Blood Hyaluronic Acid as a Marker for Canine Cirrhosis

Hideyuki KANEMOTO1)*, Koichi OHNO1), Manabu SAKAI1), Ko NAKASHIMA1), Masashi TAKAHASHI1), Yasuhiro FUJINO1) and Hajime TSUJIMOTO1)

1)Department of Veterinary Internal Medicine, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1–1–1, Yayoi, Bunkyo-ku, Tokyo 113–8657 and 2)Department of Veterinary Medicine, College of Bioresource Sciences, Nihon University, 1866 Kameino, Fujisawa, Kanagawa 252–8510, Japan

(Received 27 February 2009/Accepted 17 May 2009)

ABSTRACT. Blood hyaluronic acid (HA) concentration was measured in dogs with various liver diseases to determine its relationship with histological fibrosis of the liver. The blood HA concentration significantly increased in dogs with chronic liver diseases compared with extrahepatic diseases and control. Furthermore, the median blood HA concentration in dogs with liver cirrhosis (500 μg/l; range, 151–1970 μg/l) was significantly higher than dogs with non-cirrhotic liver diseases (153 μg/l; range, 15–477 μg/l). In histochemical analysis, HA was distributed primarily in the fibrotic area in dogs with chronic liver diseases. As a conclusion, the blood HA concentration was significantly increased in dogs with chronic liver diseases, especially those with cirrhosis. Measurement of the blood HA levels of dogs with suspected liver disease can be a useful diagnostic aid for canine cirrhosis.

KEY WORDS: canine liver, fibrosis, hyaluronic acid.

NOTE

In the case of chronic hepatitis or other chronic liver parenchymal diseases in dogs, conventional diagnostic methods such as liver enzymes (e.g., alanine transaminase, alkaline phosphatase) and abdominal ultrasound provide some information, but these methods do not always reflect the severity of the disease itself [17]. Histopathological diagnosis is the only method that estimates the severity of the disease, which is evaluated mainly by the extent of fibrosis and the degree of inflammation. However, in the clinical setting, obtaining adequate biopsy samples from patients with liver disease is sometimes difficult due to risks related to anesthesia and hemorrhage, or due to the owner’s preferences. Furthermore, repeated liver biopsies are required to evaluate the progression of the disease and the efficacy of treatment for liver fibrosis. Similarly, liver cirrhosis, the end-stage of various liver diseases, is sometimes suspected based on symptoms or biochemical profile such as albumin or serum bile acid, but liver cirrhosis is confirmed only by liver biopsy. Hypoalbuminemia and ascites, frequently observed in dogs with cirrhosis, further complicate liver biopsy to perform. Noninvasive diagnostic methods for examining the severity of canine chronic parenchymal liver diseases, especially cirrhosis, are required.

There are a number of blood markers for fibrosis in human medicine. Among them, hyaluronic acid (HA) or hyaluronan in blood is one of the reliable markers for liver fibrosis in human medicine [6, 7, 10, 11, 13] and its clinical usefulness has been reported. HA is a component of glycosaminoglycan, a major fraction of the extracellular matrix. The concentration of HA in blood increases as fibrosis develops in patients with liver disease, especially in patients with liver cirrhosis [7, 10, 11, 13, 14]. Although the mechanism underlying this elevation in HA concentration is not fully understood, the synthesis of HA in activated hepatic stellate cells, the main fibrogenic cells in the diseased liver, and the reduced uptake of HA by the hepatic sinusoidal endothelial cells may play an important role [5, 8, 14, 15]. As the structure of HA is conserved among different species, the assay system used to measure human HA can be used to measure the HA of various species, including dogs [3, 18]. Altered HA concentration in blood has been reported to be related to various pathological conditions, especially osteoarthritis, in veterinary medicine [1–3, 9, 16, 18]. However, to our knowledge, there has been no report about the relationship of circulating HA levels and hepatic fibrosis in dogs.

In this study, we compared blood HA concentration of dogs with or without liver cirrhosis to assess the clinical usefulness of HA as a marker for cirrhosis of the canine liver. We also conducted histochemical analysis for HA in these liver specimens to support the idea that a diseased condition of the liver elevates the concentration of HA in blood.

According to the availability, plasma (n=7) or serum (n=18) and liver tissue samples were collected retrospectively from patients who were histologically diagnosed as having chronic liver parenchymal disease. Samples were obtained in Veterinary Medical Center of the Tokyo University, and Animal Medical Center in Nihon University from November 2005 to December 2008. Plasma samples obtained from healthy beagles were used as controls (n=9). All of the procedures were approved by the ethical committee in the Tokyo University and we cared these animals humanely. All the blood sample of dogs with liver disease was obtained before the biopsy procedure or at the first visit after the biopsy procedure in the morning on the empty
stomach. Plasma and serum samples were obtained by centrifugation of whole blood, and stored at −20°C before measurement. Patients were excluded from the study if the size of the biopsy specimens was too small for the evaluation of the degree of hepatic fibrosis. Patients with congenital portosystemic shunt diagnosed on the basis of computed tomography (CT) or abdominal ultrasound examinations were also omitted, because this disease is not a liver parenchymal disease and can be diagnosed clinically by these kind of imaging technique. The HA level was determined using a conventional automated analyzer and a latex-agglutination assay using HA-binding proteins (HABP) [4, 12] in a commercially available kit (Fujirebio, Tokyo, Japan). According to availability, TBA-80FR neo2 (TOSHIBA MEDICAL SYSTEMS CORPORATION, Tokyo, Japan) or BM6050 (JEOL limited, Tokyo, Japan) was used as automated analyzer. HA concentration of 51 samples of stored plasma of patients with extrahepatic disease which was obtained in the same manner as the liver disease group was also measured. The liver tissue samples were obtained from the patients by laparoscopic biopsy or excisional biopsy and were obtained from the control subjects by ultrasonography (US)-guided needle biopsy. At least 2 separate specimens were obtained in each case. The specimens were fixed with 10% neutral buffered formalin and were then embedded in paraffin. Sections of the liver tissues obtained from the patients and controls were stained with H & E (HE) and Sirius red to assess the degree of fibrosis. Histological diagnoses of the patients were parenchymal liver disease (chronic hepatitis (9), primary portal vein hypoplasia (6), neutrophilic cholangitis (1), and destructive cholangitis (1)), group 1, n=17) or cirrhosis (group 2, n=8). The median and the range of the group 1 and 2 were 6 (4–10) and 5.5 (1–11), respectively.

The median concentration of HA in the blood was 73 μg/l (19–232 μg/l) in control beagles; 153 μg/l (15–477 μg/l), group 1; 500 μg/l (151–1970 μg/l), group 2; 61 μg/l (18–296 μg/l), group 3 (Fig. 1). By non-parametric multiple comparison methods using the Steel-Dwass test, the blood HA concentration was significantly different between group 1 vs. 2 (P=0.018), group 1 vs. group 3 (P=0.028), group 2 vs. group 3 (P=0.001), group 2 vs. the control (group 0) (P=0.03, Fig. 1). By using the cut-off level of 400 μg/l, the sensitivity, specificity, positive predictive value, and negative predictive value for liver cirrhosis patients with chronic liver parenchymal disease was 75% (6/8), 94.1% (16/17), 85.7% (6/7), and 88.9% (16/18), respectively. The cut-off level

Our study comprised 9 clinically healthy beagles, 25 patients with liver disease, and 51 patients with extrahepatic disease. The control group comprised 9 beagles (1 male, 7 females, and 1 castrated male; the median and the range of the age was 4(3–6)). The patient group comprised 25 dogs (10 males, 4 castrated males, 6 females, and 5 spayed females), aged 1–11 years. The patient group comprised various breeds—American cocker spaniel (7), Dobberman (3), Shih Tzu (2), miniature Schnauzer (2), miniature dachshund (1) Beagle (1), French bulldog (2), Labrador retriever (2), Maltese (1), standard poodle (1), Papillon (1), Pembroke Welsh Corgi (1), and mongrel (1). The histological diagnoses of the patients were parenchymal liver disease (chronic hepatitis (9), primary portal vein hypoplasia (6), neutrophilic cholangitis (1), and destructive cholangitis (1)), group 1, n=17) or cirrhosis (group 2, n=8). The median and the range of the group 1 and 2 were 6 (4–10) and 5.5 (1–11), respectively.

Fig. 1. Blood HA concentration in the 4 groups. Group 0: healthy beagles; Group 1: dogs with chronic liver disease without cirrhosis; Group 2: dogs with cirrhosis; Group 3: dogs with extrahepatic disease. The blood HA concentrations were significantly higher in Group 2 than in Group 0, Group 1, and Group 3. Also, Group 2 had significantly higher HA concentration than and Group 3. * P<0.05
was determined by receiver-operator curve analysis using the data of group 1 and group 2, because in the clinical setting HA would be measured for patients with suspected liver disease. The point at which the sensitivity and specificity was maximal was adopted as the cut-off level. The results of this study indicated that the concentration of the circulating HA in dogs with liver cirrhosis is higher than that of dogs with non-cirrhotic liver disease. This result is in part consistent with the reports obtained with respect to humans [6, 7, 10, 11, 13], and suggests that the concentration of HA in canine blood may be a clinically important diagnostic test for dogs and may function as markers of cirrhosis in those with hepatic disease. HA concentration of group 1 was significantly higher than dogs with extrahepatic disease (group 3), although neither did we observe that HA concentration of group 1 was higher than that of the control group (Fig. 1) nor signified the correlation between the histological extent of hepatic fibrosis and blood HA level in group 1 (data not shown). In contrast, it has been reported that most of the human patients with hepatitis have higher HA concentration than the healthy controls and the degree of HA concentration is correlated to that of histological fibrosis [6, 7, 10, 11, 13]. In this study various types of hepatitis were included. All of these reasons may contribute to the variety of HA

Fig. 2. Histological distribution of HA in the liver. Bar=100 μm. a, b, c, d: sample obtained from a healthy beagle; e, f, g, h: sample obtained from a dog with moderate to severe fibrosis, but no cirrhosis; i, j, k, l: sample obtained from a dog with cirrhosis. The stains used were as follows: first line (a, e, i): HE stain; second line (b, f, j): Sirius Red stain, collagen is stained in red; third line (c, g, k): histochemistry for HA; fourth line (d, h, l): negative control of HA. In healthy livers, the portal area was only slightly stained for HA (depicted as brown in color, c). The liver with hepatitis and moderate to severe fibrosis showed staining around the portal areas in which the fibrosis was progressing (f, g). HA was detected in a part of the fibrotic septa in the cirrhotic liver (j, k).
concentration and its lack of correlation to the degree of the histological fibrosis in group 1. Further study with a larger sample size and subdivision of fibrotic stages will be needed to clarify the usefulness of the blood HA measurement for evaluating the degree of liver fibrosis.

On histochemical staining of HA in the liver tissue from control dogs, only a weak staining was observed in the portal area. In contrast, much prominent staining of HA was observed inside the fibrotic area in both cirrhotic and non-cirrhotic liver with moderate to severe fibrosis (Fig. 2). The staining intensities and patterns were not related to the blood HA level (data not shown). These results indicated that increased HA in the liver was one of the reasons of elevated circulating HA. However, it was also possible that other factors such as impaired incorporation and degradation of HA from circulation to the liver [8, 14, 15] affects blood HA concentration.

In conclusion, the results of this study demonstrate that canine cirrhotic patients had elevated blood HA concentrations as compared to those without cirrhosis. Measurement of the circulating HA concentration can be an effective and noninvasive diagnostic aid to evaluate the presence or absence of hepatic cirrhosis.

ACKNOWLEDGMENT. The authors would like to thank for Mr. Uchida for giving us advises about the pathological diagnosis or the analysis for this study.

REFERENCES