

BLOOD HAPTOGLOBIN RESPONSE IN RABBITS WITH EXPERIMENTALLY INDUCED *STAPHYLOCOCCUS AUREUS* INFECTION

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ABSTRACT

Staphylococcus aureus is a common pathogenic bacterium which can induce severe skin infections in rabbits leading to important economic losses. The objectives of the present study included investigation of the variation profiles of blood concentrations of haptoglobin (Hp) which is considered a moderate positive APP during a 21-day-long period after inoculation and their comparison with clinical signs in order to improve diagnosis. The Hp concentrations were determined in plasma specimens from 7 male New Zealand White, 5 month-old rabbits subcutaneously injected with 100 µL of bacterial suspension of a field *S. aureus* strain (density: 8×10^8 cfu/mL) and from 6 untreated rabbits (controls). In parallel, rectal temperature and other clinical signs (skin lesions) were recorded. Abscesses and sometimes phlegmons were formed at the injection site within 48-72 hs in all infected animals and the inoculated germ was re-isolated from these lesions. The mortality rate in inoculated rabbits was 28.6%. A marked hyperthermia was observed in the inoculated rabbits after the 6 hrs and until 72 hs post-infection. Additionally, Hp concentrations were dramatically increased in infected rabbits compared to the controls between day one and 7 post-infection. These two parameters were positively but moderately correlated. Because of the great fluctuations of the body temperature in healthy rabbits, the simultaneous determination of the blood Hp concentrations may help to characterize the inflammation intensity in rabbits with experimental staphylococcal infection.

Key words: Rabbits, *Staphylococcus aureus*, inflammation, haptoglobin

INTRODUCTION

Staphylococcus aureus is an adaptive opportunistic pathogen, capable to persist and replicate under various conditions. It is the causative agent of a broad spectrum of diseases in both humans and animals (Cucarella *et al.*, 2004). In humans, *S. aureus* is the major agent associated with nosocomial infections (Francois *et al.*, 2005, Mork *et al.*, 2005). It is also involved in the aetiopathogenesis of wound and skin infections, arthritis, osteomyelitis, alimentary intoxications etc. (Gao and Stewart 2004; Wills *et al.*, 2005). Economic losses in industrial livestock husbandry attributed to staphylococcal infections are considerable at a worldwide scale (Mircheva *et al.*, 2009). It is generally acknowledged that in rabbit farms, staphylococcal infection is introduced by newly delivered animals

(Devriese *et al.*, 1981). Infection can result in poor growth and development, reduced productivity, infertility and death. Neonate rabbits may die as a consequence to agalactiae in doe rabbits due to *Staphylococcus* infection. Two types of *S. aureus* are observed in affected rabbit populations (Devriese *et al.*, 1996). One affects single animals or a small part of the population and is therefore of little economic concern. The second type causes epidemic spread of the disease in the farm, and results in chronic problems that make the farm unprofitable (Devriese *et al.*, 1996; Hermans *et al.*, 2000). The production and secretion by the liver of a number of acute phase proteins (APP), mainly glycoproteins, is one of the mechanisms involved in the response to mediators produced by leucocytes and macrophages during episodes of infection or inflammation (Eckersall and Conner, 1988). Haptoglobin (Hp) is an acute phase protein, responsive to infection and inflammation that is present in the blood plasma of all animals (Gonzales-Ramon *et al.*, 2000).

The objective of this study was to investigate the correlation between temperature and haptoglobin concentration in the infected rabbits.

MATERIALS AND METHODS

Animals and experimental design

The experimental procedure was approved by the Ethic Committee at the Faculty of Veterinary Medicine. Experiments were carried out on 13 male New Zealand White, 5 month-old rabbits. They were reared in individual sanitized metal cages (modules) with a grate floor at room temperature (20–22°C) and fed according to their age with free access to tap water. Rabbits were randomly divided into 2 groups: animals from group 1 (n = 7), with an average body weight of 3.2 kg, were experimentally infected subcutaneously with 100 µL of bacterial suspension of a field *S. aureus* strain (density: 8×10^8 cfu/mL) as described by Wills *et al.* (2005). The animals from group 2 (n = 6) (initial same body weight) were not infected and served as negative controls.

Prior to inoculation and 6, 24, 48, 72 hs as well as on the 7 day post-infection, the rectal body temperature and the presence and size of the formed abscess were recorded. Swab samples of purulent exudate were collected from 6 rabbits from Group 1 at the time of fistulisation of the abscesses. 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 h at 37°C.

Biochemical analysis

Blood samples from each rabbit were taken from v. auricularis externa prior to (0 h) and at 6, 24, 48, 72 hs and on days 7, 14, and 21 post *S. aureus* challenge into sterile heparinised tubes and were centrifuged immediately (1 500g, 10 min, 4°C) to obtain plasma. Then plasma from each animal was decanted and stored at -20°C until assayed. Haptoglobin (Hp) was measured using the patented method (ReactivLab, Glasgow, Scotland).

Statistical analysis

The statistical analysis of the data 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 Bonferroni test, because they compensate for multiple comparisons. All data are expressed as mean ± standard deviation (SD) and the differences were considered as significant when P value was less than 0.05.

RESULTS AND DISCUSSION

Alterations in rectal body temperature (Table 1) became evident on the 6th hour post-inoculation, were maximal after 6 and 24 hs and persisted elevated until the 72 hs compared to baseline values. Fourteen days after the infection, at the site of bacterial suspension application in 5 rabbits a purulent yellowishgray exudate was observed after the fistulisation of the underlying abscess. Among the most severe lesions were the spreading diffuse subcutaneous purulent inflammations (phlegmons) which affected vast areas near the site of bacterial suspension application in 3 rabbits.

Table 1: Variations of the rectal temperature (°C) in experimental (n = 7) and control (n = 6) rabbits.

Time points (post-inoculation)	Infected rabbits (n = 7 at the beginning)	Control rabbits (n = 6)
0 hour	38.94 ± 0.24 ^A	38.88 ± 0.34
6 hours	40.14 ± 1.12 ^{aC}	38.93 ± 0.19 ^b
24 hours	40.14 ± 1.25 ^{aC}	38.93 ± 0.34 ^b
48 hours	39.96 ± 0.28 ^{aC}	38.66 ± 0.31 ^b
72 hours	39.78 ± 0.28 ^{aBC}	38.81 ± 0.33 ^b
Day 7	39.78 ± 0.27 ^{BC}	39.78 ± 0.27
Day 14	39.40 ± 0.77 ^B	39.08 ± 0.17
Day 21	39.42 ± 0.76 ^B	39.06 ± 0.27

Different ^{a,b} superscripts in the same row indicate significant differences (P<0.05 or more) between the 2 groups.

Different ^{A,B,C} superscripts in the same column indicate significant differences (P<0.05) according to the time course of the infection.

As shown in Table 2, the plasma Hp concentrations measured in controls (not infected rabbits) remained roughly stable for the whole 21 day-long period (mean values remained below 1.0 g/L) although they tended to increase at hours 48 and 72. Also, values were relatively greatly dispersed. In the experimental group, the plasma Hp concentrations markedly increased at 24 h post infection and remained greatly elevated until the 7th day post-infection compared to the controls (P< 0.001) and to the initial values (P<0.001). Maximal Hp concentrations were recorded at 48 hs and were 6 times higher than the average value at 0 h (2.66 g/L vs. 0.46 g/L). After this, the biochemical parameter declined and became closely related to control values at the same time points. A significant and positive correlation was evidenced between the body temperature and the plasma Hp concentrations (r = 0.52, P<0.05).

Table 2: Variations of the plasma haptoglobin concentrations (g/L) in experimental (n = 7) and control (n = 6) rabbits

Time points (post-inoculation)	Infected rabbits (n = 7 at the beginning)	Control rabbits (n = 6)
0 hour	0.46 ± 0.15 ^A	0.43 ± 0.18
6 hours	0.46 ± 0.15 ^A	0.38 ± 0.21
24 hours	2.03 ± 0.94 ^{bB}	0.46 ± 0.16 ^a
48 hours	2.66 ± 1.03 ^{bB}	0.71 ± 0.11 ^a
72 hours	2.44 ± 0.80 ^{bB}	0.82 ± 0.23 ^a
Day 7	1.62 ± 0.42 ^{bB}	0.55 ± 0.19 ^a
Day 14	0.58 ± 0.12 ^A	0.50 ± 0.09
Day 21	0.67 ± 0.14 ^A	0.55 ± 0.14

Different ^{a,b} superscripts in the same row indicate significant differences (P<0.001) between the 2 groups.

Different ^{A,B} superscripts in the same column indicate significant differences (P<0.001) according to the time course of the infection.

Haptoglobin is considered to be a moderate APP in all kinds of domestic animals, except in ruminants. In the present study, the plasma Hp concentrations significantly increased compared to the non-infected controls between 24 hs and 7 days after *S. aureus* inoculation. This variation profile is in agreement with Hp's behaviour in other acute inflammatory reactions (Murray and Connell 1960; Mircheva *et al.*, 2009). The amplitude of the observed increase in the Hp concentrations, particularly at 48 h where values were 6 times higher than baseline values, is in accordance with previous reports (Cheftel *et al.*, 1969; Pineiro *et al.*, 2007; Segura *et al.*, 2007). Other researchers have also demonstrated that the Hp variations in plasma were synchronous with the development of the inflammatory reaction in experimental situations (Murray and Connell 1960; Cheftel *et al.*, 1969; Kurosky *et al.*, 1976; Chow *et al.*, 1984; Gutteridge 1987; Hermans *et al.*, 2000; Kristiansen *et al.*, 2001; Herrer *et al.*, 2004; Mircheva *et al.*, 2009).

Body temperature has been shown to be involved in inflammatory reactions from the initial steps and during tissue damage (Wills *et al.*, 2005). In the case of the experimental *S. aureus* infection in rabbits, this clinical parameter was significantly elevated compared to the non-infected controls between 6 and 72 hs post-inoculation. Consequently, the variation patterns of the body temperature in one hand and of the plasma Hp concentration in the other according to the time course of the infection slightly differ: the clinical parameter was altered earlier (since the 6 hs) whereas the modifications of the biochemical parameter persisted even after body temperature had reached normal values (until the 7th day post infection). The body temperature and the Hp concentrations were correlated significantly and positively but moderately ($r = 0.52$). The antioxidant activity of elevated values of Hp in this case could prevent peroxidation of lipids, which is accomplished in two discernable phases. The first phase is inhibited by binding haemoglobin to the protein haptoglobin. The second phase is stimulated by complexable iron released from the haemoglobin molecule during the process of lipid peroxidation.

CONCLUSION

The determination of blood haptoglobin concentration could be used as a fast, sensitive and significant biomarker for early detection of infection in rabbits before the appearance of the clinical signs (skin lesions, oedema, and deep alterations of the general clinical status) except of hyperthermia. As body temperature in rabbits normally varies from 38.3°C to 39.4°C (Lee R. 1939), inflammation could not be diagnosed only on the basis of the body temperature increase by 1°C and the analysis of at least one positive acute phase protein such as the haptoglobin is needed.

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