Original Contribution

EFFECT OF *STAPHYLOCOCCUS AUREUS* INFECTION ON THE ACTIVITIES OF THE ALKALINE PHOSPHATASE, ASPARTATE AND ALANINE TRANSFERASES IN THE BLOOD PLASMA OF RABBITS

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ABSTRACT

PURPOSE: The aim of this paper is to investigate the dynamics of the activities of alkaline phosphatase (AP), aspartate (ASAT) and alanine (ALAT) transferases in plasma of rabbits that are infected with *Staphylococcus aureus* (*S. aureus*). **METHODS:** The experiment was carried out with 12 male rabbits (White New Zealand), divided into two groups: experimental and control. Blood samples from each rabbit were taken before the euthanasia of *v. auricularis externa* as follows: 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. The AP, ASAT and ALAT were performed on a semi-automated biochemical analyzer, Section in Biochemistry, Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria. **RESULTS:** The results showed significant decreasing ($P < 0.05$) of AP 48h after infection and on day 14 comparing to the control group ($P < 0.01$), significant increasing of ALAT ($P < 0.01$) comparing to the control group and pre-initial level and significant increasing of ASAT on day 14 and day 21 in the experimental group comparing initial level.

Key words: ASAT, ALAT, AP, plasma, White New Zealand Rabbit

INTRODUCTION

When assessing rabbit disease, it is important to know that rabbits can mask the symptoms of the disease or show some complex and atypical clinical signs. Because physiological disorders have a significant effect on blood parameters in rabbits, it is important to analyze blood and urine in rabbits as well as in other animal species (1). Therefore, some important conclusions can be reached using hematological and biochemical parameters (2).

The most important metabolic change in infectious diseases is the strong increase in the concentration of heterogenous group of plasma proteins synthesized in the liver shortly after

pathogenic agent invasion. All they pooled in the group of acute phase proteins (APPs) (3). In this regard, we have reviewed the changes in APP after experimental *S. aureus* (4-7), *E. coli* (8, 9) infection in rabbits in previous publications.

Determining the behavior of liver enzymes in experimental reproduction of only one type of infection (staphylococcal) has an advantage because in practice the infections are usually mixed. This could give specific information about dynamics of some enzymes with clinical importance during staphylococcal skin infection, which we have not found in our available literature.

MATERIALS AND METHODS

Study design

The experiments were conducted with 12 male rabbits of White New Zealand breed. Six of the animals were infected and treated

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and constituted an experimental group. The experiment began when the rabbits were 3 months of age and infected with *S. aureus*. They were placed in separate disinfected metal cells with a lattice floor. The room temperature was maintained from 20 to 22 ° C. Their food was granulated according to age requirements. They were given free access to water. The rabbits were infected intradermally with 100 µL of the bacterial suspension of a *S. aureus* strain (density 8×10^8 cfu/mL) as described by Wills et al. (10). Twenty-one days after the infection, the rabbits of the trial group were euthanized by a lethal dose (100 mg/kg bw) of Thiopental (Sandoz GmbH Austria). The carcasses of all rabbits were subject to pathoanatomic, pathohistological and bacteriological control. 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 prior to infection, and at 6, 24, 48, 72 and on days 7, 14 and 21 after *S. aureus* infection in sterile heparinized tubes. They were centrifuged immediately (1500g, 10 min, 4°C) and plasma was obtained. Plasmas were then

decanted and stored at -20°C until the determinations of the concentrations of AP, ALAT and AsAT enzymes in semi-automatical analyzer in Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria.

Statistical analyses

The statistical processing of the results obtained in the individual experiments was done through ANOVA (Statistics for Windows, Stat Soft Ins., USA, 1993). The statistical accuracy 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

In the experimental group there was a marked decrease in AP values at and day 14 compared to the control group ($P < 0.01$), and only in the experimental group there was a significant decrease in ALP values at 48th h compared to pretreated period ($P < 0.05$) as well as on the 14th day after infection ($P < 0.01$) (**Figure 1**).

Figure 1. An activity of alkaline phosphatase in the infected (*S. aureus*) and control groups of rabbits. Significance of the differences between experimental and control groups:

** $p < 0.01$.

Significance of the differences in the experimental group comparing pretreatment period

^b $p < 0.01$

A significant increase ($P < 0.01$) in ALAT activity was found in the experimental group comparing to the control group and also the pre

-infection period in the period 14-21 day ($P < 0.01$) (**Figure 2**).

Figure 2. An activity of ALAT in the infected (*S. aureus*) and control groups of rabbits.

Significance of the differences between experimental and control groups:

** $p < 0,01$.

Significance of the differences in the experimental group comparing pretreatment period

^C $P < 0,001$

A significant increase in ASAT activity only in the experimental group against the pre-infection period was found at day 14 and day

21 ($P < 0.05$), 14 and 21 days ($P < 0.01$) respectively (**Figure 3**).

Figure 3. An activity of ASAT in the infected (*S. aureus*) and control groups of rabbits

Significance of the differences in the experimental group comparing pre-treatment period

^a $p < 0,05$; ^b $p < 0,01$

DISCUSSION

In our study, we found a significant decreasing of ALP in the experimental group on day 3 compare pre-infection time and significant decreasing versus control group comparatively late (14th day). The activities of transaminases oppositely showed significant increasing at the same time (14th day). According to Willard et al., (2004), the decreasing of ALP has no diagnostic value. According to Burtis and Ashford (12) the levels of ALP may be decreased by fasting, hypothyroidism, or pernicious anemia, but in general, ALP is increased by digestion, cholestasis, or injury to the intestinal or biliary epithelium. As a liver enzyme, ALP does not increase because of hepatocellular damage but is indicative of bile stasis (e.g., hepatic coccidiosis, liver abscesses, neoplasia, lipidosis (13-15)). Generally speaking alkaline phosphatase (EC 3.1.3.1) comprises a heterogeneous group of enzymes with a hydrolytic action, responsible for removing phosphate groups from many types of molecules, including nucleotides, proteins (11). The process of removing the phosphate group is called dephosphorylation. They are distributed in mammalian cells and often are associated with cell membranes, but their exact physiologic function is unknown (16). It is possible to find it practically in all organs. Rabbits produce 2 ALP isoenzymes in the liver (11, 17, 18). An intestinal isoenzyme is quite abundant, so serum ALP concentrations are actually the sum of these 3 isoenzymes, which may explain why many reference ranges are vague and wide and why raised ALP levels in clinically healthy animals are a common finding. ALP does have a diagnostic value because it is not altered by restraint and thus is considered a good indicator of real tissue damage (19).

We found a significant increase in ALAT and ASAT activities against the pre-infection period and comparing the control group from day 7 until day 21. In many mammalian species, alanine aminotransferase (ALT) is a useful indicator of hepatocyte damage because of its specificity for liver tissue and its long half-life (45-60 hours in dogs). In rabbits, ALT is not as useful as an indicator of liver damage as with other species because, like other herbivores (e.g., horses, cattle, guinea pigs), ALT is not liver specific and has a shorter half-life (around 5 hours). However, ALT concentrations are not affected by restraint and therefore can be used as a diagnostic tool. Slightly increased ALT levels are a common finding in apparently healthy rabbits. Mildly increased ALT levels observed in our experiment could be due to the abscesses in the skin of rabbits, because this abscess was deeply inserted into the muscle tissue and fistulated.

This could allow the absorption of substances that could travel to the liver and induce the production of pro-inflammatory cytokines and other factors of inflammation. The *S. aureus* is exotoxins producing bacteria and these toxins could impair the function of the liver and maybe a reason for the elevation of ALAT and ASAT. In the available literature, we could not find data concerning *S. aureus* infection and ASAT, but our data are comparable with the data of Petrov et al., (20), regarding the activity of liver enzymes, in rabbits with a spontaneous EPEC infection. They reported for a nearly double increase in the activity of ASAT in infected rabbits. The values of ALAT measured in our experiment prior to infection are close to those reported by Petkova et al., (21) (26 ± 0.79), but those of ASAT in our experiment are lower than those of the above-mentioned authors. This is probably due to the difference in the age and weight of the rabbits in both experiments, as the rabbit age in our experiment was 90 days, whereas that of the experiment was 45-50 days.

Some viruses, like calicivirus infection which is the reason for rabbit hemorrhagic disease (RHD) causes a huge elevation of ALT and AST in calicivirus-infected rabbits. Ferreira et al., (22) used the blood values of AST to differentiate three stages of hepatocellular degeneration in RHD: mild (up to the 20-fold increase in AST), moderate (150-200-fold elevation of AST) and severe (more than 1000-fold elevation in AST). Pamukcu et al., (23) reported about decreasing glucose concentrations and increasing total protein concentrations and ASAT, ALAT activities in serum and in cerebrospinal fluid (CSF) in infected rabbits with severe meningitis after listeria infection than in rabbits with mild damage. The authors concluded if the enzyme activity is elevated in both serum and CSF, massive parenchymal brain damage is suggested and the prognosis is poor, whereas if only the CSF level is raised, recovery is probable.

CONCLUSION

Thus, study of the dynamics of the level of the enzymes during different infection becomes an important marker for tracking their flocking. This study shows that during skin *S. aureus* infection in rabbits the chosen enzymes reached different levels - AP level decreased at day 3 and the activities of the transaminases increased 14 days after infection. Therefore, it is important to know not only that the enzymes have changed, but also the concentrations and the directions they have reached during different types of infection. We

recommend that every lab should establish its own biochemical parameters of healthy rabbits herds for a different breed, age, and gender reference values. The dynamic of enzymes could be of great importance and could be used as an additional diagnostic aid.

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