfusion injury and the underlying mechanism.
Methods A total of 66 rats were divided into three dietary groups (lipid-free, soybean oil, and fish oil groups). Each animal was administered total parenteral nutrition for 3 days, followed by induction of intestinal ischemia for 100 min. Animals subjected to sham surgery served as the controls. Intestinal tissue and blood were harvested 6 and 12 h after the surgery, then, assessment of the histological damage score, plasma-related parameters, and statistical evaluation were performed.
Results The histological damage score in the intestinal tissues was significantly lower in the fish oil group than in the soybean oil group ($P = 0.0121$). The late-phase urinary level of 8-hydroxy-2-deoxyguanosine was also significantly lower in the fish oil group as compared with that in the other groups ($P = 0.0267$). Furthermore, the plasma level of high-mobility group box 1 protein was also significantly lower in the fish oil group as compared with that in the lipid-free group ($P = 0.0398$).
Conclusion It appeared that intravenous administration of an omega-3 lipid emulsion prior to ischemia–reperfusion injury reduced the oxidative stress and severity of tissue damage. Modification of membrane fatty acids may serve as the mechanism underlying this reduction of tissue damage.

Keywords Ischemia–reperfusion injury · Omega-3 · Oxidative stress · HMGB1

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Introduction

In recent years, the beneficial effects of omega-3 lipid emulsions have been extensively studied in various pathological conditions, including in models of pancreatitis [1], ischemia–reperfusion injury of the small intestine [2, 3], and nonalcoholic steatohepatitis (NASH) [4]. On the other hand, in the clinical setting, acute and chronic administration of general-lipid-strengthened parenteral nutrition has been reported to be associated with elevated risk of liver disorders [5], coronary artery disease, inflammatory bowel disease, and NASH [6].

As an exception to the above-mentioned, omega-3 lipid emulsions have been suggested to reduce the extent of

tissue damage in intestinal ischemia–reperfusion injury by decreasing the oxidative stress. Although this protective effect has been observed in previous studies using rat models of ischemia–reperfusion injury of the small intestine [2, 3], the pathogenesis of the injury and the mechanism underlying the protective effect of omega-3 lipid emulsions against this injury remain poorly understood. The aim of the present study was to evaluate the protective effect of omega-3 lipid emulsions against tissue damage in intestinal ischemia–reperfusion injury, which is commonly seen in ischemic bowel disease, ischemia–reperfusion injury, and small bowel transplantation.

Materials and methods

Experimental animals

Male Crlj: WI rats (250–300 g, 7–8 weeks) obtained from Charles River Laboratories (Yokohama, Japan) were housed in aluminum cages at room temperature (23 ± 3 °C, humidity of 55 ± 3 %) under a 12-h light–dark cycle. All procedures were approved by the Keio University Animal Ethics Committee and Committee on the Animal Experiments of Otsuka Pharmaceutical Factory, Inc.

Experimental design

One week prior to the start of the experiment, the rats were fed a modified AIN-93G diet (Nosan Corporation, Yokohama, Japan), containing a soybean-oil-derived lipid and no fish oil. A central venous catheter (advanced silicon-body plastic tube, 0.5–1.0 mm) was inserted into the internal jugular vein of each rat after the animal had been denied access to food for 12 h, while water was still made available ad libitum. Total parenteral nutrition (TPN) was started on day 0. The rats were divided into three groups (fish oil, soybean oil, and lipid-free groups), and were administered different components of lipids, as shown in Table 1. Omegaven (Fresenius Kabi GmbH, Linz, Austria) was used as the fish-oil-enriched lipid emulsion, and Intralipos Injection 10 % (Otsuka Pharmacy, Naruto, Japan) was used as the soybean-oil-enriched lipid emulsion. TPN was administered for 3 days based upon previous observation by the co-authors of membrane fatty acid changes after 3 days of TPN [7]. Each animal was given a standard caloric supply of 210 kcal/kg/day, corresponding to 30 kcal/kg/day in humans. In the lipid mixture group, 30 % of the total calories were derived from fat. An identical amount of calories was provided by carbohydrates in the lipid-free group. The ratio of amino acids:lipids:glucose of 13:30:57 during TPN was applied according to the recommendation of the European Society for Parenteral and Enteral Nutrition and Metabolism (ESPEN) [8]. On day 3, after a 2-h infusion of extracellular fluid, the rats were subjected to intestinal ischemia–reperfusion, as described below, or sham surgery. After the surgery, the infusion of extracellular fluid, and food and water were withheld. Rats were killed 6 h or 12 h after reperfusion, and intestinal tissue, urine, and blood samples were harvested.

A total of 66 rats were randomly assigned to the following six groups:

1. lipid-free TPN undergoing sham surgery, killed after 6 h ($n = 3$) and killed after 12 h ($n = 5$)
2. soybean oil TPN undergoing sham surgery, killed after 6 h ($n = 3$) and killed after 12 h ($n = 5$)
3. fish oil TPN undergoing sham surgery, killed after 6 h ($n = 3$) and killed after 12 h ($n = 5$)
4. lipid-free TPN undergoing reperfusion surgery, killed after 6 h ($n = 6$) and killed after 12 h ($n = 8$)
5. soybean oil TPN undergoing reperfusion surgery, killed after 6 h ($n = 6$) and killed after 12 h ($n = 8$)
6. fish oil TPN undergoing reperfusion surgery, killed after 6 h ($n = 6$) and killed after 12 h ($n = 8$)

Table 1 Components of infusion solutions

	TPN with lipid-free	TPN with soybean oil	TPN with fish oil
Water volume (mL/kg/day)	260	260	260
Glucose (g/kg/day)	46	30	30
Amino acids (g/kg/day)	7	7	7
Lipids (g/kg/day)	0	7	7
Total calories (kcal/kg/day)	211	211	211

Surgical techniques

The experimental animals were handled as previously reported [9]. General anesthesia was administered by isoflurane inhalation. The superior mesenteric artery was occluded with a clamp, and the small bowels were reperfused after 100 min of ischemia.

Histological assessment of the intestine

5 cm specimens of the small intestine were randomly harvested from a region 10 cm proximal to the terminal ileum and processed for histological examination; after the specimens were fixed in formaldehyde (10 %), they were stained with hematoxylin–eosin. Each intestinal specimen was scored for evaluating the severity of tissue damage using the Park injury scoring system. The scores in this

rubric grade from 0 to 8 (Table 2; [10]). To reduce sampling error, each sample was divided into four parts, and each part was evaluated.

Biomarkers

Urine samples were collected 0–6 h after the reperfusion (early-phase urine samples) and 6–12 h after reperfusion (late-phase urine samples) and preserved in a freezer at −80 °C. Blood samples were collected from the inferior vena cava soon after the animals were killed, and the plasma specimens were stored in a freezer at −80 °C. Plasma levels of oxidative stress markers levels, including 8-hydroxy-2-deoxyguanosine (8-OHdG) and isoprostane, and also the concentrations of prostaglandin E2 (PGE2), and high-mobility group box 1 (HMGB1) protein were measured by enzyme-linked immunosorbent assay (ELISA). (The HMGB1 ELISA kit of Shino-Test Corporation, and oxidative stress marker ELISA kit of Japan Institute for the Control of Aging, NIKKEN SEIL Co., Ltd., were used for this study.)

Statistical analysis

Statistical significance was set at $P = 0.05$. In two-group comparisons, Bonferroni correction was used for adjustment of the significance level ($P = 0.05/2$). Statistical analysis was carried out using *EXSUS* (CAC Corporation), based on the *SAS* (SAS Institute Ltd.). Histopathological scores were statistically compared among the groups using Dunnett's test, F test, student's t test, and the Aspin–Welch test. Urine and blood sample scores are presented as mean ± SD. Dunnett's tests were used for two-group comparisons (i.e., comparison between the lipid-free and soybean oil groups, lipid-free and fish oil groups, and, the soybean oil and fish oil groups).

Table 2 The severity of tissue damage using the Park injury scoring system

Grading	Morphological change
0	Normal mucosa
1	Subepithelial Gruenhagen's space at villus tip
2	Extended subepithelial villus sides
3	Epithelial lifting along villus sides
4	Denuded villi
5	Loss of villus tissue
6	Crypt layer infarction
7	Transmucosal infarction
8	Transmural infarction

Results

Histology of the small intestine

There was no mucosal damage in an intestinal tissue after a sham surgery (Fig. 1). The intestinal tissue specimens from the rats in the soybean oil group exhibited severe mucosal epithelial necrosis and shedding, as well as deep-layer necrosis (Fig. 2d). The tissues from the rats in the fish oil group also showed deep-layer necrosis, but no mucosal epithelial necrosis. The rest of the damage in the fish oil group showed no or only mild intestinal tissue damage (Fig. 2f). The histological damage score was significantly lower in the fish oil group compared with that in the soybean oil group ($P = 0.0121$).

The 8-OHdG levels in the late-phase urine samples were significantly higher in the soybean oil group than in the fish oil group (101.7 ± 38.1 vs. 61.7 ± 21.3 ng/mg creatinine (CRE), respectively, $P = 0.0267$) (Fig. 3).

The PGE2 score, based on the plasma levels of inflammatory eicosanoids, after 6 h of reperfusion tended to be lower in the fish oil group than in the soybean oil group (214 ± 74 vs. 416 ± 258 pg/mL, $P = 0.1737$) (Fig. 4). At 12 h after reperfusion, plasma levels of HMGB1, a mediator of endotoxic shock and sepsis, were significantly lower in the fish oil group (0.763 ± 0.32 ng/ml) than in the lipid-free group (1.4 ± 0.63 ng/ml, $P = 0.0398$) (Fig. 5).

Discussion

Ischemia–reperfusion injury occurs frequently in small bowel transplantation, and often constitutes a major complication of this procedure.

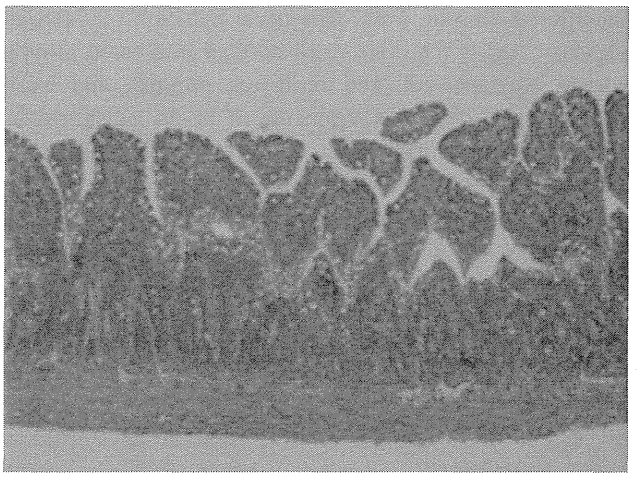

Fig. 1 Histological findings in sham surgery group. No mucosal damage was seen (hematoxylin–eosin staining×100)

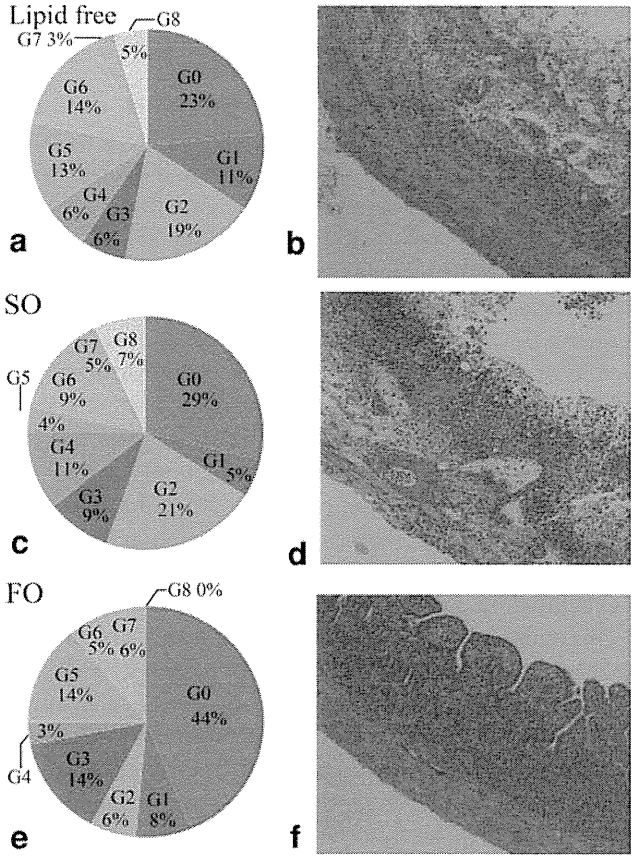

Fig. 2 Representative findings of intestinal tissue specimens after ischemia–reperfusion injury (**b**, **d**, **f**) (hematoxylin–eosin staining×100). Tissue damage scores were also shown (**a**, **c**, **e**). The histological damage score was significantly lower in the fish oil group as compared with that in the soybean oil group ($P = 0.0121$)

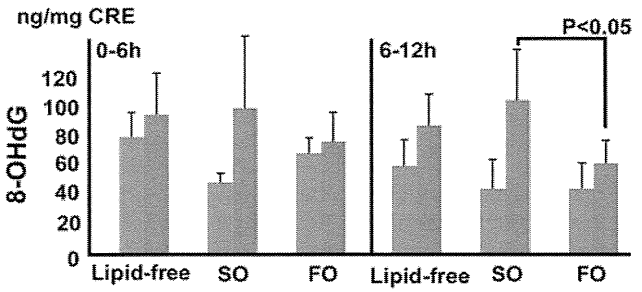

Fig. 3 The 8-OHdG levels were significantly lower in the fish oil group than in the soybean oil group in the late phase (101.7 ± 38.1 vs. 61.7 ± 21.3 ng/mg CRE, respectively, $P = 0.0267$)

In the literature, the mechanism underlying intestinal ischemia–reperfusion injury is typically described as a cascade (Fig. 6). Ischemia–reperfusion injury causes an increase in intracellular calcium, which triggers phospholipase activation and up-regulation of inflammatory eicosanoids, such as the '4' series of leukotrienes (LT), '2' series of thromboxane (TX), and prostaglandin (PG). With

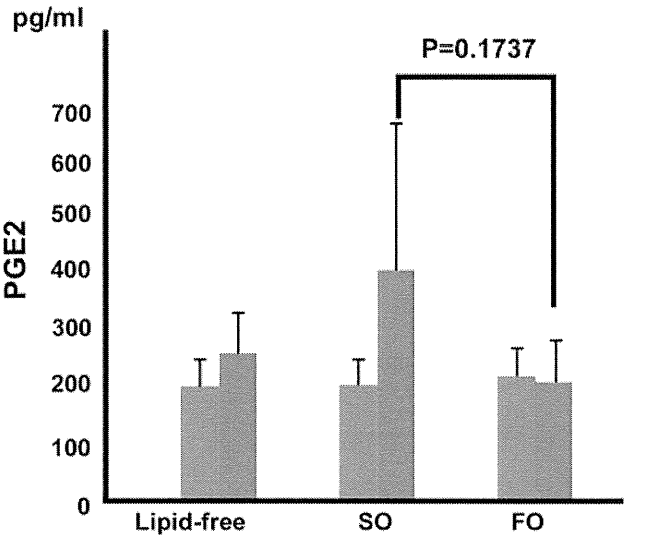

Fig. 4 The PGE2 score at 6 h tended to be lower in the fish oil group than in the soybean oil group (214 ± 74 vs. 416 ± 258 pg/mL, $P = 0.1737$)

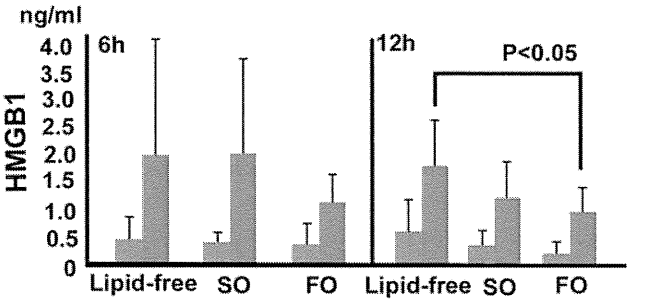

Fig. 5 The plasma levels of HMGB1 were significantly lower in the fish oil group than in the lipid-free group (0.763 ± 0.32 vs. 1.4 ± 0.63 ng/ml, $P = 0.0398$)

the formation of reactive oxygen species (ROS), including superoxide and hydroxyl radicals, nuclear oxidation occurs, resulting in the formation of 8-OHdG as a metabolite. Urinary 8-OHdG formed by nuclear peroxidase appears in the urine in the late-phase of ischemia–reperfusion injury, particularly after 24 h [11, 12]. The production of ROS is associated with induction of cell membrane damage. In addition, neutrophils and macrophage are also activated, increasing the production of inflammatory cytokines. This process also leads to the production of HMGB1, which has been regarded as a mediator of late-phase inflammatory signaling in ischemic injury of organs such as the lung and liver [13, 14]. A characteristic finding of small-intestinal injury was that the plasma HMGB1 concentrations increased more rapidly as compared with that following injury to other organs [15, 16]. Lower plasma levels of HMGB1 were found in the fish oil group as early as at 6 h after the ischemia–reperfusion injury in the present study.

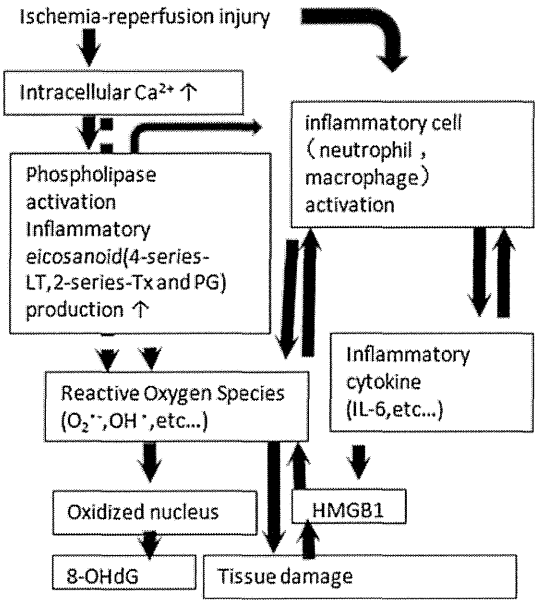

Fig. 6 The mechanism underlying intestinal ischemia–reperfusion injury is typically described as a cascade

Fatty acids derived from fish oil, such as eicosapentaenoic acid (EPA), result in the formation of the '5' series of PGs, TXs, and LTs. These substances are thought to down-regulate the inflammatory response [7]. In contrast, fatty acids derived from soybean oil, such as arachidonic acid (AA), result in the formation of the '2' series of PGs and TXs and the '4' series of LTs, which promote inflammatory responses [7]. Omega-3 lipid emulsions have been reported to down-regulate the production of inflammatory cytokines, such as IL-6 and TNFα [17]; however, only a limited amount of evidence has been accumulated. The present study was aimed at investigating the effect of fish-oil-derived fatty acids against ischemia–reperfusion injury of the intestine, as compared to other lipid components. The severity of the injury was assessed by evaluation of the changes in the plasma levels of inflammatory markers and oxidative stress markers, and the histopathologic tissue damage scores.

In the current study, fish oil administration significantly reduced the severity of histological damage in the fish oil group as compared with that in the soybean oil group. Reduction in the plasma levels of oxidative stress markers was observed, along with a decrease of the plasma HMGB1 levels. Therefore, the present observations indicate that the severity of tissue damage was reduced through down-regulation of oxidative stress and inflammatory responses. In addition, lower plasma levels of inflammatory eicosanoids observed in the present study also suggest that attenuation of the change in the omega-3/omega-6 ratio in the membranous lipid may play a major role in reducing the tissue damage. In the data, a few outliers made fairly large SD, especially in HMGB1 and PGE2. In addition to the delicate surgical animal model, dynamic changes of these parameters in vivo could result in these variabilities.

Prior administration of the omega-3 lipid emulsion reduced the plasma/urinary levels of inflammatory markers both in the early and late phases of ischemia–reperfusion injury. Consistent with the results of the current study, Byrne et al. [3] reported suppressed neutrophil adherence, which reduced the severity of ischemia–reperfusion injury, and also that omega-3 lipids mimic the early events in the injury. Furthermore, Sukhotnik et al. [2] reported decreases in the severity of intestinal mucosal injury and enterocyte apoptosis following intestinal ischemia–reperfusion injury in the rat.

These observations, including our own, suggest that the efficacy of fish oil may be attributable not only to a single step action in the late phase, but also to several steps in the inflammatory cascade. Therefore, administration of omega-3 lipids prior to intestinal ischemia may exert a significant beneficial effect against intestinal tissue injury.

Our current results indicate the clinical efficacy of omega-3 lipids in reducing intestinal ischemia–reperfusion injury commonly seen after intestinal transplantation. Future studies on omega-3 lipids are warranted for clarifying the mechanism of anti-inflammatory effect more precisely, and subsequently to establish the clinical efficacy of the lipids in a variety of critical conditions.

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Intracranial Hemorrhage Associated With Vitamin K–deficiency Bleeding in Patients With Biliary Atresia: Focus on Long-term Outcomes

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ABSTRACT

Background and Aim: The prophylactic oral administration of vitamin K to newborns has markedly reduced the incidence of vitamin K deficiency (VKD); however, intracranial hemorrhage (ICH) is still one of the complications found in biliary atresia (BA) patients and is associated with VKD bleeding. Therefore, we aimed to investigate the incidence and long-term outcome of ICH in patients with BA who previously received prophylactic vitamin K during the neonatal period.

Methods: Eighty-eight consecutive infants with BA were treated and followed up at Kyushu University Hospital from 1979 to 2009. The clinical records and imaging study results were retrospectively reviewed in the infants with BA who presented with ICH.

Results: ICH occurred in 7.95% of patients with BA. The onset of ICH occurred at 47 to 76 days after birth, before the patients underwent surgery for BA (9–37 days after the onset of ICH). Coagulopathy was found upon admission in all of the cases with available data and improved after intravenous administration of vitamin K. A craniotomy was required in 2 cases before the surgery for BA. During the 22 to 278 months of follow-up, some neurologic sequelae persisted in 5 of 7 cases. Follow-up head computed tomography scans showed a low-density area in the left hemisphere in 5 cases.

Conclusions: Although vitamin K prophylaxis had been given during the neonatal period, ICH-associated VKD bleeding was still found in 7.95% of patients with BA. Persistent neurologic sequelae were found in 5 of 7 cases, with low-density area in the left hemisphere.

Key Words: biliary atresia, intracranial hemorrhage, vitamin K–deficiency bleeding

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Biliary atresia (BA) is a rare disease with an occurrence rate of 1 in 8000 to 18,000 live births (1). The clinical symptoms of BA characteristically include jaundice and acholic stools at 1 or 2 months after birth. Although extremely rare, the presentation of a bleeding disorder as the first symptom of BA has also been reported (2). Intracranial hemorrhage (ICH) is one of the complications found in patients with BA caused by late-type vitamin K–deficiency bleeding (VKDB), which occurs most commonly at the age of 1 to 2 months (3,4). This complication causes not only mortality but also significant long-term morbidity in the survival BA before and even after liver transplantation (2,3,5,6).

Vitamin K is required for the synthesis of coagulation factors II, VII, IX, and X by the liver (7); however, the vitamin K level in the newborns is usually low because of the insufficient vitamin K stores of the newborn and low placental transfer of vitamin K (8,9). In Japan, prophylactic oral administration of vitamin K to newborns at birth, on the sixth day after birth, and 1 month after birth beginning in 1981, has markedly reduced the incidence of idiopathic vitamin K deficiency (VKD) (5). Unfortunately, this prophylaxis has no effect on secondary VKD caused by malabsorption of vitamin K caused by cholestatic disorders such as BA (6).

Although BA had been reported to be one of the major causes of secondary VKD, there are only a few reports on patients with BA presenting with ICH, especially regarding their long-term outcome. In the present study, we describe 7 patients with BA presenting with ICH. We investigated the incidence of ICH in patients with BA who previously received prophylactic oral administration of vitamin K in the neonatal period. Moreover, we also describe the management of BA-associated VKDB complicated by ICH and also focus on the long-term outcome of these patients.

METHODS

Between 1979 and 2009, 88 consecutive infants with BA underwent surgery for BA and were followed up in the Department of Pediatric Surgery of Kyushu University Hospital. Infants who presented with ICH were enrolled in the present study. Their clinical records and imaging studies were retrospectively reviewed. ICH caused by VKDB was diagnosed based on clinical and neurologic signs and symptoms, hematologic examination, and findings on computed tomography (CT) scans of the head. A confirmed case of VKDB should fulfill the diagnostic criteria of at least 2 of the following: hepaplastin level <10% and/or thrombo test <10%; prothrombin time (PT) percentage <10%; activated partial thromboplastin time (APTT) >120 seconds; protein induced by vitamin K absence (PIVKA)-II level exceeding normal controls; improvement of bleeding tendency and PT after 24 hours of vitamin K administration, and by a normal or raised platelet count (7,10).

BA was diagnosed based on clinical presentation including jaundice and acholic stools, hematologic examination, and ultrasonographic findings, and was confirmed by surgical cholangiography. BA types are classified using anatomical classification by Morio Kasai (11).

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Long-term outcomes focusing on neurologic sequelae, such as developmental delay, mental retardation, epilepsy, and hemiparesis, were observed until the end of the follow-up period. Developmental delay was defined as a subset of developmental disabilities in 1 or more developmental domains: gross/fine motor, speech/language, cognition, social/personal, and activities of daily living, on age-appropriate, standardized norm-reference testing. Mental retardation was defined as a condition characterized by intellectual ability that is significantly below average (specifically intelligence quotient [IQ] ≤70) combined with deficits in adaptive abilities. Developmental delay assessment or developmental quotient if available was done by a pediatrician in our hospital, whereas IQ and mental retardation assessment was made by a psychologist/psychiatrist. The development was evaluated in 3 aspects: motor, social, and speech development.

RESULTS

Of the 88 consecutive infants with BA, 7 infants (all girls) presented with ICH (7.95% of patients with BA). The age of onset of ICH ranged from 47 to 76 days (mean 62.0 days), with vomiting, consciousness disorders, seizures, dyspnea, anisocoria, and conjugate deviation as the major symptoms at presentation (Table 1).

All of the patients were born full-term and received oral prophylactic vitamin K during the neonatal period. Neither perinatal complications nor any history of head trauma were found for these infants. During the first 6 months of life, 4 patients received exclusive breast-feeding (BF), 1 patient formula milk (FM) containing vitamin K supplementation, and 1 patient mixed BF and FM (Table 1). Although all of the patients were screened at 1 month of age by an obstetrician, VKDB still occurred before the diagnosis of BA was established. After stabilization of their ICH, 5 patients underwent the Kasai operation and 2 patients underwent hepaticojejunostomy for definitive diagnosis of BA. The timing of the Kasai procedure was 9 to 37 days (mean 22.3) after the onset of ICH (Table 1). The types of BA found during surgery were III-b1-*v* in 3 patients, III-b2-*o* in 1 patient, III-a1-*v* in 1 patient, and I-cyst in 2 patients.

CT scans of the head demonstrated that intraparenchymal hemorrhage was found in 4 cases, subarachnoid hemorrhage in 2 cases, and subdural hemorrhage (SDH) in 3 cases. A midline shift (MS) caused by massive hemorrhage was found in 5 cases; however, only 2 of these patients underwent urgent surgical evacuation of an intracranial hematoma before laparatomy because of anisocoria. Consistent with CT evaluation upon admission, magnetic resonance imaging/magnetic resonance angiography (MRI/MRA) during hospitalization (first–fifth weeks after admission) showed a more detailed description of ICH and its complication to the brain such as the development of encephalomalacia in middle cerebral artery territory in 5 patients, cerebral atrophy in 2 patients, cerebral ventricle enlargement in 2 patients, and atrophic of corpus callosum in 1 patient. During follow-up, 2 patients underwent living-related donor liver transplantation (LDLT) and 3 patients died at the ages of 33, 36, and 278 months of liver failure because liver transplantation could not be performed (Table 2).

Upon admission, routine laboratory examination showed platelet levels within the normal range (range 20.8–61.1 × 10^4 cells/μL), whereas severe anemia was found in 2 patients (range 4.1–4.6 g/dL). Elevated direct bilirubin was found in all of the patients, and elevated alanine aminotransferase and aspartate aminotransferase were found in 6 of 7 cases (Table 3).

Significant prolonged PT and APTT were found in coagulation examination during admission in the patients for whom data were available, with a range of 36.2 to >200 seconds and 67.3 to >200 seconds, respectively. Right after the diagnosis of VKDB was made, intravenous (IV) vitamin K was administered to all 7 patients and fresh frozen plasma (FFP) was given to 5 patients. Follow-up examinations showed improvement in PT and APTT levels from 11.7 to 18.5 seconds and 26.4 to 40.3 seconds, respectively. An elevated PIVKA-II level was also noted in 4 cases (Table 3).

At follow-up examination at 22 to 24 months, developmental delay in all aspects of evaluation (motor, social, speech) was seen in 4 patients, cognitive impairment in 1 patient, and normal development in 2 patients. In 1 patient who had normal developmental evaluation, a mild hemiplegia of the right extremities was observed with upper extremity stronger than the lower 1; however, overall evaluation was normal development. At this point of age, an electroencephalography (EEG) examination was also performed in 4 patients, 3 of whom showed abnormal EEG. One of 3 patients who did not have an EEG evaluation at this time point (case 3) showed clinical epilepsy during follow-up. At this time point, evaluation of the liver function showed elevation of aspartate aminotransferase, alanine aminotransferase, and direct bilirubin in 5 patients (Table 4).

During the 22 to 278 months (mean 90.3) of follow-up, some neurologic sequelae persisted in 5 of the 7 cases. The types of neurologic sequelae found were mental retardation in 2 patients, epilepsy in 1 patient, hemiparesis in 2 patients, and developmental delay in 1 patient who showed bilateral cerebral atrophy. In 2 patients, no neurologic deficits were observed until the end of the follow-up. Follow-up CT scans of the head demonstrated significant ischemic changes shown as a low-density area (LDA) consistent with encephalomalacia in the left hemisphere were found in 5 patients (Table 5). In the present study, a comparison of head CT scans at admission and latest CT at follow-up showed that in case 6, the head CT showed SDH and MS at admission. During follow-up, head CT did not show any new hemorrhage; however,

TABLE 1. Patient characteristics and symptoms at admission

Case	Sex	Infant milk until 6 mo	Onset of ICH, d	Symptoms	Surgery for BA, d	Type of BA (10)
1	F	BF	50	Vomiting	59	III-b1-*v*
2	F	BF + FM	54	Consciousness disorder, seizures	70	III-b1-*v*
3	F	BF	66	Dyspnea, anisocoria	88	III-b1-*v*
4	F	BF	76	Dyspnea, conjugate deviation	110	III-b2-*o*
5	F	BF	47	Vomiting, seizures	84	III-a1-*v*
6	F	FM	69	Consciousness disorder, anisocoria, conjugate deviation, seizures	93	I-cyst
7	F	No data	72	Vomiting	86	I-cyst

BF = breast-feeding; FM = formula milk containing vitamin K supplementation.

TABLE 2. Types of ICH as determined by head CT scans upon admission and/or MRI/MRA during hospitalization, and subsequent procedures performed and outcomes

Case	Sex	Onset of ICH, d	Type of ICH	MRI/MRA during hospitalization (1st–5th wk after admission)	Invasive procedure	Outcome	FU duration, mo
1	F	50	IPH	NA	—	Died	278
2	F	54	IPH, MS(+)	NA	—	Died	36
3	F	66	SDH, IPH, MS(+)	P/O ICH; bilateral SDH; left EDH; bilateral cerebral atrophy; ventricle enlargement; encephalomalacia	Craniotomy	Died	33
4	F	76	SAH	Left temporal lobe encephalomalacia; old SAH	—	Alive (LDLT)	108
5	F	47	SAH, IPH, MS(+)	Laminar necrosis; ventricle enlargement; corpus callosum atrophy; bilateral brain atrophy; encephalomalacia; old SAH; and IPH	—	Alive (LDLT)	80
6	F	69	SDH, MS(+)	P/O ICH; SDH; laminar necrosis; encephalomalacia of left MCA territory	Craniotomy	Alive	75
7	F	72	SDH, MS(+)	Acute bilateral SDH; no new hemorrhage	—	Alive	22

EDH = epidural hematoma; FU = follow-up; ICH = intracranial hemorrhage; IPH = intraparenchymal hemorrhage; LDA = low-density area; LDLT = living donor liver transplantation; MCA = middle cerebral artery; MRA = magnetic resonance angiography; MRI = magnetic resonance imaging; MS = midline shift; NA = not applicable; P/O = postoperative; SAH = subarachnoid hemorrhage; SDH = subdural hemorrhage.

TABLE 3. Results of routine laboratory examinations, coagulation data, and therapy administered upon admission

	Routine laboratory examination					Coagulation data							
						Before vit K administration						After vit K administration	
Case	Hb, g/dL	Platelets, 10^4 cells/μL	AST, g/dL	ALT, g/dL	DB, mg/dL	HPT, %	INR	PIVKA-II, arbitrary unit/mL	PT, s	APTT, s	Therapy	PT, s	APTT, s
1	8.6	23.3	184	97	6.8	<10			No data	No data	Vit K 0.5 mg/kg IV	11.8	34.9
2	7.3	20.8	238	145	9.2	<5			50.3	198.7	Vit K 1 mg/kg IV + FFP	12.4	36.1
3	4.1	38.7	152	101	4.64		3.36	>40	36.2	67.3	Vit K 1 mg/kg IV + FFP	13.1	32.5
4	4.6	48	224	107	9.82				>200	>200	Vit K 1 mg IV + FFP	18.5	37.7
5	8.5	39	155	124	44.79	70.2	1.31	114	>200	>200	Vit K 1 mg IV + FFP	13	26.4
6	10.5	41.3	138	91	9.6			24.7	>50	>200	Vit K 1 mg IV + FFP	11.7	41.6
7	8.0	61.1	43	38	3.2	119	22.7	>75	No data	>150	Vit K 0.5 mg/kg IV (at other hospital)	13.6	40.3

ALT = alanine aminotransferase; APTT = activated partial thromboplastin time; AST = aspartate aminotransferase; DB = direct bilirubin; FFP = fresh frozen plasma; Hb = hemoglobin; HPT = hepaplastin; IV = intravenous; INR = international neutralized ratio; PIVKA-II = protein induced in vitamin K-II; PT = prothrombin time.

TABLE 4. Results of follow-up at 2 years of age in BA patients with ICH

Case	Sex	Development assessment	Detail developmental description (maximum ability compared with age)	EEG	Laboratory examination		
					AST, g/dL	ALT, g/dL	DB, mg/dL
1	F	Developmental delay	NA	NA	236	223	9.4
2	F	Developmental delay	NA	NA	255	135	11.2
3	F	Developmental delay	NA	NA	124	59	21.1
4	F	Cognitive impairment	Motor ≈ 2 y 4 mo, social ≈ 2 y 4 mo, speech ≈ 1 y 8 mo	Abnormal/polyspike (subclinical seizure)	151	98	2.9
5	F	Developmental delay	Motor ≈ 1 y 2'mo, social ≈ 1 y 9 mo, speech ≈ 1 y 1 mo	Abnormal EEG (poor activity in left hemisphere)	102	80	0.2
6	F	Normal development	Normal; mild right hemiplegia (upper extremity stronger)	Abnormal EEG (lazy activity in left frontal lobe)	32	19	0.0
7	F	Normal development	Normal	Normal	34	12	0.1

ALT = alanine aminotransferase; AST = aspartate aminotransferase; BA = biliary atresia; DB = direct bilirubin; EEG = electroencephalography; ICH = intracranial hemorrhage; NA = not available.

LDA was found in the left hemisphere. In addition, neurologic sequelae such as right hemiparesis were observed. In contrast, head CT in case 7 also showed SDH and MS at admission; however, during follow-up, head CT showed a complete resolution and no LDA was found. Clinically, case 7 also did not show any neurologic sequelae (Fig. 1).

DISCUSSION

Several recommendations for vitamin K administration had been proposed for all of the newborn infants (12); however, the prophylactic administration of vitamin K is not sufficient to prevent bleeding disorders in infants with BA (4,6). As described in our study, all of our cases received 2 mg prophylactic oral administration of vitamin K during the neonatal period; nevertheless, ICH still occurred in 7.95% of our patients with BA. The prophylactic failure is probably the result of their increased bleeding tendency caused by secondary late-type VKD associated with cholestasis-induced malabsorption of vitamin K in the digestive tract, as mentioned in previous studies (2,6). This is supported also by a study by Van Hasselt et al (13), which described the risk of VKDB in breast-fed infants with BA receiving 25 μg oral vitamin K from

TABLE 5. Results of follow-up head CT scan and persistent neurologic sequelae

Case	Sex	Type of ICH at admission	CT scan (last examination)	Neurologic sequelae
1	F	IPH	LDA consistent with encephalomalacia, cerebral atrophy, ventricle enlargement	Mental retardation
2	F	IPH, MS(+)	NA	Epilepsy
3	F	SDH, IPH, MS(+)	Cerebral atrophy (bilateral), LDA consistent with encephalomalacia, ventricle enlargement	Developmental delay
4	F	SAH	LDA consistent with encephalomalacia	No sequelae
5	F	SAH, IPH, MS(+)	LDA consistent with encephalomalacia, ventricle enlargement	Mild mental retardation; mild hemiparesis
6	F	SDH, MS(+)	LDA consistent with encephalomalacia, slightly enlargement of lateral ventricle	Mild hemiparesis
7	F	SDH, MS(+)	No abnormalities (LDA [−])	No sequelae

CT = computed tomography; IPH = intraparenchymal hemorrhage; LDA = low-density area; MS = midline shift; NA = not available; SAH = subarachnoid hemorrhage; SDH = subdural hemorrhage.

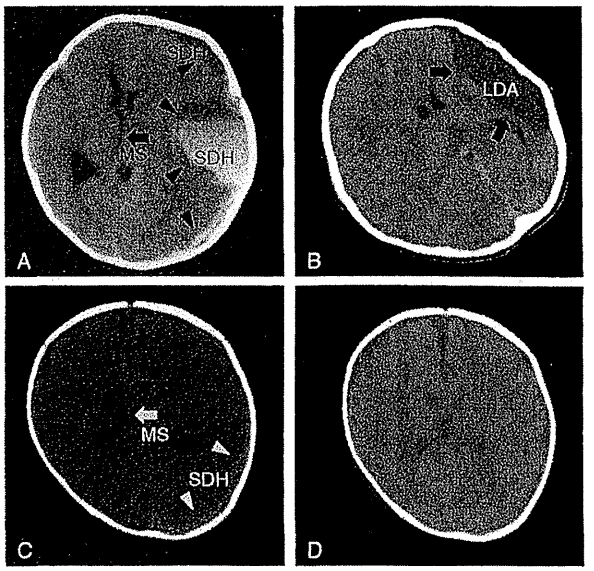

FIGURE 1. Comparison of head computed tomography (CT) scans at admission and during follow-up. A, Head CT of case 6 at admission, showing subdural hemorrhage (SDH) (arrowhead) and midline shift (MS) (arrow). B, Head CT of case 6 during follow-up, showing low-density area (LDA) consistent with encephalomalacia in the left hemisphere without new hemorrhage (arrow). C, Head CT of case 7 at admission, showing SDH (arrowhead) and MS (arrow). D, Head CT of case 7 during follow-up, showing no abnormalities.

the second week until the end of the 13th week was 8 to 10 times higher than in breast-fed infants receiving either a higher weekly oral prophylaxis dose or intramuscular (IM) prophylaxis at birth. More than 80% of the infants in that study developed VKDB by the time that cholestasis was diagnosed, and 43% presented with an ICH. A comparison study of VKD and VKDB risk in cholestatic jaundice infants who received regular infant formula and hypoallergenic formula by Van Hasselt et al (14) also reported that an increased risk is predominant in cholestasis infants receiving (whey-based) hydrolyzed formula. In the present study, we also describe that of 7 patients, 4 patients received BF only, whereas 1 patient received only regular FM and 1 patient received both BF and FM; however, in the present study we could not overestimate that BF is related to the development of ICH because of a lack of data about infant nutrition from the rest of the patients with BA who did not have ICH complication. Moreover, regarding the administration route of vitamin K, Komatsu et al also described a case of ICH in 2 infants with the late type of VKD despite 3 administrations of 2 mg oral vitamin K after birth, at 5 days old and 1 month old (15). Although the underlying diseases of these patients until hospital discharge remain undetermined, it is suggested that prophylactic oral vitamin K failed to prevent ICH in late-type VKD.

In the present study, all of the patients fulfilled the criteria of VKDB. Elevation of the PT at admission (range 36.2 to >200 seconds) was found in all of the patients with available data and was rapidly improved after administration of IV vitamin K and FFP (range 11.7–18.5 seconds). One patient (case 1) did not have exact PT data upon admission (referral information from the previous hospital was not complete); only PT and APTT were written as elevated and the patient had been administered with 2 mg vitamin K intravenously; however, hepaplastin test upon admission was <10% and PT after 24 hours' administration of vitamin K returned to the normal level, combined with improvement of bleeding tendency. Therefore, we concluded that case 1 fulfilled the VKDB criteria, and counted as ICH-associated VKDB. In addition, an elevated PIVKA-II level was also found in all 3 patients whose PIVKA-II levels were measured and 1 patient just written elevation of PIVKA-II in the referral letter. Grossly abnormal PT with a rapid normalization of coagulation test and bleeding tendency after vitamin K administration combined with an elevation of PIVKA-II level, despite a normal platelet count, were characteristic findings of laboratory examinations in patients with VKD (7,10,16,17). The severity of the coagulation disorder was not inversely proportional to the value of hemoglobin. Nevertheless, the severity of anemia was probably related to the symptoms at admission, as shown in our 2 patients with severe anemia, both of whom had dyspnea as a presenting symptom. Nevertheless, dyspnea could also occur as a cause of brain swelling or compression.

In the present study, intraparenchymal hemorrhage with MS was the most common lesion found in CT images. These findings are different from other reports, which found SDH and subarachnoid hemorrhage as the most common types of ICH (8,16). In addition, in 2 of 7 of our cases, >1 lesion was found on CT. This finding is similar to Majeed et al (8), who reported that most of the children presenting with VDKB had hemorrhage in multiple sites, and pallor was seen in all of the infants (100%). Recently, magnetic resonance (MR) imaging was widely used in clinical practice for neonates with brain injury because of its high sensitivity for depicting a developing brain. MR can provide greatly detailed images of brain structures without exposing infants to ionizing radiation (18). CT is frequently used in workup in children with sudden onset of neurologic symptoms, especially to rule out acute hemorrhage. In addition, MR demands particular care with regard to patient transport, monitoring of vital signs, and optimization of acquisition techniques (use of appropriate coils, sequences, and protocols) (18–20). In our institution, we use head CT as a diagnostic tool for emergency brain injury, especially because CT was faster as compared with MR (<5 minutes), did not require any sedation, and allows close monitoring of vital signs during evaluation of unstable neonates after ICH. During hospitalization, except for 2 patients who were born before 1995, MR imaging and MR angiography were done after patients' conditions were stabilized, to obtain an accurate evaluation, which revealed a more clear description of late brain complications as early as the first week after ICH (Table 2).

In spite of the wide variety of lesions in our cases, craniotomy was done only in 2 cases, without correlation to the lesion(s) itself, and was done before laparatomy. The indications of craniotomy of our patients were increased intracranial pressure with worsening of consciousness and the presence of anisocoria suggesting severe brain compression caused by large hemorrhage. The other cases were treated supportively or were treated for increased intracranial pressure or cerebral edema, if suspected. With or without surgical intervention for hematomas, a laparatomy was performed in all of our patients on an average of 22.3 days after the onset of evacuation of ICH and 9 days at the earliest. This was based on the fact that the Kasai portoenterostomy has become the primary surgical treatment for uncorrectable BA (21–23). In patients with BA with ICH as a presenting symptom, early Kasai portoenterostomy is also recommended. In the patients presenting with ICH and in those who have undergone craniotomy, early Kasai portoenterostomy is possible only when both hemorrhage and the bleeding tendency are well controlled. IV administration of vitamin K at 0.5 to 1.0 mg/kg, with or without addition of FFP given concurrently for more rapid restoration, is effective in patients with VKDB (16,24). Vitamin K administration had been reported to improve bleeding tendency within 1 hour and normalizes the

agulation within several hours, to facilitate even an urgent aniotomy (6). In our patients with or without craniotomy, no fficulties in hemostasis or recurrent hemorrhage were noted after itial administration of vitamin K IV and FFP. These findings iggest that surgical intervention for ICH has no adverse effects on e subsequent surgical management of BA if coagulation disorders e managed properly.

Studies on the long-term follow-up reported that some eurologic sequelae, such as developmental delays, mental tardation, and epilepsy, were observed in more than half of the atients with ICH caused by VKD (25,26). In the present study, evelopmental delay in all of the evaluation aspects showed in patients at 2 years of age, which in 3 patients remains until the end f study follow-up. At further follow-up until the end of the present udy (at 22–278 months), none of the patients died of ICH. Causes f death of our patients were end-stage liver diseases in those unable o undergo liver transplantation, in which the progressivity of the isease already could be seen at 2-year follow-up (Table 4). Follow-p imaging studies obtained using head CT scans showed an LDA onsistent with encephalomalacia in the left hemisphere in 5 cases. Until the end of our observation, some neurologic sequelae ersisted in 5 of 7 cases. The type of neurologic sequelae found /ere mental retardation in 2 cases, epilepsy in 1 case, hemiparesis 1 2 cases, and developmental disorder in 1 case, with no correlation o the substantial ischemic brain damage demonstrated on CT. Mental retardation in case 1 was determined by an IQ measurement f 47 combined with a marked decreased ability to interact with ndividuals at 20 years of age without mental retardation. These indings are in agreement with the latest head CT evaluation, which howed a marked cerebral atrophy, cerebral ventricle enlargement, nd encephalomalacia. Unfortunately, the MRI/MRA evaluation is ot available in this patient; however in case 5, mental retardation vas determined by an IQ measurement of 70 at 6 years of age, ombined with a decreased ability to speak, write, and understand onversation. In this patient, in addition to the MRI/MRA findings t 3 months of age (Table 2), a follow-up head CT showed brain trophy, ventricle enlargement, and encephalomalacia. Furthermore, MRI evaluation also showed atrophy at the splenium and he posterior part of the corpus callosum, which explains the low IQ neasurement (figure not shown). In spite of mental retardation, case 5 was enrolled in mainstream elementary school because the mental etardation was on the borderline. Compared with Akiyama et al (3), who found only 2 of 15 patients with long-term neurologic sequelae, the present study showed a higher incidence; however, the long-term outcomes observed in our cases were similar to those for other cases of ICH, which were not associated with BA. In addition, the neurologic sequelae found in our patients did not correlate with the severity of the clinical findings, the laboratory findings, or the need for craniotomy. These findings suggest that the long-term outcomes cannot be predicted from examinations during admission, and even a mild lesion should be managed properly.

In conclusion, although vitamin K prophylaxis had been given during the neonatal period, VKDB-associated ICH was still found in 7.95% of patients with BA, of whom 4 received exclusive BF. In regard to the low concentration of vitamin K in breast milk compared with regular FM, apparently healthy infants who received exclusive BF during 6 months of life and oral prophylactic vitamin K should be monitored closely. Based on our long-term observation, even though the rate of ICH in patients with BA is low, it should be managed properly to prevent long-term neurologic disability, as shown in 5 of our patients. Based on our study and other studies from Japan, we suggested that 2 mg of oral vitamin K 3 times during the neonatal period should be reevaluated for their protective dose against ICH. A study comparing routes of administration of neonatal vitamin K should be conducted to investigate the effectiveness of oral and IM administration of vitamin K in preventing the occurrence of ICH in infants with BA.

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A formula for determining the standard liver volume in children: A special reference for neonates and infants

Saeki I, Tokunaga S, Matsuura T, Hayashida M, Yanagi Y, Taguchi T. A formula for determining the standard liver volume in children: A special reference for neonates and infants.

Abstract: Accurately evaluating the ratio of GV to the SLV (GV/SLV) is important in successful pediatric liver transplantation. However, the formula used to calculate the SLV of children, including neonates and infants, has not yet been established. The aim of the current study was to estimate the SLV of children, including neonates, and to establish an accurate formula. The LV of 100 children (including 7 neonates and 15 infants) were measured using thin slice (3–5 mm) helical CT images. Their BSA was calculated from height and weight. A new formula to estimate the SLV was established as follows: SLV (mL) = 689.9 × BSA (m^2) – 24.7. The SLV of children was significantly lower than that in previous reports ($p < 0.001$). A formula for calculating the SLV of children including neonates was established. This new formula will be useful in pediatric liver transplantation.

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Key words: standard liver volume – children – neonate – computed tomography – liver transplantation

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In LDLT, the preoperative estimation of the GV is important. Either the ratio of the GV to the SLV (GV/SLV) or GRWR is used to decide on the graft type that should be used (1). In adult recipients, small-for-size grafts cause poor liver function, high risk of infection, and high morbidity (2). On the other hand, in small pediatric recipients, large-for-size graft causes insufficient blood supply to the graft and leads to higher risk of compartment syndrome because of the small size of the recipient's abdominal cavity (3–5). To prevent complications associated with large-for-size grafts, graft reduction techniques such as "reduced segment grafts" or "monosegment grafts" may be necessary (6–10). Although a GRWR >4% is reported to be a high risk factor for large-for-size syndrome (4), there are no definite criteria for a large-for-size graft. The accurate estimation of the LV in small children, including neonates, is crucial for determining the criteria for transplantation to avoid a small/large-for-size graft.

In Japan, Urata et al. (11) calculated the LV of 96 patients (65 adults and 31 children) using CT images and proposed the following formula in 1995: LV (mL) = 706.2 × BSA (m^2) + 2.4. This formula is widely accepted in many centers for liver surgery. However, the CT images used in this study were thick slices (8–10 mm), and very small infants (including neonates who a have high risk of large-for-size syndrome) were not included in this study. The reports concerning SLV from other countries also did not analyze the data from small children, including neonates (12–16). Johnson et al. (17) reported a meta-analysis of LV, and data were collected from 5036 subjects from birth to 18 yr old. Although in that report, the ways of LV measurement varies.

The purpose of this study was to establish a new formula for calculating the pediatric SLV, which is applicable to small children including neonates and infants.

Abbreviations: BSA, body surface area; BW, body weight; CT, computed tomography; GRWR, graft-versus-recipient weight ratio; GV, graft volume; LDLT, living donor liver transplantation; LV, liver volume; SLV, standard liver volume.

Patients and methods

Between January 2009 and January 2010, 100 children (0–15 yr old) who underwent an abdominal CT scan in the Department of Pediatric Surgery at Kyushu University Hospital were examined. The reasons for performing the CT examinations were as follows: acute appendicitis (20 cases), malignancy follow-up (24 cases), benign tumor of the abdomen (18 cases), intestinal diseases (19 cases), diaphragmatic hernia (7 cases), trauma (7 cases), and others (5 cases). Patients who had hepatic diseases, disturbances of growth, or patients who might have had hepatomegaly because of underlying disease were excluded from this study. We analyzed disease-free follow-up CT images in cases where patients had malignant diseases (not under medical treatment).

Liver volumetry was performed using a workshop package (AZE VirtualPlace Advance 300, AZE, Tokyo, Japan). We designated the liver area on every slice of abdominal CT scans and made 3D liver images. Each CT slices was taken at a 3 or 5 mm thickness.

The patients' height, BW, sex, and underlying disease data were obtained from their medical records. Patients' height and BW were measured at the CT examination date. Their BSA was calculated by the DuBois formula (18) (BSA $= BW^{0.425} \times Height^{0.725} \times 0.007184$).

This study was approved by the ethical committee of Kyushu University Hospital (number 22–54).

Liver volumetry

To assess the accuracy and precision of liver volumetry using the Virtual Place Advance 300 software package, we preoperatively estimated the LV of 17 recipients who received LDLT at our hospital from 2006 to 2010 and recorded the actual weight of the removed whole liver for comparison. CT examinations of these recipients were performed to assess the collateral circulation and portal vein patency before LDLT. The absolute value of the liver weight is considered to be the actual LV, because the liver organ density is almost the same as water (19).

Statistical analysis

The estimated LV, patient height, BW, and BSA were analyzed by a simple regression model and a logarithmic regression model. The accuracy of the estimations was evaluated by the mean difference between the observed and estimated values. The precision was assessed by the standard deviation of the difference between the observed and estimated values. The smaller absolute mean and standard deviation, respectively, show higher accuracy and precision. The data analysis was performed using the JMP ver8.0 (SAS Institute, Tokyo, Japan) and Stata 11.1 (Light Stone, Tokyo, Japan). A two-sided $p < 0.05$ was considered to be statistically significant.

Results

The accuracy of liver volumetry using the Virtual Place Advance 300 software program

The relationship between the actual removed whole liver weight and the preoperatively estimated LV in LDLT recipients is shown in Fig. 1a. The mean weight of 17 livers removed from recipients was 685.3 g (s.d. 408.8 g), with a range of 184.2–1450 g. The estimated LV of these livers averaged 674.4 mL (s.d. 400.2 mL) with a range of 198.2–1390 mL. The accuracy of liver volumetry was within + 1.05%, and the precision was within 7.25% (Fig. 1b). The LV estimation using our liver volumetry workstation, therefore, is considered to be highly accurate with high precision.

The age distribution of patients

Sex and age of the patients (100 in total) are summarized in Fig. 2. There were 50 boys and 50 girls, including 7 neonates and 15 infants (1–11 months old). The mean age was 5.8 with a range from 0 days to 15 yr old.

SLV in children

The relationship between the BSA and LV of all patients is shown in Fig. 3. There was a strong

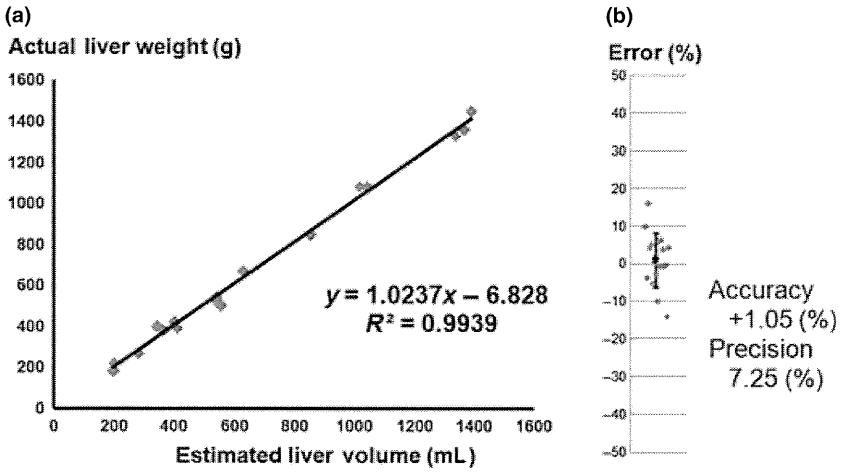

Fig. 1. (a) The relationship between the actual removed liver weight and the estimated LV in liver transplantation recipients. (b) Liver volumetry was performed with high accuracy and precision.

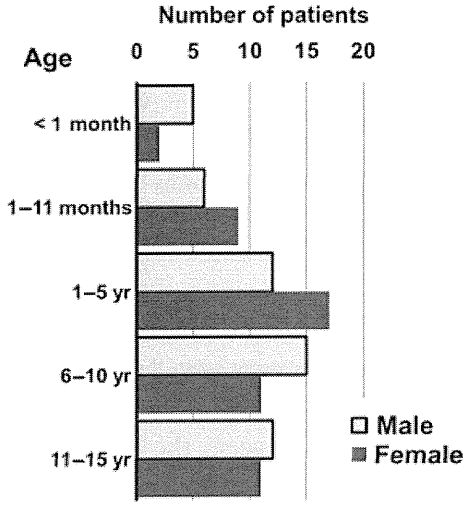

Fig. 2. The distribution of patients. The total number is 100, including seven neonates.

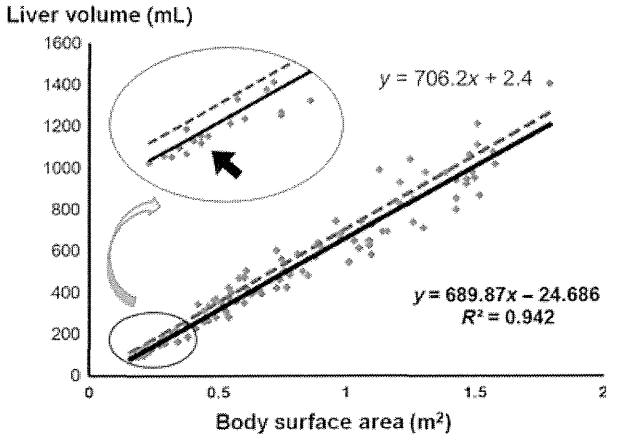

Fig. 3. The relationship between body the surface area (BSA) and LV. The regression line (—) obtained using data for 100 children is significantly smaller than that obtained using the formula proposed by Urata et al. (11) (--). ($p < 0.05$). The new formula (arrow) is more accurate, especially for small children including neonates (expansion).

relationship between the BSA and LV. The following equation was derived from the simple regression line (continuous line):

$$\text{LV (mL)} = 689.9 \times \text{BSA (m}^2\text{)} - 24.7.$$

The value of the coefficient of determination, "R^2," was 0.942. The LV calculated from the children, including neonates, was significantly smaller ($p < 0.001$) than that calculated from the formula proposed by Urata et al. (11). The Urata formula may therefore overestimate LV in children, especially in small children and neonates.

To compare the accuracy of the new formula and the Urata formula in small children, the present data were divided into six groups according to the observed LV (0–200, 200–400, 400–600, 600–800, 800–1000, and over 1000 mL), and the percent error of the estimated LV against the observed LV was analyzed (Fig. 4). In small children whose LV was 0–200 mL, the Urata formula significantly overestimated the LV.

The logarithmic scale analysis of the relationship between the BSA and LV derived the following equation: $\text{LV (mL)} = 661.7 \times \text{BSA (m}^2\text{)}^{1.0977}$.

The value of the coefficient of determination, "R^2", was 0.9662, which was almost the same as the single regression line. The single regression formula "LV (mL) = 689.9 × BSA (m^2) – 24.7" is accurate and easy to use in the clinical setting.

The relationship between the BW and LV, and the relationship between height and LV are shown in Fig. 5. The simple regression line derived from patients whose BW was above 20 kg (n = 43) was "LV (mL) = 15.5 × BW (kg) + 234.3." On the other hand, in subjects smaller than 20 kg (n = 57), it was "LV (mL) = 26.3 × BW (kg) + 27.2". There was a difference in the gradients of the lines between the two groups. This suggests that the meaning of "GRWR" changes between the BW > 20 kg and BW < 20 kg groups (Fig. 5a). Similarly, the simple regression line derived from patients whose height was above 110 cm (n = 50) was "LV (mL) = 10.7 × height (cm) – 714.2" and in patients shorter than 110 cm (n = 50), it was "LV (mL) = 6.23 × height (cm) – 151.6." There was a difference in the gradients of the lines between the two groups (Fig. 5b).

Discussion

In the past few decades, liver transplantation in pediatric patients has become a common operation with a high success rate and limited mortality compared to adult patients. However, liver transplantation in very small children, including neonates, is still associated with some difficulties, such as a large-for-size syndrome, the shape of the donor liver left lobe, and the availability of a temporary abdominal closure using a synthetic wall prosthesis.

It is sometimes necessary to perform liver transplantation in small children, including neonates and infants. In diseases such as urea cycle disorders or hemochromatosis, severe hepatic failure suddenly occurs during their neonatal period, and these patients sometimes need

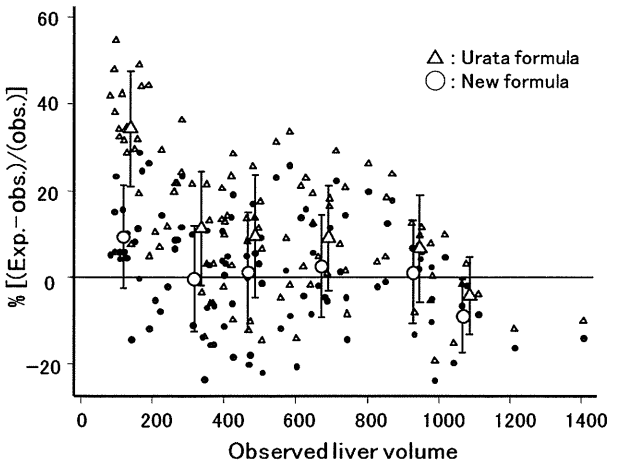

Fig. 4. The relationship between the expected LV (new formula and Urata formula) and the observed LV. The *x*-axis shows the observed LV and the *y*-axis shows the percent error (•: New formula, △: Urata formula). The data were divided into six groups according to the observed LV (0–200, 200–400, 400–600, 600–800, 800–1000, and over 1000 mL). The average and standard deviation of the percent error were calculated for each group (○: New formula, △: Urata formula). In the 0–200 mL group, the Urata formula significantly overestimated the LV. Exp., expected LV; Obs., observed liver volume.

emergency liver transplantation. In addition, some biliary atresia patients require liver transplantation during infancy because of liver cirrhosis. In fact, among the 57 LDLT performed at our institution from 1996 to 2010, seven infants received eight LDLTs because of biliary atresia, hepatoblastoma, and ornithine transcarbamylase deficiency. For preoperative risk assessment of LDLT for such small children, a precise formula to calculate the SLV that is applicable to neonates and infants is needed.

In 1995, Urata et al. (11) first reported a formula for calculating the SLV of adults and children, and this formula is now widely accepted in Japan. However, because no neonates and a little infants were included in the data used to generate this formula, it was unclear whether the Urata formula could provide acceptable accuracy for calculating the SLV of very small children including neonates, and whether it was useful for assessing the risk of large-for-size grafts.

In this report, we investigated thin slice CT images of 100 children, including 7 neonates and 15 infants, and established a new formula for calculating the SLV. The LV was well correlated to the BSA, and single regression line was "LV (mL) = 689.9 × BSA (m^2) − 24.7" (R^2 = 0.942). In the logarithmic graph, the regression line was "LV (mL) = 661.7 × BSA $(m^2)^{1.0977}$," (R^2 = 0.9662). As the sizes of the coefficient of determination were almost the same in the two formulas, the formula "LV (mL) = 689.9 × BSA (m^2) − 24.7" is considered to be accurate enough and more convenient to use in the clinical setting.

The SLV calculated for children, including neonates, in this study was significantly smaller ($p < 0.001$) using the new formula than the standard Urata formula. This is likely because our study included more children, and younger children, including neonates and infants. It is

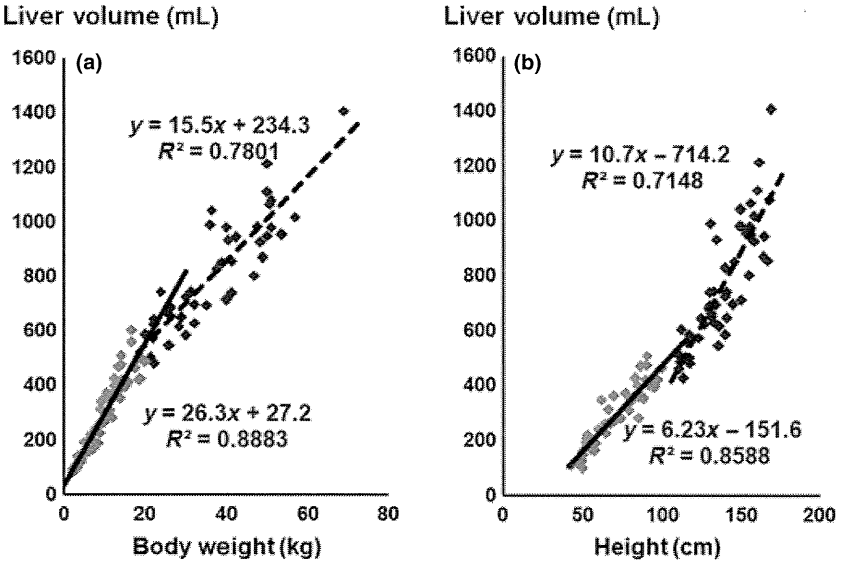

Fig. 5. (a) The relationship between BW and LV. The regression line of subjects larger than 20 kg (- -) and subjects smaller than 20 kg (—) had different gradients. (b) The relationship between height and LV. The regression line of subjects taller than 110 cm (–) and subjects shorter than 110 cm (—) had different gradients.

also possible that there may have been differences in the accuracy of the CT (thin multislice CT scanning) and LV calculation software programs.

In this study, the estimated LVs were closer to the new formula than to the Urata formula. Especially in small children including neonates, the Urata formula significantly overestimated the LV (Fig. 3). For this reason, the application of this new formula for small children is recommended. Although to confirm the accuracy of new formula compared to Urata formula, the new formula needs to be tested in a new independent cohort of children.

The graph of the relationship between the BW and LV indicated that gradients of single regression lines were different in patients with a BW > 20 kg patients and those with a BW < 20 kg patients. The meaning of GRWR is therefore suggested to be different in these two groups. The GRWR in adults or comparatively large children do not apply for small children. Furthermore, the LV is correlated more strongly with the BSA than with the BW (R^2: 0.942 vs. 0.929). For this reason, the estimated GV/SLV calculated using this newly established pediatric SLV formula should be used to precisely investigate the risk of large-for-size syndrome in pediatric liver transplantation. To estimate the risk factors of large-for-size syndrome, a subsequent clinical trial using this formula will therefore be necessary.

This pediatric SLV formula will be useful not only in LDLT, but also in liver resection of small children. More accurate prediction of remnant LV after liver resection will be possible using this formula, which will lead to safer surgery in many patients with pediatric liver diseases which require liver resection.

There are some limitations in this study. First, we compared the preoperatively estimated LV of recipients who received LDLT at our hospital and the actual weight of the removed whole disease liver to assess the accuracy and precision of liver volumetry. Although we actually measured LV of normal livers to establish SLV formula, the limitation of this study is the assumption that diseased liver weight collates well with normal LV. Secondly, DuBois formula is reported to underestimate the BSA of newborns (20). It is possible that this underestimation of BSA was the reason for the small SLV in neonates and it therefore slightly altered both the coefficients and precision.

In conclusion, the following new formula for calculating the SLV of children, including neonates, was established: LV (mL) = 689.9× BSA (m^2) − 24.7.

This new formula is accurate even in very small children and neonates. It is therefore expected to be helpful in the fields of pediatric liver transplantation and liver resection of small children.

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