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Therapeutic Effect of Equine Musculoskeletal Disease using Autogenous Platelet Rich Plasma

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ABSTRACT

This study evaluated the therapeutic effect using autogenous platelet-rich plasma (PRP) for the musculoskeletal disease of Thoroughbred horses raised in South Korea. Twenty-five Thoroughbred horses (15 adult horses more than 2 years old, 10 fostering horses less than 2 years old) were diagnosed with musculoskeletal disorders (2 horses with desmitis of the suspensory ligament, 9 horses with osteoarthritis, 14 horses with fracture) by ultrasound and X-ray were used in this study. We collected venous blood of patients by using ACP Double Syringe after sterilization of jugular site, and then autogenous PRP was taken through centrifugation. We were observed the healing process of the lesion by using ultrasonography and X-ray images after injecting 4mL of autogenous PRP into each lesion for 2-times. Results of this study, 20 (80%) of 25 damaged horses returned to normal competition. The core lesion in the ligament disappeared in 90 days and healthy tissue regenerated. The fracture line of the cortex of bone disappeared in 60 days, and no side effects such as osteophytosis during healing progress. Remodeled articular surface, and cartilage defect, a fragment in joint disappeared. It could be confirmed that applying autogenous PRP treatment is an excellent regenerative therapy for equine locomotorium lesions.

Key words: Equine, Musculoskeletal Disease, PRP, South Korea.

INTRODUCTION

According to the ministry of agriculture, food, and rural affairs, about 27,000 [12,000 improved horses breed (Thoroughbred horse), 9,000 cross horses breed (Jeju Halla horse and Jeju horse), 5,000 Jeju horse breed (a natural monument No. 347), 1,000 horses (Warmblood, Quarter horse, etc.) and about 1,000 donkeys] domestic horses are raised in South Korea (Yun et al. 2018).

The musculoskeletal system injury of racing horses in the horse industry is a highly important disease. From the soft tissue damage to complete fracture, the impairment of the musculoskeletal system is the result of chronic damage accumulated during training or racing.

Recently, the results of survey musculoskeletal disease using X-ray and ultrasound equipment for racing horses raised into Seoul Race Course of Korea Racing Authority (KRA), the ratio on surgical and internal treatment was 52.8 and 16.8%, respectively. The surgical treatment highly occurred than the internal one by 3 times. The surgical diseases have occurred in the musculoskeletal system, foreign wound, and ophthalmic cases. Injury of the musculoskeletal system in the racing horse is serious and can cause a fatal wound that may occur in any environment, including before or after the race.

Recently, regenerative medicine in the horse can be largely divided into Interleukin-1 Receptor Antagonist Protein (IRAP), PRP and stem cells. Advanced equine medicine countries like the USA developed new clinical application of renewable treatment and attempted it clinically; among these, self-platelet concentrates in plasma (PRP) is a typical treatment method (Marx et al. 1998; Dahlgren 2018; Melotti et al. 2022). PRP is a platelet phosphate-containing more platelets than normal platelets (90-350K/μL) until 1997, which was first reported in the field of surgery in soft and light tissues. PRP is considered to gradually expand to various surgical sites after promoting treatment and regeneration (Marx et al. 1998; Bhanot and Alex 2002).

It has been reported that the concentrated platelets of PRP play an important role in wound healing and release cytokines involved in the healing process of coagulation

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and soft tissue after immediate solidification in the damaged area (Margolis et al. 2001). One of the main functions of PRP is coagulated by activating the collagen, thrombin, thromboxane A2. Growth factors (Platelet-Derived Growth Factor, Transforming Growth Factor, Epidermal Growth Factor) are essential for regulating the cellular events involved in wound healing and promoting healing of normal tissues and damaged wounds (Kim et al. 2001; Kevy and Jacobson 2004).

Equine sports medicine is an innovative therapy for musculoskeletal damage. It consists of 3 stages: accurate diagnosis, appropriate treatment and rehabilitation programs. To preserve excellent equine genetic factors and economic feasibility, advanced countries have tried regenerative therapy such as autogenous PRP treatment to equine locomotorium damage. Autogenous PRP treatment is popular in advanced countries, but it is unusual in South Korea. This study aims to evaluate the therapeutic effect of autogenous PRP against musculoskeletal disease in Thoroughbred horses with locomotorium damage.

MATERIALS AND METHODS

Ethical Approval

Thermal imaging and milk sampling were carried out on the care and use of experimental animals according to the guidelines of the Animal Ethics Committee (KNU2019-0091) of Kyungpook National University in Korea.

Study Population

Twenty-five Thoroughbred sick horses (15 adult horses more than 2 years old, 10 fostering horses less than 2 years old) and 10 healthy horses for control were used in this study. These horses were diagnosed with musculoskeletal disorders (2 horses with desmitis of the suspensory ligament, 9 horses with osteoarthritis, 14 horses with fracture) by ultrasound and X-ray.

Blood Sample and PRP Production

Blood samples were collected from each sick horse (n=25) and the healthy horses for control (n=10). Performed hematological examinations for the general CBC tests (IDEXX LaserCyte®, IDEXX Laboratories, USA). The CBC tests included evaluating the red blood cell (RBC), packed cell volume (PCV), hemoglobin (Hb), mean corpuscular volume (MCV), white blood cell (WBC), neutrophil (NEU), lymphocyte (LYM), monocyte (MON), eosinophil (EOS), and platelet. Blood for PRP production is collected from the jugular vein of musculoskeletal disorders using a PRP production kit (Arthrex ACP Double Syringe, USA). According to the manufacturer's manual, PRP was obtained by centrifugation (5min at 1,500g) and analyzed using automated blood test instrument IDEXX LaserCyte (IDEXX Laboratories, USA) with the separated PRP 1mL.

PRP Treatment

Two horses with suspensory ligament desmitis were anesthetized with Detomidine hydrochloride (Domosedan Ⓡ; 10mg/mL, Provet Veterinary, Turkey), then trimmed around the affected area, and povidone-iodine (Betadine Ⓡ ; Dongindang, Korea) and alcohol (Green, Korea) were used to disinfect the targeted surgical area. PRP 2mL was injected by using the 21 G needle probe according to the manufacturer's manual of ACP kit (Arthrex Inc, USA). The bone tissue experimented on side bone fractures and stress fractures from the targeted sick horses.

In the case of horses with a stress fracture, PRP 5mL was infiltration injected directly for the lesions of cortical bone's fracture surface. In the lesions on the joint fracture, if the side bone fracture has occurred, PRP 5mL was injected into the joint after removing the fracture by performing arthroscopic surgery. In the horses with degenerative arthritis, PRP 5mL was injected into the joint with inflammation. And for the 2 weeks' intervals, in total 2 times, PRP infusion was injected, and after that monthly basis unit, the degree of healing of the lesion was observed in all horses.

Data Analysis

PRP treatment effect analysis was conducted before and after PRP treatment, with photos by radiation and ultrasound imaging in criteria for the healing of the lesions then analyzed. Analyzed the healing degree effect of symptoms by period for the individual crippled disease improvement involving locomotorium sickness.

RESULTS

PRP Production Horse's Blood Profile

Blood profile of horse indicated on red blood cell (RBC) 7.52M/mm3, packed cell volume (PCV) 42.1%, hemoglobin (Hb) 16.4g/dL, mean corpuscular volume (MCV) 51.4fL, white blood cell (WBC) 6.43M/mm3, which showed the percentage of neutrophil (NEU) 64.2%, lymphocyte (LYM) 25.2%, monocyte (MON) 6.6%, eosinophil (EOS) 3.7%, respectively. Measuring levels of WBC per item was NEU 4.13K/μL, LYM 1.62K/μL, MON 0.43K/μL, EOS 0.24K/μL, basophil 0.02K/μL and platelets 178K/μL, respectively. All blood profiles were included in the normal range in the horse (Table 1).

PRP Component Analysis

The produced PRP's blood analysis values are shown in Table 2. RBC and Hb were 0.25K/μL and 4.1g/dL, respectively. WBC was not observed. However, values on platelet (PLT) was 448K/μL, which means 2.5 times higher than the normal blood levels of PLT.

The Therapeutic Effect of PRP

As a result of the PRP therapeutic effect, 20 (80%) of 25 damaged horses returned to normal competition. The core lesion in the ligament disappeared in 90 days and healthy tissue regenerated. The fracture line of the cortex of bone disappeared in 30 days, and no side effects such as osteophytosis during healing progress (Fig. 1). Remodeled articular surface and cartilage defect, a fragment in joint disappeared (Fig. 2). As shown in Table 3, all horses (desmitis, osteoarthritis, fracture) were observed in the degree of lameness 1~4 before treatment but were recovered to degree 0~3 at two months after PRP therapy. It is difficult to distinguish the treatment effect of PRP by three groups (desmitis of the suspensory ligament, osteoarthritis, fracture). Still, it was healed about 3 months faster than traditional treatment methods and returned to racing.

Table 1: Results of blood analysis for PRP production in 35 horses

Parameters*	Values	Reference range
RBC (M/mm^3)	7.52	6.80∼12.90
PCV (%)	42.1	32.0∼53.0
Hb (g/dL)	16.4	11.0∼19.0
MCV (FL)	51.4	37.0∼58.0
WBC (M/mm^3)	6.43	5.40∼12.30
NEU (K/μL)	4.13	2.26∼8.50
LYM (K/μL)	1.62	1.50∼7.70
MONO (K/μL)	0.43	0.10∼1.00
BASO (K/μL)	0.02	0.00∼0.03
PLT (K/μL)	178	90∼350

* RBC, Red blood cell; PCV, Packed cell volume; Hb, Hemoglobin; MCV, Mean corpuscular volume; WBC, White blood cell; NEU, Neutrophil; LYM, Lymphocyte; MONO, Monocyte; BASO, Basophil; PLT, Platelet.

Table 2: Results of PRP analysis in sick horses (*n*=25) and healthy horses (*n*=10)

Parameters*	Values	
	Sick horse	Healthy horse
RBC (M/mm^3)	0.25	N/A
Hb (g/dL)	4.1	N/A
PLT (K/μL)	448	185

* RBC, Red blood cell; Hb, Hemoglobin; PLT, Platelet; N/A, not available.

Table 3: Results of degree of lameness after PRP treatment

Type	Degree of lameness(before/after)	No. of horses
Desmitis of suspensory ligament	3 / 1	2
Osteoarthritis	3 / 2	3
	1 / 0	2
	2 / 1	4
Fracture	4 / 3	2
	4 / 2	3
	3 / 1	9
Total	25	25

DISCUSSION

The body's tissue in damage is regenerated and recovered by taking steps of cells' growth, redistribution, and reconstruction, and the following three elements play an important role. The first is scaffold forming the skeleton; the second is undifferentiated cells; the third is growth factors which mean signal protein secreted from platelets, plasma, white blood cells, etc. In particular, it is reported that among three elements, the growth factors play the most important role; initiate growth and proliferation of cells; and function as catalysts forming the fundamentals of tissue through the more separated growth factors and the more accelerating of recovery of damage. This growth factor is known to be contained in platelets playing a pivotal role in the process of wound healing (Slater et al. 1995; Kim et al. 2001; Weibrich et al. 2002; Marx 2004; Everts et al. 2006). Growth factors contained in platelets are secreted in the internal particles by the process that the platelets are activated if inflammation or tissue damage such as cuts happens in a tissue, consist of platelet-derived growth factor (PDGF), transforming growth factor, and vascular endothelial growth factor (Kevy and Jacobson 2004). Among these growth factors, in particular, the one that plays an important role in the regeneration and recovery of damaged tissue is PDGF. It was found that for the same disease, the growth rate of osseous tissue in a group of treating enriched platelet plasma increases 1.62 to 2.16 times than a group of not treating so while bone density increased 15 to 30% more, and PDGF was reported to be a factor to promote the bone formation (Marx et al. 1998). Canalis et al. (1989) reported that PDGF is associated with mitosis that promotes bone activity by promoting bone cell replication and DNA synthesis in bone cells. Pierce et al. (1991) reported that PDGF stimulates gene expression, proliferation, and chemical coherence in fibroblasts and monocytes macrophages are desperately needed for amputation healing.

Currently, PRP is an emerging therapeutic application in tissue regeneration because of its high concentration of growth factors with a mitogenic and anti-inflammatory potential (Anitua et al. 2004; Gentile et al. 2012). PRP is a concentration of platelets containing multiple growth factors that are very important for regeneration and anti-inflammatory processes. Treatment with PRP has been used for various conditions in equine veterinary medicine (Gentile et al. 2012; McCarrel et al. 2012; Holmes et al.

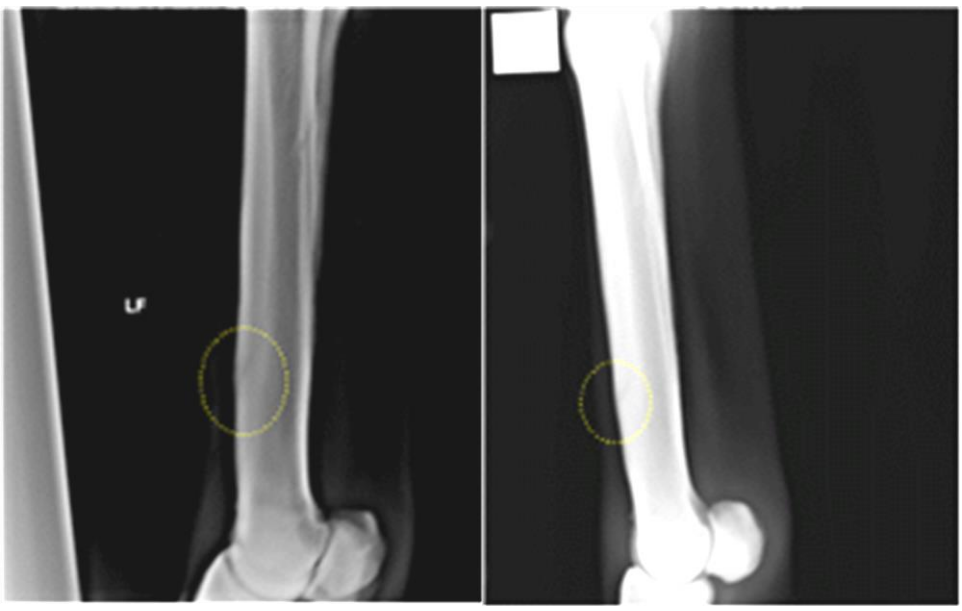

Fig. 1: Oblique saucer fracture line(circle) was in middle of metacarpal bone before (left) and after (right) PRP treatment. After 2 months PRP treatment for saucer fracture, obscure fracture line was disappeared, and bone tissue was replaced and regenerated around fracture lesion.

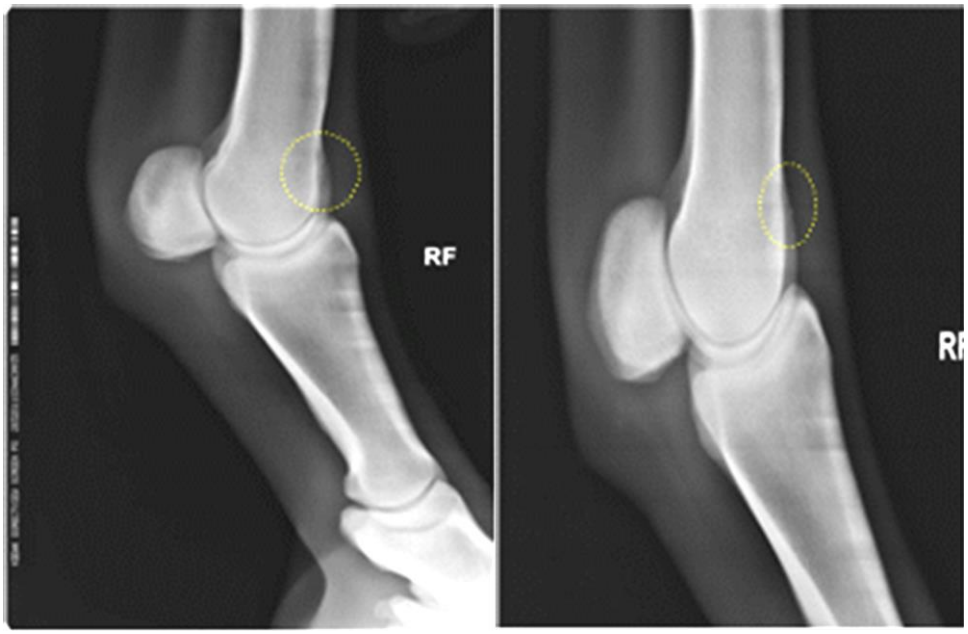

Fig. 2: Cartilage lesion of sagittal ridge (circle) of third metacarpal bone before (left) and after (right) PRP treatment. Proximal dorsal sagittal ridge of third metacarpal bone margin was irregular and radiolucent was increased and bony density decreased. After PRP treatment, irregular cartilage line was remodelled to normal shape and bone density was improved in radiology

2018; Ortved 2018; Pereira et al. 2019; Machado et al. 2019; Garbin and Olver 2020; Dawod et al. 2021; Maleas and Mageed 2021; Ribitsch et al. 2021; Perego et al. 2022) and hoof disorders (Carmona et al. 2013; Angelone et al. 2017; Carmona et al. 2018; Cengiz et al. 2018; Seidel et al. 2021). Renewable treatments that can replace conventional antibiotic treatments range from auto-PRP to auto-stem cells (Pierce et al. 1991). Bosch et al. (2009) reported that PRP was excellent in the resilience of the tendon according to the biochemical and histological analysis results after injecting its own PRP into the primary lesion of the surface digital flexor tendon.

According to Torricelli et al. (2011), the application of PRP isolated from bone marrow-induced mononuclear cells to 13 horses with musculoskeletal disorders showed excellent effects, and 84.6% returned to the race after treatment (Torricelli et al. 2011).

Recently, treatment using stem cells in parallel with autologous PRP has been attempted. The treatment using stem cells was first attempted by Smith et al. (2003) using autologous stem cells extracted from bone marrow, and it is currently commercially available as a product (Smith et al. 2003). Also, Ricco et al. (2013) reported that 89.5% of horses returned to the race after treatment using cultured mesenchymal cells extracted from fat and autologous PRP to treat 19 musculoskeletal disorders. Garbin and Olver (2020) reported that platelets play a central role in different stages of inflammation and treatment, unlike other growth factors and cytokines.

Using buffy coat plasma produced after simple centrifugation to apply the PRP system is problematic. Because buffy coat plasma contains a large amount of WBC and RBC, these factors can also delay the healing potential (Martin and Leibovich 2005). Therefore, this study confirmed that removing the base of the buffy coat by utilizing the Double Syringe can produce superior PRP with RBC and WBC removed and may improve therapeutic effectiveness.

According to the result of utilizing the ACP kit and manufacturing PRP, platelets increased per the same dose while RBC and WBC were almost removed compared with normal blood. Therefore, PRP contained the blood platelets about 1.5 to 4 times more than normal blood in the present study. But Weibrich et al. (2002) reported that the concentration ratio of platelets differs among individuals.

For two horses with desmitis of the suspensory ligament, the core lesion damaged in the ultrasound image disappeared one month after treatment. It could be identified that formed new ligament tissue on the area the hypoechogenic lesion disappeared. And it was observed that for all of 14 horses with fracture after injection of 2nd PRP, the fracture line receded and radiolucency around fracture decreased. In other words, it generated bony tissue around the fracture. Radiological examination of all nine horses with arthritis showed that low-density cartilage tissue disappeared after PRP treatment and remodeled irregular cartilage surface into an oval shape. In addition, synovial swelling and lameness symptoms have decreased.

This study has shown that horses treated with PRP have returned to the race one to two months earlier, especially for bone fractures, compared to the standardized recovery period by lesion so far, and returned to the race five months after treatment. In addition, the study confirmed that the actual concentration of platelets increased to a level suitable for the procedure by quantifying platelets within PRP using ACP kits, centrifuges and blood cell analysis.

In this study, which applied PRP therapy as a regenerative treatment for musculoskeletal disorders in South Korea, it was possible to specifically grasp each lesion's recovery period and treatment level.

Moreover, the appropriate concentration of platelets and the appropriate amount of increased factors is expected to be carried out in the future and may result in stabilizing the separation of growth factors by comparing various PRP manufacturing methods for more horses.

Conclusion

Using ACP kits, PRP contained 1.5 to 4 times more platelets than normal blood. After PRP treatment, 20 out of 25 horses recovered normally and returned to the race at an average speed of three months faster than the existing

treatment. PRP treatment is expected to be used for regenerative treatment of musculoskeletal diseases in the future.

Author's Contribution

All research protocols and animal experiments in this study designed, conducted experiments by CS Park, YJ Jo, KY Eo and TM Park, and contributed to data acquisition. GJ Cho contributed to the interpretation of the experimental results and the writing of the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

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