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Elimination Half-Lives of Acute Phase Proteins in Rats and Beagle Dogs During Acute Inflammation

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ABSTRACT—The half-lives of typical acute phase proteins in rats and beagle dogs during acute inflammation were investigated. Acute inflammation was induced by injection of turpentine oil in rats and administration of indomethacin in beagle dogs. Serum concentrations of α_2-macroglobulin (α2M) and C-reactive protein (CRP) were measured by enzyme-linked immunosorbent assay and α_1-acid glycoprotein (AAG) was measured by single radial immunodiffusion. Half-life was calculated as 0.693/elimination rate constant (*K*). The mean half-lives in the terminal elimination phase of α2M and AAG were 68.1 and 164.8 h, respectively. The half-life of AAG was significantly longer than that of α2M. Mean half-lives in the terminal elimination phase of CRP and AAG were 161.9 and 304.4 h, respectively. The half-life of AAG was significantly longer than that of CRP in beagle dogs. No significant differences in the half-life of AAG were observed between rats and beagle dogs. Furthermore, serum concentrations in the terminal elimination phase could be simulated with the *K* data acquired in this study.

KEY WORDS: CRP; AAG; α2M; half-life; beagle dogs; rats.

INTRODUCTION

Acute phase proteins are useful inflammatory markers in humans, dogs, and rats. C-reactive protein (CRP) and α_1-acid glycoprotein (AAG) are typical acute phase proteins in dogs [1–6], while α_2-macroglobulin (α2M) and AAG are typical acute phase proteins in rats [7–10]. These acute phase proteins increase after inflammatory stimulation such as administration of turpentine oil, infection with microorganisms, or surgical treatment [5, 7, 8, 11–16]. The kinetics of these acute phase proteins have been studied in rats and dogs after inflammatory stimulation. Acute phase proteins have been shown to increase during acute inflammation and to decrease gradually after reaching peak serum concentration. However, few reports are available on the elimination process of acute phase proteins. The purpose of this study was to evaluate the elimination half-life of acute phase proteins in dogs and rats after induced inflammation. The serum concentrations of CRP increased in dogs administered with indomethacin [17]. Turpentine oil has also been used to induce inflammation in many previous studies, reliably and with little individual variation [14, 18, 19]. The elimination half-lives in rats and beagle dogs induced by administration of turpentine oil or indomethacin were thus investigated in this study.

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MATERIALS AND METHODS

Animals

Five male Sprague–Dawley rats (6 weeks of age) were purchased from Charles River Laboratories Japan (Yokohama, Japan). Five beagle dogs (body weight, 12 to 15 kg)

were purchased from Saitama Experimental Animals Co. Ltd. (Saitama, Japan). Rats and beagle dogs were kept in isolation at a temperature of 23±2 °C and relative humidity of 55±10 % on a 12/12 dark (2000–0800 hours)/light (0800–2000 hours) cycle, and the air was exchanged 12 or more times per hour. Rats were fed MF (Oriental Yeast Co., Ltd., Tokyo, Japan) and were allowed free access to water. All experiments conformed to Japanese regulations with regard to animal care and use, as described in the Guidelines for Animal Experimentation (Japanese Association for Laboratory Animal Science, JALAS, 1987). The present animal experiments were approved by the Institutional Animal Care and Use Committee of Azabu University.

Animal Experimental Design

Rats

Turpentine oil (Wako Pure Chemical Industries, Co., Ltd., Osaka, Japan) was intramuscularly injected at 0.2 ml/kg body weight. Ventricular blood was collected before turpentine oil injection and at 24, 48, 72, 96, 144, 192, 240, and 336 h after injection, under slight anesthesia with pentobarbital by intravenous injection of pentobarbital (Kyoritsu Seiyaku Corporation, Tokyo, Japan) at a dose of 6 mg/kg. Serum was kept at −80 °C until analysis.

Beagle Dogs

Indomethacin (ICN Biomedicals Inc., Aurora, OH) was suspended in 0.5 % methyl cellulose (Wako Pure Chemical Industries, Ltd.) solution. After fasting for 18 h, beagle dogs were orally administered with indomethacin at 60 mg/kg using a catheter. Blood was collected from the cephalic vein before administration and at 24, 48, 72, 96, 144, 192, 240, and 336 h after administration. Serum was kept at −80 °C until analysis.

Measurement of α2M, CRP, and AAG

Serum concentrations of α2M were measured by enzyme-linked immunosorbent assay (ELISA) as described by Honjo *et al.* [12]. Serum concentrations of CRP in beagle dogs were measured by ELISA according to the procedure of Yamamoto *et al.* [16]. Serum concentrations of AAG in both rats and beagle dogs were measured by the single radial immunodiffusion method using commercial kits (Metabolic ecosystem Co., Ltd., Miyagi, Japan).

Calculation of Half-Life

Peak concentration was obtained in each individual animal. The linear slope of serum acute phase protein concentration versus time was then plotted on log-linear regression for each individual animal. The elimination rate constant (K) was calculated using a minimum of three measured serum concentrations [20]. Half-life ($t_{1/2}$) was calculated from the formula:

$$\text{Slope} = \frac{\log C^{\text{A}} - \log C^{\text{B}}}{\text{Time (A)} - \text{Time (B)}}$$
$$K\left(h^{-1}\right) = (-2.303) \times \text{slope}$$
$$\text{t1/2 } (h) = 0.693/K$$

where K is the elimination rate constant, C^{A} is serum concentration at Time (A), and C^{B} is serum concentration at Time (B)

Statistical Analysis

Half-lives of α2M, AAG, CRP, and AAG were analyzed using paired Student's t test. Half-life of AAG between beagle dogs and rats was analyzed using unpaired Student's t test. p values of <0.05 were considered to be significant.

RESULTS

Rats

The changes in mean serum concentration of α2M and AAG in rats are shown in Figs. 1 and 2, respectively. The elimination half-lives of α2M and AAG are shown in Table 1. The serum concentrations of α2M and AAG decreased in two phases. The first phase of α2M was from peak concentration to 144 h and the terminal phase was from 144 to 336 h after administration of turpentine oil. The first phase of AAG was from peak concentration to 96 h and the terminal phase was from 96 to 336 h after administration of turpentine oil. The half-life of AAG in the terminal phase was significantly longer than that of α2M (p=0.02).

Beagle Dogs

The changes in mean serum concentrations of CRP and AAG in rats are shown Figs. 3 and 4, respectively.

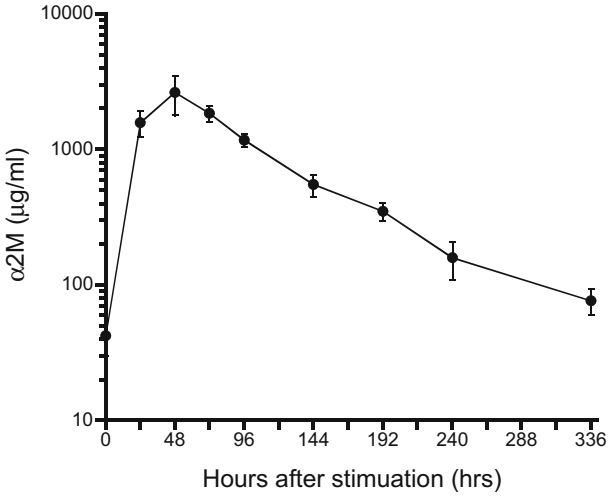

Fig. 1. Changes in serum concentrations of α_2-macroglobulin (*α2M*) in rats after administration of turpentine oil. *Each point* represents mean ± SD ($n=6$).

The elimination half-lives of CRP and AAG are shown in Table 1. Serum concentrations of CRP and AAG decreased in two phases. The first phases of CRP or AAG were from peak concentration to 144 h and the terminal phase was from 144 to 336 h after administration of indomethacin. The half-life of AAG in the terminal phase was significantly longer than that of CRP ($p=0.04$). Moreover, no significant differences were observed in the half-life of AAG between rats and beagle dogs ($p=0.12$).

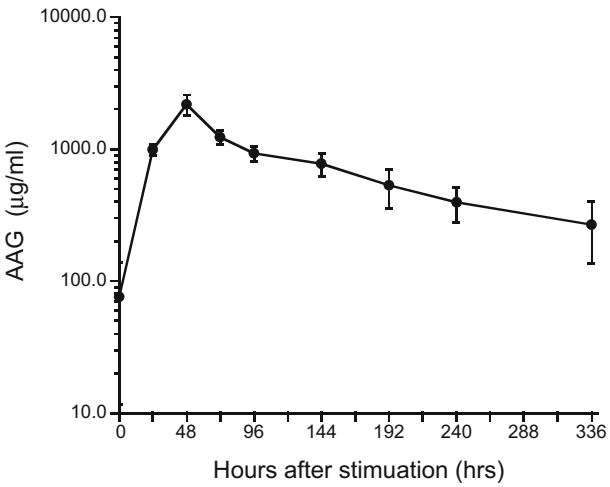

Fig. 2. Changes in serum concentrations of α_1-acid glycoprotein (*AAG*) in rats after administration of turpentine oil. *Each point* represents mean ± SD ($n=6$).

DISCUSSION

Serum concentrations of α2M, AAG, and CRP in rats and dogs increased after inflammatory stimulation [12–16, 21–23]. However, the elimination phase of these acute phase proteins has not been investigated and the half-lives are not known. Thus, the half-lives of these acute phase proteins were estimated in this study.

The ratios between pretreatment and peak concentration of α2M and CRP were larger than for AAG, similarly to previous reports [6, 10]. These results suggest that α2M and CRP increase markedly when compared with AAG in rats and beagle dogs. Peak concentrations of α2M and AAG in rats were observed at 48 h after administration of turpentine oil. Observations regarding peak concentrations were similar to previous reports [10, 12, 14]. On the other hand, the peak concentrations for CRP and AAG were observed at 48 and 72 h after administration of indomethacin, respectively. Peak concentrations of CRP were reported at 24 h [24, 25] or 48 h [26, 27] after inflammatory stimulation. The timing of peak concentration was thought to vary with differences in the methods of inflammatory stimulation. Moreover, the ratio of pretreatment and peak CRP concentration was smaller than that of AAG; CRP increased more sensitively than AAG after inflammatory stimulation. Thus, peak CRP concentration occurred more rapidly than that of AAG in beagle dogs.

The half-life of AAG between rats and beagle dogs did not differ significantly. The half-life of AAG was significantly longer than those of α2M in rats and CRP in beagle dogs. Thus, α2M and CRP showed increased sensitivity to acute inflammation, but decreased more rapidly than AAG. The half-life of CRP in humans is reported to be 18 h [28]. The half-life in beagle dogs was longer than that in humans. The serum concentration of CRP decreased in two phases and the half-life of the terminal elimination phase was longer than that of the first phase. Thus, it was assumed that 18 h refers to the half-life of the first phase [28]. The half-lives of CRP and AAG in beagle dogs ranged from 71.6 to 356.5 and 146.6 to 677.5, respectively. Half-lives showed large individual variations. Acute inflammation in beagle dogs was induced by administration of indomethacin. Gastric bleeding is known to be an adverse event of indomethcin [17, 29–31]. Otabe *et al.* reported vomiting and hematochezia, and found that serum concentrations of CRP increased in beagle dogs administered indomethacin [17]. These symptoms were also observed in beagle dogs in this study and the extent of

Table 1. Kinetic Parameters of Acute Phase Proteins in Rats and Beagle Dogs

Experimental animals	Acute phase proteins	*C*max (μg/ml)	*C*max/pre-value	Elimination rate constant	Elimination half-life (h)
Rats	α2M	2,687.3±797.0	70.8±36.8	0.0103±0.0014	68.1±9.6
	AAG	2,194.0±384.1	42.8±24.3	0.0051±0.0022	164.8[a]±86.4
Beagle dogs	CRP	153.6±64.4	53.2±40.2	0.0058±0.0029	161.9±114.5
	AAG	1,939.1±553.4	4.3±1.0	0.0031±0.0016	304.4[a]±218.5

Each value were represented mean±standard deviation (rats: $n=6$, beagle dogs: $n=5$)
α2M α_2-macroglobulin, *AAG* α_1-acid glycoprotein, *CRP* C-reactive protein
[a] Values differs significantly from α2M in rats, from CRP in beagle dogs ($p<0.05$)

these symptoms showed large individual variations. Beagle dogs showing hematochezia and vomiting also tended to show longer half-lives. Thus, half-life was considered to be extended in beagle dogs showing severe symptoms.

Half-life in the terminal elimination phase was calculated using serum concentrations on and after 96 or 144 h. Serum concentrations after 96 or 144 h may be simulated using the following formula:

$$C^1 = C^2 \times \exp(-Kt)$$

C^1 : simulated serum concentration
C^2 : serum concentration at 96 or 144 h
exp : base ofnatural logarithm
K : elimination rate constant
t : (hours at estimated serum concentration) – (96 or 144 h)

For example, the serum concentration of CRP at 336 h after administration of indomethacin in beagle dogs showing severe symptoms was 865.1 μg/ml. However, the serum concentration of CRP simulated using an elimination rate constant obtained in this study was 580.6 μg/ml at 336 h. Thus, if the actual serum concentration of C^1 was higher than the simulated serum concentration, experimental animals were considered not to have recovered from inflammation.

In conclusion, the half-lives of AAG in rats and beagle dogs were significantly longer than α2M in rats and CRP in beagle dogs. Furthermore, serum concentrations in the elimination terminal phase can be simulated using the elimination rate constant acquired in this study.

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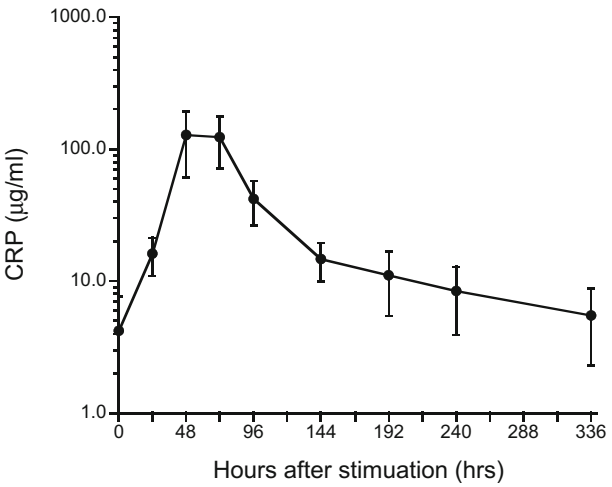

Fig. 3. Changes in C-reactive protein (*CRP*) in beagle dogs after administration of indomethacin (dose: mg/kg). *Each point* represents mean±SD ($n=5$).

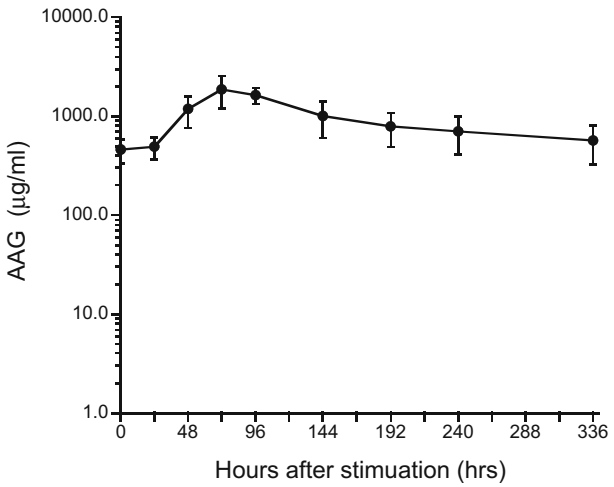

Fig. 4. Changes in concentrations of α_1-acid glycoprotein (*AAG*) in dogs after administration of indomethacin (dose: mg/kg). *Each point* represents mean±SD ($n=5$).

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