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Evaluation of tissue doppler echocardiography on detecting early myocardial relaxation abnormality in adriamycin-induced cardiomyopathy rabbits

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ABSTRACT **AIM**: To evaluate myocardial relaxation function changes in an adriamycin-induced cardiomyopathy model using the transmitral flow velocity to mitral annular velocity ratio (E/E'), a strong positive relationship with left ventricular filling pressure and a good indicator for evaluating left ventricular diastolic relaxation abnormality. **METHODS**: Twenty-eight Japanese rabbits were divided into two groups. Adriamycin was administered at a dose of 2 mg·kg^{-1} intravenous once a week for 8 weeks (total dose of 16 mg·kg^{-1}) in 20 rabbits to induce the cardiomyopathy model. 8 rabbits served as controls receiving the same amount of saline once a week for a total of 8 weeks. Conventional and tissue Doppler echocardiography (TDE) were performed at baseline, 4th, 6th, 8th, 10th and 12th week. **RESULTS**: In the adriamycin-treated group, LV chamber diameter significantly increased, while ejection fraction and fraction shortening significantly decreased in 10th and 12th week ($P < 0.05$). The significant changes were firstly found in 10th week. Mitral annulus systolic peak velocity (S') by TDE significantly decreased in 8th, 10th and 12th week ($P < 0.05$). The significant changes were firstly found in 8th week. The ratio of E/E' significantly increased in 6th, 8th, 10th and 12th week ($P < 0.05$). The significant changes were firstly found in 6th week. In the control group, no significant changes were found in all parameter by tissue Doppler conventional echocardiography ($P > 0.05$). **CONCLUSION**: Myocardial function is reduced in adriamycin-induced rabbit model of dilated cardiomyopathy. The relaxation parameter (E/E') by TDE changes is earlier than contraction indices S' by TDE and conventional echocardiography in adriamycin-induced cardiomyopathy rabbits, which provides a new sensitive and reliable method to evaluate LV relaxation function.

KEY WORDS echocardiography; rabbit; adiamycin; cardiomyopathy; tissue Doppler imaging

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Mitral inflow velocities are widely used for the evaluation of left ventricular (LV) diastolic function. However, they are closely affected by other factors such as preload and heart rate[1]. Recent reports suggest that left ventricular filling pressures can be estimated reliably by combining mitral inflow early diastolic velocity (E) and mitral annulus early diastolic velocity (E') by tissue Doppler echocardiography (TDE). An increased E/E' ratio reflects elevated filling pressures and may be useful in assessing an abnormal increase in filling pressures for patients with diastolic dysfunction, especially for relaxation abnormality[2-4]. The purpose of our study was to apply E/E' index by TDE to evaluate the temporal changes of left ventricular relaxation function in adriamycin-induced cardiomyopathy rabbit.

1 MATERIALS AND METHODS

1.1 Experimental animals Medium size adult Japanese rabbits with average body weight of 2±0.2 kg at the

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beginning of the experiment were used(TJLA-2003-203). The study was performed under the supervision of the Ethical Committee of Tongji Medical College, Huazhong University of Science and Technology in Wuhan, China.

1.2 Experimental protocol Twenty rabbits were assigned to treatment group, and the other eight rabbits were assigned to control group. Twenty rabbits were given 2 mg·kg^{-1} of adriamycin through ear-margin vein infusion once a week for 8 weeks (total dose of 16 mg·kg^{-1}). The other eight rabbits received the same amount of saline through ear-margin vein as control. For the intravenous injection, the rabbits were restrained.

1.3 Transthoracic echocardiography Transthoracic echocardiography was performed on all 28 rabbits before the first administration of adriamycin or saline as baseline, then in the week of 4, 6, 8, 10, 12 after first administration. For echocardiographic studies, 2 mg of Diazepam was injected in muscle. The rabbit chest was shaved, and then left lateral positioned. An 8.0 MHz commercially available standard pediatric transducer was connected to an echocardiography console (GE-Vingmed Vivid VII). The interrogation depth was set at 4 cm.

1.4 Conventional echocardiography parameters Left ventricular end-systolic diameter (LVESD) and left ventricular end-diastolic diameter (LVEDD) were measured at the level of the papillary muscles in parasternal long-axis. At end-diastolic inter-ventricular septal thickness (IVST) and left ventricular posterior wall thickness (PWT) were also measured. Ejection fractioning (EF) and fractional shortening (FS) were calculated as Simpson's method. Mitral inflow velocities were measured in apical four-chamber. Early diastolic velocity (E) was averaged from 3 cycles.

1.5 Tissue Doppler echocardiography parameters TDE was performed in pulse-wave in the mitral annulus velocities at its septal corner from apical four-chamber. The tissue Doppler wave was analyzed, including peak systolic velocity (S') and early diastolic velocity (E'), which averaged from 3 cycles. E/E' index was the ratio of mitral inflow early diastolic velocity (E) and early diastolic velocity (E') from septal mital annulus by TDE. All recordings were stored on a magnetic optical disk and subsequently digitized and analyzed. The total time of every examination was less than 10 minutes, whereafter rabbits were allowed to recover to end this examination.

1.6 Histological examination Histological examination was performed at 12 weeks on 2 adriamycin-treated rabbits and 2 control rabbits after being sacrificed. Histological examination included hematoxylin-eosin (HE) staining from left ventricular free wall.

1.7 Statistical analysis Data were presented as ($\bar{x} \pm s$). All statistical analyses were performed with one-way ANOVA by SPSS software 11.0 (Statistical Product and Service Solution). $P<0.05$ was considened stafistically signifiant.

2 RESULTS

2.1 Mortality None of the rabbits in the control group died during the whole period. While 4 rabbits died respectively in 2nd, 3rd, 8th and 11th week in the treatment group during the whole 12-week period. Therefore, the mortality was 20% in the adriamycin-treated group.

2.2 Experimental end-point echcardiographic indices Compared with that in the baseline in the adriamycin-treated group, LVESD and LVEDD significantly increased in the 12th week, while ejection fraction and fraction shortening significantly decreased in the 12th week ($P<0.05$). TDE S', E' decreased and E/E' significantly increased in the 12th week ($P<0.05$). There was no significant difference between the baseline and the 12th week parameters of IVST and PWT ($P>0.05$). No significant change was found in all conventional and TDE parameters between the baseline and 12th week in the control group ($P>0.05$, Tab 1).

2.3 Serial echocardiographic indices Compared with that in the baseline in the adriamycin-treated group, LVESD and LVEDD significantly increased in the 10th week and 12th week, while ejection fraction and fraction shortening decreased significantly in 10th and 12th week ($P<0.05$). The conventional echocardiography parameters significantly changed firstly in the 10th week. Compared with that in the baseline in the adriamycin-treated group, mitral annulus systolic velocity S' by TDE significantly decreased in 8th, 10th and 12th week ($P<0.05$). The S' by TDE significantly changed firstly in 8th week. The ratio of E/E' increased significantly in 6th, 8th, 10th and 12th week ($P<0.05$, Tab 2). The significant changes were firstly found in 6th week (Fig 1).

2.4 Histological examination Partially degenerated cells, cytoplasm vacuolization and focal interstitial fibrosis were shown in 2 adriamycin-treated rabbits (Fig 2). While normal rabbit cardiac histological findings were shown in 2 control rabbits.

Tab 1　Echocardiographic indices in baseline and 12th week of adriamycin-treated and control group($\bar{x}\pm s$)

Parameters	ADR group (n = 20)		Control group (n = 8)	
	baseline	12 wk	baseline	12 wk
LVEDD /mm	10.5±1.3	15.8±2.1[b]	10.6±1.2	10.7±1.4
LVESD /mm	8.7±1.0	10.3±1.1[b]	8.6±1.1	8.7±1.2
IVST /mm	1.8±0.3	1.7±0.2	1.9±0.4	1.8±0.3
PWT /mm	1.7±0.3	1.5±0.2	1.8±0.3	1.7±0.2
EF /%	63.4±8.4	38.8±7.6[b]	63.0±8.0	63.5±8.3
FS /%	37.6±7.6	21.2±6.7[b]	37.8±7.8	37.4±8.0
S' /cm·s^{-1}	8.8±1.8	3.8±1.0[b]	8.7±1.7	8.8±1.8
E' /cm·s^{-1}	8.2±1.5	5.0±1.2[b]	8.1±1.5	8.0±1.4
E /cm·s^{-1}	48.0±6.7	61.0±7.9[b]	47.0±6.6	49.0±6.8
E/E'	6.1±0.8	10.7±1.2[b]	6.0±0.7	6.1±0.8

Compared with the baseline, [b]$P<0.05$; LVEDD: left ventricular end-diastolic diameter; LVESD: left ventricular end-systolic diameter; IVST: inter-ventricular septal thickness; PWT: posterior wall thickness; EF: ejection fractioning; FS: fraction shortening; S': systolic peak velocity of inter-ventricular septal corner of mitral annulus; E': early diastolic velocity of septal corner of mitral annulus; E: early diastolic velocity of transmitral flow

Tab 2　Serial Echocardiographic indices of adriamycin-treated group($\bar{x}\pm s$, n= 20)

	0 wk	4 wk	6 wk	8 wk	10 wk	12 wk
LVEDD /mm	10.5±1.3	10.9±1.4	11.2±1.5	12.4±1.6	14.9±1.8[b]	15.8±2.1[b]
LVESD /mm	8.7±1.0	8.9±1.0	9.2±1.1	9.4±1.1	9.9±1.1[b]	10.3±1.1[b]
EF /%	63.4±8.4	59.8±8.2	51.2±8.4	48.9±8.1	42.1±7.8[b]	38.8±7.6[b]
FS /%	37.6±7.6	34.3±7.4	31.6±7.2	29.3±7.0	25.1±6.7[b]	21.2±6.7[b]
S' /cm·s^{-1}	8.8±1.8	8.4±1.7	7.6±1.6	5.8±1.5[b]	4.6±1.4[b]	3.8±1.0[b]
E/E'	6.1±0.8	6.9±0.9	8.2±1.0[b]	8.8±1.1[b]	9.6±1.2[b]	10.7±1.2[b]

Compared with the 0 wk, [b]$P<0.05$; LVEDD: left ventricular end-diastolic diameter; LVESD: left ventricular end-systolic diameter; EF: ejection fractioning; FS: fraction shortening; S': systolic peak velocity of inter-ventricular septal corner of mitral annulus; E': early diastolic velocity of septal corner of mitral annulus; E: early diastolic velocity of transmitral flow

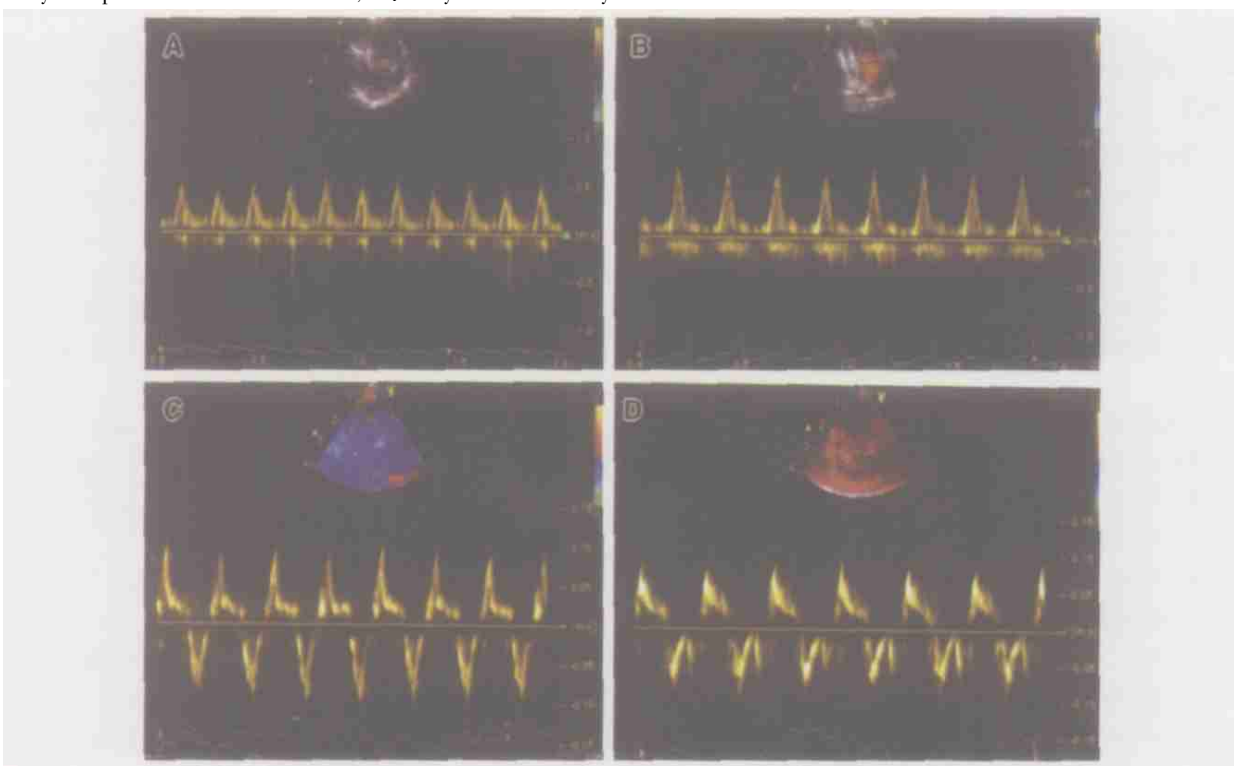

Fig 1　E and E' change at baseline and 6th week in adriamycin-treated group

A: E at baseline; B: E increased at 6th week; C: E' at baseline; D: E' decreased at 6th week; E: early diastolic velocity of transmitral flow; E': early diastolic velocity of septal corner of mitral annulus

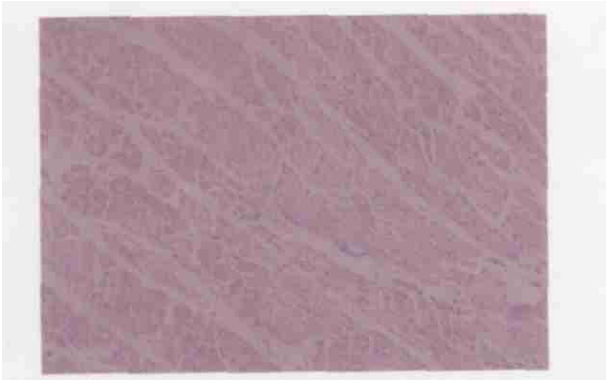

Fig 2 Histological findings in the 12th week of adriamycin-treated group(HE stained, ×60)

3 DISCUSSION

The progress makes our understanding of the pathophysiology and treatment of congestive heart failure impossible without a number of animal models of heart failure[5]. A non-invasive diagnostic technique is needed to serially evaluate morphologic or functional changes in heart model formation course. In evaluation of animal heart diseases model, echocardiography was used to evaluate heart animal model in many study because of its convenience and accuracy. Conventional echocardiography can be used to detect chamber diameter and wall thickness, cardiac global function evaluation depended on ejection fraction and fraction shortening[6]. But it is limited in evaluating early regional function changes, while in model formation is a gradually developed course. Regional function deterioration changed earlier than global function decrease. While regional diastolic relaxation function changed earlier than regional contraction function. It is more useful to early detect regional relaxation function deterioration[7]. Tissue Doppler echocardiography (TDE) has proved useful in evaluating regional systolic and diastolic myocardial function in patients and animal model[8], but experience with serial follow-up changes in adriamycin-induced cardiomyopathy rabbit is limited.

From this study, the adriamycin-induced dilated cardiomyopathy model shows progressive enlargement of LV chamber and deterioration of left ventricular (LV) systolic and diastolic function ($P < 0.05$), which is similar to human idiopathic cardiomyopathy. In our study, 8 week administration adriamycin has unambiguously caused severe pathological changes in the myocardium of the left ventricle. Cellular degeneration, cytoplasm vacuolization and focal interstitial fibrosis are typical for severe adriamycin-induced cardiotoxicity in human patients[5]. It confirmed the intravenous injection of adriamycin can be used to induce a reliable rabbit model of non-ischemic dilated cardiomyopathy with a high success rate.

From this study, mitral annulus diastolic index E/E' could evaluate a serial impairment of LV myocardial contraction function from the 6th week throughout the adriamycin-induced dilated cardiomyopathy model formation period, while the significant changes of S' by TDE were firstly found in 8th week and the conventional echocardiography parameters changed significantly firstly in the 10th week. The parameter E/E' was more useful in quantifying progressive LV dysfunction than S' by TDE and conventional echocardiographic techniques. Other study also confirmed excellent reproducibility of mitral annulus velocities by TDE in rabbit[8,9]. The noninvasive tissue Doppler method E/E' is therefore suitable for the investigation of left ventricular diastolic function in experimental studies in rabbits, while the changes of dysfunction seem to be very similar to those in human. Diastolic dysfunction is common in cardiac disease and contributes to the signs and symptoms of heart failure. Doppler echocardiography is widely used for the noninvasive assessment of diastolic filling of the left ventricle. Analysis of the mitral inflow velocity curve has provided useful information for determination of filling pressures and prediction of prognosis in selected patients[1]. However, mitral flow is dependent on multiple interrelated factors, including the rate and extent of ventricular relaxation, suction, atrial and ventricular compliance, mitral valve inertance, and left atrial pressure[2]. These factors may have confounding effects on the mitral inflow. Thus, it has not been possible to determine diastolic function from the mitral flow velocity curves in many subsets of patients. Tissue Doppler echocardiography is a new Doppler method that allows recording of the low doppler shift frequencies of high energy generated by the ventricular walls motion that are purposely filtered out high doppler shift frequencies of low energy in standard doppler blood flow[7]. Tissue Doppler echocardiography of mitral annular motion has been proposed to correct for the influence of myocardial relaxation on transmitral flows. This has been shown to be an excellent predictor of diastolic filling in subsets of patients. The early diastolic velocity of the mitral annulus (E') is reduced in patients with diastolic dysfunction and increased in filling pressures. Because transmitral inflow early velocity (E) increases progressively with higher filling pressures, E/E' has been shown to have a strong positive re-

lationship with pulmonary capillary wedge pressure (PCWP) and left ventricular end-diastolic pressure[4].

Because of the noninvasive nature of this model, we did not evaluate the temporal changes in either LA or LV pressures because of avoiding repeatedly anesthesia in this conscious rabbit study. But other studies have confirmed that (E/E') change is a strong positive relationship with left ventricular filling pressure change[2, 10].

Myocardial function was reduced in adriamycin-induced rabbit model of dilated cardiomyopathy. The relaxation parameter (E/E') by TDE changed earlier than contraction indices by TDE and conventional echocardiography in adriamycin-induced cardiomyopathy rabbits, which provides a new sensitive and reliable method to evaluate LV relaxation function.

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组织多普勒超声评价阿霉素诱导兔心肌病早期松弛功能改变的实验研究

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摘要　目的: E/E'比值(二尖瓣血流舒张早期速度/二尖瓣环组织多普勒舒张早期速度)与左室充盈压有良好的相关性, 可用来准确评价左室松弛功能异常。本研究利用该指标评价阿霉素所致兔心肌病心肌松弛功能的变化情况。方法: 28只日本白兔分为2组: 阿霉素组20只, 每周静脉注射阿霉素2 $mg\cdot kg^{-1}$, 8周共计16 $mg\cdot kg^{-1}$以期形成心肌病模型; 对照组8只, 每周注射相同剂量的生理盐水。分别在第0、4、6、8、10、12周进行常规和组织多普勒超声心动图检查。结果: 阿霉素组在10、12周左室内径显著扩大, 左室射血分数和短轴缩短率显著下降($P<0.05$)。显著变化最早发现在第10周。二尖瓣环处心肌组织收缩峰值速度在8、10、12周显著降低($P<0.05$), 显著变化最早发现在第8周。E/E'比值在6、8、10、12周显著增高($P<0.05$), 显著变化最早发现在第6周。对照组常规和组织多普勒超声指标均未发现显著变化($P>0.05$)。结论: 在阿霉素所致兔心肌病模型中心肌功能降低。E/E'变化早于组织多普勒收缩指标和常规超声心动图指标, 为评价左室松弛功能提供了一个敏感可靠的新手段。

关键词　超声心动图; 兔; 阿霉素; 心肌病; 组织多普勒