Effects of experimentally induced Staphylococcus aureus infection on blood protein fractions in obese rabbits

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SUMMARY

Staphylococcus aureus causes suppurative inflammation and is frequently isolated from infected sites in rabbits. The aim of the study was to investigate the variations of blood protein fractions during a 7 days long period after subcutaneous inoculation of Staphylococcus aureus in fattening obese castrated rabbits. For that, male New Zealand White rabbits (n = 6) were castrated at 2.5 months of age, fattened for 1.5 month and subcutaneously inoculated with S. aureus field strain (density: 8x10⁸ cfu/mL; injection volume: 100 μL) when they were 4 month 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 hours whereas abscesses were formed in all rabbits at the inoculation sites within 48-96 hours and consequently invasive suppurative phlegmons persisted until the 7th and the 14th days after inoculation. The simultaneous determination of the different fractions of blood protein may help to characterize the inflammation intensity in obese rabbits with experimental staphylococcal infection.

Keywords: Obese rabbits, Staphylococcus aureus, inflammation, albumin, globulins, α₂-globulins, β₁-globulins, β₂-globulins, abscess, hyperthermia.

RéSUMÉ

Effets sur les fractions protéiques sanguines d’une infection expérimentale par Staphylococcus aureus chez les lapins obèses

Staphylococcus aureus peut causer des inflammations à caractère suppuratif et est fréquemment isolé à partir de sites infectés chez le lapin. Le but de cette étude est d’étudier les variations des fractions protéiques sanguines durant une période de 7 jours après une inoculation sous cutanée de S. aureus chez des lapins obèses castrés. Pour cela, des lapins mâles New zélandais (n = 6) ont été castrés à 2.5 mois, engraissés pendant 1.5 mois puis inoculés par voie sous-cutanée par une souche de terrain de S. aureus (densité: 8x10⁸ cfu/mL; volume d’injection: 100 μL) à l’âge de 4 mois. L’état général, la consommation d’eau et d’aliments, la température rectale, la présence de lésions cutanées au site d’inoculation ainsi que la protéinémie, l’albuminémie et la globulinémie ont été déterminées sur une période de 21 jours alors que les fractions des protéines sanguines ont été analysées par électrophorèse sur une période de 7 jours après l’inoculation. Une importante léthargie, une forte diminution de l’appétit et une hyperthermie marquée ont été observées durant les 6 premières heures alors que des abcès se sont formés au site d’inoculation en 48-96 heures chez tous les lapins et que des lésions suppuratives et invasives (phlegmons) ont persisté chez 4 animaux du 7ème au 14ème jours. Le germe inoculé a été ré-isolé à partir de ces lésions. Une hypalbuminémie et une hyperglobulinémie significatives ont été mises en évidence le 14ème jour tandis que les fractions correspondantes aux α₂, β₁- et β₂-globulines ont été significativement augmentées par rapport aux valeurs basales du 2ème au 7ème jour après l’inoculation. La détermination simultanée des différentes fractions des protéines sanguines peut aider à évaluer l’intensité de l’inflammation induite expérimentalement par une infection staphylocooccique chez le lapin obèse.

Mots clés : Lapins obèses, Staphylococcus aureus, inflammation, albumine, globulines, α₂-globulines, β₁-globulines, β₂-globulines, abcès, hyperthermie.

Introduction

Staphylococcus aureus (S. Aureus) causes suppurative inflammation [2]. The organism is frequently isolated from infected sites in rabbits. It can also cause a fatal septicaemia [3]. S. aureus may be isolated in cases of mastitis, ulcerative pododermatitis, rhinitis, conjunctivitis, dacyrocystitis, abscesses and skin infections [11]. On the other hand, healthy rabbits can carry S. aureus in nasal cavity, conjunctiva and skin. It is often a secondary invader in tissues damaged by trauma or some other predisposing cause [3]. The severity of disease is governed by host resistance and bacterial virulence but, in rabbit colonies, staphylococcosis can cause serious losses [11]. S. aureus is also a causative agent of a broad spectrum of diseases in both men and animals [11]. In men, S. aureus is the major agent associated with nosocomial infections [4]. It is also involved in the aetiopathogenesis of wound and skin infections, arthritis, osteomyelitis, alimentary intoxications, etc [6, 16].

Economic losses in industrial livestock husbandry attributed to staphylococcal infections are considerable at a worldwide scale [9]. It is generally admitted that in rabbit farms, staphylococcal...
infection is introduced with newly delivered animals [2]. Two types of S. aureus are observed in affected rabbit populations [3]. 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 [3, 13].

Acute phase reactant proteins reported to increase in serum of rabbits in response to inflammation include C-reactive protein, haptoglobin, ceruloplasmin and fibrinogen [10]. Among these proteins haptoglobin and ceruloplasmin migrate in the alpha-2-region of plasma protein electrophoresis, while fibrinogen migrates to the beta region [12]. Increases in alpha-2-globulins are often measured when serum from animals with various inflammatory conditions is subjected to protein electrophoresis [14].

Several studies [5, 17, 18] served to indicate that body fat stores were mobilized during infectious process. Some investigations on white adipose tissue show that the tissue is not only a way to store triacylglycerols, but it also acts as an important endocrine organ, secreting a large number of biologically active substances. Some of them such as TNF-α, IL-1 and IL-6 induce production of acute phase proteins by hepatocytes [18]. Increased release of cytokines and acute phase proteins leads to activation of inflammatory signal pathways and was associated with low grade, but chronic systemic inflammation [18]. The purpose of this study was to investigate the effects of experimentally induced S. aureus infection on blood protein fractions in obese rabbits.

Material 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 [7] under general anaesthesia when they were 2.5 month old. Briefly the castration was performed with premedication, including atropine sulphate (Atropini sulfas,”Vetprom”, Radomir, Bulgaria), administrated subcutaneously (0.02 mg/kg) followed 10 minutes after by intramuscular injection of xylazine (Alfasan, Woerden, The Netherlands, 2 mg/kg). The anaesthesia was accomplished 10 minutes later by intramuscular injection of ketamine (Alfasan, 20 mg/kg).

For surgery, the rabbits were laid on their backs, the fur in the scrotal area was depilated and the skin was disinfected. The animals were bilaterally castrated through 3-cm scrotal incisions. Scrotal wounds after castration remained open. Thereafter, rabbits were fed with pelleted feed (“Bonmics”, Lovech, Bulgaria) according to their age and have had free access to the tap water. Pelleted food contained crude protein 18%, crude fat 2.7%, crude fibre 13%, crude ash 5%, lignin 0,77%, methionine + cystein 0.65%, Calcium 1%, Phosphorus 0.5%, chloride 0.45%, Cupper 20 mg/kg, vitamin A 10 000 IU/kg, vitamin D3 1000 IU/kg, vitamin E 50 mg/kg for 12% humidity and 2590 kcal/kg metabolisable energy. The fattening period lasted 1.5 months. Thereafter rabbits, with an average body weight of 4.43 kg, were experimentally infected with 100 μL of bacterial suspension of a field S. aureus strain (density: 8x10^8 cfu/mL) by subcutaneous route as described by WILLS et al. [19].

Blood samples were collected from all obese rabbits immediately before bacterial inoculation (0 hour, pre-treatment values) and 6, 24, 48, 72 hours 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 minutes, 4°C) to obtain plasma. Plasmas 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.

CLINICAL AND BACTERIOLOGICAL ANALYSES

Firstly, rabbits were monthly weighted in order to control obesity development.

Prior to inoculation and 6, 24, 48, 72 hours as well as the 7th, 14th and 21st days 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.

Swab samples of purulent exudates were collected 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). Cultures were incubated aerobically for 24 hours at 37°C. For animals slaughtered on day 21 post-infection, the identification of bacteria isolates was performed according to routine bacteriological techniques [16] from the internal organs (liver, spleen, kidney and heart), skin lesions and the site of bacteria injection.

BIOCHEMICAL 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 minutes for agarose gel electrophoresis (AGE). CAE strips and 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 were expressed as mean ± standard error (SE) and the differences were considered significant if \( P < 0.05 \).

**Results**

**CLINICAL FINDINGS**

Just before the experimental *S. aureus* infection, the body weights measured when rabbits were 4 month old (4.43 ± 0.09 kg) were markedly increased compared to weight determined when animals were 3 month old (3.20 ± 0.03 kg) \( (P < 0.001) \), confirming the obesity.

During the first 24 hours after the bacterial inoculation, rabbits were lethargic, did not eat or drink. Afterwards, they have gradually recovered: the food and water intakes progressively increased and became similar to the pre-treatment values at the 72nd hour. Rectal body temperature significantly increased at the 6th hour 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 daily ingested food was between 120 and 200 g per rabbit (Table I).

As early as the 24th hour after the inoculation, a restricted, temperate hyperaemic painful swelling has 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 after, a purulent yellowish-gray exudate was observed after the fistulisation of the underlying abscess at the site in these animals (figure 1).

**BACTERIOLOGICAL AND BIOCHEMICAL ANALYSES**

Bacteriologically, *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 II. Although plasma proteinemia slightly increased 7 and 14 days after, no significant difference according 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 also progressively declined, 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 hour (immediately before bacterial challenge) and 7 days after the subcutaneous *S. aureus* inoculation, was presented in the Table III. 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%) \( (P < 0.001) \) and were associated with significant decreases in plasma albumin concentrations during the 7 days long period (Table III). On the other hand, the percentages and the concentrations of the \( \alpha_2- \), \( \beta_1- \) and \( \beta_2- \)globulin fractions have significantly increased since the 2nd day \( (P < 0.05 \) for \( \alpha_2- \) and \( \beta_1- \)globulins, \( P < 0.001 \) for \( \beta_2- \)globulins) and remained markedly high until the 7th day \( (P < 0.01 \) for \( \alpha_2- \)and \( \beta_2- \)globulins and \( P < 0.001 \) for \( \beta_1- \)globulins). Moreover, as shown in Table III, the maximal increase in globulin concentrations was firstly observed for the \( \beta_2- \)globulins (on

### Table I: Variations of the behaviour, food and water intakes and rectal temperature in obese castrated rabbits (n = 6) after experimental *S. aureus* subcutaneous inoculation (field strain suspension density: \( 8 \times 10^8 \) cfu/mL, 100 \( \mu \)L). Results (rectal temperature) are expressed as mean ± standard error.

<table>
<thead>
<tr>
<th>Time points</th>
<th>Behaviour</th>
<th>Water/food intake</th>
<th>Rectal temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 hour</td>
<td>Normal</td>
<td>Normal</td>
<td>39.43 ± 0.05(^a)</td>
</tr>
<tr>
<td>6 hours</td>
<td>Lethargic</td>
<td>Greatly reduced</td>
<td>40.28 ± 0.15(^b)</td>
</tr>
<tr>
<td>24 hours</td>
<td>Lethargic</td>
<td>Reduced</td>
<td>39.67 ± 0.22(^ab)</td>
</tr>
<tr>
<td>48 hours</td>
<td>Improved</td>
<td>Improved</td>
<td>39.28 ± 0.12(^a)</td>
</tr>
<tr>
<td>72 hours</td>
<td>Normal</td>
<td>Normal</td>
<td>39.35 ± 0.08(^a)</td>
</tr>
<tr>
<td>7 days</td>
<td>Normal</td>
<td>Normal</td>
<td>39.75 ± 0.24(^ab)</td>
</tr>
</tbody>
</table>

\(^a,b\) Different superscripts \(^a,b\) in the same column indicate significant differences \( (P < 0.05 \) or more) according to time after bacterial inoculation.
EFFECTS OF \textit{STAPHYLOCOCCUS} INFECTION IN OBESE RABBITS

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 hours post \textit{S. aureus} infection corresponded to findings of WILLS \textit{et al.} [19].

\textbf{Discussion}

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 hours post \textit{S. aureus} infection corresponded to findings of WILLS \textit{et al.} [19].

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|}
\hline
\textbf{Time points} & \textbf{Total proteins} & \textbf{Albumin} & \textbf{Globulins} & \textbf{A/G} \\
\hline
0 hour & 65.28 ± 0.11 & 39.50 ± 0.09\textsuperscript{a} & 25.81 ± 0.07\textsuperscript{a} & 1.53 ± 0.07\textsuperscript{a} \\
6 hours & 65.73 ± 0.09 & 39.98 ± 0.08\textsuperscript{a} & 25.75 ± 0.09\textsuperscript{a} & 1.55 ± 0.07\textsuperscript{a} \\
24 hours & 64.56 ± 0.10 & 36.95 ± 0.07\textsuperscript{b} & 27.61 ± 0.06\textsuperscript{a} & 1.34 ± 0.03\textsuperscript{ab} \\
48 hours & 64.31 ± 0.14 & 36.15 ± 0.09\textsuperscript{b} & 28.16 ± 0.07\textsuperscript{ab} & 1.28 ± 0.05\textsuperscript{ab} \\
72 hours & 65.13 ± 0.22 & 36.33 ± 0.13\textsuperscript{b} & 28.80 ± 0.11\textsuperscript{ab} & 1.26 ± 0.04\textsuperscript{b} \\
7 days & 68.93 ± 0.14 & 38.13 ± 0.10\textsuperscript{b} & 30.80 ± 0.12\textsuperscript{ab} & 1.24 ± 0.07\textsuperscript{b} \\
14 days & 68.33 ± 0.12 & 35.71 ± 0.13\textsuperscript{c} & 32.61 ± 0.18\textsuperscript{b} & 1.10 ± 0.09\textsuperscript{b} \\
21 days & 66.86 ± 0.28 & 37.51 ± 0.11\textsuperscript{b} & 29.35 ± 0.30\textsuperscript{ab} & 1.28 ± 0.15\textsuperscript{ab} \\
\hline
\end{tabular}
\caption{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 \textit{S. aureus} subcutaneous inoculation (field strain suspension density: 8×10\textsuperscript{8} cfu/mL, 100 \textmu L). Results are expressed as mean ± standard error.}
\end{table}

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|c|}
\hline
\textbf{Time after bacterial inoculation (days)} & \textbf{Albumin} & & & & \\
\textbf{Day 0 (basal values)} & \textbf{Day 1} & \textbf{Day 2} & \textbf{Day 3} & \textbf{Day 7} \\
\hline
\textbf{Albumin} & & & & & \\
\% & 60.5 ± 1.3\textsuperscript{a} & 57.2 ± 1.1\textsuperscript{ab} & 56.2 ± 1.5\textsuperscript{b} & 55.8 ± 1.4\textsuperscript{b} & 55.3 ± 1.8\textsuperscript{b} \\
g/L & 39.50 ± 0.09\textsuperscript{a} & 36.95 ± 0.07\textsuperscript{b} & 36.15 ± 0.09\textsuperscript{b} & 36.33 ± 0.13\textsuperscript{b} & 38.13 ± 0.10\textsuperscript{b} \\
\textbf{α1-globulins} & & & & & \\
\% & 4.9 ± 0.3 & 5.7 ± 0.6 & 4.9 ± 0.2 & 5.4 ± 0.3 & 5.2 ± 0.3 \\
g/L & 3.21 ± 0.02 & 3.70 ± 0.04 & 3.18 ± 0.01 & 3.46 ± 0.01 & 3.58 ± 0.02 \\
\textbf{α2-globulins} & & & & & \\
\% & 8.0 ± 0.3\textsuperscript{a} & 8.3 ± 0.5\textsuperscript{ab} & 10.0 ± 0.5\textsuperscript{b} & 10.7 ± 0.6\textsuperscript{b} & 10.5 ± 0.8\textsuperscript{b} \\
g/L & 5.25 ± 0.02\textsuperscript{a} & 5.30 ± 0.03\textsuperscript{ab} & 6.43 ± 0.03\textsuperscript{b} & 6.93 ± 0.04\textsuperscript{b} & 7.25 ± 0.06\textsuperscript{b} \\
\textbf{β1-globulins} & & & & & \\
\% & 6.3 ± 0.3\textsuperscript{a} & 7.0 ± 0.2\textsuperscript{b} & 7.4 ± 0.2\textsuperscript{b} & 7.7 ± 0.3\textsuperscript{b} & 8.3 ± 0.5\textsuperscript{b} \\
g/L & 4.10 ± 0.05\textsuperscript{a} & 4.50 ± 0.02\textsuperscript{ab} & 4.78 ± 0.02\textsuperscript{b} & 5.01 ± 0.02\textsuperscript{ab} & 5.73 ± 0.04\textsuperscript{b} \\
\textbf{β2-globulins} & & & & & \\
\% & 6.0 ± 0.3\textsuperscript{a} & 8.7 ± 0.7\textsuperscript{ab} & 11.6 ± 1.4\textsuperscript{b} & 10.3 ± 1.0\textsuperscript{b} & 9.7 ± 1.0\textsuperscript{b} \\
g/L & 3.93 ± 0.02\textsuperscript{a} & 5.60 ± 0.04\textsuperscript{ab} & 7.40 ± 0.08\textsuperscript{b} & 6.63 ± 0.05\textsuperscript{b} & 6.68 ± 0.07\textsuperscript{b} \\
\textbf{γ-globulins} & & & & & \\
\% & 7.1 ± 0.6 & 6.1 ± 0.4 & 6.1 ± 0.5 & 6.4 ± 0.6 & 7.2 ± 0.6 \\
g/L & 4.63 ± 0.04 & 3.92 ± 0.03 & 3.92 ± 0.04 & 4.16 ± 0.05 & 4.93 ± 0.04 \\
\hline
\end{tabular}
\caption{Variations of the plasma protein fractions (%) and concentrations (g/L) in obese castrated rabbits (n = 6) after experimental \textit{S. aureus} subcutaneous inoculation (field strain suspension density: 8×10\textsuperscript{8} cfu/mL, 100 \textmu L). Results are expressed as mean ± standard error.}
\end{table}

\textit{The A/G ratio in obese-infected rabbits significantly decreased from the 3\textsuperscript{rd} to the 14\textsuperscript{th} day after bacterial challenge whereas significant hypoalbuminemia and hyperglobulinemia were mainly evident on day 14. The significant alterations of the A/G ratios observed earlier than alterations of the concentrations result from gradual decrease in albuminemia and gradual increase in globulinemia. Similar changes in albumin and globulin concentrations were established in chickens challenged with \textit{E. coli} on the background of a pre-existing \textit{Eimeria} infection in some previous experiments [15] and also in weaning rabbits experimentally infected with \textit{E. coli} [7-9]. Albumin has been described as a negative acute phase protein in many species [10].}
Furthermore; the plasma protein electrophoresis have shown significant increases in the proportions and concentrations of the α2-, β1- and β2-globulin fractions between the 2nd and the 7th day after the experimental S. aureus inoculation, the highest changes being firstly observed in β2-globulins (day 2), then in β2-globulins (day 3) and in β1-globulins (day 7). These results are in agreement with those of HARVEY [12], who reported a marked elevation of α2-globulin concentrations in clinically ill dogs compared to healthy ones. Most of the acute phase proteins (APPs) such as haptoglobin, ceruloplasmin and α2-macroglobulin are included in the α2-globulin fraction whereas fibrinogen migrates to the β2 region and some complement proteins (C3, C4), haemopexin, transferrin, ferritin and C reactive protein are found in the β1 fraction [14].

The temperature has been shown to be involved in inflammatory reactions from the initial step and during tissue damage [16]. In the case of the experimental S. aureus infection in non-obese rabbits, this clinical parameter was significantly increased early, with the peak values after the 6th hour. Despite the body temperature increased significantly earlier than changes in blood protein fractions it is not a so reliable criteria for the detection and time course of bacterial infection.

As a 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 it is not a so reliable criteria for S. aureus infection in rabbits as it returned to normal values after the 6th hour.

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