Table 1 Summary of patient characteristics

Case No.	Age (yrs)	Parity	AVM grade*	Lesion	Presentation	Gestation at time of presentation
1	27	G1P1	pial AVF	rt parietal	hemorrhage	21st week
2	34	G1P1	I	rt insula	hemorrhage	16th week
3	27	G1P1	II	lt parietal	hemorrhage	25th week
4	30	G0P0	II	multiple	hemorrhage	25th week
5	31	G2P2	II	lt occipital	hemorrhage	5th week
6	22	G0P0	III	rt midbrain	hemorrhage	15th week
7	31	G1P1	II	rt frontal	incidental	pre-pregnancy
8	30	G0P0	II	lt parietal	headache	pre-pregnancy
9	28	G1P1	V	rt parietal	headache	pre-pregnancy

*According to Spetzler-Martin grading scale. AVF: arteriovenous fistula, AVM: arteriovenous malformation, G: gravida, lt: left, P: para, rt: right.

Subjects and Methods

Nine patients with AVM in pregnancy aged 22 to 34 years (mean 28.9 ± 3.4 years) were treated in the National Cerebral and Cardiovascular Research Center between April 2005 and April 2011 (Table 1). Six patients presented with their first episode of intracerebral hemorrhage (ICH) during pregnancy, 2 with headache, and one with incidental finding of AVM. In the 3 patients with unruptured AVM, the diagnosis was made before pregnancy. The Spetzler-Martin grade was I in one patient, II in five, III in one, and V in one. One patient was diagnosed with pial arteriovenous fistula (AVF). In each of these cases, we examined the results of pregnancy and delivery management, and the maternal and fetal outcome with ruptured and unruptured AVMs.

Results

I. Maternal management with ruptured AVMs

Six patients presented with their first episode of ICH during pregnancy; their AVMs were previously undetected. In 3 patients (Cases 1, 2, and 3), removal of the AVM was performed prior to delivery. The ICHs occurred in the 21st week, 16th week, and 25th week of gestation, and the surgery for AVM was performed in the 21st week, 18th week, and 30th week of gestation, respectively. The interval between onset and the surgery was 0 days, 14 days, and 33 days, respectively. Emergency surgery was performed for Case 1 with severe consciousness disturbance due to the mass effect of the hematoma. In another 2 patients with Spetzler-Martin grade I and II AVM, the symptoms were mild and elective surgery for AVM was performed for the prevention of rebleeding because we expected safe resection of the AVM located in superficial lesion. The management of pregnancy after removal of the AVM was similar to a normal pregnancy, with vaginal delivery in one case and cesarean section in two cases. In Case 4, cesarean section was carried out prior to AVM treatment in the 28th week of gestation because the mother suffered from hypoxia, hemoptysis, and transient ischemic attack due to paradoxical cerebral embolism from a pulmonary AVF. The interval from the cerebral hemorrhage onset to delivery was 24 days. In this patient, the AVM lesions were small and multiple, and gamma knife surgery was conducted 4 weeks after delivery. In Case 5, the patient presented with ICH in the fifth week of pregnancy and had a miscarriage on the 11th day after ICH. Endovascular embolization and resection for AVM were performed subsequently. In Case 6, the patient presented with ICH in the 15th week of gestation, and artificial abortion was performed 18 days after onset based on the concerns of her family. Gamma knife treatment was performed subsequently. There was no rebleeding in any patient, including the puerperal period (Table 2).

II. Maternal management with unruptured AVMs

The diagnosis in 3 patients with unruptured AVMs was made before pregnancy. In Cases 7 and 8, gamma knife surgery had been performed previously, and pregnancy occurred before confirmation of the obstruction of the AVM. In Case 9 with Spetzler-Martin grade V AVM, there was no surgical indication for AVM. The vaginal delivery had been performed previously under epidural anesthesia in this patient. Case 7 had severe pregnancy-induced hypertension, and an urgent cesarean section was performed on admission to the hospital in the 28th week of gestation because her blood pressure was difficult to control. In another two cases, blood pressure management was successfully performed during pregnancy, and the patients delivered at full

Table 2 Results of arteriovenous malformation (AVM) treatment, delivery management, and mother and infant clinical outcomes

Case No.	Timing of AVM treatment	AVM treatment	Delivery (week of pregnancy)	Reasons for CS	Outcome for the mother (mRS)	Outcome for the infant
1	21st week of pregnancy	removal (emergency)	CS (36)	hemiparesis	3	infant well
2	18th week of pregnancy	removal (elective)	CS (40)	macrosomia, previous CS	0	infant well
3	30th week of pregnancy	removal (elective)	VD (40)	—	0	infant well
4	post-delivery	RS	CS (28)	pulmonary AVF	0	infant well (temporarily intubated)
5	post-abortion	EE + removal	AB (7)	—	0	—
6	post-abortion	RS	AB (18)	—	3	—
7	pre-pregnancy	RS	CS (37)	previous CS	0	infant well
8	pre-pregnancy	RS	CS (32)	severe PIH	0	infant well (temporarily intubated)
9	pre-pregnancy	conservative	VD (39)	—	0	infant well

AB: abortion, AVF: arteriovenous fistula, CS: cesarean section, EE: endovascular embolization, mRS: modified Rankin scale, PIH: pregnancy-induced hypertension, RS: radiosurgery, VD: vaginal delivery.

term. There were no bleeding complications in any of the patients over the course of the pregnancy, including the puerperal period (Table 2).

III. Method of delivery

Vaginal delivery was performed in two cases, and cesarean section in five cases. Spontaneous vaginal delivery occurred in the 40th week of gestation after removal of the AVM in Case 3, and vaginal delivery under epidural anesthesia occurred in Case 9 with Spetzler-Martin grade V AVM in the 39th week of gestation. Among the 5 patients with cesarean section, 3 had coexistent AVM. Cesarean section was performed due to the existence of the AVM in Case 7 with severe uncontrollable pregnancy-induced hypertension, in Case 4 with pulmonary AVF, and in Case 8 who had previously undergone cesarean section. Two patients underwent cesarean section after AVM resection due to maternal factors; Case 1 with limitation of abduction of the lower limbs because of hemiplegia and twin pregnancy, and Case 3 with previous cesarean section and macrosomia. Mothers and babies suffered no complications during labor (Table 2).

IV. Maternal outcome

The 6 patients with ruptured AVMs had modified Rankin scale (mRS) score of 0 in 4 cases and 3 in 2 cases. The latter resulted from initial cerebral hemorrhage. The 3 patients with unruptured AVMs had mRS score 0. There were no new maternal complications due to cerebral AVM, including bleeding complications, in all patients throughout the pregnancy, delivery, and puerperal periods after the diagnosis of AVM (Table 2).

V. Fetal outcome

One patient suffered spontaneous abortion in the 7th week of gestation (2 weeks after onset), and one patient underwent induced abortion in the 18th week of gestation (third week after onset). Two premature infants delivered by cesarean section in the 28th week and 32nd week of gestation required temporary respirator management, but their subsequent growth and development was good. In the remaining infants, the growth development was excellent (Table 2).

Discussion

I. Epidemiology of AVMs during pregnancy

The prevalence of cerebral AVMs is estimated at 0.01–0.50% of the population. AVM is generally present in patients aged between 20 and 40 years, and is more common in those over 30 years, the childbearing age for women.[3] A previous study reported 21 ischemic strokes and 11 hemorrhagic strokes among 58,429 deliveries, and 4 of 11 hemorrhagic cases resulted from AVM rupture.[18] Although the influence of pregnancy on AVM rupture is controversial among investigators,[2,7,15] in a recent report, the annual hemorrhage rate during pregnancy was 10.8%; the hemorrhage rate per pregnancy was 8.1%; and the hazard ratio for ICH during pregnancy was 7.91.[4] The frequency of rebleeding during the same pregnancy period could be as high as

27%, which is 4 times higher than for the natural course of a ruptured AVM in the first year.[15] However, conservative treatment was done in 20 of 24 cases, and surgical removal was performed during pregnancy in only 4 cases. Similarly, rebleeding of AVM occurred in one of 11 cases, and surgical treatment during pregnancy was only performed in 7 cases after delivery.[16] After AVM rupture during pregnancy, maternal mortality was 28% and fetal mortality was 14%.[2] These risks can be eliminated only by excision of the AVM. The prognosis for the mother and fetus would improve if surgical resection of the AVM is safely performed. In our ruptured cases, AVM resection was performed in 3 of 6 cases (50%) before delivery, and the rebleeding rate in the peri-pregnancy period was 0%. In view of these results, AVM in pregnant women should be treated with great care.

Table 3 Management decision chart for patients with intracerebral hemorrhage from arteriovenous malformation (AVM) during pregnancy

	Operative risk	
	Low	High
Fetus immature/ Early pregnancy	removal of AVM →delivery based on obstetrical indications	conservative maternal management →modified vaginal delivery/ cesarean section once fetus was mature →AVM treatment based on neurosurgical indications
Fetus mature/ Advanced pregnancy	modified vaginal delivery/ cesarean section →removal of AVM	modified vaginal delivery/ cesarean section →AVM treatment based on neurosurgical indications

II. Maternal management with AVMs in pregnancy

Maternal management of patients with ruptured AVMs should be based mainly on neurosurgical indications rather than on obstetrical indications.[2] When neurological deterioration occurs due to AVM rupture, emergency surgery is necessary. If the fetus is sufficiently mature, simultaneous cesarean section is possible. When there is no indication for emergency surgery for AVM, blood pressure management is important.[9] However, this is not necessarily effective for the prevention of rebleeding because patients with ruptured AVM do not always have a history of hypertension. Although radical treatment tended to be performed after delivery in many case reports and case series, some authors suggested that early surgical intervention for AVM before delivery led to improved maternal and fetal prognosis.[15,22] We agree, and try to perform AVM resection during pregnancy with an immature fetus if the surgical risk is low after considering the high risk of rebleeding (Table 3). Indeed, we performed elective AVM resection with pregnancy continuation in 2 patients, with good postoperative maternal and fetal outcomes. The average period between onset and AVM resection was 23.5 days, and no rebleeding occurred during the waiting period. In addition to the maternal and neurosurgical treatment priorities, consideration of the fetus is also necessary and cooperation between obstetricians and anesthesiologists is essential during surgery. We routinely use intraoperative fetal heart rate monitoring. If the fetus has reached the minimum age for extra-uterine life, obstetricians prepare for emergency cesarean section in case of fetal distress.

Surgery for AVM is determined primarily by the Spetzler-Martin grading scale.[19] A potential complication of surgery for AVM during pregnancy is the risk of intraoperative bleeding leading to deterioration of the uterine and placental circulation. Although preoperative embolization is possible for cases with a high risk of intraoperative bleeding, such as deep-seated AVMs, the endovascular treatment itself carries the risk of ischemic and hemorrhagic complications.[5,12,21] In addition, there is not enough evidence to presume the safety of iodinated contrast agents which cross the human placenta and enter the fetus. The potential radiation risk and the potential added risks of contrast medium should be considered in the preoperative study.[23] Previous reports of endovascular treatment for AVM during pregnancy are limited.[17] There would be wider surgical indications by discussing the efficacy and risk more about endovascular treatment for AVMs during pregnancy.

Radical treatment for ruptured AVMs in patients with a mature fetus tends to be performed in the early postpartum period.[22] It is desirable for patients with unruptured AVMs to undergo radical treatment before pregnancy due to the increasing risk of AVM rupture during pregnancy. Prior to pregnancy, multimodal therapies such as direct surgery, endovascular embolization, and radiosurgery can be performed. In patients with unruptured AVMs diagnosed during pregnancy, conservative treatment is performed based on the risk of surgical treatment.

III. Delivery management

If the AVM is completely resected during pregnancy, the method of delivery can be determined based on the obstetrical indications. Our three patients who underwent AVM surgery during pregnancy could deliver at a mature gestational age. In

patients with AVM during pregnancy, problems during labor are related to the excessive cerebral hemodynamic changes, and cesarean section tends to be performed in these circumstances.[8] Cesarean section can be provided relatively safely, and is becoming more common. Recently, the rate of cesarean section has increased with the increase in high-risk pregnancies, such as with older maternal delivery age, and complicated pregnancies, which have increased up to 15% in a recent report from the Japanese Ministry of Health, Labour and Welfare. On the other hand, the maternal risks of cesarean section were reported to be 7 times higher than those of vaginal delivery and included maternal death, massive bleeding, infection, thrombosis, and injury to organs such as the bladder, although the frequency was very low.[1,11,14] If a patient's previous delivery was performed by cesarean section, repeated cesarean section tends to be performed to prevent uterine rupture. There is no definitive evidence that cesarean section prevents the hemorrhagic complications of AVM.[2,7,13] However, it is desirable to use epidural anesthesia or to shorten the second stage of labor with forceps/vacuum delivery techniques during labor.[6] When determining the parturient method, we should understand these points and inform the patient and her family to obtain consent.

We conducted painless vaginal delivery with epidural anesthesia combination in patients with AVM. In one patient with inoperative high-grade AVM, it was possible to perform vaginal delivery safely with this method. However, cesarean section allows easy control of blood pressure during labor, and is more desirable for patients with severe pregnancy-induced hypertension syndrome, as in our Case 7. Cesarean section is also indicated in patients with consciousness disturbance or hemiplegia preventing a dorsosacral position due to the consequences of ICH.

IV. Conclusion

We achieved good maternal and fetal outcomes in our cases, excluding 2 patients with mRS 3 due to the initial ICH. Surgical intervention for ruptured AVM during pregnancy could prevent rebleeding, and allow for determination of the delivery method based on the obstetrical indications. Cooperation between neurosurgeons, obstetricians, and anesthesiologists, and sufficient information about the treatment strategy given to the patients are essential. Finally, for better maternal and fetal prognosis, guidelines for female patients with cerebral AVMs should be established.

Conflicts of Interest Disclosure

The authors declare that they have no conflicts of interest. All authors who are members of The Japan Neurosurgical Society (JNS) have registered online Self-reported COI Disclosure Statement Forms through the website for JNS members.

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A novel reproducible model of neonatal stroke in mice: Comparison with a hypoxia–ischemia model

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ABSTRACT

Neonatal stroke occurs in 1/4000 live births and leaves life-long neurological impairments, such as cerebral palsy and epilepsy. Currently, the rodent models of neonatal stroke that are available exhibit significant inter-animal variability, which makes it difficult to accurately assess the mechanisms of brain injury and the efficacy of candidate treatments. We aimed to introduce a novel, highly reproducible model of stroke, middle cerebral artery occlusion (MCAO), in immature mice, and to evaluate the reproducibility of this model compared with a conventional hypoxia–ischemia (HI) model. Postnatal day 12 CB-17 mice underwent left MCAO by direct electrocoagulation. The MCAO model exhibited excellent long-term survival; 85% up to 8 weeks after the insult. Infarct was evident in every animal with MCAO ($n = 27$) and was confined to the cortex, with the exception of some mild thalamic injury. While the % stroke volume 48 h after the insult was consistent in the MCAO group, range: 17.8–30.4% (minimum–maximum), it was substantially less consistent in the HI group, range: 3.0–70.1%. This contrasting variability between the two models was also evident in the cerebral blood flow, 24 h after the insult, and in the ipsilateral hemispheric volume, as assessed at 8 weeks after the insult. Mice with MCAO exhibited significant neurofunctional deficits in the rotarod and open-field tests. Preclinical studies for neonatal stroke could become more reliable using this model, with even a potential reduction in the number of pups required for statistical significance. The contrasting variability between the two models may provide insights into the factors that contribute to inter-animal variability in brain injury.

Introduction

Perinatal/neonatal arterial ischemic stroke occurs in 1/2800 to 1/5000 live births, has a mortality rate of 2–10%, and leaves life-long neurological impairments, such as cerebral palsy, cognitive delay, and epilepsy (Chabrier et al., 2011; Golomb et al., 2006; Nelson and Lynch, 2004). The common early symptoms are seizures, persistently altered muscle tone, and decreased consciousness (Chabrier et al., 2011). Most perinatal arterial ischemic events occur in the region of the middle cerebral artery (MCA), with a left-hemisphere predominance (Lee et al., 2005; Sreenan et al., 2000). While full-term infants tend to exhibit occlusion of the main branch, preterm infants tend to exhibit occlusions of a cortical branch or one or more of the lenticulostriate branches (de Vries et al., 1997). There is currently no evidence-based treatment for neonates with stroke (Chabrier et al., 2011). Furthermore, the average 5-year direct medical cost for neonatal stroke is approximately $52,000 US (Gardner et al., 2010).

When investigating brain injuries, it is essential to utilize a highly reproducible model of brain injury. The model has to provide: 1) an accurate neurological evaluation, 2) a detailed evaluation of the injury/neuroprotection mechanisms, and 3) limitation in the numbers of animals used. Several neonatal stroke models have been developed using artery obstruction (Ashwal et al., 1995; Comi et al., 2004; Derugin et al., 1998; Mitsufuji et al., 1996; Renolleau et al., 1998; Wen et al., 2004). Almost all of these models exhibit significant inter-animal variability in the extent of the brain injury; i.e. a subset of pups exhibit no perceivable brain injury.

Neonatal encephalopathy (NE) is a neonatal neurological syndrome with clinical features that include decreased consciousness –

Abbreviations: MCA, middle cerebral artery; MCAO, middle cerebral artery occlusion; NE, neonatal encephalopathy; HIE, hypoxic–ischemic encephalopathy; HI, hypoxic–ischemic, hypoxia–ischemia; CBF, cerebral blood flow; ANOVA, analysis of variance.

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usually associated with respiratory depression, altered muscle tone, disturbances of cranial nerve function – especially impaired feeding, and often seizures (Volpe, 2012). The most common etiology of NE is cerebral ischemia; hypoxic–ischemic encephalopathy (HIE) 50–80%, and stroke ~5–10% (Volpe, 2012), NE encompasses HIE and stroke. Recently, some authors have proposed that the term HIE should not be used in practice and should be replaced by the more general term, NE, for a number of reasons (Dammann et al., 2011), whereas other authors have opposed this proposal (Volpe, 2012). The most widely-used HIE model is the Rice–Vannucci model, which combines permanent unilateral ligation of the carotid artery in 7-day-old rat pups, along with exposure to hypoxia (Johnston et al., 2005; Rice et al., 1981). It is important to note that this model also exhibits significant inter-animal variability in the extent of the brain injury (Aden et al., 2002; Sheldon et al., 1998).

Some neonates with stroke can present signs and symptoms similar to HIE, and vice-versa. Moreover, some babies may exhibit both etiologies, and it is often difficult to isolate the cause of NE. Therefore, it is important to understand the differences between arterial ischemic stroke and hypoxia–ischemia (HI). Nevertheless, to the best of our knowledge, only one study (Ashwal et al., 2007) has directly compared the HI model in immature animals and a stroke model in immature animals to date.

We have previously developed a highly reproducible model of adult stroke induced by direct electrocoagulation of the unilateral MCA in CB-17 (CB-17/Icr-+/+Jcl) and SCID (CB-17/Icr-scid/scidJcl) mice (Taguchi et al., 2004, 2010). Recently, we adapted the same technique to immature CB-17 mice, and have succeeded in developing a model of neonatal stroke that shows remarkable consistency of the brain injury. The objectives of our study were: 1) to introduce a novel model of stroke in immature mice and 2) to test reproducibility of this model as compared to the HI model.

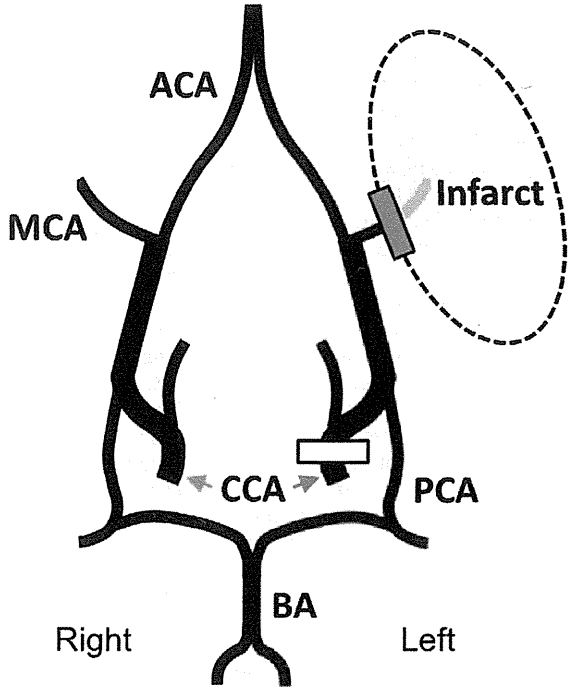

Fig. 1. Representation of the circle of Willis in rodents. The anatomic arterial system at the base of the brain in horizontal projection. ACA; anterior cerebral artery. BA; basilar artery. CCA; common carotid artery. MCA; middle cerebral artery. PCA; posterior cerebral artery. In the MCA occlusion model, the left MCA is permanently occluded (gray box). In the hypoxia–ischemia model, the left CCA is permanently occluded (open box) followed by transient systemic exposure to hypoxia.

Methods

Animals and surgeries

Postnatal day 12 (P12) male and female CB-17 mouse pups (n = 94, weight: 6.7 ± 1.2 g) (CLEA Japan Inc., Tokyo, Japan) were prepared for surgery. P8–12 mice are considered comparable to human term (P0) neonates with regard to brain maturation (Hagberg et al., 2002). All experiments were performed in accordance with protocols approved by the Experimental Animal Care and Use Committee of the National Cerebral and Cardiovascular Center.

Permanent MCA occlusion (MCAO) was produced by a modification of the adult MCAO model that we have reported previously (Taguchi et al., 2010) (Fig. 1). A skin incision was made between the left eye and ear under isoflurane anesthesia (4.0% for induction, 1.5–2.0% for maintenance). The zygoma was dissected to visualize the MCA through the cranial bone. A hole was made in the temporal bone by removing a portion of it using fine forceps. The left MCA was electrocauterized, and disconnected just distal to its crossing of the olfactory tract (distal M1 portion). The average duration of the whole procedure was approximately 15 min. HI was induced by a combination of permanent occlusion of the left common carotid artery and exposure to 8% oxygen for 30 min in the P12 CB-17 mice, as described previously (Ohshima et al., 2012) (Fig. 1). Sham-surgery controls underwent open-skull surgery without MCA electrocoagulation. To properly assess the differences in variability between the two models, a single researcher, the first author, performed all surgical procedures. All analyses were performed by investigators who were blinded to the experimental group.

Cerebral blood flow measurements

The cortical surface cerebral blood flow (CBF) was measured by a laser speckle flowmetry imaging system (Omegawave Inc., Tokyo, Japan) immediately before and 24 h after MCAO or HI, as described previously, with a minor modification (Ohshima et al., 2012). CBF was measured through the intact skull with an open-scalp.

Behavioral tests

Sensorimotor skills were evaluated 2 weeks after the insult (P26) using the rotarod test, as rodents with brain damage have been reported to exhibit behavioral impairment at this time point (Jansen and Low, 1996). The rotarod accelerated from 4 to 40 rpm over 5 min (Muromachi Kikai Co., Ltd., Tokyo, Japan). The time until the mouse fell off the rotating drum was recorded in 5 consecutive sessions, and the average time spent on the drum was used for statistical comparison.

Locomotor and exploratory behaviors were evaluated 5 weeks after the insults (almost 7 weeks of age) using the open-field test, as in our preliminary study mice began to respond to a dark environment from this age onward. Animals were allowed to search freely in a box (30 × 30 cm) for 30-min in a light environment and for the subsequent 30-min in a dark environment (Taiyo Electric Co., Ltd, Osaka, Japan). On the X-, Y-, and Z-banks of the open-field, infrared beams were mounted at specific intervals. The total number of beam crossings by the animal was counted and scored as "locomotion" for the horizontal movement, and as "rearing" for the vertical movement. Both behavioral tests were repeated one week before sacrifice at 8 weeks after the insult.

Histological analyses

Morphological evaluation of the brain injury was performed, as described previously (Tsuji et al., 2004, 2012). Forty-eight hours after the MCAO or HI insult, the brain was removed and sectioned coronally in 1-mm thick slices. The area of the viable ipsilateral and contralateral hemispheres, which stained red with 2,3,5-triphenyltetrazolium chloride (TTC) in each brain section, was measured using ImageJ software (NIH, Bethesda, USA). The hemispheric volume was estimated by integrating the hemispheric areas.

For longer-term evaluation, separate sets of animals were perfusion-fixed intracardially with 4% paraformaldehyde, 8 weeks after the insult. In assessing the hematoxylin–eosin-stained sections, neuropathological injury in the cerebral cortex was scored on a scale ranging from 0 to 4 points (0, no injury; 4, extensive confluent infarction). Neuropathologic injury in the hippocampus, striatum, and thalamus was scored on a scale ranging from 0 to 6 points. The ipsilateral and contralateral areas in the four regions and the corpus callosum were measured using ImageJ software. The ratios of the ipsilateral/contralateral areas in the five regions were calculated after summing the areas in four brain sections (cortex) or two brain sections (hippocampus, striatum, thalamus, and corpus callosum).

Statistics

The mortality rate of the animals was analyzed using Fisher's exact test with Bonferroni's correction for multiple comparisons. Hemispheric volumes, and CBF were assessed using two-way analysis of variance (ANOVA), followed by the Bonferroni test. The differences in body weight were assessed using one-way ANOVA, followed by the Bonferroni test. The injury scores were not distributed normally, so differences in injury scores were assessed with the Mann–Whitney *U* test. Ratios of the ipsilateral/contralateral areas were assessed using a Kruskal–Wallis test, followed by Dunn's multiple comparison, as the variances of the ratios were significantly different among the three groups. Pearson's product–moment correlation coefficient analysis was performed to determine the correlation between CBF and brain injury. Outcomes in the rotarod and open-field tests performed at two time points were assessed using two-way repeated measures ANOVA. Temporal changes during the course of a 60-min session in open-field test were then analyzed using two-way repeated measures ANOVA. Differences were considered significant at $P < 0.05$. The results are presented as the mean ± standard deviation (SD), unless otherwise noted.

Results

Mortality and body weight

All pups that were prepared for surgery underwent the surgery successfully. Although some pups experienced bleeding during the MCAO surgery, all pups were included in the subsequent analyses. Survival was 100% at 48 h and 85% at 8 weeks after MCAO (Table 1). Body weights at P12 and 8 weeks later did not differ among groups, including the no-surgery controls (Table 2).

Table 1
Mortality rates.

	48 h-survival cohort	8-week-survival cohort
No-surgery		0/13
Sham-surgery		2/17
HI	1/12	6/22
MCAO	0/10	3/20

None of the pups died during the surgical procedure for either MCAO (middle cerebral artery occlusion) or HI (hypoxia–ischemia). In each cohort, mortality rates did not differ significantly between groups.

Table 2
Body weights.

	Postnatal day 12	8 weeks later
No-surgery	6.5 ± 0.6	21.9 ± 2.0
Sham-surgery	6.9 ± 0.9	22.2 ± 2.1
HI	6.6 ± 1.4	20.5 ± 2.3
MCAO	6.8 ± 1.1	21.9 ± 3.2

Body weights (grams) (mean ± SD) at postnatal day 12 (the day of surgery) and 8 weeks later were not different between groups. MCAO; middle cerebral artery occlusion, HI; hypoxia–ischemia.

Morphological brain injury

Forty-eight hours after the insult, moderate-complete TTC discoloration was observed in all 10 pups that were subjected to MCAO, while discoloration was observed in only five out of 11 pups that were subjected to HI (Fig. 2A). The discoloration was confined to the ipsilateral cerebral cortex, and its location and size were consistent in all pups in the MCAO group, with the exception of one pup that exhibited discoloration extending to the striatum. In contrast, the location and size of the discoloration was markedly more variable in the HI group. The mean % stroke volume was 25.1 ± 3.6% in the MCAO group and 15.5 ± 18.6% in the HI group. The % stroke volume was calculated as follow: ((contralateral volume − viable ipsilateral volume) / contralateral volume) × 100%. Variances of the viable ipsilateral hemispheric volume and % stroke volume differed significantly between the two models ($P < 0.001$) (Fig. 2B).

Eight weeks after the insult, all 17 mice with MCAO exhibited consistent macroscopic cortical damage (Fig. 2C). The mean ipsilateral hemispheric volume was 73.0 ± 3.2 mm^3 in the MCAO group, and 72.3 ± 23.0 mm^3 in the HI group (Fig. 2D). Of note, the sham-surgery group was not different from the no-surgery group, suggesting that the open-skull surgical procedure did not cause noticeable morphological damage. No sex differences in hemispheric volumes were observed at either time point in any of the groups.

Neuropathological injury scores in the four brain regions examined differed between the two models (Fig. 3A). The ratios of the ipsilateral/contralateral areas in the four regions and corpus callosum differed among the three groups including the sham-surgery group (Fig. 3B). Interestingly, in the MCAO group, most mice exhibited mild thalamic injury, in contrast with a virtual absence of striatal or hippocampal injury. Furthermore, the thalamic damage in the MCAO model was strictly restricted to the ipsilateral ventroposterior thalamic nuclei (VPN), which contained many pyknotic cells (Fig. 3C). In contrast, the thalamic injury in the HI model was variable in terms of its distribution and severity. In both models, the ispilateral corpus callosum exhibited mild atrophy; however, this only reached statistical significance in the MCAO model.

CBF

The CBF was decreased in the MCA territory on the ipsilateral side in all pups 24 h after the HI or MCAO insult. The degree of the CBF reduction was consistent after MCAO, whereas it was variable between animals after HI (Figs. 4A, B). The CBF 24 h after the insult was compared with the morphological brain injury at 8 weeks after the insult (Fig. 4C). The reduction in CBF after the MCAO did not correlate with the subsequent morphological brain injury. In stark contrast, the reduction in CBF after the HI insult correlated strongly with brain injury ($R^2 = 0.99$), which is consistent with our previous report in P8 mice with the HI insult (Ohshima et al., 2012).

Rotarod performance

Sensorimotor performance, as assessed by rotarod treadmill at 2 and 7 weeks after the insult was analyzed by two-way repeated

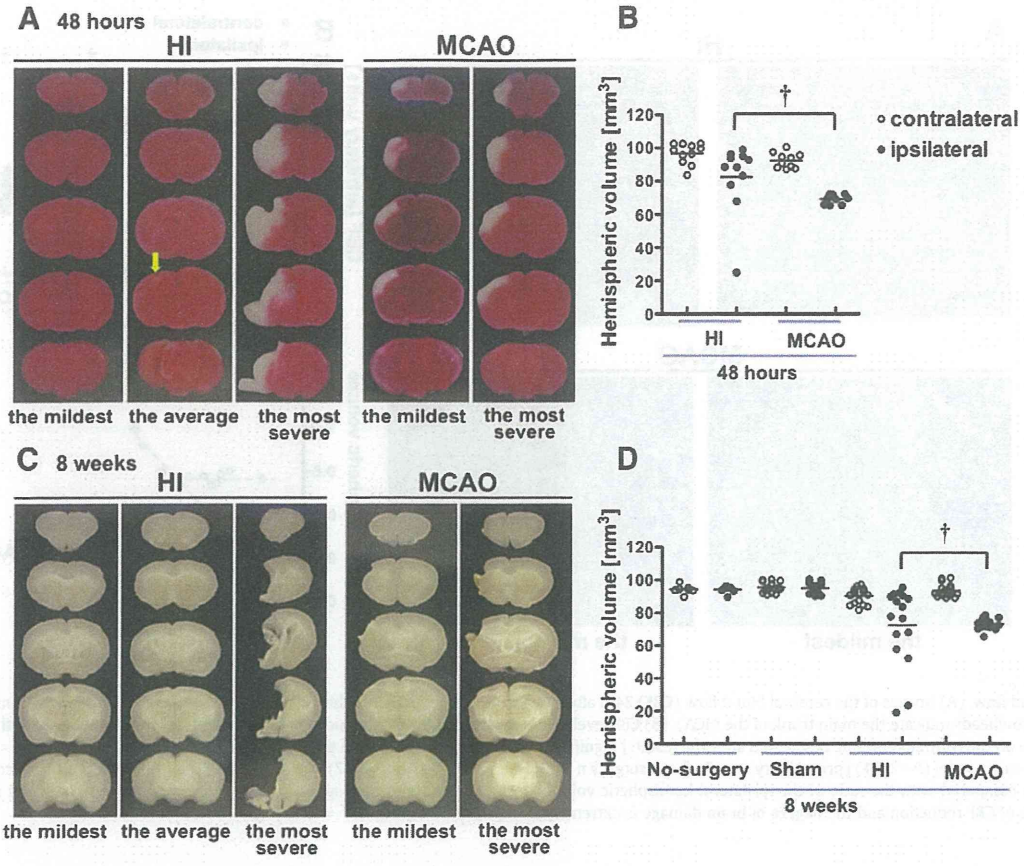

Fig. 2. Macroscopic brain injuries. (A) Images of TTC-stained brain sections 48 h after middle cerebral artery occlusion (MCAO) or hypoxia–ischemia (HI). The brains with the mildest injury and the most severe injury in the MCAO group and those with the mildest, the average, and the most severe injury in HI group are shown. The brain injury was highly consistent after MCAO. In contrast, the brain injury varied substantially after HI (the arrow indicates a small area of discoloration). (B) Hemispheric volumes of viable tissue, which stained red, examined at 48 h after the insult (HI $n = 11$; MCAO $n = 10$). (C) Images of brain slices 8 weeks after the insult. (D) Hemispheric volumes examined at 8 weeks after the insult. † Significant difference in the variances between the groups ($P < 0.001$). There were no significant differences in the ipsilateral hemispheric volumes between the no-surgery and sham-surgery groups, nor in the contralateral hemispheric volumes in the no-surgery, sham-surgery group, and MCAO groups. (no-surgery $n = 7$; sham-surgery $n = 15$; HI $n = 16$; MCAO $n = 17$).

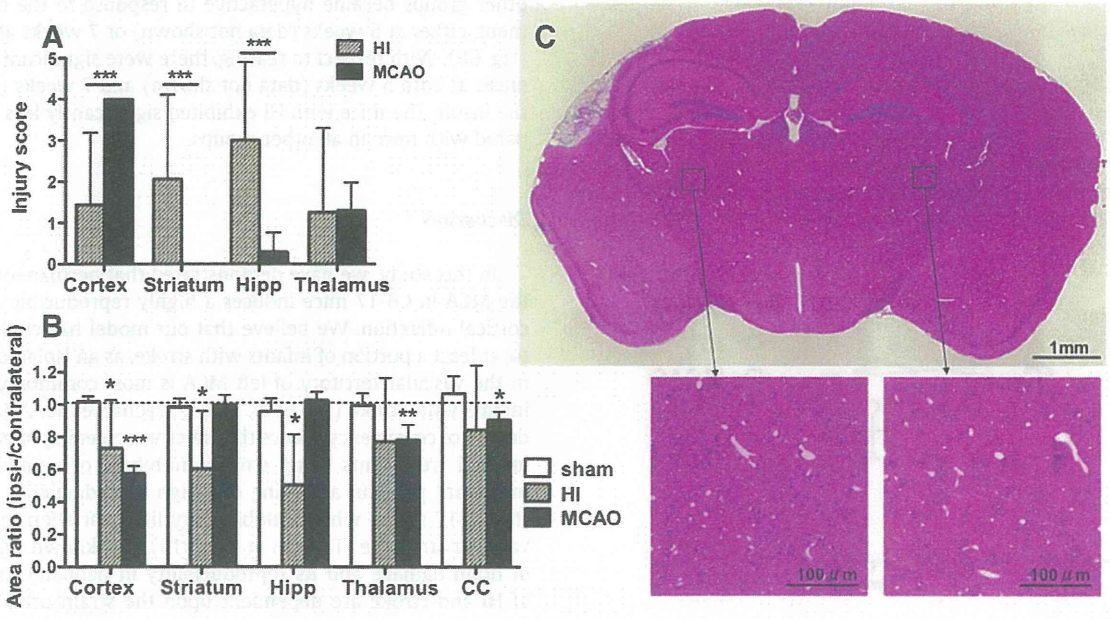

Fig. 3. Microscopic brain injuries. (A) Neuropathological injury scores examined in hematoxylin–eosin-stained sections 8 weeks after the insult. ***$P < 0.001$. (HI $n = 16$; MCAO $n = 17$) (B) The ratios of ipsilateral/contralateral areas in each region examined at 8 weeks after the insult. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, compared with sham. Note the difference in the error bars (standard deviation) between the models (sham-surgery $n = 7$; HI $n = 10$; MCAO $n = 10$). Hipp; hippocampus. CC; corpus callosum. (C) Representative image of H&E-stained sections of mice brain 8 weeks after the MCAO. There is a clearly demarcated old infarct in the ipsilateral cortex. The ipsilateral thalamus is mildly atrophic. The labeled boxes indicate the regions that were selected for higher magnification (×20). Many pyknotic neurons are observed in the ipsilateral ventroposterior thalamic nucleus (VPN). The contralateral VPN appears normal.

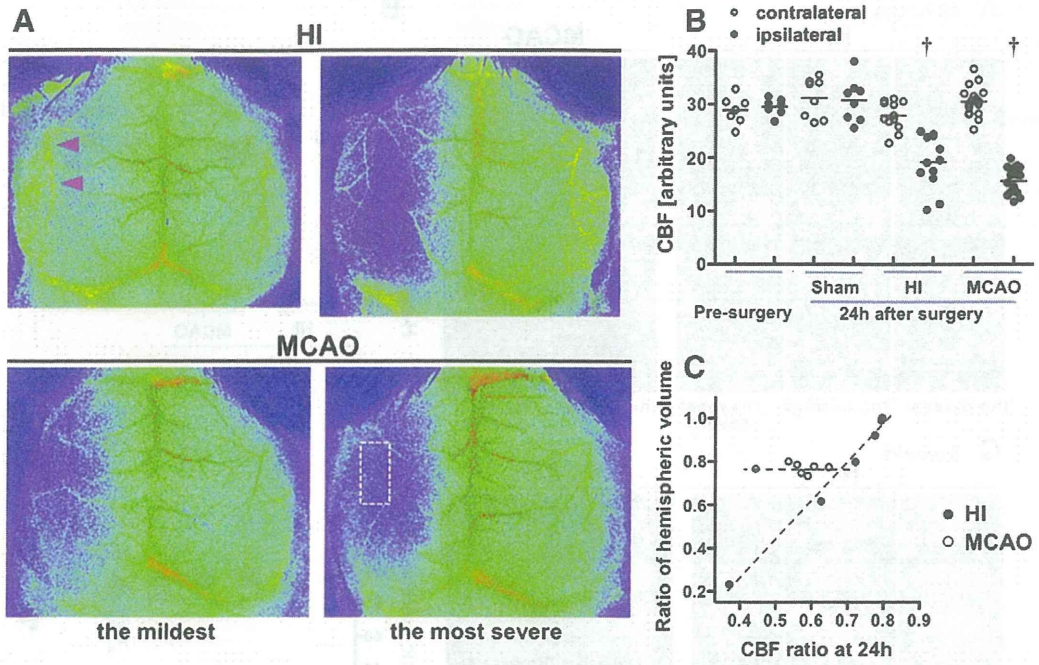

Fig. 4. Cerebral blood flow. (A) Images of the cerebral blood flow (CBF) 24 h after the insult. The reduction of the CBF, indicated by the bluish color, was consistent after MCAO, but not after HI (the arrowheads indicate the main trunk of the MCA). (B) CBF levels were measured in the ischemic core region (the box with dotted line) of the MCA territory and in the matching region on the contralateral side before and after the insult. † Significant difference compared with the pre-surgery or sham-surgery groups ($P < 0.001$), and significant difference between each model ($P < 0.01$) (pre-surgery $n = 7$; sham-surgery $n = 8$; HI $n = 12$; MCAO $n = 17$). (C) The ratio of the ipsilateral CBF to the contralateral CBF at 24 h after the insult was compared with the ratio of the ipsilateral hemispheric volume to the contralateral hemispheric volume (assessed 8 weeks after the insult). The correlation between the degree of CBF reduction and the degree of brain damage is extremely strong in the HI group ($R^2 = 0.99$). (HI $n = 6$; MCAO $n = 7$).

measure ANOVA. There were significant time and group differences; the performance in mice with MCAO was significantly impaired compared with that in the sham-surgery group (Fig. 5). The impairment in the rotarod performance in mice with HI was not statistically significant.

Open-field activities

We initially analyzed overall activities during 60-min sessions at 5 and 7 weeks after the insult using two-way repeated measures ANOVA (Figs. 6A, B). While there was no time difference with respect to either locomotion or rearing, there was a significant group difference with respect to rearing, but not locomotion; mice with HI were hypoactive compared with the mice in the other three groups.

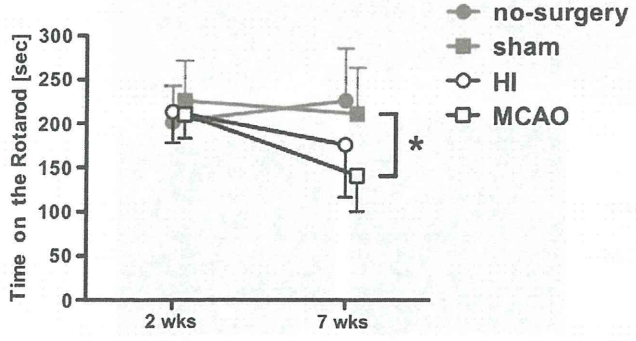

Fig. 5. Rotarod test. Repeated-measures two-way ANOVA showed significant time and group differences in sensorimotor performance, assessed 2 and 7 weeks after the insult. Performance was significantly impaired in mice with MCAO compared with the sham-surgery groups. *$P < 0.05$. (no-surgery $n = 19$; sham-surgery $n = 13$; HI $n = 16$; MCAO $n = 11$, 2 weeks after the insult. no-surgery $n = 9$; sham-surgery $n = 10$; HI $n = 13$; MCAO $n = 11$, 7 weeks after the insult).

There were no overall reductions in locomotion or rearing in the mice with MCAO.

We then analyzed the temporal changes throughout a 60-min session in 5-min increments using two-way repeated measures ANOVA. With respect to locomotion, the mice with MCAO did not respond to the change of environment from light to dark, whereas mice in all other groups became hyperactive in response to the dark environment, either at 5 weeks (data not shown) or 7 weeks after the insult (Fig. 6B). With respect to rearing, there were significant group differences at both 5 weeks (data not shown) and 7 weeks (Fig. 6C) after the insult. The mice with HI exhibited significantly less rearing compared with mice in all other groups.

Discussion

In this study, we have demonstrated that permanent occlusion of the MCA in CB-17 mice induces a highly reproducible and selective cortical infarction. We believe that our model has clinical relevance to, at least a portion of infants with stroke, as an isolated large infarct in the vascular territory of left MCA is most commonly observed in infants with stroke (Lee et al., 2005; Sreenan et al., 2000). This high degree of consistency allows the effective screening of various experimental treatments using smaller numbers of animals. The most important point in achieving this high reproducibility is the use of the CB-17 strain, which exhibits very little variation in the cerebral vascular structure (Taguchi et al., 2010). It is known that the degree of brain damage and its reproducibility in neonatal rodent models of HI and stroke are dependent upon the strain used (Comi et al., 2005; Sheldon et al., 1998). In addition to the high reproducibility, the advantages of our model are its simple procedure and high long-term survival, which provides the opportunity for long-term evaluation of neuropathological and functional outcomes. Indeed, our model exhibited significant long-term neurofunctional deficits.

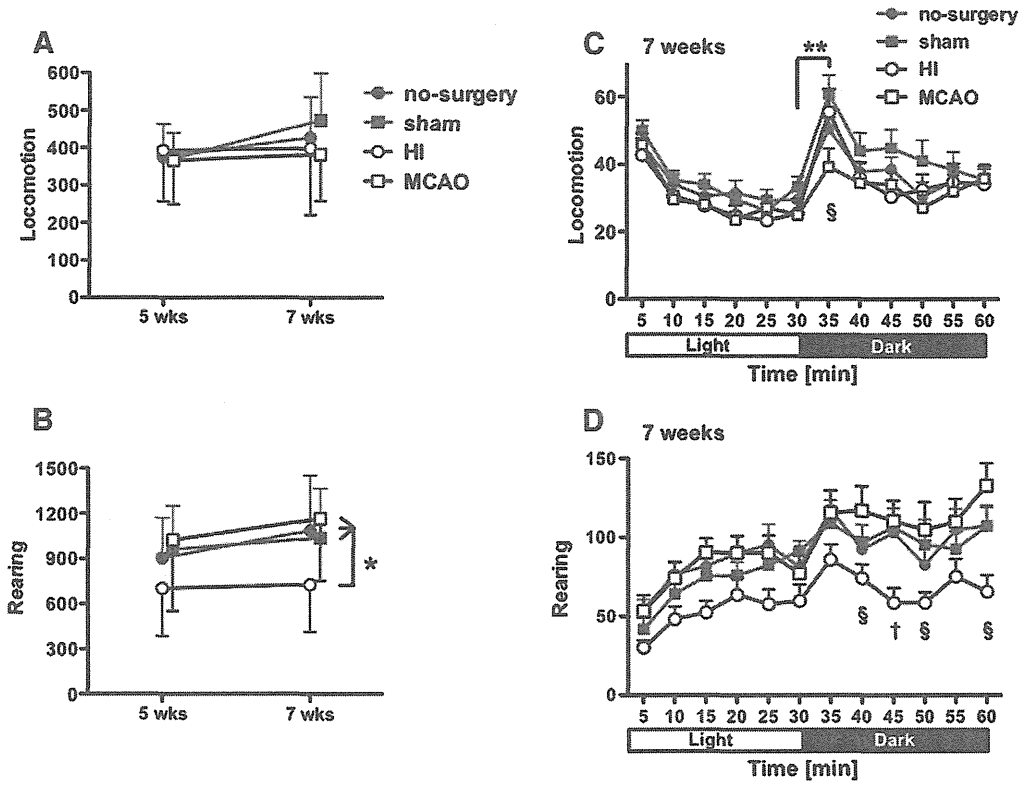

Fig. 6. Open-field test. (A, B) Overall activities during the 60-min session 5 and 7 weeks after the insult were analyzed by two-way repeated measures ANOVA. While there was no time difference with respect to either locomotion or rearing, there were significant group differences with respect to rearing, but not locomotion; mice with HI were significantly hypoactive compared with mice in the other three groups. (no-surgery $n = 14$; sham-surgery $n = 10$; HI $n = 16$; MCAO $n = 14$, 5 weeks after the insult. no-surgery $n = 13$; sham-surgery $n = 11$; HI $n = 16$; MCAO $n = 13$, 7 weeks after the insult). (C, D) Temporal changes in 5-min increments were analyzed by repeated-measures two-way ANOVA. There were significant group differences with respect to locomotion at 7 weeks after the insult. Mice in the MCAO group were significantly hypoactive during the first 5-min period in the dark than mice in the HI group. § $P < 0.05$. There were significant increases in the activity from the last 5-min period in the light environment to the first period in the dark environment in all groups except for the MCAO group. ** $P < 0.01$. With respect to rearing, there were significant group differences at 7 weeks. Mice in the HI group exhibited significantly less rearing activity. §$P < 0.05$, compared with MCAO group, †$P < 0.05$, compared with the no-surgery, sham-surgery and MCAO groups. Mean ± SEM.

Six models of neonatal stroke using artery obstruction have been developed (Ashwal et al., 1995, 2007; Bonnin et al., 2011; Comi et al., 2004; Derugin et al., 1998, 2000; Mitsufuji et al., 1996; Renolleau et al., 1998; Wen et al., 2004), and are summarized in Table 3. All models, except one, exhibit obvious inter-animal variability; some of the animals subjected to the insult do not develop infarct, as is the case in the HI model. In a permanent MCAO model developed by Wen et al. (2004), in which a tailor-made intraluminal suture embolus was placed in P7 SD rats, infarct was noted in all 10 pups that were subjected to the insult. However, the long-term survival was not reported. Taken together, among the currently available rodent models of neonatal stroke our model exhibits the highest reproducibility with excellent long-term survival. Nevertheless, those models, including ours, should be complementary, in order to lead to new understanding of the mechanisms of neonatal stroke and to find therapies for neonatal stroke. Our model has some weaknesses compared with other models. Firstly, this model does not utilize a reperfusion phase. Reperfusion may or may not occur in some patients, or the reperfusion may occur too late to activate its downstream events in other patients. Secondly, increasing or decreasing the degree of brain injury is not possible in this model. Thirdly, craniotomy results in stress to the animal and trauma to local tissues, even though the present study

Table 3
Immature rodent models of cerebral ischemia.

	Method of obstruction	Age and Species/strain	Ratio of infarct formation*	Long-term survival	Author and reference
1	t-f-MCAO	P14-18 or P10 SH rats	8/9	21% by 28 days	Ashwal et al., 1995, 2007
2	t-f-MCAO	P7 Sprague–Dawley rats	8/10, 20/31	71% by 7 days	Derugin et al., 1998, 2000
3	p-CCAO + t-CCAO†	P10 Wistar rats	NA	NA	Mitsufuji et al., 1996
4	p-MCAO + t-CCAO‡	P7 Wistar rats	10/10, 36/66	NA	Renolleau et al., 1998; Bonnin et al., 2011
5	p-CCAO	P12 CD1 mice	20/28	86% by 7 days	Comi et al., 2004
6	p-f-MCAO	P7 SD rats	10/10	NA	Wen et al., 2004
Present study	p-MCAO	P12 CB-17 mice	27/27	85% by 8 weeks	

These are unilateral cerebral ischemia models, unless otherwise noted. t-; transient. f-; intraluminal filament. p-; permanent. MCAO; middle cerebral artery occlusion. CCAO; common carotid artery occlusion. P; postnatal day. SH; spontaneously hypertensive. NA; not available. * Ratio of the number of animals presenting with obvious infarct to the number of animals that survived until the time of assessment. † Unilateral p-CCAO combined with contralateral t-CCAO. ‡ Unilateral MCAO by electrocoagulation combined with ipsilateral t-CCAO.

demonstrated that sham-surgery operated mice were not different from the no-surgery control mice, with respect to brain morphology, CBF, and behavior.

The differences in the variability between the two models (i.e., MCAO and HI) demonstrated in our study can provide insights into the mechanisms that lead to extensively variable susceptibility to HI insult by animals, even within littermates. The pivotal cause of the variation remains poorly understood. A number of explanations have been proposed for inter-animal variations in the extent of brain damage; 1) differences in collateral arteries in the brain (Rubino and Young, 1988), 2) the existence of several major MCA branching patterns (Rubino and Young, 1988), 3) subtle differences in the genetic background, 4) blood sugar level differences, which may result from variations in feeding times and amount (Chen et al., 2011; Hattori and Wasterlain, 1990), 5) temperature variation, 6) weight variation (Menzies et al., 1992), and 7) long surgery time and duration of isoflurane exposure (Chen et al., 2011). Our contrasting results in the two models suggest that these explanations are unlikely, because only the HI model exhibited substantial variability, despite the fact that all the aforementioned factors were consistent for both the MCAO and HI models. We cannot exclude the possibility that structural and physiological variations in the circle of Willis could contribute to the inconsistent brain damage after HI. Bonnin et al. (2011) reported that establishment of collateral recruitment via the basilar artery led to the presence or absence of a lesion. We also cannot exclude other possibilities, such as differences in the susceptibility to reperfusion damage, or in cardiovascular and respiratory function. As our model and the above-mentioned reproducible stroke model (Wen et al., 2004) are both permanent occlusion models, some mechanisms that occur during reperfusion may lead to large inter-animal variability.

There has only been one previous study in the literature that directly compared the MCAO and HI models (Ashwal et al., 2007). Unlike ours, variability in brain injury did not appear to be different between the two models in the previous study. The discrepancy between their results and ours may be due to the different MCAO procedures and the animals used. The previous report used a transient MCAO model in P10 spontaneously hypertensive rats, whereas we used a permanent MCAO model in P12 CB-17 mice.

We observed thalamic damage that was confined to the ipsilateral VPN in our MCAO model. As the VPN is supplied by thalamoperforating arteries originating from the basilar artery systems (Oscar and Holschneider, 2012), MCAO does not cause direct ischemic injury to this nucleus. Secondary neuronal damage in the thalamic nuclei after focal ischemia has been reported in adult rat models (Dihne et al., 2002; Schroeter et al., 2006). The damage in VPN was possibly due to retrograde degeneration of the thalamocortical projection (Dihne et al., 2002). Thalamic atrophy has been seen in children with neonatal MCA infarct (Giroud et al., 1995).

Our MCAO model exhibited neurological dysfunction in the rotarod and open-field tests; the mice with MCAO lost the response to a change of the environment from light to dark, while their overall activities were not disturbed significantly. The results in behavioral tests in immature rodent models of stroke or HI are not consistent and can often be contradictory. Rodents with ischemic insult exhibited significantly poorer rotarod performance compared with controls in some (Chen et al., 2012; Jansen and Low, 1996), but not all studies (Aden et al., 2003; Kadam et al., 2009; Lubics et al., 2005). Similarly, rodents with ischemic insult exhibited altered behavior in open-field test in some studies (Aden et al., 2002; Kadam et al., 2009; Lubics et al., 2005), but not in others (de Paula et al., 2009). The discrepancies among the reports may be due to differences in species/strain (de Visser et al., 2006), in the extent of brain damage, in the timing of the assessment (Lubics et al., 2005), and in the experimental paradigm. In the future more sensitive measures will be needed to confirm these results.

Seizure behavior, which is one of the main presenting symptoms in neonates with stroke, was not observed in our model during the 2-hour period following artery occlusion. Seizure behavior has been reported in a stroke model in immature CD1 mice (Comi et al., 2004), but not in other stroke models in immature rodents. That is likely due to strain-related differences in the susceptibility to seizures (Comi et al., 2005) or simply due to a lack of detailed assessment for seizure activities in the models. One possible reason to explain the inability to cause seizure in our model would be the distribution of the brain injury, which is confined to the ipsilateral cortex and did not involve the hippocampus. More detailed and longer observation periods will be needed before we can conclude that our model does not cause seizure activity, as the median time to seizure after the insult can be more than 2 h in some strains (Comi et al., 2005).

Conclusions

We believe that this model is useful for detailed analyses in preclinical studies of neonatal stroke using a smaller number of animals, because of its high reproducibility, excellent long-term survival rate, and measurable neurofunctional deficits, and that this model will be useful in assessing functional improvement in response to experimental therapies.

Disclosures

None.

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PERINATAL/NEONATAL CASE PRESENTATION

Serial hemodynamic assessment using Doppler echocardiography in a fetus with left ventricular aneurysm presented as fetal hydrops

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A 22-week fetus presented with a large left ventricular aneurysm, 24 × 21 × 18 mm in size, detected by abnormal four-chamber view, and severe fetal hydrops with pericardial effusion, ascites and skin edema. The aneurysm was thin-walled, hypokinetic, and had enlarged with gestational age, causing compression of the lung. Although the left ventricular function had progressively impaired as expressed by increase in Tei index, hydrops had resolved by 32 weeks of gestation, probably because of maternal digoxin therapy and successful compensation by the right ventricle, as represented by retrograde blood flow in the distal aortic arch via the patent arterial duct. Because of the significant risk of severe cardiorespiratory failure, we transported the mother to a neonatal cardiac surgical center at 38 weeks of gestation. Indeed, the baby showed severe cardiopulmonary failure after birth, showing 100% of cardiothoracic ratio on the chest X-ray film, but was saved by the successful Dor procedure, including surgical resection of the aneurysm at 10 h of life. In this case, serial echocardiographic evaluation can allow us to monitor the hemodynamics and lead to successful postnatal management.

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Keywords: ventricular aneurysm; fetal echocardiography; Doppler echocardiography; heart failure; hydrops

Congenital left ventricular aneurysm is a rare disease detected in the fetal period and may present with diverse clinical course.[1–3] Most cases of congenital left ventricular aneurysm and/or diverticulum are asymptomatic,[2,4,5] but may cause systemic embolization, heart failure,[4–9] valvular regurgitation,[1] ventricular wall rupture,[10] arrhythmia[11,12] or sudden cardiac death.[2] We describe the clinical course and hemodynamic change appreciated by fetal echocardiography in a fetus with severe form of left ventricular aneurysm causing hydrops.

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Case

A 32-year-old woman, gravida 1 and para 1, was referred to our institution at 22 weeks of gestation (GW), because fetal echocardiography, using an Aloka SSD 6500 (Aloka, Tokyo, Japan), revealed abnormal four-chamber view and hydrops, with pericardial effusion, ascitis and skin edema. There was a large aneurysm at the left ventricular free wall with a size of 24 × 21 × 18 mm (Figure 1) that compressed the ipsi-lateral lung with moderate mitral regurgitation on color Doppler mapping.

To monitor global cardiac function, we measured the Tei index,[13] which is defined as the sum of the iso-volumic contraction time and the iso-volumic relaxation time divided by ejection time. Practically, from the pulsed Doppler recordings, either mitral or tricuspid closing-to-opening time (a) was measured at the interval from the end to the onset of the mitral or tricuspid inflow velocity pattern. The corresponding ventricular ejection time (b) was measured from the onset to the end of the corresponding ventricular outflow velocity pattern. The Tei index was calculated as $(a-b)/b$. At presentation, the Tei index of the left and right ventricles of this fetus were 1.21 and 1.36, respectively, which indicated severe impairment of both ventricles.[13]

In serial echocardiograms obtained every 2 weeks, the actual size of aneurysm progressively increased, but the relative aneurysm volume, expressed by aneurysm volume divided by the left ventricular diastolic volume (LVA/LV),[5] transiently decreased from 22 to 26 GW and gradually increased afterward (Figure 2a). In parallel with the change in LVA/LV, the left ventricular Tei index transiently decreased at around 24 to 26 weeks, when the maternal oral digoxin therapy was started, and then gradually increased with GA. At 32 GW, hydrops resolved when Doppler study revealed the retrograde flow at the distal aortic arch, which indicated severely impaired left ventricular function compensated by the right ventricle, also appreciated by the discordant change in Tei index

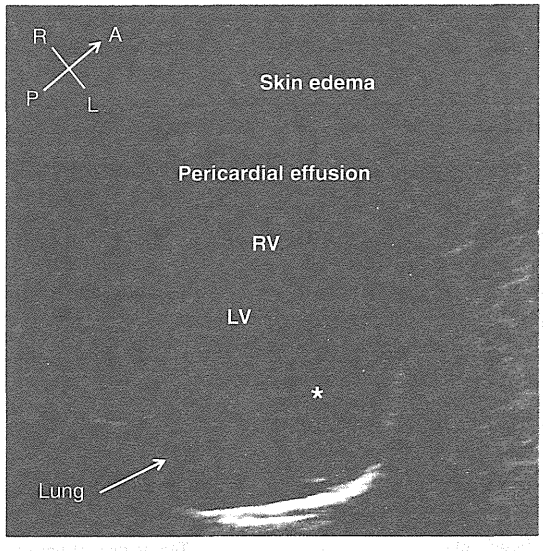

Figure 1 Two dimensional fetal echocardiography showed large thin-walled left ventricular aneurysm, 24 × 21 × 18 mm, originated from left ventricular free wall with large pericardial effusion and compressed the ipsi-lateral lung. Asterisk indicates aneurysm. R: right; L: left; A: anterior; P: posterior; RV: right ventricle; LV: left ventricle.

(Figure 2b). We expected that the baby should have severe cardiorespiratory failure because of left ventricular failure and possible pulmonary hypoplasia and decided maternal transportation to the neonatal cardiac surgery center for delivery and surgery.

At 38 weeks of gestation, the male neonate was delivered by cesarean section. Indeed, the cardiothoracic ratio on the chest X-ray was 100% and his cardiorespiratory failure rapidly progressed in spite of the intensive cardiopulmonary support. The Dor procedure,[14] which involves an endoventricular circular patch for post-infarct left ventricular wall asynergy in adults, was performed at 10 h of life. In this procedure, a large aneurysmal portion (4 × 5 cm) was resected, subendocardial circumferential purse-string suture was placed inside the left ventricle around the base of the aneurysm at the junction of scarred and normal endocardium, and a patch was then placed at this level to establish a new contour for the left ventricular cavity.

After the successful resection of the aneurysm, the left ventricle restored its function and started to pump sufficient blood to systemic circulation, with ejection fraction increasing from <10% to 53% after resection. He was discharged without any sign of congestive heart failure at 52 days of age. It has been 8 years since he had the Dor procedure and he goes to school without any symptom now.

On pathological examination of the left ventricular aneurysm, the myocardium was thin-walled, and outer part of the myocardium and the epicardium were largely replaced by fibrosis. Mural hemorrhage, hemosiderin and calcified deposits were documented. Neither evidence of neoplastic nature nor an active inflammatory process was seen.

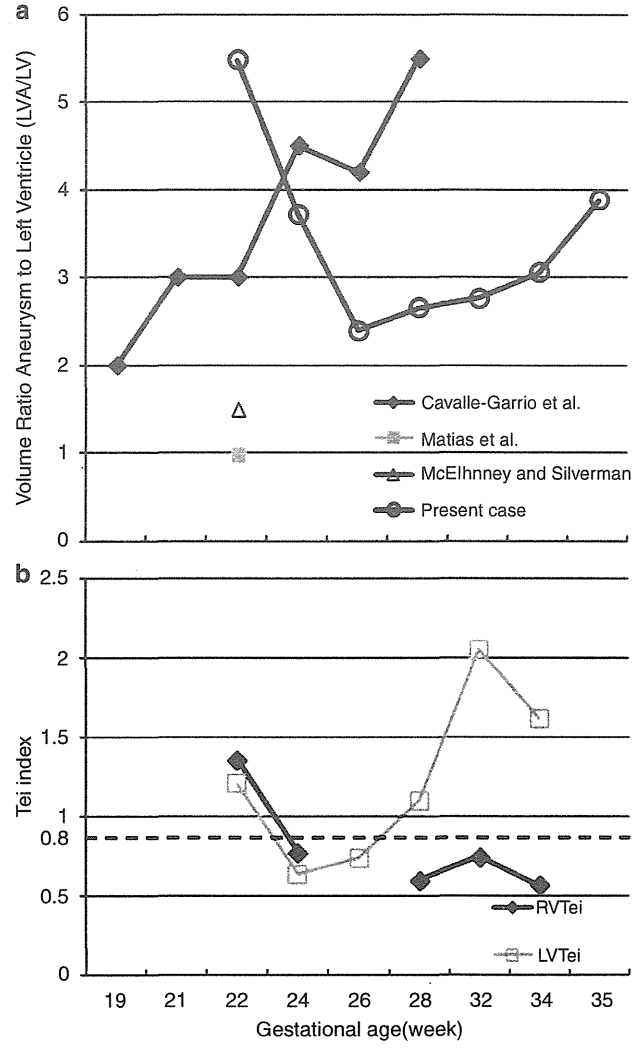

Figure 2 (**a**) Serial change in volume ratio of aneurysm to left ventricle (LVA/LV). Open circle indicates our case, LVA/LV ratio was >1 throughout the pregnancy. LVA/LV ratios of other cases were also >1, but outcome of these cases were termination of pregnancy or fetal demise. (**b**) Serial change in the Tei index. Note the discordant change in ventricular performance. Closed square indicates Tei index of the left ventricle and open circle indicates Tei index of the right ventricle.

Discussion

This case report illustrates that fetal left ventricular aneurysm can present with fetal hydrops and enlarge with gestation compromising left ventricular function. Serial echocardiographic evaluation can allow us to monitor hemodynamic change and plan appropriate postnatal management.

Owing to the rarity of congenital ventricular aneurysms and/or diverticulum, their natural history remains unclear. The prognosis of left ventricular aneurysm may vary depending on factors such as relative size in comparison to the ventricular cavity, growth on follow-up, and progression and compensation of cardiac failure.[4,5] Fetal echocardiography has made it possible to diagnose

Table 1 Prenatal diagnosis of left ventricular aneurysm/diverticulum with Hydrops

Reference	*GW (weeks)*	*Reason for referral*	*Echocardiograbic findings*	*Maximum diameter (mm)*	*Perinatal outcome*
El Kady *et al.*[7]	15	Pericaridal effusion	Large LV aneurysm (apical) (at 17 w), hydrops (at 19 w)	16	Pericardiocentesis (19 w), pericardioaminotic stent (23 w), died *in utero* (27 w)
Cavalle-Garrio *et al.* (1997)	19	Abnormal four-chamber view	Large LV aneurysm (apical), hydrops	n.d.	Died *in utero* (31 w)
	20	Pericaridal effusion, hydrops	Large LV diverticulum, (anterolateral wall), hydrops	n.d.	Died *in utero* (26 w)
Claubal *et al.* (2004)	21	Abnormal four-chamber view	Large LV aneurysm (apical), hydrops (at 27 w)	15	Died *in utero* (27 w)
Sepulve *et al.* (1996)	19	Abnormal four-chamber view	Large LV aneurysm (apical), hydrops	30	Termination of pregnancy (19 w)
Matias *et al.* (1999)	22	Fetal cardiomegaly	Large LV aneurysm (apical), hydrops	n.d.	Termination of pregnancy (23 w)
McElhnney and Silverman (1999)	22		Large LV aneurysm (apex), mild hydrops	n.d.	Termination of pregnancy (23 w)
Present case	22	Abnormal four-chamber view	Large LV aneurysm (free wall), hydrops	39	Alive, surgery at 0 day

Abbreviations: GW, gestational age; LV, left ventricle; N.d., not determined; w, weeks.

aneurysm/diverticulum prenatally with great accuracy and to monitor hemodynamics.

Table 1 shows the relevant clinical and fetal echocardiographic findings in previous cases and includes our present case.[4–9] All but our case resulted in fetal demise or termination of their pregnancy. The size of aneurysm of our case was larger than the remaining cases and progressively increased. McElhinney[5] reported that the fetuses with poor outcome showed 1 or > in LVA/LV ratio. In our case, LVA/LV ratio was >1 throughout the pregnancy that suggested poor prognosis (Figure 2a). With the relative enlargement of aneurysm, we expected poor prognosis of this fetus.

In addition, serial fetal echocardiograms including measurement of Tei index,[13] and color Doppler flow mapping, allowed us to monitor hemodynamic change precisely. Generally higher Tei index indicates cardiac dysfunction and fetuses with hydrops and higher Tei index >0.8 were reported to die either in prenatal or in early postnatal period.[13] In our case, Tei index of both sides of ventricles were over 1.0 at presentation of 22 GW, suggesting ominous prognosis. From 22 to 26 weeks, the Tei index decreased as the LVA/LV decreased (Figure 2), and we speculated that both the relative decrease in aneurysmal size and maternal digoxin treatment might lead this improvement in ventricular function. From 26 GW, both the LVA/LV ratio and the Tei index of left ventricle have increased from 0.7 to 2.0, but the Tei index of right ventricle has remained in normal range, <0.8. Substantially, the fetal hydrops resolved by 32 GW and this suggested that right ventricular function should have compensated left ventricular function with maternal digoxin administration, because color Doppler study demonstrated a retrograde flow at the distal aortic arch that is similar to hypoplastic left heart syndrome. These sequential examinations of hemodynamics and cardiac function in fetal period were very useful to determine management soon after birth. In fact, we could have saved this baby by the planned transportation and prompt operation.

The pathological findings seen in this case confirmed the diagnosis of an aneurysm, a thin myocardium and its outer part was largely replaced by fibrosis. The damage of the myocardial fibers of the aneurysm should have been considerable, with extensive transmural fibrosis and dystrophic calcifications, as found in one prenatal case early in gestation.[9]

In summary, fetal echocardiography detected very rare case of giant left ventricular aneurysm with hydrops, and the right ventricle compensated the left ventricular dysfunction with resolution of fetal hydrops. The information derived from sequential fetal echocardiography is important for planning the perinatal management.

Conflict of interest

The authors declare no conflict of interest.

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CASE REPORT

Refractory pulmonary hypertension following extremely preterm birth: paradoxical improvement in oxygenation after atrial septostomy

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Abbreviations

AcT/ET	Acceleration time relative to ejection time
BAS	Balloon atrial septostomy
CLD	Chronic lung disease
FiO_2	Inspired fractional oxygen concentration
PAH	Pulmonary arterial hypertension

Introduction

Despite newly introduced therapeutic regimens, outcomes of idiopathic pulmonary arterial hypertension (PAH) remain poor [5]. Recently, PAH associated with chronic lung disease (CLD) has been increasingly recognised, whose outcome is also unfavourable with a mortality rate of >30 % within 6 months of age [3, 7]. For idiopathic PAH refractory to pharmacological options, balloon atrial septostomy (BAS) has been proposed as a palliative intervention, aiming to prevent complete collapse of the circulation by releasing right atrial pressure through the atrial shunt [9]. However, little is known about the efficacy of BAS for PAH secondary to other clinical conditions. We present here a case of an extremely low-birth-weight infant who developed severe PAH associated with trisomy 21 and CLD, and whose PAH was permanently controlled by BAS with paradoxically improved arterial oxygenation.

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Case presentation

A 41-year-old woman, gravida 4, para 2, developed premature rupture of the membranes at 23 weeks and 5 days gestation; ritodrine hydrochloride, prophylactic antibiotics, and betamethasone were administered. A male neonate (birth weight, 725 g; Apgar scores, 5 and 7 at 1 and 5 min, respectively) was delivered by emergency caesarean section because of non-reassuring foetal status. The neonate required intubation for surfactant replacement therapy and mechanical ventilation; inspired fractional oxygen concentration (FiO_2) was swiftly reduced to <30 %. On echocardiography performed on day 0, transient pulmonary arterial hypertension (PAH) with the right-to-left dominant ductus arteriosus and trivial tricuspid valve regurgitation (TR) were observed, which resolved by day 2 when closure of the ductus arteriosus was confirmed; no cardiac defect was identified. Based on facial features characteristic of Down syndrome, a blood G banding test was performed, which confirmed trisomy 21. Mild leucopoenia and the presence of erythroblasts (with otherwise normal differential counts) were identified on a haemogram at birth, which resolved within the first week.

Chorioamnionitis was not observed on a placental histopathological examination; umbilical blood IgM was <5 mg/dL. However, the infant developed severe bronchopulmonary dysplasia (Fig. 1). Echocardiography on day 54 suggested mild PAH with shortened (28/155=0.18) acceleration time relative to the ejection time (AcT/ET) in the right ventricular outflow tract and a slightly increased (2.4 m/s) TR peak velocity. Although the infant was transiently extubated using nasal continuous positive airway pressure, abrupt and prolonged reductions in arterial oxygen saturation (SpO_2) <80 % with a poor response to 100 % oxygen (desaturation spells) were frequently observed corresponding to the patient's spontaneous activity. Despite treatments for CLD and

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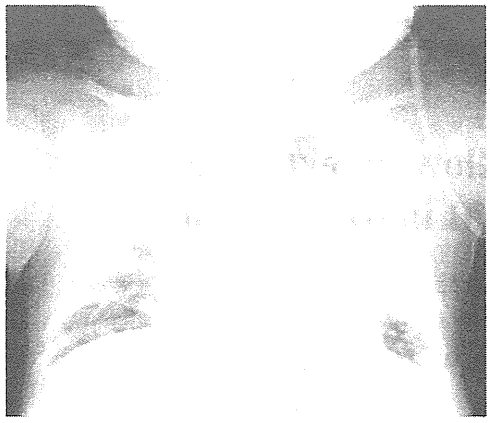

Fig. 1 Chest radiograph of the patient. Chest radiograph on day 79 showing severe lung injury with a mixed pattern of emphysema and atelectasis

PAH, including dexamethasone (started at 0.3 mg/kg/day followed by gradual weaning over 9 days), beraprost (2 μg/kg), sildenafil (3 mg/kg), and bosentan (4 mg/kg) (Fig. 2), the severity and frequency of the spells increased. Because severe spells were easily induced by spontaneous activity, continuous intravenous infusions of morphine (6 μg/kg/h) and rocuronium (4 μg/kg/min) were commenced. Following unsuccessful attempts to wean off deep sedation, tracheostomy was performed on day 282.

Echocardiography on day 376 showed consistently short AcT/ET (0.21) and a marked increase in the TR peak velocity (5.1 m/s), the latter of which was suggestive of super-systemic right ventricular pressure >100-mmHg. A foramen ovale with a diameter of 2-mm was observed, the flow of which was only visible during a desaturation spell in the direction of right to left. We concluded that an extension of conventional treatment was unlikely to improve the condition, but may merely prolong life.

After we obtained parental informed consent, on day 449, BAS was performed under general anaesthesia as a palliative treatment. Prior to BAS, the mean femoral and pulmonary artery pressures were 82 and 49-mmHg, respectively, and the left atrial pressure was 13-mmHg, giving a calculated pulmonary vascular resistance of 3.8 Wood units·m^2. With FiO_2 of 100 %, pulmonary vascular resistance decreased to 2.8 Wood

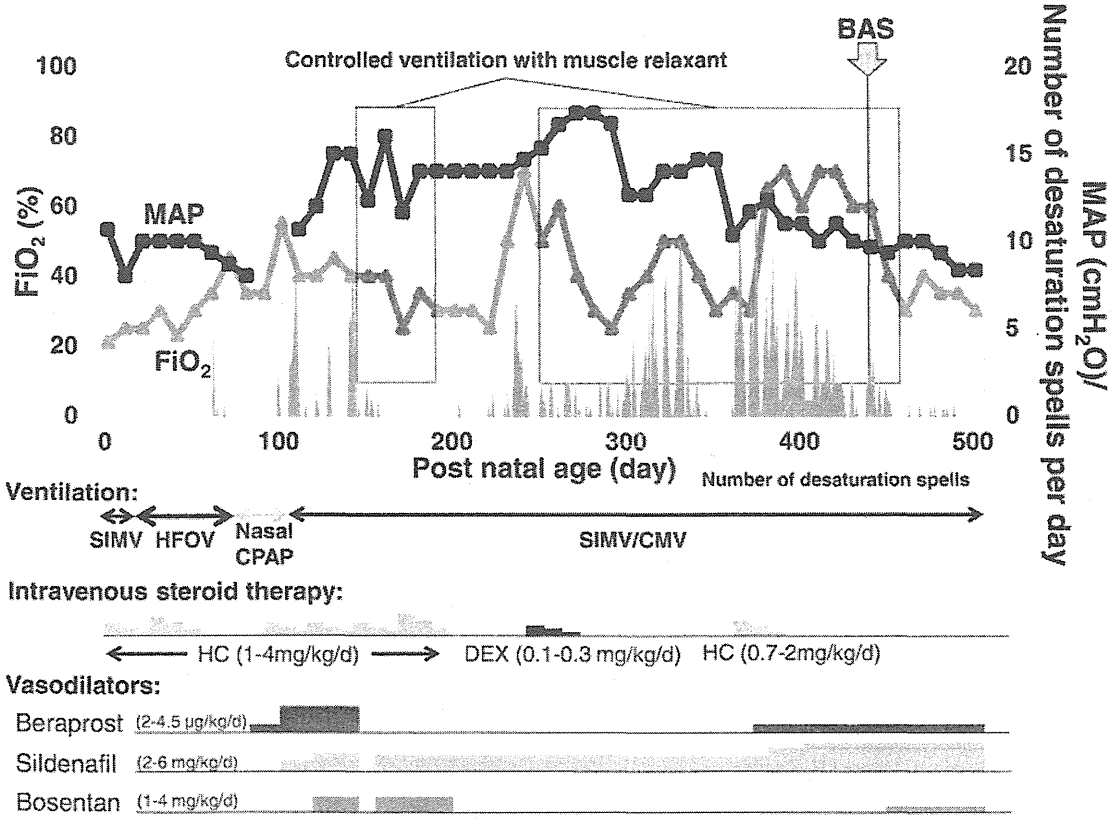

Fig. 2 Treatments and clinical variables over time. The patient required prolonged mechanical ventilation under deep sedation despite a range of medications shown in the lower panel. Minimum FiO_2 values required to maintain arterial haemoglobin oxygen saturation (SpO_2) >80 % at rest are shown. Desaturation spells are defined as SpO_2 <80 % longer than 10 min despite the use of 100 % oxygen (counted only once per hour when prolonged or repetitive). *CMV* conventional mechanical ventilation, *CPAP* continuous positive airway pressure, *DEX* dexamethasone, *FiO_2* fractional inspired oxygen, *HC* hydrocortisone, *HFOV* high-frequency oscillatory ventilation, *MAP* mean airway pressure, *SIMV* synchronised intermittent mandatory mechanical ventilation

units·m^2 with mean femoral and pulmonary artery pressures of 88 and 55-mmHg, respectively. When FiO_2 was reduced from 100 to 21 %, SpO_2 dropped from 93 to 80 %, and the mean pulmonary artery pressure increased to 118-mmHg, which was equivalent with the mean femoral artery pressure of 117-mmHg. BAS was then performed as a static balloon method using a 4×15-mm cutting balloon (Flextome®, Boston Scientific, Natick, MA, USA) with 10 atmospheres, followed by additional dilation using a 7×20-mm balloon (Ohicho II®, Kaneka Medical Products, Tokyo, Japan) with 18 atmospheres, which created a 5-mm hole at the atrial septum.

After BAS, bosentan (1 mg/kg) was re-prescribed to control the transient deterioration of PAH associated with the intervention. Enalapril (0.05 mg/kg) was used to reduce the elevated left atrial pressure (13-mmHg). Since the day of BAS, few spells of SpO_2 reduction were observed. Unexpectedly, baseline FiO_2 required to maintain SpO_2 >85 % was lowered to less than 30 % shortly after the intervention, resulting in successful weaning from deep sedation on day 465 and from mechanical ventilation on day 534. On day 595, echocardiography confirmed left to right atrial shunt and normalised AcT/ET of 0.35 (TR was too trivial to give its peak velocity), suggesting significant amelioration of PAH. The patient was discharged home on day 614 with home oxygen therapy. Severe desaturation spells were not observed until the latest visit of the patient to the follow-up clinic at 40 months old.

Discussion

We experienced a case of an extremely preterm infant with CLD and trisomy 21, who suffered from frequent episodes of severe PAH spells refractory to pharmacological treatments. However, unexpectedly, BAS permanently resolved these spells and improved baseline oxygenation, due possibly to different pathological mechanism from idiopathic PAH, where BAS is used for palliation.

CLD is a common complication of preterm birth [13]. As in our case, CLD is often accompanied by PAH [1, 3, 11]. Therapeutic regimens have been recently developed for PAH, such as prostaglandin I_2, phosphodiesterase type 5 inhibitor, and endothelin receptor inhibitor [4–6, 9–11, 14]. For PAH refractory to these options, BAS has been proposed as a palliative therapy. Micheletti et al. reported that BAS may improve right-heart function of patients with idiopathic PAH [9]. However, mortality associated with BAS (death <1 month after the procedure) is reported to be 5 to 16 % [8], highlighting the potential risk of the procedure for patients with severe PAH; it is suggested that the indication for BAS may be restricted to refractory cases with recurrent syncope and severe right-heart failure, and to patients awaiting pulmonary transplantation [9]. In our case, PAH was refractory to a range of pharmacological treatments, and the patient required 15 months of intensive mechanical ventilation under deep sedation. Together with objective data supporting the overall benefit of diverting right atrial blood to the systemic circulation, we concluded that our patient had an indication for BAS.

Based on a previous report, we initially expected that SpO_2 may be reduced by approximately 7 % after BAS because of mixing of the venous blood to the systemic flow [9]. However, BAS unexpectedly improved oxygenation during rest. In our case, a paroxysmal increase in the right-heart pressure might have been the main trigger of deleterious chain reactions, such as the release of endogenous excitatory catecholamines, inflammatory chemokines, and lung trauma due to high ventilator settings and high oxygen concentrations. Recent studies highlighted pivotal roles of inflammatory cytokines and growth factors in the progress of vascular remodelling and subsequent PAH [2]; improved control of right-heart pressure might help reduce the activation of the damaging cascade and assist the repair process of the pulmonary arterial endothelium. Future studies need to address the specific mechanism of PAH associated with underlying clinical conditions, such as CLD and Down syndrome [6, 12]. Although the benefit of BAS was obvious in our case, the indication of this procedure to young patients needs further investigation. As in patients with atrial septal defect, artificially created atrial septal communication may increase pulmonary flow. In the current case, inhaled nitric oxide was not considered because, at the time, only industrial gas was available, the use of which was strictly limited to perioperative or acute-phase patients; efficacy of inhaled nitric oxide and other pharmacological options also needs to be assessed in future studies.

Conclusions

Our findings highlighted that BAS may induce permanent remission of PAH with specific backgrounds, such as Down syndrome and CLD. However, given the risk associated with catheter interventions for patients with severe complications, the current indications of BAS for PAH should be strictly limited. Further prospective studies need to address the benefit, risk, and exact indication of this intervention for PAH secondary to Down syndrome, CLD, and other diseases.

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