Heart Lesions and Electrocardiographic Changes in DBA/2 and BALB/c Mice

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ABSTRACT. Cardiopathologic and electrocardiographic examinations were carried out on 4-, 8- and 35-week-old DBA/2 and BALB/c mice of both sexes. Both of the electrocardiographic changes (prolongation of QRS duration and PQ-interval, appearance of abnormal P wave and notch in QRS complex, deviation of "ST-segment", and shift of mean electrical heart axis to the left) and pathologic alterations (scatteringly observed small focal myocardial calcification and marked calcification restricted to the myocardium under the right ventricular epicardium) were significantly more frequent and clear in DBA/2 mice than in BALB/c mice, and they became more apparent at 8 weeks of age. Such cardiac disorders as observed in the present study were thought to be free from hypercalcemia because of unchanged serum total calcium concentration.—**KEY WORDS:** BALB/c, ECG, DBA/2, heart lesion.

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Both of the morphologic and functional examinations are necessary to understand correctly the pathologic conditions of the heart. As to the spontaneous cardiac calcinosis in DBA/2 and BALB/c mice, there were many reports of histopathology [1, 2, 4–7, 10–12, 14] but no ones of cardiac function up to the present time.

The purpose of this study is to investigate the correlation between the electrocardiographic and pathologic changes in DBA/2 and BALB/c mice of both sexes with advancing age.

MATERIALS AND METHODS

Four-, 8- and 35-week-old DBA/2NCrj (DBA/2) and BALB/cAnNCrj (BALB/c) mice of both sexes were obtained from Charles River Japan Inc., Kanagawa (Table 1). Examinations were carried out several days after their arrival in our laboratory. Mice were fed CRF-1 pellets (Charles River Japan

Inc.) and water *ad libitum*.

At 10 minutes after each mouse was anesthetized with pentobarbital sodium (50 mg/kg, i.p.), electrocardiogram (ECG) by standard limb leads was recorded in a supine position using a visigraph (NEC San-ei, 5L36ME, Tokyo). Paper speed for ECG recording was 200 mm/sec and time constant of bioamplifier (NEC San-ei, 1205c, Tokyo) was selected in 1.5 sec.

Immediately after ECG recording, mice were sacrificed by heart pucture in the left ventricle. Hearts were weighed and fixed in 10% neutral buffered formol solution. Paraffin sections were stained with hematoxylin and eosin. Some of them were also stained by Gomori's one-step trichrome technique or by von Kossa's procedure.

Colorimetric determination of serum total calcium concentration was done by OCPC method (Ca-SET, Yatron Ltd., Tokyo). For statistical evaluation, the Student's *t*-test was applied.

Table 1. Outline of clinicopathological findings in DBA/2 and BALB/c mice

Age (week)	4				8				35			
Strain	D ^{a)}		B ^{b)}		D		B		D		B	
Sex	M	F	M	F	M	F	M	F	M	F	M	F
No. of mice	11	11	11	11	11	8	11	8	12	10	10	10
Body weight (g)	13.3 ^{c)} ±0.8	12.4 ±0.7	19.4 ±1.0	16.2 ±0.8	24.9 ±1.2	17.8 ±0.6	26.7 ±1.2	21.5 ±0.9	28.1 ±1.4	28.8 ±2.6	32.8 ±2.6	32.7 ±3.5
Heart weight (g/100 g B.W.)	0.68 ±0.07	0.75 ±0.04	0.60 ±0.06	0.73 ±0.38	0.60 ±0.04	0.69 ±0.07	0.60 ±0.04	0.59 ±0.04	0.73 ±0.06	0.64 ±0.07	0.63 ±0.14	0.53 ±0.03
Serum calcium concentration (mg/dl)	11.1 ±1.2	10.0 ±0.9	11.1 ±0.8	11.0 ±1.3	9.1 ±0.5	8.6 ±0.6	8.9 ±0.8	8.2 ±1.4	9.5 ±1.0	9.3 ±1.0	9.7 ±1.1	10.3 ±1.5
Incidence of gross heart lesions (%)	0.0	9.1	0.0	9.1	100.0	63.6	37.5	0.0	100.0	90.0	0.0	0.0
incidence of microscopic heart lesions (%)	72.7	90.9	9.0	18.2	100.0	100.0	37.5	0.0	100.0	100.0	10.0	0.0

a) DBA/2NCrj.

b) BALB/cAnNCrj.

c) Mean±SD.

RESULTS

Body weight, heart weight and serum total calcium concentration: As shown in Table 1, heart weight (g/100 g body weight) was relatively constant while body weight increased with age. Serum total calcium concentration showed no apparent age-, sex- and strain-related changes.

Electrocardiographic findings: Table 2 shows the conduction time of ECG at three stages of age in DBA/2 and BALB/c mice. No significant difference in PQ interval, QRS duration and QT interval were observed between the both strains of mice at 4 weeks of age. However, at 8 and 35 weeks of age, the conduction times became significantly longer than those at 4 weeks of age, especially in male DBA/2 mice. Prolongation of PQ interval and QRS duration was significantly greater in 8-week-old DBA/2 mice than in the same aged BALB/c mice, irrespective of their

sex.

As for the abnormalities of ECG configuration, abnormal P waves implying pulmonary or mitral wave, notch in QRS complex and deviation, mainly elevation, of "ST-segment" were recognized at such appearance rate as shown in Table 3. Abnormal P waves and notch in QRS complex were found even at 4 weeks of age in almost all groups of mice excepting female BALB/c mice, and their appearance rate including deviation of "ST-segment" increased remarkably at 8 and 35 weeks of age. Such abnormalities of ECG configuration were apparently greater in DBA/2 mice than in BALB/c mice, and so in males than in females.

As shown in Fig. 1, the shift of mean electrical axis to the left with advancing age was observed more clearly in DBA/2 mice than in BALB/c mice, irrespective of their age.

Pathologic findings: At necropsy, calcifi-

Table 2. Comparison between DBA/2 and BALB/c mice in the conduction time of electrocardiograms

Age (week)	Strain	Sex	No. of mice	PQ. interval (msec)	QRS duration (msec)	QT interval (msec)
4	D ^{a)}	M	10	36.1±3.2	9.9±0.9	47.7±4.0
		F	11	36.2±3.2	10.1±0.8	55.1±7.9
	B ^{b)}	M	10	35.7±1.7	9.2±0.8	45.0±2.9
		F	7	33.4±1.2	10.3±0.5	50.7±5.7
8	D	M	10	40.1±4.8 ^{c), d)}	11.2±0.9 ^{c), d)}	59.3±6.5 ^{d)}
		F	11	39.1±2.3 ^{c)}	10.4±0.6 ^{c)}	59.0±9.4
	B	M	8	35.7±2.9	10.0±0.8	62.3±5.6 ^{d)}
		F	8	35.7±3.4	9.4±0.9	55.9±3.6
35	D	M	10	41.7±4.9 ^{c), d)}	11.7±1.4 ^{c), d)}	61.7±8.1 ^{d)}
		F	10	40.1±3.2 ^{d)}	11.0±0.9 ^{d)}	57.8±3.6
	B	M	10	36.9±4.0	10.1±0.7 ^{d)}	59.8±4.1 ^{d)}
		F	7	37.5±3.3 ^{d)}	10.3±0.5	60.9±1.9 ^{c), d)}

a) DBA/2NCrj.

b) BALB/cAnNCrj.

c) Significant difference between DBA/2 and BALB/c mice at the same age ($p<0.05$).d) Significant difference between 4-week-old and 8 or 35 weeks old mice of the same strain ($p<0.05$). Each value represents the mean±SD.

Table 3. Appearance rate of abnormalities of ECG configuration in DBA/2 and BALB/c mice

Age (week)	Strain	Sex	No. of mice	Abnormal ^{c)} P waves (%)	Notch in QRS complex (%)	Deviation of "ST-segment" ^{d)} (%)
4	D ^{a)}	M	10	60.0	50.0	0.0
		F	11	45.5	27.3	0.0
	B ^{b)}	M	10	10.0	10.0	0.0
		F	7	0.0	0.0	0.0
8	D	M	10	100.0	100.0	20.0
		F	11	45.5	63.6	0.0
	B	M	8	0.0	50.0	0.0
		F	8	0.0	0.0	0.0
35	D	M	10	70.0	100.0	50.0
		F	10	20.0	60.0	30.0
	B	M	10	0.0	30.0	10.0
		F	7	0.0	20.0	0.0

a) DBA/2NCrj.

b) BALB/cAnNCrj.

c) Expressed as a high voltage or bifid P wave suggesting pulmonary or mitral P wave.

d) Mouse ECG has no true ST-segment, however, the term "ST-segment" is used here to describe the junction of QRS complex and T wave [8].

Fig. 1. Changes in the mean electrical axis in DBA/2 and BALB/c mice. Arrows and circular arcs represent the mean and standard deviation respectively.

cation appeared as a white patch- or plate-like lesion which covered one- to three-fourth of the outer surface of the right ventricle. No gross lesions of the heart were observed in other regions. As shown in Table 1, the incidence of grossly visible heart lesions was very low in both strains at 4 weeks of age. At 8 and 35 weeks of age, DBA/2 mice showed very high incidence while BALB/c mice presented low frequency.

Microscopically, two types of lesions were recognized in DBA/2 mice (Fig. 2). One was a small focal myocardial calcification (Fig. 3), which involved a small number of myocardial cells and was found in many animals

Fig. 2. Schema of distribution of calcified lesions in the hearts of DBA/2 and BALB/c mice. a. Scattered small focal lesion. b. Massive lesion in myocardium under right ventricular epicardium. c. Focal lesion in right ventricular epicardium.

at as early as 4 weeks of age (Table 1). Such small focal lesions revealed no specific distribution and maximum of seven foci per section scattered among the left and right ventricular walls and interventricular septum. The atria, atrioventricular and aortic valves, and papillary muscles were free from lesions in all cases examined.

The other was a massive myocardial calcification which was detected in many DBA/2 mice of 8 and 35 weeks of age in addition to the above-mentioned small focal lesions. Corresponding to the gross findings, it was found to be localized in the myocardial layer under the right ventricular epicardium (Fig. 4) showing the same frequency with that of grossly visible calcification.

Organization of such massively calcified myocardium started at 8 weeks of age, and large parts of the lesions had been replaced by collagen fibers at 35 weeks of age (Fig. 5).

In comparison with DBA/2 mice, BALB/c mice had apparently lower percentage of microscopic calcified lesions (Table 1), most of which were localized in the right ventricular epicardium (Figs. 2 and 6). In a few cases, a maximum of three focal lesions per section

Fig. 3. 4-week-old DBA/2 mouse. Focal myocardial calcification. HE stain. $\times 300$.

Fig. 4. 8-week-old male DBA/2 mouse. Marked myocardial calcification under the right ventricular epicardium. HE stain. $\times 150$.

Fig. 5. 35-week-old female DBA/2 mouse. Large parts of calcified region are replaced by collagen fibers. HE stain. $\times 60$.

Fig. 6. 8-week-old male BALB/c mouse. Focal calcification in the right ventricular epicardium. HE stain. $\times 150$.

was also found sporadically among the right and left ventricular walls and interventricular septum. Similar to the case of DBA/2 mice, the atria, atrioventricular and aortic valves, and papillary muscles exhibited no changes in BALB/c mice.

DISCUSSION

This study is characterized by the fact that cardiopathologic and electrocardiographic examinations were simultaneously carried out on DBA/2 and BALB/c mice which are well known to show spontaneous cardiac calcinosis [1, 2, 4, 6, 7, 10, 12, 14]. Histopathologic nature, distribution and incidence of cardiac lesions observed in the present materials were almost the same as those of our previous reports [5, 11]. The characteristic ECG

changes in the present study were prolongation of conduction time and appearance of abnormalities in ECG configuration.

It is considered that prolongation of QRS duration observed at 8 and 35 weeks of age in both strains might result from not the cardiac hypertrophy but rather the left bundle block, because the relative heart weight did not show age-related changes and notch in QRS complex appeared at high incidence in coincidence with the shift of mean electrical heart axis to the left [3]. The appearance rate of notch in QRS complex at each stage of age was closely related to the incidence of microscopic heart lesions. Consequently, it suggests a possibility that various levels of conduction system in the ventricular walls might be injured by small focal myocardial calcification scatteringly seen among the ventricular

walls [15].

The cause of abnormal P waves and prolongation of PQ interval observed in this study is inexplicable histopathologically because the lesions located in the atria, atrioventricular valves, atrioventricular node and bundles of His could not be clearly pointed out in any sections examined. However, there remains a possibility that a small focal lesion may be detectable in such regions as mentioned above when serial sections are made and examined in detail. On the other hand, the deviation of "ST-segment" [8] might be related to massive myocardial calcification, as it appeared clearly at 8 and 35 weeks of age.

It is said that QT interval shortens under hypercalcemia while lengthens under hypocalcemia [9]. In the present results, serum calcium concentration exhibited no age-related changes and QT interval lengthened at 8 and 35 weeks of age in both strains. Consequently, it is undoubtful that cardiac disorders in the present study do not result from hypercalcemia, but the cause for prolongation of QT interval is unknown.

The shift of mean electrical axis to the left with advancing age correlated well with the incidence of massive myocardial calcification with subsequent organization restricted to the right ventricular wall. Therefore, as for its genesis, it is considered that the electrical vector to the direction of the right might be reduced relatively with the appearance of marked calcified or organized lesions in the right ventricular myocardium.

The existence of some discrepancy between electrocardiographic and histopathologic changes pointed out in this experiment has also been described in hereditary cardiomyopathic KK mice [13], and further studies will be needed to elucidate the strict correspondence between functional and morphologic conditions.

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要 約

DBA/2 および BALB/c マウスにおける心臓病変と心電図学的変化: 前田尚之・菅野 茂¹⁾・土井邦雄・桑原正貴¹⁾・山本龍生¹⁾・光岡知足 (東京大学農学部実験動物学教室, ¹⁾家畜環境生理学教室)——4, 8 および 35 週齢の DBA/2 と BALB/c マウスについて, 心臓の病変および心電図学的検索を行った。心電図所見として, QRS 持続時間と PQ 間隔の延長, 異常 P 波や QRS 群におけるノッチの出現, ST 部分の偏位および平均電気軸の左方偏位が認められた。病変としては, 散在性の小巣状の心筋石灰沈着と右心室心外膜下心筋層に局限した高度の石灰沈着がとくに DBA/2 マウスにおいて認められた。これら心電図学および病理学的変化はともに 8 週齢で明らかとなり, また, BALB/c マウスに比べ DBA/2 マウスで顕著であった。このような心臓障害は, 血清カルシウム値に変動がみられないことから, 高カルシウム血症に起因するものではないと考えられた。