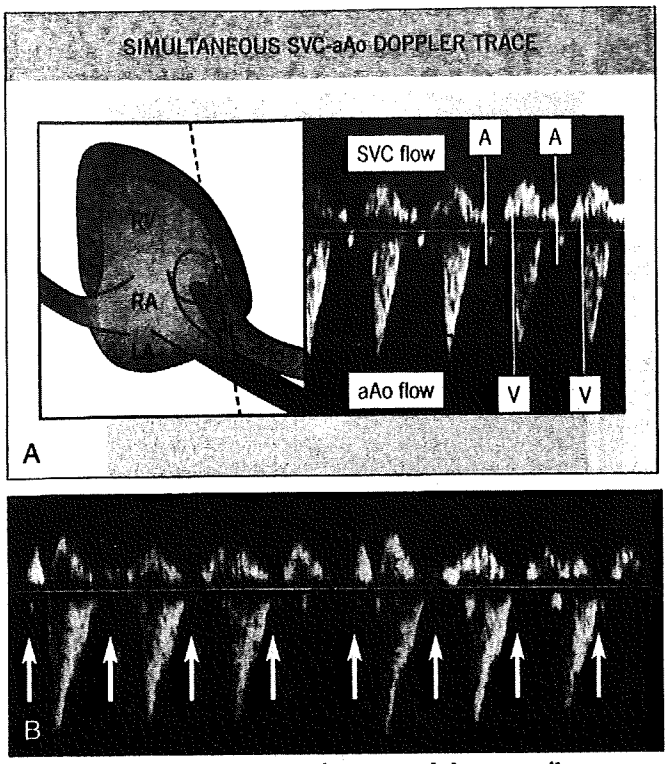

Fig. 104.3 Simultaneous Doppler trace of the ascending aorta (aAo) and the superior vena cava (SVC). A, Beginning of reverse flow at SVC (A) and forward flow at aAo (V) represent the timing of atrial and ventricular contractions, respectively. **B,** A case with a Wenckebach type of atrioventricular block revealed atrial contraction with regular rhythm (*arrows*), atrioventricular conducting time of gradual prolongation, and block with pose of ventricular contraction.

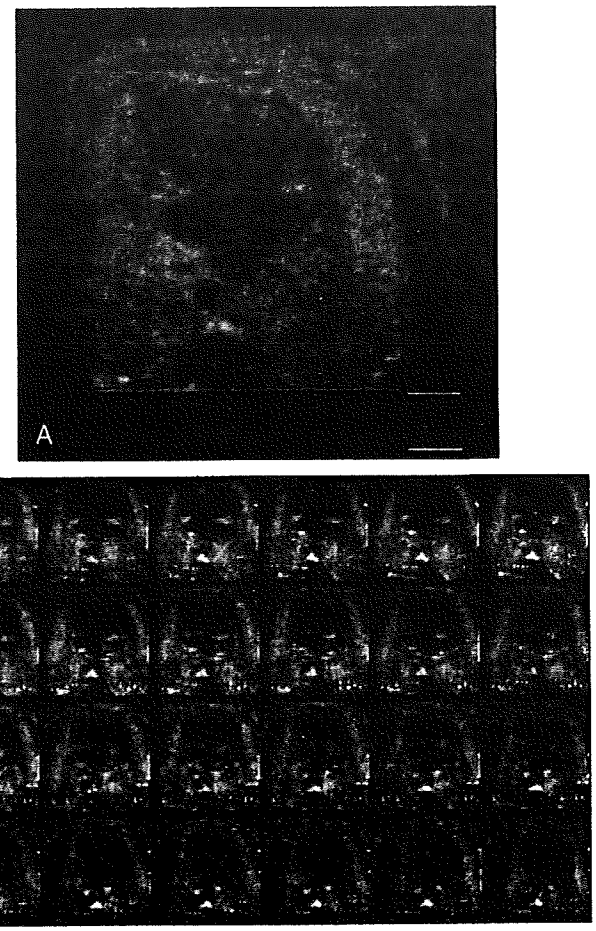

Fig. 104.4 Three-dimensional echocardiography in a case with common atrioventricular valve (asplenic syndrome). A, Optimal 2D cutting plane can be created from the 3D data set and demonstrated from optimal angle. **B,** Multiple parallel cutting planes obtained from 3D data set demonstrate the connection from the cardiac chambers to the vessels.

contraction are interpreted as the beginning of P and Q waves by electrocardiography, respectively. Hence, relation of the atrial and ventricular contraction can be assessed from this wave-form.

Color Doppler mapping is useful in the detection of abnormal blood flow. Color Doppler is examined at each valve to detect obstruction or regurgitation. Color Doppler mapping can also be used to detect abnormal vessels that are suspected in the abnormal heart, such as a coronary artery fistula[20] or, in the case of pulmonary atresia with ventricular septal defect, collateral vessels from the descending aorta to the lung.

Three-Dimensional Echocardiography

Three-dimensional echocardiography has become a powerful tool for screening and making detailed diagnosis of fetal cardiac anomalies.[11,21] For the 2D echocardiography, fetal movement and limited window caused by the fetal position often limit a detailed anatomic assessment of the fetal heart. Whereas with 3D echocardiography, any optimal 2D cutting image can be obtained without fetal movement once the 3D data set of the fetal heart is acquired and saved into the hard disk (Fig. 104.4). Especially for the spatio-temporal image correlation (STIC) method, 3D images with heartbeat can be created by automatic calculation of fetal heart rate from the acquired data set. Display of multiple parallel cutting planes is useful to assess the cardiac anatomy. Volume of the chambers and ventricular function are also accurately analyzed from this 3D data set.[21]

Interpretation of the Examination

The most important benefit of the prenatal diagnosis of CHD is making optimal plans for the perinatal management to improve the mortality and morbidity. Once the fetal cardiac problem is detected, consideration of the following issues is useful to optimize perinatal management:

- the precise anatomic and functional diagnosis, especially with respect to structures that affect the postnatal management strategy and outcome;
- anticipation of possible anatomic and functional progression of the disease during the prenatal period and planning of perinatal follow-up;
- indications for chromosome analysis;
- the plan for delivery; and
- the plan for postnatal management and estimation of the outcome.

Precise Anatomic and Functional Diagnosis

Precise anatomic assessment is essential for prenatal diagnosis. Detailed information is required about cardiac structures that affect perinatal management and postnatal outcome. Hence, possible postnatal management options are always anticipated while echocardiography is being performed. For example, when repair of a single ventricle is expected postnatally, the presence of atrioventricular valve regurgitation, cardiac function, and the size of the pulmonary arteries must be checked. The presence of subaortic stenosis is also looked for in such cases, because it presents a risk if a Damus-Kaye-Stansel (DKS) type of repair is anticipated. To give another example, when a fetus has a double-outlet right ventricle, an associated mitral valve abnormality, especially straddling of the chordae, must be excluded, because the presence of this lesion may change the surgical repair from biventricular repair to single-ventricle repair.

Doppler echocardiography provides valuable information for the investigation of detailed cardiac anatomy. Identifying the presence of valvular regurgitation or an obstructive lesion is important for an accurate diagnosis and to plan perinatal management. However, interpretation of the Doppler findings requires knowledge of fetal hemodynamics. Flow from the left atrium to the right atrium at the foramen ovale or flow from the aorta to the pulmonary artery at the ductus arteriosus, which are the complete antithesis of the normal direction of flow in the prenatal period, represents the presence of a significant congenital cardiac abnormality.[17] The pressure gradient of a stenotic aortic or pulmonary valve tends to underestimate the postnatal pressure gradient, because the main pulmonary artery and aorta, being connected by the large ductus arteriosus, have the same pressure regardless of the presence of obstructed outflow.

Functional assessment using M-mode and Doppler echocardiography is also important. The cardiac output and ventricular systolic and diastolic function are checked, and the presence of congestive heart failure should be detected before fetal hydrops develops.

Prenatal Progression

The prenatal cardiac anatomy, chamber size, and function will change during gestation, so that the appearance of the cardiac structures may be different from that usually seen postnatally. In addition, several abnormal lesions may progress during the prenatal period. The ascending aorta or the main pulmonary artery may not grow in the presence of outflow tract obstruction.[20,22] Even if the ventricle is enlarged in a fetus with severe outflow tract obstruction with intact ventricular septum, the ventricle may not grow and may become hypoplastic at birth.[22] The degree of regurgitation at the semilunar and atrioventricular valves tends to increase during gestation. Arrhythmia may develop in utero; in particular, atrioventricular block may develop in fetuses with left isomerism or discordant atrioventricular connection.[23] Restriction of the foramen ovale may appear in utero in fetuses with left-sided heart obstruction[24] or transposition of the great arteries.[25] Knowledge of all these possible developments is important when plans for follow-up and perinatal management are being made, when outcome is being predicted, and when the family is counseled.

Indication of Chromosomal Analysis

Once the presence of a congenital cardiac anomaly is diagnosed, the indication for chromosomal analysis should be considered.[26,27] In particular, when the termination of pregnancy is considered, chromosomal analysis may provide additional information to assist the family in their decision making. However, when the particular cardiac anomaly is unlikely to result from a chromosome abnormality (e.g., heterotaxy), or when the diagnosis of a chromosome abnormality will not change the management plan or the family's decision, the need for chromosome analysis should be discussed with the family, because the amniocentesis required for chromosome analysis carries some risk of premature birth.

Plan for Delivery

The delivery must take place at the tertiary care center, preferably close to the pediatric cardiac center, in all cases of major cardiac disease with some risk of deterioration at delivery. In particular, fetuses with a ductus-dependent lesion, or those in whom there is a possibility of severe hypoxemia because of a restricted foramen ovale, such as a left-heart obstructive lesion or transposition of the great arteries, must be delivered in conditions in which prostaglandin infusion and emergency balloon atrial septotomy are readily available. Prenatal echocardiographic assessment in later gestation may be able to detect some of these high-risk cases.[25]

INDICATIONS

There are two different indications for prenatal echocardiography:

- screening the fetal heart for anatomic and functional abnormality; and
- detailed prenatal echocardiography performed or assessed by a pediatric cardiologist with particular knowledge of fetal cardiology.

Screening

Effective screening of anatomic and functional abnormality of the fetal heart is essential for prenatal echocardiography. Unlike in the newborn period, the presence of severe cardiac disease in the fetus—such as heart murmur and cyanosis—cannot be detected by physical examination. Therefore fetal ultrasonographic screening of a completely healthy mother with an uneventful pregnancy is the only method of identifying the majority of cases of congenital heart disease in utero. In this regard, appropriate fetal echocardiographic assessment is indicated for all pregnant women.

The first screening ultrasound should be performed between 18 and 22 weeks of gestation if termination of pregnancy is to be considered as an option in complex cardiac problems. Screening has to be performed sufficiently early to make it possible to refer the case to a fetal cardiac center and to allow the parents to make their decision. Because some cardiac abnormalities become more obvious later in gestation, a

second screening for cardiac disease should be performed at 28 to 32 weeks of gestation.

Detailed Fetal Cardiac Assessment

Detailed fetal cardiac assessment is indicated for all cases in which a structural, rhythmic, or functional cardiac abnormality is detected by screening, and those with extracardiac abnormality. The purpose of this detailed assessment is not only to make an accurate diagnosis of any cardiac disease, but also to allow an appropriate plan for perinatal management to be drawn up. From this examination, the plan for prenatal follow-up and preparation for delivery and postnatal management, such as early neonatal cardiac surgery, must be decided. Therefore the fetal echocardiography should be performed and/or reviewed by physicians with specialized skill in fetal cardiology. The indication and timing of follow-up ultrasound must be decided, in each case, on the basis of the likelihood and severity of progression of the cardiac problems.

SPECIFIC STRUCTURAL ABNORMALITIES

Atrioventricular Septal Defect

The common valve in atrioventricular septal defect can usually be easily diagnosed by the four-chamber view (Fig. 104.5a) and the short-axis view at the level of the atrioventricular valve. This anomaly is often associated with other complex cardiac anomalies. In particular, any associated right or left atrial isomerism has to be checked carefully.[2] In addition, the presence of left or right ventricular outflow obstruction, straddling of the mitral valve, and atrioventricular valve regurgitation must be checked to predict postnatal mortality and morbidity. The association of atrioventricular heart block, with or without left atrial isomerism, is also important for prognosis.[23,28]

Fetuses with this anomaly need careful follow-up during gestation, because several aspects of the fetal heart may change in utero. The size of either the ventricular or the atrial component of the septal defect may change (it may spontaneously close), and obstruction at either side of the

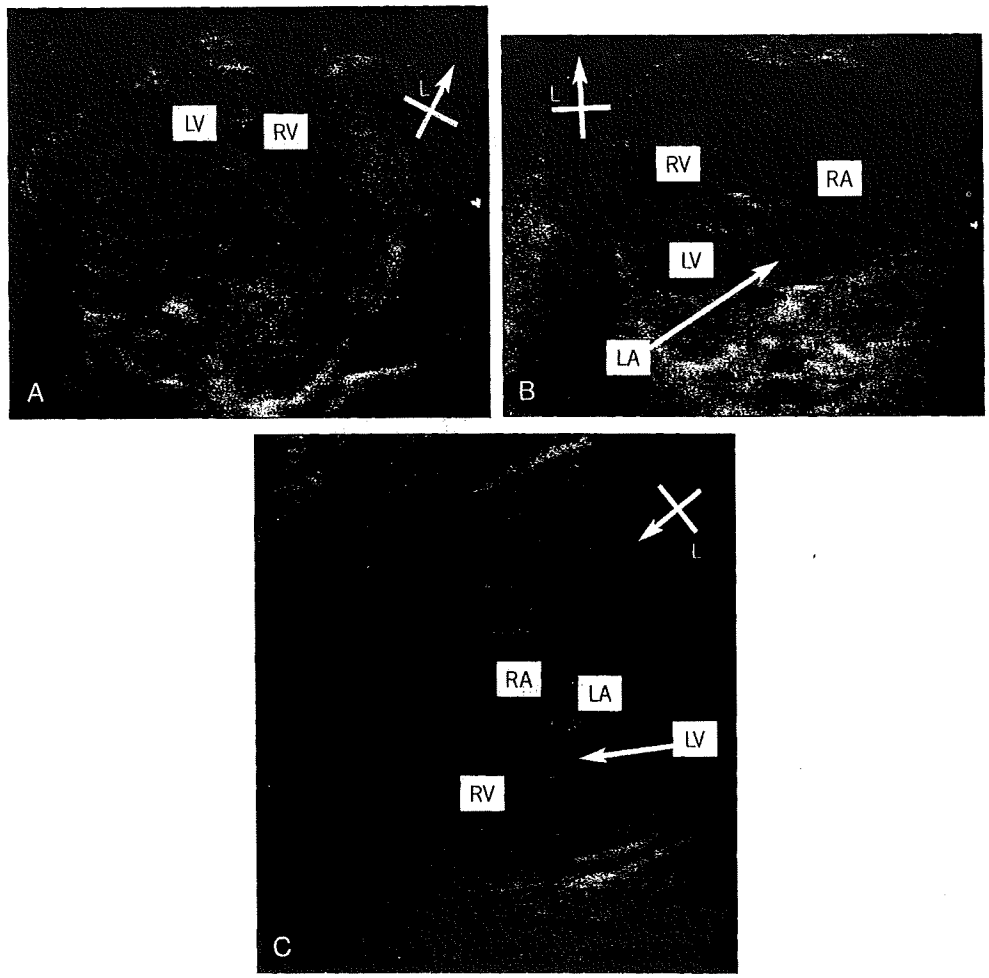

Fig. 104.5 Four-chamber view of a fetus with **(A)** atrioventricular septal defect, **(B)** tricuspid valve dysplasia, and **(C)** hypoplastic left heart syndrome. **(A)** Atrio-ventricular valve is not separated to left and right portion, and creates common atrioventricular valve. **(B)** The heart is quite enlarged and fills almost the entire chest. The right atrium (RA) is enlarged because of severe tricuspid valve regurgitation. **(C)** The left ventricle (LV) is small and endocardium of LV is high echogenic due to endocardial fibroelastosis. LA, left atrium; LV, left ventricle; RA, right atrium; RV, right ventricle.

outflow tract may appear and progress during gestation. In particular, worsening atrioventricular valve regurgitation or development of complete heart block may lead to sudden deterioration of the fetus in utero.

Complete atrioventricular septal defect, especially in the cases associated with intrauterine growth restriction, is often associated with trisomy 21. The existence of extracardiac anomalies, such as duodenal atresia and hygroma, should be carefully checked. Pleural effusion due to chylothorax and enlarged high-echogenic liver due to the transient myeloproliferative disorder are rare but important complications associated with trisomy 21.

Ebstein's Malformation/Tricuspid Valve Dysplasia

Both Ebstein's malformation and tricuspid valve dysplasia involve significant tricuspid valve regurgitation.[29,30] The severe form of these diseases produces an enlarged right atrium, which is easily detected by the four-chamber view (see Fig. 104.5b). The right atrium is sometimes considerably enlarged and fills almost the entire fetal chest. The septal leaflet of the tricuspid valve in Ebstein's malformation is displaced toward the apex of the heart. Tricuspid valve dysplasia produces a thick and nodular tricuspid valve. Both diseases show a spectrum of severity and may overlap.

Severe tricuspid valve regurgitation is often associated with functional or anatomic right ventricular outflow tract obstruction.[29,30] In the case of functional atresia, right ventricular pressure estimated from the tricuspid valve regurgitation jet is low. The presence of pulmonary valve regurgitation in the systolic phase represents penetration of the pulmonary valve.

Severe tricuspid valve regurgitation causes two serious conditions in the fetus: fetal hydrops and lung hypoplasia.[29,30] Severe regurgitation causes increased central venous pressure, in turn causing congestive heart failure and fetal hydrops. Lung hypoplasia develops secondary to the enlargement of the fetal heart, especially the right atrium, which sometimes almost fills the chest.

The fetus with these tricuspid valve abnormalities should be carefully followed during gestation to detect early signs of fetal hydrops. If fetal hydrops is already present during the middle trimester, the prognosis is extremely poor and the risk of intrauterine death is quite high.

Right Ventricular Outflow Tract (RVOT) Obstruction with Intact Ventricular Septum

Pulmonary atresia with intact ventricular septum and pulmonary valve stenosis occur in various degrees of severity.[20,31,32] Severe forms of this type of abnormality, with a severely hypoplastic right ventricle or enlarged right ventricle with severe tricuspid valve regurgitation, are easily detected by screening using the four-chamber view. However, prenatal detection of milder tricuspid regurgitation cases or those with a normal-sized right ventricle may not be possible. Critical pulmonary valve stenosis is very difficult to distinguish from pulmonary atresia.[2,20]

For fetuses in this category with associated hypoplastic right ventricle, the size of the right ventricle, the presence or absence of the outflow portion of the right ventricle, and the connections between the right ventricle and the coronary artery should be carefully assessed by prenatal echocardiography.[20] The diameter of the annulus of the tricuspid valve should be measured to assess the size of the right ventricular cavity indirectly.

The severity of pulmonary valve stenosis may progress during gestation, and, in some fetuses, valvular atresia may develop.[32] Unless the fetus develops severe tricuspid valve regurgitation, congestive heart failure may not develop and the incidence of fetal death is quite low.[2,32]

Balloon pulmonary valvuloplasty by direct puncture of the fetal heart under echocardiographic guidance has been tried in an attempt to prevent hypoplastic right ventricle at birth.[33] Although some cases of successful valvuloplasty have been reported, in others valvular atresia redeveloped.

Because the pulmonary circulation is dependent on the ductus arteriosus in all neonates with pulmonary atresia and some with pulmonary stenosis, infusion of prostaglandin should be started immediately after birth. In particular, cases of pulmonary stenosis with retrograde flow in the ductus arteriosus in the prenatal period always require a patent ductus arteriosus postnatally.

Left Ventricular Outflow Tract (LVOT) Obstruction

Left ventricular outflow tract obstruction occurs in various degrees of severity.[2,22,24,34,35] The most severe form is complete or severe obstruction of the aortic valve, associated with obstruction of the mitral valve, endocardial fibroelastosis of the left ventricle, and severe hypoplastic left ventricle (so-called hypoplastic left heart syndrome).

Most fetuses in this category have a hypoplastic or enlarged left ventricle, with or without poor cardiac function, and can be easily detected by the simple four-chamber view (see Fig. 104.5c). The enlarged, poorly contractile left ventricle may not grow during gestation and is likely to be hypoplastic at birth, especially when there is significant retrograde flow from the left to the right atrium at the foramen ovale, or a closed foramen ovale.[23] Systolic retrograde flow at the isthmus of the aortic arch represents significant obstruction at the aortic valve and probable ductus-dependent systemic circulation postnatally. In fetuses with mitral valve regurgitation, the severity of the condition may change and some of them develop fetal hydrops.

Balloon aortic valvuloplasty before 29 weeks of gestation by direct puncture of the fetal heart using echocardiographic guidance has been reported to prevent hypoplastic left ventricle at birth.[36,37] Once the left ventricle progresses to hypoplastic, Norwood-type palliative repair and single ventricular type of repair, which has much higher morbidity and mortality compared with biventricular repair, is required. Hence, prevention of the progression of hypoplastic left ventricle is very important. Successful balloon valvuloplasty and postnatal biventricular repair have been reported in recent years. To decide the indication for this procedure, accurate prediction of which fetuses develop hypoplastic left ventricle in later gestation is essential.

D-TRANSPOSITION OF THE GREAT ARTERIES

D-transposition of the great arteries cannot be detected on the four-chamber view unless there is a large ventricular septal defect, but the three-vessel view clearly demonstrates the

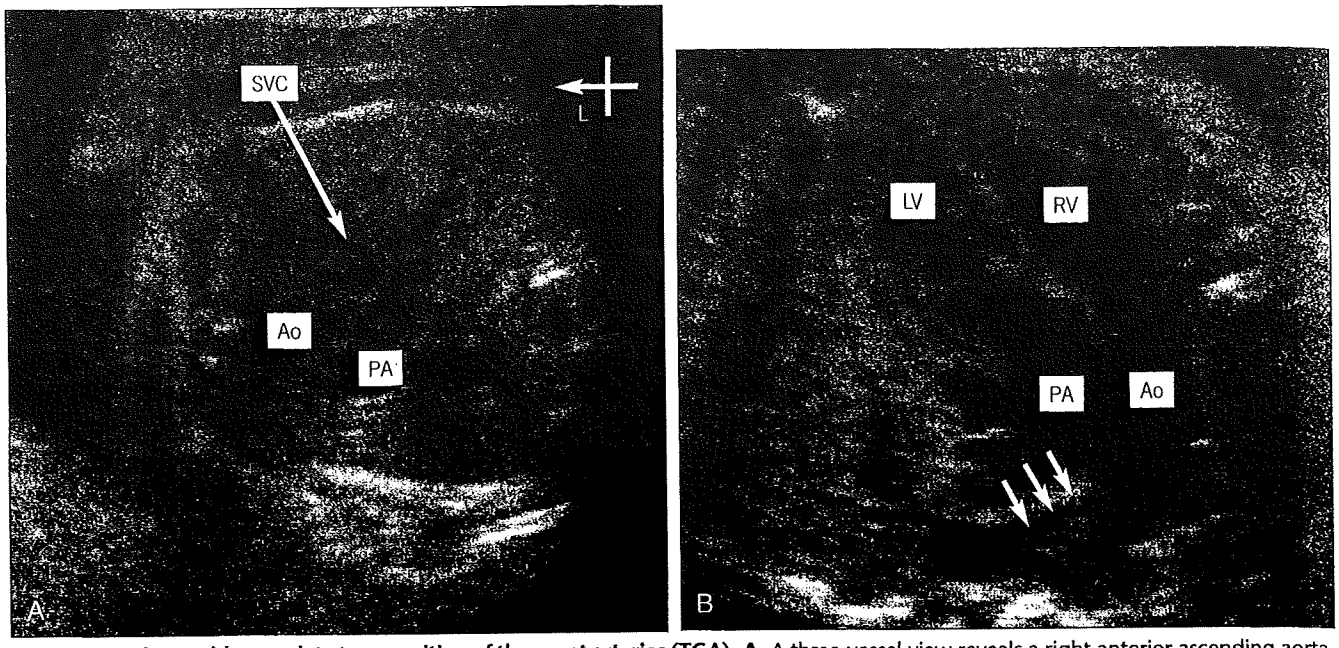

Fig. 104.6 A fetus with complete transposition of the great arteries (TGA). A, A three-vessel view reveals a right anterior ascending aorta (Ao) and left posterior main pulmonary artery (PA). **B,** A view of the outflow tract clearly demonstrates the ascending Ao arising from the right ventricle (RV) and the main PA arising from the left ventricle (LV). The great vessels lying parallel to one another can never be obtained in normal cardiac anatomy, in which the relationship between the great vessels is spiral. Of note, the ductus arteriosus (*arrows*) is narrow in many fetuses with TGA, which may lead to severe hypoxicemia immediately after birth. L, left; SVC, superior vena cava.

abnormal position of the great vessels (i.e., a right anterior ascending aorta and a left posterior main pulmonary artery) (Fig. 104.6).[6] Oblique sections visualizing the ventricular outflow tract can demonstrate the abnormal connection of the great arteries directly.[25,38] The ascending aorta and the pulmonary artery run parallel, unlike the normal heart, in which the great vessels have a spiral relation.

Once the diagnosis of transposition is made, it is very important to check for the presence of ventricular septal defect and the appearance of the foramen ovale and ductus arteriosus.[25,39] Color Doppler flow mapping is helpful in detecting the ventricular septal defect. Stenosis of the foramen ovale and the ductus arteriosus may develop during the prenatal period. High-velocity flow from the pulmonary artery to the aorta represents the presence of constricted ductus arteriosus and possible associated pulmonary hypertension. It is important to note that, even when there is a small ductus arteriosus, the Doppler velocity of the flow through the ductus may not increase if the amount of flow is reduced or pulmonary hypertension is absent. Neonates with severe constriction at either site develop severe hypoxemia soon after birth and may die within a short period. Although immediate balloon atrial septotomy in the delivery room is indicated in such cases,[25,38] exact prediction of such cases by fetal echocardiography is still difficult.[39]

Tetralogy of Fallot

The presence of tetralogy of Fallot is easily missed by the four-chamber view, but the three-vessel view demonstrates the small main pulmonary artery positioned slightly more posteriorly than usual.[6] Oblique sections to visualize both the RVOT and LVOT are the most valuable views with which to demonstrate the anteriorly deviated infundibular septum and other anatomic features.[40,41]

The patency of the pulmonary valve is sometimes difficult to ascertain in cases of severely hypoplastic RVOT obstruction. In addition, some progress in utero to pulmonary atresia.[2,40] The presence of retrograde ductal flow from the aorta to the pulmonary artery, however, indicates a postnatal ductal-dependent pulmonary circulation, regardless of the patency of the pulmonary valve. The size of both branches of the pulmonary arteries is an important factor for postnatal surgical treatment.

There is a strong relationship between tetralogy of Fallot and the microdeletion of 22q; hence, the presence of associated cleft lip or cleft palate should be checked. Associations with other chromosomal abnormalities, such as trisomy 18, or other extracardiac anomalies, have also been reported.[41] Therefore counseling of the family may require particular attention, because the prognosis of fetal tetralogy of Fallot is not always favorable.

Tetralogy of Fallot with Absent Pulmonary Valve

Tetralogy of Fallot with absent pulmonary valve complex is a relatively rare CHD, but easily detected by screening because of aneurysmally enlarged main and branch pulmonary arteries (Fig. 104.7). The ductus arteriosus is absent in most of the cases. Some of the fetuses develop to fetal hydrops and fetal death.[42,43] Postnatal mortality is high because of respiratory failure. The prediction of these worse outcomes is difficult. About 20% of the cases are associated with the microdeletion of 22q.

Univentricular Heart

Univentricular heart usually has an obviously abnormal appearance on the routine four-chamber view and is easily detected by screening. The presence of heterotaxy, such as

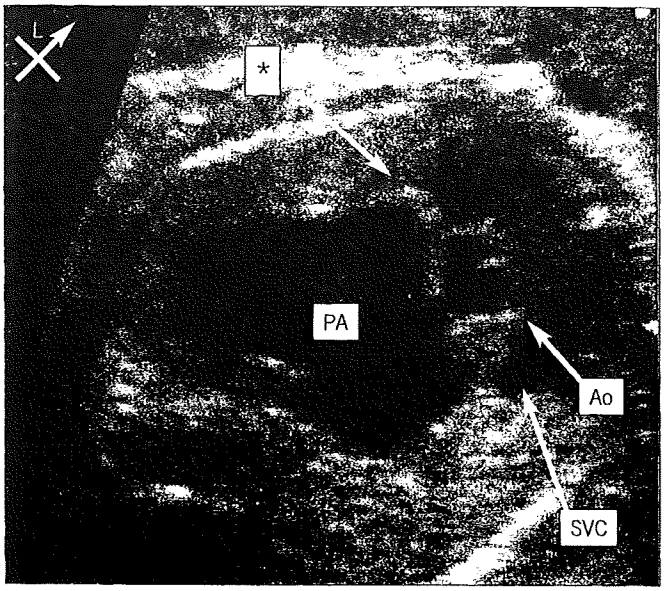

Fig. 104.7 A fetus with tetralogy of Fallot with absent pulmonary valve complex. The pulmonary valve is not well developed (*). The main and branch pulmonary arteries (PA) are aneurysmally enlarged. Ao, aorta; SVC, superior vena cava.

abdominal situs and the presence of azygos continuation, should be checked.[28,44]

The cardiac anatomy should be carefully examined to predict dependence of the postnatal circulation on the ductus arteriosus, the foramen ovale, or both. The risks of postnatal repair must also be checked during the prenatal period. This is important not only for perinatal management but also for counseling the family. In cases in which the usual type of single ventricle repair is indicated, the presence of regurgitation at the atrioventricular valves, the size of both branches of the pulmonary arteries, and ventricular function should be checked. Cases associated with subaortic or valvular aortic obstruction or arch obstruction may require a Norwood type or DKS type of arch repair during the early neonatal period and are at additional risk for postnatal morbidity and mortality.

Coarctation of the Aorta

Prenatal diagnosis of coarctation of the aorta has several limitations. When the diameter of the ascending aorta is visibly smaller than that of the main pulmonary artery, there is some possibility to develop coarctation of the aorta postnatally.[6,45-48] However, even fetuses with a relatively large isthmus may develop coarctation of the aorta if there is extension of ductal tissue into the aortic wall and ductal closure narrows the aorta, and prediction of such postnatal development is difficult.

Some fetuses with persistent left superior vena cava and enlarged coronary sinus are known to develop coarctation of the aorta.[49,50] Hence, in such cases, the diameter of the isthmus has to be carefully checked, and close follow-up is required postnatally. Although the reason for this coexistence is not clearly understood, it is speculated that enlarged coronary sinus reduces the size of the foramen ovale and mitral valve annulus and reduces the left ventricular inflow, which reduces the size of the left ventricle as well as the ascending aorta and isthmus.

Truncus Arteriosus

Although truncus arteriosus can be detected by abnormal three-vessel view during screening, differentiation from pulmonary atresia with ventricular septum is sometimes difficult.[51,52] The truncal valve is frequently stenotic, incompetent, or both. In the cases with significant regurgitation at the truncal valve, the fetus may develop fetal hydrops and may die in utero. Postnatal prognosis is also poor in such cases. About 30% to 40% of the fetuses with truncus arteriosus also have a chromosomal abnormality, such as 22q11.2, an extracardiac anomaly, or both.

Vascular Rings

There are various types of vascular rings, and the most common type is the one associated with right aortic arch, which can be detected by three-vessel and trachea view (Fig. 104.8).[7,10] Postnatally, the vascular rings may cause stenosis at the trachea, esophagus, or both. However, prediction of which cases develop stenosis after birth is not established. Nevertheless, prenatal detection is very useful because postnatal diagnosis of this vascular anomaly is often difficult and often delayed.

OTHER CARDIAC PROBLEMS

Fetal Arrhythmia

Fetal arrhythmia is found at the time of fetal heart monitoring or routine prenatal ultrasound examination. Sometimes, the mother realizes that fetal movement has decreased. Prenatal ultrasound can detect arrhythmia with or without enlarged heart and fetal hydrops. Arrhythmias are of three kinds[53,54]:

- bradycardia (heart rate <100 beats per minute);
- tachycardia (heart rate >180 beats per minute); and
- ectopic beats.

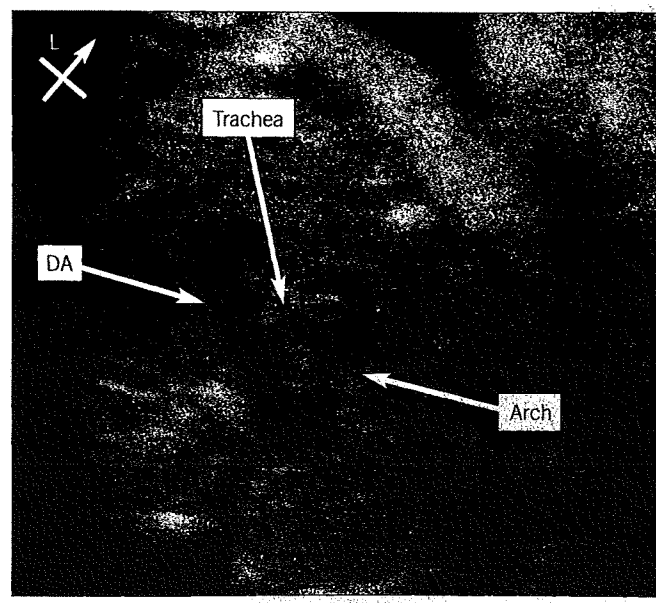

Fig. 104.8 Three-vessel and trachea view of a fetus with right aortic arch and vascular ring. The aortic arch is running on the right side of the trachea and is connected to the ductus arteriosus (DA), which is running left of and posterior to the trachea, at the right side of the spine. Of note the trachea is surrounded by the arch and the ductus arteriosus creating the vascular ring.

Detailed analysis for the type of arrhythmia in utero has become possible using M-mode (see Fig. 104.2), Doppler (see Fig. 104.3), and tissue Doppler methods.[55-57]

Bradycardia

The most common cause of fetal bradycardia is complete atrioventricular block. About 33% to 50% of cases of complete atrioventricular block are caused by associated congenital cardiac anomalies, such as left atrial isomerism, discordant connection, and septal defect, and prognosis for these fetuses is generally poor.[23,58] Most of the remaining cases of isolated atrioventricular block are caused by maternal SS-A or SS-B antibody. A heart rate of less than 55 beats per minute carries a risk for the development of fetal hydrops. It is important that transferred maternal IgG can cause pleural, pericardial, and peritoneal effusion—in addition to myocarditis and poor cardiac function—in the fetus, even if there is no hydrops.

When such atrioventricular block is detected shortly after it develops, maternal steroid treatment is reported to improve the situation.[58] Maternal steroid treatment may also improve fetal myocarditis or effusions. When this kind of atrioventricular block is discovered later in gestation, maternal administration of sympathomimetic agents appears to increase the fetal heart rate.[58,59] When the fetal heart rate progressively decreases and fetal hydrops starts to develop, early delivery and direct pacing of the ventricle may be the only option to improve congestive heart failure. Nevertheless, in cases with significant myocarditis, congestive heart failure may not improve even after pacing.

Atrial bigeminy and blocked premature atrial beats cause bradycardia and mimic 2:1 atrioventricular block. A blocked premature atrial beat has an irregular interval of atrial contraction on M-mode, whereas 2:1 atrioventricular block has a regular interval. Long QT syndrome also causes fetal bradycardia, hence a postnatal electrocardiogram to measure the QT interval is essential.

Tachycardia

Common causes of fetal tachycardia are paroxysmal supraventricular tachycardia (PSVT) and atrial flutter.[13,60] Although the tachycardia is sometimes intermittent during prenatal examination, the chance of hemodynamic complications and the development of fetal hydrops is still high.[61] Most cases of both PSVT and atrial fibrillation are successfully treated in utero by transplacental administration of antiarrhythmic drugs.

Most PSVT is caused by atrioventricular re-entry tachycardia (AVRT) caused by Wolff-Parkinson-White syndrome. A simultaneous M-mode record of atrial and ventricular contraction shows 1:1 atrioventricular conduction, with heart rates ranging from 200 to 300 beats per minute (Fig. 104.9). Measurement of the time interval from the ventricular contraction to the following atrial contraction (VA interval) is useful to distinguish AVRT (short VA interval) from the other tachycardias (long VA interval).[55] The VA interval can be measured from the simultaneous M-mode record of atrial and ventricular contraction, or from a simultaneous Doppler waveform record of the superior vena cava and the ascending aorta (Fig. 104.10).[56]

It is difficult to predict when the fetus will develop hydrops. Several Doppler echocardiographic parameters that demonstrate congestive heart failure cannot be used at this extremely high heart rate. Serial measurement of the cardiothoracic ratio may be useful for monitoring the degree of heart failure. The presence of atrioventricular valve regurgitation, especially at the mitral valve, may represent severe congestive heart failure. Usually, the fetus does not deteriorate quickly even after developing fetal hydrops. Maternal administration of digoxin, the first choice for treating AVRT, may be indicated for fetuses with early signs of heart failure or sustained tachycardia lasting more than 8 hours in a day. Sotalol, procainamide, propranolol, amiodarone, and flecainide are used as second-line drugs when digoxin fails to achieve conversion to sinus rhythm. These second-line drugs can be used from the beginning in fetuses with a long VA interval or in fetuses with severe congestive heart failure. Early delivery and administration of ATP or electrical conversion is the option when intrauterine conversion cannot be achieved.

M-mode recording of atrial and ventricular contraction for fetuses with atrial fibrillation shows a regular atrial rate of 400 to 500 beats per minute with 2:1 to 4:1 atrioventricular conduction, resulting in a ventricular rate of 200 to 300 beats

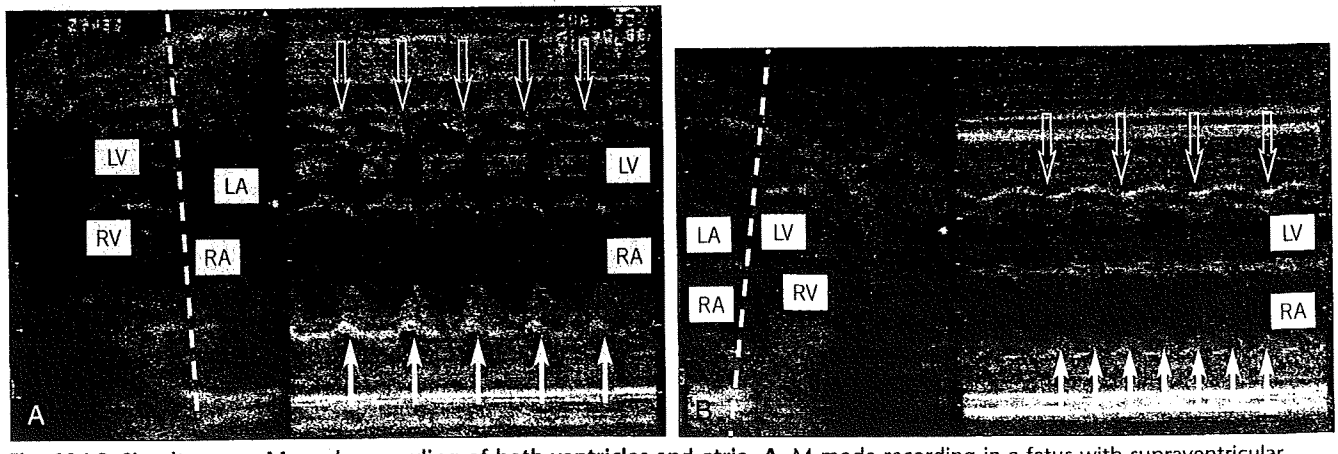

Fig. 104.9 Simultaneous M-mode recording of both ventricles and atria. A, M-mode recording in a fetus with supraventricular tachycardia reveals 1:1 relation of atrial (*solid arrow*) and ventricular contraction (*open arrow*) with a ventricular rate of 210 beats per minute. **B,** M-mode recording in a fetus with atrial flutter and 2:1 atrioventricular conduction, with an atrial (*solid arrow*) rate of 510 beats per minute and a ventricular (*open arrow*) rate of 255 beats per minute. LA, left atrium; LV, left ventricle; RA, right atrium; RV, right ventricle.

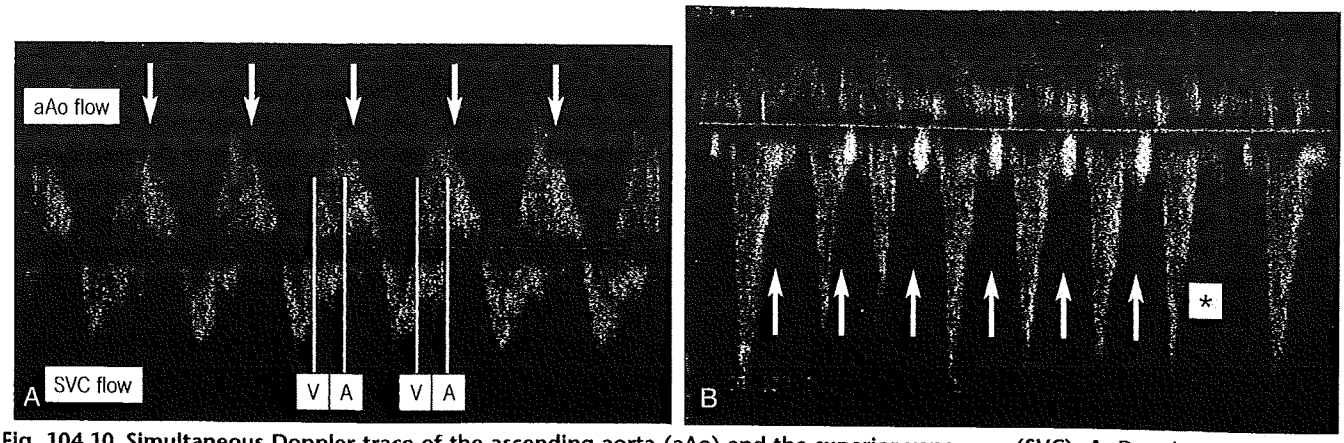

Fig. 104.10 Simultaneous Doppler trace of the ascending aorta (aAo) and the superior vena cava (SVC). A, Doppler trace of a fetus with supraventricular tachycardia with short VA interval. Although high-velocity reversal flow of SVC is almost over-rapped to the flow of Ao, the starting point of the reversal flow can be detected from the interrupted forward flow of the SVC. **B,** Doppler trace of the fetus with 1:1 atrioventricular conduction tachycardia with long VA interval. Tachycardic atrial contraction (*arrows*) disappears after the seventh ventricular contraction (*), and the tachycardia is stopped.

per minute.[62] Associated cardiac structural abnormality is more common than in PSVT. There is some tendency to develop 1:1 AV conduction and sudden deterioration; hence, all fetuses with atrial fibrillation should be treated after diagnosis. The selection of antiarrhythmic drug is similar to that for PSVT. However, the decision to start a second-line drug may be made earlier than for PSVT because of the possibility of sudden deterioration.

Fetal Cardiomyopathies

Although fetal echocardiography detects cardiac dysfunction, little is known regarding the cardiomyopathies and myocarditis in fetal life.[63] On routine fetal echocardiographic examination, the four-chamber view clearly detects the dilated, poorly contractile heart in dilated cardiomyopathy or the ventricular hypertrophy in hypertrophic cardiomyopathy. Signs of fetal hydrops are present in the case of developed heart failure. Serial assessment of systolic and diastolic cardiac function by M-mode and Doppler echocardiography is important for managing the affected fetuses.

Fetal cardiomyopathy is etiologically a heterogeneous condition, which can be the result of intrinsic pathology in addition to extrinsic factors. Noonan's syndrome, familial cardiomyopathy, α-thalassemia, and metabolic disease had been reported as intrinsic causes. Extrinsic causes include infectious disease, maternal autoantibodies,[64] and others. Although treatment for the various causes of cardiomyopathy and management of cardiac failure have been reported, the outcome of the affected fetuses is generally poor.

ACCURACY

The detection rate for congenital heart disease by screening fetal ultrasound and the accuracy of prenatal echocardiography are directly related to the quality of the examiner's technique and the reviewer's interpretation. Hence, teaching programs and sufficient experience specific to prenatal echocardiography are very important to make a significant impact on practice.

At the more advanced prenatal echocardiographic centers, quite accurate diagnosis has been reported.[2] With the new high-resolution echocardiographic machines, morphologic diagnosis has become more and more accurate. At about 18 to 28 weeks of gestation, the large volume of surrounding amniotic fluid and the less calcified fetal spine and ribs make it possible to visualize quite detailed cardiac anatomy. Even small cardiac structures with small blood flows, such as pulmonary venous return and small ventricular septal defects, can be detected by detailed prenatal echocardiographic examination combined with advanced color Doppler mapping.

COMPLICATIONS

The ultrasound technique is known to be a noninvasive imaging modality. However, there remain some concerns regarding possible adverse effects on the fetus of exposure to ultrasound, especially for Doppler echocardiography, in which the energy levels are greater. Possible adverse effects are both heat-related and non–heat-related. Although no epidemiologic report has demonstrated any adverse effects of prenatal ultrasonographic examination, particular attention should be paid to minimizing the energy level during prenatal examination. The examination time should be as short as possible, using a low power level, and use of pulse, continuous, and color Doppler should be limited to as short a time as possible.

PITFALLS AND ALTERNATIVES

Although high-resolution echocardiography provides quite accurate morphologic information, there remain some limitations, and correct knowledge of these may prevent possible misinterpretation and misdiagnosis. The limitations are divided into three categories:

- technical limitations;
- fetal hemodynamic limitations; and
- limitations resulting from prenatal and perinatal progression of disease.

The images of prenatal echocardiography are not always sufficient to provide detailed information. Movement and position of the fetus, location of the placenta, and maternal obesity are the most typical causes of inadequate images.

Less amniotic fluid and more heavily calcified bones in later gestation also limit the amount of information obtained. For fetuses in a bad position, changing the mother's position to lie on her side, having her fill her bladder by drinking water and letting her take a walk for several minutes may change the fetal position. Three-dimensional echocardiography can overcome some of these position limitations. When the position is unsuitable even after various attempts, repeat examination on a different day may be another option. When maternal obesity limits image quality, positioning the probe in the mother's navel where there is less lipid thickness may provide a better image. Despite the limitations of prenatal echocardiography, there is no alternative clinically applicable imaging modality.

The difference between fetal and postnatal hemodynamics often makes it difficult to diagnose cardiac problems in utero. Because the ductus arteriosus is widely patent, the pressures in the aorta and the pulmonary artery are the same. Hence, the presence of aortic or pulmonary stenosis may not cause a significant pressure gradient at the stenotic lesion. Functional pulmonary atresia as a result of increased pulmonary artery pressure is also difficult to distinguish from anatomic atresia. It may not be possible to diagnose patent ductus arteriosus and atrial septal defect prenatally.

Some cardiac anatomic abnormalities may progress in utero. The ventricles and the great arteries may become hypoplastic, the degree of valve stenosis and regurgitation may increase, and arrhythmia may develop. In particular, the fetus may develop atrioventricular block in left isomerism or discordant atrioventricular connection. At the present time, there is no clear method of predicting such progression. Therefore awareness of possible progression and serial prenatal examinations are important.

SUMMARY

Prenatal diagnosis of congenital heart disease has had an impact on its clinical management in several ways.[2,65] High-risk cases are delivered under cover of prostaglandin infusion and balloon atrial septostomy and are transferred to the pediatric cardiac center without deterioration. The possibility for family counseling is a clear benefit. Parents can be provided with sufficient information to make decisions and work out a strategy for perinatal management.

Prenatal pharmacologic and surgical treatment of heart disease has already begun. Fetal tachyarrhythmia has been successfully managed by pharmacologic treatment in utero.[13,58,60] Intrauterine treatment using interventional catheterization for LVOT and RVOT has also been started.[33,36,37] A number of research projects for use in future prenatal cardiac intervention and surgery, such as a very small cardiopulmonary pump for the fetus and fetoscopic surgery, have already begun.[66-69]

For the benefits of prenatal diagnosis to have a significant impact on practice, effective screening for congenital heart disease is essential.[3] The prenatal sonographic screening system for each lesion must be established. Practical methods, simple techniques, and clear interpretations within short examination times need to be taught in prenatal screening centers.

Finally, ethical problems are a very important issue in prenatal diagnosis.[70] Even before screening, the fetal ultrasonographic examination may require appropriate informed consent. Parents must be made aware that there is some chance that an anomaly in the fetus will be detected, and they may not want to know about this until the child's birth. Once the fetus is diagnosed as having congenital heart disease, the parents have to decide the management plan. The decision as to whether to continue with the pregnancy may be the most important ethical issue for prenatal diagnosis. In addition, many prenatal treatments are still experimental; hence, their efficacy and possible adverse effects on both the fetus and the healthy mother are still unknown. However, the opinion of the physician who tells the parents about fetal heart problems tends to influence the parents' decision. The support system to help parents with their decision making must be established for each lesion.

Pediatr Cardiol
DOI 10.1007/s00246-008-9273-5

ORIGINAL ARTICLE

Outcome of Prenatally Diagnosed Isolated Congenital Complete Atrioventricular Block Treated with Transplacental Betamethasone or Ritodrine Therapy

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Received: 26 March 2008 / Accepted: 6 July 2008

Abstract The effectiveness of transplacental drug therapy for prenatally diagnosed isolated congenital complete atrioventricular block (CCAVB) is controversial. Nine cases of prenatal isolated CCAVB were treated from 2002 to 2007. Ritodrine was administered transplacentally to all fetuses and betamethasone to those whose mothers tested positive for maternal anti-SSA/Ro antibodies. Six of the nine patients had an anti-SSA/Ro-positive mother and received transplacental betamethasone 4 mg/day at a median gestational age of 28 weeks (range, 24–31 weeks). No patients exhibited an improvement in the degrees of complete heart block, and one patient died in utero. No serious adverse events occurred. After the mean follow-up period of 1.7 ± 1.3 years, all five patients treated with transplacental betamethasone experienced a good cardiac function, whereas one of the three patients not treated with transplacental betamethasone experienced cardiomyopathy and died at the age of 4 months. Pacemaker implantation was required for seven of the eight live-born infants. Transplacental betamethasone therapy for the patients with isolated CCAVB neither improved the degree of atrioventricular block nor decreased the rate of patients requiring pacemaker implantation, but it probably reduced the risk for the development of myocardial disease.

Keywords Anti-SSA/Ro antibody · Atrioventricular block · Betamethasone · Congenial · Fetal hydrops

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Congenital complete atrioventricular block (CCAVB) is a relatively rare disease among children with normal heart structures, with an estimated incidence of 1 in 14,000–20,000 live births [18]. Isolated CCAVB often is associated with maternal anti-SSA/Ro or anti-SSB/La antibodies. Among mothers with positive test results for anti-SSA/Ro or anti-SSB/La antibodies, the rate of bearing an infant with isolated CCAVB is 1–2% [2, 7, 10], and 16% mothers with a previous affected offspring had a second affected child [4].

A previous study found that patients with positive maternal antibodies had a worse prognosis [20]. The deposition of the antibodies along the conduction system and the myocardium of the fetus may trigger immune-mediated inflammation and result in fibrosis of the atrioventricular node and endocardium [12]. Regardless of the maternal antibody status, pacemaker implantation was required for 63–95% of patients, and death due to dilated cardiomyopathy was not uncommon [4, 13, 20].

Transplacental steroid therapy is reported to be ineffective in terms of reversing the severity of atrioventricular block, but it probably could reduce fetal and neonatal

morbidity and mortality [14, 17]. However, reports on the clinical outcome of fetuses with isolated CCAVB who have received transplacental therapy are limited.

This study aimed to review the clinical course of infants with a prenatal diagnosis of isolated CCAVB who were treated with transplacental ritodrine, betamethasone therapy, or both.

Materials and Methods

We studied all cases of isolated CCAVB diagnosed prenatally at our institution from 2002 to 2007. Complete atrioventricular block was diagnosed when no mechanical relation existed between atrial and ventricular contraction on fetal M-mode echocardiography. Patients with complex cardiac anomalies such as heterotaxy syndrome and congenitally corrected transposition of the great arteries were not included in the study. Nine cases eventually were identified and included in the study.

The medical records of the affected patients were reviewed. The collected data included status at presentation, maternal symptoms of collagen disease, type and duration of transplacental therapy, changes in fetal heart rates, and outcome of pregnancy. Postnatal electrocardiograms were reviewed for the degree of atrioventricular block, heart rates, QRS widths, and QTc intervals. Data regarding postnatal treatment, presence or absence of cardiomyopathy, and status at the most recent follow-up assessment also were collected.

All mothers were tested for the presence or absence of anti-SSA/Ro and anti-SSB/La antibodies using the Ouchterlony double immunodiffusion test.

Statistical Analysis

The data are expressed as frequencies, mean ± standard deviation, and median and range, as appropriate. Comparisons between groups were performed using Fisher's exact test for categorical variables and the Mann–Whitney U test for continuous variables. All p values less than 0.05 were considered statistically significant.

Results

Clinical Features

Table 1 summarizes the clinical features of all nine cases included in the study. Five mothers had been pregnant previously, but none of their offspring had a history of complete atrioventricular block. The median gestational age at diagnosis was 27 weeks (range, 23–30 weeks), and all mothers were referred to our institution after fetal bradycardia was detected using a routine fetal ultrasound examination. Fetal hydrops was diagnosed in three fetuses at the time of diagnosis, and no other fetus had hydrops thereafter.

Eight mothers (89%) delivered live offspring, whereas one mother had an intrauterine fetal death. Two neonates (cases 3 and 7) had structural cardiac anomalies: small

Table 1 Characteristics of the nine cases with a prenatal diagnosis of isolated congenital complete atrioventricular block (CCAVB)

Case	Sex	Maternal antibodies	GA at diagnosis (weeks)	Prenatal treatment		Hydrops	GA at birth (weeks)	Birth weight (g)	CHD	Postnatal treatment	Pacemaker age (days)	Mode	Outcome (follow-up years)
				Ritodrine	Betamethasone								
1	M	SS-A	23	+	+	–	32	2,338	–	ISP	24	VVI	Alive (3.9)
2	F	–	30	+	–	+	30	1,624	–	ISP, DOA	4	VVI	Alive (2.9)
3	M	–	27	+	–	+	28	1,332	VSD	ISP, DOA	4	VVI	Alive (2.8)
4	F	SS-A	24	+	+	–	35	1,634[a]	–	ISP, DOA			Alive (1.6)
5	M	SS-A	29	+	+	–	36	2,300[a]	–	ISP	40	VVI	Alive (1.0)
6	F	SS-A	27	+	+	–	37	2,460	–	ISP	297	VVI	Alive (0.8)
7	F	–	28	+	–	–	37	2,816	PS	ISP	11	VVI	DCM, died at 4 months
8	F	SS-A	29	+	+	–	36	1,884[a]	–	ISP	3	VVI	Alive (0.4)
9	–	SS-A	23	+	+	+	–	–	–	–	–	–	IUFD at 30 weeks

GA, gestational age; CHD, congenital heart disease; SS-A, anti SS-A/Ro antibody; ISP, isoprenaline; VVI; DOA, dopamine; IUFD, intrauterine fetal death

[a] Small for gestational age

muscular ventricular septal defect in the former and pulmonary valve stenosis in the latter. Postnatal electrocardiography confirmed the diagnosis of CCAVB in all live-born infants.

Six mothers tested positive for anti-SSA/Ro antibodies, whereas none tested positive for anti-SSB/La antibodies. Among the six mothers with positive anti-SSA/Ro antibodies, four experienced some symptoms of collagen disease. However, only one mother had a previous diagnosis of collagen disease. None of the mothers had been treated with steroids at the time of referral to our institution.

Transplacental Therapy and Outcome of Pregnancy

Beta-mimetic agent (ritodrine) was administered to all nine mothers as soon as CCAVB was diagnosed. Five fetuses (56%) showed a persistent increase in their heart rate by more than 5 bpm for at least 2 weeks after initiation of the beta-mimetic agent (Fig. 1).

Betamethasone was additionally administered if the mothers tested positive for anti-SSA/Ro antibodies. All six mothers with anti-SSA/Ro antibodies received betamethasone therapy at the median gestational age of 28 weeks (range, 24–31 weeks). Betamethasone therapy was initiated at a dose of 4 mg/day in all cases. In two cases, the same dose of betamethasone was maintained for 10 weeks (case 4) or 6 weeks (case 9) until the end of pregnancy. In four cases, the dose was tapered after 2–4 weeks.

The betamethasone therapy did not improve the degree of atrioventricular block in any patients treated. Three mothers exhibited adverse effects attributable to the transplacental betamethasone therapy, which included mood disorder, insomnia, and increased appetite. However, betamethasone therapy was not discontinued for any of these mothers because serious adverse effects did not occur.

Of the three fetuses with hydrops, one (case 9) died in utero at the gestational age of 30 weeks. In this case, the fetal heart rate was 42 bpm at the time of diagnosis—the lowest among all the cases. It even decreased to 35 bpm, indicating absence of response to transplacental therapy with ritodrine and betamethasone. In the other two fetuses, the initial fetal heart rate was 55 bpm (case 3) or less (case 2) at the time of referral to our institution. Because the maternal anti-SSA/Ro antibodies were negative, both of these fetuses were treated with transplacental ritodrine alone. However, increases in the fetal heart rates were subtle, and these two fetuses showed no improvement in fetal hydrops. Their mothers underwent preterm cesarean section at the gestational age of 30 weeks (case 2) and 28 weeks (case 3).

Of the six fetuses without hydrops, all but one infant were born after the gestational age of 35 weeks. In case 1, the fetus was delivered by cesarean section at 32 weeks gestation because of placenta previa and vaginal bleeding.

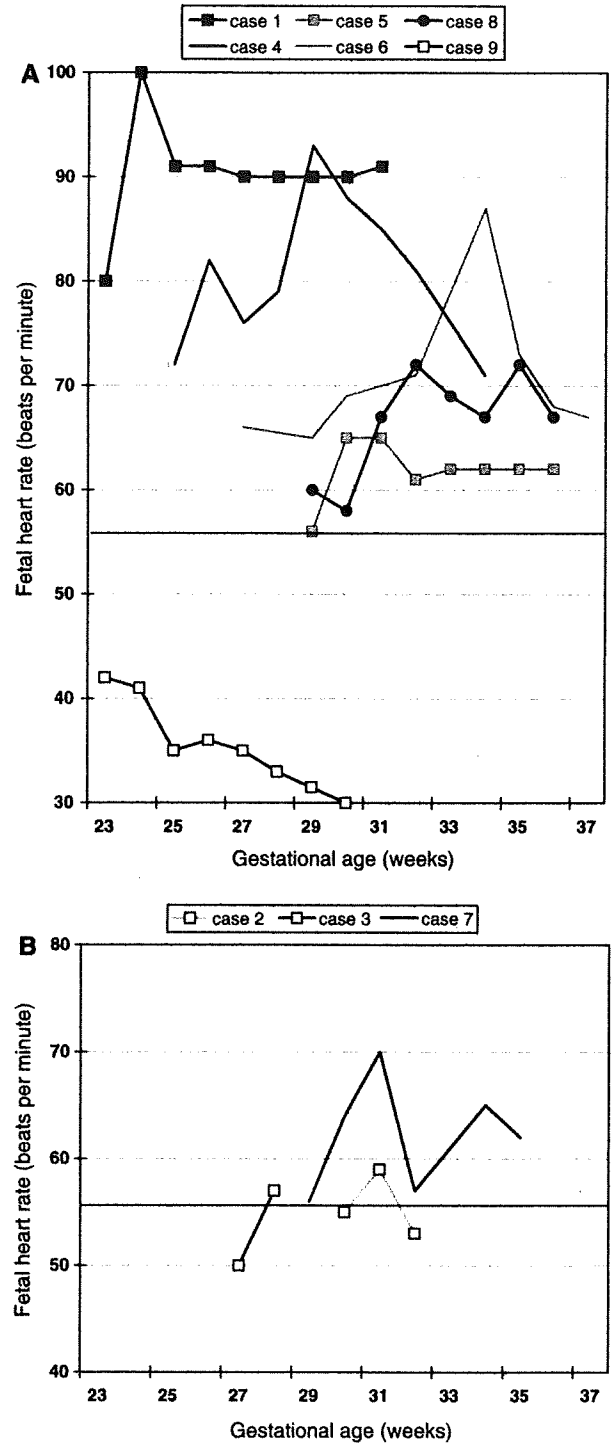

Fig. 1 Fetal heart rate of patients treated transplacentally with betamethasone and ritodrine (**a**) and with ritodrine alone (**b**). Five (56%) of the fetuses had a persisting increase in heart rate of more than 5 bpm for at least 2 weeks after initiation of the beta-mimetic agent. No cases showed improvement in the degree of complete heart block. In the cases with fetal hydrops (open square), the fetal heart rates were lower than 55 bpm at least once. In the cases without fetal hydrops, the fetal heart rates never fell below 55 bpm throughout pregnancy

In none of the six cases did the fetal heart rate fall below 55 bpm throughout pregnancy (Fig. 1). The combined rate of intrauterine fetal death and premature delivery (before 35 weeks gestation) was significantly higher in the fetuses with hydrops than in those without hydrops (100% vs. 17%; $p = 0.047$).

The mean birth weight was 2,049 ± 507 g (range, 1332–2816 g). Three infants were small for gestational age, and all these infants tested positive for maternal anti-SSA/Ro antibodies.

Postnatal Treatment

Eight pregnancies resulted in live births, and isoprenaline infusion was initiated during the neonatal period for all eight infants. Isoprenaline therapy was successfully tapered for two infants (cases 4 and 6), both of whom tested positive for maternal anti-SSA/Ro antibodies. The remaining six infants needed pacemaker implantation at a median age of 7.5 days (range, 3–40 days). The indications for pacemaker implantation were bradycardia with ventricular rates less than 55 bpm in five infants and ventricular dysfunction with a wide QRS escape rhythm in one infant (case 7).

Three patients with birth weights less than 2,000 g were treated initially with temporary epicardial pacing. Two of these three patients (cases 2 and 3) were born prematurely due to fetal hydrops. We had adopted a staged approach for hydropic fetuses. Before placing temporary epicardial leads, we stabilized their respiratory and circulatory condition by isoprenaline and dopamine infusion, supplementation of pulmonary surfactant, and if necessary, surgical drainage of pulmonary effusion. Although pericardial effusion developed in one patient, requiring surgical drainage, temporary pacing could be successfully switched to permanent pacemaker implantation in all the patients when their body weights increased to 2,500 g.

For the patients discharged without pacemaker implantation, the QRS widths and QTc intervals tended to be shorter than for those discharged after pacemaker implantation (QRS widths, 64 ± 5.7 vs. 97 ± 28 ms; $p = 0.05$; QTc intervals, 374 ± 67.9 vs. 454 ± 379 ms; $p = 0.08$). However, we found no statistically significant differences.

Outcome of Affected Infants

After the mean follow-up period of 1.7 ± 1.3 years (range, 0.4–3.9 years), seven patients were alive and one patient (case 7) had died of dilated cardiomyopathy at the age of 4 months. In this case, fetal echocardiography performed at the 28th gestational week had shown myocardial hypertrophy. Because the maternal anti-SSA/Ro antibody was negative, the patient had not received transplacental steroid therapy. At birth, echocardiography showed pulmonary valve stenosis and severe heart failure, with a left ventricular ejection fraction of 50% and a left ventricular end-diastolic diameter of 22 mm. Although the baby was treated postnatally with pacemaker implantation and methylprednisolone pulse therapy in addition to pharmacologic therapy with diuretics, vasodilators, and inotropic agents, the cardiac function continued to deteriorate, and the patient died at the age of 4 months.

The remaining seven patients experienced a good cardiac function without the development of any significant valve insufficiency. Of the two patients discharged initially without pacemaker implantation, one (case 6) experienced an abrupt pause in ventricular rhythm that exceeded 3.7 s on Holter monitoring at the age of 9 months and therefore received pacemaker implantation. Finally, at the mean follow-up period of 1.7 ± 1.3 years, one of the eight patients (13%) had experienced dilated cardiomyopathy, and the remaining seven (88%) had required a permanent pacemaker.

Discussion

With fetal echocardiography coming into wide use, cases of isolated CCAVB are increasingly being identified prenatally, mostly before the gestational age of 30 weeks [13]. Because prenatally diagnosed CCAVB cases are reported to have higher mortality and require pacemaker implantation more frequently [13], various types of transplacental therapy have been attempted [1, 5]. Although transplacental therapy with steroids, beta-mimetics, or both is becoming commonplace, reports on the outcome of these therapies are limited, and there is no widely accepted regimen of transplacental therapy to date.

Maternally administered steroids have been used for the treatment of isolated CCAVB. Although several studies [9, 19] have reported that heart block of less advanced degrees reverted to normal sinus rhythm after transplacental steroid therapy, reports documenting the reversal of complete atrioventricular block are rare [12]. However, it has been suggested that transplacental steroid therapy may reduce the incidence of more severe myocardial disease. Jaeggi et al. [14] reported that the rate of immune-mediated postnatal complications such as hepatitis, myocarditis, and endocardial fibroelastosis was significantly lower for cases treated with transplacental dexamethasone administration. These authors recommended transplacental dexamethasone therapy for all isolated CCAVB cases regardless of the maternal antibody status.

Our study also suggests the efficacy of transplacental steroid therapy in preventing myocardial disease. In our series, all the six mothers testing positive for anti-SSA/Ro antibodies had received transplacental betamethasone

therapy. One fetus with hydrops died in utero. Although the transplacental betamethasone therapy neither improved the degree of complete atrioventricular block nor prevented postnatal pacemaker implantation, none of the five surviving infants experienced dilated cardiomyopathy, and all experienced a good cardiac condition with normal left ventricular ejection fraction. The only patient who died of dilated cardiomyopathy in our series had not received betamethasone prenatally.

It is reported that transplacental steroid therapy may cause oligohydramnios, which sometimes prompts premature delivery or fetal death [14]. No patients in our series experienced such serious adverse effects. Prenatal exposure to dexamethasone may lead to adverse obstetric events such as spontaneous abortion, stillbirth, and neonatal adrenal insufficiency [6]. In addition, there is concern about the adverse neurodevelopmental effects of prenatal steroid exposure. Brucato et al. [3] studied 14 children with isolated CCAVB who had been exposed prenatally to a high dosage of dexamethasone and found that all these patients had normal intelligence at the mean age of 5 years.

One recent large study of extremely low-birth-weight infants by Lee et al. [16] showed that prenatal betamethasone exposure was associated with increased likelihood of unimpaired neurodevelopmental status at corrected ages of 18 to 22 months compared with prenatal dexamethasone exposure or no prenatal steroid exposure. Although the dose and the duration of steroids administered in transplacental therapy for CCAVB differ from those for extremely low-birth-weight infants, prenatal dexamethasone exposure may be more harmful than prenatal betamethasone exposure in terms of neurodevelopmental status. Therefore, transplacental therapy with betamethasone currently seems to be better than therapy with dexamethasone. The efficacy and safety of transplacental betamethasone therapy for CCAVB should be evaluated in a prospective randomized trial.

Transplacental therapy with beta-mimetics is reported to increase the fetal ventricular heart rate in some CCAVB cases, but this approach does not seem to be universally effective [14, 17]. Whereas Jaeggi et al. [14] used beta-mimetics only for cases with a fetal ventricular rate less than 55 bpm, we administered beta-mimetics to all the mothers regardless of the fetal ventricular rate because we consider that all affected fetuses will benefit from an increase in their ventricular rate. In our series, five (56%) of nine fetuses showed a persistent increase in their heart rate by more than 5 bpm for at least 2 weeks after the initiation of the beta-mimetic agent. Unfortunately, the transplacental ritodrine therapy was not effective for three fetuses with hydrops in our series, suggesting the limitations of the therapy in severely affected cases.

Former reports have shown that risk factors for poor prognosis for isolated CCAVB patients include fetal hydrops, positive maternal anti-SSA/Ro antibodies, and endocardial fibroelastosis [13, 20]. In our series, the combined rate of intrauterine fetal death and premature delivery (before 35 weeks gestation) was significantly higher for fetuses with hydrops than for fetuses without hydrops. We did not find any differences in prognosis between fetuses born to mothers with and those without anti-SSA/Ro antibodies, partly because of too few cases. However, it is noteworthy that one fetus experienced dilated cardiomyopathy and two experienced hydrops despite negative maternal anti-SSA/Ro antibodies. It seems that patients without maternal anti-SSA/Ro antibodies do not always have a better prognosis than those with maternal anti-SSA/Ro antibodies.

No patients in our series experienced endocardial fibroelastosis. The heart rates of the three fetuses with hydrops were 55 bpm or less at the time of referral to our institution, and the fetus with the lowest heart rate eventually died in utero. Thus, a low fetal heart rate could possibly predict poor prognosis. Grove et al. [11] reported that a fetal heart rate lower than 55 bpm before 28 weeks gestation was associated with greater likelihood of a poor outcome. Jaeggi et al. [13] reported that the majority of fetuses with a heart rate lower than 55 bpm did not survive the perinatal period, but this observation did not reach statistical significance.

A large proportion of patients with isolated CCAVB require pacemaker implantation. Previous reports have shown that pacemaker implantation was required for 63% to 95% of patients [4, 13, 20]. In our series, pacemaker implantation was necessary for seven patients (88% of the live-born infants) after the mean follow-up period of 1.7 ± 1.3 years. Of the five live-born infants who had received the transplacental betamethasone therapy, four (80%) required pacemaker implantation. Five patients underwent pacemaker implantation during the neonatal period, one at 40 days of age and one more patient at the age of 9 months.

For the premature hydropic fetuses, we adopted the staged pacing approach, as formerly reported [8, 21]. Before placing the temporary epicardial leads, we stabilized the respiratory and circulatory condition of the patients by isoprenaline and dopamine infusion, supplementation of pulmonary surfactant, and if necessary, surgical drainage of pulmonary effusion. This approach successfully bridged the interval until a permanent pacemaker could be implanted. A previous report described temporary epicardial pacing for a neonate weighing as little as 930 g [21]. Although the decision of performing premature delivery must be carefully considered, the initial staged pacing approach with the use of temporary

epicardial pacing can improve the outcome of premature hydropic fetuses with isolated CCAVB.

Although all our patients, except for one who experienced dilated cardiomyopathy and died at 4 months of age, had a good cardiac condition, a close long-term follow-up is essential. Kurosaki et al. [15] reported that the total mortality rate for patients with isolated CCAVB who underwent pacemaker implantation during the neonatal period was 30% after a median follow-up period of 5.6 years, and that the cumulative probability of freedom from dilated cardiomyopathy at 10 years was 59%.

The limitations of our study need to be addressed. Our series included only a small number of patients, and the follow-up periods were relatively short. The gestational ages at which the transplacental drug therapy was initiated may have been too late in some cases. In addition, the ways of tapering betamethasone varied across cases, although the initial dose was 4 mg/day in all cases.

In conclusion, transplacental betamethasone therapy for the patients with isolated CCAVB neither improved the degree of atrioventricular block nor decreased the rate of patients requiring pacemaker implantation, but it probably decreased the risk for the development of myocardial disease. Fetal hydrops in patients with isolated CCAVB relates to intrauterine fetal death and premature delivery. A large proportion of patients with isolated CCAVB require pacemaker implantation. The initial staged pacing approach with temporary epicardial pacing can improve the outcome for premature hydropic fetuses with isolated CCAVB.

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Clinical & Experimental Immunology
The Journal of Translational Immunology
immunology

Clinical and Experimental Immunology ORIGINAL ARTICLE doi:10.1111/j.1365-2249.2010.04094.x

Inhibition of transforming growth factor-β signalling attenuates interleukin (IL)-18 plus IL-2-induced interstitial lung disease in mice

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Accepted for publication 15 December 2009
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Summary

Interstitial lung disease (ILD) is an intractable disease induced by various factors in humans. However, there is no universally effective treatment for ILD. In this study, we investigated the role of transforming growth factor (TGF)-β signalling in the pathogenesis of ILD by using model mice. Injection of interleukin (IL)-18 plus IL-2 in C57BL6 (B6) mice resulted in acute ILD by infiltration of natural killer (NK) cells and a significant increase of TGF-β mRNA in the lung. To examine the pathogenetic role of TGF-β in ILD mice, we used SB-431542 (4-[4-(1,3-benzodioxol-5-yl)-5-(2-pyridinyl)-1H-imidazol-2-yl]-benzamide), which is a potent and selective inhibitor of TGF-β receptor I (TβRI), also known as activin receptor-like kinase 5 (ALK5). Treatment of B6-ILD mice with SB-431542 resulted in improvement of ILD, delay in mortality, reduction of the expression of interferon (IFN)-γ and IL-6 in the lungs. The same treatment also decreased significantly the percentage of natural killer (NK) cells in the lungs ($P < 0.05$) and mRNA expression levels of certain chemokines such as CCL2, CCL3, CCL4, CCL5 and CXCL10 in B6-ILD. These findings were confirmed by IL-18 plus IL-2 treatment of Smad3-deficient (Smad3$^{-/-}$) mice ($P < 0.05$). Our results showed that inhibition of TGF-β signalling reduced the percentage of NK cells and the expression of certain chemokines in the lungs, resulting in improvement of ILD. The findings suggest that TGF-β signalling may play an important role in the pathogenesis of IL-18 plus IL-2-induced ILD in mice.

Keywords: activin receptor-like kinase 5, chemokines, interstitial lung disease, pathogenesis, SB-431542, TGF-β signalling

Introduction

Interstitial lung disease (ILD) is an intractable disease induced by various factors such as autoimmune diseases, drugs, occupational and environmental exposure [1]. However, there is no universally effective treatment for ILD. On the other hand, chemotherapy with bleomycin (BLM) and busulphan is reported to cause lung fibrosis in some patients [2]. Histopathologically, diffuse infiltration of mononuclear and polymorphonuclear leucocytes is observed in the lung in the early stages of human ILD. Following the interstitial inflammation, florid fibroblast proliferation within both the interstitium and alveolar space is often detected. The same pathology is observed in BLM-induced ILD in mice [1]. Previous studies suggested that various mediators, such as cytokines and chemokines, including tumour necrosis factor (TNF)-α, transforming growth factor (TGF)-β, interleukin (IL)-1β, macrophage inflammatory factor (MIP)-1α/CCL3, monocyte chemoattractant protein (MCP)-1/CCL2, reactive oxygen species (ROS) and Fas/Fas ligand interactions, are associated with BLM-induced ILD and fibrosis in mice [3–9].

IL-18, a member of the IL-1 family, is a proinflammatory cytokine [10,11] known to induce interferon (IFN)-γ production synergistically by stimulation with IL-12, IL-2, antigens and IFN-α. Previous studies reported that IL-18 can potentially induce Th2 cytokines from T cells, natural killer (NK) cells, NK T cells, basophils and mast cells [11–16]. Thus, IL-18 can act as co-factor for both T helper type 1 (Th1) and Th2 cell development. In BLM-induced ILD mice models, there were two conflicting reports on the effect of IL-18. Nakatani-Okuda *et al.* [17] reported that IL-18 played a protective role in BLM-induced ILD in mice. In contrast, Hoshino *et al.* [18] reported that IL-18 played a

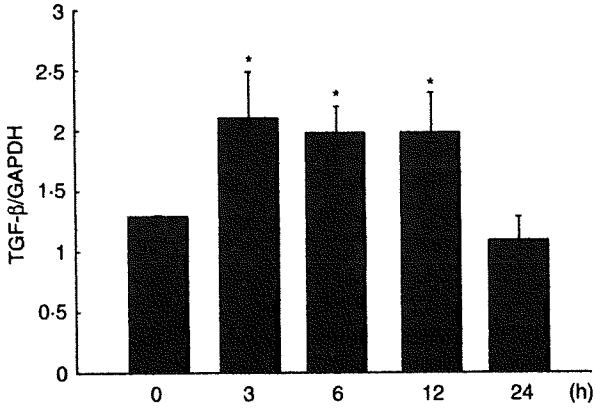

Fig. 1. Expression of transforming growth factor (TGF)-β mRNA after injection of interleukin (IL)-18 plus IL-2. Expression of TGF-β mRNA was analysed by real-time–polymerase chain reaction (RT-PCR). C57BL/6 (B6) mice were injected intraperitoneally with a single dose of IL-18/IL-2. At 3, 6, 12 and 24 h after injection, mice were killed and lung mRNA was extracted. Data are mean ± standard error of the mean; $n = 3$ mice per group. $^*P < 0{\cdot}05$; one-way analysis of variance.

pathologenetic role in the ILD. Therefore, the intimate role of IL-18 in ILD was controversial. Recently, Okamoto *et al.* [19] reported a new mouse model of ILD induced by IL-18 plus IL-2 (IL-18/IL-2). Daily administration of IL-18 with IL-2, but not IL-18 or IL-2 alone, produced a synergistic effect and induced ILD in mice. Unlike BLM-induced ILD, lung fibrosis was not caused in IL-18/IL-2-induced ILD. The pathological condition of BLM-induced ILD was mainly fibroblastic proliferation [20]. However, little fibroblastic proliferation was found in IL-18/IL-2-induced ILD. This model of ILD is characterized by severe infiltration of NK cells, mononuclear cells and polymorphonuclear leucocytes in the lung. Furthermore, the mortality in this two ILD mice models was different. Whereas 60% of mice died at 30 days after BLM treatment [21], 100% of mice died at 7 days after IL-18/IL-2 injection. Based on rapid and severe cell infiltration in IL-18/IL-2-induced ILD, the mouse model is considered suitable for early-phase human ILD.

Various chemokines, such as CCL2, CCL3, CCL4, CCL5, CXCL1 and CXCL8, are induced by activated fibroblasts in

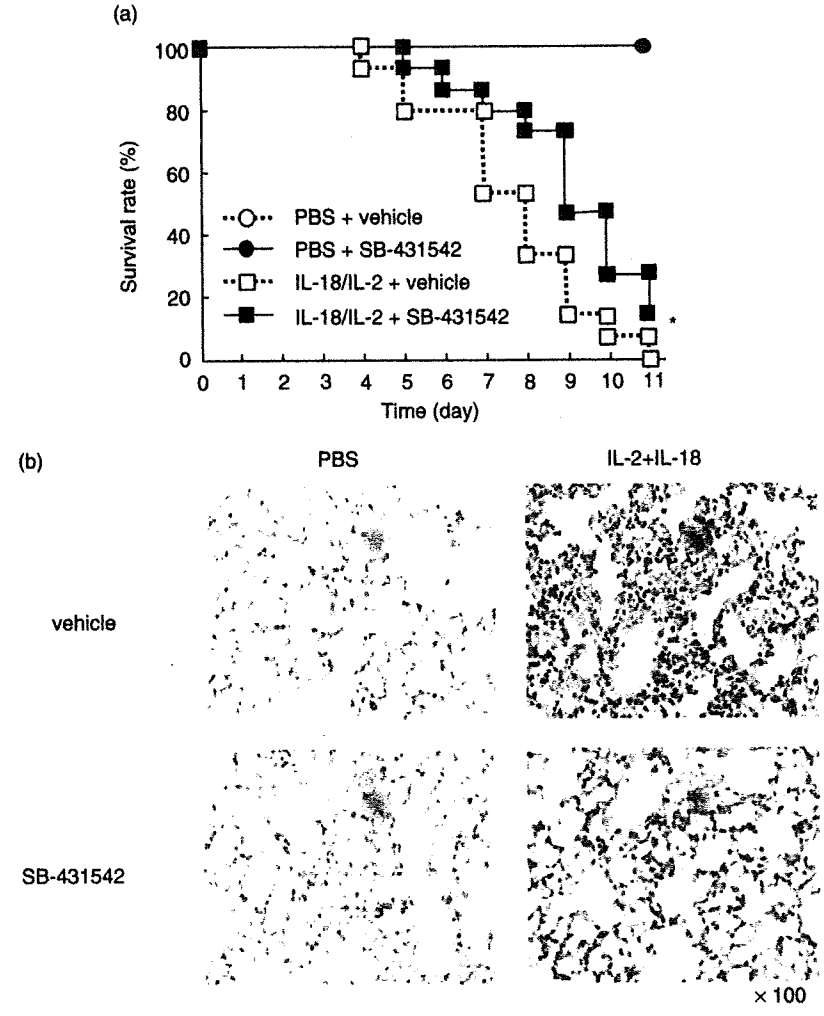

Fig. 2. Effects of SB-431542 in interleukin (IL)-18 plus IL-2-induced interstitial lung disease (ILD) mice. (a) B6 mice were injected with IL-18/IL-2 with or without SB-431542 for 10 days, as described in Materials and methods. ○, phosphate = interstitial lung disease (ILD) –buffered saline (PBS) + vehicle; ●, PBS + SB-431542; □, IL-18/IL-2 + vehicle; ■, IL-18/IL-2 + SB-431542; $n = 5$ mice per group. Data are representative of three independent experiments and graph shows pooled data of three experiments. $^*P < 0{\cdot}05$; Kaplan–Meier method. (b) Lungs were harvested from B6 mice at 24 h after injected with IL-18/IL-2 with or without SB-431542 for 3 days. Lung tissues were stained with haematoxylin and eosin. Original magnification: ×100. (c) Lungs were harvested from B6 mice at 6 h after injection with IL-18/IL-2 with or without SB-431542 for 3 days. (d) The sera were harvested from B6 mice at 6 h after injection with IL-18/IL-2 with or without SB-431542 for 3 days. Serum cytokine levels were measured by enzyme-linked immunosorbent assay (ELISA). ELISA assayed the lung tissue supernatant as described in Materials and methods. Date are mean ± standard error of the mean; $n = 3$ mice per group. $^*P < 0{\cdot}05$; Student's *t*-test.

the lung tissue [22]. Mice with BLM-induced pulmonary fibrosis exhibit up-regulation of CCL2, CCL5, CCL3 and CXCL1 in the lung and such overexpression is associated with enhanced fibroblast proliferation and collagen production [23]. Thus, these chemokines are thought to be involved in the pathogenesis of ILD and subsequent fibrotic process. In contrast, the functional roles of chemokines in IL-18/IL-2-induced ILD in mice remain elusive, although lymphotactin (Ltn), CCL2, CCL3, CCL4, CCL5, CCL11, CXCL1 and CXCL10 are increased in the lung [19].

TGF-β is thought to be one of the pathogenic factors in ILD. Previous reports suggested that TGF-β acts as a regulatory molecule with pleiotropic effects on cell proliferation, differentiation, migration and survival [24]. TGF-β mediates its biological functions via binding to TGF-β receptor II (TβRII) and phosphorylation of TGF-β receptor I (TβRI), also known as activin receptor-like kinase 5 (ALK5). After forming the TGF-β–TβRII-ALK5 complex, ALK5 phosphorylates intracellular signal mediators Smad2/3. The importance of ALK5-mediated Smad2/3 activation in TGF-β signalling has been confirmed both *in vitro* and *in vivo* [24–29].

SB-431542 (4-[4-(1,3-benzodioxol-5-yl)-5-(2-pyridinyl)-1H-imidazol-2-yl]-benzamide) is a potent and selective inhibitor of ALK-5 [30–32]. Inhibition of ALK-5 suppressed BLM-induced pulmonary fibrosis [33]. However, the therapeutic potential of ALK-5 inhibitor in IL-18/IL-2-induced ILD has not yet been clarified.

In the present study, we examined the role of TGF-β signalling in early-stage ILD. For this purpose, we used SB-431542 and Smad3-deficient (Smad3$^{-/-}$) mice for IL-18/IL-2-induced ILD. The results demonstrated that inhibition of TGF-β signalling suppressed accumulation of NK cells and reduced mRNA expression of CCL2, CCL3, CCL4, CCL5 and CXCL10 in the lung. The main message of this study is that Smad-mediated TGF-β signalling seems to play an important role in the pathogenesis of IL-18/IL-2-induced ILD.

Materials and methods

Mice

C57BL/6 (B6) mice were purchased from Charles River Japan Inc. (Tokyo, Japan). Smad3-deficient (Smad3$^{-/-}$) mice were kindly provided by Dr Chuxia Deng (National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD, USA) [34]. The genotypes of both B6 and Smad3$^{-/-}$ mice were determined by polymerase chain reaction (PCR) analysis on tail DNA obtained from 4-week-old animals. Female mice were used in this study. The animals were kept under specific pathogen-free conditions and studied at 4–5 weeks of age. The Institutional Animal Care and Use Committee at Tsukuba University approved the experimental protocol.

Cell isolation and purification

Pulmonary lymphocytes were isolated as described previously [35], with the following modifications. The lung was

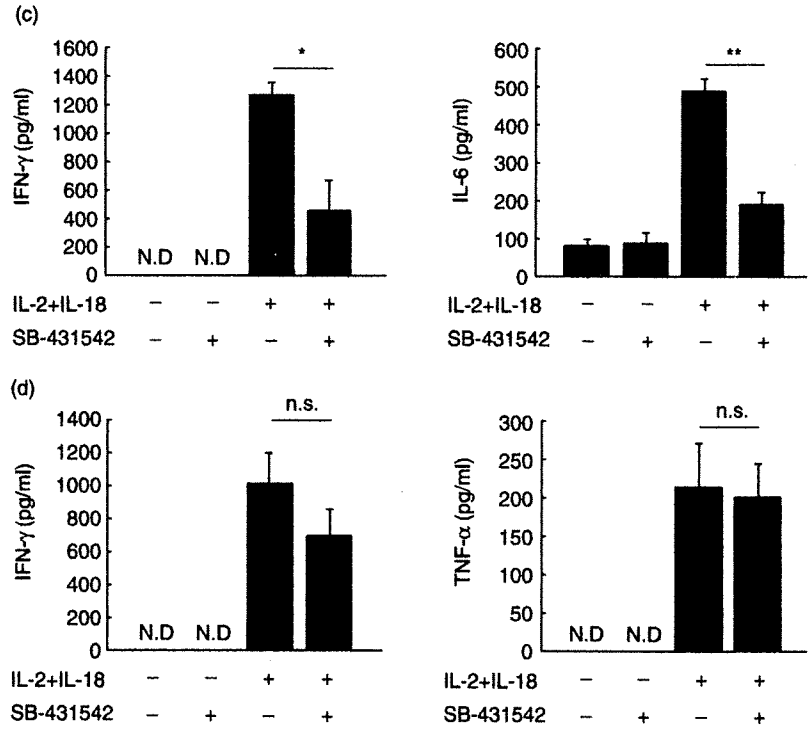

Fig. 2. *Continued*

perfused thoroughly with phosphate-buffered saline (PBS) to remove circulating blood cells. The dissected lung was minced in PBS containing 1 mM ethylenediamine tetraacetic acid (EDTA). The minced lung tissue was suspended in RPMI-1640 medium (Sigma-Aldrich, St Louis, MO, USA) containing 10% fetal bovine serum (FBS) (BioWest, FL, USA), 1 mM EDTA and 1 mM dithiothreitol (DTT). The suspension was incubated at 37°C for 45 min with gentle shaking. The resultant suspension was passed through nylon mesh to remove debris. The washed and recovered cells were subjected to Lympholyte (Cedarlane, Ontario, Canada) at 1100 ***g*** at room temperature for 20 min. The resultant interface containing pulmonary lymphocytes was recovered and washed with RPMI-1640 medium containing 10% FBS, 100 units/ml penicillin, 100 μg/ml streptomycin and 50 μM 2-mercaptoethanol. Spleens were harvested and haemolyzed with PBS. Single-cell suspensions were prepared in RPMI-1640 medium containing 10% FBS, 100 units/ml of penicillin, 100 μg/ml streptomycin and 50 μM 2-mercaptoethanol.

Antibodies and flow cytometry

All antibodies were used according to the recommendations of the respective manufacturers. For flow cytometric analysis, cells were preincubated with anti-CD16/32 (eBioscience, San Diego, CA, USA) to block Fc receptors. The following antibodies were used in this study: phycoerythrin (PE)-conjugated anti-natural killer (NK)1·1 (PK136) (Biolegend, San Diego, CA, USA) and PE/cyanine 7 (Cy7)-conjugated anti-CD3ε (145-2C11) (Biolegend). The stained cells were analysed on CyAn advanced digital processing (ADP) (Dako, Glostrup, Denmark) and data were processed using Summit4·3 (Dako).

Induction of lung fibrosis with IL-18 and IL-2

Recombinant human IL-2 (rhIL-2) and recombinant mouse IL-18 (rmIL-18) were obtained from MBL (Nagoya, Japan). Mice were treated once a day with an intraperitoneal (i.p.) injection of rhIL-2 (100 000 U) and/or rmIL-18 (1 μg). These cytokines were suspended in sterile 200 μl PBS. Mice treated with 200 μl PBS served as the control group. Following treatment for 3 days, mice were bled and killed. Pulmonary lymphocytes and splenocytes were analysed by flow cytometry.

Treatment of mice with SB-431542

ALK-5 inhibitor, SB-431542 (4-[4-(1,3-benzodioxol-5-yl)-5-(2-pyridinyl)-1H-imidazol-2-yl]-benzamide) (SB-431542) was obtained from Tocris Bioscience (Park Ellisville, MO, USA). It was suspended in sterile dimethyl sulphoxide (DMSO) at 20 mg/ml. Mice were treated twice a day (0 h and 12 h after IL-18/IL-2 treated) by i.p. injection of 50 μl (0·2 mg) SB-431542 or vehicle for 3 days.

Reverse transcription polymerase chain reaction (RT-PCR) analysis

Total RNA was extracted from the lung, and was reverse transcribed into cDNA using RevertAid™ first-strand cDNA synthesis kit (Fermentas, Burlington, Ontario, Canada), according to the manufacturer's protocol. For amplification of chemokine cDNA, after an initial denaturation step at 94°C for 4 min, 35 cycles were conducted each at 94°C for 30 s followed by 60°C for 30 s and 72°C for 30 s, and further extension at 72°C for 7 min. For amplification of glyceraldehyde-2-phosphate dehydrogenase (GAPDH) cDNA, PCR assays were performed for 30 cycles (94°C for 30 s followed by 60°C for 30 s and 72°C for 30 s). At the end of cycles, samples were stored at 4°C until analysed. After amplification, the PCR products were separated by electrophoresis in 2·0% agarose gels. The primer sequences were as follows and the PCR product sizes [base pairs (bp)] were indicated: CCL2, 5′-AGGTCCCTGTCATGCTTCTG, 3′-TC TGGACCCATTCCTTCTTG (249 bp); CCL3, 5′-AGATTC CACGCCAATTCATC, 3′-CTCAAGCCCCTGCTCTACAC (223 bp); CCL4, 5′-CCCACTTCCTGCTGTTTCTC, 3′-GA GGAGGCCTCTCCCTGAAGT (238 bp); CCL5, 5′-CCCTC ACCATCATCCTCACT, 3′-CCTTCGAGTGACAAACACGA (185 bp); CCL11, 5′-TCCACAGCGCTTCTATTCCT, 3′-CTA TGGCTTTCAGGGTGCAT (178 bp); CXCL1, 5′-GCTGGG ATTCACCTCAAGAA, 3′-TCTCCGTTACTTGGGGACAC (180 bp); CXCL10, 5′-GGATGGCTGTCCTAGCTCTG, 3′-ATAACCCCTTGGGAAGATGG (211 bp); and GAPDH, 5′-CGTCCCGTAGACAAAATGGGT, 3′-GAATTTGCCGT GAGTGGAGT (177 bp).

Quantification of gene expression by RT-PCR

The cDNA samples were amplified with specific primers and fluorescence-labelled probes for the target genes. Specific primers and probes for TGF-β and GAPDH were purchased from Applied Biosystems Japan (Tokyo, Japan). The amplified product genes were monitored on an ABI 7700 sequence detector (Applied Biosystems Japan). The quantitative PCR master mix was purchased from Applied Biosystems Japan. The final concentrations of the primers were 200 nM for each of the 5′ and 3′ primers, and the final probe concentration was 100 nM. The thermal cycler conditions used were 50°C for 2 min, 95°C for 10 min, then 50 cycles of 95°C for 15 s and 60°C for 1 min. Serial dilutions of a standard sample were included in every assay, and standard curves for the genes of interest and GAPDH genes were generated. All measurements were performed in triplicate. The level of gene expression was calculated from the standard curve, and expressed relative to GAPDH gene expression.

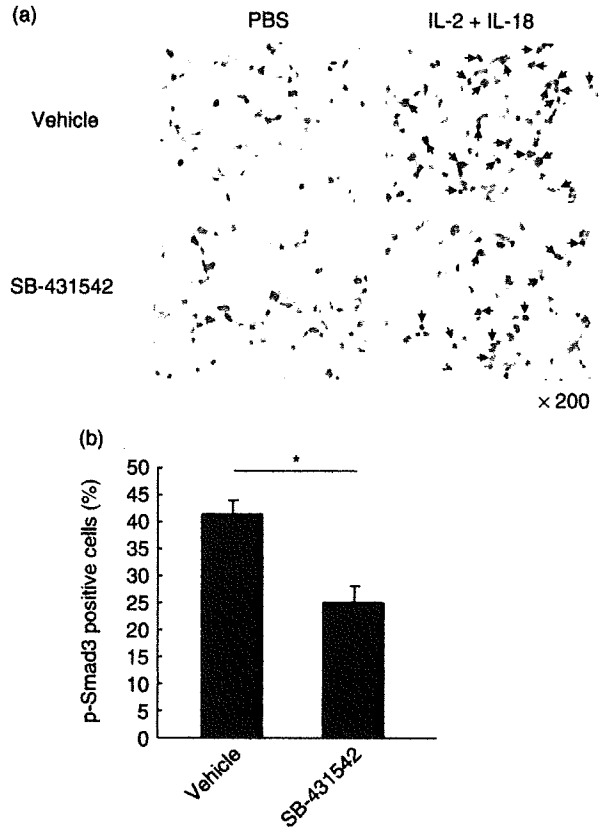

Fig. 3. Immunohistochemical findings of phosphorylated Smad3 (p-Smad3) in lungs of B6 mice treated with SB-431542. (a) Lungs were harvested from B6 mice at 6 h after treatment with interleukin (IL)-18/IL-2 with or without SB-431542 for 3 days. The tissues were stained immunohistochemically with anti-pSmad3 antibody. Arrow: pSmad3-positive cells. Original magnification: ×200. (b) In lungs of mice treated with IL-18/IL-2 with or without SB-431542, the percentage of p-Smad3-positive cells per total cells was calculated in five fields under ×200 magnification. Data are mean ± standard error of the mean of three mice per group. *$P < 0.05$; Student's *t*-test.

Histological examination

For histological analysis, mice were euthanized by isopropanol and lung was fixed with 4% paraformaldehyde. Lung tissues were stained with haematoxylin and eosin (H&E).

Immunohistochemistry

In the present study, anti-phosphorylated Smad3 were used (Rockland, Gilbertsville, PA, USA). For detection of immunocomplexes, Histofine (Nichirei Corporation, Tokyo, Japan) for phosphorylated Smad3 was used using the manufacturer's instructions. Substitution of the primary antibody with irrelevant immunoglobulin G (IgG) served as negative controls. Staining was repeated for each sample at least three times. After counterstaining with haematoxylin, sections were mounted with mounting agent, PARAmount-D (Falma, Tokyo, Japan).

Measurement of cytokines in the lung and serum

For enzyme-linked immunosorbent assay (ELISA), the lung tissue was suspended and homogenized in sterile PBS and centrifuged at 10 000 *g* for 10 min. The cytokine levels in the lung tissue supernatants and the serum were evaluated by ELISA (R&D Systems, Minneapolis, MN, USA).

Statistical analysis

Data are expressed as median or mean ± standard error of the mean (s.e.m.). Data were analysed using a statistical software package (StatView 5·0; SAS Institute Inc, Cary, NC, USA). The survival rates were analysed by Kaplan–Meier method. Differences between groups were examined for statistical significance using Student's *t*-test. For multiple group comparisons, one-way analysis of variance (ANOVA) was performed followed by a *post-hoc* Dunnett's test. A *P*-value less than 0·05 denoted a statistically significant difference.

Results

Overexpression of TGF-β mRNA in IL-18/IL-2-induced ILD

Figure 1 shows significant up-regulation of TGF-β mRNA in the whole lung tissues from mice at 3, 6 and 12 h after injection of IL-18/IL-2 compared with 0 h ($P < 0.05$, $P < 0.01$ and $P < 0.05$, respectively). However, at 24 h after injection of IL-18/IL-2, the expression of TGF-β mRNA returned to the level at 0 h.

SB-431542 ameliorated ILD and reduced the expression of IFN-γ and IL-6 in the lung

Treatment with SB-431542 was employed to examine the effect of TGF-β inhibition on IL-18/IL-2-induced ILD. As shown in Fig. 2a, treatment with SB-431542 delayed mortality significantly compared with control on the 11th day ($P < 0.05$). Histological examination showed inhibition of cell infiltration in the lungs of SB-431542-treated ILD mice compared with the control (Fig. 2b). Furthermore, we analysed the expression of cytokines in sera and the lung tissues from SB-431542 and vehicle-treated ILD-induced mice. In the lung of SB-431542-treated mice, the expression of IFN-γ and IL-6 was significantly lower than control mice ($P < 0.05$, $P < 0.01$, Fig. 2c). However, in sera, the expression of IFN-γ and TNF-α was not different in each group (Fig. 2d). The lung wet–dry ratio was not significantly different between SB-431542-treated ILD mice and vehicle-treated ILD mice (data not shown).