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Original article

Serum concentrations of PIIINP aminopeptide in dogs with liver fibrosis

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Abstract

The aim of the study was to evaluate the serum concentration of the type III procollagen aminopeptide in dogs, and to assess its utility in the diagnosis of liver fibrosis. The study was carried out on 20 dogs of different breeds and of both genders, between 7 and 15 years old. Based on the results of the histopathological examination and the evaluation of the degree of liver fibrosis, the dogs were divided into five groups. The mean serum PIIINP concentration in the group of dogs with stage 1 and 2 liver fibrosis (groups 2 and 3) was five-fold higher than in healthy dogs (group 1). In turn, the mean PIIINP concentration in the group of dogs with stage 3 (group 4) and stage 4 (group 5) fibrosis was 10-fold higher than that of the control group (group 1). Based on the results, we found that the serum PIIINP concentration correlated with the degree of liver fibrosis, assessed based on a histopathological examination. Therefore, PIIINP serum concentration tests may be a promising non-invasive diagnostic technique that could be used in veterinary hepatology to assess the degree of liver fibrosis.

Key words: dog, serum PIIINP, liver fibrosis

Introduction

Currently, liver diseases in veterinary medicine pose a serious diagnostic challenge for the clinician. The most common causes of liver damage are viral and bacterial infections as well as toxic substances. The liver suffers from damage, remodelling and fibrosis in response to a toxic and infectious agent.

The term „liver fibrosis” refers to a constant, excessive deposition of components of the extracellular matrix (ECM) and a change in the proportion of its components, leading to tissue hypertrophy, hardening

and scarring (Kruś 2007, Guarino et al. 2009, Pinzani and Macias-Barragan 2010).

In most cases, a core needle biopsy is performed to correctly assess the degree of liver inflammation and fibrosis. Currently, in human medicine, there are numerous studies under way aimed at developing a panel of non-invasive markers, which may be used to assess the degree of liver fibrosis. These markers are required to have high liver specificity, a high sensitivity to fibrinolysis and synthesis of components of the ECM, and are expected to give repeatable results (Gutkowski et al. 2007). Based on those studies, we attempted to

evaluate some non-invasive markers of liver fibrosis in dogs. One such marker is the type III procollagen aminopeptide (PIIINP), which indicates an accumulation of the extracellular matrix. To date, the usefulness of PIIINP in the diagnosis of liver fibrosis has not been studied in veterinary medicine. In 2013, Heikkilä et al. assessed the utility of analyzing serum PIIINP in the diagnosis of lung and bronchial disease. Hazzeli et al. (2014) evaluated serum PIIINP concentrations in myxomatous mitral valve disease.

The aim of our study was to evaluate the serum concentration of the type III procollagen aminopeptide in dogs and to assess its utility in the diagnostics of liver fibrosis.

Materials and Methods

The study was carried out on 20 dogs of different breeds (ten mixed-breed dogs, two Labrador retrievers, three cocker spaniels, three German shepherds, one miniature poodle and one Yorkshire terrier) and of both genders (15 males, 5 females) between 7 and 15 years old. All dogs were suspected of having a chronic liver parenchyma damage and were referred to the Clinic of the Department of Internal Diseases with Clinic of Horses, Dogs and Cats for a liver biopsy. Systemic diseases and diseases of other organs were excluded in each animal based on a clinical examination, laboratory test results and an abdominal ultrasound examination. Blood samples were drawn from each dog, and a basic blood biochemistry (AST, ALT, ALP, GGT, total bilirubin) was carried out. Additionally, serum samples were obtained by centrifuging EDTA anticoagulated blood and stored at -20°C . The Serum PIIINP level was determined using a Canine Procollagen Type III N-Terminal Propeptide ELISA Kit (MyBioSource, San Diego, USA).

A core needle biopsy of the liver under ultrasound guidance was carried out in all animals using a Tru – Cut needle (20 cm long, 16G). In all the animals, numerous hepatic lesions were visible by ultrasound. Consequently, a left-lobe and right-lobe biopsy was obtained from each dog. The specimens were fixed in a 10% buffered formalin solution. They were then transferred to the histopathology laboratory and underwent an assessment of the degree of liver fibrosis.

A 5 point scale approved by the Hepatology Group of the Polish Society of Gastroenterology was used to assess hepatic fibrosis:

Stage 0 (F0) – normal – no fibrosis, single collagen fibres in portal spaces

Stage 1 (F1) – fibrosis within portal spaces with an extension of the portal tracts

Stage 2 (F2) – peri-portal fibrosis and possibly single bridging fibrosis while maintaining lobular structure,

Stage 3 (F3) – the presence of multiple fibre spans, a destruction of the lobular architecture, no regeneration reaction.

Stage 4 (F4) – disseminated fibrosis or cirrhosis (Goodman 2007).

Using the above classification, we were able to assess the concentration of the type III procollagen aminopeptide depending on the degree of changes in the liver tissue.

The dogs were divided into five groups based on the degree of liver fibrosis:

– group 1, comprising 5 dogs that did not have liver fibrosis (F0)

– group 2, comprising 3 dogs that had first degree liver fibrosis (F1)

– group 3, comprising 4 dogs that had second degree liver fibrosis (F2)

– group 4, comprising 3 dogs that had third degree liver fibrosis (F3)

– group 5, comprising 5 dogs that had fourth degree liver fibrosis (F4).

Statistical analyses were performed using version 10 of STATISTICA (StatSoft Inc., Poland). Data are expressed as median values. The correlation between serum PIIINP levels and the liver fibrosis were determined with the Students-t test. A p-value of ≤ 0.05 was considered statistically significant.

Results

The mean PIIINP serum concentration was 634.25 ± 618.41 pg/ml in group 1, 3077.91 ± 894.046 pg/ml in group 2, 3030.93 ± 827.11 pg/ml in group 3, 5685.83 ± 1630.33 pg/ml in group 4 and 6574.5 ± 2983.46 pg/ml in group 5. The results of the chosen biochemical parameters, the serum PIIINP concentrations and the degree of liver fibrosis determined by histopathology of the liver biopsy specimens for each animal and group are shown in Table 1.

Statistically significant differences in the concentrations of PIIINP were found between group 1 and group 2 ($p=0.0035$), 3 ($p=0.012127$), 4 ($p=0.0027$) and 5 ($p=0.011817$). The PIIINP concentration was higher in group 5 than in all the remaining groups. Statistically significant differences in the concentration of PIIINP were also found between group 3 and 4 ($p=0.0352$).

A graphic display of the PIIINP serum concentrations in each study group is presented in Fig. 1.

Table 1. Results of chosen biochemical parameters, stage of liver fibrosis and PIIINP serum concentrations in each animal and study group.

Study group	Animal	AST [U/l]	ALT [U/l]	ALP [U/l]	GGT [U/l]	Total bilirubin [mmol/l]	Stage of Fibrosis	PIIINP [pg/ml]
1	1	78	54	170	5	1.8	0	322.5
	2	32	77	170	1	1.8	0	311.25
	3	52	24	41	4	1.8	0	173.75
	4	31	53	86	2	2.6	0	1690
	5	56	138	287	12	2	0	673.75
2	1	81	632	61	13	3	1	3787.5
	2	43	213	56	1	1.8	1	2073.75
	3	117	205	160	18	1	1	3372.5
3	1	214	412	214	10	1	2	3306.25
	2	214	412	214	10	1	2	1798.75
	3	43	34	43	1	2.6	2	3493.75
	4	64	30	74	13	2	2	3525
4	1	160	587	382	13	17	3	4925
	2	47	117	468	2	8.9	3	7557.5
	3	198	277	411	27	5.4	3	4575
5	1	311	1187	442	29	56	4	9070
	2	234	411	242	2	23	4	5047.5
	3	233	1314	452	16	32	4	3095
	4	320	715	438	20	24	4	5402.5
	5	234	411	242	2	76	4	10257.5

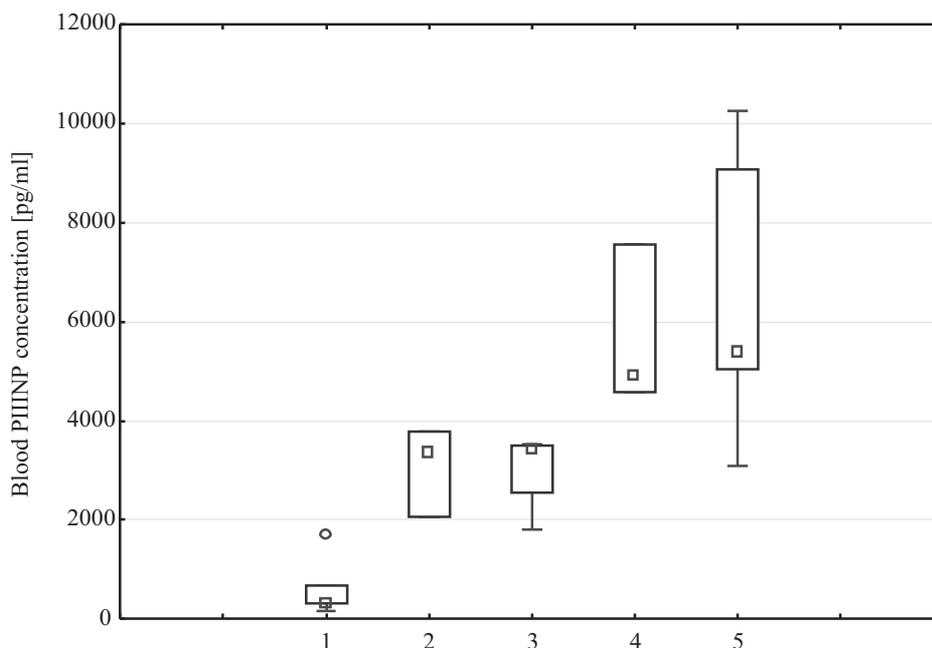


Fig. 1. Box plots of serum PIIINP concentrations in each study group.

Discussion

Type III procollagen aminopeptide (PIIINP) is a marker that enables an assessment of the function of fibroblasts and type III collagen. During the synthesis of type III collagen, the amino-terminal peptide

is released from the collagen molecule and enters circulation. The amount of released peptide is proportional to the amount of the collagen produced (Prockop et al. 1979). In human medicine, numerous studies have been performed to assess the utility of PIIINP in the diagnostics of fibrosis. They revealed

that this parameter may be useful in the diagnosis of idiopathic pulmonary fibrosis (Norris et al. 2005), obstructive pulmonary disease (Harju et al. 2010), sarcoidosis, (Lammi et al. 1997) and the respiratory distress syndrome (Meduri et al. 1998). In humans, PIIINP has also been found to be associated with the myocardial fraction of type III collagen and may be used as a marker of myocardial fibrosis (Izawa et al. 2005, Klappacher et al. 2005). Apart from its wide use in pulmonology and cardiology, PIIINP has been found to correlate well with the results obtained from microscopic examinations of liver biopsy specimens in patients with alcoholic liver disease, viral hepatitis and primary biliary cirrhosis. Therefore, it is a sensitive marker of liver fibrosis (Gutkowski et al. 2007). In recent years, a commercial enhanced liver fibrosis (ELF) test that non-invasively measures the degree of liver fibrosis has been introduced in human medicine. It assesses three main markers of liver fibrosis – hyaluronic acid (HA), type III procollagen aminopeptide (PIIINP) and the inhibitor of the precursor of metalloproteinase-1 (TIMP1) (Rosenberg et al. 2004, Parkes et al. 2010, Pinzani 2010).

To date, two studies assessing this marker in veterinary medicine have been performed. One evaluated the utility of PIIINP concentrations in the serum and bronchoalveolar lavage fluid (BALF) by radioimmunoassay in healthy dogs and in dogs with idiopathic pulmonary fibrosis (IPF), chronic bronchitis (CB) and eosinophilic bronchopneumopathy (EBP) (Heikkilä et al. 2013). The other study determined the associations between type III N-terminal procollagen, fibrosis and echocardiographic indices in dogs that died due to myxomatous mitral valve disease (Hazzell et al. 2014). The study conducted by Heikkilä (Heikkilä et al. 2013) revealed that serum PIIINP values did not differ between groups, indicating that serum PIIINP is not useful in evaluating respiratory diseases in dogs. However, BALF PIIINP concentrations were significantly higher in dogs with EBP than in dogs with CB or in healthy dogs. In turn, the study by Hazzell (Hazzell et al. 2014) showed that serum PIIINP concentrations increased with the fibrosis score, although those relationships were not strong enough to be clinically useful. In veterinary medicine, there are no available studies assessing PIIINP role in the diagnosis of canine liver fibrosis. In view of the usefulness of this parameter in human medicine, we attempted to assess the suitability of PIIINP in the diagnosis of liver fibrosis in dogs with varying degrees of liver damage.

In 1986, Torres-Salinas (Torres-Salinas et al. 1986) conducted one of the first studies on the utility of analysing serum PIIINP levels to diagnose liver fibrosis. He measured the PIIINP concentrations in two groups of patients with alcoholic liver disease. Based

on the results, he concluded that patients who developed severe liver fibrosis had significantly higher concentrations of PIIINP (1208 ± 154 cpm/mg) than patients who did not develop liver fibrosis (734 ± 138 cpm/mg) (Torres-Salinas et al. 1986). His findings were confirmed by other scientists in various research centers (Bentsen et al. 1978, Nouchi et al. 1987, Trinchet et al. 1992, Oberti et al. 1997, Tran et al. 2000, Stickel et al. 2001). However, we failed to find a correlation between the concentrations of PIIINP and the degree of liver damage in the available literature. Our study showed that dogs with severe liver fibrosis had significantly increased concentrations of PIIINP. The mean PIIINP concentrations in group 2 and 3 were almost five-fold higher than in healthy dogs (group 1). The mean PIIINP concentrations in group 4 and 5 were 10-fold greater than those in the control animals. In addition, based on the results, we found no statistically significant differences in the concentrations of serum PIIINP between groups 2 and 3 (dogs with stage 1 and 2 liver fibrosis) and groups 4 and 5 (dogs with stage 3 and 4 liver fibrosis).

Based on the results, we found that the serum PIIINP concentrations correlated with the degree of liver fibrosis assessed histopathologically. Therefore, the determination of serum concentrations of PIIINP seems as a promising non-invasive diagnostic technique that can be used in veterinary hepatology to assess the degree of liver fibrosis. However, further research is needed on a larger study group including animals of different ages.

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