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Clinical evaluation of pentosan polysulfate as a chondroprotective substance in native Mongolian horses

Mijiddorj Tsogbadrakh[1)], Takafumi Sunaga[1, *)], Eugene Bwalya[1)], Suranji Wijekoon[1)], Ekkapol Akaraphutiporn[1)], Yanlin Wang[1)], Carol Mwale[1)], Adiya Naranbaatar[2)], Sangho Kim[1)], Kenji Hosoya[1)], Damdinsuren Alimaa[2)] and Masahiro Okumura[1)]

[1)]Laboratory of Veterinary Surgery, Department of Veterinary Clinical Sciences, Faculty of Veterinary Medicine, Kita18, Nishi9, Kita-ku, Sapporo, Hokkaido 060-00818, Japan

[2)]Department of Veterinary Surgery and Theriogenology, School of Veterinary Medicine, Mongolian University of Life Science, Zaisan, Khan-uul District, Ulaanbaatar 17024, Mongolia

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Abstract

Pentosan polysulfate (PPS) is widely used as therapeutic intervention for joint diseases in humans and animals, while objective confirmation has not been established yet. The purpose of this study was to provide the objective measure of the efficacy of PPS. Twenty-five healthy Mongolian horses were randomly assigned in three groups. Three different doses of PPS, 0/1.2/3.0 mg/kg, were injected intramuscularly one a week for consecutive 4 weeks. On 14 and 28 days after the initial administration, relative ratios of serum COMP/CPII were 97.9/87.6/61.8 and 94.2/104.3/88.1 in 0/1.2/3.0 mg/kg PPS, respectively. The results revealed that balance of cartilage metabolism could be significantly brought to an anabolism dominant state by PPS injections in dose dependent manor in field fed horses in Mongolia.

Key Words: pentosan polysulfate, chondroprotection, horse

Pentosan polysulfate (PPS) is a semi-synthetic polysulfated xylan derived from beechwood[8)]. PPS is weak heparinoid indicating slight anticoagulant activities[9)] and anti-inflammatory properties, which are widely used for symptomatic managements for selective diseases, including interstitial cystitis[16)], osteoarthritis[1)] and mucopolysaccharidosis[6)] in human beings and veterinary patients. The pathobiology pathways, to which PPS could interfere as therapeutic interventions in osteoarthritis, were speculated to include induction and maintenance of synovitis, microcirculation in subchondral bones and surrounding structures resulting to bone sclerosis, and degradation of articular cartilage[5)]. Despite popularities of use of PPS for equine patients have been increased[18)], clinical factual observations are surely very limited to prove the evidence for PPS to restrain any of the above-mentioned pathways. The objective of the present study was to provide reliable clinical confirmation that PPS could control those pathways, particularly degradative activity of articular cartilage metabolism in horses with ordinally field-feeding.

* Corresponding author: Takafumi Sunaga
Address: Kita18, Nishi9, Kita-ku, Sapporo, Hokkaido 060-0818, Japan
Fax number: 011-706-5228 (Japan) Email: sunaga@vetmed.hokudai.ac.jp
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Table 1. Blood biochemistry examination on ALT, LDH, BUN and Cr

	days after injection	PPS 3.0 mg/kg bw	PPS 1.2 mg/kg bw	PPS 0 mg/kg bw
ALT(U/l)	0	16.6±6.5	18.4±14.9	11.8±2.8
	14	9.8±2.2	10.3±2.5	8.2±2.2
	28	10.6±2.2	9.2±6.1	7.2±1.2
LDH(U/l)	0	481±105	634±165	465±119
	14	620±112	452±157	363±173
	28	535±73	542±144	351±103
BUN(mg/dl)	0	19.1±2.6	20.9±4.6	19.0±5.2
	14	20.9±3.3	19.8±4.8	17.1±4.1
	28	19.2±1.8	17.3±4.3	11.3±3.4
Cr(mg/dl)	0	0.52±0.24	0.65±0.10	0.54±0.12
	14	0.88±0.08	0.84±0.15	0.60±0.13
	28	0.84±0.05	0.93±0.16	0.59±0.14

Blood specimens were collected every 2 weeks, and serum biochemistry profiles, including total alanine aminotransferase (ALT), lactate dehydrogenase (LDH), blood urea nitrogen (BUN) and creatinine (Cr), were measured (Fuji DryChem NX500, Fujifilm, Tokyo, Japan) after sera being separated and prepared. Parameters of serum biochemistry examined were all in the normal reference ranges. Values were described as mean±standard deviation.

In the present study, a total of 25 native Mongolian horses (average body weight±standard error: 242±9 kg; 14 males and 11 females), which were two years of age and all after breaking, were randomly selected at a feeder in one plain in Khentii province, Mongolia, in September, 2017. All the horses had free access to grass and water daily in the same pasture condition. Before being assigned to this study, horses were examined to prove its general physical condition, serum biochemistry profiles including hepatic and renal functional measures and orthopedic fitness being normal and healthy. Four doses of PPS (OJI-200EI, Oji Holdings Co., Tokyo, Japan) at 3.0 mg/kg or 1.2 mg/kg were injected intramuscularly one a week in 20 horses, as of PPS dosage, injected each group of horses was consisted with 10 horses. In the rest five horses, phosphate-buffered saline solutions were injected in the same course of PPS administrations in other 20 horses. Horses were randomly assigned in each treatment group and entire procedures of the administration of PPS in an open label fashion. Use of PPS to these horses assigned in this study was on the basis of the owners' preference of its clinical use for prevention of osteoarthritis and maintained skeletal health[18]. Evaluation of horses in general physical and orthopedic conditions was done every week when PPS was injected. Blood specimens were collected every 2 weeks, and serum biochemistry profiles, including total alanine aminotransferase (ALT), lactate dehydrogenase (LDH), blood urea nitrogen (BUN) and creatinine (Cr), were measured (Fuji DryChem NX500, Fujifilm, Tokyo, Japan) after sera being separated and prepared. Serum cartilage metabolic markers, including cartilage oligomeric matrix protein (COMP; My BioSource., San Diego, CA, U.S.A.) and procollagen II C-propeptide (CPII; IBEX Pharmaceuticals Inc., Montreal, QC, Canada), were quantified by respective commercially available enzyme immune assay kits in accordance to manufacturer's instructions. All procedures were done by registered veterinary professionals in Mongolia under the approval of School of Veterinary Medicine, Mongolian University of Life Science as ethical standards for animals. Analysis of variance (ANOVA) was used to compare relative ratio of COMP/CPII among different PPS dose groups of horses on 14 or 28

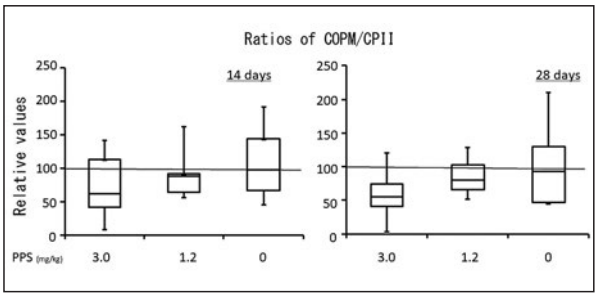


Fig. 1. The ratio of COMP/CPII at 14 and 28 days after the initial injection of PPS.

The ratios of COMP/CPII in horses with 3.0 mg/kg PPS showed some tendency to be lower than those with 0 mg/kg PPS on both 14 and 28 days after the initial injection (P =0.2517; P =0.1762, respectively).

Relative values of each cartilage metabolic marker represented %values in comparison with ones at the initial administration. Analysis of variance (ANOVA) was used to compare ratios of relative values of COMP/CPII among different dose groups of PPS on 14 or 28 days after the initial injections. Significant difference was defined as $P < 0.05$.

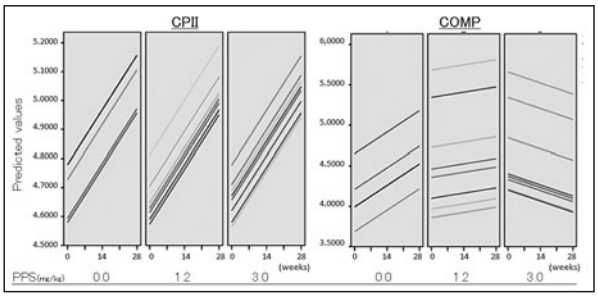


Fig. 2. Analysis of effects of sequential administration of PPS on cartilage metabolism by using the linear mixed effects models.

On the analysis, PPS was positively correlated to values of CPII, an anabolic marker of cartilage metabolism (Left). PPS dose and sequential injections showed negative correlation to vales of COMP with certain significance (Right) (P =0.027).

Each bar represents respective horse on the basis of concentration markers.

Significance of PPS administration on values of the markers in the course was evaluated by the linear mixed effects models fit by REML.

Significant difference was defined as $P < 0.05$.

days after the initial administration. Significance of PPS administration on values of the markers in the course was on the basis of dose-dependent manner, which was evaluated by the linear mixed effects models fit by residual maximum likelihood estimation. P value estimating statistical significance was set less than 0.05.

All horses showed no unbeneficial presentations in relation to series of PPS administration during the observation period. No symptomatic pathologies in joints, its adjacent structures and injection sites in the neck were found, and parameters of serum biochemistry examined were all in the normal reference ranges (Table 1). After 14 and 28 days from the initial administration, the relative ratios of serum relative values of two markers, COMP to CPII, representing balance of articular cartilage metabolism were 97.9/87.6/61.8 and 94.2/104.3/88.1 in three different dosages of PPS including 0, 1.2 and 3.0 mg/kg, respectively (Fig. 1). The ratios in 3.0 mg/kg PPS showed some tendency to be lower than those of 0 mg/ml PPS in both 14 and 28 days after the initial injection (P=0.2517; P=0.1762, respectively) (Fig. 1). The reduction of the ratio of COMP/CPII in different doses of PPS was simulated on the linear mixed effect models (Fig. 2). On the analysis, sequential injections of PPS showed the tendency to be positively correlated to values of CPII, an anabolic marker of cartilage metabolism (P=0.167). PPS dose and sequential injections showed negative correlation to vales of COMP with statistical significance (P=0.027).

This investigation suggested a clinical evidence to prove one of pathobiology pathways, degradative activities of cartilage, where PPS would effectively suppress in the course of joint diseases[5]. In previous studies, PPS is suggested to have suppressive effects on some of classic mitogen activated protein kinase pathways in cultured chondrocytes[1,15], to regulate phenotype of chondrocytes in appropriate condition[4], to down-regulate induction osteoclastic differentiation of hematopoietic stem cells simulating inflamed synovitis[2,17] and to show suppressive inflammatory changes of synovium in rodent models *in vivo*[19]. In horses, while much attention, especially for owners and trainers of racing horses, is being paid to PPS as both

preventive and therapeutic use to joint fitness, some studies suggest some positive opinions but none of them reached to provide clinical evidence using objective measures[3,7]. This would suggest that values of cartilage metabolic markers from large animals would have much variation, which could compromise to comprehend the obtained data properly. Statistical analysis using means or medians were not suitable to absorb the physiological variation of results.

The results on the balance of anabolic (CPII) and catabolic (COMP) markers suggested tendency, which PPS would bring it to more less catabolic in comparison with negative control (Fig. 1). The results analyzed by using the linear mixed effects models fit by REML revealed that degradation marker changes were significant to correlate with doses and sequential uses of PPS, proving that PPS would reduce degradative activities of cartilage in the course of joint diseases (Fig. 2). Domestic Mongolian horses are being fed by nomads in a traditional fashion customized for the specific climate and environment in Mongolian steppe, resulted in excess athletic and regular migration activities in summer and less movement and very limited nutritional intake in winter. On its life style, native horses are forced to have much physical stress on the skeletal system through active movement on the rough surface of the ground in warm season[11,13]. This would surely increase degradative activity of cartilage on joint surfaces causing future osteoarthritis, which would be one of the most prevalent diseases in athletic horses[12]. In human trial to use PPS for mild knee osteoarthritis, significant reduction of C2C, one of degradation markers of cartilage, was observed, which promised suppressive effects for PPS on degradation of cartilage[10]. Our observation of two cartilage metabolic markers would be the first evidence of anti-degradative effects of PPS in horses at a clinical setting.

No adverse effects were seen in serum biochemistry profiles and orthopedic examinations. Basically, this semi-synthetic substance is believed to be very safe and orally used for the only medical treatment for human interstitial cystitis for lifelong use to relief pain under the approval and registration by United States Food and drug Administration[19]. However, recently some ophthalmologists reported the augmentation that long time use of oral PPS for 15 years or more might cause vision threating pathologies in human beings aged more than 60 years old[14]. While short time use of PPS would not be expected to lead any serious adverse events, more knowledge and experience related to long time use of PPS in horses are necessary to establish its safety.

In conclusion, through our results from the analysis of cartilage metabolic markers related to PPS injections, reliable clinical confirmation that this substance could reduce degradative activity of articular cartilage metabolism in native Mongolian horses with ordinally field-feeding.

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References

1) Bwalya EC, Kim S, Fang J, Wijekoon HMS, Hosoya K, Okumura M. Pentosan polysulfate inhibits IL-1β-induced iNOS, c-Jun and HIF-1α upregulation in canine articular chondrocytes. PLoS ONE 12, e0177144, 2017.
2) Bwalya EC, Kim S, Fang J, Wijekoon HMS, Hosoya K, Okumura M. Effects of pentosan polysulfate and polysulfated glycosaminoglycan on chondrogenesis of canine bone marrow-derived mesenchymal stem cells in alginate and micromass culture. J Vet Med Sci 79, 1182-1190, 2017.

3) Cruz AM, Hurtig MB. Multiple pathways to osteoarthritis and articular fractures: is subchondral bone the culprit? Vet Clin North Am Equine Pract 24, 101–116, 2008.
4) Francis DJ, Hutadilok N, Kongtawelert P, Ghosh P. Pentosan polysulphate and glycosaminoglycan polysulphate stimulate the synthesis of hyaluronan in vivo. Rheumatol Int 13, 61–64, 1993.
5) Ghosh P. The pathobiology of osteoarthritis and the rationale for the use of pentosan polysulfate for its treatment. Semin Arthritis Rheum 28, 211-267, 1999.
6) Hennermann JB, Gökce S, Solyom A, Mengel E, Schuchman EH, Simonaro CM. Treatment with pentosan polysulphate in patients with MPS I: results from an open label, randomized, monocentric phase II study. J Inherit Metab Dis 39, 831-837, 2016.
7) Jambaldorj S. Horse treasure book (Морин эрдэнэ судар), 1st ed. Central library of Mongolian, Ulaanbaatar, Mongolia. pp. 11-14, 1996. (in Mogolian)
8) Koenig TJ, Dart AJ, McIlwraith CW, Horadagoda N, Bell RJ, Perkins N, Dart C, Krockenberger M, Jeffcott LB, Little CB. Treatment of experimentally induced osteoarthritis in horses using an intravenous combination of sodium pentosan polysulfate, N-acetyl glucosamine, and sodium hyaluronan. Vet Surg 43, 612-22, 2014.
9) Kramer CM, Tsang AS, Koenig T, Jeffcott LB, Dartb CM, and Dartc AJ. Survey of the therapeutic approach and efficacy of pentosan polysulfate for the prevention and treatment of equine osteoarthritis in veterinary practice in Australia. Australian Vet J 92, 482-487, 2014.
10) Kumagai K, Shirabe S, Miyata N, Murata M, Yamauchi A, Kataoka Y, Niwa M. Sodium pentosan polysulfate resulted in cartilage improvement in knee osteoarthritis--an open clinical trial. BMC Clin Pharmacol 28, 7, 2010.
11) Losonczy H, Nagy I, Menyhei G. Management of chronic venous insufficiency with the combination of coumarin (Syncoumar) and oral pentosan polysulfate (PPS, SP 54) (preliminary report)]. Orv Hetil 134, 291–295, 1993.
12) McIlwraith CW, Frisbie DD, Kawcak CE. Evaluation of intramuscularly administered sodium pentosan polysulfate for treatment of experimentally induced osteoarthritis in horses. Am J Vet Res 73, 628–633, 2012.
13) Orgil D, Lo Y. Racehorse lameness and treatment (Хурдан морь доголох эмгэг ба эмчилгээ), 1st ed. Mongolian University of Life Science, Ulaanbaatar, Mongolia. pp. 16–21, 2019. (in Mongolian)
14) Pearce WA, Chen R, Jain N. Pigmentary maculopathy associated with chronic exposure to pentosan polysulfate sodium. Ophthalmology 125, 1793-1802, 2018.
15) Sunaga T, Oh N, Hosoya K, Takagi S, Okumura M. Inhibitory effects of pentosan polysulfate sodium on MAP-kinase pathway and NF-κB nuclear translocation in canine chondrocytes in vitro. J Vet Med Sci 74, 707–711, 2012.
16) van Ophoven A, Vonde K, Koch W, Auerbach G, Maag KP. Efficacy of pentosan polysulfate for the treatment of interstitial cystitis/bladder pain syndrome: results of a systematic review of randomized controlled trials. Curr Med Res Opin 35, 1495-1503, 2019.
17) Wijekoon S, Bwalya EC, Fang J, Kim S, Hosoya K, Okumura M. Chronological differential effects of pro-inflammatory cytokines on RANKL-induced osteoclast differentiation of canine bone marrow-derived Macrophages. J Vet Med Sci 79, 2030-2035, 2017.
18) Wijekoon HMS, Bwalya EC, Fang J, Kim S, Hosoya K, Okumura M. Inhibitory effects of sodium pentosan polysulfate on formation and function of osteoclasts derived from canine bone marrow. BMC Vet Res 14, 152, 2018.

19) Wijekoon HMS, Kim S, Bwalya EC, Fang J, Aoshima K, Hosoya K, Okumura M. Anti-arthritic effect of pentosan polysulfate in rats with collagen-induced arthritis. Res Vet Sci 122, 179-185, 2019.