

Evaluation of serum and milk amyloid A in some inflammatory diseases of cattle

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Summary

This study was designed to evaluate serum and milk amyloid A (SAA) as an inflammatory indicator in inflammatory diseases. Twenty clinically healthy cows and 100 cows with various inflammatory diseases were selected for this study. Blood samples were collected from the coccygeal vein of both healthy and diseased groups. Milk samples were taken from four quarters of both groups. Milk samples of four quarters from each cow were mixed, then one sample was taken from pooled milk. There was significant difference in concentrations of serum and milk AA between clinically healthy cows and diseased cows ($P < 0.05$). The difference of SAA and MAA between cows with subclinical mastitis and other inflammatory disease of cows was also significant ($P < 0.05$). In conclusion, serum and milk amyloid A are useful diagnostic indicators in the inflammatory diseases of cows.

Key words: Amyloid A, Milk, Serum, Inflammatory diseases, Clinically healthy cows

Introduction

Serum amyloid A (SAA) is an apolipoprotein of high-density lipoprotein (Nakayama *et al.*, 1993; Gruys *et al.*, 1994; Husby *et al.*, 1994). As acute phase protein, it is thought to influence high-density lipoprotein-cholesterol transport. In tissues, it attracts inflammatory cells and inhibits the respiratory burst of leukocytes (Linke *et al.*, 1991) and modulates the immune response (Gruys *et al.*, 1994). It is described to bind lipopolysaccharide, comparable to lipopolysaccharide binding protein (Schroedl *et al.*, 2001). Several isotypes of SAA are found; types 1 and 2 represent positive acute phase proteins. In the bovine, also a negative protein cross-reacting with anti-SAA serum has been described (Yamamoto *et al.*, 1998). The reference value for SAA in apparently healthy cows was determined as < 8.8 mg/L (Horadagoda *et al.*, 1999).

Determination and evaluation of SAA showed that this protein could be valuable

factor in the diagnosis of infections (Alsemgeest *et al.*, 1994). Gronlund *et al.* (2003) reported that in cows infected with staphylococcal mastitis, SAA elevated rapidly in milk and serum in acute phase. Whole herd screening using SAA can be of value to the veterinarian in identifying cows with inflammatory diseases (Karreman *et al.*, 2000). The stages of disease can be better evaluated by monitoring more than one acute phase protein, so chronic as well as acute conditions should be evaluated and characterised by acute phase protein profiling (Eckersall, 2004). Measurement of SAA could be a useful tool for evaluation of health in calves (Ganheim *et al.*, 2007). There are no published reports about the comparison of serum and milk amyloid A concentrations in post-surgical abdominal infection, acute metritis, acute local traumatic reticuloperitonitis (TRP) and theileriosis due to *Theileria annulata*. Therefore, the present study was conducted to evaluate serum and milk AA as inflammatory indicators in various

inflammatory diseases of cows.

Materials and Methods

Data were obtained from an observational clinical study conducted in Veterinary Clinic of Islamic Azad University, Kazeroon, Iran and six dairy farms in Fars province of Iran. Twenty clinically healthy adult cows were selected randomly as control cases for this study. All cows that were selected for this study were in milking period. Barely, corn and concentrates were used in the diet of dairy cows. All cows had a history of vaccination against foot and mouth disease, brucellosis and anthrax about five months ago. One hundred cows with various inflammatory diseases were examined for common infectious diseases such as acute local TRP (20 cases), subclinical mastitis (20 cases), theileriosis due to *Theileria annulata* (20 cases), post-surgical abdominal infection (20 cases) and acute metritis (20 cases). Diseased cows were thoroughly examined and appropriate samples were collected for haematology, clinical biochemistry and other relevant analysis (Nazifi *et al.*, 2008). The presence of inflammation and disease was assessed on the basis of clinical examination and laboratory findings. Blood samples were collected from the coccygeal vein of both clinically healthy and diseased groups. Milk samples were collected from the quarters of mammary glands. For determination of SAA, blood samples were collected into plain vacutainers and the serum was separated following centrifugation for 15 min at 750 g. Serum samples were stored at -20°C until analysed. Serum and milk amyloid A concentrations were measured using a solid phase sandwich enzyme-linked immunosorbent assay

(ELISA) (Tridelta Development Plc, Co. Wicklow, Ireland). The sensitivity of this test has been determined as 0.3 µg/ml in serum and 0.10 µg/ml in milk. Detection of significant differences between groups conducted by one way ANOVA and Duncan's multiple comparison test. Relationship between AA of serum and milk was compared by Pearson correlation test. SPSS/PC software version 11.5 was used for statistical analysis (SPSS-PC software, Chicago, Illinois). All values were expressed as mean ± standard error (SE), and $p < 0.05$ was considered as statistically significant.

Results

The mean (\pm SE) concentrations of serum and milk AA and their correlation in cows with common infectious diseases and clinically healthy cows are presented in Table 1.

There were significant differences in mean concentrations of serum and milk AA between clinically healthy cows and diseased cows and also, between subclinical mastitis and other infectious diseases ($P < 0.05$). Statistical evaluations showed that there are no significant correlation between serum and milk AA in each of inflammatory diseases ($P > 0.05$; Table 1).

Discussion

In the present study there were significant differences in serum and milk amyloid A between clinically healthy cows and cows with different inflammatory diseases ($P < 0.05$). That is due to increase in the production of cytokines that are responsible for the increase in synthesis of acute phase proteins in cows with

Table 1: Concentrations of serum and milk amyloid A (mean \pm SE) and their correlation in cows with common infectious diseases and clinically healthy cows

Groups	Number	SAA (µg/ml)	MAA (µg/ml)	Correlation coefficient
Clinically healthy cows	20	72.71 \pm 5.95 ^a	6.96 \pm 0.34 ^a	0.41*
Acute local traumatic reticuloperitonitis (TRP)	20	250.39 \pm 7.76 ^b	228.80 \pm 6.89 ^c	-0.23
Subclinical mastitis	20	162.19 \pm 6.81 ^c	54.53 \pm 2.96 ^b	-0.34
Post-surgical abdominal infection	20	239.91 \pm 7.62 ^b	482.74 \pm 10.83 ^d	0.12
Theileriosis	20	218.44 \pm 7.97 ^b	206.94 \pm 5.10 ^c	-0.03
Acute metritis	20	227.50 \pm 7.49 ^b	364.93 \pm 7.87 ^d	0.009

Means within a column with different superscript letters (a, b, c, d) denote significant differences ($P < 0.05$).

*Indicates $P < 0.05$

inflammatory diseases. Gronlund *et al.* (2003) reported that in cows infected with staphylococcal mastitis, SAA elevated rapidly in milk and serum in acute phase. Gronlund *et al.* (2005) stated that a substantial variation in SAA concentrations in milk was observed in udder quarters with chronic subclinical mastitis. Previous studies have been revealed that the concentration of SAA was higher in serum and milk of the cows with mastitis than in the cows with extramammary inflammatory conditions (Nielsen *et al.*, 2004). However, in this study the mean concentrations of SAA and MAA was lower in serum and milk of the cows with subclinical mastitis than in the cows with extramammary inflammatory disease. The reason may be that the severity of subclinical mastitis is lower than other inflammatory disease. SAA concentrations in milk increased significantly with increasing somatic cell count, suggesting that they may be indicators of the severity of the infection (Nielsen *et al.*, 2004). SAA concentrations below the detection limit were considered as a good indicator of healthy udder quarters (Gronlund *et al.*, 2005). Serum SAA and iron profiles reflected the course of inflammation and their levels correlated with the clinical severity of the inflammation. Lehtolainen *et al.* (2004) reported that during experimental endotoxin-induced mastitis, SAA concentrations increased both in serum and in milk. Pedersen *et al.* (2003) reported that the parallel development of inflammation and increased concentration of SAA in milk points to this acute phase protein as potential diagnostic marker for the early detection of *Streptococcus uberis*- associated mastitis.

The results of the present investigation showed that there are no correlation between concentration of SAA and MAA in each of the inflammatory diseases that had been studied. These results can be due to intramammary synthesis of SAA3. The mammary gland is a well known source of a SAA3 variant (Eckersall *et al.*, 2001; McDonald *et al.*, 2001; Larson *et al.*, 2005). It secretes in colostrum and mastitis milk and has beneficial functions for the gut mucosa of the offspring (Larson *et al.*, 2003a, b; Mack *et al.*, 2003).

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display a profound acute-phase protein response. SAA inhibit the local inflammatory response to *Acinetobacter baumannii* pneumonia, which may facilitate bacterial outgrowth (Renckens *et al.*, 2006). In contrast, fever and changes in the leukocyte numbers, which are usually considered to be hallmarks of inflammation and infection, were not useful for monitoring post-operative recovery. Measurement of SAA may improve post-operative monitoring (Jacobsen *et al.*, 2005). SAA has the greatest role in bacterial and pyogenic infections and increases in common infectious diseases such as metritis, haematologic, respiratory and digestive infections and TRP. Circulating concentrations of SAA were significantly higher at admission in horses with colic attributable to conditions having a primary inflammatory cause (e.g., enteritis, colitis, peritonitis, or abdominal abscesses) and were higher in horses that failed to survive the episode of colic, compared with horses that survived. Evaluation of SAA concentrations may be helpful in identifying horses with colic attributable to diseases that have inflammation as a primary component of pathogenesis (Vandenplas *et al.*, 2005). Post-operative concentration of SAA provide valuable information on the subsidence of the inflammatory response during the uneventful post-operative period. SAA could be a useful diagnostic marker of early post-operative complications (Dabrowski *et al.*, 2007).

In the present study, the concentration of SAA in cows infected with *Theileria annulata* was significantly higher than in the clinically healthy cows ($P < 0.05$). In this respect, Glass *et al.* (2003) reported that following experimental infection with *Theileria annulata* in cattle, serum amyloid A appeared first, followed by a rise in alpha-1-acid glycoprotein in all animals, whereas haptoglobin only appeared in some of the animals, and generally at a low level. The production of SAA and alpha-1-acid glycoprotein correlated strongly with each other, and also with some clinical measures of the severity of the disease, including the time to fever, development of leukopenia, parasitaemia and mortality. In the present study, the concentration of SAA in cows

with acute metritis was significantly higher than clinically healthy cows ($P < 0.05$). Humblet *et al.* (2006) reported that serum amyloid A should be used with caution as a marker of inflammation in the week after calving. Poor sensitivity in other postpartum periods could be related to the higher incidence of chronic (vs acute) inflammation.

The results of the present study reveal that serum and milk amyloid A are useful indicators of various inflammatory conditions in cows. The sensitivity of evaluation of this acute phase protein in diagnosis the disease is more than haematological and clinical tests. Haematological factors are very variable in different stages of inflammatory diseases, and clinical tests diagnose the disease when it has been developed. However, acute phase proteins increase during the development of disease and decrease in the recovery stage and can diagnose the disease in the early stages.

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