Assessment of plasma brain natriuretic peptide concentration in Boxers with arrhythmogenic right ventricular cardiomyopathy

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Objective—To determine whether Boxers with a clinical diagnosis of arrhythmogenic right ventricular cardiomyopathy (ARVC) have increased plasma concentrations of brain natriuretic peptide (BNP), compared with concentrations in clinically normal dogs.

Animals—13 Boxers with ARVC, 9 clinically normal Boxers, 10 clinically normal non-Boxer dogs, and 5 hound dogs with systolic dysfunction.

Procedure—All Boxers were evaluated via 24-hour ambulatory electrocardiography and echocardiography; the number of ventricular premature contractions (VPCs) per 24 hours was assessed. Hound dogs with cardiac pacing-induced systolic dysfunction (positive control dogs) and clinically normal non-Boxer dogs (negative control dogs) were evaluated echocardiographically. Three milliliters of blood was collected from each dog for measurement of plasma BNP concentration by use of a radioimmunoassay.

Results—Mean ± SD plasma BNP concentration for the ARVC-affected Boxers, clinically normal Boxers, negative control dogs, and positive control dogs was 11.0 ± 4.6 pg/mL, 79 ± 3.2 pg/mL, 115 ± 4.9 pg/mL, and 100.8 ± 56.8 pg/mL, respectively. Compared with findings in the positive control group, plasma BNP concentration in each of the other 3 groups was significantly different. There was no significant difference in BNP concentration between the 2 groups of Boxers. A significant correlation between plasma BNP concentration and number of VPCs per 24 hours in the ARVC-affected Boxers was not identified.

Conclusions and Clinical Relevance—A significant difference in BNP concentration between Boxers with ARVC and clinically normal Boxers was not identified. Results suggest that BNP concentration may not be an indicator of ARVC in Boxers. (Am J Vet Res 2005;66:2086–2089)

Brain natriuretic peptide (BNP) is secreted in response to stretching of the myocytes in the ventricles of the heart (with a minor portion secreted from the atria); in humans with right or left ventricular dysfunction, plasma concentrations of BNP are increased, compared with concentrations in clinically normal individuals.5-8 Brain natriuretic peptide has natriuretic, diuretic, hypotensive, and smooth muscle relaxant effects.11,12 Humans with ARVC have high plasma concentrations of BNP that correlate with right ventricular ejection fraction measurements obtained via computed tomography.12 Additionally, BNP immunoreactivity has been detected in endomyocardial biopsy specimens from ARVC-affected humans but not in specimens collected from individuals with idiopathic right ventricular outflow tract tachycardia.13,14 Augmented expression of BNP in ventricular myocytes has been detected in areas of interstitial fibrosis located in the perivascular regions and subendocardium of the left ventricle in humans with dilated cardiomyopathy.15 It has been suggested that measurement of plasma BNP concentration may be useful for detection and assessment of the severity of ARVC in humans.12 Compared with clinically normal dogs, plasma BNP concentration is high in dogs with experimentally induced heart failure14,15 and acquired valvular endocardiosis and in dogs with Golden Retriever muscular dystrophy cardiomyopathy.16,17 The purpose of the study reported here was to compare plasma concentrations of BNP in Boxers with ARVC with concentrations detected in clinically normal Boxers and non-Boxer dogs and dogs with cardiac pacing-induced systolic dysfunction. We hypothesized that Boxers with ARVC would have increased plasma BNP concentrations, compared with clinically normal Boxers.

Materials and Methods

Four groups of dogs were selected for evaluation. The groups of Boxers with ARVC and clinically normal Boxers were derived from a population of dogs that were enrolled in an ongoing study of ARVC. Adult hound dogs with myocardial failure and decreased fractional shortening that were enrolled in a long-term pacing study were selected as the positive control group. Ten clinically normal non-Boxer dogs (1 German Shepherd Dog, 1 Australian Shepherd, 1 Corgi, 1 Catahoula, 1 Labrador Retriever, 1 Beagle, and 4 mixed-breed dogs) were selected as the negative control group. All dogs were assessed via echocardiography.18 Each echocardiographic examination was conducted by use of a standard clinical technique and without sedation. Data including M-mode measurements of the left ventricular internal diastolic diameter and left ventricular internal systolic diameter were obtained. Boxers with abnormal left ventricular fractional shortening (< 25%), increased left ventricular chamber size with respect to body size, or notable valvular regurgitation were excluded from the study.19

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Echocardiographic recordings were made with a simultaneous ECG, and all raw data were captured digitally to maintain optimal fidelity for off-line measurement.

Boxers that were currently receiving antiarrhythmic medication were not excluded from the study. Seven dogs in the ARVC-affected category were being treated with antiarrhythmic medications. Drugs and the mean ± SD dosages administered were as follows: sotalol (n = 4), 2.56 ± 0.45 mg/kg, PO, every 12 hours; mexiletine and atenolol (2), 6.31 ± 0.76 mg/kg, PO, every 8 hours and 0.53 ± 0.06 mg/kg, PO, every 12 hours, respectively; and sotalol and propranolol (1), 2.93 mg/kg, PO, every 12 hours and 12.20 mg/kg, PO, every 8 hours, respectively. No inclusion or exclusion criterion was placed on the ARVC-affected Boxer group with regard to having had syncopal events, although a history of syncope was recorded for those dogs.

A blood sample (3 mL) was collected from a jugular vein of each dog and placed in polyethylene tubes with EDTA (1 mg of EDTA/mL of blood) and aprotinin® (500,000 U of aprotinin/mL of blood) at 0°C. Samples were then centrifuged at 1,600 × g for 15 minutes at 0°C, plasma was extracted and stored at −80°C for batch analysis. Samples were thawed only once, at the time of analysis. A commercially available canine specific BNP-32 radioimmunoassay kit was used following the assay recommendations for extraction and quantification of the peptide. All BNP assays were run in duplicate. The minimal detectable quantity of canine BNP in the assay is 1 pg/mL. A 3-channel ambulatory 24-hour ECG (AECD) system was attached to each Boxer immediately after drawing the blood sample. The monitor was removed after 25 hours. A technician under the guidance of a veterinary cardiologist analyzed AECD recordings by use of an analysis system. Any recordings that did not have at least 20 hours of readable data were excluded from analysis. Analysis of AECD data included determination of the number of ventricular premature complexes (VPCs) per 24 hours.

For purposes of analysis, dogs were allocated to 1 of 4 groups. The first group consisted of Boxers that were defined as affected with ARVC on the basis of ≥1,000 VPCs/24 h detected on a 24-hour AECD recording and echocardiographic parameters that were within reference limits. The second group consisted of Boxers that were defined as clinically normal on the basis of ≤6 VPCs/24 h detected on a 24-hour AECD recording and echocardiographic parameters that were within reference limits. The third group consisted of adult hound dogs with myocardial failure and a decreased (32% to 40%) fractional shortening; the fourth group consisted of Boxers that were defined as clinically normal on the basis of physical examination and echocardiographic examination findings. Dogs in this group were used as negative controls.

Statistical analysis—All calculations, statistical analyses, and graphing were done by use of commercial software. Descriptive statistics were obtained for plasma BNP concentrations, age, weight, and echocardiographic parameters for all 4 groups as well as total VPCs per 24 hours for the Boxer groups. A 1-way ANOVA on ranks and Dunn method of multiple comparisons were performed to assess differences in plasma BNP concentrations among the 4 groups. A Mann-Whitney rank sum test was performed to determine whether the numbers of VPCs per 24 hours between the clinically normal and ARVC-affected Boxer groups differed significantly. A 1-way ANOVA and Holm-Sidak method of multiple comparisons was performed to assess differences in fractional shortening between the 4 dog groups. A Pearson correlation was performed to determine whether the numbers of VPCs detected via AECD per 24 hours correlated with plasma BNP concentrations for the 2 Boxer groups. Significance was set at α = 0.05 for all statistical tests.

Results—Thirty-eight total dogs were evaluated including 10 clinically normal non-Boxer dogs, 5 hound dogs, and 23 Boxers. Thirteen ARVC-affected Boxers, 9 clinically normal Boxers, 5 adult hound dogs with systolic dysfunction, and 10 clinically normal non-Boxer dogs were included in the present study (Table 1). One ARVC-affected Boxer dog was excluded because of a low shortening fraction.

Mean ± SD fractional shortening for the ARVC-affected Boxers, clinically normal Boxers, negative control dogs, and positive control dogs was 31.9 ± 3.7%, 35.3 ± 5.5%, 33.1 ± 2.9%, and 11.8 ± 2.8%, respectively. A significant difference was detected in fractional shortening between the positive control group and each of the other 3 groups; however, a significant difference in fractional shortening was not identified between the ARVC-affected and clinically normal Boxer groups or between the negative control group and each of the Boxer groups. Mean ± SD left ventricular internal diastolic diameter for the ARVC-affected Boxers, clinically normal Boxers, negative controls, and positive control dogs was 4.0 ± 0.4, 5.4 ± 0.3, 3.7 ± 1.0, and 5.2 ± 0.7, respectively. Mean ± SD left ventricular internal systolic diameter for the ARVC-affected Boxers, clinically normal Boxers, negative controls, and positive control dogs was 2.9 ± 0.4, 2.4 ± 0.6, 2.4 ± 0.7, and 4.6 ± 0.6, respectively.

Table 1—Demographic data and descriptive statistics regarding age, weight, sex, and history of syncope in clinically normal non-Boxers (negative control group), clinically normal Boxers, Boxers with arrhythmogenic right ventricular cardiomyopathy (ARVC), and adult hound dogs with systolic dysfunction (positive control group) in which plasma brain natriuretic peptide concentration was assessed.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Negative control dogs (n = 10)</th>
<th>Clinically normal Boxers (9)</th>
<th>ARVC-affected Boxers (13)</th>
<th>Positive control dogs (5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>2.8 ± (1.0–4.5)</td>
<td>3.8 ± (2.0–7.0)</td>
<td>6.6 ± (3.0–12.0)</td>
<td>2.1 ± (2.0–2.1)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>22.4 ± (4.6–41.0)</td>
<td>26.8 ± (21.9–34.7)</td>
<td>29.7 ± (25.0–41.0)</td>
<td>26.1 ± (24.2–28.4)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sexually intact males</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Neutered males</td>
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<td>0</td>
</tr>
<tr>
<td>Sexually intact females</td>
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<td>5</td>
<td>0</td>
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<tr>
<td>Neutered females</td>
<td>8</td>
<td>1</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>No. of dogs with history of syncope*</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>0</td>
</tr>
</tbody>
</table>

Age and weight values are given as mean (range).
*Syncopal history indicating at least 1 prior syncopal episode.
The median number (range) of VPCs detected via AECG groups. The number of VPCs per 24 hours for the ARVC-affected Boxers was significantly greater ($P < 0.001$) than the value for the clinically normal Boxers. Data were analyzed to determine whether the number of VPCs detected via AECG per 24 hours correlated with plasma BNP concentrations in the 2 Boxer groups. A significant correlation between plasma BNP concentration and number of VPCs per 24 hours for the ARVC-affected Boxers was not identified (correlation coefficient = 0.04; Figure 2).

**Discussion**

In the present study, Boxers with a clinical diagnosis of ARVC did not have increased plasma concentrations of BNP, compared with values in clinically normal Boxers. Investigation of plasma BNP concentration was performed because results of a study in humans with ARVC indicated that plasma BNP concentration was increased in ARVC-affected individuals but not in individuals with idiopathic right ventricular outflow tract tachycardia. Furthermore, endomyocardial biopsy specimens obtained from the right ventricles of ARVC-affected humans had strong BNP immunoreactivity in areas in which tissue was being replaced by fibrofatty material. Two mechanisms were hypothesized for this increase in plasma BNP concentration: local wall stress on myocytes around the atrophic area or secretion of vasoactive substance by fibroblasts, adipocytes, and vascular endothelium that would stimulate BNP release from myocytes. Brain natriuretic peptide synthesis and secretion is stimulated in vivo and in vitro by arginine, vasopressin, endothelin-1, and angiotensin II. Because Boxers with ARVC have histopathologic lesions similar to those detected in humans with ARVC, we hypothesized that Boxers would have similar increases in plasma BNP concentration, compared with clinically normal dogs. Our results do not mimic findings in humans despite reported similarities between the human and canine forms of ARVC. It is possible that the degree of fibrofatty infiltration and right ventricular dysfunction in the ARVC-affected Boxers was less than that in ARVC-affected humans and not severe enough to result in increases in plasma BNP concentration. Although fibrofatty infiltration has been previously reported in Boxers with ARVC, histologic evaluation was not specifically performed for the dogs in our study. Additionally, given the difficulty in assessing right ventricular function via echocardiography, this was not performed and the selection criterion was based on the detection of VPCs rather than on assessment of right ventricular function. However, a recent evaluation of Golden Retrievers with muscular dystrophy cardiomyopathy identified increases in plasma BNP concentration, compared with values in clinically normal dogs, even before echocardiographic evidence of dysfunction was detected. This may suggest that an increase in plasma BNP concentration in Boxers with ARVC might be expected even before echocardiographic changes are detectable. Finally, the small number of dogs in the sample populations of our study may have prevented the detection of significant differences.
It is possible that concurrent drug treatments could affect wall stress or vasoactive substances that may interfere with the release of BNP. Dogs that were currently receiving antiarrhythmic medication were not excluded from our study because it was assumed that the substrate (fibrofatty infiltrate) would not regress as a result of administration of β-adrenoreceptor blockers or class I antiarrhythmic agents and because of concerns for the well-being of the dogs. The effect of β-adrenoreceptor blockade associated with the antiarrhythmic medications on the expression of BNP from ventricular myocytes in dogs is not fully understood at this time.

Boxers with ARVC were selected on the basis of detection via AECG of ≥1,000 VPCs/24 h. This was a somewhat arbitrary cutoff for defining ARVC status. At present, it is not known what number of VPCs in Boxers represents an acceptable limit or defines clinical normalcy. However, in 1 study,21 the median number of VPCs per 24-hour period in clinically normal non-Boxer dogs was 2. The criterion of ≥1,000 VPCs/24 h was chosen for selection of the ARVC-affected Boxer group in the hope of inclusion of Boxers that would have convincing evidence of arrhythmic disease.

The positive control dogs were selected for inclusion in our study because previous studies of rapid pacing models in dogs and pigs have revealed increased plasma BNP concentrations, compared with clinically normal animals. We selected these dogs because we believed that they would likely have increased plasma concentrations of BNP, compared with clinically normal dogs. Increased plasma concentration of BNP would likely be detected if the BNP assay was done correctly, as opposed to using data from this group for comparison with that from the ARVC-affected Boxers.

A propeptide (N-terminal-proBNP) has been used recently as a marker of ventricular dysfunction in humans.22 It is not known at this time whether measurement of plasma N-terminal-proBNP concentration is more useful than measurement of plasma BNP concentration as an indicator of ventricular dysfunction in dogs. In humans, plasma N-terminal-proBNP concentration is influenced by both age and renal function.22 Our data did not indicate a significant increase in plasma BNP concentration in Boxers with ARVC, compared with the clinically normal Boxers or the non-Boxer dog groups. This may indicate that evaluation of plasma BNP concentration is not an accurate indicator of ARVC in Boxers or that it is perhaps useful only in dogs with disease that is more advanced than that of the Boxers in the present study. To determine whether the latter is correct, assessment of ventricular fibrofatty infiltration either noninvasively (eg, via magnetic resonance imaging) or invasively (eg, via endomyocardial biopsy procedures) may enable researchers to select populations of ARVC-affected Boxers in which disease progression is considerably advanced and in which increases in plasma BNP concentrations may therefore be detected.

References