

EFFECTS OF EXPERIMENTALLY INDUCED *STAPHYLOCOCCUS AUREUS* INFECTION ON BLOOD PROTEIN FRACTIONS IN OBESE RABBITS

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ABSTRACT

Staphylococcus aureus causes suppurative inflammation and is frequently isolated from infected sites in rabbits. The aims of the study was to investigate the variations of blood protein fractions during a 7 days long period after subcutaneous inoculation of *S. aureus* in fattening obese castrated rabbits. Male New Zealand White rabbits (n=6) were castrated at 2.5 months of age, fattened for 1.5 month and subcutaneously inoculated with a *S. aureus* field strain (suspension concentration: 8×10^8 cfu/mL, injection volume: 100 μ L) when they were 4 months old. General health status, water/food intake, rectal temperature and skin lesions at the inoculation site as well as proteinemia, albuminemia and globulinemia were determined for 21 days whereas plasma protein fractions were analysed by electrophoresis for 7 days after the bacterial challenge. Lethargy, a strong decrease in food consumption and a marked hyperthermia were observed during the first 6 h whereas abscesses were formed in all rabbits at the inoculation sites within 48-96 h and consecutive invasive suppurative phlegmons persisted until the 7 and the 14 days in 4 rabbits. The inoculated germ was re-isolated from these lesions. Significant hypoalbuminemia and hyperglobulinemia were noticed on day 14 whereas α_2 -, β_1 - and β_2 -globulin fractions were significantly increased from day 2 to day 7, after inoculation compared to the basal values. The simultaneous determination of the blood protein fractions may help to characterize the inflammation intensity in rabbits with experimental staphylococcal infection.

Key words: Rabbits, *Staphylococcus aureus*, Inflammation, Abscess, Albumin, Globulins

INTRODUCTION

Staphylococcus aureus is a versatile opportunistic pathogen that causes a wide spectrum of pathologies (Corpa *et al.*, 2009). In rabbits, this bacterium infects dermal lesions causing suppurative dermatitis, and invades subcutaneous tissues, causing different well-known disease conditions such as fatal septicaemia, mastitis, ulcerative pododermatitis, rhinitis, conjunctivitis, dacryocystitis, abscesses (subcutaneously or affecting internal organs) and skin infections (Devriese *et al.*, 1981; Segura *et al.*,

2007; Corpa *et al.*, 2009). Economic losses in industrial livestock husbandry attributed to staphylococcal infections are considerable at a worldwide scale. It is generally admitted that in rabbit farms, staphylococcal infection is introduced with newly delivered animals. Two types of *S. aureus* are observed in affected rabbit populations (Devriese *et al.*, 1981). The one affects single animals or a small part of the population and induces therefore weak economic losses whereas the second type causes epidemic spread of the disease in the farm, and results in chronic problems that make the farm unprofitable. Acute phase reactant proteins reported to increase in serum of rabbits in response to inflammation include C-reactive protein, haptoglobin, ceruloplasmin and fibrinogen (Gruyse *et al.*, 1994). Among these proteins haptoglobin and ceruloplasmin migrate in the alpha-2-region of plasma protein electrophoresis, while fibrinogen migrates to the beta region. Increases in alpha-2-globulins are often measured when serum from animals with various inflammatory conditions is subjected to protein electrophoresis. Several studies indicate that body fat stores are mobilized during the infectious process. Investigations of white adipose tissue show that it is not only a depot of triacylglycerols, but it is also an important endocrine organ, secreting a large number of biologically active substances. Part of these substances (TNF- α , IL-1, IL-6), induce production of acute phase proteins by hepatocytes. Increased levels of cytokines and acute phase proteins lead to activation of inflammatory signal pathways and associated with low grade, but chronic systemic inflammation.

The objective of this study was to investigate the effects of experimentally induced *S. aureus* infection on blood protein fractions in obese rabbits.

MATERIALS AND METHODS

Animals and experimental design

The experimental procedure was approved by the Ethic Committee at the Faculty of Veterinary Medicine. Male New Zealand White rabbits were born from healthy doe rabbits. They were reared in individual sanitized metal cages (modules) with a grate floor at room temperature (20-22 °C). In order to become obese, 6 rabbits were castrated as described earlier under general anaesthesia when they were 2.5 month old, then fed with standard pelleted feed (Bonmix, Lovech, Bg) according to their age and had free access to tap water. The composition of the pelleted feed was as following: crude protein 18%; crude fat 2,7%; crude fibre 13%; crude ash 5%; lysine 0,77%; methionine + cystine 0,65%; calcium 1.0%; phosphorus 0,5%; chlorides 0,45; energy 2590 kcal/kg; vitamins: A-10 000 IU/kg; D₃-1000 IU/kg; E-50 mg/kg, Cu as (CuSO₄)-20 mg/kg. The fattening period lasted 1.5 months. Thereafter rabbits, with an average body weight of 4.43kg, were experimentally infected with 100 μ L of bacterial suspension of a field *S. aureus* strain (suspension concentration: 8×10^8 cfu/mL) by subcutaneous route as described by Wills *et al.* (2005). Blood samples were collected from all obese rabbits immediately before bacterial inoculation (0 h, pre-treatment values) and 6, 24, 48, 72 h and 7, 14 and 21 days, after by puncture of the *v. auricularis externa* into sterile heparinised tubes and were immediately centrifuged (1 500g, 10 min, 4° C) to obtain plasma. Plasma samples were decanted and stored at -20°C until assayed. All animals survived until 21 days post infection, were euthanized by intravenous injection of a lethal thiopental dose (Sandoz GmbH Austria, 100 mg/kg) and were subjected to gross anatomy, histopathological and bacteriological examinations. Firstly, rabbits were monthly weighted in order to control obesity development. Prior to inoculation and 6, 24, 48, 72 h as well as on the 7, 14 and 21 day post-infection, the rectal body temperature and the presence and size of the formed abscesses were recorded. Some parameters related to the general conditions of the rabbits, i.e. behaviour, intake of food and water, were also monitored.

Bacteriological Analysis

Swab samples of purulent exudates were collected aseptically when fistulisation of the abscesses occurred. Samples were inoculated on Blood agar with 8-10% sheep blood (BUL-BUO base, National Institute of Infectious and Parasitic Diseases, Sofia). Cultures were incubated aerobically at 37°C for 24 h. For animals slaughtered on day 21 post-infection, the identification of bacteria isolates was performed according to routine bacteriological techniques (Abdel-Gwad *et al.*, 2004) from the internal organs (liver, spleen, kidney and heart), skin lesions and the site of bacterial inoculums injection.

Blood Protein Analysis

Protein electrophoresis was performed in agarose gels using an automated Hydrasys system and Hydragel Protein15/30 agarose gels (Sebia Hispania). Running conditions were 200 Volts for 30 minutes for cellulose acetate electrophoresis (CAE) and at 33V-h for 7 min for and agarose gel electrophoresis (AGE) CAE strips (AGE) gels were stained with Amido Black (Cellogel) at the Veterinary Clinical Biochemistry Laboratory, Faculty of Veterinary Medicine, Barcelona, Spain.

Statistical analysis

Data statistical analysis was performed using one way analysis of variance (ANOVA). The significance of differences of means between post infection and base line values was evaluated by LSD test. All data are expressed as mean \pm standard error (SE) and the differences were considered significant if $P < 0.05$.

RESULTS AND DISCUSSION

Just before the experimental *S. aureus* infection, the body weights measured when the rabbits were 4 months old ($4.43 \pm 0.09\text{kg}$) were markedly increased compared to weight determined when the animals were 3 months old ($3.20 \pm 0.03\text{kg}$) ($P < 0.001$), confirming the obesity. During the first 24 h after the bacterial inoculation, rabbits were lethargic and did not eat or drink. Afterwards, they gradually recovered, the food and water intakes progressively increased and became similar to the pre-inoculation values at the 72 h. Rectal body temperature significantly increased at the 6 h compared to pre-treatment value ($P < 0.001$) then declined and remained closely related to the basal values until the end of the experimental period (day 7). The ingested food per day was between 120 and 200 g per rabbit. All the rabbits were with common reservoir for water supply. As early as the 24 h after the inoculation, a restricted, temperate hyperaemic painful swelling appeared at the injection site. Spreading diffuse subcutaneous purulent skin lesions (phlegmons) which affected vast areas near the site of bacterial inoculation were observed on day 7 in 4 rabbits. Fourteen days post-inoculation, a purulent yellowish-gray exudate was observed after the fistulisation of the underlying abscess at the site in these animals. The appearance of abscesses at the site of inoculation in all experimental rabbits, as well as the re-isolation of the challenging strain from abscesses provided evidence for the successful reproduction of the experimental infection. Abscess formation within 48-96 h post *S. aureus* infection corresponded to findings of Wills *et al.* (2005).

Bacteriological and Biochemical Analyses

S. aureus with the characteristics of the challenging strain was isolated from all swab abscess samples. No *S. aureus* was isolated from the visceral organs. Plasma concentrations of total proteins, albumin and globulins as well as the albumin/globulin (A/G) ratios according to time after *S. aureus* subcutaneous inoculation were summarized in Table 1.

Table 1: Variations of the plasma concentrations (g/L) of total proteins, albumin and globulins and of albumin/globulin (A/G) ratios in obese castrated rabbits (n = 6) after experimental *S. aureus* subcutaneous inoculation (field strain suspension density: 8×10^8 cfu/mL, 100 μ L).

Time points	Total proteins	Albumin	Globulins	A/G
0 hour	65.28 \pm 0.11	39.50 \pm 0.09 ^a	25.81 \pm 0.07 ^a	1.53 \pm 0.07 ^a
6 hours	65.73 \pm 0.09	39.98 \pm 0.08 ^a	25.75 \pm 0.09 ^a	1.55 \pm 0.07 ^a
24 hours	64.56 \pm 0.10	36.95 \pm 0.07 ^{ab}	27.61 \pm 0.06 ^a	1.34 \pm 0.03 ^{ab}
48 hours	64.31 \pm 0.14	36.15 \pm 0.09 ^{ab}	28.16 \pm 0.07 ^{ab}	1.28 \pm 0.05 ^{ab}
72 hours	65.13 \pm 0.22	36.33 \pm 0.13 ^{ab}	28.80 \pm 0.11 ^{ab}	1.26 \pm 0.04 ^b
7 days	68.93 \pm 0.14	38.13 \pm 0.10 ^a	30.80 \pm 0.12 ^{ab}	1.24 \pm 0.07 ^b
14 days	68.33 \pm 0.12	35.71 \pm 0.13 ^b	32.61 \pm 0.18 ^b	1.10 \pm 0.09 ^b
21 days	66.86 \pm 0.28	37.51 \pm 0.11 ^{ab}	29.35 \pm 0.30 ^{ab}	1.28 \pm 0.15 ^{ab}

Different superscripts (a, b) in the same column indicate significant differences ($P < 0.05$ or more) according to time after bacterial inoculation.

Although plasma proteinemia slightly increased 7 and 14 days post-inoculation, no significant difference related to time was recorded in obese rabbits. By contrast, albuminemia slowly declined after bacterial inoculation and reached a significantly lowered value compared to the basal one on day 14 ($P < 0.05$) whereas globulin concentrations gradually increased in parallel and were significantly higher on day 14 than basal concentrations ($P < 0.05$). Consequently, the A/G ratios progressively declined and were significantly lowered compared to the initial values on days 3 and 7 after bacterial challenge ($P < 0.05$) and reached minimal values on day 14 ($P < 0.01$).

The analysis of the plasma protein electrophoresis performed between 0 h (immediately before bacterial challenge) and 7 days after the subcutaneous *S. aureus* inoculation was presented in the Figure 1. The proportions of albumin have dramatically declined after bacterial challenge compared to basal values (approximately 61%) on days 2, 3 and 7 (around 56-55%) ($P < 0.001$) (Figure 1), but the corresponding plasma concentrations have not significantly differed from initial values, because of slight parallel variations in proteinemia. On the other hand, the percentages and the concentrations of the α_2 -, β_1 - and β_2 -globulin fractions have significantly increased since the day 2 ($P < 0.05$) for α_2 - and β_1 -globulins ($P < 0.001$) for β_2 - globulins) and remained markedly high until day 7 ($P < 0.01$) for α_2 - and β_2 -globulins ($P < 0.001$) and for β_1 -globulins. Moreover, as shown in Figure 1, the maximal increase in globulin concentrations was firstly observed for the β_2 -globulins (on day 2), then for the α_2 -globulins (on day 3) and finally for the β_1 -globulins (on day 7). Furthermore, the plasma protein electrophoresis has shown significant increases in the proportions and concentrations of the α_2 -, β_1 - and β_2 -globulin fractions between the 2 and the 7 day after the experimental *S. aureus* inoculation, the highest changes being firstly observed in β_2 -globulins (day 2), then in α_2 -globulins (day 3) and in β_1 -globulins (day 7). These results are in agreement with those of Harvey (1986), who reported a marked elevation of α_2 -globulin concentrations in clinically ill dogs compared to healthy ones. The appearance of abscesses at the site of inoculation in all experimental rabbits, as well as the re-isolation of the challenging strain from abscesses provided evidence for the successful reproduction of the experimental infection. Abscess formation within 48-96 h post *S. aureus* infection corresponded to findings of Wills *et al.* (2005). The A/G ratio in obese-infected rabbits significantly decreased from the 3 to the 14 day after bacterial challenge whereas significant hypoalbuminemia and hyperglobulinemia were only recorded on day 14. Most of the acute phase proteins (APPs) such as haptoglobin, ceruloplasmin and α_2 -macroglobulin are included in the β_2 -globulin fraction whereas fibrinogen migrates to the β_2 region and some complement proteins (C3, C4), haemopexin, transferrin, ferritin and C reactive protein are found in the β_1 fraction.

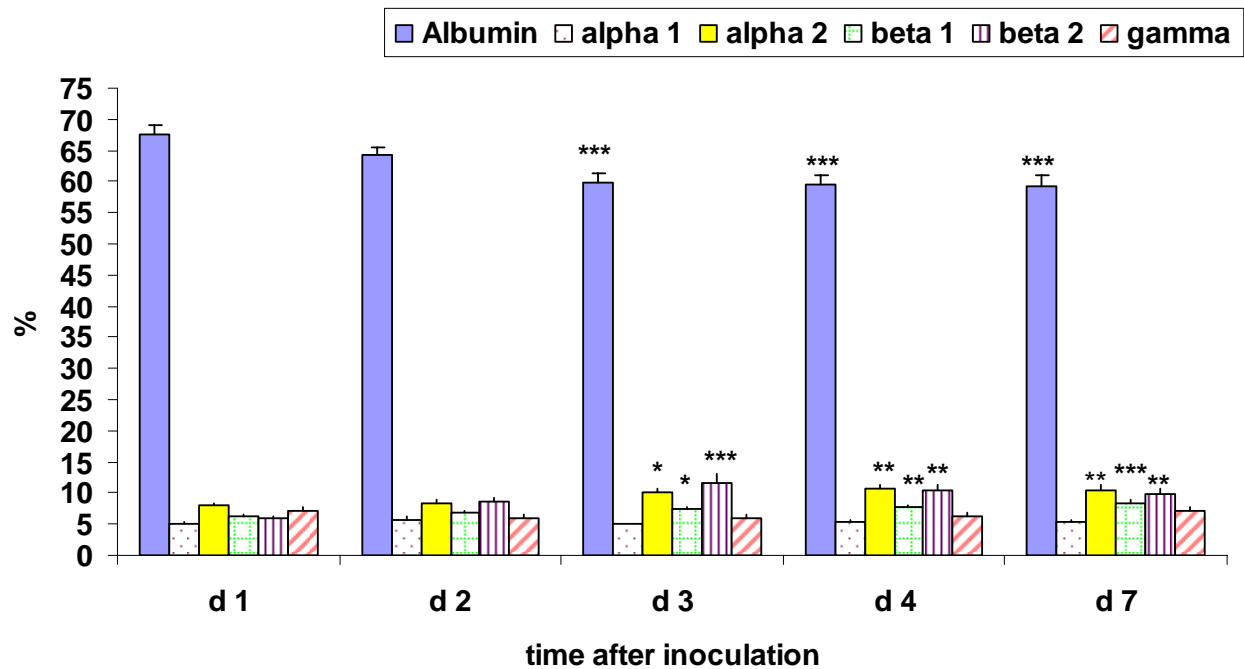


Figure 1: Variations of the plasma protein fractions (%) in obese castrated rabbits (n = 6) after experimental *S. aureus* subcutaneous inoculation (field strain suspension density: 8×10^8 cfu/mL, 100 μ L). (* P < 0.05; ** P < 0.01; *** P < 0.001)

CONCLUSIONS

In conclusion, the determination of the blood protein fractions, especially albumin, A/G ratio, globulin concentrations could be considered as moderate, sensitive and significant biomarkers for detection of *S. aureus* infection in obese rabbits before the appearance of the typical clinical signs (severe skin lesions and deep alterations of the general clinical status). Despite the body temperature increased significantly earlier than changes in blood protein fractions the rectal temperature is not a so reliable criteria for *S. aureus* infection in rabbits as it returned to normal values after the 6th h.

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