The effect of three different doses of sodium pentosan polysulphate on haematological and haemostatic variables in adult horses

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Objective To evaluate the effects of three different doses of sodium pentosan polysulphate (PPS) on haematological and haemostatic variables in adult horses.

Design Eight adult standardbred horses were used. All horses received a single injection of 0, 3, 6, and 10 mg/kg of PPS at the beginning of each treatment week for 4 weeks so that by the end of the study all horses had received all four doses of PPS. Blood samples were collected at 0, 1, 2, 3, 4, 6, 8, 12, 24, 48, and 168 h after each weekly injection of PPS. Variables measured were packed cell volume, haemoglobin, red blood cell count, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, platelet count, white cell count, neutrophil count, lymphocyte count, eosinophil count, monocyte count, serum protein, fibrinogen, prothrombin time, and activated partial thromboplastin time (PTT). Data were analysed using an ANOVA. Significance was set at $P < 0.05$.

Results There was a dose-dependent increase in PTT. A significant increase in PTT occurrred in all treatment groups when compared to horses receiving 0 mg/kg in which there was no change over time. The PTT values all returned to baseline by 48 h after treatment. The mean neutrophil count was higher 3 h after treatment when compared to time 0. Horses receiving 3 mg/kg of PPS had a higher lymphocyte count 4 h after injection, and those receiving 6 and 10 mg/kg had higher counts at 3,4,6 and 8 h after injection when compared to time 0. At 8 h after injection horses receiving 6 and 10 mg PPS had higher lymphocyte counts than horses not receiving PPS.

Conclusions PPS causes a dose-dependent prolongation of PTT in horses. At the dose rates currently recommended for treatment of joint problems in horses this increase was small and remained elevated from baseline for up to 24 h. Based on these findings doses of PPS up to 3 mg/kg should not be administered to horses within 24 h of high stress activities or where physical injury may occur.

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Sodium pentosan polysulphate is a polysulphated polysaccharide, heparin analogue derived from beechwood hemicellulose, with a well documented anticoagulant effect in various species.[1-5] It has been used in Europe as an antithrombotic and antilipidaemic agent for over 30 years.[2] More recently PPS has become registered in Australia as a chondroprotective agent for use in the management of osteoarthritis in dogs and horses.[2-5]

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Non-steroidal anti-inflammatory agents have traditionally been the mainstay of osteoarthritis management.[4-6] These drugs reduce signs of pain and inflammation through the inhibition of the arachidonic acid cascade. In contrast, PPS modifies the underlying pathophysiological process. PPS has weak anti-inflammatory activity attributed to its ability to stabilise the peripheral vascular system thereby improving the microcirculation in inflamed tissues and by modifying the release of cytokines such as interleukin-6 and tumour necrosis factor.[7] Even at low concentrations PPS enhances synthesis of proteoglycans,[2-5,8] increases production of hyaluronic acid,[3] and inhibits a number of the enzymes responsible for cartilage matrix degeneration incuding stromeolysin.[5,8-10] Other proposed beneficial effects stem from weak anticoagulant but strong fibrinolytic effects which facilitate the clearing of vascular occlusions in the subchondral vasculature and synovium.[6] Studies using dogs affected with osteoarthritis of various aetiologies have consistently shown PPS to be efficacious in alleviating clinical signs of lameness.[5,11,12]

Although the effects of PPS have been well documented in a variety of species there are no published studies describing the use of this agent for equine degenerative joint disease.[3] Anecdotally it is reported that treatment reduces synovial effusion and lameness particuarly after racing.[2,3] The effects on the vascular system described in other species are proposed to also decrease the rate of subchondral bone necrosis or sclerosis in the horse.[3]

EIPH is a common disorder in performance horses, especially racing Thoroughbred and Standardbred horses. Studies have suggested that up to 100% of racing horses have some degree of EIPH during high intensity exercise.[13] The use of PPS is currently widespread among performance horses, including those populations at greatest risk of EIPH. However, the administration of a drug with anticoagulant properties to athletic horses prior to exercise could potentially increase the severity of EIPH or other haemorrhagic disorders, with potentially fatal consequences.

ANOVA	Analysis of variance
EDTA	Ethylenediaminetetraacetic acid
EIPH	Exercise induced pulmonary haemorrhage
Hb	Haemoglobin (g/L)
MCH	Mean corpuscular haemoglobin (pg)
MCHC	Mean corpuscular haemoglobin concentration (g/L)
MCV	Mean corpuscular volume (fL)
MSerror	Mean square error
PCV	Packed cell volume (L/L)
PPS	Sodium pentosan polysulphate
PT	Prothrombin time (seconds)
PTT	Activated partial thromboplastin time (seconds)
RBC	Red blood cell count (10^{12}/L)
WCC	White blood cell count (10^{9}/L)

The purpose of this study was to evaluate the effects of three different doses of PPS on haematological and haemostatic variables in adult horses.

Materials and methods

Six thoroughbred mares and two geldings aged between 8 and 12 years and weighing between 400 and 600 kg were used in the study. Horses were identified by a numbered brand. An anthelminthic and a tetanus toxoid were administered 14 d before beginning the experiment.

The study was designed as a duplicated Latin square. The eight horses were randomly assigned into four groups, each consisting of two horses. Each group of two horses received four single doses (0 mg/kg, 3 mg/kg, 6 mg/kg, 10 mg/kg) of PPS (Cartrophen®; Biopharm Australia, Bondi Junction, New South Wales) over a 4-week period. Each group received one of the four doses of PPS at the beginning of each week so that by the end of the study all horses had received all four doses of PPS.

Horses were housed in a paddock between treatments. The night before the beginning of each treatment week horses were brought into small yards and weighed. On the same morning of each treatment week horses were bought into individual standing stocks and a 14-gauge, over-the-needle jugular catheter was placed and connected to minimal volume extension tubing and a three-way stopcock. The catheter and tubing were secured to the skin with quick-set adherence glue and the lumen of the catheter kept patent during the experimental period by flushing with 5 mL sterile saline every hour.

A blood sample was collected and baseline clinical variables (heart rate, respiratory rate and rectal temperature) were recorded after the catheter had been placed and the horses appeared to be relaxed in the standing stocks (0 h). Following collection of baseline clinical variables and a blood sample, the assigned dose of PPS was administered. Where 0 mg/kg was assigned, the horses were given 20 mL of sterile isotonic saline. All injections were administered into the gluteal muscles using a 3.75 cm, 18-gauge needle and syringe. Additional clinical and haematological data were collected 1, 2, 3, 4, 6, 8, 12, 24, 48 and 168 h after injection. After the measurements at 8 h, the catheters were removed and the horses placed in small yards and offered food and water. At 12, 24 and 48 h the horses were quietly caught in the yard, clinical variables measured and recorded and a blood sample collected from the jugular vein using a syringe and needle. Horses were then returned to the paddock until the following week. Clinical variables and a venous blood sample was collected at 168 h (7 d). This measurement point coincided with the pretreatment sample collected during the experimental protocol in the subsequent week.

Blood was collected in a plain tube and tubes containing EDTA and sodium citrate. At each sampling time 5 mL of blood was drawn from the catheter and tubing and discarded before drawing the sample to ensure retrieval of a representative sample. Haematological variables measured using Coulter automated techniques (Technicon H-1™ System; Bayer Diagnostics, Australia) were PCV, Hb, RBC, MCV, MCH, MCHC, platelet count and WCC. The differential WCC was determined manually from a blood smear stained with Diff Quick® (Lab Aids, Sydney, New South Wales). Fibrinogen concentration was measured using a heat precipitation technique.[14] Serum protein was measured with an automated analysing unit using spectrophotometric techniques (Cobas Mira; Roche Pharmaceuticals, Switzerland). Haemostatic variables (PT and PTT) were determined using commercially available tests following manufacturer's instructions (Organon Teknika Corporation, Durham, North Carolina, USA).

Statistical Analysis

Clinical variables remained within normal reference ranges for horses so were not subjected to statistical analysis.[15,16] One horse died during the study. Necropsy determined the cause of death to be unrelated to the treatment protocol. Data from this animal was excluded from the analysis.

Data were analysed using an ANOVA for repeated measures. Two variables (PT and PTT) required an inverse square root transformation to normalise data. Each dependant variable (RBC, Hb, PCV, MCV, MCH, MCHC, WCC, neutrophils, lymphocytes, monocytes, eosinophils, serum protein, fibrinogen, platelet count, PT, PTT) was entered into an ANOVA. Independent variables were horse identity, time (hours after PPS treatment), treatment group and week. Week was treated as a blocking factor in all analyses. Horses were treated as a random factor and nested within treatment group for the purposes of the analysis. All variables with significant effects of within subject factors (time and the time by treatment group interaction) were then examined for sphericity using Mauchly's test. If Mauchly's test indicated violation of the sphericity assumption, a multivariate analysis of repeated measures using Wilks' Lambda test of significance was used to determine if the dependant variable was associated with either a time or time by treatment interaction effect. In this case subsequent pairwise comparisons consisted of simple *t*-tests with a Bonferroni correction. When Mauchly's test indicated sphericity was not violated, univariate ANOVA tests for repeated measures were performed. Follow-up tests were *t*-tests using the appropriate MS error term derived from the ANOVA. Bonferroni corrections were applied. Where both time and time by treatment interaction were found to be significant, follow-up tests were only performed for the interaction term. All follow-up tests were performed at the alpha = 0.05 level of significance. All tests were performed using SPSS version 9 for windows.

Results

The univariate ANOVA found a significant time effect or time by treatment interaction for seven variables, namely WCC, lymphocytes, fibrinogen, serum protein, MCV, MCHC, and neutrophils, and a time effect, time by treatment interaction and a treatment group effect for PTT. The assumption of sphericity was not violated when data were analysed using lymphocytes and fibrinogen as dependent variables. Data from the other six variables violated the sphericity assumption.

Lymphocytes

There was a significant time ($P < 0.001$) and time by treatment interaction ($P < 0.001$). Horses receiving 3 mg/kg of PPS had a higher lymphocyte count at 4 h postinjection than at time 0. Horses reciving 6 and 10 mg/kg PPS had higher lymphocyte counts at 3, 4, 6, and 8 h post-injection. At 8 h postinjection horses receiving 6 and 10 mg/kg PPS had higher lymphocyte counts than those horses not receiving PPS (Figure 1).

Fibrinogen

There was a significant time ($P < 0.007$) and time by treatment ($P < 0.022$) interaction. No significant differences were found when the Bonferroni adjusted pairwise comparison was used to evaluate time by treatment interaction means for fibrinogen.

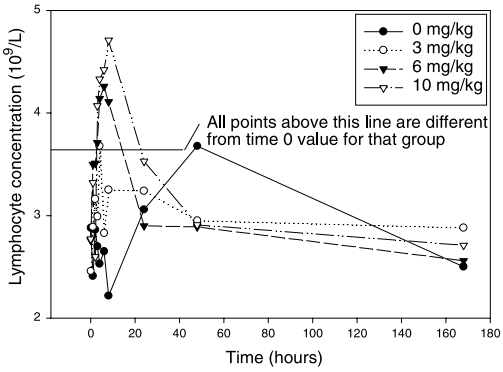

Figure 1. Changes in the mean lymphocyte count with time following treatment with sodium pentosan polysulphate. Points above the line are significantly different from time 0 for that treatment group (P < 0.05).

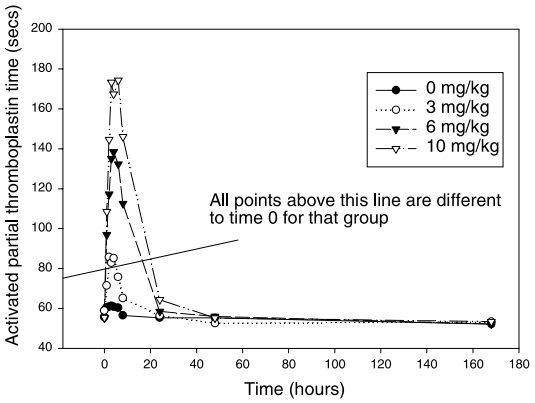

Figure 2. Changes in the geometric mean values of activated partial thromboplastin time with time following treatment with pentosan polysulphate. Points above the line are significantly different from time 0 for that treatment group (P < 0.05).

PTT

There was a significant time (P < 0.001), time by treatment interaction (P < 0.001) and treatment group effect (P < 0.001). Comparisons were performed on transformed data. The geometric means for the four treatment group means plotted over time are shown (Figure 2). There was a dose-dependent increase in PTT. A significant increase occurred in all treatment groups when compared to horses receiving 0 mg/kg of PPS where there was no change in PTT over time. The PTT values in treated horses returned to baseline times by 48 h after treatment.

Serum protein, MCV, MCHC and WCC

There was a significant effect of time only for these variables (P < 0.011; P < 0.001; P < 0.001; P < 0.001, respectively). No significant differences were found for any of the variables using Bonferroni adjusted *t*-tests.

Neutrophils

There was a significant effect of time (P < 0.001). The mean neutrophil count was significantly higher 3 h after administration of PPS than at time 0 (P = 0.023) however, the mean neutrophil count at all times was within the normal laboratory reference range. There were no other differences.

Discussion

The effects of PPS on haematological and haemostatic variables in species other than the horse are well reported,[4-6,11,17-28] however, the effects of PPS on these variables in the horse are largely unknown.[2,3,18] It has been suggested that PPS offers considerable potential for the treatment of joint problems in the horse,[2] therefore further investigation in potential side effects in this species is warranted. The dose of PPS commonly used for the treatment of joint problems in this species is approximately 2 to 3 mg/kg.[2] We elected to evaluate the effects of the recommended dose on haemotological and haemostatic variables in the horse and the effects of two higher doses where overdosing might occur.

The anticoagulant and fibrinolytic properties of PPS have been reported.[17-24] The mode of action of PPS is similar to that of heparin in that it inhibits Factor Xa and its precursors in the intrinsic coagulation pathway.[17,18] However, heparin acts primarily by catalysing the formation of the thrombin-antithrombin III and thrombin-heparin cofactor II complexes while PPS acts by catalysing the formation of the thrombin-heparin cofactor II complex independent of antithrombin III.[19] PPS also shows much weaker effects than heparin on prothrombin activation and catalysis of factor Xa inhibition by antithrombin III.[20] The net result is that in vivo heparin is approximately six times more potent as an anticoagulant than PPS.[2,20] PPS was shown to increase the PTT in the horse within 10 min of injection of 1.3 mg/kg. However, these increases were transient and no longer significantly different from baseline times 1 h after injection. Using higher doses we demonstrated a dose-dependent increase in PTT. At the recommended dose rate for joint problems in the horse (2 to 3 mg/kg[2,3]) PTTs increased within 1 h of injection and remained significantly elevated for 4 to 5 h. PTT did not return to baseline times until 24 h after administration of the drug. Higher doses of 6 and 10 mg/kg led to more prolonged PTTs which remained elevated for up to 48 h. There was no demonstrable effect of PPS on PT's confirming that there was no effect on the extrinsic clotting pathway.

Published effects of PPS on platelet counts in humans vary depending on methods used, however the in vitro and in vivo effects do not always correlate.[21-24] It has been suggested that the conflicting results reflect that PPS has a variety of effects on the many feedback mechanisms of haemostasis, which are operative in vivo but not in vitro.[25] However the available literature would indicate that after initial administration, some degree of spontaneous platelet aggregation leads to a transient reduction in the platelet count, however with time, platelet numbers recover and may even increase with further administration of the drug.[22-24] We were unable to find any effect of PPS on the platelet count in the horse, however our data was collected after a single injection with a 7-day washout period between doses, in contrast to studies in other species investigating multiple injections or continuous infusions.[22-24]

An elevation in lymphocyte numbers with higher doses of PPS has been reported to occur after administration of polysulphated polysaccharides.[26,27] A transient increase in lymphocyte numbers was reported after intramuscular administration of PPS to

human subjects with osteoarthritis.[28] This effect was thought to be mediated by the interaction of PPS with adhesion proteins such as the selectins which are present on cell surfaces.[29] These adhesion proteins are responsible for the binding of lymphocytes to post-capillary endothelial cells and control the passage of these cells through lymph nodes.[30] When PPS interacts with the binding proteins lymphocytes pass through the lymph nodes more rapidly leading to a concomitant elevation in their numbers in the peripheral circulation for up to 24 h following treatment. There was a significant increase in lymphocyte numbers in horses receiving 6 and 10 mg/kg of PPS within 4 h of treatment. These counts remained significantly elevated for 7 to 8 h. The effect of 3 mg/kg was less pronounced. Increases in lymphocyte counts are suggested to be beneficial, providing a source of antibodies and anti-inflammatory cytokines including interleukin-4, interleukin-10 and transforming growth factor ß.[31,32] Potent anti-inflammatory activity has been shown to occur in animal models of inflammation following subcutaneous administration of PPS at dose rates up to 50 mg/kg.[33] However, in this study total lymphocyte counts in treated horses were not substantially elevated outside of normal reference ranges and were not sufficient to significantly influence total WCC suggesting that any beneficial effects of increased lymphocyte counts following administration of recommended dose rates of PPS could be expected to be minimal.

The most clinically relevant effects of PPS on horses in this study were on the haemostatic system. PPS caused a dose-dependent prolongation of PTT but not PT demonstrating an effect on the intrinsic pathway of haemostasis. At the doses commonly used in horses there was a small but significant increase in PTT by 3 h post-injection. This increase was transient and PTT had returned to baseline values by 24 h. Higher doses had more profound and protracted effects however no horses showed clinical manifestations of prolonged bleeding following administration of up to 10 mg/kg. This would suggest that the drug at recommended doses is safe to administer to sedentary horses. However, based on the findings of this study, the horse's haemostatic mechanism is temporarily compromised, and it appears logical to recommend that doses of PPS up to 3 mg/kg should not be administered to horses within 24 h of high stress activities or where physical injury may be sustained. It is also suggested that PPS not be administered concurrently with other drugs that may have anticoagulant effects.

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