Pathological Features of Arrhythmogenic Right Ventricular Cardiomyopathy in Middle-Aged Dogs

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ABSTRACT. The hearts of four dogs (a 4-year-old Shetland sheepdog, a 4-year-old Labrador retriever, a 5-year-old English Bulldog, and a 6-year-old Dalmatian; three males and one female), that had died suddenly and had been clinically diagnosed as having arrhythmogenic right ventricular cardiomyopathy (ARVC), were studied post mortem. At the cut surface, all four hearts showed mild to moderate hypertrophy of the left and right ventricular free walls and ventricular septum, with grayish-white tissue replacement of the myocardium to various degrees. Histologically, all had typical right ventricular features of ARVC and morphological evidence of left ventricular and ventricular septal involvement. Two main histological patterns were identified: a fatty type (two cases) and a fibrofatty type (two cases). With either type, myocardial replacement by fatty or fibrofatty tissue were detected in both ventricles, but were more severe in the right ventricle, where they usually became transmural. Furthermore, this myocardial replacement was more severely seen in the epicardium and midmyocardium; the endomyocardium was less severely affected. On the basis of the present observation, it is evident that, in dogs, the disease process of ARVC affects both the right and left ventricles, although the striking pathological feature is right ventricular involvement. The pathological evidence of biventricular involvement in these canine cases of ARVC may represent a wider spectrum of the disease than has previously been recognized, suggesting that, in dogs, this disease should no longer be considered as limited to the right ventricle.

KEY WORDS: arrhythmogenic right ventricular cardiomyopathy, canine, sudden death, ventricular tachycardia.

FULL PAPER

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Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a primary heart muscle disease characterized in humans by progressive fatty or fibrofatty replacement of the right ventricular myocardium, initially within a typical region, and later with global right and, sometimes, left ventricular involvement [3, 11, 20, 23]. These structural changes account for the electrical instability of the right ventricle and could provide the substrate for reentrant circuits [23]. Patients typically suffer lethal ventricular arrhythmias and sudden death, or less commonly develop systolic dysfunction of the right ventricle and congestive right-sided heart failure [5, 6, 9, 12, 23]. Cardiac sudden death usually occurs during physical exertion [5, 9, 23].

Although the etiology and pathogenesis of the condition remain to be established, several causes have been tentatively suggested in humans. Autosomal recessive forms of ARVC (e.g., Nagos and Carvajal syndromes caused by mutations in genes encoding plakoglobin and desmoplakin, respectively) are recognized, but the majority of cases are considered to be caused by autosomal dominantly inherited mutations in genes encoding plakophilin 2 and other proteins of the desmosome of cardiomyocytes [7, 16]. Mutations in the TGF-β and ryanodine receptor 2 genes may be associated with an ARVC phenotype [7, 16].

In the early 1980s, Harpster first described a unique myo-cardial disease in Boxer dogs, which was characterized by fatty or fibrofatty replacement of the right ventricular myocardium and named ‘Boxer dog cardiomyopathy’ [13]. Since then, careful evaluation of the disease has demonstrated that it has striking similarities to ARVC in humans, and therefore it has been reclassified as ‘Boxer ARVC’ [17]. Meanwhile, five isolated cases of ARVC have been reported in young to middle-aged dogs except for Boxer dogs: a 7-month-old Siberian husky [8], an 8-month-old Labrador retriever [18], an 18-month-old Bull mastiff [4], a 3-year-old Dachshund [22], and a 5-year-old Bull dog [21]. These dogs developed congestive right-sided heart failure or suffered lethal ventricular arrhythmias and sudden cardiac death. On histological examination, fatty or fibrofatty, segmental or diffuse replacement of the myocardium with hypertrophy and degeneration of the remaining myocytes were observed within the right ventricular wall.

The purpose of the present study was to characterize the pathological features of this condition occurring in various breeds of middle-aged dogs, except for Boxer dogs.

MATERIALS AND METHODS

Dogs: The hearts of four dogs (cases 1 to 4) that had died suddenly and had been clinically diagnosed as having ARVC were sent to our laboratory for detailed morphological study. The four dogs comprised of three males (case 1, a 4-year-old Shetland sheepdog; case 2, a 4-year-old Labrador retriever; case 3, a 5-year-old English Bulldog) and one female (case 4, a 6-year-old Dalmatian). At necropsy, thor-
ough examination had excluded any extracardiac causes of sudden death.

**Gross examination and histopathology:** Gross examination of the heart following fixation in formaldehyde included a measurement of heart weight. The heart was serially cross-sectioned from the atrioventricular valves to the apex. For histological examination, myocardial samples were obtained from a transverse section through the entire heart at the level of the midventricular wall. Full-thickness tissue blocks were taken from the anterior, lateral, and posterior left ventricle; anterior, lateral, and posterior right ventricle; and anterior, medial, and posterior ventricular septum. In addition, 10 sections were taken from the atria transversely across the atrial appendages.

Tissue sections were embedded in paraffin wax, sectioned at a thickness of 5 µm, and stained with hematoxylin and eosin (HE) and Masson’s trichrome for light microscopic examination. To evaluate myocardial inflammatory infiltration immunohistochemically, the avidin-biotin-peroxidase method (Vectastain; Vector Laboratories, Burlingame, CA, U.S.A.) was employed on paraffin-wax sections of the heart, with monoclonal antibodies (Dako, Santa Barbara, CA, U.S.A.) directed against T lymphocytes (CD3), B lymphocytes (CD79α), and macrophages (lysozyme).

**RESULTS**

**Clinical findings:** The main clinical information for the present four canine cases of ARVC was as follows. All the dogs had suffered sudden and unexpected death (during excitement or startle in cases 1 and 3, and during exercise in cases 2 and 4). The duration of the clinical disease ranged from a few days (cases 1, 2 and 3) to several months (case 4). For cases 3 and 4, there was a history of sudden death occurring in the family pedigree. There was no significant family history in case 2, and no detailed family history was available in case 1.

All the dogs had shown symptoms: syncope in two (cases 2 and 4), collapse in one (case 3), and collapse and dyspnea in 1 (case 1). Syncope or collapse was usually associated with physical exertion. Electrocardiograms (standard limb leads) were available for all cases, and these commonly showed repetitive non-sustained monomorphic ventricular tachycardia with left bundle branch block morphology (Fig. 1). Sinus beats showed a normal QRS complex configuration, with prolonged duration (0.08–0.12 s) and slurring in lead II.

**Macroscopical findings:** The heart weight in these ARVC cases ranged from 68 to 285 g (mean ± SD 193.9 ± 88.6 g); the mean relative heart weight (i.e., heart weight in g/body weight in kg) was greater (9.7 ± 2.0 g/kg) than that in normal dogs (7.7 ± 0.6 g/kg) [14]. The hearts were enlarged and globular in shape in all cases, and focal or diffuse subepicardial grayish-white infiltrates were observed from the exterior in cases 1 and 2. At the cut surface, all four hearts showed mild to moderate hypertrophy of the left and right ventricular free walls and ventricular septum, with grayish-white tissue replacement of the atrial and ventricular walls to various degrees (Fig. 2). The grayish-white tissue appeared to be an extension of the epicardium towards the endocardium in the right and left ventricles. The changes were patchy or multifocal and distributed across the full thickness of the wall, extensively affecting areas located between the base and the apex. The ventricular septum was affected mainly in the right half in cases 1, 2 and 4 (Fig. 2), but ventricular septum involvement was not obvious in case 3. There was no anatomic valvular involvement, or evidence of congenital heart disease.

**Microscopical findings:** Two main histological patterns were identified in the hearts of these four dogs; a fatty type or a fibrofatty type (Fig. 3). With either type, myocardial replacement by fatty or fibrofatty tissue were detected in the left and right ventricular free walls and ventricular septum (Figs. 4 and 5), but were more evident in the right ventricle, where they usually became transmural. Furthermore, myocardial replacement was more severe in the epimyocardium and midmyocardium; the endomyocardium was less severely affected. Myocyte loss with fatty or fibrofatty replacement was observed to various degrees in the left and right atrial walls in all cases.

The fatty type lesions were characterized by substantial myocardial replacement with mature adipose cells, often in association with little fibrosis (Fig. 3A). This type of lesions was detected in cases 1 and 2. The lesions of fatty replacement of the myocardium were distributed diffusely in the left and right ventricular free walls, with variously sized islands of persisting muscular tissue within the regions of the fatty tissue (Figs. 4A and 4C). The fatty tissue was con-
tiguous with the preexisting epicardial fat and infiltrated from the epicardium toward the endocardium. In the ventricular septum, fatty replacement was focal or patchy and present on in the right third to half (Fig. 4B).

The fibrofatty type lesions consisted of focal or diffuse myocardial replacement with interstitial fibrosis accompanied by fatty tissue infiltration (Fig. 3B). This type of lesions was detected in cases 3 and 4. These lesions had a band-like or laminar appearance with almost confluent transmural areas of involvement in the right ventricular free wall (Fig. 5C). They were focal or patchy and distributed across the full thickness of the left ventricular free wall (Fig. 5A) and mainly in the right half of the ventricular septum (Fig. 5B). Residual myocytes were embedded within the regions of fibrofatty tissue, showing various degrees of hypertrophy and degeneration. There was obvious continuity between fibrofatty replacement of the myocardium in the right ventricular free wall and that in the right side of the ventricular septum. Focal or patchy inflammatory mononuclear cell infiltration (more than 10 cells per focus), consisting of large numbers of CD79α-positive cells and occasional CD3- or lysozyme-positive cells, associated with myocyte death and dropout, were identified in both ventricular free walls in these cases. Such lesions of myocarditis were observed most frequently adjacent to the region of fibrofatty replacement (Fig. 6).

Fig. 2. Longitudinal section of the heart of the 6-year-old Dalmatian (case 4), showing grayish-white tissue replacement of the ventricular myocardium to various degrees.

Fig. 3. (A) Right ventricular myocardium from the 4-year-old Shetland sheepdog (case 1), showing fatty replacement with clusters of myocytes entrapped within the fatty tissue. Masson’s trichrome. Bar=80 µm. (B) Right ventricular myocardium from the 5-year-old Bulldog (case 3), showing fibrofatty replacement with interstitial fibrosis. Masson’s trichrome. Bar=80 µm.

Fig. 4. Histological sections of the left ventricle (A), ventricular septum (B) and right ventricle (C) from the 4-year-old Shetland sheepdog (case 1), showing myocardial replacement by fatty tissue. In the ventricular septum, the areas of fatty replacement are located mainly in the right half (top). Masson’s trichrome. Bar=1 mm.
DISCUSSION

Based on the clinical and histopathological similarities of human ARVC, the heart of our four cases were diagnosed as canine ARVC [7]. Extensive loss of myocytes with replacement by fatty or fibrofatty tissue in the right ventricle was a characteristic feature in the four dogs that had shown syncope or collapse, monomorphic ventricular tachycardia with left bundle branch block morphology, and sudden death. Structural change of the myocardium was also detected in the left ventricle and ventricular septum, but was less severe than in the right ventricle. There were no apparent differences in the clinical features among the four cases. The pathological hallmark of human ARVC is fatty or fibrofatty replacement of the right ventricular myocardium, which is usually associated with ventricular arrhythmias and sudden death [10, 23, 24]. Left ventricular involvement has been also reported in human ARVC, and the biventricular nature of this cardiomyopathy has been assessed on the basis of pathologic background [3, 11, 15, 19]. The combined clinical profile (syncope or collapse, ventricular tachycardia of suspected right ventricular origin, and sudden death) and histopathological findings (fatty or fibrofatty replacement of the myocardium mainly in the right ventricle) provide compelling evidence that these four cases were consistent with ARVC, and are not shared by any other cardiomyopathies including hypertrophic and dilated types.

Canine ARVC has been previously described in five isolated case reports [4, 8, 18, 21, 22] as well as two large case series in Boxer dogs [1, 17]. These reported cases included young to middle-aged three male dogs (Dachshund, Bull mastiff and Siberian husky) [4, 8, 16] and two females (Labrador retriever and Bulldog) [18, 21]. Postmortem examination was performed in four of the five cases, and all four dogs revealed myocardial replacement by fibrofatty tissue [4, 8, 21, 22]. In the Bull mastiff, the right ventricle and atrium were partially affected [4]. In the Dachshund, generalized thinning of the right ventricle was evident and myocardial replacement associated with mononuclear cell inflammatory infiltration was present in the right ventricle and ventricular septum [22]. In the Siberian husky, the right ventricle showed myocardial replacement and the most severe lesions were located in the same area as the human ‘triangle of dysplasia’ comprising the right ventricular inflow, outflow, and apex [2, 23]. The left ventricle and ventricular septum were also affected, but less severely than the right ventricle [8]. In the Bulldog, only the distal part of the right ventricular outflow tract showed transmural replacement with myocardial atrophy [21]. In Boxer dogs, ARVC is a primary familial myocardial disease apparently
inherited as an autosomal dominant trait [17]. In a case series of 23 Boxer dogs with ARVC comprising 12 males and 11 females aged 4.5 to 13.7 years (mean 9.1 ± 2.3 years), severe myocyte loss with replacement by fatty (n=15) or fibrofatty (n=8) tissue was consistently present in the right ventricle. Focal fibrofatty lesions were also present in both atria (n=8) and the left ventricle (n=11). In addition, myocarditis was evident in the right ventricle (n=14) and left ventricle (n=16) [1].

In the current study, ARVC occurred in middle-aged dogs of various breeds except for Boxer dogs (three males and one female). Extensive fatty (n=2) or fibrofatty (n=2) substitution was consistently observed in the right ventricle. The left ventricle and ventricular septum and both atria were also involved, although less intensively than the right ventricle. Lymphocytic myocarditis was detected in both two cases showing fibrofatty type lesions, but not in the two cases showing fatty type lesions. Based on the canine cases reported in the literature and the present four cases, it is considered that ARVC may show different morphological aspects, with constant alteration of the right ventricle and quite a variable degree of extension to the left ventricle and ventricular septum. This may be related to a genetic mechanism of transmission with differences in phenotypic expression [3]. However, there were different descriptions with regard to myocardial damages, including myocarditis, among the previous cases. This may reflect a very wide spectrum of disease and/or the possibility of superimposition of different etiological agents [3].

In human ARVC, it is believed that fibrofatty replacement of the myocardium reflects a healing process in the setting of chronic myocarditis [11, 25]. The disappearance of the ventricular myocardium might be a consequence of inflammatory necrotic injury followed by fibrofatty repair [11, 25]. Whether the inflammation is a primary event or a reaction to myocyte cell death is unresolved [2]. In the present series, mononuclear cell inflammatory infiltration was detected in both two cases showing fibrofatty replacement, but not in the two cases showing fatty replacement. Although the precise role of myocarditis in the pathogenesis of ARVC remains unclear, these findings support the view that the inflammatory process modulates the morphology and progression of at least the fibrofatty type lesions [1, 11].

Human ARVC is defined histologically by the presence of progressive replacement of the so-called ‘triangle of dysplasia’ in the right ventricle [2, 23]. Although the condition of ARVC is believed to be a selective disorder involving the right ventricle, concomitant involvement of the left ventricle and ventricular septum has been reported in numerous instances [3, 11, 20, 23]. Thus several authors [3, 11] have proposed that ARVC should simply be termed “arrhythmogenic right ventricular cardiomyopathy”. On the basis of the present observation, it is evident that, in dogs, the disease process of ARVC affects both the right and left ventricles, although the striking pathological feature is right ventricular involvement. The pathological evidence of biventricular involvement in the present canine cases of ARVC may reflect a wider spectrum of the disease than has previously been recognized, suggesting that the disease should no longer be considered as limited to the right ventricle.

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