TRADITION AND MODERNITY IN VETERINARY MEDICINE, 2018, vol. 3, No 2(5): 3–8

EFFECT OF *STAPHYLOCOCCUS* AUREUS INFECTION ON THE DYNAMICS OF CREATININE AND UREA CONCENTRATIONS IN THE BLOOD PLASMA OF RABBITS

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ABSTRACT

The aim of this paper is to compare the dynamics of urea and creatinine, in rabbits that are infected with *Staphylococcus aureus* (experimental) and in control groups. Blood samples from each rabbit were taken as follows: at 3 months of age, coinciding with 0 hours before infection, and 6, 24, 48, 72 hours and on days 7, 14, and 21 after *S. aureus* infection. The results showed significant decreasing between groups ($P < 0.05$) of urea since 72 hours after infection to day 14. Significant increase in creatinine between groups was established on days 7 and 14 ($P < 0.01$; $P < 0.05$ respectively), and on the 6th hour after infection until the end of the experiment in the experimental group.

Key words: Urea, creatinine, plasma, New Zealand rabbit.

Introduction

When assessing rabbit diseases, it is important to know that the rabbits may mask the symptoms of the diseases or manifest some complex clinical signs. As disorders have considerable effects on blood parameters in rabbits, it is important to analyse blood and urine in rabbits as well as in other animal species (Özkan et al., 2012; Archetti et al., 2008; Betacrount et Alonso, 2011). Hence, some important findings may be achieved using haematological and biochemical parameters (Lepitzki and Woolf, 1991) and to add something novel to the profession.

The most important metabolic change in infectious diseases is the strong increase in the concentration of plasma proteins synthesized in the liver and pooled in the group of acute phase proteins (Kushner 1982, Eckersall & Conner, 1988, Pannen & Robotham, 1995, Baumann & Gauldie, 1994, Gruys et al., 1994, Raines, 1994). In this regard, we have reviewed the changes in APP (Georgieva et al., 2012, Georgieva et al., 2017, Vlaykova et al., 2011, Georgieva et al., 2012; Dyshlianova et al.,2011) and hematology (Petrov et Georgieva, 2013; Petrova et al., 2017) in experimental *St. Aureus* and *E.Coli* infection in rabbits in previous publications (Georgieva et al., 2008; Georgieva et al., 2009).

Determination of urea and creatinine in the experimental reproduction of staphiloccocal infection could provide information not only for the liver and the kidney condition, but also for the intensity of metabolic changes in the body.

Materials and Methods

The experiment was conducted with 12 male rabbits (White New Zealand), 6 of which were infected and treated in the experimental group. The experiment began when the rabbits were 3 months old and infected with *S. aureus*. They were placed in individual disinfected metal cells with grate floor and placed in a room with a temperature (20–22 °C). Their food was pelleted according to age requirements with free access to water.

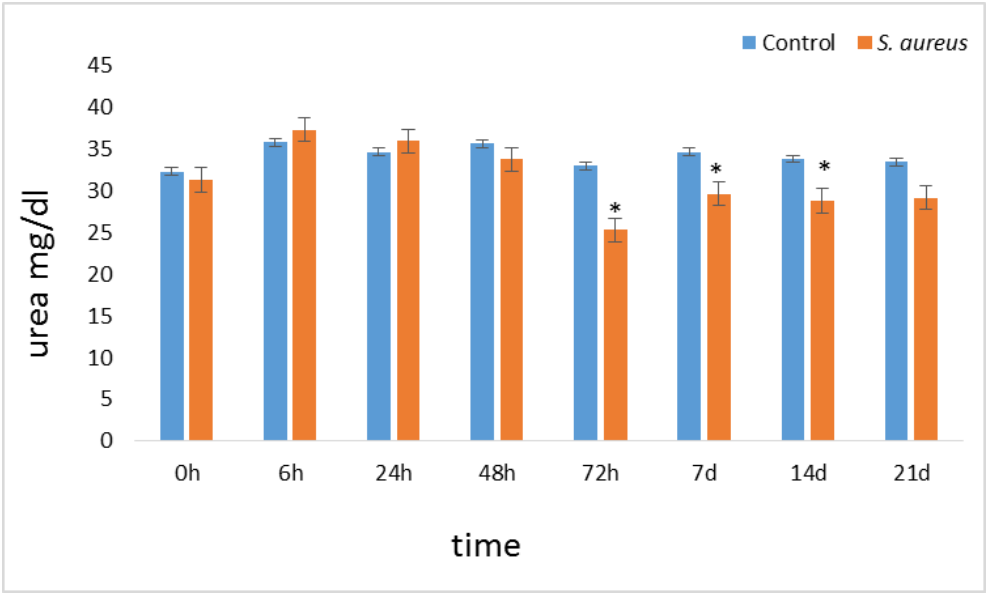
The rabbits were infected intradermally with 100 μL of the bacterial suspension of a *S. aureus* strain (density: 8x108 cfu / mL). The blood samples from each rabbit were taken before the euthanasia of v. auricularis externa as follows: 1) at 3 months of age, coinciding with 0 hours before infection, and at 6, 24, 48, 72 hours and on Days 7, 14 and 21 after *S. aureus* infection in sterile heparinized tubes. They were centrifuged immediately (1500 g, 10 min, 4 °C) and plasma was obtained. The plasma was then decanted and stored at -20 °C until the determinations were performed, except for the fibrinogen which was determined at 2 hours.

The determination of urea, creatinine, was performed by a semi-automatic biochemical analyst at the Faculty of Veterinary Medicine at the Thracian University, Stara Zagora, Bulgaria.

The statistical processing of the results obtained in the individual experiments was performed through ANOVA (Statistics for Windows, Stat Soft Ins., USA, 1993). The statistical reliability of the difference within and between groups was determined by the Posthoc procedure LSD test (Stat Soft Ins., USA, 1993). The level of statistical significance of the differences was $p < 0.05$.

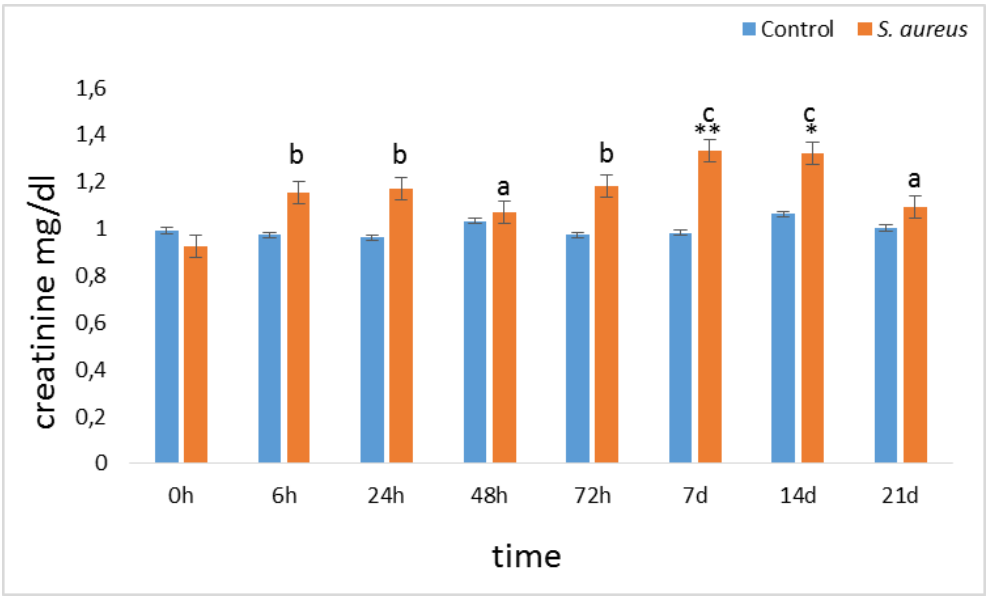
Results

The level of urea in the blood of the infected rabbits significantly decreased compared to the control group over the period since 72 hours to 14 days after infection (Figure 1).



Significance of differences between groups: * p < 0,05

Figure 1: Concentration of urea in the experimental and control groups in rabbits.



Significance of differences between groups: * p < 0,05, ** p < 0,01
Significance of differences in the experimental group comparing 0 hour (before infection)
a p<0,05 b p<0,01 c p<0,001

Figure 2: Concentration of creatinine in the experimental and control groups in rabbits.

During the infection, the level of creatinine fluctuated between 1.07 ± 0.04 and 1.32 ± 0.06 mg / dL, and throughout the experimental period the concentrations were significantly higher than the pre-infection period. A significant increasing compared the control group was observed at day 7 ($p < 0.01$) and 14 ($p < 0.05$).

Discussion

Unlike other small animals, such as dogs and cats, where urea elevation is associated with kidney disease and an inability to radiate from the body, we observed a decrease in urea from day 4 to day 14 in rabbits infected with staphylococcus, which was associated with liver and ornithine cycle disorders, which take place in the liver. We also take in account that lower level of urea is connected with reduced synthesis of protein, because of defective liver function or the reduced intake of the protein food during infection time. Concova et al., 1999 found a significant decrease in urea level in rabbits infected with *Encephalitozoon cuniculi* and treated with albendazole only on day 60. A pronounced elevation of urea and creatinine clearance was found in rabbits infected with coccidian oocysts (Tambur et al.,1999). According to Kumar & Josh (1993), who studied a longer period (3 months after infection), creatinine and urea levels increased after experimental infection of rabbits with coccidia of the species *Staria cervi*. In our study, urea decreased from 72 hours to 14 days after infection with *S. aureus*, whereas the studies of the abov ementioned authors showed a credible urea increase of values of 19.62 ± 2.78 to 47.85 ± 5.54 mM/L during the 30^{th} - 70^{th} day of infection. We could assume that the differences in these results were due to the difference in the infectious agent. The same authors found a creatine increase of 81.7 ± 5.6 µM / L

to 92.3 ± 4.6 μM / L on the 15th day after infection. Creatinine during *S. aureus* infection in our studies was also increased from 1.06 ± 0.04 to 1.32 ± 0.06 mg/dL.

Urea is an end product of the detoxification of toxic ammonia during ornithine cycle and ammonia is produced by the deaminiation of aminoacids during protein catabolism, and deaminiation of purine bases of nucleic acids. Moreover, the metabolic changes during infection time include muscle loss and negative nitrogen balance. The urea is synthezised in the liver, but excreted by the kidneys into the urine. *S.aureus* produces two types of exotoxins pyrogenic toxin superantigens (PTSAgs) and hemolysins exotoxins (Dinges et al., 2000) which are absorbed and transported by the blood into the liver and rens. PTSAgs may impair liver endotoxin clearance functions through direct cytotoxic effects on liver cells (Canonico et al., 1971) which leads to damaged hepatocells and destroyed the function of the above mentioned organs. As we observed staphylococcal abscesses concerned not only skin, but also liver. We have to mension that it is very difficult to interpret small changes in urea levels because urea levels in rabbits depend on some physiological factors like: the circadian rhythm (peak in late afternoon and early evening), quantity and quality of proteins in the diet, nutritional status, liver function, intestinal absorption, urease activity of the caecal flora, and hydration status. Moreover, the reference ranges have been determined from laboratory rabbits fed on a standardized diet and bled at the same time of the day, whereas clinicians see pet rabbits fed on a variety of foods and samples are taken at random (Hackness, 2013). According to Petkova et al., 2011 the urea concentration in New Zealand rabbits at 45-50 days of age is 5.62 ± 0.35 mM / L in males and 6.4 ± 0.35 mM /L in females, which is coincided with our results.

In our study, the initial concentration of creatinine was below 1mg/dl, which falls in the reference range for it according Hackness, 2013, who reported this range between 0.5 to 2.2 mg /dL and according Ozkan et al., 2012 the reference range of creatinine are between 0,06 и 0,14 mmol/L. The level of creatinine was significantly elevated since 6th hour after infection, which remained until the end of the experimental period compared to the 0 hour in the infected rabbits, and when compared to the control, a significant increase was obtained since day 14 to day 21. Orhue & Nwanze, (2005) found a significant increase in creatinine levels in rabbits infected with trypanosome on the 14th day after invasion, and explained this with impaired functional status of the kidneys, especially glomeruls. Moreover, creatinine is not influenced by non-renal factors (Hackness, 2013). In addition, rabbits have a limited ability to concentrate urine and only a few hours of lack of drinking water or diarrhea lead to increased levels of urea and creatinine, but their levels are rapidly returning to normal if dehydration ceases (Hackness, 2013).

A significant increase is indicative of a reduction in glomerular filtration rate, which may be due to infection of the glomeruli or renal pelvis. Creatinine measurement in our study was performed when there was a suspicion of disruption of glomerular urine filtration rate. Its elevated levels are an indicator of impaired kidney function (Javadi et al., 2014); (Solomon et al., 2015). Creatinine is a catabolite that is produced from the dephosphorilation of muscle creatinephospate and excreted by glomerular filtration at a constant rate. This reaction is occur when energy from the catabolism of ATP is unadequite and the organism needs an additional energy, because after infection the rabbits suffered from decreasing of appetite and the consumed food. This leads them to starvation and activation their reserves of creatinephosphate with formation of creatinine.

Therefore, we recommend that all the biochemical parameters of the herds of healthy rabbits be investigated on each farm and that the breed's reference age and gender differences should be

determined, as well as the nutrition, hydration status and response from different times of taking the blood.

Conclusion

During staphylococcal infection of rabbits the level of urea is decreased and the level of creatinine is increased which could be due to the impair function of the liver and kidneys.

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