

Effects of hydrocortisone on platelet aggregation in jumper horses

Giuseppe Piccione^{1*}, Stefania Casella¹, Claudia Giannetto¹, Vanessa Messina¹,
Pietro P. Niutta², and Elisabetta Giudice²

¹ Dipartimento di Scienze Sperimentali e Biotecnologie Applicate, Facoltà di Medicina Veterinaria Università degli Studi di Messina, Polo Universitario dell'Annunziata, Messina, Italy

² Dipartimento di Sanità Pubblica Veterinaria, Sezione Clinica Medica, Facoltà di Medicina Veterinaria Università degli Studi di Messina, Polo Universitario dell'Annunziata, Messina, Italy

PICCIONE, G., S. CASELLA, C. GIANNETTO, V. MESSINA, P. P. NIUTTA, E. GIUDICE: Effects of hydrocortisone on platelet aggregation in jumper horses. Vet. arhiv 81, 153-162, 2011.

ABSTRACT

Research conducted in human sports medicine showed the variable effect of exercise on platelet aggregation parameters, which consist, for some authors, in a significant increase of aggregation, while for others, in its decline as result of physical activity. The purpose of our study was to test, in athletic horses, the effect of hydrocortisone as an anti-inflammatory steroid drug as an inhibitor of platelet aggregation *in vitro*. In our research a total of 12 jumper horses, 4 females and 8 geldings (Sella Italiana breed), clinically healthy, specifically trained and in good nutritional condition, were divided into two groups (A and B). From all the blood samples, collected in tubes containing sodium citrate (1 part sodium citrate to 9 parts blood), platelet aggregation was evaluated adding adenosine diphosphate (ADP) as a platelet-activating agent, and also after incubation for 20 minutes with hydrocortisone (IDR) as an inhibitor of platelet aggregation. Using an aggregometer platelet aggregation curves were defined and from these the aggregation rate and the slope of aggregation were evaluated. Multivariate ANOVA showed the significant effect of time ($P < 0.05$) on platelet aggregation and on the average speed of aggregation ($P < 0.05$). The effect of hydrocortisone was observed only on the aggregation slope ($P < 0.01$). Further research should be conducted to assess the effective sensitivity of the slope, and certainly, given the absent effect of hydrocortisone on the platelet aggregation, another kind of anti-aggregation substance should be used to compare our data.

Key words: athletic horse, platelet aggregation, hydrocortisone, adenosin diphosphate, jumper horse

Introduction

Platelets, upon activation, stimulate thrombus formation and recruit additional platelets. Their activation is essential for several physiological and pathological reactions and depends upon their adhesion to the vessel wall and attachment to each other in the

*Corresponding author:

Prof. Giuseppe Piccione, Dipartimento di Scienze Sperimentali e Biotecnologie Applicate, Facoltà di Medicina Veterinaria, Università degli Studi di Messina, Polo Universitario dell'Annunziata, Postal code 98168, Messina, Italy, Phone: +39 90350 3584; Fax: +39 90350 3975; E-mail: giuseppe.piccione@unime.it

ISSN 0372-5480
Printed in Croatia

aggregation process (SAWICKI et al., 1997). Plasma constituents and blood cells other than platelets affect aggregation and secretion in an agonist-dependent manner (THOMAS, 1996). Adenosin diphosphate (ADP) is used as a promoter of aggregation because it stimulates intracellular calcium flux and thereby establishes platelet activation, which leads to the formation of the “haemostatic plug” (JOHNSTONE, 1983). The aggregation response is then enhanced by the secretion of products of arachidonic acid metabolism and by further ADP release (JOHNSTONE, 1983; CATTANEO and GACHET, 1999).

Research conducted in human sports medicine has showed the variable effect of exercise on aggregation parameters, which consist, for some authors, in a significant increase of aggregation, while for others, in its decline as result of the physical activity (HENDRA et al., 1988; PRISCO et al., 1993; HURLEN et al., 2000; PETIDIS et al., 2008; HONG et al., 2009).

The effects of exercise on platelet aggregation and activation have been extensively studied, but the results are still highly variable. Platelet activation and aggregation seem to be directly related to the intensity of physical effort, and the duration and degree of training of the subject (EL SAYED, 2004). Certainly strenuous exercise causes an increase in the number of circulating platelets through the release of platelets from the spleen, and increased concentration of the von Willerbrand factor, but the effect on coagulation is also expressed through other mechanisms not yet well clarified (LIPPI and MAFFULLI, 2009; LIN et al., 1999). The increased fibrinolytic activity appears to counterbalance the exercise-induced increase in coagulability (PICCIONE et al., 2004a and b; PICCIONE et al., 2005). There is evidence that the reduction in bleeding time after exercise is due to the involvement of the platelets (PRISCO et al., 1993). However, long term physical endurance training seems to suppress platelet adhesiveness and aggregation (LEE and LIP, 2003). Some studies have been conducted in horses to test the effectiveness of various non steroidal anti-inflammatory drugs (JOHNSTONE, 1983), but no studies are available in the current literature which compare platelet aggregation after exercise and the use of a selective inhibitor of platelet aggregation in athletic horses. The purpose of our study was to test the effect of hydrocortisone as an inhibitor of platelet aggregation *in vitro* in athletic horses.

Materials and methods

In our study, a total of 12 jumper horses were used, 4 females and 8 geldings (Sella Italiana breed), clinically healthy, specifically trained and in good nutritional state. All the subjects were divided into two groups (A and B) and examined to exclude general phenomena of dehydration. No medication was administered for one month before the study. Group A was composed of 6 horses, 5 years old and with an average weight of 450 ± 10 kg. Group B was composed of 6 horses aged between 7 and 9 years and with an

average weight of 490 ± 40 kg. Both sets of exercises consisted of a standardized obstacle course length of 400 meters preceded by warming-up for 15 minutes. The warming-up consisted of walk, trot, canter and three obstacles of increasing height, 0.6-1.00 meters for Group A and 0.8 - 1.25 meters for the Group B. The obstacle course length of 400 m height of 10 obstacles 1 meter respectively for Group A and 125 for group B, was divided into 4 vertical, 5 wide, and a combination of vertical and wide. From all subjects blood samples were taken by jugular venipuncture using Vacutainers tubes (Terumo Corporation, Japan) containing K3-EDTA and sodium citrate (1 part sodium citrate to 9 parts blood). Blood samples collected in K3-EDTA were analyzed using an automatic multiparametric analyzer for haematology (HeCoVet C, SEAC, Italy) in order to obtain a complete cell blood count. Blood samples collected in sodium citrate were treated to prepare platelet-rich plasma (PRP) and platelet-poor plasma (PPP). To prepare the PRP, the samples were centrifuged at $300 g \times 20'$, then the PRP removed and transferred into Eppendorf. To prepare the PPP the blood samples were again centrifuged at $3000 g \times 10'$ and also the PPP obtained was transferred into Eppendorf. Subsequently, all serum samples obtained were divided into two aliquots. After the addition of $10 \mu\text{M}$ adenosine diphosphate (ADP) as the platelet-activating agent, the sera were analyzed. One of the aliquots was incubated for 20 minutes with $10 \mu\text{L}$ of hydrocortisone (Flebocortid Richter, 500 mg/5 mL) as the inhibitor of platelet aggregation (IDR). Using an aggregometer (Clot 2S, Radim-SEAC, Florence, Italy) the curves of platelet aggregation were defined and the aggregation rate (percentage of maximum amount of clotted platelets) and the slope of aggregation (percentage of platelets that aggregate in 1 minute) were evaluated from these curves.

Statistical analysis. Statistical analysis of data was performed from the values of platelet aggregation and the speed of aggregation obtained in different experimental conditions. Multivariate ANOVA was applied to assess the statistically significant effect of time in different experimental conditions (at rest, after exercise, after 30 min and after 60 min from the end of exercise) and statistical differences due to the addition of hydrocortisone. Bonferroni's test was applied as a post-hoc comparison. A P value <0.05 was considered statistically significant. Statistica software version 7.0 (Stat Soft Inc.) was used to perform the analysis. All results were expressed as mean \pm standard deviation of the mean (SD).

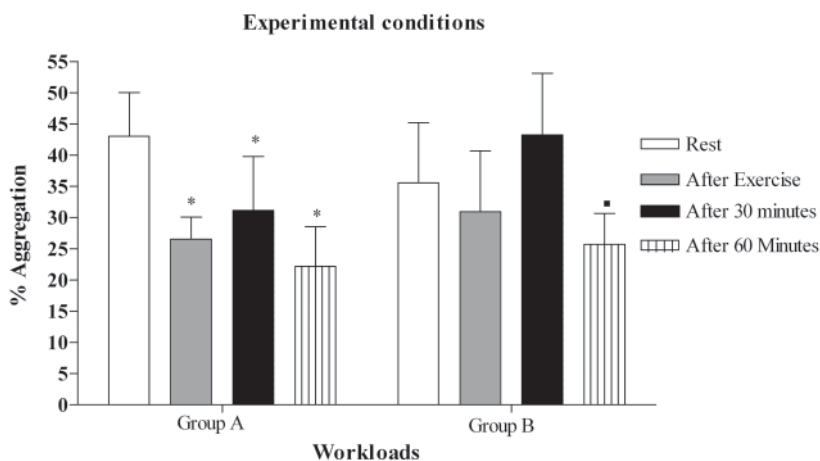
Results

No significance differences emerged in the hemochrome-cytometric parameters. In particular the platelet count no showed significant variations during the experimental conditions studied. Table 1 shows mean values and standard deviations of platelet aggregation and the slope of aggregation, expressed in their conventional units of measurement, observed during our research.

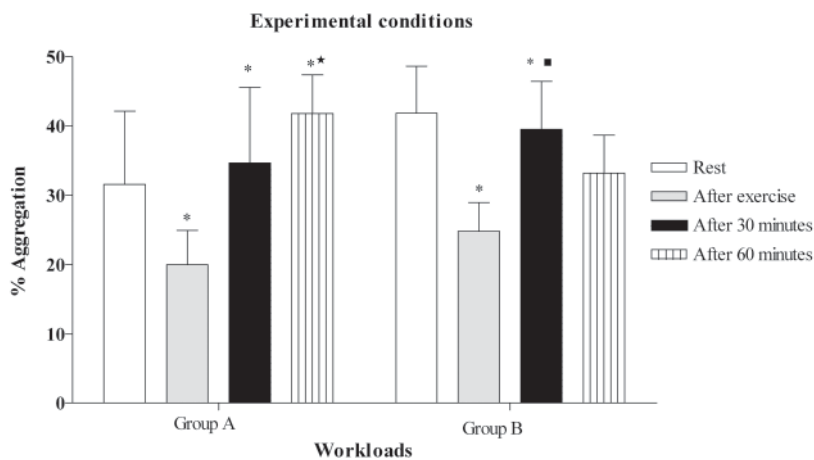
Table 1. Average values of the parameters considered, expressed in their conventional units of measurement with the related standard error, observed in different experimental condition in 12 jumper horses

| Parameter | Experimental conditions | | | | |
|--------------------------------|-------------------------|-------------------|------------------|-------------------|------------------|
| | Groups | Rest | After exercise | After 30 | After 60 |
| % Aggregation (10 μ M ADP) | A | 43.00 \pm 7.04 | 26.55 \pm 3.50 | 31.16 \pm 8.58 | 22.23 \pm 6.26 |
| | B | 35.53 \pm 9.63 | 30.93 \pm 9.71 | 43.22 \pm 9.87 | 25.73 \pm 4.85 |
| % Aggregation IDR | A | 31.57 \pm 10.58 | 20.01 \pm 4.91 | 34.66 \pm 10.93 | 41.79 \pm 5.59 |
| | B | 41.86 \pm 6.78 | 24.75 \pm 4.16 | 39.5 \pm 6.97 | 33.16 \pm 5.49 |
| Slope (%/min) | A | 3.05 \pm 1.02 | 2.91 \pm 1.06 | 3.91 \pm 0.86 | 1.50 \pm 0.63 |
| | B | 4.95 \pm 1.01 | 3.63 \pm 0.74 | 3.93 \pm 0.78 | 3.33 \pm 0.81 |
| Slope % IDR (%/min). | A | 3.86 \pm 0.87 | 2.40 \pm 0.45 | 3.00 \pm 0.89 | 3.60 \pm 0.88 |
| | B | 3.13 \pm 1.12 | 2.99 \pm 0.20 | 5.05 \pm 0.91 | 5.86 \pm 0.61 |

Multivariate ANOVA showed the significant effect of time ($F_{(3,80)} = 26.41$, $P < 0.0001$) on platelet aggregation. No effect of hydrocortisone was observed on this parameter. In Group A, a significant decrease of platelet aggregation was observed after exercise and after 60 min *vs.* rest, whereas a significant decrease was observed after 60 min *vs.* after 30 min. The value recorded 30 min after the end of exercise, however, showed an increase *vs.* after exercise. The same significant pattern was observed in Group B. On samples treated with IDR from Group A, a significant decrease after exercise *vs.* rest and a significant increase after 30 and 60 min *vs.* after exercise was observed. In samples treated with IDR from Group B, a significant decrease after exercise *vs.* rest and a significant increase 30 min after exercise *vs.* rest and after exercise was observed. The value recorded after 60 min showed a significant increase *vs.* after 30 min. The application of multivariate ANOVA on the average speed of aggregation, showed the significant effect of time ($F_{(3,80)} = 4.97$, $P \leq 0.003$) and hydrocortisone ($F_{(1,80)} = 8.41$, $P \leq 0.004$). In Group A the value recorded after 60 min showed a statistically significant decrease *vs.* rest, after exercise and 30 min from the end of exercise. In Group B, ANOVA showed a significant decrease after exercise, after 30 min and after 60 min from the end of exercise *vs.* rest. On samples treated with IDR from Group A, a statistically significant decrease was observed after exercise *vs.* at rest and an increase and after 30 min *vs.* rest, whereas a statistical increase after 60 min *vs.* after exercise was shown. On samples treated with IDR from Group B a statistically significant increase after 30 min and 60 min *vs.* rest and after exercise was observed. The comparison of samples with and without IDR showed a significant increase after 60 min with the addition of IDR both in Group A and in Group B.

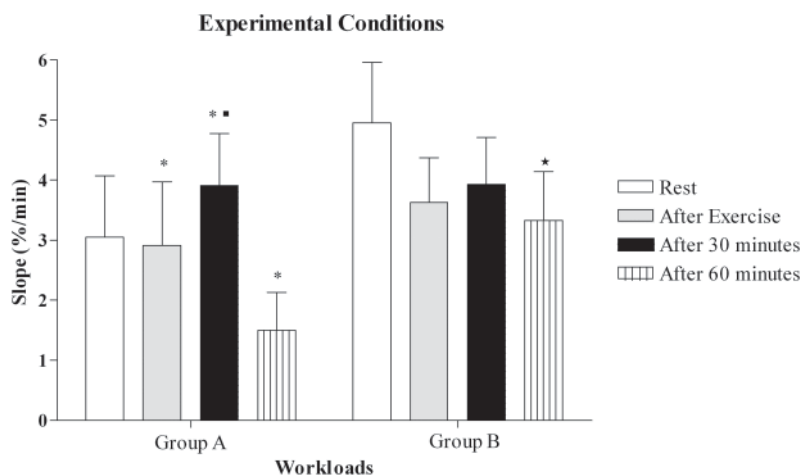


Significances: *Vs Rest (P<0.05); *Vs After exercise (P<0.005); *Vs After 30 min (P<0.001); *Vs After 60 min (P<0.0001).

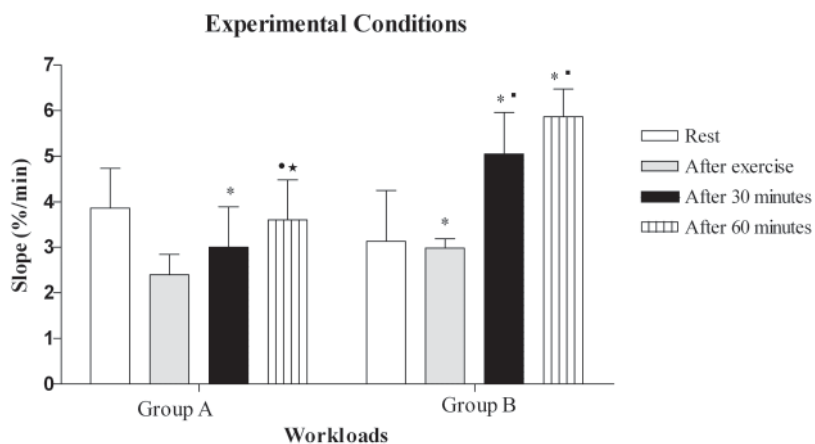


Significances: *Vs Rest (P<0.005); *Vs After exercise (P<0.005); *Vs After 30 min (P<0.001); *Vs After 60 min (P<0.0001).

Fig. 1. Graphical representation of average mean values of platelet aggregation during different experimental conditions. a. Platelet aggregation of groups A and B; b. Platelet aggregation of groups A and B with addition to samples of 10 μ L of hydrocortisone (Flebocortid Richter, 500 mg/5 mL).



Significances: *Vs Rest (P<0.05); ■Vs After exercise (P<0.005); •Vs After 30 min (P<0.001); *Vs After 60 min (P<0.0001).



Significances: *Vs Rest (P<0.05); ■Vs After exercise (P<0.005); •Vs After 30 min (P<0.001); *Vs After 60 min (P<0.0001).

Fig. 2. Graphical representation of average mean values of slope of aggregation during different experimental conditions. a. Slope of aggregation of groups A and B; b. Slope of aggregation of groups A and B with addition to samples of 10 µL of hydrocortisone (Flebocortid Richter, 500 mg/5 mL).

Discussion

Figures 1-2 represent the average values of platelet aggregation and the slope of aggregation during different experimental conditions both with and without the addition

of 10 µL hydrocortisone (Flebocortid Richter, 500 mg/5 mL). Our results showed no statistically significant changes in platelet count, as previously observed by other authors (KINGSTON et al., 1999; WEISS et al., 1998; LEPHERD, 1977). Authors showed that moderate, rather than intense exercise in humans causes the increased release of catecholamines from the adrenergic system, as well as the increased release of nitric oxide by the cells' vascular epithelium (WANG, 2006). This phenomenon causes the inhibition of thrombus formation, even under the action of an intense "shear flow", and a decrease in platelet aggregation in response to exercise, even with the addition of ADP as an aggregation agonist (WANG, 2006; JOHNSTONE et al., 1991). Moreover, results from our previous studies show that training enhances fibrinolytic activity. Some studies in humans have shown that the effect of physical conditioning in the long term, such as from continuous and aerobic exercise, also involves platelet reactivity in general as well as other blood components, and results in a decrease in aggregation following exercise (PETIDIS et al., 2008). This should be considered in the light of research conducted in humans, which demonstrate that, in trained subjects, in contrast to sedentary ones, exercise causes a change in the ability of platelets to aggregate (DAVIS et al., 1990). Usually jumper horses undergo an intensive program of training before the start of their careers. In this study a decrease in aggregation occurred in both Group A and Group B. This means that the workloads used do not cause significant changes in the behaviour of aggregation. In fact, the result of both types of exercise was a decrease in platelet aggregation. This platelet aggregation behaviour is attributable to the increased release of catecholamines during exercise and to the activation of the hypothalamic-pituitary axis (CAYADO et al., 2006; HENDRA et al., 1988; HURLEN et al., 2000; SAKITA et al., 1997).

The trend shown by platelet aggregation can be explained by the substance used as aggregating agent. These observations were made by some authors who demonstrated that in equine platelets aggregation is only significantly decreased in response to ADP (KINGSTONE et al., 1999). Other research previously showed that different types of agonists or the ADP itself provide different results at higher amounts (PETIDIS et al., 2008; JOHNSTONE, 1983).

Several authors wanted to test on platelets the effect of the addition of substances to samples that antagonize the adhesion of platelets *in vitro* (JOHNSTONE, 1983; BAYLY et al., 1983; JARVIS and EVANS, 1996). The substances mostly used for this purpose are inhibitors of cyclooxygenase, i.e. non steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids. These substances, by blocking the conversion of arachidonic acid into prostaglandins, prostacyclin and thromboxanes, act by preventing the platelet activation mediated by them. However, the action of hydrocortisone did not show any influence on platelet aggregation. Hydrocortisone has been used in some research as an anti-aggregation drug, with various results, especially dose-dependent. Hydrocortisone has a variety of immunological and haematological effects. Against platelets are known effects

of a decrease in aggregate dose-dependent, although are also known opposed effects. In particular in the case of ADP, a higher dose of hydrocortisone is needed to inhibit platelet aggregation than the dose required to inhibit aggregation mediated by other antagonists, such as collagen and prostaglandins (GLASS et al., 1981; SCHUERHOLZ et al., 2007).

The slope of the aggregation curve showed a significant decrease related to exercise and the anti-aggregation drug. This should be read in the light of the work of other authors, where it is proposed that the aggregation parameter alone may be measured as a function of the slope of the aggregation curve and for this reason it may be seen as a more sensitive parameter in detecting and recording changes in physical conditions such as temperature, pH, sample preparation and the presence of electrolytes (SEE et al., 1992; DE PAULA et al., 2009). We can assume, that in fact the presence of hydrocortisone, which did not significantly affect the platelet aggregation, caused a decrease in the values of the slope.

Further research should be conducted to assess the effective sensitivity of the slope, and certainly, given the absent effect of hydrocortisone on the platelet aggregation, another kind of anti-aggregation substance should be used to compare our data. Moreover, to clarify the results obtained, it would be interesting to test different workloads, to see how far the exercise interferes with the function of platelets in the equine species.

References

- BAYLY, W. M., K. M., MEYERS, M. T. KECK, L. J. HUSTON, B. D. GRANT (1983): Effects of exercise on the haemostatic system of thoroughbred horses displaying post exercise epistaxis. *J. Equine Vet. Sci.* 3, 191-193.
- CATTANEO, M., C. GACHET (1999): ADP receptors and clinical bleeding disorders. *Arteriosclerosis, Thrombosis and Vascular Biology* 19, 2281-2285.
- CAYADO, P., B. MUNOZ-ESCASSI, C. DOMINGUEZ, W. MANLEY, B. OLABARRI, M. SANCHEZ DE LA MUELA, F. CASTEJON, G. MARANON, E. VARA (2006): Hormone responses to training and competition in athletic horses. *Equine Vet. J. Suppl.* 36, 274-278.
- DAVIS, R. B., D. G. BOYD, M. E. MCKINNEY, C. C. JONES (1990): Effects of exercise and exercise conditioning on blood platelet function. *Med. Sci. Sports Exe.* 22, 49-53.
- DE PAULA, E. V., T. F. G. S. MACHADO, M. S. PEREIRA, F. ORSI, J. M. ANNICHILO-BIZZACCHI (2009): Maximal aggregation or slope: which one should be used in platelet aggregation reports? *Proceedings of the XXII International on Thrombosis and Haemostasis*, July 2009.
- EL-SAYED, M. S., A. Z. EL-SAYED, S. AHMADIZAD (2004): Exercise and training effects on blood haemostasis in health and disease: an update. *Sports Med.* 34, 181-200.
- GLASS, F., H. LIPPTON, P. J. KADOWITZ (1981): Effects of methylprednisolone and hydrocortisone on aggregation of rabbit platelets induced by arachidonic acid and other aggregating substances. *Thromb. Haemost.* 46, 676-679.

- HENDRA, T. J., J. OUGHTON, C. C. SMITH, D. J. BETTERIDGE, J. S. YUDKIN (1988): Exercise-induced changes in platelet aggregation; a comparison of whole blood and platelet rich plasma techniques. *Thromb. Res.* 52, 443-451.
- HONG, S., A. ADLER, R. VON KANEL, J. NORDBERG, M. G. ZIEGLER, P. J. MILLS (2009): Prolonged platelet activation in individuals with elevated blood pressure in response to a moderate exercise challenge. *Psychophysiol.* 46, 276-284.
- HURLEN, M., I. SELJEFLOT, H. ARNESEN (2000): Increased platelet aggregability during exercise in patients with previous myocardial infarction. Lack of inhibition by aspirin. *Thromb. Res.* 99, 487-494.
- JARVIS, G. E., R. J. EVANS (1996): Platelet-activating factor and not thromboxanes A2 is an important mediator of endotoxin-induced platelet aggregation in equine heparinised whole blood *in vitro*. *Blood Coagul. Fibrinolysis* 7, 194-198.
- JOHNSTONE, I. B. (1983): Comparative effects of phenylbutazone, naproxen and flunixin meglumine on equine platelet aggregation and platelet factor 3 availability *in vitro*. *Canadian J. Comp. Med.* 47, 172-179.
- JOHNSTONE, I. B., L. VIEL, S. CRANE, T. WHITING (1991): Haemostatic studies in racing standardbred horses with exercise-induced pulmonary haemorrhage. Haemostatic parameters at rest and after moderate exercise. *Can. J. Vet. Res.* 55, 101-106.
- KINGSTON, J. K., S. N. SAMPSON, L. A. BEARD, K. M. MEYERS, D. C. SELTON, W. M. BAYLY (1999): The effect of supramaximal exercise on equine platelet function. *Equine Vet. J. Suppl.* 30, 181-183.
- KINGSTON, J. K., W. M. BAYLY, K. M. MEYERS, D. C. SELTON, K. J. WARDROP (2002): Evaluation of binding of fibrinogen and annexin V to equine platelets in response to supramaximal treadmill exercise. *Equine Vet. J. Suppl.* 34, 502-505.
- LEE, K. W., G. Y. LIP (2003): Effects of lifestyle on hemostasis, fibrinolysis, and platelet reactivity: a systematic review. *Arch. Intern. Med.* 163, 2368-2392.
- LEPHERD, E. E. (1977): Effect of exercise on platelet size and number in ponies. *Vet. Rec.* 101, 488.
- LIN, X., M. S. EL SAYED, J. WATERHOUSE, T. REILLY (1999): Activation and disturbance of blood haemostasis following strenuous physical exercise. *Int. J. Sports Med.* 20, 149-153.
- LIPPI, G., N. MAFFULLI (2009): Biological influence of physical exercise on haemostasis. *Semin. Thromb. Hemost.* 35, 269-276.
- PETIDIS, K., S. DOUMA, M. DOUMAS, I. BASAGIANNIS, K. VOGIATZIS, C. ZAMBOULIS (2008): The interaction of vasoactive substances during exercise modulates platelets aggregation in hypertension and coronary artery disease. *BMC Cardiovascular Disorders* 8, 11.
- PICCIONE, G., A. ARCIGLI, A. COSTA, F. FAZIO, G. CAOLA (2004a): Changes in clotting times and fibrinogen concentrations in horses during a show jump. *Slove. Vet. Res.* 41, 41-45.
- PICCIONE, G., F. FAZIO, E. GIUDICE, F. GRASSO, G. CAOLA (2004b): Changes in haematological parameters and clotting times in the horse during a long distance running. *Med. Weter.* 60, 587-590.

- PICCIONE G., F. FAZIO, E. GIUDICE, F. GRASSO, G. CAOLA (2005): Exercise induced changes in the clotting times and fibrinolytic activity during official 1600 and 2000 meters trot races in standardbred horses. *Acta Vet. Brno* 74, 509-514.
- PRISCO, D., R. PANICCIA, V. GUARNACCIA, G. OLIVO, T. TADDEI, M. BODDI, G. F. GENUINI (1993): Thrombin generation after physical exercise. *Thromb. Res.* 69, 159-164.
- SAKITA, S., Y. KISHI, F. NUMANO (1997): Acute vigorous exercise attenuates sensitivity of platelets to nitric oxide. *Thromb. Res.* 87, 461-471.
- SAWICKI, G., E. SALAS, J. MURAT, H. MISZTA-LANE, M. W. RADOMSKI (1997): Release of gelatinase A during platelet activation mediates aggregation. *Nature* 386, 616-619.
- SCHUERHOLZ, T., O. KEIL, T. WAGNER, S. KLINZING, R. SUMPELMANN, V. OBERLE, G. MARX (2007): Hydrocortisone does not affect major platelet receptors in inflammation *in vitro*. *Steroids* 72, 609-613.
- SEE, T. T., H. Y. LEE, S. P. LEE (1992): ADP-induced platelet aggregation in abdominal obese and normal females. *J. Med. Sci.* 12, 398-403.
- THOMAS, S. J. (1996): Comparison of platelet aggregation and adenosine triphosphate secretion in whole blood and platelet-rich plasma from normal dogs. *Comp. Haematol. International* 6, 70-76.
- WEISS, D. J., O. A. EVANSON, D. McCLENAHAN, J. FAGLIARI, B. WALCHECK (1998): Shear induced platelet activation and platelet-neutrophil aggregate formation by equine platelets. *Am. J. Vet. Res.* 59, 1243-1246.
- WANG, J. S. (2006): Exercise prescription and thrombogenesis. *J. Biomed. Sci.* 13, 753-761.

Received: 22 December 2009

Accepted: 9 July 2010

PICCIONE, G., S. CASELLA, C. GIANNETTO, V. MESSINA, P. P. NIUTTA, E. GIUDICE: Učinci hidrokortizona na nakupljanje krvnih pločica u preponskih konja. *Vet. arhiv* 81, 153-162, 2011.

SAŽETAK

Istraživanja poduzeta u humanoj športskoj medicini pokazala su da postoji različit učinak tjelovježbe na nakupljanje (agregaciju) krvnih pločica pa tako neki autori smatraju da tjelovježba značajno povećava njihovo nakupljanje dok drugi smatraju da ona smanjuje nakupljanje krvnih pločica. Svrha je ovog rada u športskih konja istražiti učinak protuupalnoga steroidnoga lijeka hidrokortizona kao inhibitora nakupljanja krvnih pločica *in vitro*. U istraživanje je bilo uzeto ukupno 12 preponskih konja, četiri kobile i osam kastrata pasmine Sella Italiana. Svi su bili klinički zdravi, uvježbani i dobroga gojnoga stanja, a bili su podijeljeni u dvije skupine (A i B). Uzorci krvi bili su im uzeti u epruvete s natrijevim citratom (jedan dio natrijeva citrata na devet dijelova krvi). Nakupljanje pločica bilo je određivano dodavanjem adenozin-difosfata (ADP) kao aktivatora nakupljanja i, nakon inkubacije od 20 minuta, dodavanjem hidrokortizona kao inhibitora nakupljanja pločica. Krivulje nakupljanja pločica bile su određene uporabom agregometra te je tako vrednovan stupanj nakupljanja. ANOVA analizom pokazan je značajan učinak vremena ($P < 0,05$) na nakupljanje i na prosječnu brzinu nakupljanja ($P < 0,05$). Učinak hidrokortizona ustanovljen je samo na krivulji nakupljanja ($P < 0,01$).

Ključne riječi: športski konji, nakupljanje krvnih pločica, hidrokortizon, adenozin-difosfat, preponski konji
