(5) 肺血栓塞栓症の診断・治療に習熟する

症例検討会で検討した50例の中で4例が肺血栓塞栓症（pulmonary thromboembolism：PTE）での死亡であった．

深部静脈血栓症（deep vein thrombosis：DVT）はこれまで本邦では比較的稀であるとされていたが，生活習慣の欧米化などに伴い近年急速に増加し，その発症頻度は欧米に近づいている．DVTとそれに起因するPTEは一度発症するとその症状は重篤であり致命的となるので，急速な対処が必要となる．

そのためには，リスク評価をきちんと行い，予防をすることが重要である．委員会では予防をしっかりと行っていてもPTEを起こすことがあり，診療に当たるチーム全員が常にPTEが起こる可能性を意識し，実際に起こった際に速やかに対応するということも重要であるとの指摘があった．

継続の重要性

母体安全への提言の目的は母体の安全を究極まで高めることである．全例を検討することでこれまで気づかれていなかった重要な問題が数多く浮かび上がってきた．Saving Mothers' Livesでは過去血栓塞栓症が大きな問題になった際に対策を行い，明らかな効果を挙げることができた．しかし2006～2008年ではSepsisが最も大きい原因となり，問題となってきていることが明らかになった．

本邦でも，今後医療技術の発達や社会の変化などで今後本邦でも将来妊産婦死亡を取り巻く状況が大きく変わる可能性もある．これからもこの努力を継続して行くことが重要であると考えている．

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Special Theme Topic:
Stroke During Pregnancy or Delivery

Pregnancy and Delivery Management in Patients With Cerebral Arteriovenous Malformation: A Single-Center Experience

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Abstract

We described pregnancy and delivery management in 9 patients with cerebral arteriovenous malformation (AVM). Six patients presented with intracerebral hemorrhage (ICH) during pregnancy (first hemorrhagic episode); 2 patients presented with headache; and 1 patient with incidental detection of AVM. In the 3 patients with unruptured AVM, the diagnosis was made before pregnancy. In 3 of 6 patients who presented with ICH, AVM removal was performed during pregnancy. One patient required emergency surgery for the mass effect of the hematoma, and 2 patients with Spetzler-Martin grade I and II AVMs underwent elective surgery for the prevention of rebleeding. Radiosurgery for multiple AVMs was performed after delivery in one patient. Surgical resection and radiosurgery were performed after abortion in two patients. Of 3 patients with unruptured AVM, 2 patients became pregnant after radiosurgery and conservative treatment was initiated in 1 patient for Spetzler-Martin grade V AVM. Cesarean section was performed in 5 patients (one with severe uncontrollable pregnancy-induced hypertension) and vaginal delivery in 2 patients (one with grade V AVM). Delivery by obstetrical indication was possible in patients who underwent AVM resection during pregnancy. No rebleeding during pregnancy occurred. The maternal outcome was good except for the 2 patients with consequences of the initial ICH. The fetal outcome was good except for 2 cases of abortion. Pregnancy and delivery management in patients with AVM was successful in our institution. Early surgical intervention for AVM presenting as ICH during pregnancy could prevent rebleeding and improve the maternal and fetal prognosis.

Key words: arteriovenous malformation, pregnancy, delivery, surgery

Introduction

Cerebral arteriovenous malformations (AVMs) may affect the prognosis for both mother and fetus because they may result in fatal intracranial bleeding during pregnancy.[2,4,7,15,16,18,22] The natural history of AVMs is poorly understood, and even less understood in pregnant patients, because the frequency is rare and changes in the maternal body are complicated during pregnancy. No definitive guidelines for the treatment of AVMs during pregnancy exist and the management of cerebrovascular disease in pregnancy is under discussion.[4,10,20,24] We examined the results of pregnancy and delivery management in patients with AVMs in a single institution.

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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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Panel Data Analysis of Cardiotocograph (CTG) Data

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Abstract and Objective

Panel data analysis is a statistical method, widely used in econometrics, which deals with two-dimensional panel data collected over time and over individuals. Cardiotocograph (CTG) which monitors fetal heart rate (FHR) using Doppler ultrasound and uterine contraction by strain gage is commonly used in intrapartum treatment of pregnant women. Although the relationship between FHR waveform pattern and the outcome such as umbilical blood gas data at delivery has long been analyzed, there exists no accumulated FHR patterns from large number of cases. As time-series economic fluctuations in econometrics such as consumption trend has been studied using panel data which consists of time-series and cross-sectional data, we tried to apply this method to CTG data. The panel data composed of a symbolized segment of FHR pattern can be easily handled, and a perinatologist can get the whole FHR pattern view from the microscopic level of time-series FHR data.

Keywords: Cardiotocogram, Fetal heart rate, Panel data analysis, FHR pattern

Introduction

Intrapartum CTG monitoring is used to prevent non-reassuring fetal status in most perinatal medical facilities and the intrapartum management guidelines based on FHR classification were proposed in major countries. In literatures, there were many individual relationship studies between characteristics of FHR waveform pattern and outcome such as umbilical arterial blood gas data at delivery. Although these previous methods were not enough flexible to deal with the accumulated large number of time-series FHR waveforms, it will be easy to handle as panel data which are filtered from the FHR waveforms to symbolized data.

Table 1 - 134 patterns for FHR classification

Moderate Variability (Amplitude 6-25 bpm)

Deceleration			Variable			Late			Prolonged		
Baseline FHR	None	Early	Mild	Moderate	Severe	Mild	Moderate	Severe	Mild	Moderate	Severe
over 160 bpm	a1	b1	c1	d1	e1	f1	g1	h1	i1	j1	k1
110-160 bpm	a2	b2	c2	d2	e2	f2	g2	h2	i2	j2	k2
80-110 bpm	a3	b3	c3	d3	e3	f3	g3	h3	i3	j3	k3
70-80 bpm	a4	b4			e4		g4	h4			k4
below 70 bpm	a5	b5			e5			h5			k5

Minimal Variability (Amplitude 3-5bpm)

Deceleration			Variable			Late			Prolonged		
Baseline FHR	None	Early	Mild	Moderate	Severe	Mild	Moderate	Severe	Mild	Moderate	Severe
over 160 bpm	a10	b10	c10	d10	e10	f10	g10	h10	i10	j10	k10
110-160 bpm	a20	b20	c20	d20	e20	f20	g20	h20	i20	j20	k20
80-110 bpm	a30	b30	c30	d30	e30	f30	g30	h30	i30	j30	k30
70-80 bpm	a40	b40			e40		g40	h40			k40
below 70 bpm	a50	b50			e50			h50			k50

Absent Variability (Amplitude ≈2bpm)

Deceleration			Variable			Late			Prolonged		
Baseline FHR	None	Early	Mild	Moderate	Severe	Mild	Moderate	Severe	Mild	Moderate	Severe
over 160 bpm	a100	b100	c100	d100	e100	f100	g100	h100	i100	j100	k100
110-160 bpm	a200	b200	c200	d200	e200	f200	g200	h200	i200	j200	k200
80-110 bpm	a300	b300	c300	d300	e300	f300	g300	h300	i300	j300	k300
70-80 bpm	a400	b400			e400		g400	h400			k400
below 70 bpm	a500	b500			e500			h500			k500

Marked Variability (Amplitude≥26bpm)	M	Sinusoidal FHR Pattern	S

Methods

In 1909 consecutive low risk pregnancies, each 10 min-segment of the last 60 min. of vaginal delivery was retrospectively labeled into 134 patterns (Table 1) by expert perinatologist. These were used to two dimensional panel data of 10 min. patterns and individuals with the umbilical arterial blood gas data, pH, base excess (BE), $PaCO_2$ and PaO_2 at birth to analyze the relationship between the FHR pattern sequence and the outcome.

Results

Using the panel data, it is clear to handle each time-series FHR data as the representative segments from beginning to end of FHR monitoring for further analysis. A result was shown in Figure 1. In this analysis, each number of occurrences of deceleration in 1552 cases which had moderate variability and 110-160 bpm baseline was plotted with the pH at birth. Although all of these cases had normal FHR baseline and variability, the number of the deceleration occurrence had a significant difference with the pH at birth. Although the panel data of this study was only one hour data, it compressed the amount of time series FHR to character based length, and a perinatologist can overview the whole FHR data under the standard interpretation.

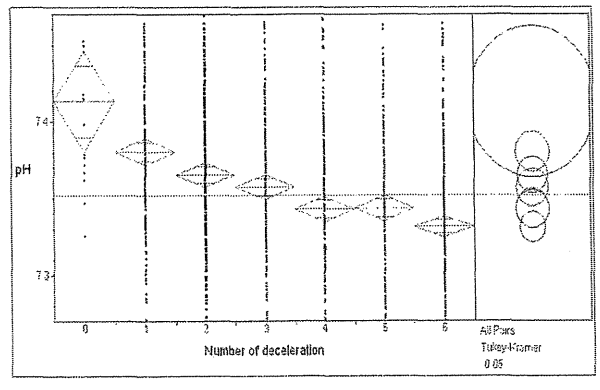

Figure 1. Relationship between umbilical arterial blood gas pH at delivery and the number of deceleration before delivery from FHR panel data.

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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.

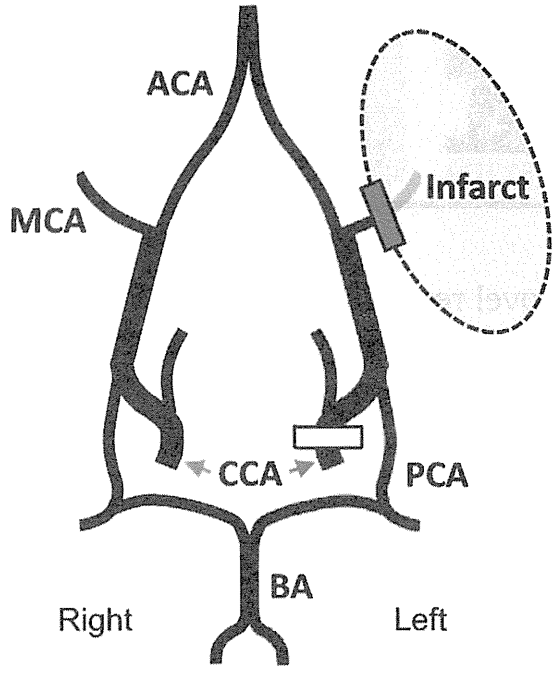

Fig. 1. Representation of the circle of Willis in rodents. The anatomic arterial system at the base of the brain in horizontal projection. ACA; anterior cerebral artery. BA; basilar artery. CCA; common carotid artery. MCA; middle cerebral artery. PCA; posterior cerebral artery. In the MCA occlusion model, 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

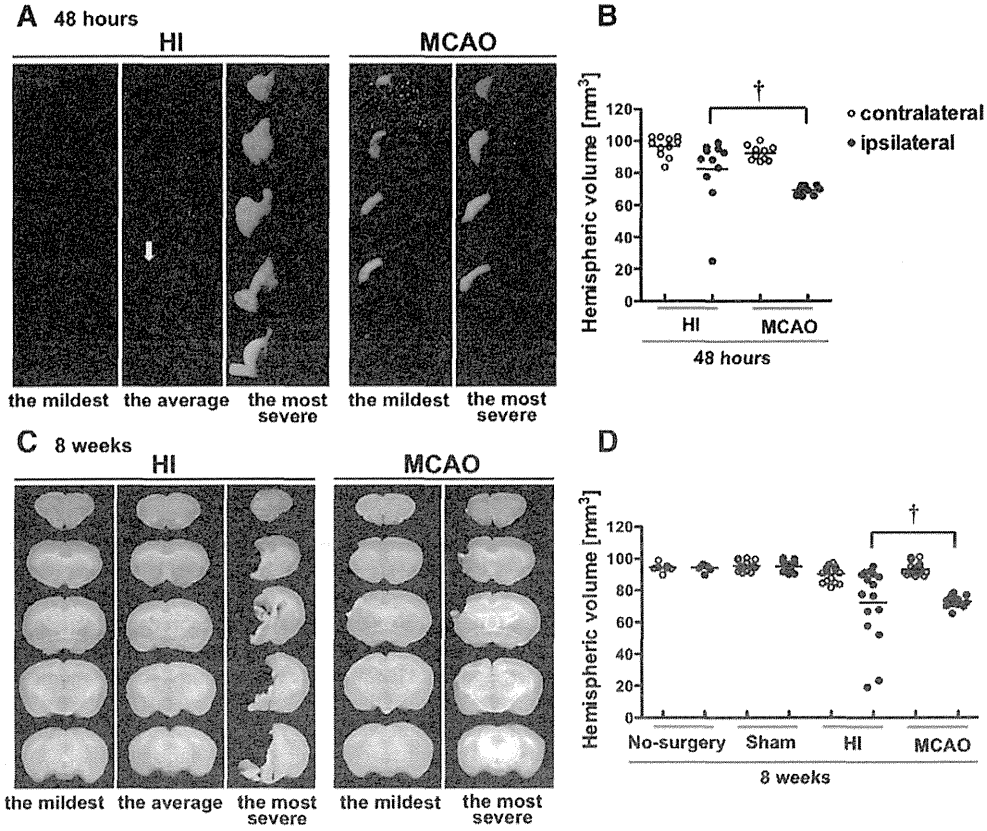

Fig. 2. Macroscopic brain injuries. (A) Images of TTC-stained brain sections 48 h after middle cerebral artery occlusion (MCAO) or hypoxia–ischemia (HI). The brains with the mildest injury and the most severe injury in the MCAO group and those with the mildest, the average, and the most severe injury 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 $n = 17$).

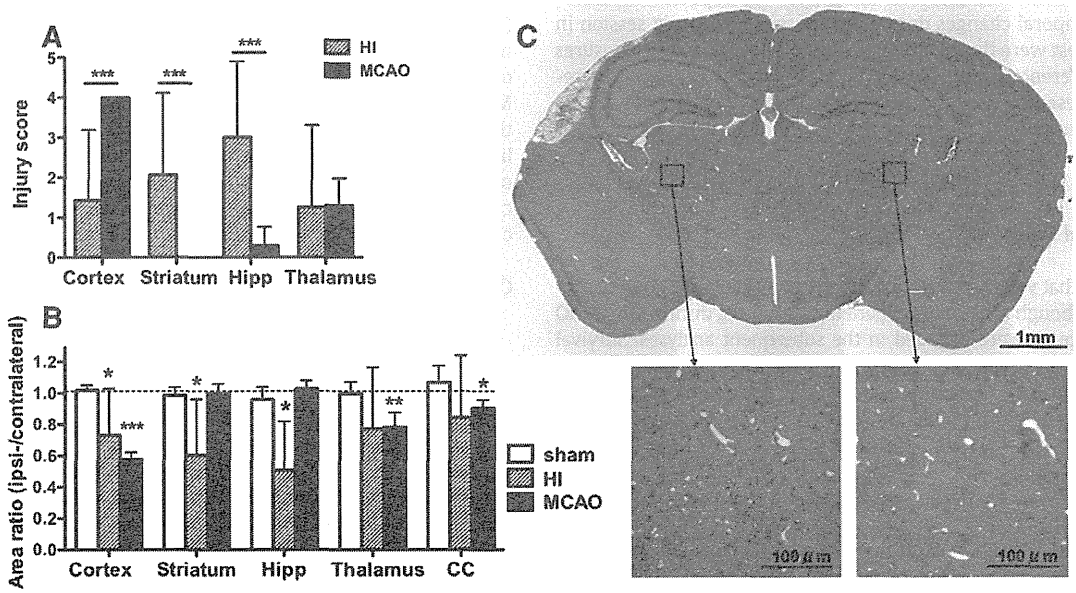

Fig. 3. Microscopic brain injuries. (A) Neuropathological injury scores examined in hematoxylin–eosin-stained sections 8 weeks after the insult. ***$P < 0.001$. (HI $n = 16$; MCAO $n = 17$) (B) The ratios of ipsilateral/contralateral areas in each region examined at 8 weeks after the insult. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, 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.

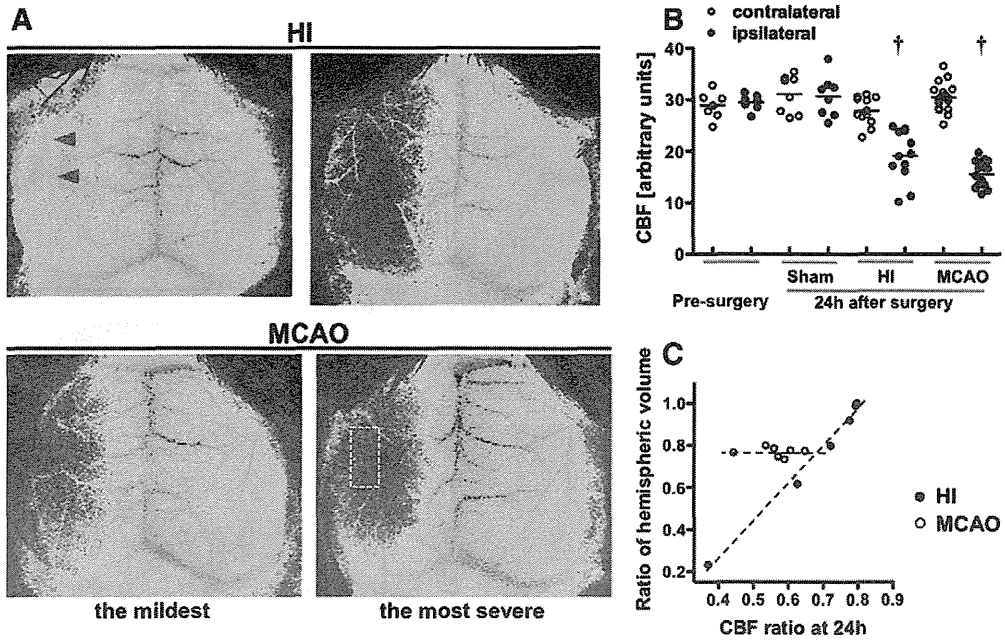

Fig. 4. Cerebral blood flow. (A) Images of the cerebral blood flow (CBF) 24 h after the insult. The reduction of the CBF, indicated by the bluish color, was consistent after MCAO, but not after HI (the arrowheads indicate the main trunk of the MCA). (B) CBF levels were measured in the ischemic core region (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. 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.

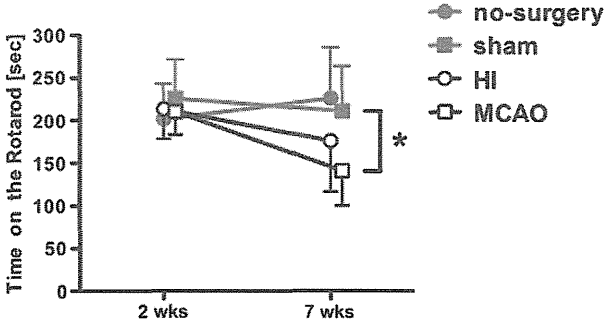

Fig. 5. Rotarod test. Repeated-measures two-way ANOVA showed significant time and group differences in sensorimotor performance, assessed 2 and 7 weeks after the insult. Performance was significantly impaired in mice with MCAO compared with the sham-surgery groups. *$P < 0.05$. (no-surgery $n = 19$;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

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.

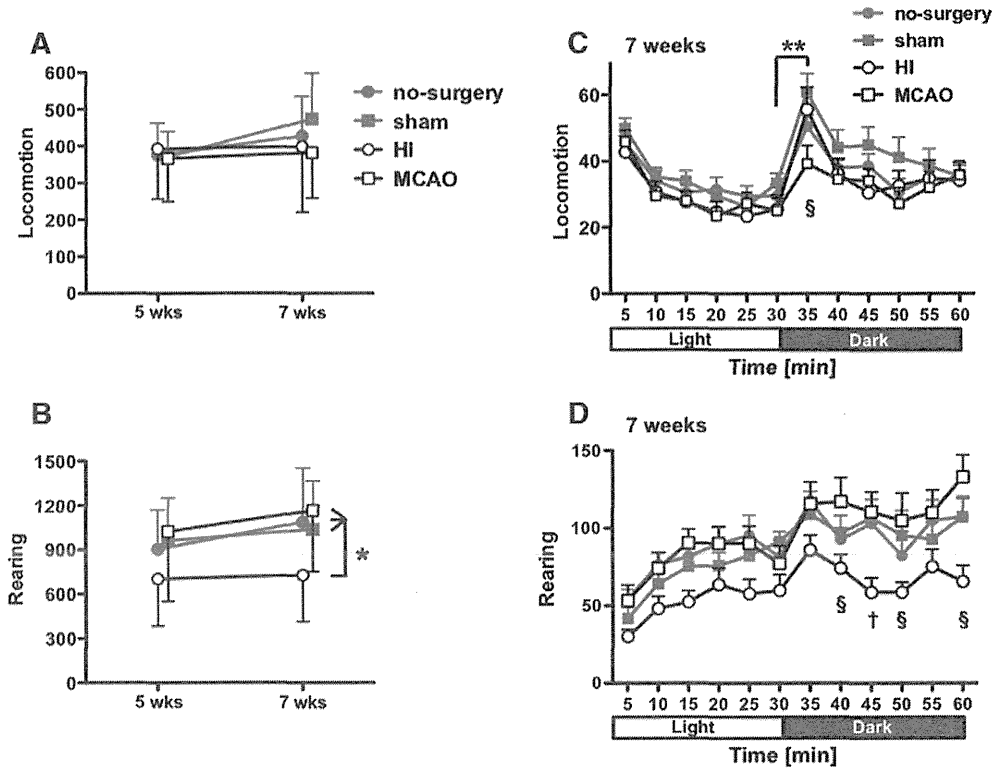

Fig. 6. Open-field test. (A, B) Overall activities during the 60-min session 5 and 7 weeks after the insult were analyzed by two-way repeated measures ANOVA. While there was no time difference with respect to either locomotion or rearing, there were significant group differences with respect to rearing, 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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OBSTETRICS

Risk factors for maternal and fetal outcome in pregnancy complicated by Ebstein anomaly

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OBJECTIVE: The goal of the study was to examine risks in pregnancy in patients with Ebstein anomaly.

STUDY DESIGN: Data were examined retrospectively for 13 patients (27 pregnancies, 21 live births) with Ebstein anomaly during pregnancy who were treated at our institution from 1985 to 2011. The associated anomalies in these patients were atrial septal defect (ASD) (n = 4) and the Wolff-Parkinson-White syndrome (n = 6).

RESULTS: Before pregnancy, 2 patients underwent ASD closure and 1 received tricuspid valve replacement (TVR). In all patients, the cardiothoracic ratio increased from 55.1 at conception to 57.0 during pregnancy and 58.0 postpartum ($P < .05$). Cesarean sections were performed in 3 cases: 1 with ventricular tachycardia and orthopnea (New York Heart Association [NYHA] III) preterm; at full term, and the third in a patient with a mechanical tricuspid valve who developed maternal cerebellum hemorrhage at 27 weeks. The baby died of prematurity in the third case. In all other cases (20 of 21), neonatal prognoses were good without congenital heart diseases. There were 6 spontaneous abortions. Recurrent paroxysmal supraventricular tachycardia occurred during pregnancy in 2 cases and was treated with adenosine triphosphate or verapamil. In 17 pregnancies, NYHA remained in class I and all had full-term vaginal delivery.

CONCLUSION: Maternal and fetal outcomes are good in patients with Ebstein anomaly and NYHA class I. However, pregnancy in Ebstein anomaly can be complicated with tachyarrhythmia or cardiac failure. In post-TVR cases, meticulous care is required for these complications during pregnancy and delivery.

Key words: arrhythmia, cardiac function, Ebstein anomaly, New York Heart Association, pregnancy

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Ebstein anomaly occurs in about 0.5% of cases of congenital heart disease.[1-4] This congenital malformation is characterized by downward displacement of tricuspid valve into the right ventricle because of an anomalous attachment of septal and posterior leaflets. The abnormally situated tricuspid orifice divides the right ventricle into a proximal atrialized segement and a distal functional small ventricular chamber. Ebstein anomaly usually revealed tricuspid regurgitation with right atrial enlargement and is often associated with atrial septal defect (ASD) and the Wolff-Parkinson-White (WPW) syndrome type that leads to cyanosis and arrhythmias such as supraventricular tachyarrhythmia.[3]

Many women with Ebstein anomaly reach child-bearing age, but there is little information about the risks of pregnancy and delivery,[5-7] with only 2 reports of a relatively large series of pregnancy complicated with Ebstein anomaly.[7,8]

In these reports, there were a total of 56 patients and 153 pregnancies, in which miscarriage occurred in 16%, neonatal death in 2%, preeclampsia in 1%, preterm delivery in 29%, vaginal delivery in 90%, heart failure of New York Heart Association (NYHA) class III or above in 0.7%, and life-threatening arrhythmia in 0.7%.[7,8] Thus, pregnancy in women with Ebstein anomaly is generally well tolerated, but some patients are undiagnosed until the onset of symptoms such as dyspnea and atrial flutter in the latter half of the pregnancy.[5,8] Furthermore, some patients with Ebstein anomaly are asymptomatic, even after multiple deliveries, and the condition may not be diagnosed until the age of 50 years or later.[8]

The variety of symptoms and disease severity and differences in complications such as cyanosis, arrhythmia, and stroke make a uniform evaluation difficult. Therefore, the current retrospective study of pregnancy complicated with Ebstein anomaly was carried out to determine factors that influence maternal and fetal prognosis.

Materials and Methods

Maternal and fetal mortality and morbidity in 13 patients with Ebstein anomaly (27 pregnancies) were examined as a case series based on charts in our hospital from January 1985 to December 2011. Cases associated with congenital heart diseases other than ASD and patent foramen ovale were excluded.

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TABLE 1
Patient characteristics

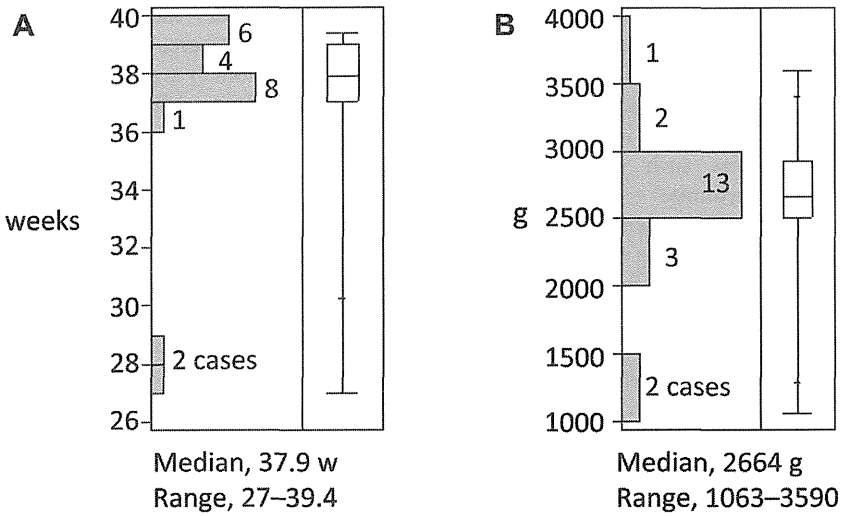
Case	Associated lesions		Age at onset, mo	Age at initial diagnosis, mo	Age at delivery, y
1			9	9	25
2	ASD		3	5	35, 37
3	ASD	WPW	5	26	35
4	ASD		6	28	31
5			6	10	28
6	ASD		7	7	30, 33
7		WPW	7	35	35, 39
8		WPW	18	38	38
9		WPW	10	10	31
10		WPW	13	21	25, 29
11		WPW	22	22	26, 28, 36
12			25	25	27, 36
13			30	30	32, 36

Each age except 9 months (case 1) is shown by age in years. In case 1, the first symptom was heart murmur.

ASD, atrial septal defect; *WPW*, Wolff-Parkinson-White syndrome.

Katsuragi. Ebstein anomaly in pregnancy. Am J Obstet Gynecol 2013.

FIGURE 1
Time of delivery and birthweight



A, The median (range) gestational weeks at delivery was 37.9 weeks (range, 27–39.4 weeks). Most cases delivered between 37 and 40 weeks, but 2 cases ended in preterm delivery at 27 and 28 gestational weeks. The number on the bar indicates the number of cases. **B**, The median (range) neonatal birthweight was 2664 g (1063–3590 g). Most cases were included in the standard normal distribution between 2000 and 4000 g, but in 2 cases the birthweight was less than 1500 g.

Katsuragi. Ebstein anomaly in pregnancy. Am J Obstet Gynecol 2013.

Cardiac function was evaluated by echocardiography performed before pregnancy, at conception, in each trimester, and postpartum. Cardiothoracic ratio (CTR) was measured on chest X-rays taken before pregnancy, in the third trimester, postpartum, and at other times if needed. A Holter electrocardiogram was recorded in each trimester and postpartum. Vaginal delivery was attempted for women with spontaneous labor, whereas cesarean section was selected for those with a need for early delivery for both fetal and maternal indications. The NYHA classification was used to evaluate cardiac status.[9]

Data collection

Data were collected for maternal age; age at diagnosis; CTR; grade of tricuspid valve regurgitation (TR); NYHA functional class during and after pregnancy; delivery mode (cesarean section or vaginal delivery); treatment before, during, and after delivery; time of delivery (gestational weeks); and birthweight. Echocardiography was performed for all babies to evaluate associated congenital heart diseases.

Statistical analysis

For continuous variables, a Student *t* test was performed for analysis of normally distributed data; otherwise a Wilcoxon test was used. All statistical analyses were performed using JMP 7 (SAS Institute, Cary, NC). $P < .05$ was considered statistically significant.

RESULTS

There were 27 pregnancies and 21 live births in 13 patients during the study period. Six pregnancies were spontaneously aborted before 10 weeks of gestation. The median (range) ages at the time of initial symptoms, diagnosis of Ebstein anomaly, and delivery were 10 (0–30), 21 (0–38), and 32 (25–29) years old, respectively (Table 1). In 2 patients, heart disease was not diagnosed at the time of conception (cases 7 and 8). Four patients had ASD, 6 had WPW syndrome, and 1 had both of these conditions. No patients had cyanosis.

The patients with ASD developed symptoms of Ebstein anomaly earlier than those with WPW (5.3 vs 11.2 years

old, $P < .05$), but there was no difference in the age of diagnosis (12.2 vs 10.2 years old, P = not significant) or delivery (33.5 vs 32.2 years old, P = not significant). The median time of delivery was 37.9 (27–39.4) gestational weeks, and the neonatal weight was 2664 (1063–3590) g (Figure 1). There were 2 early deliveries at 27 and 28 weeks, respectively, and in these 2 cases, the babies were extremely low birthweight infants (between 1000 and 1499 g) (Figure 1). There was 1 small-for-gestational-age baby. The other 20 babies were appropriate for gestational age. No babies had congenital heart diseases. One baby born with a birthweight of 1063 g at 27 weeks died of prematurity. All other babies showed normal growth and no neurological disability.

Treatment before pregnancy

Ablation therapy for paroximal supraventricular tachycardia (PSVT) accompanied with WPW syndrome) was performed in 2 cases before pregnancy (cases 9 and 11) (Table 2), and there was no other arrhythmia event during and after delivery. One patient (case 2) with mechanical tricuspid valve replacement (TVR) developed maternal cerebral hemorrhage at 27 weeks of gestation and cesarean section was performed.

Treatment during pregnancy

Nonsustained ventricular tachycardia (NSVT) was terminated with bisoprolol fumarate (case 1). Diuretics and oxygen were administered when dyspnea caused by cardiac failure occurred (cases 1 and 3) (Table 2). Two patients had recurrent PSVT during pregnancy (cases 7 and 10). Each arrhythmia was treated effectively with verapamil or adenosine triphosphate. Each patient underwent ablation therapy after delivery.

Treatment after delivery

The patient with ASD and severe TR (case 4) developed cardiac failure (NYHA class worsened to III) at 16 years after delivery, and ASD closure and tricuspid valvoplasty were performed (Table 2). In 2 cases, diuretics were given because of worsening of cardiac failure (cases 6 and 12).

TABLE 2
Treatment before, during, and after pregnancy

Case	TR before pregnancy	CTR (%) in 1st pregnancy			Delivery week	CV event	Treatment before pregnancy
		Before	Third	Post			
2	Mild	53	27	58	27	CHF	ASD closure, TVR (3 y)
9	Mild	46	39	53	39	PSVT	Ablation (15 y)
11	Mild	42	39	52	39	PSVT	Ablation (22 y)
							During pregnancy
1	Mod	62	28	66	28	CHF	Furosemide, oxygen
3	Mod	58	37	58	37	CHF	Furosemide, oxygen
7	Mod	nd	39	62	39	PSVT	Verapamil
10	Mild	47	39	55	39	PSVT	ATP
							After pregnancy
4	Sev	55	37	62	37	CHF	ASD closure, TVP
6	Mod	59	37	58	37	CHF	Furosemide
12	Sev	62	37	61	37	CHF	Furosemide
5	Mod	54	38	52	38	No	None
8	Mod	55	38	58	38	No	None
13	Mod	57	61	58	37	No	None

Furosemide; Bristol Laboratories, Luton, UK.

ASD, atrial septal defect; *ATP*, adenosine triphosphate; *CHF*, congestive heart failure; *CV*, cardiovascular; *mod*, moderate; *PSVT*, paroximal supraventricular tachycardia; *sev*, severe; *TVP*, tricuspid valvoplasty; *TVR*, tricuspid valve replacement.

Katsuragi. Ebstein anomaly in pregnancy. Am J Obstet Gynecol 2013.

Change in CTR

The median (range) CTR significantly increased from before to during pregnancy (55.1% [42–62%] vs 57.0% [51–72%], $P < .05$) and from before pregnancy to after delivery (55.1% [42–62%] vs 58.0% [52–62%], $P < .05$) (Figure 2).

Changes in TR

TR was severe in 3, moderate in 10, and trivial to mild in 8 pregnancies and did not change in any of the 21 cases throughout the study period. Enlargement of CTR greater than 0.6 during pregnancy and postpartum was more common in cases with moderate (8 of 10, 80%) and severe (3 of 3, 100%) TR, compared with mild TR cases (0 of 8, 0%).

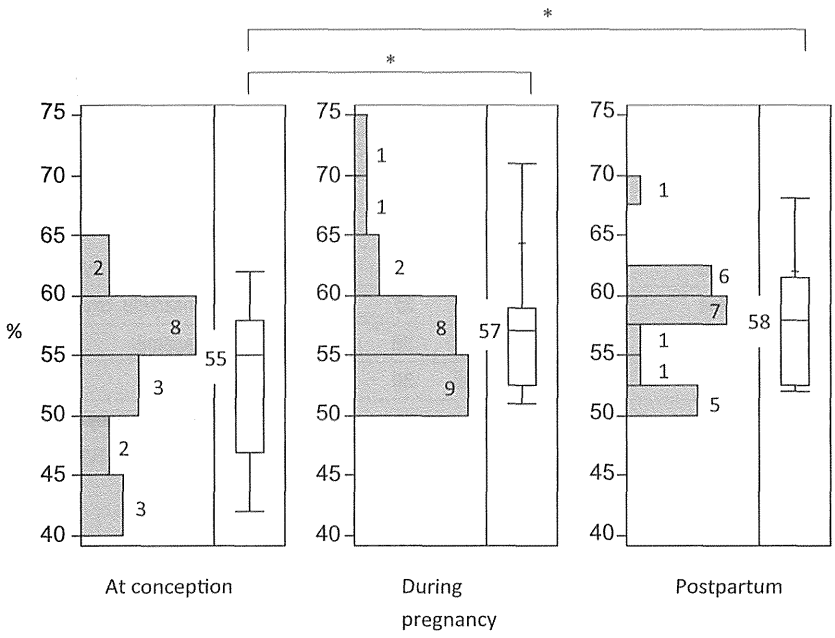
Changes in NYHA functional class

In 18 of the 21 pregnancies, NYHA class I was maintained throughout pregnancy (Figure 3). In 17 of these pregnancies, full-term vaginal delivery was possible. The other case was terminated at 27 weeks because of maternal cerebellum hemorrhage (case 2). One case with severe TR and ASD deteriorated in cardiac function from NYHA class I to III 16 years after delivery (case 4). Two cases dropped by 1 NYHA class (1 with class I, the other with class II) temporarily during pregnancy but returned to the original classes after delivery (cases 3 and 1).

COMMENT

We investigated the maternal and fetal outcomes in 21 pregnancies in 13 patients with Ebstein anomaly. There were 2 preterm cesarean sections and 1 neonatal death. Postdelivery courses were good, but 1 case with severe TR and ASD deteriorated in cardiac function from NYHA class I to III, and another patient with a severely enlarged right heart remained in class II but with transient deterioration to class III during pregnancy. Two patients needed diuretics

FIGURE 2
Changes in the cardiothoracic ratio



The median (range) of cardiothoracic ratios at conception, during pregnancy, and postpartum were 55.1% (46–62%), 57.0% (51–72%), and 58% (52–68%), respectively. The *asterisk* indicates a value of $P < .05$ (the Wilcoxon test) for conception vs during pregnancy and for conception vs postpartum.

Katsuragi. Ebstein anomaly in pregnancy. Am J Obstet Gynecol 2013.

postpartum because of worsening of edema. One patient transiently deteriorated to NYHA class II during pregnancy.

Maternal cyanotic congenital heart disease has been associated with prematurity and low birthweight and with infant survival rates of 50-55%.[10-12] In the current study, there were 3 pregnancies in 2 patients with unrepaired ASD, but neither patient was cyanotic and the mother did not have hypoxemia. One fetus showed intrauterine growth restriction that was not related to maternal cyanosis. Obstetric complications were infrequent in this series and no patient had preeclampsia. The miscarriage rate of 21% was similar to the standard rate of 15-20%.[13,14]

During pregnancy cardiac output increases to 150% with increased heart rate, and the autonomic nerve system is activated.[15] These changes increase the risk of cardiac failure in patients with Ebstein anomaly because of superimposition of hyperdynamic and hypervolemic circulatory stress on the preexisting enlarged right heart, decreased right ventricular function with increased TR, and a compressed left ventricle. The median CTR significantly increased during pregnancy and postpartum, and this was especially apparent in symptomatic cases such as those with dyspnea or worsening of edema and in those with worsened NYHA class. Attenhofer Jost et al[16] also found that CTR greater than 0.65 was related to a poor prognosis in Ebstein anomaly, and women with increase in CTR had the worst outcomes of pregnancy and postpartum. Therefore, our findings and those of Attenhofer Jost et al indicate that worsening of CTR is useful for the prediction of maternal outcome.

However, it has been reported that Ebstein patients tolerate pregnancy well.[5,7,8] In an investigation of 12 pregnancies complicated by Ebstein anomaly, Donnelly et al[8] found that mild dyspnea was a common symptom in the third trimester but was no more troublesome than that described in normal pregnancy, except for 1 case. However in our series, there were 2 early terminations of pregnancy because of heart failure and complications of a mechanical valve, 3 patients needed diuretics because of progression of edema, 1 dropped to NYHA class III in the postdelivery course, and 2 showed recurrent PSVT during pregnancy. In total, 7 patients showed some cardiac symptoms during pregnancy and 1 had deteriorated cardiac function after delivery in our series. Therefore, unique strategies for reducing the risk in pregnancy could include arrhythmia control by catheter ablation and bioprosthetic valve replacement before pregnancy in a case with symptoms because of these complications.

In our patients, even some in NYHA class I at conception, developed PSVT and NSVT, which indicates that arrhythmia during pregnancy is a concern in patients with Ebstein anomaly and that the outcome of pregnancy may not always be good in these patients.

In our study, the severity of TR did not progress during pregnancy and postpartum. In a normal pregnancy, systemic vascular resistance decreases to 70% of the level of the nonpregnant state until 16 weeks and then maintains this level until the end of the second trimester.[17] This decreased afterload is beneficial for patients with Ebstein anomaly with mild cardiomegaly and mild TR, and such patients tolerated pregnancy well. Most cases with moderate to severe TR developed cardiomegaly with CTR of greater than 0.6 during pregnancy and postpartum in our series, and Celermajer et al[18] found that late hemodynamic deterioration in Ebstein anomaly may be due to increased right or left heart failure or both.

Our findings and those of Celermajer et al[18] indicate that NYHA functional class and prepregnancy severity of TR may be predictive of worsening of maternal and fetal outcome. And echocardiography is the most important diagnostic test in Ebstein's anomaly, permitting accurate assessment of the anatomy and distal attachments of the tricuspid valve, the size and contractility of the functional right ventricle, and the overall grade of disease