Determination of Serum C-Reactive Protein (CRP) in Healthy Beagle Dogs of Various Ages and Pregnant Beagle Dogs

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Abstract: Serum C-reactive protein (CRP) concentrations in healthy beagle dogs of various ages and in pregnant beagles were measured by enzyme-linked immunosorbent assay (ELISA). Serum CRP concentrations were 1.5–16.0 µg/ml (mean 7.9 ± 3.4 µg/ml) in male, and 1.8–18.9 µg/ml (mean 8.3 ± 4.0 µg/ml) in female dogs. No significant sex-related differences were observed in the values. Further, there were no significant age-related differences either. Serum CRP concentrations increased during pregnancy. The concentration of serum CRP in pregnant dogs peaked at 70.2–90.4 µg/ml (mean 77.5 ± 7.1 µg/ml) 30 or 45 days after ovulation, demonstrating two characteristic features of CRP concentration change in pregnant dogs.

Key words: CRP, dogs, physiological concentrations, pregnancy

Introduction

Human C-reactive protein (CRP) is one of the acute-phase proteins produced in response to inflammation following an infection or tissue damage [6, 12, 14]. Since CRP is a representative, typical acute-phase protein, the dynamics of its concentration during a disease have been described quite well [9, 12, 14], and it is recognized to be one of the essential clinical examination items in human medicine. The physiological properties of human CRP have been investigated already. The changes of canine CRP concentration have been reported to accompany changing pathological conditions. Like human CRP, canine CRP markedly increases during pneumonia [15, 18], surgical trauma [1, 2, 16], and various other inflammatory disorders [7, 14, 16, 19].

Prior investigators reported the use of canine CRP in veterinary medicine as an inflammatory marker, in the toxicology of drugs and in clinical diagnostics. Very few reports, however, are available on physiological CRP concentrations in beagle dogs. Intra-day/daily
variations of CRP [10] and production of CRP in young and adult dogs [5] have only been described as physiological characteristics. In spite of CRP concentration being routinely examined against various pathologic backgrounds, not much has been done to clarify the physiological CRP concentration in dogs. Further, very few researchers have reported on the concentration changes of acute phase proteins during pregnancy in humans and dogs [4, 8, 13]. Determining the physiological concentrations and properties of CRP in dogs is very important for diagnosis in veterinary medicine and in the toxicology of drugs.

Systematic research on the physiological CRP concentrations was conducted in the course of the present study for two groups of beagle dogs: healthy dogs of various ages and pregnant dogs.

### Materials and Methods

#### Experimental dogs and serum

Seventy healthy male beagle dogs and 74 healthy female beagle dogs, with ages ranging from 3 to 72 months in one month intervals (except for 60 months in male), were used to establish age-related CRP properties. Eight healthy 1 to 6-year-old pregnant beagles weighing 8 to 13 kg were used for CRP determination in pregnant dogs. The dogs were kept in isolators at the Breeding Division of Hongo Beagle Farm, Kitayama Labes Co., Ltd. (Yamaguchi, Japan) at a temperature of 23 ± 2°C, relative humidity of 55 ± 10%, on a continuous 12/12 dark/light cycle (6:00–18:00), with air exchanged 12 times or more per hour. The dogs were fed solid feed DS-E 200 g/dog per day (Oriental Yeast Co., Ltd., Tokyo, Japan), and allowed free access to water. All experiments conformed to the Japanese regulations concerning animal care and use, following the Guideline for Animal Experimentation (Japanese Association for Laboratory Animal Science, JALAS, 1987), and were approved by the Institutional Animal Care and Use Committee of the Kitayama Labes Co., Ltd.

Serum was collected from 8 pregnant dogs at 15 day intervals during pregnancy. Calculation of the ovulatory day was determined retrospectively as the 63rd day before delivery [3]. All serum samples were stored at –80°C until ready for use.

#### Measurement of CRP

The serum samples were diluted with 0.01 M phosphate-buffered saline (PBS, pH 7.2) prior to analysis. The serum CRP concentrations were measured by sandwich enzyme-linked immunosorbent assay (ELISA) according to Yamamoto et al. [16].

#### Statistical analysis

Student’s unpaired t test was used to evaluate sex differences, while age-related differences were compared with one-way analysis of variance (ANOVA) at a significance level of p<0.05.

### Results

#### CRP concentration in healthy beagles of various ages

Serum CRP concentrations in healthy male and female beagle dogs are shown in Table 1. The serum CRP concentrations ranged from 1.5 to 16.0 µg/ml (mean 7.9 ± 3.4 µg/ml) in male and 1.8–18.9 µg/ml (mean 8.3 ± 4.0 µg/ml) in female dogs. No significant sex-/age-related differences were observed.

#### CRP concentration in pregnant dogs

The change of serum CRP concentrations in pregnant dogs are shown in Fig. 1. Two characteristic patterns of this parameter were confirmed: the maximum serum CRP concentration ranged from 70.2–90.4 µg/ml (mean 77.5 ± 7.1 µg/ml), and the maximum concentration was observed 30 or 45 days after ovulation.

### Discussion

Similar to its function in humans, CRP is recognized as a useful inflammation marker in dogs [11]. Canine CRP has already been used in veterinary medicine, as well as in toxicology, diagnosis of inflammation due to drugs [2, 16–18]. The concentrations from normal dogs kept in private households and those kept in kennels by breeders have been reported [18], however, few have reported on the physiological CRP concentrations systematically. Furthermore, little has been described of the CRP concentration in pregnant dogs. Determination of physiological CRP concentrations in beagles, therefore, is a new and important area of research.

The present study defines physiological CRP concentrations in healthy beagle dogs of various ages and
pregnant beagle dogs by the method of quantitative immunoassay. The mean serum CRP concentration determined in male dogs was 7.9 µg/ml, and in female dogs was 8.3 µg/ml. These values are consistent with previous reports with regard to the concentrations of CRP in normal dogs kept in private households or in kennels by breeders [18]. No significant sex- or age-related differences were observed in the course of the present study, and the obtained CRP concentrations are considered to be the physiological CRP concentrations of healthy beagle dogs. The results of the CRP concentrations are suggested for use as a reference in toxicological examinations.

In pregnant beagles the serum CRP concentration increased to 70.2–90.4 µg/ml (mean 77.5 ± 7.1 µg/ml) at 30 to 45 days after ovulation. These were the two characteristic features of CRP change in pregnant dogs. Ekersall et al. reported that the change of serum CRP concentration in dogs during pregnancy could be defined by three characteristic features [4]. The result of our study was consistent with two of those three features, while the third feature, a serum CRP concentration increase during the post-parturition period, was not observed. Further, while Ekersall et al. reported serum CRP concentration increased again during the post-parturition period as a feature, we detected only a single case among the 8 dogs [4]. These results suggest that the serum CRP concentration during pregnancy should be carefully monitored, because the elevation pattern may differ individually. While the underlying causes of the CRP concentration increase during pregnancy are not understood, it is known that endocrine hormones affect acute phase proteins (such as alpha 1-glycoprotein) during pregnancy [13]. Though further confirmation is needed, we presume that the serum CRP concentration increase during pregnancy is also due to the influence of endocrine hormones.

We determined the physiological CRP concentrations in healthy beagle dogs, and the CRP increase in the middle of gestation, establishing reference values for the physiological properties of dogs for use in examinations of the CRP concentration.

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**Table 1.** Serum C-reactive protein (CRP) concentrations (µg/ml) in healthy male and female beagle dogs

<table>
<thead>
<tr>
<th>Age (months-old)</th>
<th>Range</th>
<th>Mean ± SD</th>
<th>Range</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>4.1–15.9</td>
<td>9.4 ± 3.2</td>
<td>4.0–13.9</td>
<td>8.8 ± 3.3</td>
</tr>
<tr>
<td>6</td>
<td>6.7–13.9</td>
<td>9.6 ± 2.3</td>
<td>2.6–18.9</td>
<td>9.0 ± 4.7</td>
</tr>
<tr>
<td>9</td>
<td>3.1–13.2</td>
<td>8.2 ± 3.1</td>
<td>5.5–13.1</td>
<td>10.0 ± 2.4</td>
</tr>
<tr>
<td>12</td>
<td>2.4–11.4</td>
<td>7.9 ± 3.3</td>
<td>4.4–14.2</td>
<td>9.6 ± 3.3</td>
</tr>
<tr>
<td>18</td>
<td>1.5–11.6</td>
<td>7.3 ± 3.2</td>
<td>2.4–15.3</td>
<td>7.8 ± 3.5</td>
</tr>
<tr>
<td>24</td>
<td>2.0–16.0</td>
<td>7.5 ± 4.1</td>
<td>2.7–10.6</td>
<td>5.9 ± 2.6</td>
</tr>
<tr>
<td>60</td>
<td>—</td>
<td>—</td>
<td>1.8–15.7</td>
<td>6.5 ± 5.4</td>
</tr>
<tr>
<td>72</td>
<td>1.6–12.6</td>
<td>5.8 ± 3.4</td>
<td>3.9–18.3</td>
<td>9.5 ± 6.4</td>
</tr>
<tr>
<td>Total</td>
<td>1.5–16.0</td>
<td>7.9 ± 3.4</td>
<td>1.8–18.9</td>
<td>8.3 ± 4.0</td>
</tr>
</tbody>
</table>

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Fig. 1. Change of serum CRP concentrations (µg/ml) in pregnant dogs.
Acknowledgments

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References