EVALUATION OF DESMIN EXPRESSION
BY IMMUNOHISTOCHEMISTRY IN MYOCARDIAL CELLS
OF DOGS WITH DILATED CARDIOMYOPATHY

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Abstract

The experimental material consisted of 20 dogs: 15 Doberman pinschers with diagnosed dilated cardiomyopathy (DCM), and five healthy dogs (three mixed breeds, one Doberman pinscher, one Boxer). DCM was confirmed by electrocardiography and echocardiography before euthanasia. After euthanasia, an autopsy was performed and myocardial samples were collected from the right and left ventricles, intraventricle septum, and right and left atrium. The samples for the histopathological and immunohistochemical analysis were fixed in with 7% formalin in PBS buffer. The presence of desmin was semiquantitatively evaluated under a light microscope. The normal levels of desmin expression in immunohistochemical staining were observed only in the control dogs. The desmin expression was elevated in 15 dogs with severe DCM. The immunohistochemical method allowed for the evaluation of desmin activity in cardiomyocytes, and showed three patterns of its expression (increase expression, decrease expression, heterogeneous expression). The desmin expression was abnormal in the myocardial samples in dogs with DCM. We observed an increase expression of desmin in 10 dogs with moderate and a decrease expression of desmin in five dogs with advanced DCM. The relationship between the desmin expression and the progression of heart failure secondary to DCM requires further studies.

Key words: dog, desmin, dilated cardiomyopathy.

Desmin was isolated for the first time in 1977 by Lazardes and Hubbard (9). It is a specific cell protein of the myocardium, striated muscles, and smooth muscles. Desmin plays a role in muscle cells, fulfilling mechanical, structural, and control functions. It comprises approximately 2% of the weight of myocardial cells, and 0.35% of the cell weight in skeletal and smooth muscles (12). Desmin appears in the preliminary stages of myogenesis, as proven by studies on mice in which a gene for desmin is activated in cardiac primordium around the 7th d of embryonal development (5). The early appearance of desmin provides evidence for its critical role in the development of normal myocyte function (12). The intracellular distribution of desmin changes in the course of its development, starting as thick fibres distended throughout the cell to scattered junctions along the Z line. Desmin fibres form a densely intertwined network around myofibrilles and Z lines. Desmin surrounds the Z lines, links the neighbouring lines with each other, with sarcolemma and with the nuclear envelope. Within a myofibril, it is responsible for the lateral links, and entwines the myofibrils, forming a lattice-skeleton attached to Z lines (6, 17). Due to its localisation and links, the protein is thought to integrate the mechanical contractile activity of muscle fibres (6). A physiologically-increased amount of desmin is observed in the fibres of the impulse-conducting system (17). The altered content of desmin in cardiomyocytes, and its abnormal distribution, is noted in several myopathies, termed desminopathies. The clinical signs/symptoms are linked to both the weakened skeletal muscles and to abnormalities in the heart, which may acquire the form of restrictive cardiomyopathy, cardiomyocyte hypertrophy, dilatation of ventricular lumen, conduction blocks, or cardiac insufficiency. Hypoplasia of the aorta and the thinning of the vascular walls were also described in the course of desmin disturbances (3, 10). Changes in the desmin content and in its distribution were observed in humans and animals in the course of cardiac failure, independently of its primary cause (7, 18, 19). A change in desmin was observed in mitral myxomatous valves. Interstitial cells from myxomatous valves showed progressive positive staining for α-actin.
and desmin, but were negative for smooth muscle myosin (i.e., myofibroblast phenotype). Normal canine mitral valve interstitial cells were negative for desmin (4).

The present study is aimed at evaluating desmin expression by immunohistochemistry in myocardium samples originating from dogs with dilated cardiomyopathy, and at the determination of a relationship between the extent of the desmin expression and the advancement of cardiac failure in dogs with dilated cardiomyopathy.

Material and Methods

The material for the studies consisted of 20 dogs of either sex, aged from 5 to 9 years, including 15 Doberman pinschers with DCM and five healthy dogs (three mixed breeds, one Doberman pinscher, one Boxer). Due to the significant advancement of the disease, the animals were subjected to euthanasia with the consent of their owners, and one of the dogs died suddenly. The control group included five dogs, aged from 7 to 11 years, which was subjected to euthanasia due to numerous injuries experienced in traffic accidents. In all of the dogs, the blood morphology and biochemistry was analysed (ALT, AST, urea, creatinine, Na⁺, K⁺, Ca²⁺, Mg²⁺, Cl⁻), and electrocardiographic, echocardiographic, and chest X-ray examinations were conducted in order to corroborate DCM diagnosis. The echocardiographic examination was performed with a six-channel BLT machine with the dogs in a standing position. The echocardiographic examination was conducted using an Aloka SSD 4000 machine. The left-ventricular end-systolic dimension (LVEDd), the thickness of the left ventricular posterior wall in the systole (LVPWs) and in the diastole (LVPWd), and the thickness of the interventricular septum in the systole (IVSs) and diastole (IVSd) were recorded. The measurements were taken in parasternal projection on the short and long axis. The measurements conducted allowed for an automatic calculation of the left ventricular ejection fraction (LVEF) and of the fraction shortening (FS). The widths of the aorta and of the left atrium, as well as the ratio of the two parameters, were estimated in the short axis view of the heart base. Eleven Doberman Pinschers with DCM were treated due to heart failure for a period of 6 to 24 months at the Cardiology Clinic, Department of Internal and Parasitic Diseases with Clinic for Horses, Dogs and Cats at the Wroclaw University of Environmental and Life Sciences. Ten of these dogs were subjected to euthanasia due to a rapid deterioration in the heart, which developed despite treatment. One of the dogs died during a clinical examination, due to the abrupt cessation of the heart function induced by arrhythmia. Four dogs came to the Cardiology Clinic with advanced signs of heart failure and, following the diagnosis of DCM and informing the owners as to the prognosis, they were subjected to euthanasia. The euthanasia was followed by an autopsy, and myocardium samples from the right and left ventricles, the intraventricule septum, and the right and left atrium were collected for the evaluation of desmin expression by the immunohistochemical technique. The tissue samples were fixed in a buffered 7% formalin, dehydrated, and then embedded in paraffin blocks. Four micrometre-thick sections were placed on Superfrost type microscopical glasses (Menzel Gläser, Germany) and the activity of the endogenous peroxidase was blocked using 3% H₂O₂. Subsequently, the paraffin sections were subjected to boiling in a microwave oven (250 W, 15min) in the Antigen Retrieval Solution to unblock the antigenic determinants. For the detection of the desmin expression in the paraffin sections, mouse monoclonal antibodies (clone DE-R-11, DakoCytomation, Denmark) diluted 1:50 were used. The studied antigens were visualised using the LSAB2 reagent set and diaminobenzidine (DAB). Every test was accompanied by a negative control in which the Primary Negative Control was used. All the reagents came from DakoCytomation, Denmark.

The obtained preparations were photographed under a microscope and the microphotographs were subjected to a computer-assisted image analysis on a stand consisting of a computer coupled to an Axiosph model microscope (Carl Zeiss). The set has the capacity to record and digitally analyse images. The measurements were taken using MultiScaneBase V 14.02 software with Windows.

The desmin expression was evaluated using a modified semiquantitative IRS Remmele scale (Table 1) (14). The method takes into account both the percentage of positive cells (A) and the intensity of the colour reaction (B), and the final result represents the product of the two parameters (A x B).

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 pts – no cells with positive reaction</td>
<td>0 pts – no colour reaction</td>
</tr>
<tr>
<td>1 pt – to 10% cells with positive reaction</td>
<td>1 pt – low intensity of colour reaction</td>
</tr>
<tr>
<td>2 pts – 11%-50% cells with positive reaction</td>
<td>2 pts – moderate intensity of colour reaction</td>
</tr>
<tr>
<td>3 pts – 51%-80% cells with positive reaction</td>
<td>3 pts – intense colour reaction</td>
</tr>
<tr>
<td>4 pts – &gt; 80% cells with positive reaction</td>
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</table>

Table 1

Semiquantitative IRS Remmele scale for the evaluation of the desmin expression. The Remmele scale takes into account both the percentage of positive cells (A) and the intensity of the colour reaction (B), and the final score represents the product of the two parameters (A x B).
Table 2
Echocardiographic parameters in the dogs studied

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVESd (mm)</td>
<td>6.84 ± 0.22</td>
</tr>
<tr>
<td>LVEDd (mm)</td>
<td>6.47 ± 0.31</td>
</tr>
<tr>
<td>LVPWs (mm)</td>
<td>1.0 ± 0.12</td>
</tr>
<tr>
<td>LVPWs (mm)</td>
<td>1.13 ± 0.09</td>
</tr>
<tr>
<td>IVSd (mm)</td>
<td>1.21 ± 0.08</td>
</tr>
<tr>
<td>IVSs (mm)</td>
<td>1.24 ± 0.11</td>
</tr>
<tr>
<td>EF(%)</td>
<td>13.4 ± 5.41</td>
</tr>
<tr>
<td>FS(%)</td>
<td>6.1 ± 6.46</td>
</tr>
<tr>
<td>Ao (mm)</td>
<td>2.0 ± 0.22</td>
</tr>
<tr>
<td>LA (mm)</td>
<td>5.9 ± 0.38</td>
</tr>
</tbody>
</table>

Fig. 1. Third degree atrio-ventricular block with a ventricular escape rhythm of ~40/min and a paroxysmal of multiform ventricular tachycardia. 1mV=10 mm, paper feed of 50 mm/s.

Fig. 2a. Dilation of cardiac cavities in the echocardiographic examination confirming the DCM diagnosis. (a) Dilation of the left atrium. Right parasternal long axis view.

Fig. 2b. Dilation of the left ventricular lumen, thinning of the left ventricular walls, poor contractility, atrial fibrillation. Right parasternal short axis view.

Fig. 3. Moderately intense (++) expression of desmin in the cardiomyocytes of a healthy dog. 400x.

Fig. 4. High (+++) expression of desmin in the cardiomyocytes of a dog affected with DCM. 200x.

Results

In the twelve dogs with DCM, the ECG examination conducted before euthanasia showed atrial fibrillation with a rapid ventricular action of 200-240/min; the remaining two dogs manifested a sinus rhythm of 150-180/min, with numerous ventricular extrasystoles and attacks of nonstable monomorphic ventricular tachycardia (nsVT). In one of the dogs, the ECG examination recorded a third degree atrio-ventricular block with a ventricular escape rhythm of ~40/min, and paroxysmal multiform ventricular
tachycardia (Fig. 1). The recorded rhythm disturbances represented the probable cause of death shortly after the examination. In the dogs with a preserved sinus rhythm, the duration of the P wave was 0.055 ± 0.01 s, the amplitude of the P waves was 0.55 ± 0.1 mV, and the duration of the PQ was 0.1 s. In all the dogs with DCM, the duration of the QRS complex was 0.08 ± 0.012 s, the amplitude of the R waves was 2.84 ± 0.3 mV, and the duration of the ST-T segment was 0.23 ± 0.02 s.

In all the dogs with DCM, the echocardiographic examination demonstrated a dilation of the heart cavities and the poor contractility of the left ventricle (Table 2, Fig. 2).

In the dogs examined, no deviations were shown in the blood’s morphological and biochemical parameters.

The expression of desmin from the myocardial samples according to the Remmele scale equalled 4 pts in the control group, with a relatively uniform distribution (Fig. 3). In all the dogs with DCM, the disturbances of various extent were noted in the desmin expression, both in its intensity and distribution in the studied regions. In 10/15 dogs with DCM, the desmin expression was elevated (6-8 pts on the Remmele scale) (Fig. 4). In 5/15 dogs with DCM, the desmin expression showed no quantitative alterations; however, there were zones with elevated and decreased protein expressions. The elevated expression of desmin was manifested in the dogs, which suffered longer from cardiac insufficiency (the dogs treated due to DCM-related heart failure), and the need for euthanasia reflected the increasingly severe pulmonary oedema. A normal amount of desmin with an uneven expression was noted in the untreated dogs subjected to euthanasia due to DCM-induced heart failure, and in the dog, which died due to arrhythmias.

Discussion

Desmin plays an important role in the cell and any alterations in its amount and/or distribution may be related to dilated cardiomyopathy. In the study conducted, an increased expression of desmin was identified in 10/15 dogs with DCM. The results are consistent with studies conducted on dogs with cardiac insufficiency secondary to DCM, and on humans with heart failure (HF). In studies on dogs with experimental HF induced by acute myocardial ischaemia resulting from the microembolisation of coronary vessels (the left ventricular ejection fraction of 20-25%), an augmented expression of desmin was noted, particularly in the regions of accentuated fibrosis (15). The studies also demonstrated the disorganisation of cytoskeleton proteins. In certain regions, the disorganisation was linked to the loss of myofilaments. The authors have suggested that such a pattern corresponded to the cardiomyocyte remodelling process and changes in the structural proteins, including changes in the desmin content, and that it promoted the development of left-ventricular insufficiency (15). Furthermore, in the dogs with the experimental tachycardiomyopathy and with the arrhythmogenic right ventricular dysplasia, increased amounts of desmin and changes in cytoskeleton proteins were detected (8, 11). Augmented amounts of desmin were also described in Portuguese water dog puppies, with a recessive form of taurin-dependent dilated cardiomyopathy (2). It should be added that an abnormal expression of desmin was also detected and confirmed in humans with heart failure induced by ischaemia, inflammation or tachyarrhythmias (13). Moreover, studies by Stabej et al. (16) excluded the possibility of a disturbed expression of desmin in Doberman Pinschers with DCM, which was, however, observed in the present study (16). Therefore, the changes observed in this study are thought to be related to the developing heart failure. The changes, which induce HF, independently of the cause, are associated with the accumulation of desmin, and are encountered in all types of cardiomyopathy, i.e. in hypertrophic, restrictive or dilation cardiomyopathy (1, 9). The problem of whether the accumulation of desmin represents the cause or the consequence of the disease still remains a matter of dispute. Our study and data from the literature indicate that the accumulation of tubulin, desmin, and membrane-associated proteins represents a compensatory mechanism, typical for heart injury, independent of the type of the principal cardiac disease. The analyses of the accessible studies argue for two stages in the transition from cardiac hypertrophy to cardiac insufficiency: an early one in which the accumulation of cytoskeletal proteins results due to the increasing tension in the myocardium, and a second one, involving the irreversible loss of contractile fibres in the presence of an increased density of microtubules and desmin disorganisation. Such a process may be advantageous, as it compensates for the loss of myofilaments but, in parallel, it may exert a deleterious effect on the cells because it overloads the myocardial cells in dogs, which already have damaged cells (8). Our results point to the role of desmin in the development of cardiac insufficiency. In dogs treated due to DCM, the expression of desmin increased, which is consistent with the theory that the protein accumulates in response to increasing myocardial tension. Despite the abrupt deterioration of cardiac insufficiency, no desmin disorganisation was noted in the dogs due to the short period separating the time of decompensation from death. Dogs, which demonstrated no increase in the desmin content might have already reached the stage of microtubule and desmin disorganisation, since a non-uniform expression of desmin has been observed. The observations confirm that the disturbances in desmin, the basic cytoskeleton protein responsible for the normal function of a muscle cell, may provide an interesting marker of the advancement in cardiomyocyte damage. This may directly affect the prognosis.

In conclusion, immunohistochemistry allows for a rapid and effective evaluation of the desmin expression in the cardiomyocytes of dogs with DCM. The expression of desmin is disturbed in myocardium samples of dogs with DCM. The relationship between the desmin expression and the advancement of cardiac insufficiency deserves further studies.
References


