Evaluation of Feline Serum Amyloid A (SAA) as an Inflammatory Marker

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ABSTRACT. The concentration of feline serum amyloid A (fSAA) was determined by a direct enzyme-linked immunosorbent assay (ELISA) by using fSAA specific monoclonal antibodies, to evaluate the fSAA as an inflammatory marker in cats. The mean concentration ± standard deviation of fSAA was found to be 0.60 ± 1.06 µg/ml and 33.65 ± 67.59 µg/ml in serum samples from normal cats (n=45) and cats (n=312) with various diseases and disorders, respectively. A significant difference (p<0.001) was found between the two groups. It was also found that the concentration of fSAA begins to increase rapidly at approximately 3–6 hr after spay, and increases up to significantly high levels in some disorders, like injury, renal failure, infectious diseases, etc.

KEY WORDS: feline, inflammatory marker, serum amyloid A (SAA).

The acute phase response is the initial response to inflammatory stimuli such as infection, trauma, tumors and surgery. One characteristic feature of this response is increased hepatic synthesis of a heterogeneous group of plasma proteins termed as acute phase protein, which include C-reactive proteins (CRP), serum amyloid A (SAA), haptoglobin, and α1-acid glycoprotein, etc. CRP and SAA, the major acute phase proteins in human, exhibit a dramatic increase in serum concentration in response to inflammatory stimuli, thus have been used as inflammatory markers in human medicine [18].

SAA, a serum precursor of amyloid A which is the main fibrillar component in reactive amyloid deposits [7], has been characterized as a heterogeneous protein in human [16] and other several species [3, 5, 13, 15, 19, 25]. The high degree of conservation of the SAA genes and proteins that have been maintained during evolution of eutherian mammals [20], provides further evidence that they are likely to have important biological functions. The normal physiological function of SAA, which constitutes a major component of the high density lipoprotein 3 complex [1], remains unclear, though it is assumed to have a crucial, yet undefined, protective role during inflammation [21].

Plasma SAA levels can be increased up to 100-fold of the basal level in inflammatory disorders in human [22] and other species [6, 24], suggesting it to be an important indicator of disease status. However, whether feline SAA (fSAA) can be used as an inflammatory marker or not, has not been well evaluated yet. Earlier, we have expressed the recombinant feline SAA (rfSAA) [12], and produced the fSAA specific monoclonal antibodies [14]. Those materials gave us an idea to evaluate if fSAA could be used as an inflammatory marker in cats. In order to measure the concentration of SAA, a considerable number of assays, such as laser immunoensephelometric assay [4], latex agglutination nephelometric immunoassay [23], sandwich enzyme-linked immunosorbent assay (ELISA) [10, 24], single radial immunodiffusion [11], non-competitive chemiluminescence enzyme immunoassay [6] and direct ELISA [17] have been developed in human and other species. However, a method for the measurement of SAA concentration in cats has not been developed yet. We considered that direct ELISA is a rapid and simple method, so would be useful in routine clinical diagnosis and clinical management. Therefore, a direct ELISA was developed and used for evaluation of feline SAA as an inflammatory marker. A number of factors crucial for the direct ELISA, such as type of ELISA plate, composition of dilution buffer for serum samples, coating time and temperature, dilution rate of serum samples, and type of blocking buffer were carefully optimized and set up as follows. Serum samples were diluted 1:70 in PBS-T (phosphate-buffered saline with 0.05% Tween 20) containing 1% of Gelatin, and coated on Nunc-Immu MaxiSorp™ surface plates (Nalge Nunc International, Denmark) at 37°C for 4 hr. The plates were incubated with horseradish peroxidase (HRP)-conjugated monoclonal antibodies against fSAA (Boehringer Mannheim Biochemical, Germany) at 37°C for 1 hr, after washing 4 times with PBS-T. After incubation, the plates were washed again 3 times. The reaction was initiated by adding ABTS Microwell Peroxidase Substrate (Kirkegaard and Perry Laboratories, Gaithersburg, U.S.A.), and absorbance at 405 nm was determined on a bichromatic microplate reader. To make a standard curve for measuring fSAA, the purified rfSAA was serially diluted and used. Available range of fSAA concentrations based on the standard curve was 0.34 to 350 µg/ml. The SAA concentrations of serum samples were calculated from this standard curve.

Serum samples were collected from cats suffering from various diseases and disorders, and from clinically normal cats. The sera were stored at −20°C until use. Serum samples obtained from clinically healthy cats were consid-
ered as normal serum samples. The concentrations of fSAA in the normal serum samples (n=45) ranged from ≤0.34 to 3.6 μg/ml, and the mean concentration ± standard deviation (SD) was 0.60 ± 1.06 μg/ml. The serum samples from cats with various diseases and disorders were also tested by the direct ELISA. The concentration range of fSAA in those serum samples (n=312) was from ≤0.34 to 350 μg/ml, and the mean ± SD was 33.65 ± 67.59 μg/ml. A significant difference (p<0.001) between the normal serum samples and the serum samples from cats with various diseases and disorders was found by Welch’s t-test. A positive cutoff value was provisionally estimated as 3.79 μg/ml calculated by using the following formula: mean + 3SD, based on the concentrations of normal serum samples (Fig. 1). The concentration of fSAA in the serum samples from normal cats found to be 0.60 ± 1.06 μg/ml in the present study, differs from that of 16.6 ± 11.4 μg/ml detected by Sandwich ELISA [8], and that of 74.4 ± 23.2 μg/ml detected by Radial Immunodiffusion [2]. Similar results have been reported in humans, in which, the mean concentration of SAA in normal serum samples ranges from <0.05 μg/ml to 2.5 μg/ml detected by different methods [9]. This difference in results within various reports is possibly due to the use of different detection methods and the specificity of the antibodies used.

Among the serum samples from cats with various diseases and disorders, injury (n=46, 61.63 ± 97.48 μg/ml, Fig. 2), renal failure (n=75, 30.98 ± 55.67 μg/ml, Fig. 2) and infectious diseases (n=48, 47.22 ± 86.40 μg/ml, Fig. 2) showed significant difference (p<0.001) to the normal samples. The serum samples from feline lower urinary tract diseases (n=27, 27.99 ± 62.59 μg/ml, Fig. 2), tumors (n=24, 16.57 ± 28.40 μg/ml, Fig. 2) and diabetes (n=6, 7.85 ± 4.37 μg/ml, Fig. 2) showed difference (p<0.05) to the normal samples. In contrast, enteritis (n=9, 40.18 ± 82.72 μg/ml, Fig. 2), oral disorder (n=14, 2.90 ± 4.25 μg/ml, Fig. 2) and liver disorder (n=21, 13.90 ± 31.28 μg/ml, Fig. 2) showed no difference to the normal samples (Fig. 2). These results suggest that fSAA could be a useful marker for some, but not for all diseases and disorders. The group of enteritis, oral disorder and liver disorder did not show any significant difference to the normal samples. This may probably be because disordered liver casts a negative effect on the synthesis of the acute phase proteins, including SAA. For the group of enteritis and oral disorder, more serum samples and more detailed classification is needed, so as to study the changes of concentrations of fSAA in those diseases, and make the results more clear.

To measure the response time of SAA, serum samples collected from healthy cats (n=3) undergoing spay under general anaesthesia, before and after the operation, were evaluated. The concentrations of SAA were found to begin increase at approximately 3–6 hr, and reaching to the peak values at approximately 21–24 hr after the operation (Fig. 3). This result is in accordance with the previous report [8], suggesting that fSAA reacts rapidly to inflammatory stimuli, thus, can be a useful marker for indicating inflammatory responses.

As SAA was reported responding rapidly to inflammatory diseases in humans [22] and some species of animals [6, 24], it can also be a useful marker for indicating the status of some inflammatory diseases of cats. It was found that the concentration of fSAA begins to increase rapidly at approximately 3–6 hr after spay, and increases up to significantly high levels in some disorders, such as injury, renal...
failure, infectious diseases, feline lower urinary tract diseases, tumors and diabetes. These positive results would be useful for studying the role of fSAA as an inflammatory marker in various diseases in detail.

REFERENCES