Serum C-reactive protein concentrations in dogs with idiopathic epilepsy

C-reactief proteïneconcentraties in het serum van honden met idiopathische epilepsie

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SAMENVATTING

Inflammatory reactions in dogs are associated with systemic changes in serum, called the acute phase response; changes in the concentration of acute phase proteins in the serum take place. C-reactive protein (CRP) is a positive acute phase protein, which increases during inflammation. The role of inflammation in epilepsy remains unclear. In this study, the inflammatory response in dogs with idiopathic epilepsy (IE) was investigated. The aims of the study were: 1. to measure serum CRP concentrations in dogs with IE and in healthy dogs, 2. to measure serum CRP concentrations in dogs with acute cluster seizures and in dogs with isolated seizures and 3. to observe the evolution of serum CRP concentrations in time after the last seizure. This study showed no significant differences in serum CRP concentrations between dogs with IE (7.8 mg/l) and dogs of the control group (8.3 mg/l). Furthermore, the results showed higher mean serum CRP concentrations in dogs with IE exhibiting cluster seizures (11,8 mg/l) than in dogs with isolated seizures (5.7 mg/l). However, these results were not statistically significant (P = 0,077). Finally, no statistically significant decrease in serum CRP concentrations was seen with time after the last epileptic seizure in dogs with IE (P = 0,077).

ABSTRACT

Inflammatoire reacties bij honden zijn geassocieerd met systemische veranderingen in het serum, de acute fase respons genaamd. Deze gaat gepaard met veranderde concentraties acutefase-eiwitten in het serum. Het C-reactief proteïne (CRP) is een positief acutefase-eiwit en stijgt bijgevolg bij een ontsteking. Er bestaat veel onduidelijkheid over de relatie tussen epilepsie en ontsteking. Daarom werd in deze studie de rol van ontsteking onderzocht bij honden met idiopathische epilepsie (IE). Drie doelstellingen werden vooropgesteld: 1. het meten van serum-CRP-concentraties bij honden met IE en bij gezonde honden, 2. het meten van serum-CRP-concentraties bij honden met acute clusteraanvallen en bij honden met geïsoleerde aanvallen en 3. het opvolgen van de evolutie in serum-CRP-concentraties in de tijd na de laatste epilepsieaanval. In deze studie kon geen significant verschil in serum-CRP-concentratie tussen honden met IE en de honden van de controlegroep worden aangetoond. Verder waren de gemiddelde serum-CRP-concentraties hoger bij honden met clusteraanvallen (8,3 mg/l) dan bij honden met geïsoleerde aanvallen (7,8 mg/l). Deze resultaten waren echter statistisch niet significant (P = 0,077). Ten slotte werd een statistisch niet-significante daling in serum-CRP-concentraties gezien na de laatste epileptische aanval bij de honden met IE (P = 0,077).

INTRODUCTION

Inflammatory reactions are associated with systemic changes, also called the “acute phase response”, which leads to an increase of inflammatory cells in the blood, consequently resulting in raised serum cytokines. These cytokines stimulate the synthesis of serum CRP, a positive acute phase protein, in hepatocytes (Kushner, 1993; Gabay and Kushner, 1999; Volanakis, 2001; Mantovani et al., 2008; Lech et al., 2013). Research has been performed on serum CRP changes in several human inflammatory diseases, but

little is known about the role of serum CRP in epilepsy (Alapirtti et al., 2012). Furthermore, there are still a lot of questions on the effect and role of inflammation in epilepsy. It is currently known that epileptic activity leads to inflammatory reactions in cerebrospinal fluid (CSF) and serum, both in humans and rodents. (Peltola et al., 1998; Vezzani et al., 2013). This results in an increase of interleukin-6 (IL-6) in both CSF and serum, and consequently, in a higher serum CRP concentration in humans (Lehtimäki et al., 2004). Epileptic seizures can also provoke systemical inflammatory reactions, which might cause structural cortical changes that can be responsible for propagation of the seizure. These changes, in particular an increased permeability for inflammatory cells through the brain-blood barrier, appear due to chronic stimulation of cytokines (Penkowa et al., 2001; Janigro, 2012).

Three hypotheses were investigated in this study. A higher serum CRP concentration was expected in dogs with IE than in healthy dogs of a control group, since in both the human and veterinary literature, it has been confirmed that epileptic activity leads to systemical inflammatory reactions (Peltola et al., 1998; Vezzani et al., 2013). In human medicine, serum CRP alterations were investigated in patients with epilepsy (Peltola et al., 2002; Alapirtti et al., 2012; Sutter et al., 2013; Ishikawa et al., 2015), whereas veterinary studies are much more limited (Nakamura et al., 2008; Holtman et al, 2013). Secondly, a higher serum CRP concentration was expected in dogs with cluster seizures (two or more seizures within 24 hours), than in dogs with isolated seizures (Lorenz et al., 2011). Probably, there is a more severe form of inflammation in cluster seizures, because of the high frequency of seizures during a short time-span. In only one study, serum CRP concentrations in humans with status epilepticus (SE), which is also an acute and severe seizure type, has been investigated. The results of this study showed no consistent association between serum CRP concentration and the outcome of status epilepticus (Sutter et al., 2013). So far, no other human or veterinary studies have already compared serum CRP concentrations in patients with cluster seizures/SE and patients with isolated seizures. Finally, a higher serum CRP concentration was expected when the sample was taken closest to the last seizure, because a decrease in systemical inflammation could have occured in the period after seizure. This presumption has already been demonstrated in humans and rats, but not in dogs (Peltola et al., 2002; Alapirtti et al., 2012; Holtman et al., 2013).

MATERIALS AND METHODS

Patient selection

Serum samples were collected at the Small Animal Department of the Faculty of Veterinary Medicine, Ghent University (2015-2016). Serum CRP measurement was only performed in dogs with IE whose blood had to be taken for other purposes such as generalized blood work or serum concentration monitoring for standard follow-up of antiepileptic treatment. Inclusion criteria for dogs with IE were: 1. onset of seizures between six months and six years of age, 2. normal physical and neurological examination interictally (except for the neurological abnormalities due to the treatment with anti-epileptic drugs or due to seizure induced cerebral hypoxic/excitoxic damage), 3. normal hematology, 4. normal biochemistry (Na, K, Cl, Ca, P, ALT, AST, AF, Gamma-GT, bile-acids (preprandial), NH_3, bilirubin, urea, creatinine, total protein, albumin, glucose, cholesterol, triglycerides, total T4 (if total T4 was low, TSH was measured, and if still doubtfull, a TSH stimulation test was added) 5. no abnormal findings on magnetic resonance imaging of the brain and cerebrospinal fluid analysis (cell count and protein measurement), if available. The timing between blood sampling, the last seizure and the fact whether the dog had isolated or cluster seizures, was tracked. The dogs in the control group were all healthy Beagles (aged between five and seven years) having no neurological abnormalities. These serum samples had been collected for another study at the Small Animal Department, Faculty of Veterinary Medicine, Ghent University.

To complete the first and second part of the study, the sample closest to the seizure was used when more than one serum sample was available of the same dog. The evolution of serum CRP concentration in time after the last seizure was evaluated as a last part of this study. Serial samples, if available, were all included. The time after the seizure was described in four categories: 0-3 days, 4-14 days, 15 to 31 days, and more than 1 month after the last seizure.

Sample processing

Each sample was collected in a serum tube and centrifuged afterwards. A part of the serum was transferred into 2 ml tubes and preserved in a -80°C freezer awaiting further analysis. The CRP measurements were carried out at Idexx laboratories® using immunoturbidimetry. Normal values ranged between 0 and 10.7 mg/L.

Statistical analysis

The analytical program SPSS® was used to process the data. For the first and second part of the study, an unpaired student T-test was conducted. One mg/L was added to each CRP concentration and was followed by logarithmic transformation to obtain a value for each CRP concentration and to acquire a normal distribution. The mean CRP concentration (for the first part both in the IE and the control group, for the second part both in the group of dogs with cluster seizures and in the group of dogs with isolated seizures) and 95%-confidence intervals were calculated. After-

wards, every mean concentration was again converted to the actual CRP concentration and decreased by 1 mg/L. For the third part of this study, a linear mixed regression model was built in order to take into account clustering of repeated observations. In every test, $P < 0.05$ was considered significant.

RESULTS

An overview of the number of dogs that was included in each part of this study is displayed in Table 1. In the first part of this study, in which serum CRP concentrations in dogs with and without IE were compared, a total amount of 66 samples were collected including 38 samples of dogs with IE and 28 samples of the dogs in the control group. In the second part of the study, 16 samples of dogs with cluster seizures and 22 samples of dogs with isolated seizures were included so that a total amount of 38 samples were used in this part of the study. For the group of dogs with cluster seizures, the time between the seizure and the blood sample ranged from samples taken during the seizure to 53 days after the last seizure (mean: 9 days). For the group of dogs with isolated seizures, time ranged from 15 minutes to 304 days (mean: 49 days). For the last part of the study, in total, 50 samples of 37 dogs were included. There were 27, 7 and 3 dogs that produced respectively 1, 2 and 3 samples. Each group contained respectively 13, 13, 12 and 12 dogs.

ln each part of this study, there was a normal distribution of the data after logarithmic transformation. An overview of mean serum CRP concentrations is displayed in Table 1. The mean serum CRP concentration in dogs with IE [7.8 mg/ml ± standard error of the mean (SE) 0.09] was not different from the mean CRP concentration in the dogs of the control group (8.3 mg/ml ± 0.08) ($P = 0.8$). The results of the second part of this study showed higher mean serum CRP concentrations in the dogs with cluster seizures (11.8 mg/ml ± 0.08) than in the dogs with isolated seizures (5.7 mg/ml ± 0.13); however this was not statistically significant ($P = 0.077$). Finally, the serum CRP concentrations of four groups were compared, with the first group representing the shortest time and the fourth group the longest time after the last seizure. For the linear mixed regression model that was built, an overall P-value of 0.2 was obtained, which means that the time after the last seizure had no significant influence on the logarithmic serum CRP concentrations in this study. However, when comparing mean serum CRP concentrations of group 1 (i.e. blood sample taken 0 to 3 days after last seizure) and group 4 (i.e. blood sample taken more than one month after last seizure), a higher mean serum CRP concentration was present in group 1 (10.2 mg/l) than in group 4 (3.4 mg/l). However, this result was not statistically significant ($P = 0.077$) (Figure 1).

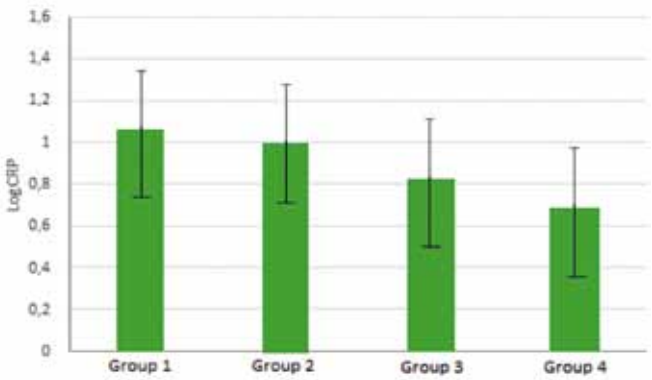

Figure 1. Mean logarithmical serum CRP concentrations of each group (group 1: 0-3 days after a seizure; group 2: 4-14 days after a seizure; group 3: 15-31 days after a seizure; group 4: >1 month after a seizure). The error bars are representing the range of the 95%-confidence interval.

DISCUSSION

The hypothesis that dogs with IE have increased serum CRP concentrations could not be confirmed in this study. This is in contradiction with certain human

Table 1. Number of dogs and mean serum CRP concentration (in mg/l).

	Groups	Number of dogs	Mean CRP concentration (in mg/l)
IE vs. control	**Total**	**66**	
	IE group	38	7.8
	Control group	28	8.3
Isolated vs. cluster seizures	Total	38	
	Isolated seizures	22	5.7
	Cluster seizures	16	11.8
Time after seizure	**Total**	**50**	
	0 - 3 Days after seizure	13	10.2
	4 - 14 Days after seizure	13	9.2
	15 - 31 Days after seizure	12	5.4
	> 1 Month after seizure	12	3.4

studies where significantly higher serum CRP concentrations were found in patients with epilepsy (Alapirtti et al., 2012; Ishikawa et al., 2015). Moreover, these studies showed higher serum CRP concentrations in humans with generalized seizures than in humans with focal seizures. In the present study, these different types of seizures were not compared. However, higher serum CRP concentrations were expected, due to the high frequency of generalized seizures in dogs with IE (Schwartz-Porsche, 1994; Knowles, 1998). On the contrary, in some human studies, no significant differences in serum CRP concentrations in patients with or without epilepsy could be found (Peltola et al., 2002; Sutter et al., 2013). In one veterinary study, serum CRP concentrations between dogs with epilepsy and healthy dogs were compared, but no differences in serum CRP concentrations were detected (Nakamura et al., 2008). In that study, dogs with all types of epilepsy were included (Nakamura et al., 2008), whereas only dogs with IE were included in the present study, leading to a more homogenous population. It is possible that a significant difference in serum CRP concentrations in dogs with and without IE could not be found because the blood samples were not taken at the same time after seizure. The results suggest potentially lower serum CRP concentrations when samples were taken later after the last seizure. Since the majority of the samples were not taken soon after the last seizure, this might have influenced the results. The chance to detect a higher CRP concentration in dogs with IE than in the dogs of the control group, might have been higher when the serum sample would have been taken shortly after the last seizure. Another reason might be that the dogs of the control group might have had a higher serum CRP concentration due to presence of a subclinical inflammation, but as all dogs of the control group appeared clinically healthy, this hypothesis is less likely.

The results of the second part of this study suggest a potential difference in logarithmic transformed serum CRP concentrations between dogs with cluster seizures and dogs with isolated seizures, although the results are not significant. It may be assumed that dogs with cluster seizures show a more severe form of inflammation than dogs with isolated seizures, as seizures are more frequent over a short-term period and there is less time for the body to reduce the inflammation. So far, nothing has been described about serum CRP concentration in dogs with cluster seizures and dogs with isolated seizures. In a study by Sutter et al. (2013), a clear association between CRP levels and outcome in human patients with SE, another acute and severe seizure type, could not be demonstrated. Two other studies demonstrated higher serum CRP concentrations in humans with generalized versus focal seizures (Alapirtti et al., 2012; Ishikawa et al., 2015), but this was not investigated in the present study.

Finally, the numerical differences in logarithmic serum CRP concentrations between group 1 (10.2 mg/l) and group 4 (3.4 mg/l), indicate that lower serum CRP concentrations might be expected when the sample is taken longer after the last seizure. However, this result was statistically not significant. In the literature, an increase in serum CRP concentrations has been described in the first hours after an epileptic seizure both in humans and rats (Peltola et al., 2002; Alapirtti et al., 2012; Holtman et al., 2013). In the present study, not all CRP concentrations were measured this soon after a seizure. The cause of a decrease in serum CRP concentrations during the days after the seizure could be due to a reduction of the inflammatory response (Otabe et al., 2000). However, when CRP concentrations between all the groups were compared, no significant differences could be found, which may have multiple causes. 1. There is no difference in serum CRP concentration between dogs with and without IE. 2. The confidence intervals cover a wide range. When using a larger sample size, they may become more dense (Figure 1). 3. The amount of serial samples was not the same for all of the dogs. 4. It might take more time for the CRP concentrations to decrease, but previously published work shows that the CRP molecule has a fast elimination halftime, which makes this hypothesis less likely (Young et al., 1991; Shrive et al., 1996).

As the sample size in this study was limited, future research using a larger patient group is necessary to support the results of this study.

CONCLUSION

In conclusion, inflammatory reactions might play a role in dogs with IE. In this study, no significant differences in serum CRP concentrations in dogs with IE and dogs of the control group were shown. The results demonstrated lower mean CRP concentrations in dogs with cluster seizures than in dogs with isolated seizures and lower mean CRP concentrations in samples taken longer after the last seizure. However, this could not be demonstrated with statistical significance. Notwithstanding these findings, a larger sample size in each of the tested groups is required before final conclusions can be drawn.

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OP DIJNSDAG 19 DECEMBER, om 9 uur

in de Crevestraat, nabij de smederij De Waele, in plaats van op Maandag 18 December, gelijk het aangekondigd was door onzen aanplakbrief van 8 December 1916.

J. MARIEN.

VII-68 Foto: Paarden branden in de Crevestraat bij hoefsmid De Waele.