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ORIGINAL ARTICLE

Interleukin-6 (IL-6) serum concentrations in dogs with hepatitis and hepatic tumours compared with those with extra-hepatic inflammation and tumours

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Abstract Cytokines are part of pathogenesis in many diseases. Their measurement could be interesting for diagnostic purposes. One cytokine which participates in different inflammatory and neoplastic diseases is interleukin-6 (IL-6). The aim of this study was to investigate the IL-6 serum concentration in dogs with different liver diseases to show if there is any association between the cytokine serum level and the disease aetiology or the degree of the disease. IL-6 was measured in dogs with acute hepatitis, chronic hepatitis of different degrees and primary and secondary liver tumours. The data were compared with clinically healthy dogs and dogs with extra-hepatic diseases. For measurement, a commercial ELISA Kit (R&D Systems) was used. Compared with clinically healthy dogs and dogs with diabetes mellitus, all dogs with an intra- or extra-hepatic inflammatory or neoplastic disease have increased serum levels of IL-6. Dogs with acute hepatitis have significantly increased IL-6 serum concentrations compared with dogs with chronic hepatitis ($P<0.05$). No significant difference between mild and moderate chronic hepatitis exists ($P>0.05$). Dogs with secondary liver tumours have significantly increased IL-6 serum concentrations in comparison to dogs with primary liver tumours ($P<0.01$), but both groups have comparable IL-6 serum concentration to dogs with extra-hepatic tumours. Measurement of IL-6 serum concentration may help differentiate between acute and chronic hepatitis and between primary and secondary liver tumours. Further information about the aetiology of the liver disease cannot be obtained by measuring IL-6 in the serum.

Keywords Liver · IL-6 · Dog

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Introduction

Cytokines are mediators of information transfer between cells, and in this way, they regulate physiological and pathological mechanisms in the body. They are potentially of use in diagnosis. As a result of its function during inflammatory processes and regeneration, interleukin-6 (IL-6) could be an interesting marker for liver diseases in dogs. IL-6 is a protein that consists of 184 amino acids. It is produced by lymphoid cells and by a variety of non-lymphoid cells, including endothelial cells, epithelial cells and fibroblasts (Kishimoto et al. 1995).

The synthesis and release of IL-6 are influenced by various other cytokines and hormones. For example, tumour necrosis factor-α, interleukin-1 and catecholamines stimulate the synthesis of IL-6, whereas glucocorticoids suppress it (Papanicolaou et al. 1998).

The target cells of IL-6 are lymphoid cells and other cells such as hepatocytes and bone marrow cells (Barton 1997; Papanicolaou et al. 1998). IL-6 acts on its target cells via different transduction pathways, including those that involve protein kinase C, cAMP/protein kinase A and the calcium release pathway (Kamimura et al. 2003; Mitsuyama et al. 2006). The cytokine plays a role in different disease mechanisms. Release of IL-6 occurs in cases of infection, trauma and neoplasia, and its functions range from induction

of acute-phase proteins to cell growth and differentiation (Jakab and Kalabay 1998; Barrientos et al. 2008). A role is described for IL-6, for example, in different autoimmune and inflammatory diseases. In addition, IL-6 appears to have a function in different diseases of bones and liver (Linker-Israeli et al. 1991; Kotake et al. 1996; Chen et al. 2009; Haedo et al. 2009; Tacke et al. 2009).

In hepatic diseases of humans, increased serum concentrations of IL-6 have been found in cases of hepatitis, hepatic tumours and regeneration of the liver after different insults (Lacour et al. 2005; Avrămescu et al. 2008; Wong et al. 2009). Given its function in different disease mechanisms, IL-6 could be an important parameter in clinical pathology to describe the aetiology, severity and course of diseases, and to determine the prognosis.

In this study, the serum concentration of IL-6 was measured in dogs with different diseases of the liver. The aim was to investigate the association of different liver diseases and the serum concentration of IL-6. Furthermore, the presence of a correlation between the degree of liver disease and the serum concentration of IL-6 was investigated. Finally, the serum concentration of IL-6 associated with hepatic disease was compared with that in cases of extra-hepatic inflammatory disease, tumours and metabolic diseases.

Materials and methods

Animals

Thirty-five dogs with hepatic disease were included in this study. The diagnosis of liver disease was based on the anamnesis, the clinical investigation, clinical pathological examination [alanine transferase (ALT); aspartate transferase (AST); glutamate dehydrogenase (GLDH); alkaline phosphatase (ALP); fasting bile acids (BA); bilirubin (BILI) and albumin (ALB)], ultrasound investigation and histopathological diagnosis from a biopsy. The definitive diagnosis was based on the histological investigation, and the disease was classified according to the recommendations of the World Small Animal Veterinary Association (WSAVA Liver Standardization Group 2006).

The IL-6 values of these dogs were compared with those of 18 clinically healthy dogs. These dogs were presented for vaccination and other routine examinations and had no abnormalities in anamnesis, clinical investigation, clinical biochemistry (ALT, AST, GLDH, ALP, BA, ALB, BILI, creatinine, urea, phosphorus, calcium and glucose) and haematology (e.g. white blood cells, packed cell volume). Furthermore, no abnormalities were found in the liver during ultrasound investigation of these dogs. Both groups were compared with a group of 30 dogs with extra-hepatic diseases. Ten dogs of this group had diabetes mellitus, ten dogs had extra-hepatic inflammation and ten dogs had an extra-hepatic tumour. All procedures of this study conformed to the German Protection of Animals Act.

Blood sampling and analysis

Blood samples were collected from the cephalic vein with a 22-g needle and were harvested in a plastic serum tube. The samples were kept at room temperature for 15 min to allow them to clot and were then centrifuged (10 min, 3,000×*g*). Analysis of ALT, AST, GLDH, ALP, fasting BA, BILI and ALB was performed according to standardised procedures using an analyser (Konelab, Thermo Fisher Scientific) and commercial kits. The serum for measurement of IL-6 was frozen (−20°C) directly after clotting. The serum samples were stored for no longer than 3 months before measurements were made.

The concentration of IL-6 was measured using the Canine IL-6 Duoset ELISA (catalogue number DY1609; R&D Systems, Inc.). The analysis included plate preparation and assay procedure and was performed according to the manufacturer's ELISA protocol. Finally, the microtitre plate was read using a microtitre reader (SLT Spectra, Tecan) at 450 nm (correction wavelength 540 nm).

Biopsy and histology

The liver of each dog was evaluated by ultrasonography (Megas, Esaote). The livers were examined for size, shape and structure. Using a biopsy needle (True-cut, Surgivet) and guided by ultrasound, three to four tissue samples were taken from each structural lesion for further examination. The tissue core specimens were 0.5–1.0 cm long and 2 mm thick. In some cases, tissue samples were obtained at laparotomy. In these cases, the dogs were investigated under general anaesthesia (levomethadone, L-Polamivet®, Intervet; xylazine, Rompun®, Bayer; isoflurane, Isoflurane®, Abbott).

Each tissue sample was fixed in 10% formaldehyde solution. Tissue samples for histology were prepared and stained with haematoxylin and eosin. The histological diagnosis was performed by a board-certified pathologist. The histopathological diagnosis corresponded to the recommendations of the WSAVA Liver Standardization Group (2006). The degree of hepatitis was assessed semi-quantitatively.

Statistical analysis

The data from the dogs were split into different groups for further statistical analysis. Group 1 comprised clinically healthy dogs; group 2, dogs with acute hepatitis; group 3, chronic hepatitis of mild degree; group 4, chronic hepatitis

of moderate degree; group 5, liver tumours; group 5a, primary liver tumours; group 5b, secondary liver tumours; group 6, extra-hepatic inflammation; group 7, extra-hepatic tumours and group 8, diabetes mellitus. The statistical analysis was performed with the programme SAS System, univariate procedure, version 8.1 (SAS Institute Inc.). The data were checked for normal distribution with the Shapiro–Wilk test.

The serum concentrations of IL-6 in all groups were compared using the Mann–Whitney *U* test. The level of significance was $P<0.05$.

The precision of the IL-6 ELISA was determined using three serum samples on which ten consecutive runs were performed during a single day. The day-to-day precision was determined using three serum samples that were evaluated once daily for 5 days. The mean, standard deviation and coefficients of variation were calculated.

Results

The dogs with liver disease were divided into five groups, depending on their histopathological diagnoses. Six dogs had acute hepatitis, 13 dogs had chronic hepatitis and 16 dogs had a liver tumour. The six dogs with acute hepatitis could be divided into three cases of acute purulent hepatitis, one case of hepatitis associated with adenovirus infection, one case associated with parvovirus infection and one case associated with leptospirosis. The serum concentrations of IL-6 in this group varied between 1 and 202 pg/mL, with an average of 102 pg/mL (Fig. 1).

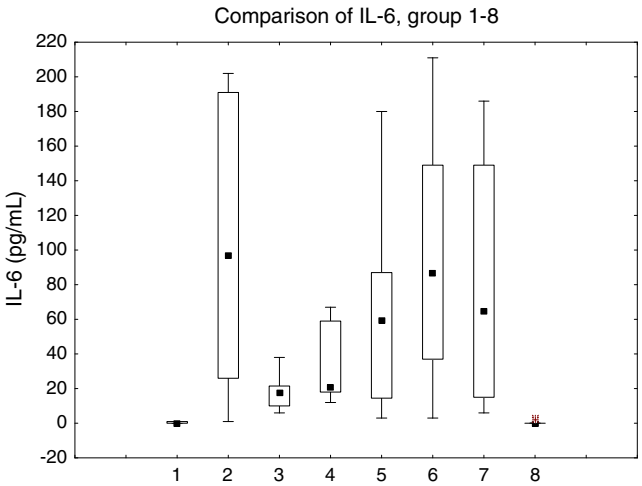

Fig. 1 Minimum, median, maximum and first and third quartiles of IL-6 serum concentrations in groups *1* to *8* (group *1*=healthy controls, group *2*=acute hepatitis, group *3*=mild chronic hepatitis, group *4*=moderate chronic hepatitis, group *5*=liver tumours [group 5a=primary liver tumours, group 5b=secondary liver tumours], group *6*=extra-hepatic inflammation, group *7*=extra-hepatic tumours and group *8*=diabetes mellitus)

The group with chronic hepatitis consisted of 13 dogs, of which eight had a mild degree of hepatitis and five had a moderate degree. Reactive hepatitis, as a reaction of an extra-hepatic disease, was excluded by histopathology; those dogs were not considered in this study. The concentration of IL-6 was between 6 and 67 pg/mL. The average concentration in this group was 25 pg/mL (Fig. 1).

Sixteen dogs with liver tumours of different aetiologies were included in this study. Hepatocellular carcinoma was most frequently seen, with seven cases. Two cases of cholangiocarcinoma were observed. In addition, two cases of haemangiosarcoma, two cases of fibrosarcoma and three cases of lymphoma were also included in the study. The range of IL-6 concentrations in this group was from 3 to180 pg/mL, with an average concentration of 58 pg/mL (Fig. 2). The average concentration of IL-6 in dogs with a primary liver tumour (hepatocellular carcinoma, cholangiocellular carcinoma) was 31 pg/mL and that of dogs with secondary liver tumours (lymphoma, haemangiosarcoma, fibrosarcoma) was 91 pg/mL (Fig. 3).

We compared these data between the liver disease groups and also compared them with data from healthy controls. The serum concentrations of IL-6 in dogs with different liver diseases were highly significantly ($P<0.001$) increased compared with those of clinically healthy dogs. The difference between acute and chronic hepatitis was also significant ($P<0.05$). On the other hand, there was no significant difference between dogs with mild and moderate chronic hepatitis (Fig. 2).

The serum concentrations of IL-6 in dogs with different liver tumours were highly significantly increased compared with those of healthy dogs ($P<0.001$; Fig. 1). Dogs with secondary liver tumours had significantly higher serum concentrations of IL-6 than dogs with primary liver

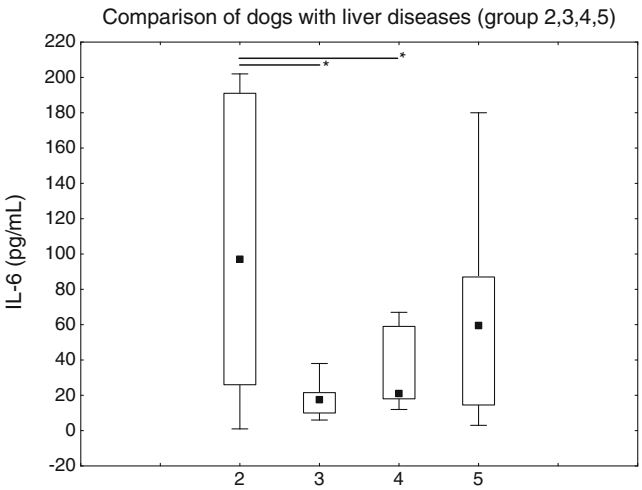

Fig. 2 Minimum, median, maximum and first and third quartiles of IL-6 serum concentrations in groups *2* to *5*. Significant differences are indicated by *asterisks*

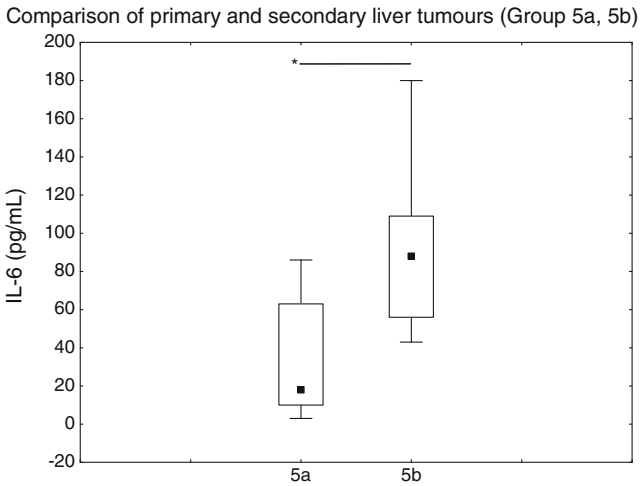

Fig. 3 Minimum, median, maximum and first and third quartiles of IL-6 serum concentrations in groups *5a* and *5b*. Significant differences are indicated by an *asterisk*

tumours ($P<0.01$; Fig. 3). There was no significant difference between dogs with acute hepatitis and dogs with primary and secondary liver tumours ($P>0.05$; Fig. 2); however, the difference between dogs with chronic hepatitis and those with secondary liver tumours was highly significant ($P<0.001$).

To detect differences in the serum concentration of IL-6 between dogs with intra- and extra-hepatic inflammatory diseases or tumours, we compared the dogs with liver disease in this study with dogs that had extra-hepatic diseases. This group of dogs included ten dogs with an extra-hepatic inflammatory process; two dogs in this group had prostatitis and eight had pyometra. A further ten dogs with an extra-hepatic tumour were included in this comparison, comprising five cases of mammary carcinoma, three cases of splenic haemangiosarcoma, one case of bronchial carcinoma and one case of carcinoma of the stomach. Finally, all data were compared with those from a group of ten dogs with diabetes mellitus that had no inflammatory or neoplastic disease.

No significant difference could be found between dogs with acute hepatitis and those with extra-hepatic inflammation ($P>0.05$); in contrast, the serum concentrations of IL-6 in dogs with chronic hepatitis were significantly lower than those in dogs with extra-hepatic inflammation ($P<0.01$). The serum concentration of IL-6 in dogs with intra- or extra-hepatic tumours showed no significant difference ($P>0.05$). However, dogs with an extra-hepatic inflammation or tumour had highly significantly increased serum concentration of IL-6 compared with dogs with diabetes mellitus ($P<0.001$; Fig. 1). Finally the intra- and inter-assay precision of the IL-6 assay was 2.9% and 5.9%, respectively.

Discussion

The measurement of cytokines in diseased dogs is a new field in clinical pathology. Given their function as mediators, cytokines can help to identify disease mechanisms, to describe the course of disease and to assess prognosis. This study focused on IL-6 in dogs with liver disease because IL-6 has been shown to be critical for the acute-phase response, for protection against hepatic injury and for liver regeneration (Taub 2005; Berasain et al. 2009).

In clinically healthy dogs, the serum concentration of IL-6 is very low. In this investigation, no healthy dog was found with a serum concentration above 1 pg/mL. Unfortunately, there is no study in which a reference interval for IL-6 in dogs has been calculated. However, in human studies with healthy controls, comparable serum concentrations of about 1 pg/mL were reported (Benoy et al. 2008; Salamon et al. 2008). Given this very low reference range, the elevations of IL-6 associated with different diseases can be recognised easily.

The results of this study showed that liver diseases of different aetiologies were associated with elevated serum concentrations of IL-6. This is in agreement with the results of investigations in humans, which have involved measurement of IL-6 in the serum of patients with different neoplastic diseases, including metastases, and those with non-neoplastic liver diseases. In both types of diseases, the serum concentrations of IL-6 are increased compared with those of controls (Matzaraki et al. 2007; Cheng et al. 2009).

The serum concentration of IL-6 in cases of acute hepatitis is significantly higher than that in chronic hepatitis. The pro-inflammatory function of IL-6 may explain this finding. The release of different cytokines, including IL-6, indicates the early beginning of an inflammatory process (Eklund 2009). Measurement of a significant difference in the level of IL-6 between acute and chronic liver disease may help to differentiate between these processes. Currently, there is no reliable blood indicator of acute hepatitis. The results of this study show that IL-6 could be helpful for identification of acute hepatitis in dogs; however, the number of dogs with acute hepatitis in this study was low, so further studies are recommended with a larger number of patients.

Unfortunately, there was no significant difference in the level of IL-6 between patients with mild and moderate chronic hepatitis. In human patients with chronic hepatitis, the serum concentration of IL-6 correlates with the degree of fibrosis (Migita et al. 2006). Given that all the dogs with chronic hepatitis in this study had a low degree of inflammation and no or only mild fibrosis, dogs with severe chronic hepatitis and fibrosis should be observed in further studies to investigate the correlation between the serum concentration of IL-6 and the degree of hepatitis.

All dogs with hepatitis in this study, both acute and chronic, had comparable serum concentrations of IL-6 to dogs with inflammatory diseases outside the liver, such as prostatitis and pyometra. This supports the usefulness of measuring IL-6 for identification of inflammatory processes. The concentration of IL-6 in dogs with chronic hepatitis was lower than in the control group with extra-hepatic inflammatory processes. One reason for this could be a lower degree of inflammation in the group with chronic hepatitis.

The dogs with liver tumours in this study also had elevated serum concentrations of IL-6. The levels were comparable to those in cases of acute hepatitis but higher than in cases of chronic hepatitis. An explanation for this could be the acute initiation of inflammation by mediators in hepatic tumours and the resulting destruction of the liver parenchyma. It could also be caused by attempts of the liver to regenerate its tissue during neoplastic alteration. Elevation of IL-6 has also been reported in humans with liver tumours of different aetiologies (Goydos et al. 1998; Cheng et al. 2009).

Studies of liver tumours and serum concentrations of IL-6 in dogs have not been published previously. It is interesting that the concentrations of IL-6 in dogs with secondary liver tumours were significantly higher than those in dogs with primary liver tumours. In cases of lymphoma, the release of IL-6 from lymphoid tumour cells could explain this observation. In humans, serum concentrations of IL-6 are increased in patients with lymphoma, and the levels fall during chemotherapy (Serebriakov et al. 1998).

To confirm that non-inflammatory and non-neoplastic diseases have little influence on the serum concentration of IL-6, we measured IL-6 in cases of diabetes mellitus and compared these results with those from all cases of inflammation or tumour. Like the healthy controls, the dogs with diabetes mellitus have very low concentrations of IL-6 in the serum. It can be concluded that the absence of inflammatory processes is associated with a lack of IL-6 activation. On the other hand, in humans, secondary inflammation associated with diabetes mellitus can induce elevations of IL-6 (Goldberg 2009). This observation requires investigation in further studies.

In conclusion, the results of our study show that IL-6 is measurable in dogs and that diseases of the liver are associated with elevations of this cytokine. Differentiation between acute and chronic hepatitis may be possible, but the assessment of the degree of hepatitis is not possible in milder cases. Dogs with liver tumours of different aetiologies have elevated serum concentrations of IL-6, and those with secondary liver tumours showed significantly higher levels than those with primary tumours or inflammatory diseases. The reason for this is not clearly understood, but production of IL-6 by the tumour cells could be one explanation. Further studies of IL-6 and its involvement in liver disease in dogs are necessary to investigate the prognostic use of IL-6 in patients with liver diseases.

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