Characteristics of Increased Serum Amyloid A (SAA) and α_1•Acid Glycoprotein (AAG) Concentrations in Cats Subjected to Experimental Surgical Treatments or Inoculated with *Bordetella bronchiseptica*

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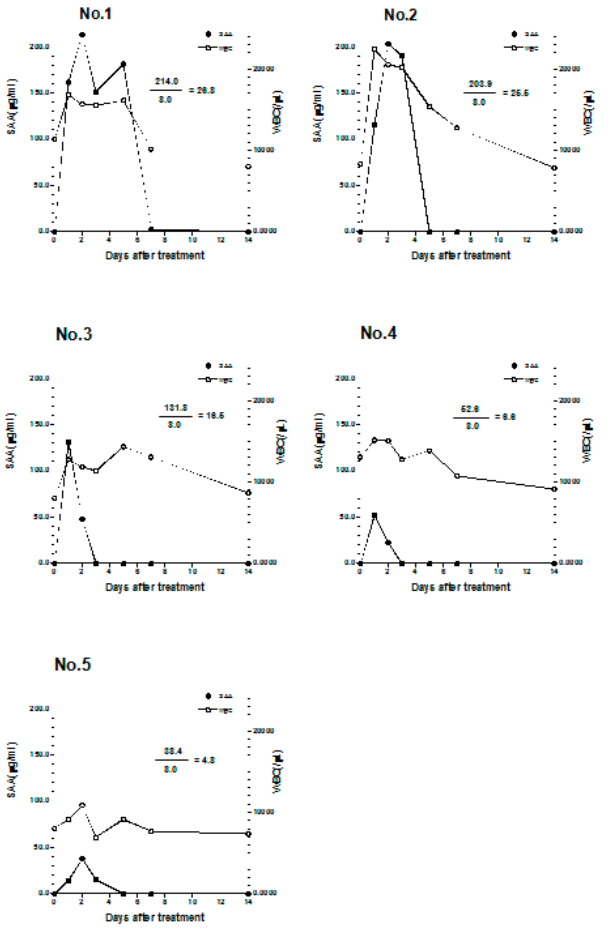
ABSTRACT

The concentrations of serum amyloid A (SAA) and α1•acid glycoprotein (AAG) in the serum of 11 healthy cats subjected to oophorohysterectomy (5 females) and gastrotomy (3 males, 3 females) were measured by turbidimetric immunoassay (TIA) for human SAA (human SAA-TIA) and single radial immunodiffusion (SRID) kits for feline AAG, respectively. Six healthy cats (3 males, 3 females) were inoculated intrabronchially with 2 × 109 live, avirulent cells of *Bordetella bronchiseptica* as a model of transient bronchopneumonia in order to investigate serum concentrations of SAA and AAG, as well as white blood cell (WBC) counts and antibody responses to *B. bronchiseptica* in sera by indirect fluorescence antibody technique (IFA). Peak concentrations of SAA after oophorohysterectomy and gastrotomy were 4.8 to 26.8 times and 11.5 to 23.4 times higher than pre-

***Figure 1**. Changes in serum concentrations of SAA in cats subjected to oophorohysterectomy. The values given in the figure are the ratios between pre-treatment and peak concentrations. Pre-treatment values were calculated as the detection limit (8.0 μg/ml).*



treatment concentrations, respectively, while those of AAG were 1.7 to 4.1 times and 1.8 to 2.6 times higher than pre-treatment concentrations, respectively. The ratio of pre-treatment and peak serum concentrations of SAA and AAG in cats inoculated with *B. bronchiseptica* ranged from 0.4 to 20.1 and 1.3 to 2.9, respectively. In these experiments, peak serum concentrations of SAA and AAG were observed at 1 or 2 days after surgical treatments and inoculation with *B. bronchiseptica*. SAA was not detected at 5 days after these treatments, except in 1 cat after oophorohysterectomy. Strong individual variations in SAA and AAG concentrations were observed in the present study.

INTRODUCTION

In cats, serum amyloid A (SAA) and α1•acid glycoprotein (AAG) are typical acute-phase proteins whose behavior has been studied in various diseases[1-3]. The concentrations of SAA4,5 and AAG[1,2] in healthy feline serum have been reported. On the other hand, feline SAA concentrations increase in feline infectious peritonitis (FIP) and Coronavirus infection6, pancreatitis7, various diseases[1,5,8] and after surgery[1,8,9]. Feline AAG concentrations also increase in cats with neoplasm[2], lymphoma[3] and feline infectious peritonitis[10]. However, detailed data regarding SAA and AAG concentrations after surgery in cats are scarce, and the magnitude of the SAA increase in cats is lower than in other species[11]. The changes in SAA and AAG concentrations after surgery, and the relationship between SAA and AAG concentrations and the progression of pneumonia are therefore poorly understood.

In this study, the increases in serum SAA and AAG concentrations were evaluated in healthy cats subjected to oophorohysterectomy and gastrotomy, and in healthy cats after induction of transient bronchopneumonia by intrabronchial inoculation with *Bordetella bronchiseptica*[12,13], a pathogen known to be responsible for canine infectious tracheobronchitis.

MATERIALS AND METHODS

Experimental Cats and Sera

Seventeen 2- or 3-year-old clinically healthy American shorthair cats (6 males and 11 females) weighing 3-4 kg were used for experimental surgery (6 males and 5 females) and infection (3 males and 3 females). Cats were kept in isolators within a facility kept at a temperature of 22 ± 2°C with a relative humidity of 60 ± 10% and a 12-h light/dark cycle (07:00-19:00). Concentrations of SAA and AAG were measured in sera from all 17 experimental cats. White blood cell (WBC) counts were obtained for all blood samples collected. All serum samples were stored at -80°C until use. All experiments conformed to Japanese regulations concerning animal care and use, as laid out in the Guidelines for Animal Experimentation14, and were approved by the Institutional Animal Care and Use Committee of Azabu University.

Hematological Examination

White blood cell counts were obtained using an automatic analyzer (MEK-63; Nihon Koden Corp., Tokyo, Japan).

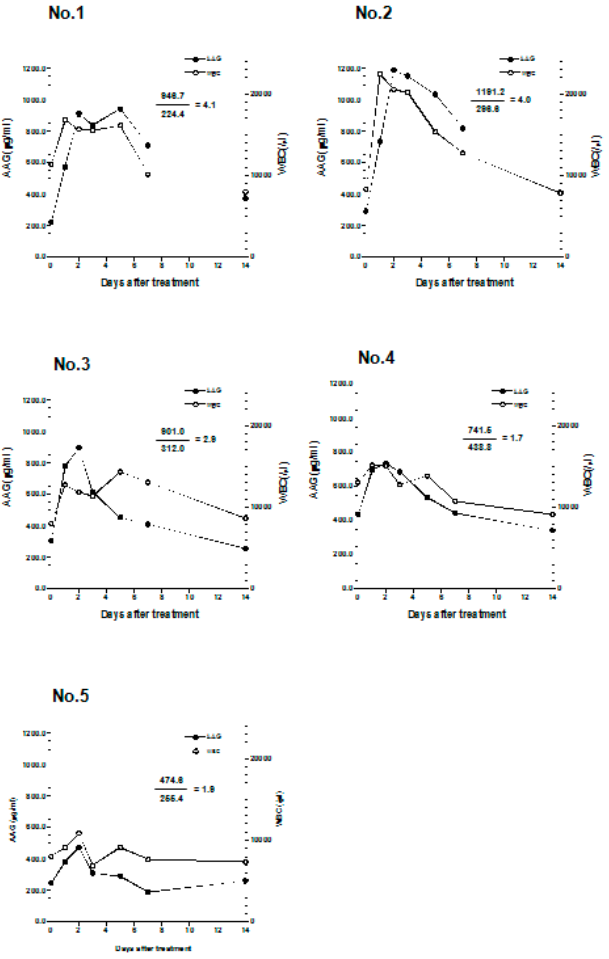
Experimental Surgery

Eleven cats (6 males and 5 females) were subjected to experimental surgery under anesthesia with isoflurane. Incisions of approximately 2 cm for oophorohysterectomy and approximately 10 cm for gastrotomy were made. Blood (1.5 ml) was collected before treatment and at 1, 2, 3, 5, 7 and 14 days after oophorohysterectomy or gastrotomy. Sera from cats subjected to oophorohysterectomy (n=5) and gastrotomy (n=6, 6 males) were tested.

Measurement of SAA and AAG

Serum SAA concentrations in cats were measured by human SAA turbidimetric immunoassay (human SAA-TIA) using LZ-SAA (Eiken Chemical Co., Ltd., Tokyo, Japan) in accordance with previous reports[5,9]. Serum AAG concentrations in cats were measured according to the method of Ohwada and Tamura[15] using a commercial single radial immunodiffusion (SRID) kit (Institute for Metabolic Ecosystem Co., Ltd., Furukawa, Japan) with an AAG detection limit of 40 υ∴μg/ml.

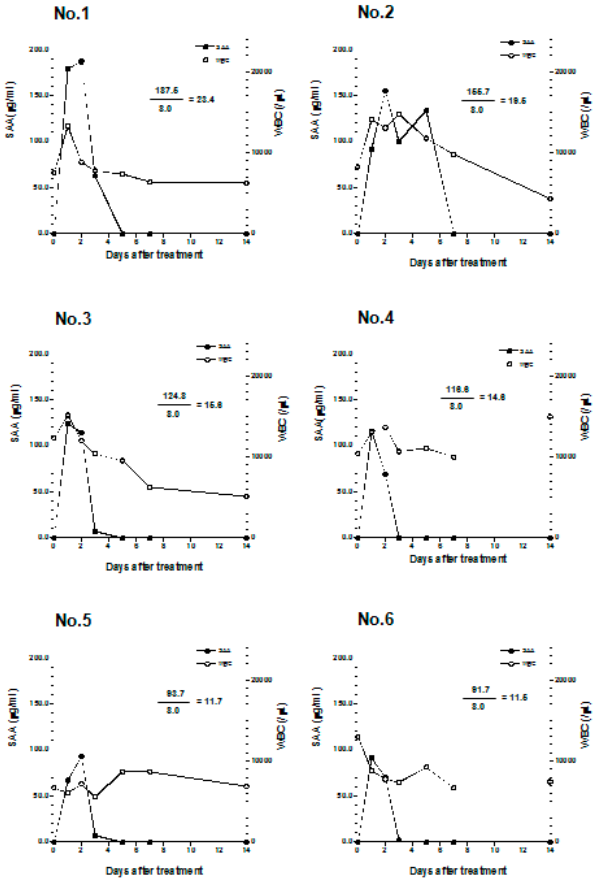
Figure 2. Changes in serum concentrations of AAG in cats subjected to oophorohysterectomy. The values given in the figure are the ratios between pre-treatment and peak concentrations.



Culture of *B. bronchiseptica*

B. bronchiseptica supplied by Dr A. Kiuchi, Laboratory of Veterinary Microbiology, Azabu University, was cultured at 37°C for 20 h in trypticase soy agar containing 5% bovine serum13. Cultured *B. bronchi-*

Figure 3. Changes in serum concentrations of SAA in cats subjected to gastrotomy. The values given in the figure represent the ratios between pre-treatment and peak concentrations. Pre-treatment values were calculated as the detection limit (8.0μg/ml).



septica was suspended at 109 cells/ml in sterile physiological saline containing 10% trypticase soybroth in order to prepare live, avirulent cells for inoculation. A suspension of *B. bronchiseptica* in physiological saline containing 3% formalin was used to prepare IFA slides.

Inoculation with *B. bronchiseptica*

After induction of anesthesia, a catheter was inserted into the bronchial branch[13]. In the control group, 2 ml of sterile physiological saline was inoculated via this catheter. In the other group, 2×10^9 living *B. bronchiseptica* was inoculated in 2 ml of solution. Blood (1.5 ml) was collected from experimental cats before inoculation and at 1, 2, 3, 5, 10 and 26 days after inoculation with *B. bronchiseptica* for measurement of SAA and AAG concentrations, and indirect fluorescence antibody titers.

Indirect Fluorescence Antibody Technique (IFA) for *B. bronchiseptica*

IFA for *B. bronchiseptica* in the sera collected at 26 days after inoculation was performed by a modification of the method of Killinger and colleagues16 using fluorescein isothiocyanate (FITC)-conjugated goat anti-cat IgG antibody (Southern Biotechnology Associates Inc., Birmingham, AL, USA).

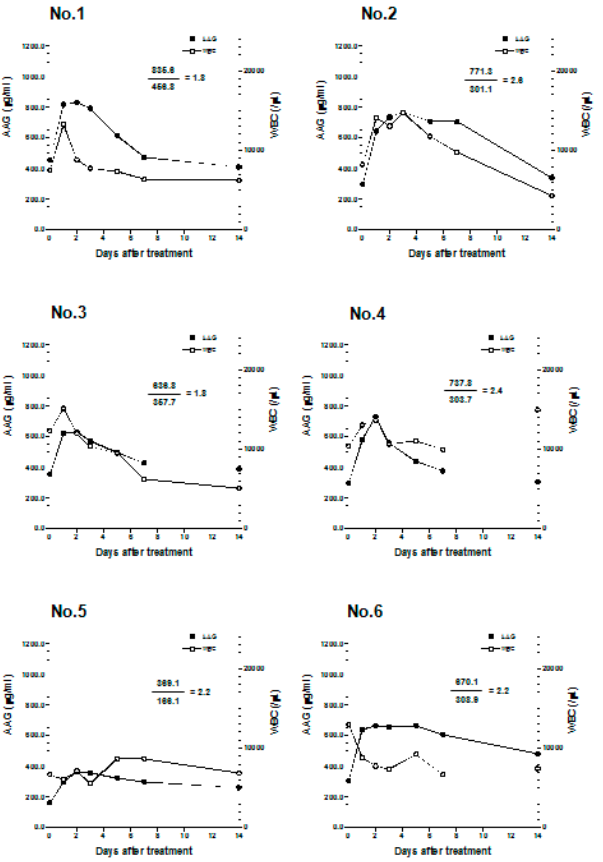
RESULTS

Serum concentrations of SAA and AAG, and WBC counts in cats subjected to oophorohysterectomy are shown Figure 1 and 2, respectively. The ratio of pre-treatment and peak serum concentrations of SAA and AAG ranged from 4.8 to 26.8 and from 1.7 to 4.1, respectively. Pre-treatment values of SAA were below the detection limit (8.0 μg/ml). Serum concentrations of SAA and AAG, and WBC counts in cats subjected to gastrotomy are shown Figure 3 and 4, respectively. The ratio of pre-treatment and peak serum concentrations of SAA and AAG ranged from 11.5 to 23.4 and from 1.8 to 2.6, respectively. Changes in serum SAA concentrations in cats subjected to oophorohysterectomy and gastrotomy varied widely in these experiments. We therefore considered mean values for each point to be inappropriate, and instead show individual concentrations in Figure 1 and 3. Serum concentrations of SAA and AAG, and titers in felines after

infection with *B. bronchiseptica* are shown Figure 5. No signs of bronchopneumonia were observed in bronchitis on auscultation. However, *B. bronchiseptica* IFA titers against *B. bronchiseptica* ranged from 1:64 to 1:258 at 26 days after inoculation. The ratio of pre-treatment and peak serum concentrations of SAA and AAG ranged from 1.2 to 20.1 and from 1.3 to 2.9, respectively.

Peak serum concentrations of SAA and AAG were observed at 1 or 2 days after oophorohysterectomy or gastrotomy. On the other hand, peak serum concentrations of SAA were observed sooner after infection with *B. bronchiseptica* when compared to AAG. Serum concentrations of SAA decreased more quickly than AAG, with SAA not being detected at 5 days after oophorohysterectomy, except in 1 cat. WBC counts increased after treatment and peak concentrations were observed at 1 or 2 days in all cases.

Figure 4. Changes in serum concentrations of AAG in cats subjected to gastrotomy. The values given in the figure are ratios between pre-treatment and peak concentrations.

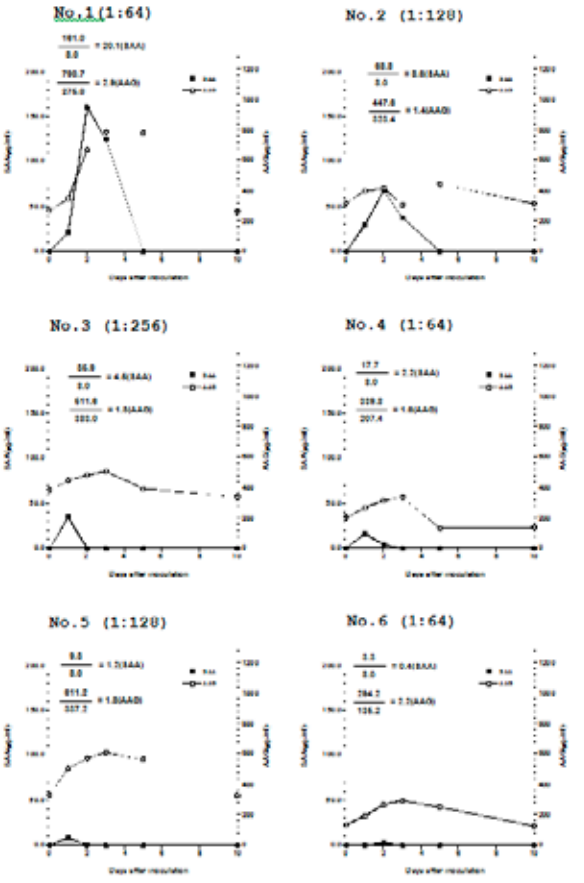


DISCUSSION

In cats, most acute-phase protein studies have focused on SAA and AAG. These proteins have been shown to work as major acute-phase proteins in cats17. However, there is little information on the relationship between surgical treatment or bronchopneumonia, and SAA and AAG in cats[1,8,9].

Peak concentrations of SAA were observed at 1-2 days after surgical treatment, and these recovered to almost pre-treatment levels as pre-treatment at 3-7 days after surgical treatment, similarly to previous reports[1,8,9]. However, increases in serum SAA concentrations in cats subjected to experimental surgery varied widely in these experiments (Figures 1 and 2) and the peak concentrations after oophorohysterectomy and gastrotomy were 4.8 to 26.8 times and 11.5 to 23.4 times higher than pre-treatment concentrations, respectively. These results are different from those reported previously[1,8,9]. The relatively small increase in SAA was not considered to be attributable to measurement by human SAA-TIA, but was thought to be a characteristic of SAA as a feline acute-phase protein. In other words, because SAA increased >10-fold in all cats subjected to gastrotomy, in which a large skin incision was made, increases in SAA were thought to be related to the degree of tissue destruction as in humans[19] and dogs1. On the other hand, although

Figure 5. *Changes in serum concentrations of SAA and AAG in cats after inoculation with Bordetella bronchiseptica. Data in parentheses indicate IFA titers against B. brochiseptica. The values given in the figure are the ratios between pre-treatment and peak concentrations. Pre-treatment values were calculated as the detection limit (8.0 μg/ml).*



the rate of increase in AAG was higher for oophorohysterectomy, for which the surgical wound was small, the reason for this finding is unknown. In addition, because it took many days for AAG concentrations to return to preoperative levels after surgery, SAA is considered to be a more suitable inflammatory marker in cats. The increase rate of AAG in cats subjected to oophorohysterectomy in this study and in cats under inflammation induced by intramuscular injection of turpentine oil in another report were similar to observations in dogs[18]. This suggests that the slight increases in AAG concentrations in cats are similar to the AAG characteristics observed in other animals[15, 18]. WBC count peaked one day after surgery earlier than SAA and AAG in most cats, but because trends in its subsequent reduction varied, it was considered to be useful for determining pathological status when used in combination with SAA.

Diagnosis of individual pathologic conditions was therefore considered to be difficult based on fragmentary SAA or AAG concentrations, in contrast to CRP concentrations in humans[19,20] and dogs[21,22]. Therefore, it is difficult to determine cut off values for feline SAA and AAG, and individual daily SAA and AAG concentrations were necessary to diagnosis pathological status after surgical treatment in cats.

B. bronchiseptica is a pathogen responsible for canine infectious tracheobronchitis, commonly known as kennel cough, which is a highly contagious respiratory tract disease in dogs[12,23]. Induction of artificial bronchopneumonia by single inoculation of *B. bronchiseptica* was achieved in cats. Therefore, IFA titers against *B. bronchiseptica* were observed (1:64-1-256), and serum SAA and AAG concentrations in these cats were slightly elevated. However, onset of typical bronchopneumonia was not observed. This suggests that variations in SAA and AAG are a useful indicator for evaluating the pathology that develops in bronchopneumonia following spontaneous infection.

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