PATHOGENETIC ROLE OF MYOCARDIAL FIBER DISARRAY IN THE PROGRESSION OF CARDIAC FIBROSIS IN NORMAL HEARTS, HYPERTENSIVE HEARTS AND HEARTS WITH HYPERTROPHIC CARDIOMYOPATHY

MASARU TANAKA, M.D., HISAYOSHI FUJIWARA, M.D., TOMOYA ONODERA, M.D.
DER-JINN WU, M.D., MITSUO MATSUDA, M.D., YOSHIHIRO HAMASHIMA, M.D.*
AND CHUICHI KAWAI, M.D.

To define the pathogenetic role of myocardial fiber disarray in the progression of cardiac fibrosis, the percent area of fibrosis in tissue with disarray was compared with that in tissue without disarray. Thirty autopsied hearts, 10 from patients with hypertrophic cardiomyopathy, 10 from patients with hypertension and 10 from normal adults, were studied. The percent areas of fibrosis in tissues with and without disarray were significantly different ($p < 0.01$) among hearts with hypertrophic cardiomyopathy (12.6 ± 4.0 and $8.2 \pm 3.3\%$), hypertensive hearts (6.6 ± 3.6 and $2.5 \pm 1.4\%$) and normal hearts (2.8 ± 1.2 and $1.0 \pm 0.4\%$). The percent area of fibrosis in tissue with disarray was greater than in that without disarray in all 3 groups and the ratios of these percentages were similar in the 3 groups: 2.9 ± 4.2 in hypertrophic cardiomyopathy 2.5 ± 1.7 in hypertensive hearts and 2.5 ± 1.8 in normal hearts. The conclusions are: 1) disarray promotes fibrosis to a similar degree, not only in hypertrophic cardiomyopathy, but also in hypertensive hearts and normal hearts; 2) the increased level of fibrosis in hearts with hypertrophic cardiomyopathy and in hypertensive hearts, together with widespread fibrosis in hearts with hypertrophic cardiomyopathy in particular cannot be explained by disarray alone.

IN hypertrophic cardiomyopathy, myocardial fiber disarray and fibrosis are important morphologic findings¹⁻³ as is myocytic hypertrophy^{4,5}. Quantitative analyses of myocardial fiber disarray⁶⁻⁸ and fibrosis⁹ have disclosed that in hypertrophic cardiomyopathy,

the extent of fibrosis is greater in the ventricular septum, where widespread disarray is present, than it is in the left ventricular free wall. This suggests a close relationship between fibrosis and disarray. However, little is known about the exact role of disarray in the pathogenesis of cardiac fibrosis. Myocardial fiber disarray itself is not pathognomonic in hypertrophic cardiomyopathy but is also present in other pathologic hearts and normal hearts, although it is less extensive^{3,7,10}. The pathologic role of

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The Third Division, Departments of Internal Medicine and *Pathology Kyoto University, Kyoto, Japan

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Mailing address: Hisayoshi Fujiwara, M.D., The Third Division, Department of Internal Medicine, Faculty of Medicine, Kyoto University, Sakyo-ku, Kyoto 606, Japan

TABLE I AUTOPSY DATA

Hypertrophic cardiomyopathy

	Age	Sex	H.W. (g)	VS/FW (mm)	Cause of Death
1. #	20	M	415	23/11 = 2.1	SD
2.	37	M	680	40/25 = 1.6	SD
3.	42	M	600	22/11 = 2.0	SD
4. #	26	M	560	17/12 = 1.4	SD
5. #	25	F	970	27/15 = 1.8	SD
6.	17	M	530	22/10 = 2.2	SD
7.	44	M	340	28/15 = 1.9	SD
8.	76	M	430	19/11 = 1.7	malig. lymphoma
9.	47	M	570	28/16 = 1.8	accident
10. #	66	M	700	24/15 = 1.6	CVA
<i>m ± sd</i>	40 ± 19		580 ± 179	25 ± 6/14 ± 4 = 1.8 ± 0.2	

Hypertensive heart disease

	Age	Sex	H.W. (g)	VS/FW (mm)	Cause of Death
1.	65	M	520	15/13 = 1.2	CVA
2.	31	M	600	19/17 = 1.1	CVA
3.	68	M	500	19/16 = 1.2	CVA
4.	62	F	520	13/13 = 1.0	CVA
5.	35	M	450	15/15 = 1.0	CVA
6.	76	F	360	16/14 = 1.1	CVA
7.	43	F	460	15/13 = 1.2	CVA
8.	59	M	420	14/13 = 1.1	CVA
9.	61	M	410	14/14 = 1.0	cancer
10.	30	F	470	17/14 = 1.2	renal failure
<i>m ± sd</i>	53 ± 17		471 ± 68	16 ± 3/14 ± 1 = 1.1 ± 0.2	

Normal controls

No. of cases	Sex (M/F)	Age (years) range	H.W. (g) <i>m ± sd</i>	VS/FW (mm) <i>m ± sd</i>
10	8/2	25-74	44 ± 11	10 ± 1/10 ± 2

H.W. = heart weight; VS = ventricular septum; FW = left ventricular free wall; SD = sudden cardiac death; CVA = cerebrovascular attack; *m ± sd* = mean ± standard deviation.

Left ventricular outflow tract obstruction (+)

disarray in hearts without hypertrophic cardiomyopathy also remains unknown.

The purpose of this study is to examine the pathogenetic role of myocardial fiber disarray in the progression of cardiac fibrosis in hypertrophic cardiomyopathy by comparison with another form of left ventricular hypertrophy (hypertensive hearts) and with normal adult hearts.

MATERIALS AND METHODS

Thirty autopsied hearts were studied, 10 from patients with hypertrophic cardiomyopathy, 10 from hypertensive patients and 10 from normal adults. There were no significant differences in age or sex among the 3 groups. Nor was there any in heart weight between hearts with hypertrophic cardiomyopathy and hypertensive hearts.

Autopsy data for the 30 patients are listed in Table I.

Eight of the 10 cases of hypertrophic cardio-

Fig.1 a. A tracing of myocardial fibrosis in a sector at $\times 10$ magnification. Areas encircled by continuous and dotted lines show those with and without disarray, respectively. The percent areas of fibrosis were calculated in these areas (%FD(+)) and %FD(-)) and the ratio of these percentages was used as an index for the fibrogenic effect of disarray. In this sector, %FD(+)/%FD(-) was 12.1/6.4% and the index was 1.9. A bar indicates 1 cm. b. The left ventricular wall was divided into 38 sectors (9 each of the anterior (A), lateral (L), posterior (P) and septal (S) wall areas, and single anterior and posterior triangle sectors (T)). To equalize factors other than disarray which influence the degree of fibrosis, %FD(+)/%FD(-) was calculated separately within each of the sectors. For the regional evaluation of the fibrogenic effect of disarray, the average value for all %FD(+)/%FD(-) ratio values of the sectors composing that region was calculated and compared.

%FD(+) = percent area of fibrosis in the tissue with disarray

%FD(-) = percent area of fibrosis in the tissue without disarray

LV = left ventricle, RV = right ventricle

myopathy were diagnosed by both clinical and pathological criteria. In the remaining two cases the diagnosis was based on pathological findings. The pathological criterion for hypertrophic cardiomyopathy was the presence of asymmetric septal hypertrophy with widespread myocardial fiber disarray in the absence of hypertensive, valvar, congenital, or infectious diseases that could have produced left ventricular hypertrophy. None of the cases showed important stenosis of the coronary arteries or acute or old myocardial infarction. Sudden death from cardiac causes occurred in seven patients while others died of extracardiac causes.

Hypertensive heart disease was diagnosed on the basis of left ventricular concentric hypertrophy due to long term overt hypertension ($> 160/95$ mmHg) in a heart weighing > 350 g. Hearts with hypertensive disease and severe coronary arterial stenosis ($> 75\%$ luminal narrowing) were excluded from this study.

A heart was regarded as normal if there was no clinical or pathological evidence of heart disease; if the coronary arteries did not show $> 50\%$ luminal narrowing; and if it weighed < 350 g. In all patients with hypertensive heart disease and in normal controls death resulted from extracardiac causes.

The hearts were fixed in 10% formalin and cut transversely from the base to the apex serially at 1 cm intervals. In the hearts with hypertrophic cardiomyopathy, the slice showing maximal septal hypertrophy was used. In the normal and hypertensive hearts, a slice from the middle third of the heart was used. Each slice was divided into 6–8 blocks, which were sectioned at a thickness of 4μ . These sections were stained with Masson's trichrome.

These preparations were enlarged ten times by a photo-enlarger and the blue areas showing fibrosis were carefully traced on white papers with a fine black pen in the entire area of each section. A transparent overlay (transparent film 632, Staedtler Lumocolor Company) was then laid over the coverglass of these preparations. Using an inverted microscope (Nikon TMS) with a magnification of $\times 100$, areas of myocardium occupied by disorganized cardiac muscle cells and those of normally aligned myocytes were distinguished and outlined separately on the overlay with a sharp stainless steel needle.¹¹ Only the areas with definite disarray and the areas clearly identified as normal were outlined; borderline areas were excluded. This

Fig. 2. The percent area of fibrosis in the tissue with, D(+), and that without, D(-), disarray in the left ventricular wall of hypertrophic cardiomyopathy (HCM), hypertensive heart disease (HHD) and normal controls. Both the percent areas of fibrosis in D(+) and D(-) were significantly different among the 3 groups. The percent area of fibrosis was significantly greater in D(+) than in D(-) in all of the 3 groups.

outlined overlay was enlarged by a photo-enlarger, projected and superimposed on the original paper to distinguish between the areas with and without disarray (Fig. 1a). This procedure was performed for each section cut from the examined transverse slice. To equalize factors other than disarray which influence the degree of fibrosis (wall stress, myocytic hypertrophy, etc), the left ventricular wall was divided into 38 sectors as shown in Fig. 1b and the relationship between fibrosis and disarray was evaluated in each of these sectors in the following manner. Using a multi-purpose color image processor (model VIP-21c, Olympus Co.), the ratio of the fibrotic area to the total tissue area (percent area of fibrosis) was calculated and became the index of the degree of fibrosis. In each sector, the percent areas of fibrosis in the tissues with and without disarray were calculated separately and the ratio of these percentages was used as an index showing fibrogenic effect of disarray. To make a regional evaluation of fibrogenic effect, the average value of all ratios

Fig. 3. The ratio of the percent areas of fibrosis in the tissue with, %FD(+), and that without, %FD(-), disarray in the left ventricular wall. Though it varied among individuals, there were no significant differences in %FD(+)/%FD(-) ratio values among the 3 groups, hypertrophic cardiomyopathy (HCM), hypertensive heart disease (HHD) and normal controls.

derived from the sectors composing that region was calculated and compared.

Data analysis

Statistical analysis was performed using one-way analysis of variance with Neuman-Keuls multiple comparison test to compare regional fibrogenic effect of disarray in each group, and to compare global fibrogenic effect of disarray or myocardial fibrosis among the 3 groups. Statistical significance was determined at the level of $p < 0.05$.

RESULTS

The extent of fibrosis in the tissues with and without disarray (Fig. 2)

The percent areas of fibrosis in the tissues with and without disarray were 12.6 ± 4.0 (mean $\pm$ sd) and $8.2 \pm 3.3\%$ in hypertrophic cardiomyopathy; 6.6 ± 3.6 and $2.5 \pm 1.4\%$ in hypertensive hearts; and 2.8 ± 1.2 and $1.0 \pm 0.4\%$ in normal hearts, respectively. The percent areas of fibrosis in tissues with and without disarray were significantly different among the 3 groups

Fig. 4. The ratio of the percent areas of fibrosis in the tissue with, %FD(+), and that without, %FD(-), disarray in the ventricular septum (VS) and left ventricular free wall (FW). There was no significant difference in %FD(+)/%FD(-) ratio values between VS and FW in any of the 3 groups, hypertrophic cardiomyopathy (HCM), hypertensive heart disease (HHD) and normal controls.

($p < 0.01$). The percent area of fibrosis in tissue with disarray was greater than in that without disarray in all of the 3 groups ($p < 0.01$).

The ratio of percent areas of fibrosis in the tissues with and without disarray

The ratios of percent areas of fibrosis in tissues with and without disarray in the left ventricular wall were 2.9 ± 4.2 in hypertrophic cardiomyopathy, 2.5 ± 1.7 in hypertensive hearts and 2.5 ± 1.8 in normal hearts (Fig. 3). There were no significant differences in the ratios of the 3 groups, although the ratio varied among individuals.

The ratios of percent areas of fibrosis in tissues with and without disarray were 3.5 ± 5.5 and 2.8 ± 3.5 in the ventricular septum and left ventricular free wall in hypertrophic cardiomyopathy, 2.6 ± 1.5 and 2.3 ± 1.7 in hypertensive hearts, 3.2 ± 2.6 and 1.9 ± 1.2 in normal hearts, respectively (Fig. 4). In the right, middle and left thirds of the ventricular septum, the ratios were 4.0 ± 8.0 , 4.3 ± 3.1 and 2.2 ± 2.0 in hypertrophic cardiomyopathy, 2.5 ± 1.2 , 2.4 ± 2.4 and 2.8 ± 1.3 in hypertensive hearts, 4.4 ± 2.8 , 2.4 ± 2.3 and 1.3 ± 1.1 in normal hearts,

Fig. 5. The ratio of the percent areas of fibrosis in the tissue with, %FD(+), and that without, %FD(-), disarray in the right (R), middle (M) and left (L) thirds of the ventricular septum (VS)/ in the inner (I), middle (M) and outer (O) thirds of the left ventricular free wall (FW); and in the triangle region (T). There were no significant differences in %FD(+)/%FD(-) ratio values among these 7 regions in any of the 3 groups, hypertrophic cardiomyopathy (HCM), hypertensive heart disease (HHD) and normal controls.

respectively. In the inner, middle and outer thirds of the left ventricular free wall, the ratios were 1.7 ± 1.5 , 3.7 ± 4.2 and 3.0 ± 4.1 in hypertrophic cardiomyopathy, 2.0 ± 1.2 , 2.5 ± 1.9 and 3.4 ± 3.2 in hypertensive hearts, 1.9 ± 1.1 , 2.0 ± 1.6 in normal hearts, respectively. In the outer third of normal hearts, there was no area with disarray and ratios were not obtained

(Fig. 5). There were no significant differences in the regional ratios of the percent areas of fibrosis in tissues with and without disarray in any hearts.

DISCUSSION

Our data revealed that 1) the percent area of fibrosis in tissue with disarray was greater than in that without disarray in all 3 groups, i.e., hearts with hypertrophic cardiomyopathy, hypertensive hearts and normal hearts; and 2) there were no significant differences in the ratios of these two percent areas among the 3 groups. This indicates that disarray promotes fibrosis, not only in hypertrophic cardiomyopathy, but in hypertensive and normal hearts as well, and that the fibrogenic effect of disarray is approximately the same among these 3 groups, and not necessarily higher in hypertrophic cardiomyopathy. However, as the extent of disarray is less in hypertensive and normal hearts than in hearts with hypertrophic cardiomyopathy,⁶⁻⁸ the global effect of disarray on the progression of fibrosis is not so high in hypertensive and normal hearts. In contrast, at the convergence of the ventricular septum, right and left ventricular free wall (triangular zone), disarray is also widely distributed even in hearts without hypertrophic cardiomyopathy,^{3,10} and relatively widespread fibrosis was evident in this study even in hypertensive and normal hearts.

Previously, we reported that the extent of fibrosis was greater in hypertrophic cardiomyopathy ($10.5 \pm 4.3\%$) than in hypertensive hearts ($2.6 \pm 1.5\%$) or in normal hearts ($1.1 \pm 0.5\%$).⁹ The present findings indicate that disarray is closely related to cardiac fibrosis in all hearts. However, this study also revealed that in tissue without disarray, the extent of fibrosis was the greatest in hypertrophic cardiomyopathy, less in hypertensive hearts and least in normal hearts. This indicates that the difference in the extent of fibrosis among these 3 groups, and the widespread fibrosis in hypertrophic cardiomyopathy in particular, cannot be explained by disarray alone; other factors are also involved in its pathogenesis.

It was reported that the extent of fibrosis varies among individuals and that within each individual heart it differs greatly depending on the sites.⁹ This study showed that the ratios of percent areas of fibrosis in tissues with and without disarray also varies widely among

individuals. Probably, such variability in the extent of fibrosis among individuals and the nonuniform distribution of fibrosis in the left ventricular wall are partly due to the variability in the fibrogenic effect of disarray and/or the nonuniform distribution of disarray. However, in this study, the variability in the fibrogenic effect of disarray could not be explained by their morphological differences and the pathogenesis remains unclear.

It was concluded that 1) both the extent of fibrosis in tissues with and without disarray were the greatest in hearts with hypertrophic cardiomyopathy, less in hypertensive hearts and least in normal hearts; 2) disarray promotes fibrosis, not only in hypertrophic cardiomyopathy, but also in hypertensive and normal hearts, and there is no difference in the fibrogenic effect of disarray among these hearts; 3) the increased level of fibrosis in hearts with hypertrophic cardiomyopathy and hypertensive hearts, and the widespread fibrosis in hearts with hypertrophic cardiomyopathy in particular cannot be explained by disarray alone.

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