Preclinical research

Tissue Doppler imaging detects early asymptomatic myocardial abnormalities in a dog model of Duchenne’s cardiomyopathy

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Aims Early diagnosis of Duchenne’s dilated cardiomyopathy remains a challenge for conventional echocardiography. We sought to determine whether tissue Doppler imaging (TDI) could detect early alteration in myocardial function in a dog model of Duchenne muscular dystrophy, i.e. the Golden Retriever Muscular Dystrophy (GRMD).

Methods and results Myocardial function was assessed by TDI in 20 dogs with normal conventional parameters of systolic function (eight controls and 12 GRMD, 25 ± 11 weeks) without knowledge of the genotype. M-mode TDI was recorded from a short-axis view for measurement of epicardial and endocardial velocities and myocardial velocity gradient (MVG) within the posterior wall.

Controls and GRMD dogs were comparable regarding left ventricular fractional shortening (37 ± 2 vs 42 ± 3%, p = ns). Conversely, TDI showed, in all GRMD dogs, a dramatic decrease in systolic MVG (0.8 ± 0.1 vs 2.9 ± 0.3 s⁻¹, p < 0.0001) and early diastolic MVG (2.3 ± 2.2 vs 10.8 ± 1.1 s⁻¹, p < 0.0001). This MVG alteration was related to a significant decrease in endocardial velocities in GRMD whereas epicardial velocities were comparable in the two groups.

Conclusion These results show that TDI is more sensitive than conventional echocardiography in detecting pre-clinical myocardial abnormalities before occurrence of left ventricular dilation and dysfunction. TDI should be part of the screening techniques for the early diagnosis of cardiomyopathy.

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Introduction

Duchenne’s muscular dystrophy is related to a dystrophin mutation, resulting in a dysfunctional protein. Dystrophin is a key linker protein between the sarcolema of the myocyte and the contractile apparatus, the sarcomere.1 In dystrophinopathies, in addition to the skeletal muscle disease, cardiac alteration is common but asymptomatic over a variable period, before the occurrence of a dilated cardiomyopathy resulting from widespread fibrosis and ending with fatal outcome.2

As cardiac involvement does not parallel the muscular disease and its severity is not predicted by the genetic analysis,3 an alternative approach should use tissue Doppler imaging (TDI) to diagnose subtle and asymptomatic myocardial abnormalities early.4,5 TDI allows quantification of systolic and diastolic regional myocardial velocities and deformation indices such as myocardial velocity gradient (MVG) or strain rate. Both myocardial velocities and deformation indices such as systolic and diastolic strain rate are less dependent on loading conditions than ejection fraction.6,8 Endocardial to epicardial velocity gradients may identify left ventricular dysfunction before ejection fraction is altered.9 Recent studies have shown that deformation indices are less dependent on loading conditions than ejection fraction is.6,8

As in human Duchenne dystrophy, the Golden Retriever Muscular Dystrophy (GRMD) is related to a spontaneous X-linked mutation of the dystrophin gene in dogs10 and is characterized by myocardial lesions, including continuing muscular necrosis and replacement by fibrosis, fat and mineralization, leading to progressive myocardial dysfunction and heart failure.11-12 The aim of this study was to determine the accuracy of TDI to detect dystrophin mutant GRMD dogs early, before the occurrence of congestive heart failure and myocardial dysfunction as determined by conventional echocardiographic parameters.

Methods

All procedures performed in this study conformed to the Guiding principles in the care and use of animals approved by the American Physiological Society.

Animals

Twenty asymptomatic male dogs (eight controls with a normal genotype and 12 GRMD dogs, 25 ± 11 weeks old) were recruited from the cohort of GRMD dogs bred in the Neurobiology Laboratory of the National Veterinary School of Alfort. The two groups were similar in age, gender and strain (all Golden Retriever). GRMD mutation is related to a single base mutation in the 3’ consensus splice site of intron 6 of the canine dystrophin gene. The diagnosis of GRMD was based on DNA analysis when the dogs were 1 month old.13

Echocardiography

Conventional and TDI echocardiography were performed in awake dogs using a Sequoia system (Acuson, Mountain View, CA, USA) with a 7 MHz phased-array transducer. Data were analysed by an observer blinded to the results of genotype. Left ventricular (LV) dimensions, posterior wall (PW) and interventricular septal (IVS) wall thicknesses were measured. Left ventricular fractional shortening (LVFS%) and PW and IVS thickening were then calculated. Pulsed Doppler of the mitral valve inflow was used for measuring the ratio of early to late diastolic flow velocity (E/A).

Measurement of myocardial velocities was obtained from a short-axis view at the level of the papillary muscles. Using M-mode TDI, endocardial and epicardial velocities were calculated during systole and diastole within the posterior wall, as previously described.14 The posterior wall was arbitrarily divided from the endocardial to epicardial borders into two layers of equal thickness by manual tracing of endocardial and epicardial boundaries. Endocardial and epicardial mean velocities were defined as the average value of the velocity estimates measured along each M-mode scan line throughout the thickness of the inner and outer layers of myocardial walls. MVG was defined as the difference between endocardial and epicardial velocities divided by wall thickness.

Endomyocardial biopsies

Under echocardiographic control, left ventricular biopsies were performed within the PW in five control and eight GRMD anaesthetised dogs with a 20 mm, 14 G Tru-Cut biopsy needle. Tissue samples (five per animal) were frozen and sectioned at a thickness of 10 ± 3 μm. Histological slices were stained with Sirus red for the determination of interstitial collagen density. Perivascular collagen was excluded from this measurement. The presence of apoptotic cells was detected by in situ end-labelling of DNA fragmentation (TUNEL, Roche Diagnostics).

Statistical analysis

Data are expressed as means ± SEM. Echocardiographic parameters were compared between controls and the GRMD group using a two-sided, unpaired Student’s t test. In order to find the best TDI index to discriminate GRMD from controls, sensitivity and specificity were calculated from ROC curves for both systolic and diastolic velocities and MVGs. P values of less than 0.05 were considered statistically significant.

Results

Control and GRMD dogs were similar regarding their age and haemodynamic data (Table 1). All animals had conventional echocardiography and TDI studies, which were satisfactory for analysis.
Conventional parameters

GRMD dogs showed strictly normal standard echocardiographic parameters compared with controls. No difference was found between the two groups regarding left atrial and LV end-systolic and end-diastolic dimensions, LVFS% and wall thickening and E/A (Table 1).

Table 1 Conventional echocardiographic parameters of controls and GRMD dogs

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Controls (n = 8)</th>
<th>GRMD (n = 12)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (weeks)</td>
<td>23 ± 4</td>
<td>25 ± 2</td>
<td>0.56</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>114 ± 12</td>
<td>121 ± 22</td>
<td>0.43</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>166 ± 4</td>
<td>164 ± 3</td>
<td>0.74</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>89 ± 5</td>
<td>89 ± 4</td>
<td>0.69</td>
</tr>
<tr>
<td>Aortic diameter (mm)</td>
<td>24 ± 1</td>
<td>21 ± 5</td>
<td>0.45</td>
</tr>
<tr>
<td>Left atrial diameter (mm)</td>
<td>19 ± 4</td>
<td>17 ± 2</td>
<td>0.27</td>
</tr>
<tr>
<td>Aortic diameter/left atrial diameter</td>
<td>0.74 ± 0.15</td>
<td>0.82 ± 0.08</td>
<td>0.67</td>
</tr>
<tr>
<td>LVEDD (mm)</td>
<td>30 ± 2</td>
<td>34 ± 2</td>
<td>0.22</td>
</tr>
<tr>
<td>LVESD (mm)</td>
<td>19 ± 1</td>
<td>20 ± 2</td>
<td>0.65</td>
</tr>
<tr>
<td>LVFS%</td>
<td>37 ± 2</td>
<td>42 ± 3</td>
<td>0.19</td>
</tr>
<tr>
<td>PW%</td>
<td>75 ± 8</td>
<td>66 ± 4</td>
<td>0.44</td>
</tr>
<tr>
<td>IVS%</td>
<td>73 ± 10</td>
<td>67 ± 12</td>
<td>0.71</td>
</tr>
<tr>
<td>E/A</td>
<td>1.34 ± 0.4</td>
<td>1.56 ± 0.7</td>
<td>0.43</td>
</tr>
</tbody>
</table>

GRMD, Golden Retriever Muscular Dystrophy; BP, blood pressure; LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; LVFS, left ventricular fractional shortening; PW, posterior wall; IVS, inter-ventricular septum.

Table 2 Tissue Doppler imaging parameters of controls and GRMD dogs

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Controls (n = 8)</th>
<th>GRMD (n = 12)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_S$ endocardium (cm/s)</td>
<td>7.7 ± 0.7</td>
<td>5.0 ± 0.2</td>
<td>0.0005</td>
</tr>
<tr>
<td>$V_T$ epicardium (cm/s)</td>
<td>4.8 ± 0.6</td>
<td>4.3 ± 0.7</td>
<td>0.39</td>
</tr>
<tr>
<td>MVG$G_1$ (s$^{-1}$)</td>
<td>2.96 ± 0.3</td>
<td>0.82 ± 0.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>$V_S$ endocardium (cm/s)</td>
<td>14.6 ± 1.3</td>
<td>6.4 ± 2.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>$V_T$ epicardium (cm/s)</td>
<td>5.4 ± 2.1</td>
<td>4.8 ± 1.2</td>
<td>0.09</td>
</tr>
<tr>
<td>MVG$G_2$ (s$^{-1}$)</td>
<td>10.8 ± 1.1</td>
<td>2.3 ± 2.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>$V_S$ endocardium (cm/s)</td>
<td>4.5 ± 0.5</td>
<td>7.6 ± 0.9</td>
<td>0.005</td>
</tr>
<tr>
<td>$V_T$ epicardium (cm/s)</td>
<td>3.4 ± 0.8</td>
<td>4.1 ± 1.2</td>
<td>0.07</td>
</tr>
<tr>
<td>MVG$G_A$ (s$^{-1}$)</td>
<td>1.5 ± 0.2</td>
<td>3.8 ± 0.7</td>
<td>0.0005</td>
</tr>
<tr>
<td>MVG$G_2$/MVG$G_A$</td>
<td>6.1 ± 0.9</td>
<td>0.8 ± 1.1</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

GRMD, Golden Retriever Muscular Dystrophy; MVG, myocardial velocity gradient; $V_S$, systolic velocity; $V_T$, early diastolic velocity; $V_A$, atrial contraction velocity.

Conventional parameters

GRMD dogs showed strictly normal standard echocardiographic parameters compared with controls. No difference was found between the two groups regarding left atrial and LV end-systolic and end-diastolic dimensions, LVFS% and wall thickening and E/A (Table 1).

Fig. 1 Upper panel: TDI-M-mode in control and GRMD dogs. Lower panel: LV collagen density (left) and apoptotic cells (right) in control and GRMD dogs. Note the decrease in myocardial velocity in the GRMD group associated with fibrosis and apoptotic cells.
**TDI parameters**

Fig. 1 shows representative recordings of TDI colour-encoded posterior wall in a control dog and in a GRMD dog. In all GRMD dogs, TDI demonstrated alterations in systolic and diastolic regional myocardial function. Both systolic and diastolic MVG were indeed significantly lower in GRMD than in control dogs (Table 2, Fig. 1). Therefore, the two groups could be accurately distinguished based on systolic MVG, but not LVFS% (Fig. 2). This severe alteration in systolic and diastolic MVG was related to a significant decrease in both systolic and diastolic endocardial velocities in GRMD dogs whereas epicardial velocities were comparable in the two groups (Table 2).

In addition, the ratio of early to late diastolic myocardial velocity gradient was significantly lower in GRMD dogs than in controls (Table 2).

Among systolic and diastolic velocities and myocardial velocity gradient, the best index to discriminate GRMD from controls was the systolic MVG that demonstrated a sensitivity of 100% and a specificity of 93% for a value <1 s⁻¹.

**Endomyocardial biopsies**

In GRMD dogs, endomyocardial biopsies demonstrated a slight but significant increase in interstitial collagen den-
Conversely, cardiac lesions are detected in the neonatal period and then extending to the other segments of the heart [11]. Cardiac lesions are detected in the posterobasal part of the left ventricular free wall of the heart. These lesions are described as myocardial fibrosis, initially localized within the sarcoplasmic reticulum. Our previous study [10] has shown the ability of TDI to detect myocardial lesions early.

In this study, we demonstrated that TDI parameters of regional myocardial function were altered early whereas global ventricular function, as assessed by conventional echocardiographic parameters, was still preserved. Early detection of myocardial abnormalities is important in Duchenne's disease because early treatment might be effective in preventing the development of myocardial fibrosis and further left ventricular remodelling leading to congestive heart failure. Therefore, TDI might be a useful tool for early detection of contraction and relaxation alteration when LV global systolic and diastolic function is still preserved.

Duchenne's muscular dystrophy is a model of progressive cardiac dysfunction and early detection and treatment of the myocardial disease is important in order to prevent irreversible LV remodelling and its clinical course ending in death. Genetic, histological, echocardiographic and radionuclide findings have demonstrated the cardiac involvement of GRMD dogs to be similar to Duchenne's human dystrophy.11,12,16,17 Prior to the occurrence of heart failure, a skeletal muscle atrophy appears as early as during the neonatal period.11 Conversely, cardiac lesions are detected in the later stages of the GRMD disease: histological changes are generally not present up to 3 months of age,16 and several months or years are required to observe progressive systolic dysfunction and left ventricular dilation.12,16 Therefore, this dog model of Duchenne's human dystrophy is very helpful to provide new insights in the comprehensive study of patients, regarding both markers of cardiac function and potential new treatments. In this study, we selected young animals in order to analyse specifically the first stage of the disease for determining the accuracy of TDI to detect myocardial lesions early. Despite normal conventional parameters, both systolic and diasatic TDI indices were severely impaired in these young GRMD dogs, indicating subtle alterations in contraction and relaxation. As demonstrated by endomyocardial biopsies, these abnormalities might be related to interstitial fibrosis and, to a lesser extent, apoptosis, in addition to the previously described abnormal cytoskeletal links between sarcolemma and sarcomere.18 Our pathological findings are consistent with the previously described myocardial fibrosis, initially localized within the posterobasal part of the left ventricular free wall and then extending to the other segments of the myocardium.16

Previous studies have shown the key role of TDI for screening and pre-clinical diagnosis of familial hypertrophic cardiomyopathy before and independently of the development of left ventricular hypertrophy.5,19,20 Similar to subclinical cardiac involvement, these abnormalities might be related to interstitial fibrosis and, to a lesser extent, apoptosis, in addition to the previously described abnormal cytoskeletal links between sarcolemma and sarcomere.18 Our pathological findings are consistent with the previously described myocardial fibrosis, initially localized within the posterobasal part of the left ventricular free wall and then extending to the other segments of the myocardium.16

Discordant muscular dystrophy (6.3 ± 0.3 vs 2.1 ± 0.2%, p < 0.05) and a few apoptotic cells (Fig. 1).

Discussion

In this dog model of Duchenne’s disease, we demonstrated that TDI parameters of regional myocardial function were altered early whereas global ventricular function, as assessed by conventional echocardiographic parameters, was still preserved. Early detection of myocardial abnormalities is important in Duchenne’s disease because early treatment might be effective in preventing the development of myocardial fibrosis and further left ventricular remodelling leading to congestive heart failure. Therefore, TDI might be a useful tool for early detection of contraction and relaxation alteration when LV global systolic and diastolic function is still preserved.

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Previous studies have shown the key role of TDI for screening and pre-clinical diagnosis of familial hypertrophic cardiomyopathy before and independently of the development of left ventricular hypertrophy.5,19,20 Similarly, subclinical cardiac involvement has also been recently described and revealed by decreased myocardial velocities in patients with myotonic dystrophy.21 Regarding dystrophinopathic cardiomyopathy, TDI has also been shown to accurately identify early systolic dysfunction in patients with Becker muscular dystrophy and normal LV function.22 In this study, regional myocardial function was assessed by pulsed Doppler velocities.

Although encouraging data were obtained in determining regional myocardial function from velocity data sets, interrogation of velocities alone has some limitations since overall heart motion, rotation and tethering effects also influence velocity estimates. In order to overcome these problems, the rate of regional myocardial deformation (or strain rate) may be assessed by spatial gradients in myocardial velocities. A recent study by Mori et al.23 demonstrated that peak systolic and early diastolic MVGs were superior to mitral annulus velocities in differentiating young patients with Duchenne’s muscular dystrophy than in age-matched control subjects. In our study, myocardial velocities could not discriminate GRMD dogs and controls as accurately as deformation parameters, i.e., MVG. Systolic MVG was the best indicator of early myocardial alteration with a sensitivity of 100% and a specificity of 93%.

Limitations

We only analysed the short-axis view and therefore the radial component of myocardial function. We did not get information on longitudinal function since, in these awake dogs, we often had an angle between the Doppler interrogation and the long axis of the left ventricle in the apical views. Invasive assessment of left ventricular systolic and diastolic function was not performed. Therefore, we cannot state that the decrease in systolic and diastolic MVG precisely reflects abnormal contractility and diastolic dysfunction. However, both peak systolic velocity and deformation indices have been shown to correlate well with changes in fractional shortening as assessed by sonomicrometry.6,24 Moreover, peak systolic strain rate has been reported to correlate well with peak elastance, a load-independent index of myocardial contractility.6 Finally, based upon the transmural distribution of myocardial velocity, previous studies had also shown the ability of MVG to accurately quantify regional contractile dysfunction.7,19,20

Conclusion

Our results clearly indicate that TDI is able to early detect alterations in myocardial function that are not detectable by current techniques. This early diagnosis has important clinical implications since it should allow an early institution of drug therapy in order to prevent the left ventricular remodelling and the subsequent development of heart failure.
Acknowledgements

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References