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Serum big endothelin-1 as a clinical marker for cardiopulmonary and neoplastic diseases in dogs

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ABSTRACT

Aims: Many studies of human subjects have demonstrated the utility of assessing serum levels of endothelin-1 (ET-1) and big ET-1 as clinical biomarkers in cardiopulmonary and neoplastic diseases. In this study we explored the feasibility of using serum big ET-1 as a reliable veterinary marker in dogs with various cardiopulmonary and neoplastic diseases.

Main methods: Serum big ET-1 levels were measured by ELISA in dogs with cardiopulmonary ($n = 21$) and neoplastic diseases ($n = 57$). Dogs exhibiting cardiopulmonary disease were divided into two groups based on the velocity of tricuspid valve regurgitation ($3.0 > \text{m/s}$) measured by ultrasound: without and with pulmonary hypertension. Big ET-1 levels for the dogs with the diseases were compared with levels in normal healthy dogs ($n = 17$).

Key findings: Dogs with cardiopulmonary disease ($4.6 \pm 4.6 \text{ pmol/l}$) showed a significantly ($P < 0.01$) higher level of big ET-1 than healthy control dogs ($1.1 \pm 0.53 \text{ pmol/l}$). Serum levels in the dogs with pulmonary hypertension ($6.2 \pm 5.3 \text{ pmol/l}$) were significantly ($P < 0.01$) higher than those without pulmonary hypertension ($2.0 \pm 0.6 \text{ pmol/l}$). Dogs with hemangiosarcoma ($5.6 \pm 2.2 \text{ pmol/l}$), adenocarcinoma ($2.0 \pm 1.8 \text{ pmol/l}$), histiocytic sarcoma ($3.3 \pm 1.9 \text{ pmol/l}$), chondrosarcoma or osteosarcoma ($3.0 \pm 1.6 \text{ pmol/l}$) and hepatocellular carcinoma ($2.7 \pm 1.8 \text{ pmol/l}$) showed significantly ($P < 0.05$) higher levels than healthy control dogs.

Significance: These findings point to the potential of serum big ET-1 as a clinical marker for cardiopulmonary and neoplastic diseases in dogs.

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Introduction

There have been many studies reporting the importance of evaluating endothelin-1 (ET-1) or big ET-1 in blood as a clinical marker for various diseases, especially cardiopulmonary and neoplastic diseases in human patients (Simpson et al., 2000; Yildirim et al., 2008; Ishikawa et al., 1995a, 1995b; Miyauchi et al., 1989). In cardiopulmonary diseases including cardiac valvular insufficiency, cardiomyopathy and primary pulmonary hypertension, ET-1 and big ET-1 blood levels have been reported to correlate with disease severity (Ishikawa et al., 1995a, 1995b; Miyauchi et al., 1989). In patients with tumors including breast, hepatocellular, gastric and prostate cancer, blood levels have been shown to elevate at an advanced stage with distant metastasis to the lymph nodes or lungs (Yildirim et al., 2008; Nakamuta et al., 1993; Ferrari-Bravo et al., 2000; Nelson et al., 1995). Prompted by these findings, clinicians have investigated the feasibility of using blood ET-1 and big ET-1 levels as clinical markers for predicting prognosis, evaluating

therapeutic benefit and diagnosing these diseases (Yildirim et al., 2008; Nakamuta et al., 1993; Ferrari-Bravo et al., 2000; Nelson et al., 1995). In veterinary medicine, however, there is less information on the clinical significance of blood ET-1 and big ET-1 levels.

In this study we investigated the potential of big ET-1 as a clinical marker for canine cardiopulmonary and neoplastic diseases by comparing serum levels in canine patients diagnosed with cardiopulmonary or neoplastic disease through clinical and pathological examinations. Serum levels of N-terminal pro-brain natriuretic peptide (NT-proBNP), which is a reliable clinical marker for cardiac disease (Hori et al., 2012), was also measured and compared with big ET-1 levels in dogs with cardiopulmonary disease.

Materials and methods

Dogs

Twenty-one dogs (eight female and thirteen male, average age 7.8 ± 3.6 years old, age range 5–13 years old) with cardiopulmonary

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disease and 57 dogs with neoplastic disease (twenty five female and thirty two male, average age 8.9 ± 4.1 years old, age range 6–14 years old), diagnosed at Rakuno Gakuen University Veterinary Teaching Hospital during a period from April 2010 to July 2013, were enrolled in this study. Seventeen healthy dogs (eleven female and six male, average age 8.3 ± 3.4 years old, age range 4–10 years old) kept at Kitasato University or Rakuno Gakuen University were used as controls. There was no significant difference in the results based on age or sex among cardiopulmonary, neoplastic and control groups.

All experimental procedures in this study progressed under approval by ethics committees in Kitasato and Rakuno Gakuen Universities (ethics committee approval numbers 10-028 and VH24A13, respectively).

Diagnosis of cardiopulmonary and neoplastic diseases

Cardiopulmonary diseases were diagnosed by ultrasound with sector probes (3 to 7.5 MHz) in conjunction with a scanner (EUB-6500, HITACHI Medical Corporation, Tokyo, Japan) by three experienced veterinarians in accordance with commonly recognized ultrasound criteria. The cardiopulmonary disease studied was valvular (mitral and tricuspid valves) insufficiency (twenty-one cases). The presence of pulmonary hypertension was confirmed based on tricuspid valve regurgitation (TR) velocity ($3.0 > \text{m/s}$) in accordance with previous reports (Hori et al., 2012). Neoplastic diseases were diagnosed by histopathological examination using excised tumor tissues after surgery. The neoplastic diseases enrolled in this study were hemangiosarcomas (eight cases), malignant melanomas (eight cases), adenocarcinomas (nine cases), histiocytic sarcoma (six cases), lymphomas (seven cases), chondrosarcoma or osteosarcoma (four cases), mast cell tumors (four cases), transitional cell carcinomas (three cases), hepatocellular carcinomas (four cases), gastrointestinal stromal tumor (one case), granulosa cell tumor (one case), multiple myeloma (one case) and schwannoma (one case). Because a decrease in renal excretion lengthens the half-life of big ET-1 in blood (Rossi et al., 2013), dogs with renal disease were excluded from this study based on blood tests and urinalysis.

Serum big ET-1 levels

We prepared serum samples according to methods described in previous studies (Yildirim et al., 2008; Rossi et al., 2013; Gruber et al., 2000; Glaus et al., 2004). Briefly, venous blood was collected in a serum separating plastic tube. Immediately after centrifugation at 1200 g for 10 min at 4 °C, serum samples were stored at –80 °C until measurement.

Serum big ET-1 concentrations were assessed with a commercial ELISA kit for human big ET-1 (Big ET-1 EIA Kit, IBL, Japan) after solid phase extraction using Sep-Pak C18 cartridges (Waters, USA) according to the manufacturer's protocol. This assay kit is demonstrated by the manufacturer to have no significant cross-reactivity (<0.1%) with ET-1, ET-2, ET-3, big ET-2 or big ET-3. To show the feasibility of using a human ELISA kit for estimation of canine big ET-1, we performed a dilution experiment (2^n dilution) using canine serum samples. The resulting curves obtained from the dilution experiment were parallel to the standard curve. Linearity of the curves was observed between 0.18 and 23 pmol/l. To evaluate the intra-assay and inter-assay coefficients of variance, we measured three samples five times for three alternating days. The intra-assay and inter-assay coefficients of variance were 3.9% and 2.5% respectively. Serum NT-proBNP levels were measured by a commercial laboratory (IDEXX Laboratories, Japan).

Statistical analysis

The Mann–Whitney *U* test was used to analyze differences in serum big ET-1 or NT-pro BNP levels with $P < 0.05$ considered significant. Spearman's rank correlation coefficient was used to analyze the correlation between serum big ET-1 levels and TR speeds. Data analyses were

carried out with Excel Toukei 2010 (SSRI, Tokyo, Japan). All data in this study are shown as mean \pm standard deviation.

Results

Serum NT-pro BNP and big ET-1 levels in cardiopulmonary disease

Dogs with cardiopulmonary disease were divided into two groups: without and with pulmonary hypertension based on TR velocity measured by ultrasound. No significant difference was observed in serum NT-pro BNP levels between the two cardiopulmonary disease groups: those without (848 ± 1018 pmol/l) and with (1398 ± 1746 pmol/l) pulmonary hypertension. In contrast, serum big ET-1 levels were significantly ($P < 0.01$) elevated in dogs with pulmonary hypertension (6.2 ± 5.3 pmol/l) compared to those without pulmonary hypertension (2.0 ± 0.6 pmol/l) (Fig. 1). Regardless of the presence or absence of pulmonary hypertension, dogs with cardiopulmonary disease (4.6 ± 4.6 pmol/l) showed a significantly ($P < 0.01$) higher level of big ET-1 than healthy controls (1.1 ± 0.49 pmol/l). Through correlation analysis between the serum levels of NT-pro BNP or big ET-1 and TR velocity values obtained by ultrasound, which represent systolic pulmonary artery pressure, big ET-1 levels ($R^2 = 0.74$, $P < 0.01$) were shown to correlate better with the severity of pulmonary hypertension than NT-pro BNP levels ($R^2 = 0.32$, $P = 0.24$). These results suggest that serum big ET-1 could be a more reliable clinical marker than serum NT-pro BNP for detecting cardiopulmonary disease with pulmonary hypertension in dogs.

Serum big ET-1 levels in neoplastic disease

To investigate the potential of big ET-1 as a tumor maker, we assessed serum levels in dogs exhibiting various tumors. Serum big ET-1 levels in dogs with hemangiosarcoma (5.6 ± 2.2 pmol/l), adenocarcinoma (2.0 ± 1.8 pmol/l), histiocytic sarcoma (3.3 ± 1.9 pmol/l), chondrosarcoma or osteosarcoma (3.0 ± 1.6 pmol/l) and hepatocellular carcinoma (2.7 ± 1.8 pmol/l) were significantly ($P < 0.05$) higher than those in healthy control dogs (1.1 ± 0.53 pmol/l) (Fig. 2). Interestingly, hemangiosarcoma, a tumor derived from vascular endothelial cells, produced the highest level of all tumors examined.

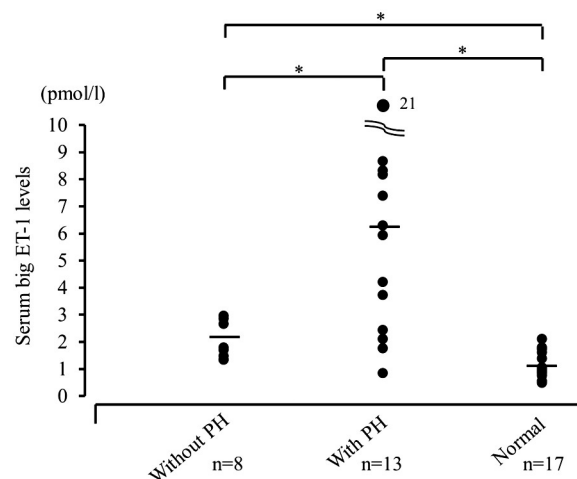


Fig. 1. Serum big ET-1 levels in dogs with cardiopulmonary diseases. Dogs with cardiopulmonary diseases were divided into two groups, dogs without and with pulmonary hypertension (PH), based on TR velocity measured by ultrasound. Serum big ET-1 levels were assessed in the groups without and with PH (groups with cardiopulmonary disease), compared to those of healthy control group. Serum big ET-1 levels in the dogs with the disease were significantly ($P < 0.01$) higher than those of control dogs. Significant ($P < 0.01$) and remarkable increase was observed in dogs with PH compared to the group without PH and healthy group. Each point represents an individual serum level. The horizontal lines represent the means for each group.

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