Lysozyme and complement response to exercise in horses with booster vaccination against influenza virus and equine herpes virus 4/1

L.SOTIROV, D.GOUNDASHEVA1 and P.DZHELEBOV1

Department of Animal Genetics and 1General and Clinic Pathology, Faculty of Veterinary Medicine, Trakia University, 6000 Stara Zagora, Bulgaria

SUMMARY

The aim of this study was to determine the influence of exercise on innate immunity factors, complement response and lysozyme, during booster vaccination against influenza virus and Equine herpes virus 4/1 in horses.

Twelve healthy Hannover horses (4-9 year old) were subjected to booster vaccination and 6 of them (assay group) were submitted to exercises (jumping hurdles on four consecutive days).

Regular exercises during the period of vaccinal antibody production did not significantly change lysozyme concentrations, but on the contrary improved the intensity of classical pathway of complement activation (CPCA) on the 2nd day after exercise (19th day post vaccination) and induced sustained increases of alternative pathway complement activation (APCA) within the 4th and the 11th days after exercise (21st - 28th days post vaccination). These results show that exercise has no negative effect on innate immunity factors (lysozyme, APCA and CPCA) and by contrast, could promote the vaccinal response by increasing complement activation.

KEY WORDS : horse, booster vaccination, lysozyme, complement.

Introduction

Complement system and lysozyme are important components of innate immunity. Some pathogenic microorganisms induce activation of complement response directly by activating the alternative pathway, while others need specific antibodies to activate the function of the classical pathway of complement [4,15]. Both pathways play important role in the induction and regulation of immune response, opsonisation, inactivation of viruses, lysis of cells and bacteria, immunopathology and outcome of host-parasite relationships [5, 19, 20].

Lysozyme (mucopeptidase) is considered as a component of the earlier protective mechanisms, and is active mainly against Gram-positive bacteria [1, 11] but it can also be active against E.coli [2]. Effects of viruses on lysozyme concentrations are unknown.

Infections caused by some viruses as influenza and equine herpes virus-1 may induce also neurological disorders and abortion in pregnant mares [10]. The fore-mentioned viral infections cause changes in hematological parameters - decrease in total blood leucocytes, neutrophile and lymphocyte counts -, and an increase in antibody production [9, 16, 18]. Preventing these viral respiratory tract diseases in horse by means of vaccination applied every year, is of prime importance to the equine industry.

Studies of the stress influence on complement system [6, 21] and on lysozyme concentration [12] are scarce. How stress modifies the innate immunity and particularly lysozyme and complement concentrations has not been explored in horses. Similar investigations of these parameters after viral vaccination are also very few [8]. Simultaneous effect of exercise and booster vaccination on lysozyme and complement has not been studied. That is why the aim of present experiment is to study the changes in complement and lysozyme activity after exercise, in horses vaccinated with a killed virus vaccine (booster vaccination) against IV (IV: Influenza virus) and EHV4/1 (EHV4/1: Equine Herpes Virus).

RéSUMÉ

Effet de l’effort physique sur le lysozyme et le complément chez des chevaux ayant subi une vaccination de rappel contre le virus grippal et les herpes virus 4 et 1. Par L. SOTIROV, D. GOUNDASHEVA et P. DZHELEBOV.

L’objectif de cette étude a été d’évaluer l’influence d’un exercice sur l’immunité innée (activation du complément et lysozyme) durant une vaccination de rappel contre le virus grippal et les herpes virus équins types 1 et 4 chez les chevaux.

Douze chevaux sains, âgés de 4 à 9 ans, de race Hanovre ont subi la vaccination de rappel et 6 d’entre eux (groupe expérimental) ont été soumis à des efforts physiques (sauts d’obstacles durant 4 jours consécutifs).


MOTS CLÉS : cheval, rappel vaccinal, lysozyme, complément.
Materials and methods

1) ANIMALS

Our experiment was carried out on twelve male Hannover horses (from Experimental Equine Base of the Trakia University - Stara Zagora 4-9 years old, weighing 400-600 kg, and previously vaccinated against IV and EHV 4/1. They were vaccinated against with commercially available combined influenza-herpesvirus vaccine (Fluvac EHV 4/1 Plus, Ford Dodge Laboratories Inc., Ford Dodge, Iowa, USA). Each horse received booster dose (1 ml) of the oily adjuvant vaccine, applied aseptically and intramuscularly in the neck. The animals were divided into 2 group of 6 horses: in group I, horses were subjected to exercise (experimental group), whereas horses of group II, served as controls.

The horses were kept in box stalls, were fed with commercially pellets and mixed hay and had access to water and salt. They were not submitted to strenuous exercise up to the 14th day after booster vaccination. They were left free in paddock for a few hours a day.

2) EXERCISE PROTOCOL

The exercise, to which horses were submitted, was a simulated competition. It consisted in 15 min walk at a speed of 100-200 m/min, then 15 min trot at 250 m/min and 5 min canter at 350 m/min. Afterwards the horses performed 7 preliminary jumps followed by another 15 jumps during a course which the height of obstacles gradually increased from 0,90 to 1,10 m. The height was increased to eliminate the habituation of the hypothalamic-pituitary-adrenal axis, leading to attenuated response. The exercise procedure was repeated for four consecutive days between 8 am. and 11 a.m. beginning on the 14th day after booster vaccination. Thereafter, the animals were not subjected to any other physical stretch up to the end of the study.

3) BLOOD COLLECTION

Blood samples were collected from v. jugularis externa into test tubes without anticoagulant. The samples were kept at room temperature for 1 hour to coagulate and were then centrifuged at 1200 x g for 10 minutes at 4°C. The serum was then separated and used to quantify lysozyme concentration and to examine the Complement Activation by Alternative Pathway (APCA) or by Classical Pathway (CPCA).

The blood samples from both groups were collected before exercise (BE), immediately (hour 0) and 2 hours after the last exercise procedure, and on days 1, 2, 4 and 11 after the exercise, corresponding to days 18, 19, 21 and 28, after booster vaccination.

4) DETERMINATION OF SERUM LYSOZYME CONCENTRATION

The lysozyme content was determined by the method of LIE et al.[13]. Briefly, 20 ml of 2% agarose (ICN, UK, Lot 2050) dissolved in phosphate buffer (70 mM Na₂ HPO₄, pH=6.2) was mixed with 20 ml suspension of 24 hours culture of Micrococcus lysodeikticus at 67°C. This mixture was poured out in Petri’s dish (140 mm diameter). After solidifying at room temperature 32 wells were made (5 mm diameter). Fifty microliters of undiluted sera were poured out in each well. Eight standard dilutions (from 0,025 to 3,125 mg/l) of lysozyme (Veterinary Research Institute, Veliko Tarnovo, Bulgaria) were used in the same quantity as well. The samples were incubated for 20 hours at 37°C and lytic diameters were measured.

5) DETERMINATION OF THE ALTERNATIVE PATHWAY COMPLEMENT ACTIVATION (APCA)

The APCA was studied by the method of SOTIROV [17], adapted for horses. For this aim, we used veronal- veronal Na buffer (0.97 M NaCl; 12,13 mM 5,5-Diethylbarbiturate Natrium salt; 20,82 mM 5,5-Diethylbarbituric acid; 6,7 mM EGTA; 5,3mM MgCl₂ , pH: 7,5). From each serum sample, seven dilutions were made: 8/10; 7/10; 6/10; 5/10; 4/10; 3/10 and 2/10. Then 50µl of diluted 1/5 veronal- veronal Na buffer were added to each well containing 100 µl of diluted samples. Finally 100 µl of 1% rabbit erythrocyte suspension (target cells) were dropped and were incubated at 37°C for 1 hour. Optical density was measured by « Sumal - PE2 » ELISA reader (Karl Zeiss, Jena, Germany) at 540 nm.

6) DETERMINATION OF THE CLASSICAL PATHWAY COMPLEMENT ACTIVATION (CPCA)

The classical pathway complement activation was determined according to the method of MAYER [14] adapted for horse [3]. After washing in veronal buffer, a 5% sheep red blood cell suspension (SRBC) (at 541 nm, the optical density was 0.700) was sensitized with rabbit antisheep haemolysin, diluted 1/40 in veronal buffer (National Center of Infectious and Parasite Disease, Bulgaria) during 30 minutes at 37°C under constant agitation, then cooled down in ice for 30 minutes.

Serial dilutions of horse serum in ice - cold veronal buffer were slowly added to the continuously mixed SRBC suspension. Samples were incubated and frequently resuspended for 1 hour at 37°C. Haemolytic reactions were stopped by a 5 min incubation in an ice bath and subsequent refrigerated (4°C) centrifugation for 10 min at 500 x g. The supernatant was spectrophotometrically analyzed (540 nm) for quantitative determination of haemolysis. Percentage of lysis was calculated and compared to control sample (SRBC in distilled water). The results for complement activation by alternative pathway (APCA) or by classical pathway (CPCA) were expressed as 50% haemolytic units (CH50). CH50 was reciprocal of the serum dilution that lysed 50% of sensitized SRBC.

7) STATISTICAL ANALYSIS

The results were expressed as mean ± SEM and submitted to a statistical analysis of variance (ANOVA). Post-hoc comparisons of individual group means were carried out by the
least significant difference test (LSD). Differences were considered statistically significant when p values were less than 0.05.

Results

The results for lysozyme activity are presented in figure 1. In controls, progressive insignificant decrease of lysozyme activity was observed during the study. Like in control group, a progressive and not significant decline of lysozyme concentrations was noticed from initial values to 2 hours after exercise in horses from experimental group. Thereafter, lysozyme concentrations markedly decreased in comparison to initial values on 1st day to the 4th day after exercise (p<0.05, and p<0.001 respectively). The observed decreases were maximal on day 4 (0.302 ± 0.010 mg/l vs. 0.980 ± 0.100 mg/l, p<0.001), then lysozyme concentrations rose again on day 11 but they significantly stayed below the values obtained before exercise (p<0.01).

The APCA (figure 2) in controls was slightly lower on the 1st day and returned to initial values on the 2nd day. In experimental group, APCA showed a statistically significant decrease at the 2nd hour compared to the initial values (p<0.01). Afterwards its activity returned to values obtained before exercise between the 1st and the 2nd day. On the 4th day, there was a dramatic and statistically significant increase of APCA (p<0.001), which has persisted until the 11th day compared to initial values and to APCA results in control horses (p<0.001 and p<0.05 respectively).

The CPCA in the control group (figure 3) increased from the 2nd hour and were maximal on day 2 (vs. initial values p<0.05 and p<0.001 respectively), thereafter returned to basal values on the 4th day. In experimental horses, CPCA has presented similar variations with maximal increase on the 2nd day after exercise (vs. initial values, p<0.001). Besides, on the 2nd day, the CPCA was more elevated in assay group (29.52 ± 7.13 CH50) than in control group (22.51 ± 1.25 CH50) (p<0.05).

Discussion

Reduction of lysozyme concentration and of APCA occurred in control horses, while CPCA increased during the same period. Although not significant, the observed decline of lysozyme activity was progressive and was more evident within the 4th and the 11th days corresponding to the 21st and the 28th days after booster vaccination. A marked decrease of APCA was obtained on day 1 (the 18th day post-vaccination) and a rise in CPCA was evidenced on day 2 (the 19th day post-vaccination). Because classical pathway of complement activation required formation of antibody-antigen complexes, increases of serum antibodies probably occurred in horses in this period following booster vaccination. This hypothesis was confirmed by our previous studies. Indeed, we have detected marked increases of antibodies against influenza viruses types A1 and A2 and against equine herpes viruses 1 and 4 within the 14th and the 21st days after booster vaccination [8].
A significant reduction of lysozyme concentrations from basal values was observed from the 1st day to the 11th day after exercise in the assay group. Although for each time, the differences between groups were not statistically significant, the recorded variations were greater in experimental horses, and could result from exercise-induced stress. Probably the applied exercise leads to changes in neuroendocrine and immune system-derived substances, which could influence the innate immune factors, but a detailed explanation of these mechanisms needs some additional studies. Such decrease in lysozyme activity was observed after immobilization stress [6]. The potential effects of stress on lysozyme activity are highly controversial since, on the contrary, other researchers [5, 12] have reported increases of this marker after stress induced by isolation and trauma in other animal species.

An early decrease of APCA compared to basal values was also seen in assay group on the 2nd hour after exercise corresponding to the 17th day after booster vaccination, whereas this variation occurred on the 18th day in control group. Thereafter, marked increases of APCA within the 21st and the 28th day post vaccination were only seen in assay group. A peak of CPCA was obtained in experimental animals on this variation occurred on the 18th day in control group. Also seen in assay group on the 2nd hour after exercise corresponding to the 17th day after booster vaccination against influenza and equine herpes virus 4/1 on some parameters of the immune response in horses. Rev. Med. Vet., 2002, 153(8-9): 569-574.