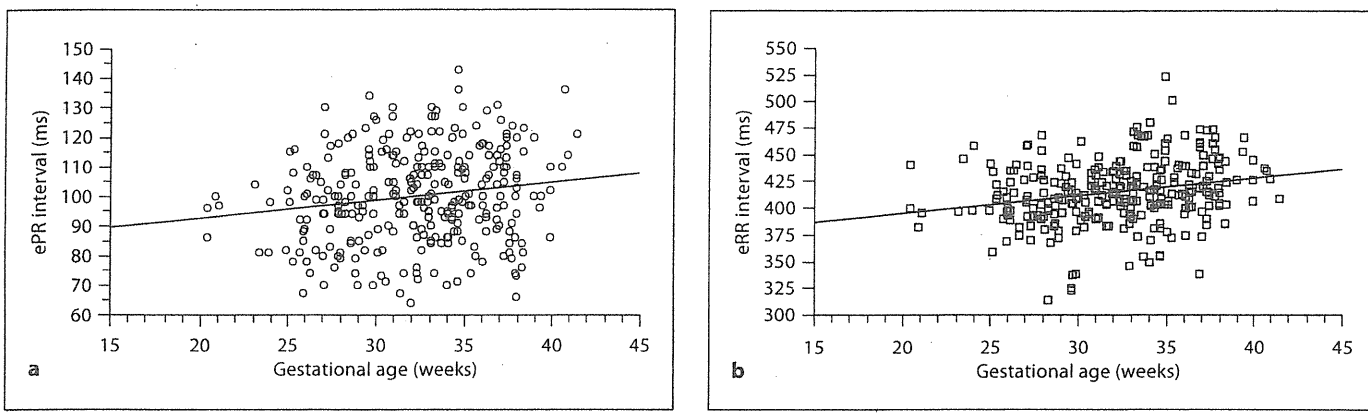

Fig. 2. Relationship between gestational age and the ePR interval (**a**) and eRR interval (**b**) by fMCG. The ePR and eRR intervals correlated significantly but weakly with gestational age (ePR: n = 295, y = 80.7 ± 0.60x, r = 0.162, p = 0.0053; eRR: n = 295, y = 362.6 ± 1.65x, r = 0.232, p < 0.0001).

Table 2. Comparisons of RR and PR intervals measured by fMCG and fUCG (LV in/out) (n = 120)

	fMCG	fUCG	Paired t test
RR interval, ms	414.8 ± 24.3	419.0 ± 27.6	p = 0.120
PR interval, ms	101.7 ± 15.8	119.6 ± 12.4	p < 0.0001

Table 3. Comparisons of RR and PR intervals measured by fMCG and fUCG (SVC/aAo) (n = 79)

	fMCG	fUCG	Paired t test
RR interval, ms	411.0 ± 5.0	414.1 ± 29.5	p = 0.381
PR interval, ms	102.0 ± 14.1	120.2 ± 12.1	p < 0.0001

Student's paired t test revealed significant differences between the ePR and mPR determined by LV in/out ($p < 0.0001$) and SVC/aAo ($p < 0.0001$). Bland-Altman analysis for comparison of mPR and ePR showed a mean difference of 14.6% (95% limits of agreement −10.7, 39.9) for LV in/out and 14.7% (95% limits of agreement −8.6, 38.0) for SVC/aAo (fig. 3a, b). On the other hand, there were no significant differences between mRR and eRR (fig. 3c, d). Stepwise multiple regression analysis indicated that the difference between the ePR and the mPR was not significantly affected by any factor.

The mean interobserver difference in PR interval was 1.0% (95% limits of agreement −6.0, 8.1) for fMCG, 0.4% (95% limits of agreement −10.6, 11.4) for LV in/out, and 2.3% (95% limits of agreement −10.6, 15.1) for SVC/aAo. The mean intraobserver difference in PR interval was −0.1% (95% limits of agreement −6.4, 6.2) for fMCG, 1.0% (95% limits of agreement −8.9, 10.9) for LV in/out, and 2.2% (95% limits of agreement −8.0, 12.3) for SVC/aAo.

Discussion

The PR interval encompasses the conduction time of the atrium (sinus node to entrance of atrioventricular node), the atrioventricular node, and the His-Purkinje system. These conduction properties depend on various factors such as heart size, the extent of autonomic nervous system development, and myocardial damage. Our results demonstrated that ePR showed a significant, but weak, correlation with gestational age. Previous studies described prolongation of ePR measured by fMCG [2–4] and mPR measured by fUCG [17, 18] with increase in gestational age. This phenomenon seems to be reasonable because the fetal heart size becomes larger and parasympathetic function develops with increasing gestational age, as is the case with normal postnatal development. However, some controversy exists on this point. Some reports showed that PR intervals are independent of gestational age [9, 19, 20].

Our results demonstrated overestimation of pulsed Doppler-derived mPR compared with ePR determined by fMCG. This finding was similar to those of previous

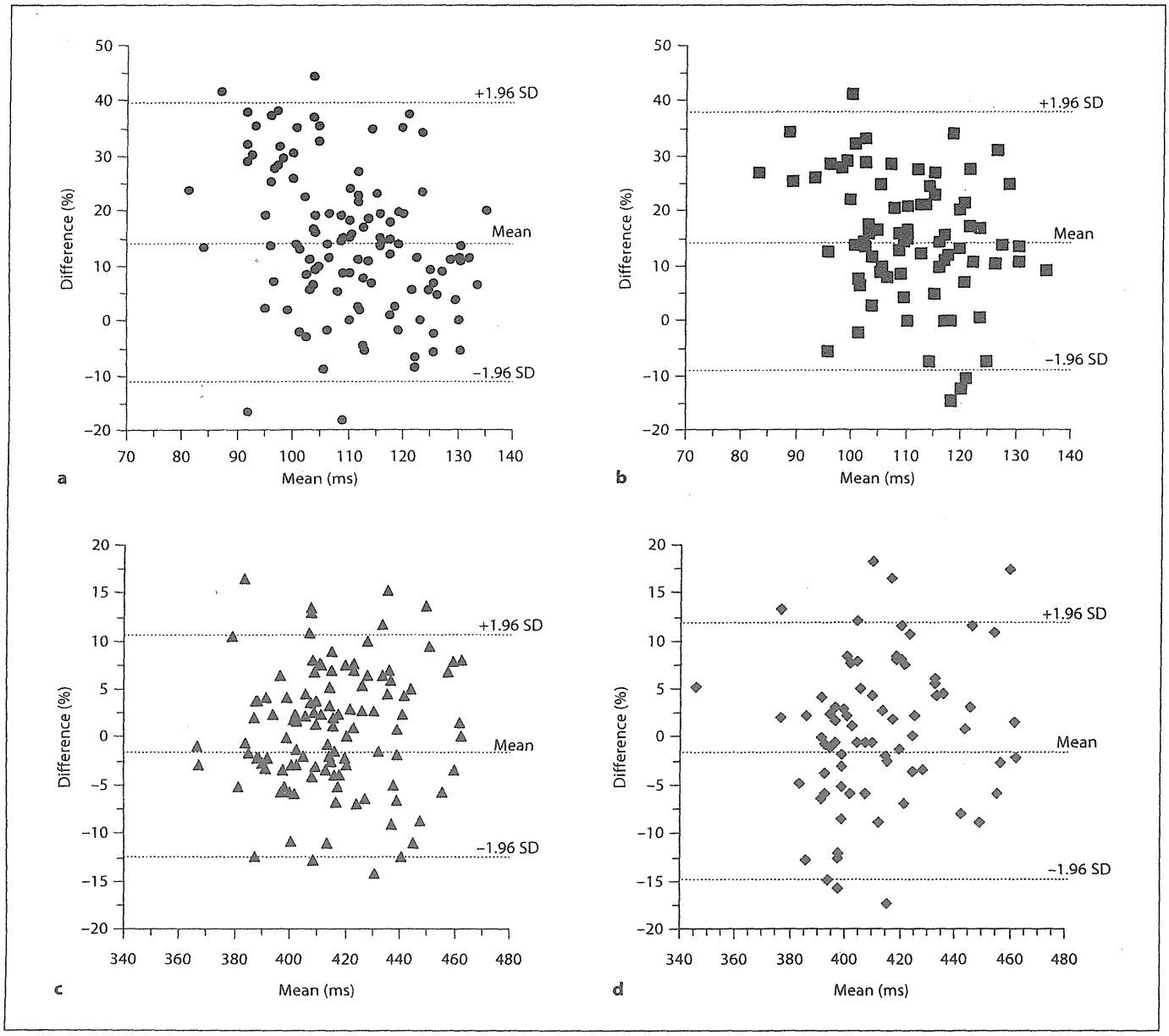

Fig. 3. Results of the Bland-Altman plot analysis for agreement between PR and RR intervals determined by fUCG and fMCG. **a** mPR measured by LV in/out versus ePR measured by fMCG. **b** mPR measured by SVC/aAo versus ePR. **c** mRR measured by LV in/out versus eRR by fMCG. **d** mRR measured by SVC/aAo versus eRR. The mean differences between mPR and ePR were 14.6% (95% limits of agreement −10.7, 39.9) for the LV in/out method and 14.7% (95% limits of agreement −8.6, 38.0) for the SVC/aAo method, and they were significantly different from 0. On the other hand, the mean differences between mRR and eRR were not significantly different from 0. The mean differences between mRR and eRR were 0.6% (95% limits of agreement −0.6, 1.8) for the LV in/out method and 0.4% (95% limits of agreement −0.4, 2.2) for the SVC/aAo method.

studies performed with fetal electrocardiography [12, 13]. Such a difference in the estimated values is not unreasonable because fUCG directly reflects the mechanical properties of the heart, rather than its electrical properties. The sum of the electromechanical delay (the interval between electrical myocardial excitation and myocardial contraction) and the isovolumic contraction time (the time between the onset of myocardial contraction and blood flow) should explain the time difference between the onsets of the P wave and A wave, as well as the QRS

complex and V wave [21]. This electro-hemodynamic delay, the preejection period, is influenced by various hemodynamic factors, such as heart rate, preload and afterload conditions, and myocardial contractility. Comparison of the simultaneously recorded ePR and mPR in neonates indicated that the preejection period of the ventricle was longer than that of the atrium [21]. Based on the data of neonates, this difference in the preejection period of the atrium and ventricle may be the main factor for the time difference between ePR and mPR. Therefore, the prolonged mPR might be due not only to delays in atrioventricular conduction but also to changes in the hemodynamic condition. Several studies have reported the advantages of tissue Doppler imaging compared with pulsed Doppler assessment: the former is less influenced by the isovolumic contraction time in assessment of the atrioventricular conduction time [12, 22]. Although there is no established standardized method for fetal assessment, especially with regard to the location of the sampling points for the ventricle [12, 22], tissue Doppler imaging may be a potentially suitable alternative method for estimation of ePR.

In our study, the absolute difference between ePR and mPR ranged from −18.0 to 59.0 ms in LV in/out and from −16.0 to 52.0 ms in SVC/aAo; these values are similar to those of other studies using signal-averaged fetal electrocardiography [12, 13]. These wide variations could not be fully explained by differences in electrical and mechanical properties. Although there was no significant difference between the eRR and mRR values, ePR and mPR were measured on different heartbeats, and it is possible that these intervals fluctuated beat by beat. Mensah-Brown et al. [23] succeeded in simultaneous recording of fMCG and fUCG to calculate the fetal ventricular preejection time. However, this method is not easy to apply for determination of the atrial preejection time. The combination of fetal electrical and hemodynamic data on the same heart beats should provide a detailed description of the electro-hemodynamic relations, such as the preejection period, and should clarify the mechanism underlying the difference between ePR and mPR.

Standard values for ePR and mPR have been proposed for each gestational period [2–4, 17, 18]. However, the diagnostic criteria for the first-degree atrioventricular block in the fetus are still not clear. Such criteria are important in the fetuses of anti-SSA/SSB antibody-positive mothers because early detection of congenital atrioventricular block would be useful for prevention of its progression to a higher grade [24, 25]. We believe that fMCG is a powerful tool for the detection of atrioventricular block with less effect of hemodynamic factors; however, fMCG is available only in a limited number of hospitals, and fUCG is still the most practical modality to detect atrioventricular block in the fetus. When the mPR becomes longer during the course of pregnancy, investigators should examine the presence of not only damage of the conduction system but also changes in hemodynamics.

Limitation of the Study

Although there was no relationship between gestational age and the success rate of determination of pulsed Doppler-derived mPR in the present study, the number of subjects was not enough due to the retrospective nature of the study. As the LV in/out method was relatively independent of fetal position and not time-consuming compared with the SVC/aAo method, we first applied the former method and then attempted the latter method if time permitted. To compare the values of mPR obtained by both methods, a prospective study in a large number of subjects is needed.

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Evaluation of Transplacental Treatment for Fetal Congenital Bradyarrhythmia

– Nationwide Survey in Japan –

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Background: There are few large studies of fetal congenital bradyarrhythmia. The aim of the present study was to investigate the effects and risks of transplacental treatment for this condition.

Methods and Results: Using questionnaires, 128 cases of fetal bradyarrhythmia were identified at 52 Japanese institutions from 2002 to 2008. Of the 128 fetuses, 90 had structurally normal hearts. Among these 90 fetuses, 61 had complete atrioventricular block (CAVB), 16 had second-degree AVB, 8 had sinus bradycardia, and 5 had other conditions. The 61 CAVB fetuses were divided into those who did (n=38) and those who did not (n=23) receive transplacental medication. Monotherapy with β-sympathomimetics, steroid monotherapy, and combination therapy with these agents was given in 11, 5 and 22 cases, respectively. Beta-sympathomimetics improved bradycardia (P<0.001), but no medication could significantly improve the survival rate. Fetal hydrops was associated with a 14-fold increased risk of perinatal death (P=0.001), and myocardial dysfunction was a significant risk factor for poor prognosis (P=0.034). Many adverse effects were observed with steroid treatment, with fetal growth restriction increasing significantly after >10 weeks on steroids (P=0.043).

Conclusions: Treatment with β-sympathomimetics improved bradycardia, but survival rate did not differ significantly in fetuses with and without transplacental medication. It is recommended that steroid use should be limited to <10 weeks to avoid maternal and fetal adverse effects, especially fetal growth restriction and oligohydramnios. (*Circ J* 2012; **76:** 469–476)

Key Words: Anti-Ro/SSA antibody; Congenital atrioventricular block; Pregnancy; Steroids; Transplacental treatment

Fetal congenital bradyarrhythmia is an uncommon but life-threatening disease, especially in the case of complete atrioventricular block (CAVB), which has a poor prognosis because of fetal hydrops, endocardial fibroelastosis and late-onset dilated cardiomyopathy.[1–9] Predominantly untreated CAVB has a significant mortality rate of 14–34%, while congenital CAVB is irreversible and requires a pacemaker in approximately 66% of cases after birth.[10] The association of CAVB with maternal anti-Ro/Sjögren's syndrome A (SSA) antibodies is well established, but the trigger for the maternal antibody interaction with the fetal Ro particle is unknown in some cases of antibody-exposed babies.[2,7–9,11,12]

There is limited evidence for the clinical efficacy of transplacental treatment of congenital AVB.[13–19] Steroids and i.v. immunoglobulins are given as anti-inflammatory treatment, while β-sympathomimetics are used for fetal pacing.[20] A recent

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Table 1. Baseline Characteristics of CAVB Fetuses

	Medication group (n=38)	No medication group (n=23)	P value
Maternal anti-SSA antibodies	29 (76.3)	11 (47.8)	<0.05‡
Gestational age at diagnosis (weeks)	24±3.2	28±5.7	<0.005†
Fetal heart rate at diagnosis (beats/min)	58±7.9	63±14.7	NS†
Fetal hydrops	16 (42.1)	6 (26.1)	NS‡
Fetal myocardial dysfunction	13 (34.2)	7 (30.4)	NS‡
Gestational age at initiation of therapy (weeks)	26±3.6	–	
Fetal heart rate at initiation of therapy (beats/min)	56±8.4	–	
Gestational age at delivery (weeks)	34±4.0	35±4.5	NS†
Birth weight (g)	2,120±620	2,528±653	<0.001†
Delivery mode			
Vaginal	8	7	NS‡
Cesarean section	30	16	NS‡
Permanent pacemaker implantation	14 (46.7)	6 (35.3)	NS‡
Neonatal survival	30 (78.9)	17 (73.9)	NS‡

Data given as mean±SD or n (%). P<0.05, significant difference.
†Student's t-test; ‡chi-square test and Fisher's exact test.
CAVB, complete atrioventricular block; SSA, Sjögren's syndrome A.

cohort study found an improved survival rate of >90% with initiation of maternal high-dose dexamethasone at the time of CAVB detection, and maintenance of this drug during pregnancy with use of β-sympathomimetics to keep fetal heart rates at >55 beats/min.[9,21] It was also suggested that prolonged use of dexamethasone might render fetuses with congenital CAVB less likely to develop the additional manifestations of myocarditis, cardiomyopathy, and hydrops fetalis, thus improving the overall outcome. Use of steroids, however, is controversial because of the potential risks for the fetus, including problems with neurological development, growth retardation, and oligohydramnios.[22–25]

Few large studies of fetal congenital bradyarrhythmia have been performed in Japan. The aims of the present study were to determine the features of fetal congenital bradyarrhythmia in Japan, and to examine the effects and risks of transplacental treatment for this condition.

Methods

Subjects

Data were collected using questionnaires sent to Departments of Perinatology and Pediatric Cardiology at 750 institutions in Japan over 7 years (2002–2008). The response rate was 60.7% (455 institutions). Fetal bradyarrhythmia was defined as ventricular heart rate <100 beats/min at the time of diagnosis.[4] The following perinatal data were also collected: gestational age at diagnosis and delivery, presence or absence of a congenital heart defect (CHD), type of bradyarrhythmia, method of diagnosis, presence or absence of maternal autoantibodies such as anti-Ro/SSA antibodies, presence or absence of fetal hydrops, presence or absence of fetal myocardial dysfunction, fetal ventricular and atrial heart rate at presentation, prenatal treatment, mode of delivery, and outcome. Adverse effects related to prenatal treatment were also evaluated.

Statistical Analysis

Statistical analysis was performed using STATA 11.1 (StataCorp, College Station, TX, USA) and JMP 9 (SAS Institute, Cary, NC, USA). Data are presented as mean±SD or number of patients and were analyzed using Student's t-test. Categorical variables were evaluated on chi-square test and Fisher's exact test. Logistic regression was used to adjust for baseline variables known to be associated with fetal ventricular heart rate, fetal hydrops, fetal myocardial dysfunction, and maternal anti-Ro/SSA antibody. Time to fetal or neonatal death was analyzed using the Kaplan-Meier method with a log-rank test and a Cox proportional hazard model. P<0.05 was considered significant.

Results

Baseline Characteristics

A total of 128 cases were registered from 52 institutions during 7 years (2002–2008). All cases of fetal bradyarrhythmia were diagnosed during fetal life using echocardiography. In 8 cases, magnetocardiography was performed due to fetal bradyarrhythmia and family history of long QT syndrome (LQTS). Of the 128 fetuses, 38 (29.7%) had CHD, 15 had left atrial isomerism, 1 had right atrial isomerism, 5 had atrioventricular septal defect, 4 had corrected transposition of the great arteries, 4 had pulmonary stenosis, and 9 had other conditions. Patent ductus arteriosus and atrial septal defect were categorized as an absence of CHD. Ninety fetuses (70.3%) had a structurally normal heart, of whom 61 had CAVB, 16 had second-degree AVB, 8 had sinus bradycardia, 3 had sick sinus syndrome. Nine LQTS cases occurred in combination with another condition.

CAVB

Of the 61 fetuses with a structurally normal heart and CAVB (Table 1), 38 received transplacental medication. No fetus showed improvement of heart block. Monotherapy with β-sympathomimetics was given in 11 cases, steroids were given in 5 cases, and combination therapy with these agents was used in 22 cases. No transplacental medication was given in 23 cases. Ritodrine hydrochloride was used as the β-sympathomimetic agent. Steroids tended to be used in fetuses that were positive for maternal anti-Ro/SSA antibody throughout pregnancy, but the chosen steroid differed among institutions. Maternal i.v. immunoglobulin was not used. After birth, a pacemaker was implanted based on the Japanese guidelines of syncope, ventricular heart rate <50 beats/min, decreased cardiac function, LQTS, and a sudden pause longer than 2–3-fold the regular ventricular heart rate.

Table 2. Factors in Improvement of Bradycardia

	OR	95%CI	P value
β-sympathomimetics	49.02	5.18–464.02	<0.005
Steroids	1.32	0.24–7.20	0.745
β-sympathomimetics+steroids	725,448.8	0	0.996
Fetal heart rate	1	0.93–1.08	0.924
Fetal hydrops	0.41	0.07–2.39	0.319
Fetal myocardial dysfunction	1.14	0.20–6.60	0.883
Maternal anti-Ro/SSA antibodies	0.22	0.04–1.36	0.105

P<0.05, significant difference.
Logistic regression was used to adjust for baseline variables known to be associated with fetal ventricular heart rate, fetal hydrops, fetal myocardial dysfunction, and maternal anti-Ro/SSA antibody.
OR, odds ratio; CI, confidence interval; SSA, Sjögren's syndrome A.

Table 3. Factors in Fetal or Neonatal Death

	HR	95%CI	P value
β-sympathomimetics	1.16	0.37–3.63	0.792
Steroids	0.56	0.20–1.58	0.273
Fetal heart rate	0.98	0.92–1.05	0.546
Fetal hydrops	13.84	3.12–61.44	0.001
Fetal myocardial dysfunction	2.44	0.71–8.40	0.157
Maternal anti-Ro/SSA antibodies	1.07	0.33–3.47	0.906

P<0.05, significant difference.
Logistic regression was used to adjust for baseline variables known to be associated with fetal ventricular heart rate, fetal hydrops, fetal myocardial dysfunction, and maternal anti-Ro/SSA antibody.
HR, hazard ratio; CI, confidence interval; SSA, Sjögren's syndrome A.

Table 4. Factors in Development of Fetal Hydrops

	OR	95%CI	P value
β-sympathomimetics	2	0.35–11.50	0.439
Steroids	0.27	0.04–1.97	0.198
Fetal heart rate	1.01	0.94–1.08	0.813
Fetal myocardial dysfunction	5.71	1.14–28.62	0.034
Maternal anti-Ro/SSA antibodies	0.71	0.13–3.90	0.698

P<0.05, significant difference.
Logistic regression was used to adjust for baseline variables known to be associated with fetal ventricular heart rate, fetal myocardial dysfunction, and maternal anti-Ro/SSA antibody.
OR, odds ratio; CI, confidence interval; SSA, Sjögren's syndrome A.

The anti-Ro/SSA antibody-positive rate was significantly higher in fetuses treated with transplacental medication compared to those who did not receive this medication (76.3% vs. 47.8%; P=0.031). Gestational age at diagnosis was significantly lower in those receiving transplacental medication (24.0 weeks vs. 28.3 weeks; P=0.003). Fetal ventricular heart rate at diagnosis did not differ between the 2 groups, but the ventricular heart rate was significantly lower in fetuses treated with transplacental medication (56 beats/min vs. 63 beats/min; P=0.034). Birth weight was also significantly lower in fetuses treated with transplacental medication (2,120 g vs. 2,528 g; P=0.006). Gestational age at delivery, neonatal survival rate, and pacemaker implantation rate did not differ between the 2 groups.

Multivariate analysis was performed with adjustment for baseline variables with a known association with fetal ventricular heart rate, fetal hydrops, fetal myocardial dysfunction, and the presence of maternal anti-Ro/SSA antibodies (**Tables 2–4**). In this analysis, β-sympathomimetic treatment was significantly associated with improved bradycardia (odds ratio [OR], 49.02; 95% confidence interval [CI]: 5.18–464.02; P<0.001), whereas steroids were ineffective, and no evidence of a synergistic effect was obtained. The presence of maternal anti-Ro/SSA antibodies may inhibit improvement of bradycardia, but this effect was not significant (OR, 0.22; 95%CI: 0.04–1.36; P=0.105). Drug therapy had no significant effect on survival. Fetal ventricular heart rate and the presence of maternal anti-Ro/SSA antibodies also had no influence on prognosis, but fetal hydrops was associated with a 14-fold increased risk of perinatal death (hazard ratio [HR], 13.84; 95%CI: 3.12–61.44; P=0.001).

Kaplan-Meier survival curves are shown in **Figure**. The primary endpoint was intrauterine death or neonatal death. Beta-sympathomimetic treatment was not associated with improved prognosis. Steroid also did not improve the prognosis (HR, 0.56; 95%CI: 0.20–1.58; P=0.273). Fetal myocardial dysfunction was a significant risk factor for fetal hydrops (OR, 5.71; 95%CI: 1.14–28.62; P=0.034). Fetal ventricular heart rate and the presence of maternal anti-Ro/SSA antibodies were not associated with fetal hydrops. Beta-sympathomimetic treatment did not inhibit development of fetal hydrops. Steroids tended to inhibit fetal hydrops, but again this effect was not

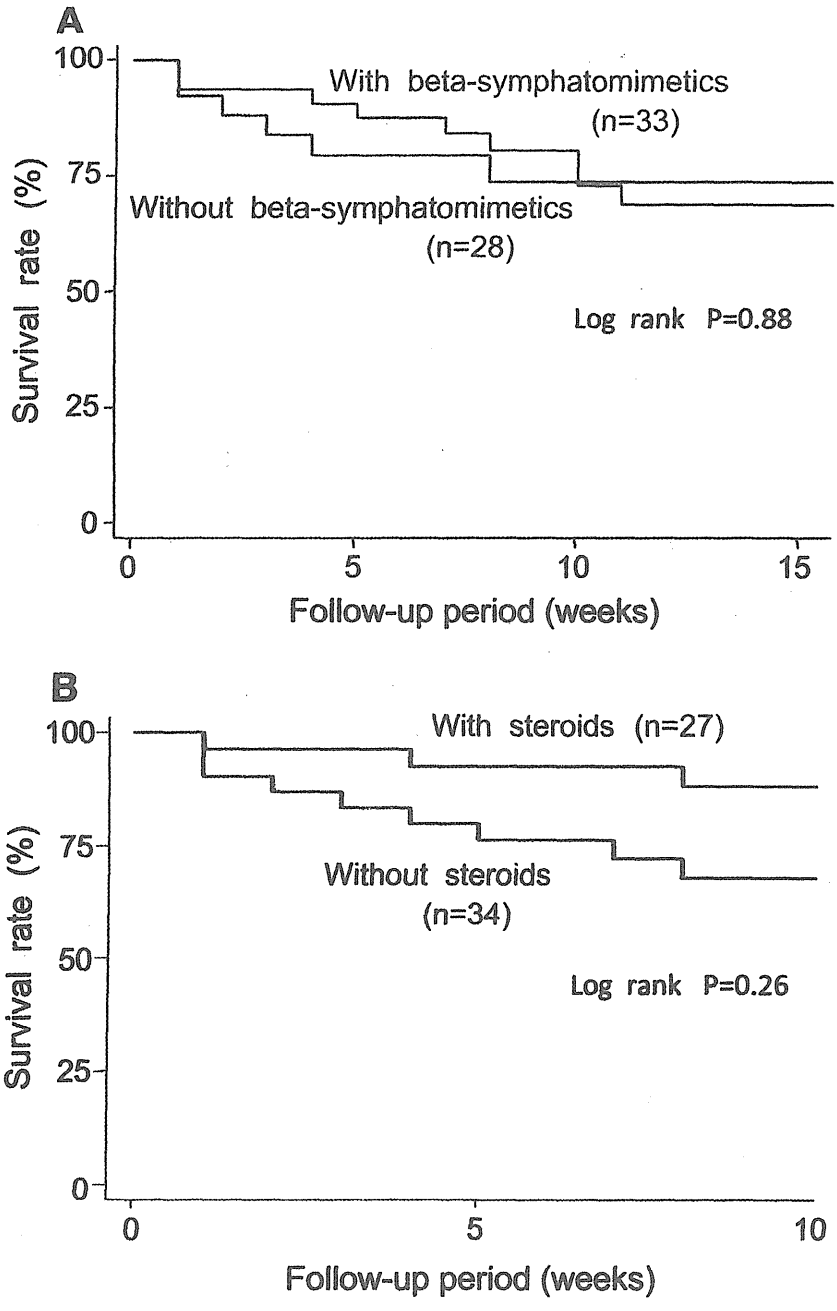

Figure. Kaplan-Meier survival curves (log-rank test and Cox proportional hazard model) for the primary endpoint (fetal death or neonatal death) in fetuses treated (A) with and without β-sympathomimetics and (B) with and without steroids. Follow-up period, time from diagnosis in utero to fetal or neonatal death. Significant difference defined as $P<0.05$.

statistically significant (OR, 0.27; 95%CI: 0.04–1.97; P=0.198). Drug therapy had no significant effect on improvement of fetal myocardial dysfunction.

Second-Degree AVB With Bradycardia

Of the 90 fetuses with a structurally normal heart, second-degree AVB was present in 16 cases (Table 5). Transplacental medication was given in 8 of these cases: β-sympathomimetic monotherapy in 4, steroids in 3, and a combination of these therapies in 1. In the 8 medication cases, fetal ventricular heart rate at diagnosis was significantly lower than that in the non-medication cases (70 beats/min vs. 79 beats/min; P=0.017). No other clinical characteristics differed significantly between the 2 groups. Of the 8 medicated fetuses, 3 developed CAVB, 3 maintained second-degree AVB, 1 improved to first-degree AVB, and 1 had no AVB at the time of delivery. Of the 8 non-medicated fetuses, 2 developed CAVB, 3 maintained second-degree AVB, and 3 had no AVB at the time of delivery. Survival rate did not differ between the groups (87.5%).

Adverse Effects of Transplacental Treatment

Treatment-related adverse events were examined in the 63 fetuses with a structurally normal heart and no fetal hydrops (Table 6). Steroids were given in 23 cases, drugs other than steroids were given in 10 cases, and no treatment was given in 30 cases. Gestational age at delivery did not differ among these 3 groups. In the steroid group, birth weight was significantly lower than in the non-treatment group (2,201 g vs.

Table 5. Second-Degree AVB Fetus Baseline Characteristics vs. Medication

	Medication (n=8)	No medication (n=8)	P value
Maternal anti-Ro/SSA antibodies	4	3	NS‡
Gestational age at diagnosis (weeks)	28±4.3	26±5.0	NS†
Fetal heart rate at diagnosis (beats/min)	70±9.0	79±10.4	<0.05†
Fetal hydrops	2	2	NS‡
Fetal myocardial dysfunction	3	2	NS‡
Gestational age at initiation of therapy (weeks)	29±4.8	–	
Fetal heart rate at initiation of therapy (beats/min)	70±10.0	–	
Gestational age at delivery (weeks)	35±3.8	37±2.1	NS†
Birth weight (g)	2,207±688	2,533±544	NS†
Delivery mode			
Vaginal	2	5	NS‡
Cesarean section	6	3	NS‡
Degree of AVB at delivery			
Complete	3	2	NS‡
Second	3	3	NS‡
First	1	0	NS‡
None	1	3	NS‡
Neonatal survival	7 (87.5)	7 (87.5)	NS‡

Data given as mean±SD or n (%).
P<0.05, significant difference. †Wilcoxon test; ‡chi-square test and Fisher's exact test.
AVB, atrioventricular block; SSA, Sjögren's syndrome A.

Table 6. Baseline Characteristics vs. Treatment Type

	Steroid treatment (n=23)	Non-steroid treatment (n=10)	No treatment (n=30)
Treatment (weeks)	8.8±4.4	5.6±3.2	–
Gestational age at delivery (weeks)	36±2.6	35.8±2.6	36.8±3.0
Birth weight (g)	2,201±525*	2,413±552	2,713±512*
Fetal arrythmia: CAVB	21	6	23
Fetal arrythmia: Second-degree AVB	1	2	5
Maternal diabetes	1 (4.3)	0	0
Fetal growth restriction	6 (26.1)	0	2 (6.7)
Fetal oligohydramnios	2 (8.7)	0	0
Neonatal adrenal insufficiency	1 (4.3)	0	0

Data given as mean±SD or n (%).
†For fetuses without fetal hydrops and with a structurally normal heart. *P<0.05 (Student's t-test).
CAVB, complete atrioventricular block; AVB, atrioventricular block.

Table 7. Baseline Characteristics vs. Length of Steroid Treatment

	<10 weeks (n=12)	≥10 weeks (n=11)	P value
Treatment (weeks)	5.4±2.7	12.5±2.5	<0.01†
Gestational age at delivery (weeks)	35±3.2	36±1.7	NS†
Birth weight (g)	2,184±569	2,218±503	NS†
Maternal diabetes	0	1 (9.1)	NS‡
Fetal growth restriction	1 (8.3)	5 (45.5)	<0.05‡
Fetal oligohydramnios	0	2 (18.2)	NS‡
Neonatal adrenal insufficiency	0	1 (9.1)	NS‡

Data given as mean±SD or n (%). P<0.05, significant difference.
†Student's t-test; ‡chi-square test and Fisher's exact test.

2,713 g; P=0.001) and fetal growth restriction was close to being significantly higher than in the non-steroid (26.1% vs. 0%; P=0.050) and non-treatment (26.1% vs. 6.7%; P=0.074) groups. Adverse effects that might have been attributable to the use of steroids included development of oligohydramnios in 8.7% of cases, maternal diabetes in 4.3%, and neonatal adrenal insufficiency in 4.3%. All these adverse effects were observed in cases of steroid use >10 weeks (**Table 7**). In particular, fetal growth restriction increased significantly after steroid use >10 weeks (45.5% vs. 8.3%; P=0.043).

LQTS

Of the 90 fetuses with a structurally normal heart, 9 (10.0%) were diagnosed with LQTS, including 4 diagnosed on electrocardiography after birth and 5 diagnosed on magnetocardiography during fetal life. The background of the LQTS fetuses included a family history of LQTS (n=2), maternal anti-Ro/SSA antibody (n=2), fetal hydrops (n=3), myocardial dysfunction (n=2), CAVB (n=6), second-degree AVB with bradycardia (n=1), and sinus bradycardia (n=2). In 4 of the 9 cases of LQTS, emergency cesarean section was performed because of fetal ventricular tachycardia/torsades de pointes (VT/TdP) at 33–36 weeks of gestation. In 2 of the 9 cases, fetal hydrops caused neonate death.

Discussion

This is the first large-scale study to investigate the effects and risks of transplacental treatment for fetal congenital bradyarrhythmia in Japan. The results indicate that fetal hydrops is associated with a 14-fold increased risk of perinatal death, and that fetal myocardial dysfunction is a significant risk factor for fetal hydrops. Fetal ventricular heart rate and the presence of maternal anti-Ro/SSA antibodies were not associated with neonatal prognosis. Beta-sympathomimetics improved bradycardia, but survival rate did not differ significantly with regard to transplacental medication. Maternal and fetal adverse effects were observed in cases of steroid use. In particular, fetal growth restriction increased significantly after steroid use >10 weeks.

Evaluation of Anti-Ro/SSA Antibodies

Ro/SSA is one of the major immunogenic ribonucleoproteins, and antibodies against these proteins are found in a number of connective diseases, especially in Sjögren's syndrome (SS) and systemic lupus erythematosus (SLE). Anti-Ro/SSA antibodies are detected in 60–90% of SS cases and in 30–50% of SLE cases.[26,27] Interestingly, these antibodies are relatively common and are detected in 1–2% of randomly tested pregnant women.[28] Currently, the outcome of anti-Ro/SSA-positive pregnancies is very good when prospectively followed by multidisciplinary teams with experience in this field.[1] Transplacental passage of anti-Ro/SSA antibodies from mother to fetus, however, is associated with a risk of development of neonatal lupus erythematosus (NLE).[2,11,12] NLE is an uncommon but life-threatening disease of the fetus and neonate, with important cardiac complications of CAVB, sinus bradycardia, QTc interval prolongation, endocardial fibroelastosis, and late-onset dilated cardiomyopathy.[3–5] Congenital CAVB develops in 1–5% of anti-Ro/SSA antibody-positive pregnancies, typically between 18 and 24 weeks of gestational age. Predominantly untreated CAVB has a mortality rate of 14–34%,[1–9] consistent with the untreated CAVB mortality rate of 26% in the current study.

The association of NLE with maternal anti-Ro/SSA antibodies is well established, but the trigger of the maternal antibody interaction with the fetal Ro particle is unclear in some antibody-exposed babies. The percentage of maternal anti-Ro/SSA antibody-positive fetuses with CAVB diagnosed in utero is unknown. Brucato et al and Jaeggi et al found maternal anti-Ro/SSA antibodies in 92% of 37 CAVB cases,[7,9] whereas in the present study maternal anti-Ro/SSA antibodies were detected in only 66% of 61 CAVB fetuses with a structurally normal heart. Jaeggi et al also reported that CAVB occurred in 5% of prospectively screened pregnancies with anti-Ro/SSA ELISA levels >100 U/ml, but did not occur in pregnancies with levels <50 U/ml.[6] Approximately two-thirds of anti-Ro/SSA antibody-positive mothers had low anti-Ro/SSA levels and probably little risk of development of fetal cardiac NLE.[8] It is unclear why the anti-Ro/SSA-positive rate in the present study was lower than in other reports. It is unlikely to be due to the sensitivity of the laboratory methods, but it is possible that other undetectable antibodies associated with congenital AVB are present in the Japanese population. Brucato et al and Lopes et al found similar mortality rates in the anti-Ro/SSA-positive and -negative groups,[7,8] and in the present multivariate analysis anti-Ro/SSA antibodies were not associated with prognosis.

Benefits and Risks of Transplacental Treatment

Congenital AVB is a progressively developing disease that evolves through 2 fundamental phases: an early phase characterized by the occurrence of still reversible AV conduction abnormalities (first- or second-degree AVB) and a final phase in which development of irreversible damage of the conduction system leads to the appearance of CAVB.[29] The specific pathogenetic mechanisms involved in the 2 phases have not been clarified, but there are 2 main theories. The first is based on an inflammatory-driven injury elicited by interaction between anti-Ro/SSA antibodies and specific antigens expressed in the conduction tissue of the fetal heart (inflammatory theory). The second theory involves electrophysiologic interference of anti-Ro/SSA antibodies with heart conduction (electrophysiological theory).[20] Consistent with these respective theories, steroids and i.v. immunoglobulins are used for anti-inflammatory treatment, while β-sympathomimetics are given for fetal pacing.

Several studies have found that a ventricular heart rate <55 beats/min is a risk factor for fetal and neonatal death,[4,14] and have recommended transplacental treatment with β-sympathomimetics to increase the heart rate. Jaeggi et al and Maeno et al, however, found that fetuses with CAVB without CHD and with a ventricular heart rate of <55 beats/min were not at risk.[30,31] In the present study, fetal ventricular heart rate did not influence fetal hydrops and prognosis, but treatment with a β-sympathomimetic agent was significantly associated with improved bradycardia.

To date, evidence of clinical efficacy of transplacental treatment has been limited to cases of congenital AVB.[13–19] Jaeggi et al reported a significant improvement in the outcome of fetal CAVB simultaneously with the introduction of routine perinatal treatment guidelines in 1997.[9] Hutter et al obtained an improved survival rate of >90% by initiation of maternal high-dose dexamethasone at the time of CAVB detection and maintenance of this dose during pregnancy, with addition of β-sympathomimetics to keep the fetal heart rate above 55 beats/min.[21] It was also suggested that prolonged use of dexamethasone might render a fetus with congenital CAVB less likely to develop additional manifestations of cardiac NLE such as myocarditis, cardiomyopathy, and hydrops fetalis, thus improving the overall outcome. The present findings suggest that use of steroids might render the affected fetus less likely to develop fetal hydrops, but that the neonatal survival rate improved only to 79%. The reason for the relatively bad prognosis in the present study may have been the difference in the rate of fetal hydrops compared to the Hutter et al study (42% vs. 10%). Undetectable autoantibodies or virus infection may be related to the increased rate of fetal hydrops in the Japanese population. Furthermore, Hutter et al initiated maternal high-dose dexamethasone at the time of CAVB diagnosis, at a mean gestational age of 24 weeks. The mean age of diagnosis was similar in the present study, but mean gestational age at which steroids were started was 26 weeks. In addition, the percentage of steroids used in transplacental treatment was

lower in the present patients (71% vs. 95%). These findings suggest that sufficient steroid dose at an early stage is very important to prevent fetal hydrops and to improve prognosis.

Use of steroids is controversial because of the potential risks for the fetus and mother, including problems with fetal growth restriction, oligohydramnios, and neurological development. Animal models suggest that repeated antenatal steroid doses can interfere with the growth and development of the immature brain, and human studies suggest that antenatal and postnatal dexamethasone may negatively affect a child's neuropsychological development.[22–24] In contrast, Brucato et al found no negative effects on neuropsychological development and intelligence in a cohort of preschool- and school-age children with CAVB who had been prenatally exposed to maternal anti-Ro antibodies and prolonged dexamethasone treatment.[25] The association of fetal growth restriction and oligohydramnios with antenatal steroids is well established, but the amount and length of steroid treatment that can be used safely is unclear. We note that development of fetal growth restriction and oligohydramnios are dose-related complications of steroids. Consequently, we recommend limiting steroid use to <10 weeks to avoid maternal and fetal adverse effects.

Prevention of Progression to Congenital CAVB

There are many case reports describing prevention of congenital CAVB, and first- or second-degree AVB is also relatively common and often normalizes spontaneously before or soon after delivery.[32] Recent prospective studies suggest that steroids and i.v. immunoglobulins are not beneficial for preventing progression to congenital AVB.[33,34] Similarly, the present study found a lack of superiority of transplacental treatment for second-degree AVB with bradyarrhythmia.

LQTS

Recent evidence has shown that anti-Ro/SSA antibodies are associated with prolongation of the QTc interval.[35] Although the exact arrhythmogenic mechanisms have not been clarified, anti-Ro/SSA antibodies may trigger rhythm disturbances through inhibition of cross-reactions with several cardiac ionic channels, including calcium channels and the hERG potassium channel.[36,37] Beta-sympathomimetics may trigger life-threatening arrhythmia such as VT/TdP in patients with LQTS, and therefore use of these drugs should be avoided in fetuses with QTc interval prolongation.[38,39] In the present study, in 4 of the 9 LQTS cases, emergency cesarean section was performed because of fetal VT/TdP at 33–36 weeks of gestational age. Oka et al also recently described atrioventricular block-induced TdP.[40] With this background, we recommend avoidance of β-sympathomimetics in a fetus with a heart rate >55 beats/min. Furthermore, assessment of QTc interval prolongation on magnetocardiography may be required to evaluate the risk of fetal congenital bradyarrhythmia.

Study Limitations

There were several limitations in the present study due to retrospective data selection bias and the relatively small sample size. The nature of a multicenter retrospective observational study using a questionnaire is such that the clinical data obtained vary among cases, so treatment bias may exist. Only ritodrine hydrochloride was used as β-sympathomimetic treatment, but was given in cases involving fetal heart rate >55 beats/min at some institutions, while dexamethasone, betamethasone and prednisolone were used as steroids at different doses among institutions. The follow-up period after birth was insufficient to permit analysis of long-term morbidity and mortality, and this prevented evaluation of potential long-term benefits and risks of transplacental medication. Finally, the sample size might have been too small to detect the effects of steroids on fetal congenital bradyarrhythmia. The steroid effect may become significant in a study with a higher number of cases.

Guidelines are required for transplacental treatment of fetal congenital bradyarrhythmia and follow-up after birth. We expect to analyze long-term outcome of fetal congenital bradyarrhythmia in a future study. Further large prospective studies are also needed to establish the most appropriate treatment strategies in Japan.

Conclusion

Beta-sympathomimetics improved bradycardia, but survival rate did not differ significantly in fetuses treated with and without transplacental medication. We recommend limiting steroid use to <10 weeks to avoid maternal and fetal adverse effects, with fetal growth restriction and oligohydramnios being of particular concern.

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Disclosures

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特集　リウマチ性疾患における自己抗体に関する新たな知見

抗SS-A抗体・抗SS-B抗体陽性者の妊娠リスク*

村島温子**

Key Words：anti-SS-A/B antibodies, pregnancy, neonatal lupus erythematosus, congenital heart block

はじめに

抗SS-A抗体はシェーグレン症候群(SS)や全身性エリテマトーデス(SLE)などの自己免疫疾患で高頻度に出現する自己抗体であるが，健常者でも約1％で本抗体を持つとされる．抗SS-B抗体は抗SS-A抗体と併存し，単独で陽性となることは稀であり，抗SS-A抗体の陽性例の約3割で陽性となる．抗SS-B抗体は抗SS-A抗体に比較してシェーグレン症候群に特異性が高いという特徴を持つ．

新生児ループスは母体血中の自己抗体が胎児に移行し，SLE様の症状を呈することからついた名前である．主な症状は皮疹，血球減少，肝機能障害，心ブロック(CHB)であり，抗SS-A抗体が深くかかわっていると考えられていて，この抗体を持つ患者から出生した児の約10％に出現するとされている[1]．皮疹，血球減少は母親からの移行抗体の消失とともに軽快するが，CHBは不可逆的な場合が多い．膠原病と診断されていて本抗体を持っていることがわかっている場合と，無症候の女性が新生児ループス児を出産したあとこの抗体の存在が明らかになる場合がある．CHBについては本抗体陽性妊娠例の約1～2％にみられるといわれている．その多くは妊娠18週から24週までに出現するといわれ，重症例では心不全などにより子宮内ないしは新生児死亡となる．無事出産したとしても約3分の2は成人するまでにペースメーカーの植え込みが必要となる重症の病態であり[2]，その予測ならびに予防方法が検討されてきているが，まだ確立していない．抗SS-B抗体の併存，抗SS-A/52kDa抗体の存在などがリスク因子として提案されてきたが，現時点で明らかになっているのはCHB児出産(死産も含めて)の既往があると約10倍の発生率になるだろうということだけである[3]．

抗SS-A抗体陽性女性の妊娠に際し，われわれはどのように考えてどのように対応すべきか，この疑問を明らかにするため，文献ならびに筆者が代表者を務める研究班の途中経過を用いて，現時点でわかっていること，これからの課題について述べる．

CHBの病態

抗SS-A抗体の対応抗原は分子量で52kDa，60kDaに分けられる．そのうち，52kDa蛋白のleucine zipper amino acid(aa200-239)が抗原決定基(P200)となっている[4]と考えられている．この抗原は核や細胞質内に存在するため，なんらかの機序が働いて細胞表面に表出されるのか，あ

* Anti-SS-A/B antibodies and pregnancy.

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表1　全国症例調査

実施施設
　2008～2009年：研究者所属施設
　2010～2011年：全国の膠原病関連，周産期専門施設
収集した情報
　母体の情報
　・分娩年齢，妊娠歴(新生児ループス児出産の既往を含む)，膠原病様症状(既往)，診断名
　・抗SS-A抗体(DID, ELISA)，抗SS-B抗体
　・抗52kDa抗体，抗60kDa抗体(WB and/or ELISA)
　・他の自己抗体
　・妊娠前・妊娠中の治療内容
　・心ブロック診断時の治療介入の有無・内容
　児の情報
　・出生の妊娠週数，性別，生下時体重，アプガースコア
　・新生児ループス発症の有無

るいはこの抗原と交差反応性のある物質が心筋細胞表面にあって，抗体と反応しているのかもしれない．CHBの発症に母体の抗体の存在は必要ではあるが，そのほかに環境因子や胎児側の因子もなんらかの形でかかわっているはずである．その候補の1つに胎児の遺伝的要因があるが，一卵性双生児で必ずしも一致しない[5]ということから子宮内の環境も1つの要因になっている可能性が示唆される．

発生学的には房室結節とヒス束は独立して発生し，胎生8週から16週(妊娠10週から18週)で刺激伝導系として完成すると考えられている．胎盤を介して胎児に移行した抗SS-A抗体が胎児の心筋(刺激伝導系)細胞のカルシウムチャンネルに直接結合し，カルシウムの流入の障害ないしはカルシウムの過負荷により第一段階のブロックが生ずるというのが現在のところ最も有力な仮説である[6]．伝導系障害がここで回復すれば一過性の不完全心ブロックということになるが，伝導障害に終わらず炎症や線維化といった第二段階に進むと不可逆性となる．CHBの病理組織所見では刺激伝導路にIgG, TNFやTGF mRNAを発現したアポトーシス細胞，大量のコラーゲンの沈着を認めることから以下の仮説が考えられている[7]．アポトーシスを起こしている心筋細胞には胎生15週前後をピークとして52kDa, 60kDa蛋白が表出される．母体が抗SS-A抗体を持っていると，経胎盤的に胎児に移行した本抗体がこれらに結合することによって生理的なアポトーシスが障害され，Fcレセプターを介してマクロファージに貪食される．そして，炎症や線維化に関係するサイトカインが放出され，心筋線維芽細胞の増殖やコラーゲンの過剰産生をひき起こし，結果的に瘢痕化を強めることになると考えられている[8]．

抗SS-A抗体陽性女性の妊娠管理に関する班研究について

新生児ループスは内科(膠原病)，産科，小児科(循環器)と，複数の科がかかわっている病態であるが，それだけにその実態を把握することは難しかった．その実態を明らかにすることにより，CHB発症のリスク因子，予防方法を探るとともに，CHB発症の早期診断ならびに診断時の治療方法を呈示することを目標に本研究を開始した．当該症例の集積施設を中心に症例調査(表1)をお願いしたところ，多くの先生方のご協力により約750例にも及ぶ症例データベースが構築でき，現在解析中である．解析途中であり，不十分な記述になるとは思うが，この研究からみえてきたことを文献考察も含めながら述べてみたい．

内科の立場からの驚きは，児のCHB発症をきっかけに抗SS-A抗体陽性が判明するケースが多いことである．それらの多くは無症候女性である．これらの女性を拾いあげていくためには肝炎ウイルスなどと同様に妊娠初期検査に組み込むということも案としてはあがるが，全妊娠女性の本抗体の保有率は約1％，そのうちのCHB発症率は約1～2％であることから現実的なものにはならないだろう．したがって，本研究では，抗SS-A抗体陽性とわかっている女性が妊娠する場合の，CHB発症リスクの予測方法，CHBの発症予防方法，CHB発症時の治療方法を呈示し，最終的には自己抗体陽性女性の妊娠管理指針を作成することを目標としている．

抗SS-A抗体が陽性とわかって妊娠管理されている症例でのCHB発症が少ない印象を持っていたので，妊娠前の治療とCHB発症が関係しているのかを評価したが，両者の間に関連は認めなかった．では，妊娠してからの治療がCHBに影

響しているかどうか，すなわちCHB発症を抑止しているのかどうかの解析を行ったところ，ステロイド剤が抑止できる可能性が伺えた．このデータベースで使われているステロイド剤のほとんどが胎盤移行性の低いプレドニゾロンないしは，中等度の移行性を持つベタメサゾンであった．胎児への影響を考えるとプレドニゾロンで予防できるにこしたことはないが，症例が増えるに従って，プレドニゾロンを投与していた症例の中でのCHB発症例が複数認められるようになった一方で，ベタメサゾンを投与していた症例では1例もCHBを発症していない状況をみると維持量のプレドニゾロンでのCHB予防は難しいのかもしれない．結論は今後の統計的解析結果が出るまで待つしかないだろう．

これまでにも抗SS-A抗体のプロフィールからCHBのリスクを予測する試みはいろいろと行われてきた．まず，抗体価との関連について気になるところだが，それについて明らかにした報告はほとんどない．抗SS-A抗体は二重免疫拡散法(DID)と酵素抗体法(ELISA)で測定されているが，検査の利便性から近年はELISA法が主流になりつつある．しかし，ELISA法のキットは複数のメーカーが発売しているため，まとめて解析することは不可能である．今回の症例データベースを十分な症例数を対象としたままで解析するためには抗SS-A/B抗体(ELISA法)の標準化が必要であると考えて試みた．結果的には単純な標準化は難しいと判断されたが，ELISA法を「定性判定」と捉えて，DID法での確認が必要な基準案を作成した(現在，投稿中)．

抗SS-A抗体の力価の解釈にはこのような問題点があるものの，研究班で集積した症例データベースではDID, ELISAともに，その力価が高いほどCHBの発症率が高くなる傾向は確認できた．しかし，力価が高くてもCHBを発症しない例も多い一方で，力価が低いにもかかわらずCHBを発症した例があるのも事実であり，カットオフ値を設定することの難しさを実感している．

従来，抗SS-A抗体の対応抗原である52kDa，60kDaに対する抗体がimmunoblotting法，ELISA法で測定され，その結果がCHBの予測に利用できないかと検討されてきた．そのなかで，52kDaに対する抗体が陽性あるいは60kDaに対する抗体も含めてある一定以上の値だとCHBのリスクが高まることを示す研究報告が多かった[9][10]．われわれの症例データベースをもとにした解析でもその傾向は認めたが，優れた感度，特異度を持つカットオフ値を示すことは不可能そうである．また，抗SS-B抗体の併存でCHBのリスクが高まるという結果は得られそうにない．

さらに最近では前述した52kDaの抗原決定基と考えられているleucine zipper amino acid(aa200-239)(P200)に対する抗体がCHBのリスク予測に有用である[11]という報告があるが，これを否定する報告もあり，その評価は確立していない．

このように，抗SS-A抗体のプロフィールのみでCHBのリスクを予測することは不可能であり，他の臨床データと合わせて解析していく必要がある．

CHBの治療

CHBが成立するまでの時間は短いと考えられ，完全房室ブロックの状態で発見されることが多い．完全房室ブロックになってから母体に胎盤移行率の高いデキサメサゾン，ベタメサゾンなどフッ化ステロイド剤を投与しても戻る可能性はほとんどない．また，ステロイド剤を投与してもペースメーカー装着率や死亡率に差がなかったという報告がある[12]．一方で，CHB発症後であってもステロイド剤を投与することにより死亡率を下げることができるのではないかという報告がある[13]．その理由として心ブロックだけでなく心筋障害の進展を抑制する効果があるからではないかと推測されている．また，心嚢水，胸水，腹水，胎児水腫の改善が期待できるともいわれており，完全房室ブロックへの効果は期待できなくてもCHB出現時には，母体に胎盤移行性の高いステロイド剤を投与することは容認されると考える．Buyonは，その適応として不完全心ブロックないしは心不全徴候が認められたときと提案している[2]．

フッ化ステロイド剤を母体に投与することにより，胎児には子宮内発育遅延，羊水過少，副腎抑制などのリスクが，母体には糖尿病，高血圧，感染症などのリスクがあることを考慮して判断しなければならない．デキサメサゾンとベ

タメサゾンの胎盤移行性を比較した場合，前者は母体の血中濃度と胎児のそれがほぼ等しいが，後者では3分の1程度になるというデータもあり前者ほど移行率は高くないと考える．

CHBの予防

これまでにもステロイド剤，血漿交換療法，大量ガンマグロブリン療法など，いくつかの候補があがってきた．このうち，ステロイド剤，血漿交換療法についてわれわれのデータベースから私見を述べる．CHB児出産の既往のあるハイリスク群17例にベタメサゾンが投与されたが，そのなかからは1例もCHBを発症しなかったことよりベタメサゾンがCHBを予防できる可能性が示唆された．しかし，効果ありと判断するには例数が少ないこと，プレドニゾロンに比べて胎児移行性が高く胎児へのリスクが否定できない現状では，CHB児出産の既往のあるハイリスク群においてリスクとベネフィットに関する情報が不十分であることを理解して，それでも望む場合にのみ容認できるのではないかと考えている．血漿交換療法を施行した17例中2例でCHBを発症しており，本療法単独でCHBを予防することは難しいと考える．

Kaaja[14]らはCHBの既往のあるハイリスク群を対象に大量ガンマグロブリンを妊娠12週から24週まで3週間おきに投与するというプロトコールで臨床試験を行った．対象となった54例のうち，6例にCHBを発症したという結果から本療法がCHBの予防に有効ではないという判断がなされた．最近，Buyonらは，クロロキンがCHB予防に効果があることを報告した[15]．本邦で使用できる状況になれば1つの候補にあげても良いかもしれない．

現時点ではCHB児出産歴のある場合を除いて予防の対象にはならないと考えて良いだろう．しかし，今後われわれの症例データベースの解析が進み，ほかにハイリスク群の抽出が可能になれば，それらに対しても予防を拡大していくことになるだろう．その場合にも備え，われわれにとって最も身近な予防方法と思われるベタメサゾンの児への安全性に関する検証も同時に行っていくべきと考え現在取り組んでいるところである．

抗SS-A抗体陽性妊娠例では，たとえハイリスク群と認定できなくてもCHBのリスクについて説明し，産科医など胎児心エコーの熟達した医師に慎重な観察を求める必要がある．特にCHBの好発する妊娠18週から24週の期間は，通常なら1回/4週の通院で十分な，胎児エコーも頻回に施行しない期間であり，産科医への周知は必須である．

抗SS-A抗体と新生児ループス，特にCHBとの関係が取り上げられるようになって30年あまりがたった．この間，免疫学の進歩は著しく，新生児ループスをはじめさまざまな病態が明らかになってきたが，抗SS-A抗体を持つ女性の妊娠管理をどうしていけばよいのかという臨床的核心がつかめていないのが現状である．基礎的なアプローチもさることながら症例からみえてくることも少なくないはずだという確信を持って，この研究を進めてきた．本研究の進行にあたっては全国の内科，産科，小児科の先生方に，多大なご協力を賜った．この紙面を借りて感謝申しあげるとともに，ご尽力に報いるような結果を出していくことをお約束したい．

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酵素免疫測定法による抗SS-A/B抗体標準化の検討

Key words: Congenital heart block, anti-SS-A/B antibody, double immunodiffusion, enzyme immunoassay, standardization

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要 旨

現在，日本国内での抗SS-A抗体及び抗SS-B抗体の測定は，ほとんどが酵素免疫測定法を原理とする検査試薬で行われており，数値データとして報告されている．しかし，その値は試薬メーカーごとに値付け方法が異なり，標準化されていない．抗SS-A抗体と関連するCHB(congenital heart block）のリスクをその抗体価から推測することを目的として，試薬メーカー6社7試薬の測定値の関係を明らかにし，一定の基準を設けることで各試薬の測定値を一致させることができるか検討した．

Pool血清，CDC標準品，臨床検体を用いて酵素免疫測定法による抗SS-A/B抗体の標準化を試みたが現状では難しいことがわかった．多施設共同研究の症例データベースの解析から，抗SS-A抗体がDID (double immunodiffusion) 法で32倍以上であることが，CHBのリスクを推測する因子として抽出されているため[1)18)]，DID法力価32倍に相当する各試薬の測定値を推定した．しかし，この推定値において陰性と判定されるCHB例が認められたこと，また，酵素免疫測定法は主な臨床での使用目的が「定性判定」であるため，各社試薬での測定値が本来の抗体力価として報告されていないことが多い（特に高力価例について）現状を考え，DID法での確認が必要な基準案を作成した．その結果，Bio-Radで100 EU以上，INOVAで80 units以上，CosmicでIndex値100以上，TFBで300 U/ml以上，Phadiaで240 U/ml以上，MBL MESACUPでIndex値100以上，MBL STACIAで500 U/ml以上を示した検体についてはDID法での確認が必要であることがわかった．

はじめに

抗SS-A抗体は全身性エリテマトーデス（SLE）やシェーグレン症候群（SjS）の患者が保有しているばかりでなく[16)]，無症候女性の1%が本抗体を保有する[5)]．抗SS-A抗体陽性母体から出生した児に皮疹や血球減少，心ブロック（CHB）がみられることがあり，これらを新生児ループスと呼んでいる．特に抗SS-A抗体陽性妊娠例の1%前後に出現すると考えられて

Investigation of anti-SS-A/B antibody standardization using enzyme immunoassay.
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いるCHBは[2]生涯ペースメーカー装着となる場合が多く，死亡にいたる症例もある重篤な病態である。CHBは第1子に発症した場合，第2子の発症率が高くなると報告されており，前児がCHBであった場合は特に注意が必要である[10]。

近年，SS-A抗原のアイソフォームである52-kDa（Ro52）と60-kDa（Ro60）に対する抗体を測定する試薬が開発され，CHB発症の可能性を示唆する点でその有用性が検討されている[14)20)]。CHBが重症なほど母親の抗Ro52抗体価が高いという報告がある[8)13)17)]。さらに，Ro52を構成するサブユニットのひとつであるRo52 p200 peptideで免疫されたラットにおいて生まれる仔がCHBを生じたという報告[15]がある。

我々は，尿素誘導体（European patent EP0875761, Japanese patent application No. 10-121896）を用いた抗体の質（avidity）[11]の研究で，抗Ro52抗体はその児のCHBを，抗Ro60抗体はCHBを予想できる可能性を示した。その後の一般試薬である8M尿素を用いた検討[12]では，妊婦におけるRo52とRo60の抗体価及びRo60 AI（avidity index）はCHB群において有意に高値であったこと，また，Ro52 AIとRo60 AIの和のデータを比較したところCHB群において有意に高値であったことを示した。しかし，2項ロジスティック回帰分析においてRo60 AIにのみに有意差が認められる回帰式が得られた。また，妊婦の抗Ro52抗体単独陽性（抗Ro60抗体陰性）や低いRo52 AIでもCHBが生じたことなどからRo52とRo60に対する抗体価のみでCHBを予測することは難しいのが現状である。

このたび，母体の抗SS-A抗体が胎児へ移行して引き起こされる新生児ループス，特にCHBの実態を明らかにし，妊娠管理指針を作成することを目的に全国症例調査が行われた[7]。その解析において抗SS-A抗体の抗体価がDID（double immunodiffusion）法で32倍以上であることが，CHBのリスクを推測する因子として抽出された[1)18)]。しかし最近の臨床現場では，抗SS-A抗体及び抗SS-B抗体は酵素免疫測定法（ELISA）を原理とする検査試薬で測定される傾向にあり，本法による測定結果を用いてリスク評価をする必要があると考えた。ところが，ELISA測定用のキットは複数の試薬メーカーが製造しているため，多施設共同研究で各施設から報告された測定値はそのままでは集計・解析ができないことが判明した。そこで，試薬メーカー6社7試薬の測定値の関係を明らかにし，一定の基準を設けることで各試薬の測定値を一致させることができるか検討した。

この研究では，試薬メーカー6社（Bio-Rad, INOVA, Cosmic, TFB, Phadia, MBL）を含め標準化委員会を組織し，7試薬の測定値の関係を明らかにし，一定の基準を設けることで各試薬の測定値を一致させることができるか検討した。また，多施設共同研究の症例データベースの解析から，抗SS-A抗体がDID（double immunodiffusion）法で32倍以上であることが，CHB（congenital heart block）を推測する因子として抽出されているため[1)18)]，DID法力価32倍に相当する各試薬の測定値を算出することを目的とした。

対　　象

1．標準化委員会

大阪府立母子保健総合医療センター，国立成育医療研究センター，バイオ・ラッド　ラボラトリーズ（Bio-Rad，東京），アイ・エル・ジャパン（INOVA，東京），コスミック　コーポレーション（Cosmic，東京），テイエフビー（TFB，東京），ファディア（Phadia，東京），医学生物学研究所（MBL，名古屋）で標準化委員会を組織し研究を遂行した。

2．Pool血清

各施設の倫理委員会承認のもとに国立成育医療研究センター及び順天堂大学からの－30°C以下で保存された患者血清49例を，MBLの試薬を用いて抗SS-A/B抗体（DID法とELISA法），抗Ro52，抗Ro60抗体（ELISA法）を測定後，その結果から以下のPool血清を分類・作製した。

1）抗SS-A抗体（抗Ro52，抗Ro60抗体を含

む）

SS-A No. 1：抗 SS-A 抗体陽性（DID 法で1～64倍），抗 SS-B 抗体陰性かつ，抗 Ro52 抗体陰性または弱陽性の 9 例。

SS-A No. 2：抗 SS-A 抗体陽性（DID 法で16～512倍），抗 SS-B 抗体陰性かつ，抗 Ro52 抗体陽性の20例。

SS-A No. 3：抗 SS-A 抗体陽性（DID 法で32～128倍），抗 SS-B 抗体陽性かつ，抗 Ro52 抗体陽性の 5 例。

SS-A No. 1 と 2 の血清は国立成育医療研究センターから，No. 3 は順天堂大学から分与された。

2）抗 SS-B 抗体

SS-B No. 1：抗 SS-A 抗体陽性，抗 SS-B 抗体陽性（DID 法32～256倍）かつ，抗 Ro52 抗体陽性の 5 例。

SS-B No. 2：抗 SS-A 抗体陽性，抗 SS-B 抗体陽性（DID 法32～64倍）かつ，抗 Ro52 抗体陽性の 5 例。

SS-B No. 3：抗 SS-A 抗体陽性，抗 SS-B 抗体陽性（DID 法64倍）かつ，抗 Ro52 抗体陽性の 5 例。

すべての血清は順天堂大学から分与された。

3．CDC（米国疾病予防管理センター）標準抗体[4]

CDC 標準抗体：

抗 SS-A 抗体；ANA HUMAN REFERENCE SERUM # 7

(Catalogue # IS2105 Lot # 83-0026)

抗 SS-B 抗体；ANA HUMAN REFERENCE SERUM # 2

(Catalogue # IS2073 Lot # 82-0008)

4．臨床検体

各施設の倫理委員会承認のもとに国立成育医療研究センター及び順天堂大学からの93例の膠原病患者血清を用いた（一部 Pool 血清に使用した患者を含む）。性別は女性，平均年齢は34歳（23歳～47歳）で，SjS40例，systemic lupus erythematosus (SLE) 20例，SS-A 抗体陽性のみで臨床症状なし14例，rheumatoid arthritis (RA) 7 例，CHB 既往 7 例，mixed connective tissue disease (MCTD) 2 例，anti-phospholipid antibody syndrome（APS） 2 例，橋本病 1 例を含む。

これらの検体は，測定まで－30°C以下で保存され，DID 法にて抗 SS-A 抗体陽性93例と，その中の抗 SS-B 抗体陽性45例を用いた。

方　　法

1．測定試薬

表 1 に抗 SS-A 抗体測定用 7 キットにおける測定法の特徴を示した。INOVA，MBL MESACUP, Cosmic, Bio-Rad は定量法ではなく，TFB，Phadia，MBL STACIA は定量法である。各キット名は，表 1 上段より，AI シリーズ Anti-SS-A/Ro テスト Bio-Rad，Premmune 抗 SS-A 抗体 ELISA「コスミック」，クアンタライト SS-A，MESACUP-2 テスト SS-A，ステイシア MEBLux テスト SS-A，エリア SS-A/Ro，抗 SS-A/Ro 抗体（E）［S］である。

また，抗 Ro52 抗体と抗 Ro60 抗体は MBL と Phadia のキットを用い，それぞれ抗 Ro52 抗体のキット名は，MESACUP 52K SS-A/Ro，エリア Ro52 で，抗 Ro60 抗体のキット名は，MESACUP 60K SS-A/Ro，エリア Ro60 である。

表 2 に抗 SS-B 抗体測定用キットにおける測定法の特徴を示した。各キット名は，表 2 上段より，AI シリーズ Anti-SS-B/La テスト Bio-Rad，Premmune 抗 SS-B 抗体 ELISA「コスミック」，クアンタライト SS-B，MESACUP-3 テスト SS-B，ステイシア MEBLux テスト SS-B，エリア SS-B/La，抗 SS-B/La 抗体（E）［S］である。

DID 法測定試薬には ENA-2 テスト（MBL，名古屋）または SRL（TFB，東京）を用いた。

2．Pool 血清測定

Pool 血清 6 種を表 1，2 の各試薬の検体希釈液にて10倍，100倍，300倍，1,000倍，3,000倍，10,000倍希釈して測定し，最終力価を求めた。各試薬の最終力価の総平均値を基準とし，各試薬の測定値との比率を換算係数として換算し，換算前後の各試薬の測定値の一致性を評価し