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Visceral Heterotaxy Syndrome Induced by Retinoids in Mouse Embryo

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Visceral heterotaxy syndrome causes abnormal arrangement of thoracoabdominal organs and severe complex cardiac anomalies by abnormal laterality. The purpose of the present study is to analyze the incidence and pattern of heterotaxy syndrome in etretinate and all-tran retinoic acid treated pregnant DDY mice. Pregnant DDY mice were intragastrically given a single dose of 15mg/kg of etretinate at day 6, 7 of gestation, 30mg/kg of etretinate at day 7 of gestation and 20mg/kg of all-trans retinoic acid at day 7 of gestation. The incidence of visceral heterotaxy was highest in the etretinate 15mg/kg treated group on day 7 of gestation (38.5%). The major cardiovascular anomalies in heterotaxy syndrome were common atrium, common atrioventricular valve, atrioventricular septal defect, transposition of great arteries, pulmonary atresia, pulmonary artery hypoplasia and aortic arch anomalies. Atrial situs of heterotaxy syndrome were right isomerism, solitus-like, inversus-like and left atrial aplsia, but right isomerism was observed most frequently. The results suggest that retinoic acid exerts a significant effect on the determination of atrial situs during the development of mouse embryo.

Key Words : *Heterotaxy, Etretinate, All-trans retinoic acid*

INTRODUCTION

Visceral heterotaxy syndrome, also known as asplenia and polysplenia syndromes is a disease that gives rise to derangement of thoracoabdominal organs and severe congenital cardiac malformations stemming from abnormal laterality (Layman et al., 1967 ; Van Mierop and Winglesworth,1962 ; Moller et al., 1967). Although visceral heterotaxy is relatively rare among congenital heart diseases, it causes high mortality in early life from severe complex cardiac anomalies(Van Praagh et al., 1992).

There have been two major approaches to the morphologic classification of visceral heterotaxy syndrome. The first is the tendency to group into asplenia and polysplenia according to the connections of systemic veins and other visceral morphologies. The second is to devide into right and left isomerisms based on atrial appendages. But there is continuing controversy about the use of nomenclature itself(Sharma et al., 1988 ; Van Praagh et al., 1990 ; Becker and Anderson, 1990 ; Van Praagh and Van Praagh, 1990 ; Phoon and Neill, 1994). Most asplenia show morphologic characteristics similar to right isomerism, where-

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as polysplenia show those similar to left isomerism. The previous studies regarding visceral heterotaxy in experimental animals have been done through administration of drugs to pregnant dams. Some of the studies reported heterotaxy syndrome in experimental animals by the use of isotretinoin(Miura, 1989) and all-trans retinoic acid(Irie et al., 1990), in embryos of pregnant NOD mice during diabetic state(Morishima et al., 1991), and in some in iv/iv mice as the genetic animal model(Layton, 1985 ; Icardo and Sanchez de Vega, 1991 ; Seo et al., 1992). Of these reports, the highest incidence of heterotaxy syndrome was reported in NOD mice. We conducted this study to assess the prevalence of visceral heterotaxy and developmental modality of cardiovascular anomalies induced by the use of etretinate and all-trans retinoic acid in comparison with the results of previous studies.

MATERIALS AND METHODS

Female DDY mice weighing about 30gm at 10~14 weeks' of age were housed with mating partners for 4 hours from 8 AM to 12 MD. The day on which a vaginal plug was found was defined as day 0 of gestation and onset of gestation was regarded as 10 AM. Pregnant dams were divided into five groups consisting of 1 control group and 4 treated groups. The control group were given only olive oil on day 7 of gestation. The treated groups were further divided into 4 subgroups according to kind, dosages, and administrating time of etretinate(retinoic acid ethylester, Hoffmann La Roche Co., Tigason, abbreviated as ET) and all-trans retinoic acid(Sigma Co., abbreviated as AT). Group ET 6-15 was given ET 15mg/kg at day 6 of gestation, group ET 7-15 was given ET 15mg/kg at day 7, group ET 7-30 was given ET 30mg/kg at day 7 and group AT 7-20 was given AT 20mg/kg at day 7. The drugs were administrated intragastrically mixed with olive oil. On day 18 of gestation, fetuses were delivered by cesarean section under anesthesia with sodium pentothal and weighed. The heart was exposed through dissection of the thoracoabdominal wall under a dissecting microscope. The left ventricle of the heart was punctured with a 27-gauge needle and saline was injected to wash out the blood in the heart, followed by 1ml of 2.5% glutaraldehyde for fixation. The fixed embryos were observed for external anomaly and then dissected for detection of anomalies of the heart and other internal organs.

Atrial situs was defined by the type of atrial appendage and develpment of the sinus venosus. The right atrium was characterized by an appendage wall with fine trabeculation which extended to the orifice of the inferior vena cava and has a broad triangular appendage and a wide junction with the venous component of the atrium, the demarcation between the two being the terminal groove(sulcus terminalis). The left atrium was characterized by a restrictive appendage localized anteriorly with the posterior pulmonary venous chamber having a wall devoid of trabeculation, but the junction between the appendage and venous component is narrow and deeply excavated. The left superior vena cava runs across the posterior margin to the left atrium to connect to the venous component of the morphological right atrium through the coronoray sinus (Fig. 1).

Spleens were classified into normal, hypoplastic, and absent according to the size.

RESULTS

A. Incidence of anomalies

There was no cardiac anomaly in 52 cases in the control group. Heterotaxy syndrome was not observed in group ET 6-15 in which etretinate was given on day 6 of gestation. 66.7% (2/3) of viable embryos in group ET 7-30 showed heterotaxy syndrome, but because of the insufficient number of

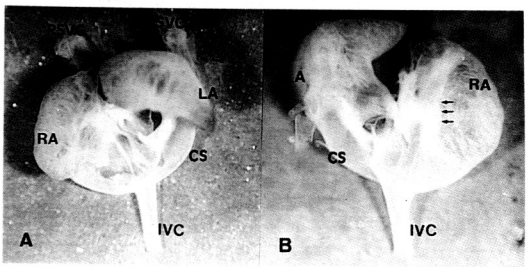

Fig. 1. A dissected normal atrium. A : Anterior aspect of the atrium. The junction between the atrial portion and venous component is wider in the right atrium.
B : Posterior aspect of the atrium. The left superior vena cava runs across the posterior margin of the left atrium and connects to the right atrium through the coronary sinus. The arrow indicates terminal groove.
RA : right atrium, LA : left atrium, IVC : inferior vena cava, RSVC : right superior vena cava, LSVC : left superior vena cava, CS : coronary sinus.

Table 1. Cardiovascular anomalies induced by Retinoids in mouse embryo(%).

	No.of litters	Resorption	Live fetuses	Fetuses with CHD	Heterotaxia	CHD without Heterotaxia
ET 6-15	7	4(6.8)	55	4(72.7)	-	4(72.7)
ET 7-30	5	42(93.3)	3	2(66.7)	2(66.7)*	-
ET 7-15	12	24(31.6)	52	29(55.8)	20(38.5)* #	9(17.3)
AT 7-20	6	10(17.2)	48	12(25)	4(8.3)#	8(16.7)
Control	6	2(3.7)	52	-	-	-

CHD : Congenital heart disease
- : none of the cases
* : $x^2(df=1)=0.132$ $p>0.1$
# : $x^2(df=1)=10.824$ $p<0.01$

cases derived from a high resorption rate (93.3%, 42/45), the incidence was not statistically higher than group ET 7-15. 38.5% (20/52) of group ET 7-15 showed heterotaxy syndrome. On the other hand, in group AT 7-20, only 8.3% (4/48) showed heterotaxy syndrome despite a high dosage, with a lower incidence than the etretinate-given groups (Table 1). Cardiovascular anomalies other than heterotaxy syndrome were ventricular septal defect, transposition of the great arteries, right aortic arch, double outlet right ventricle, interruption of aortic arch in decreasing order and there was one case of mirror-image dextrocardia (Table 2).

B. Pattern of heterotaxy syndrome

Right isomerism was predominant constituting 50% of heterotaxy. Among others, inversus like type was 19.2%, undetermined group was 15.4%, and solitus like group was 15.4%. In the undetermined group, morphologic left atrium was not identifiable and cardiovascular anomalies showed complex types similar to right isomerism. The solitus like and inversus like groups showed abnormal arrangement of atrial situs and other organs with incomplete solitus or incomplete inversus and all of these groups were accompanied by complex cardiovascular anomalies (Fig. 2) (Table 3).

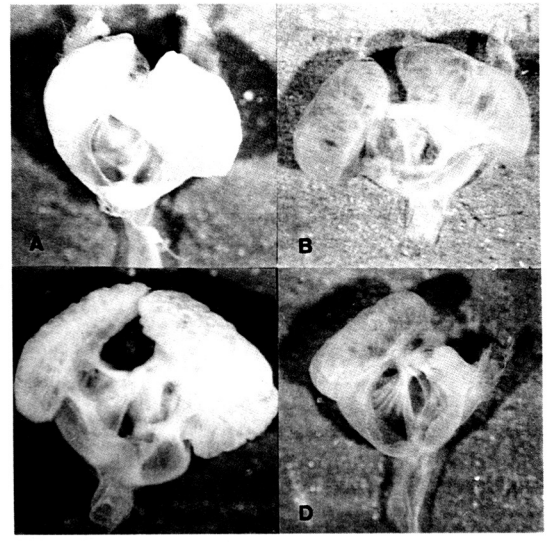

Fig. 2. Anterior view of atrial situs.
A : right isomerism, B : solitus like,
C : inversus like, D : left atrial aplasia

Table 2. Cardiovascular anomalies in mouse embryo without heterotaxy syndrome(%).

Type of anomaly	No. of cases(n=21)
Ventricular septal defect	12(57.1)
Transposition of great artries	9(42.9)
Right aortic arch	5(23.8)
Double outlet right ventricle	2(9.5)
Interruption of aortic arch	2(9.5)
Right subclavian artery from pulmonary artery	1(4.8)
Circumflex retroesophageal aortic arch	1(4.8)
Vascular ring	1(4.8)
Mirror image dextrocardia	1(4.8)

Table 3. Types of heterotaxy syndrome in study groups(%).

	Right isomerism	Solitus like	Inversus like	Undetermined	Total
ET 7-30	1	-	-	1	2(7.7)
ET 7-15	11	2	4	3	20(76.9)
AT 7-20	1	2	1	-	4(15.4)
Total	13(50)	4(15.4)	5(19.2)	4(15.4)	26(100)

C. Cardiovascular anomalies

The most prevalent cardiac anomalies were common atrium and common atrioventricular valve which were present in every case of the right isomerism, solitus like and undetermined groups. A superior-inferior ventricle was observed in the right isomerism and undetermined groups respectively. Among great vessel anomalies, transposition of great arteries was the most common and the others were pulmonary atresia, pulmonary artery hypoplasia and double outlet right ventricle in decreasing order. Aortic arch anomalies were right aortic arch and right ductus arteriosus. The axis of the ventricle was normal in most cases, but dextrocardia was observed in all of the inversus like group and in 15.4% of the right isomerism group(Table 4).

D. Relationship with other organs

In the distribution of pulmonary lobes, most of the right isomerism group showed identical patterns of left and right sides ; 4 lobes on both sides in 76.9%, 3 lobes on both sides in 7.7%. In the solitus like group, 25% showed 4 lobes on both sides and 50% (2 cases) showed normal lobes. Inverted lobes (1 lobe on the right side, 4 lobes on the left side) were predominant with an incidence of 40% (2 cases) in the inversus like group. In the undetermined group, 50% (2 cases) showed 3 lobes on both sides and another 50% (2 cases) showed normal lobes.

In the bifurcation pattern of the bronchus and pulmonary arteries, 92.3% of the right isomerism group showed right bronchus bifurcation pattern on both sides and 80% of the inversus group showed

Table 4. Incidence of cardiovascular anomalies in heterotaxy syndrome in mouse embryo(%).

Anomalies	Right isomerism (n=13)	Solitus like (n=4)	Inversus like (n=5)	Undetermined (n=4)
Dextrocardia	2(15.4)		5(100)	1(25)
Intracardiac				
common atrium	13(100)	4(100)	4(80)	4(100)
common AV valve	13(100)	4(100)	4(80)	4(100)
AVSD	13(100)	4(100)		
absent coronary sinus	13(100)	4(100)		1(25)
superior-inferior ventricle	1(7.7)			1(25)
Venous system				
IVC with contralateral hepatic vein	3(23.1)			
Great vessels				
transposition of great arteries	9(69.2)	2(50)	3(60)	3(75)
double outlet right ventricle		2(50)	1(20)	
pulmonary artery hypoplasia	3(23.1)	1(25)		
pulmonary atresia	4(30.7)			1(25)
Aortic arch anomalies				
right aortic arch	2(15.4)	1(25)	3(60)	2(50)
right ductus arteriosus	2(15.4)			
interrupted aortic arch		1(25)		
aberrent subclavian arteries	1(7.7)			
duplication of aortic arch				1(25)
circumflex retroesophageal aortic arch			2(40)	

AV : Atrioventricular, AVSD : Atrioventricular septal defect, IVC : Inferior vena cava

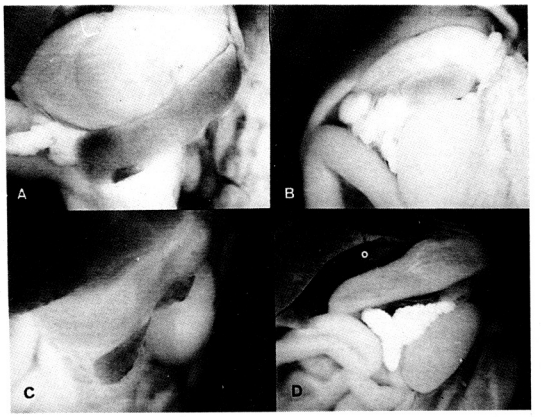

Fig. 3. Morphology of the spleen.
A : normal, B : hypoplastic,
C : bilobed, D : asplenia

inverted bronchus bifurcation pattern.

Although the stomach was located variously, it was in a normal position (left side) in the majority of groups.

In the splenic morphology, hypoplasia was observed in 76.9% (10 cases) of the right isomerism groups and asplenia in 23% (3 cases). Asplenia was most prevalent (50%) in the undetermined group(Fig. 3).

The liver was located symmetrically in most cases in these groups (Table 5).

DISCUSSION

Among retinoids, derivatives of vitamin A, etretinate has been used as a drug for psoriasis treatment under the trade name of Tigason and isotretinoin has had popular use in the treatment of acne under the trade name of Accutane. Some of retinoic acid are being administered experimentally in leukemia therapy. Retinoic acid has been known to be an essential material for the normal development of vertebrates so that excessive or deficient states of retinoic acid cause various anomalies in developing verteb-

Table 5. Non-cardiovascular anomalies in heterotaxy syndrome in mouse embryo(%).

	Right isomerism (n=13)	Solitus like (n=4)	Inversus like (n=5)	Undetermined (n=4)
Lobulation of lung				
Rt 4, Lt 4	10(76.9)	1(25)		2(50)
Rt 3, Lt 3	1(7.7)			
Rt 1, Lt 4	1(7.7)		2(40)	
Rt 3, Lt 1		1(25)		2(50)
Rt 4, Lt 1		2(50)		
Rt 2, Lt 1			1(20)	
Rt 2, Lt 2	1(7.7)			
Rt 2, Lt 4			1(20)	
Rt 1, Lt 3			1(20)	
Bronchus				
bilateral Rt	12(92.3)	1(25)		2(50)
bilateral Lt	1(7.7)		1(20)	
inverted			4(80)	
Normal		3(75)		2(50)
Stomach				
Lt	4(30.7)	4(100)	3(60)	2(50)
Rt	7(53.8)			
mid portion	2(15.4)		2(40)	2(50)
Spleen				
normal			2(40)	
hypoplasia	10(76.9)	4(100)	2(40)	2(50)
absent	3(23)		1(20)	2(50)
Liver				
normal	1(7.7)	4(100)		
symmetrical	12(92.3)		4(80)	4(100)
inverted			1(20)	

Rt : right, Lt : left

rates(Wilson et al., 1953 ; Morris, 1972 ; Shenefelt, 1972 ; Lammer et al., 1985 ; Rosa et al., 1986). Such anomalies have been reported to be caused by interaction between the binding protein of intracellular retinoic acid and intranuclear receptors of retinoic acid with resultant abnormal metabolism and coordination of retinoic acid(Morris-Kay, 1993). It has been also well known that all retinoids have a high teratogenic effect and cause craniofacial, cardiac, thymic and CNS anomalies in man, cardiac anomalies develop mainly from the conotruncus and aortic arch(Lammer et al., 1985) and in experimental animals, various forms can be induced according to the kinds of drugs given, experimental animals, and administering time. Namely, in vitamin A given hamsters, great vessel anomalies such as aortic hypoplasia and double aortic arch were prevailing(Davis and Sadler, 1981), whereas in 62% of retinoic acid given groups, double outlet right ventricle occurred(Shenefelt, 1972). In isotretinoin treated mice, 20% developed interruption of the aortic arch(Johnston et al., 1985), in the all-trans retinoic acid treated group, 64% developed transposition of great arteries(Irie et al., 1990), and in the etretinate treated group, 46% developed transposition of great arteries(Kim et al., 1995), most of the cardiac anomalies being conotruncal or aortic arch anomalies. In the animal models of heterotaxy syndrome, a low incidence of 4.8% was reported in iv/iv mice(Seo et al., 1992), an incidence of 36.1% in the embryos of pregnant NOD mice with diabetes mellitus(Morishima et al., 1991), and Miura et al.(1989) reported an incidence of 16.7% in isotretinoin treated Wistar rats. But the our study showed an incidence of 38.5% in etretinate (15mg/kg) given mice on day 7 of gestation, higher than other reports.

There are two methods in classifing heterotaxy syndrome, of which the first divides into asplenia and polysplenia according to systemic venous connection and splenic morphology(Van Praagh et al., 1992 ; Phoon and Neill, 1994), and the second divides into right and left isomerisms according to atrial morphology(Sharma et al., 1988 ; Becker and Anderson, 1990). Although asplenia shows the pattern of right isomerism and polysplenia that of left isomerism, there is still controversy on the use of terms for abnormal lateralilty of the heart and other organs. Van Mierop et al.(1962, 1964, 1971) originally reported the concepts of atrial isomerism, but Van Praagh(1990) contradicted this concept because of the absence of reports in the literature about the simultaneous presence of anatomical right atrium or left atrium and contended that atrial situs can be determined by the connecting pattern of IVC. Landing et al.(1971) and Van Mierop et al.(1970) reported that the bifurcation pattern of bronchi tend to be compatible with atrial isomerism rather than splenic morphology, namely asplenia and polysplenia, but some investigators reported that all cases did not show this compatability(Caruso and Becker, 1979). Seo et al.(1992) and Becker et al.(1990) classified heterotaxy syndrome according to isomerism of atrial appendage, contradicting Van Praagh et al's concepts(1990) because of the fact that some cases with interruption of IVC show atrial morphology of solitus or inversus types.

The authors classify heterotaxy syndrome by atrial morphology which were divided into four types in man such as normal, mirror image, right isomerism, and left isomerism. In this study, some cases showed heterotaxy syndrome with atrial situs being solitus or solitus-like, and other cases showed heterotaxy syndrome with atrial situs being inversus or inversus-like, similar to Morishima et al's reports(1991). We also observed left atrial aplasia in undetermined group that has not been reported in other studies. The other organs in cases with left atrial aplasia showed similar patterns of right isomerism but some cases were not compatible with right isomerism. That is to say, developed atrial situs were diverse patterns such as solitus, inversus, solitus like, inversus like and right isomerism. So these findings suggest that retinoic acid plays a crucial role in determining atrial situs.

As in previous reports(Irie et al., 1990 ; Morishima et al., 1991 ; Seo et al., 1992), cardiovascular anomalies were common atrium, common atrioventricular valve, atrioventricular septal defect, coronary sinus defect, transposition of great arteries, pulmonary atresia, pulmonary artery hypoplasia, double outlet right ventricle, right aortic arch, right ductus arteriosus and so on, but abnormality of pulmonary venous return was not found. In this study, regarding connection between the ventricle and great vessels, the incidence of transposition of great arteries was higher and that of double outlet right ventricle lower than in other reports, but this result was similar to Irie et al's study(1990) using all-trans retinoic acid in which transposition of great arteries was relatively predominant in heterotaxy syndrome.

It has been well known that morphologic abnormality of the spleen has a correlation with abnormal laterality of the heart and other organs. In man,

splenic abnormality dividing into asplenia and polysplenia has a correlation with atrial appendage but with some inconsistency(Caruso and Becker, 1979 ; Becker and Anderson, 1990) and morphology of the lungs and bronchi also has a correlation with atrial laterality but with some inconsistency(Caruso and Becker, 1979). So there has been controversy in classifying heterotaxy syndrome by the morphology of these organs.

Also in this study, splenic morphology showed variation in each group and lung and bronchi patterns were not consistent in some cases, all of which was similar to other reports. Normal vertebrates becomes asymmetric due to laterality, the heart being also an asymmetric organ. The mechanism controlling laterality has been studied but still remains to be elucidated(Brown et al., 1990 ; Brown and Wolpert, 1990). Of hypotheses on the controlling mechanism of atrial and organ laterality, Stanger et al.(1977) hypothesized that there are separate factors for controlling the development of morphologic right and left structures from paired lateral isomer. When both factors are present, the resulting body configuration is either situs solitus or inversus depending on the interrelationship of the factors. When the factors controlling right morphology are present bilaterally, asplenia syndrome may be expected to occur, and with duplicate left factors, polysplenia syndrome will be present. Layton(1976) hypothesized that positional abnormality in iv/iv mice resulted from deficiency of the developmental control mechanism which normally determines situs solitus. The author observed left atrial hypoplasia not having been reported in other studies. It seemed that retinoic acid exerted influence on developmental factor controlling left morphology of the atrium, resulting in growth failure and aplasia of the left atrium. This finding underlies the hypothesis that factors controlling atrial development exist in separate forms of right and left sides.

In this study, a high resorption rate was shown when the embryos were observed at day 18 of gestation. Considering the report that when observed earlier, lower mortality and higher incidence of anomalies were seen(Seo et al., 1992), we can speculate that there may be many early deaths due to deranged hemodynamics from severe cardiac anomalies, which means that high a incidence of heterotaxy syndrome will develop with earlier observation.

Observation at the onset of atrial morphogenesis is thought to be of great help in clarifying the mechanism determining atrial situs and the developmental mechanism of cardiac anomalies in heterotaxy syndrome. Viewing the results of this study in which the incidence of heterotaxy syndrome was higher than that of other reports, we hope this study will serve as a useful model of experimental animals in the study of the morphogenic mechanism of heterotaxy syndrome.

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