

Comparison of platelet counts in platelet-rich plasma (PRP) obtained by two methods from dogs¹⁾

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Summary

Platelet-rich plasma (PRP) is widely used in human medicine, but seldom used in veterinary practice. Even so, the efficacy of PRP in osteoarthritis, dental implants, bone defects and wound healing in dogs and horses is well documented. There are many commercially available PRP tubes and kits for horses and humans, but studies on the effectiveness of these products in dogs are limited. Researches have demonstrated that systems developed for humans and horses do not always produce the same results in dogs. In the present study, two prevailing systems, a sodium citrate-based system and a gel-based system, were compared in terms of their platelet output. The data obtained revealed that, although there was no statistically significant difference between the two systems, the sodium citrate-based kit is probably more efficient in practice due to its superiority in volume output. PRP is an important component of regenerative therapy. It is autologous and practical, but has not been sufficiently implemented in veterinary practice, but promising clinical satisfaction and must be considered in various conditions.

Keywords: autologous, dog, platelet-rich plasma (PRP), regenerative medicine

Platelet-rich plasma (PRP) may be defined as a highly concentrated plasma solution derived from the patient's own blood. The first report on PRP efficacy in tissue healing was published in 1998 by Marx R. E., a dentist, who described its use in the treatment of human mandibular defects (17). Today PRP is used widely to stimulate the healing of complex wounds, in oral surgery, (22, 29), in the treatment of tendinopathies, ligament injuries (7), bone fractures (19), and arthritis (5), in anti-inflammatory and acne treatments, as well as in esthetic dermatology, to treat facial wrinkles and hair baldness (11, 14, 20, 23). The method acts by activation of local cells, so applications are local.

There are three types of granules identified among platelets: alpha granules, dense granules and lysosomes (8, 16, 27). These growth factors modulate cell proliferation, differentiation, angiogenesis and chemotaxis. PRP has been used in regenerative medicine since the discovery of its effectiveness in tissue regeneration. It is widely used due to its commercial availability, its autologous character avoiding immune response, fast and easy application, low cost compared

to other regenerative medicine methods and high effectiveness (23).

PRP is relatively new in veterinary medicine (3), but is occasionally used in dogs and horses with osteoarthritis (1, 9), dental implants (25), bone defects (12) and wounds (13). PRP injections are highly efficacious especially in decubitis, dermal ulcers, burns, extensive post-operation wounds, wounds due to systemic disorders (diabetes mellitus, etc.), delayed healing, and recurrent wounds in behavioral disorders, such as tail chasing syndrome.

The key factor for a successful PRP application is the cell count in the end product. Studies show that an ideal PRP product must include 4-7 times as many platelets as circulating blood does (6, 18, 21). Apart from the platelet number, mononuclear cell count, as well as neutrophil and erythrocyte numbers also affect the anti-inflammatory effects and therapeutical success of PRP injections (6, 15, 21). Generally, pure platelet preparations, without erythrocyte and leucocyte cells, are preferred in order to avoid inflammatory effects (2, 26).

There are many commercially available PRP kits prepared for use in humans and horses by different PRP

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separation systems, and different systems result in end products with different platelet, leucocyte and growth factor concentrations (4, 21). There are limited studies on the usefulness of these human and horse kits in dogs, since research demonstrated that these kits may not create the same results in dogs (24). There are very few studies investigating the usefulness of human PRP kits in dogs (3, 10).

The aim of this study was to compare the end products obtained from dogs by two different (gel-based and sodium citrate-based) PRP kit systems developed for human medicine.

Material and methods

Ethical approval. This study was approved by the Ondokuz Mayıs University Animal Studies Local Ethical Committee (approval number B.30.2.ODM.0.20.09.00-050.04).

Animal material. The study material consisted of 15 dogs referred with recurrent wounds or delayed healing due to various causes, such as decubitis, skin ulcers, burns, post-operative wounds, diabetes complications and tail chasing syndrome or traumas. Indications for PRP were determined in 15 dogs following detailed history taking, physical and dermatological examinations, including wound inspection for contamination and complications, and detailed laboratory examinations for systemic disorders.

PRP kit systems. Two different PRP systems were used in the study. The first system, a T-Lab PRP kit (T-Biyoteknoloji Ltd), was a 10 ml tube containing sodium citrate for the prevention of coagulation and it was licensed for clinical applications. Tubes were gamma-sterile and did not contain ficoll or gel.

The second system, a DPG PRP kit (Derμοaroma Inc., Italy), contained alkyl acrylate crosspolymer-based separating gel (inert separator gel) and was also licensed for clinical use.

Both systems are available globally.

Preparation of the PRP product. Venous blood was collected into each tube in both systems simultaneously and processed according to the producers' manual. Cell counts for each tube were recorded by a cell counter, and the end products were applied with dermal needles locally at the site of wounds.

Statistical analyses. Standard error, standard deviation, and average values for both groups and differences between the two groups were calculated by Student's t-test (14).

Results and discussion

Platelet counts for both systems and a statistical comparison of the two systems are presented in Tables 1 and 2, respectively.

There are a number of commercially available PRP tubes and kit systems for humans and horses. These kits differ in their cell separation systems, and therefore also in the platelet, white blood cell, red blood cell and growth factor concentrations in the end products (4, 21). There are limited studies on the effectiveness

Tab. 1. The amount of platelets obtained by using the Tlab system and the DPG system in/ μ L

Specimen	Tlab (Sodium citrate-based system)/ μ L	DPG (Gel-based system)/ μ L
1	572000	388000
2	604000	876000
3	156000	214000
4	150000	194000
5	110000	152000
6	288000	280000
7	266000	254000
8	252000	264000
9	550000	462000
10	800000	300000
11	440000	360000
12	620000	410000
13	580000	400000
14	720000	510000
15	550000	420000

Tab. 2. Statistical comparison of the systems

n	TLAB (Sodium citrate-based system) ($\bar{x} \pm SD$)	DPG (Gel-based system) ($\bar{x} \pm SD$)
15	443866.7 \pm 222112.5	365600 \pm 174206.3

Explanation: the difference is statistically significant at $p \leq 0.05$ level.

of these systems, developed for humans and horses, in canine species, and some studies demonstrated that these systems may not produce the same results in dogs (24). The literature suggests that an ideal PRP product must contain 4-7 fold of the normal platelet count (18, 21). In the present study, the optimal number was achieved by both systems, so both of them are suitable for dogs in that respect.

Few studies have been published on the effectiveness of commercially available human kit systems in dogs (3, 10). Carr et al. (3), performed the most extensive study and put emphasis on obtaining blood from the same dog by different systems. In their study, too many systems were compared, so sampling could not be achieved from the same dog, and the authors underlined this fact as the major limitation of the study. In the present study, only two systems were compared, so reliable results were obtained.

One of the crucial points in PRP techniques is acquiring a sterile product, and manual methods without kits present a bacterial contamination risk during the preparation procedure (20, 28). Many products do not contain anticoagulant, so it needs to be added. This creates an important contamination risk together with a risk of preparing a wrong amount of the anticoagulant. In the present work, the citrate-based system included anticoagulant, which eliminated the con-

tamination risk and can be considered as an important advantage.

During the centrifugation process, cells are subjected to a gravity force of 1.500G and are separated according to their molecular weights. This procedure makes it possible to obtain PRP, but it also causes platelet aggregation, which is a risk for therapeutic success (5, 15, 16, 27). With this aggregation, a homogenous distribution cannot be achieved. In the citrate-based system used in the present study, the re-suspension tube provided in the kit ensures a homogenous distribution of platelets, which is another therapeutic advantage.

Different amounts of blood must be obtained in different systems, but the volume of blood obtained and the number of platelets acquired do not always correlate. For example, Carr et al. (3), who compared five different systems, reported that blood sample volumes were 50, 16, 50, 9, and 55 ml, whereas PRP product volumes derived were 10, 4-7, 4, 4-5, 6-8 ml, respectively. On the other hand, regarding the platelet numbers obtained, the first, second, and third systems yielded the most efficient products, respectively. Yet, according to the blood volume obtained, the system yielding the highest volume of the product was the third system, which was the worst option in terms of efficiency. In the present study, no statistical differences were recorded between the two systems regarding the number of platelets derived.

In conclusion, although there were no statistical differences between the two systems regarding the platelet content, the sodium citrate-based system appeared more beneficial considering the end product volume and lower contamination risk. Also, in PRP application, the key factors for a successful therapy in practice are the cell count procedure, product selection according to the species, and maximum attention to asepsis.

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