TÜBİTAK

Turkish Journal of Medical Sciences

http://journals.tubitak.gov.tr/medical/

Research Article

Turk J Med Sci
(2017) 47: 570-576

doi:10.3906/sag-1602-60

Meloxicam and diclofenac do not change VEGF and PDGF-AB serum levels of platelet-rich plasma

Burkay UTKU[1,*], Gürhan DÖNMEZ[2], Gülriz ERİŞGEN[3], Şenay AKIN[4], Haydar Ali DEMİREL[2,4], Feza KORKUSUZ[2], Mahmut Nedim DORAL[2,5]

[1]Department of Sports Medicine, Ankara Atatürk Educational and Research Hospital, Bilkent, Ankara, Turkey
[2]Department of Sports Medicine, Faculty of Medicine, Hacettepe University, Ankara, Turkey
[3]Department of Physiology, Faculty of Medicine, TOBB ETU University, Ankara, Turkey
[4]Faculty of Sports Sciences, Hacettepe University, Ankara, Turkey
[5]Department of Orthopedics and Traumatology, Faculty of Medicine, Hacettepe University, Ankara, Turkey

Received: 11.02.2016 • **Accepted/Published Online:** 22.09.2016 • **Final Version:** 18.04.2017

Background/aim: Platelet-rich plasma (PRP) application has gained widespread interest for musculoskeletal injuries. Nonsteroidal antiinflammatory drugs are frequently used in sports medicine before and/or after PRP application. Our study seeks to determine whether serum levels of platelet-derived growth factor-AB (PDGF-AB) and vascular endothelial growth factor (VEGF) levels of PRP would be affected by nonsteroidal antiinflammatory drugs.

Materials and methods: Two different final concentrations of diclofenac (0.5 μg mL^{-1} and 2.5 μg mL^{-1}), meloxicam (0.8 μg mL^{-1} and 2.0 μg mL^{-1}), and acetylsalicylic acid (final concentration 450 μm) were obtained in separate tubes with PRPs prepared from 20 healthy male volunteers. Medicine-free PRP was the control group. Growth factors were measured using ELISA.

Results: PDGF-AB and VEGF serum levels did not change with diclofenac, meloxicam, or acetylsalicylic acid addition. PDGF-AB and VEGF serum levels correlated with each other.

Conclusion: Diclofenac, meloxicam, and acetylsalicylic acid did not affect PDGF-AB and VEGF serum levels.

Key words: Platelet-rich plasma, PDGF-AB, VEGF, nonsteroidal antiinflammatory drugs

1. Introduction

Platelet-derived growth factor (PDGF), endothelial growth factor (EGF), insulin-like growth factor (IGF-I), transforming growth factor-β-1 (TGF-β-l), vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), and basic fibroblast growth factor (bFGF) (1,2) that contain platelet-rich plasma (PRP) are frequently used to treat musculoskeletal injuries (3,4).

Like other injections, PRP applications could be painful, and clinicians may prefer to apply local anesthetics before or after injections for relieving pain (5). There are conflicting results concerning the interaction of local anesthetics and PRP (5,6). In addition, nonsteroidal antiinflammatory drug (NSAID) usage is also not uncommon in athletes with acute or chronic pain (7,8). NSAIDs are known to inhibit platelet aggregation by inhibiting cyclooxygenase (COX) enzymes (Figure 1); however, to our best knowledge, there are no clear data about whether NSAIDs affect platelet activity. Since PDGF and VEGF stimulate cell proliferation and angiogenesis and contribute to tissue regeneration (9,10), the research questions of this study were as follows: whether reversible COX-1 and COX-2 inhibitors, as well as irreversible acetylsalicylic acid (ASA), affect serum PDGF and VEGF concentrations, and whether PDGF and VEGF levels of PRP correlate with each other according to different doses of the drugs used in the study.

2. Materials and methods

2.1. Design

A cross-sectional in vitro controlled experiment was designed. Independent variables were groups (n = 6) (Table), and dependent variables were PDGF-AB and VEGF levels of PRP measured with ELISA.

2.2. Participants

Twenty healthy males voluntarily participated. The average age of participants was 24.0 ± 3.2 years (range:

* Correspondence: burkay.utku@gmail.com

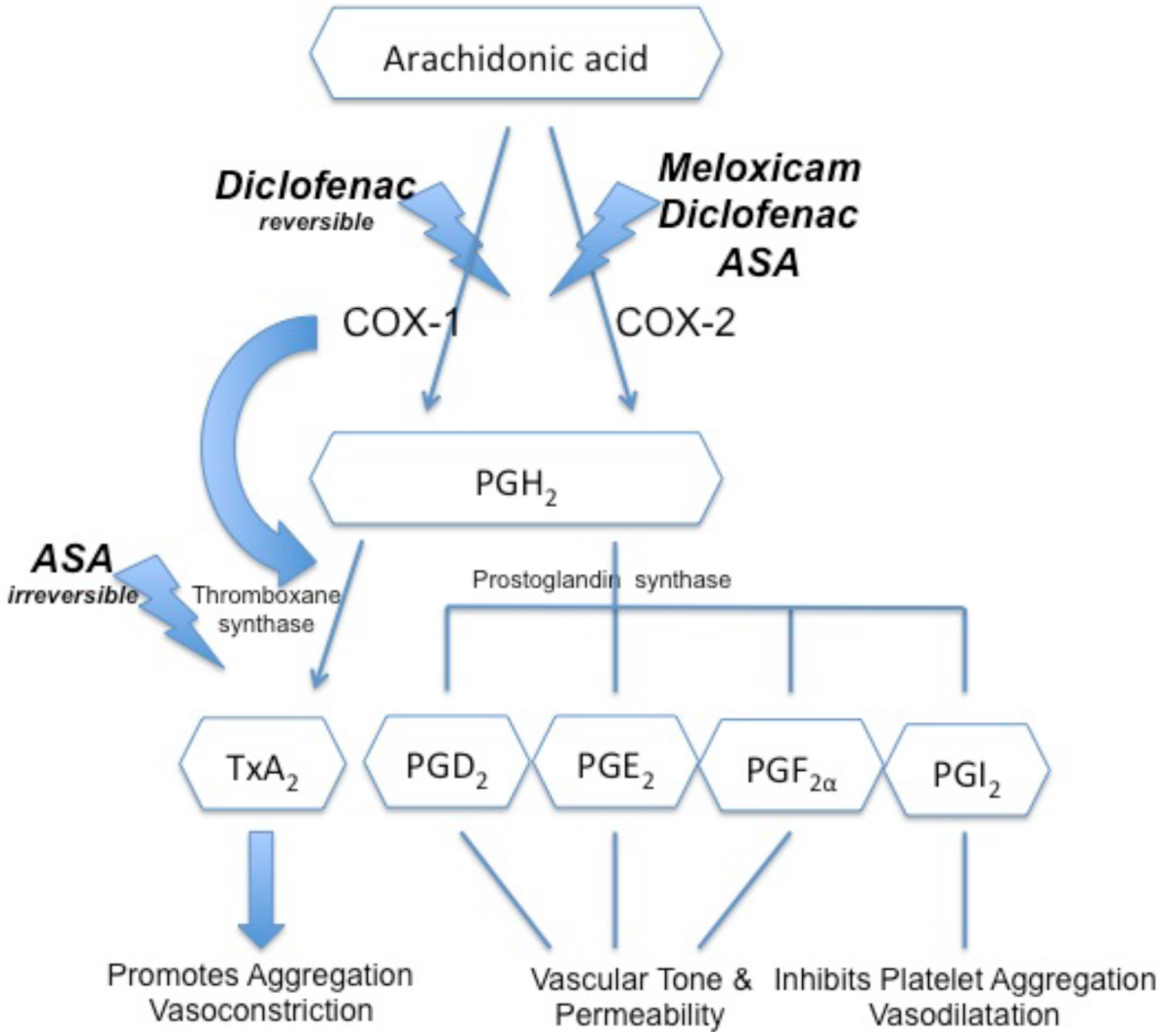

Figure 1. Mechanism of the effects of diclofenac, meloxicam, and acetylsalicylic acid on platelet aggregation (ASA: acetylsalicylic acid, COX-1: cyclooxygenase-1, COX-2: cyclooxygenase-2, PGH_2: prostaglandin H_2, TxA_2: thromboxane A_2, PGD_2: prostaglandin D_2, PGE_2: prostaglandin E_2, $PGF_{2\alpha}$: prostaglandin $F_{2\alpha}$, PGI_2: prostaglandin I_2).

Table. Preparation of samples for each group. The final volume was 550 µL for each suspension.

Groups	PRP (µL)	Medicine (µL)	Saline (µL)	Final volume (µL)
Control	500	---	50.0	550
Diclofenac 0.5 µg/mL	500	5.5	44.5	550
Diclofenac 2.5 µg/mL	500	27.5	22.5	550
Meloxicam 0.8 µg/mL	500	11.0	39.0	550
Meloxicam 2.0 µg/mL	500	27.5	22.5	550
Acetylsalicylic acid (450 µm)	500	50.0	---	550

18–35). Exclusion criteria included smoking, medication for thrombocyte disorders, and alcohol consumption. The institutional review board of the Hacettepe University Ethics Committee approved the study (08.01.2014/16969557-15).

2.3. Platelet-rich plasma preparation

We briefly collected 20 mL samples of venous blood using 21 gauge butterfly needles (Dahlhausen Scalp Vein Set CE, Cologne, Germany) into polypropylene tubes containing 3.2% buffered citrate (BD Vacutainer Systems, Plymouth, UK) from the antecubital vein of each participant. This was done by compressing the vein without applying a vacuum following at least 3 h of fasting. Blood samples were centrifuged at 580 × *g* for 8 min (Heraeus Labofuge 400R, Thermo Scientific, Waltham, MA, USA), and 4 mL of PRP was obtained according to the Anitua technique (11). Platelet and leukocyte counts of peripheral blood and PRP were analyzed with a Beckman Coulter LH 780 device (Galway, Ireland). Platelet counts of PRP samples were in the range of 244–692 × 10^6 mL^{-1} (mean: 456.1 ± 10.6 × 10^6 mL^{-1}). Blood or PRP specimens of 250 µL taken in polypropylene tubes were loaded into the device for cellular count. Pearson's correlation coefficient for platelet counts of PRP and whole blood samples was $r_p = 0.91$. These data indicate that PRP preparations were successful (11).

2.4. NSAIDs and supernatant collection

Reversible COX-1 and COX-2 inhibitor diclofenac, reversible COX-2 inhibitor meloxicam, and irreversible COX-1 inhibitor ASA were added to the test tubes containing PRP.

Each PRP sample was divided into 6 tubes. We added two different dosages of diclofenac (Diclomec, Abdi İbrahim Pharmaceuticals, İstanbul, Turkey) and meloxicam (Melox, Nobel İlaç, Düzce, Turkey) into tubes to obtain 0.5 µg mL^{-1} and 2.5 µg mL^{-1} final concentrations of diclofenac and meloxicam (12–14) in order to evaluate the dose response. The same volumes of ASA (a final concentration of 450 µm) and saline were added to other PRP samples to have ASA and medicine-free groups as the control (14–16). All test tubes were incubated for 20 min at room temperature. PRP clots were formed by adding 27.5 µL of 10% calcium chloride to 0.55 mL of PRP. Clots were allowed to retract for 20 to 30 min and were then centrifuged at 1000 × *g* for 10 min (Hettich Mikro 200R Centrifuges, Tuttlingen, Germany). Supernatants were stored at –20 °C for subsequent ELISA analysis.

2.5. Growth factor concentration measurements

PDGF-AB and VEGF concentrations were measured using a Quantikine Colorimetric Sandwich ELISA kit (R&D Systems Inc., Minneapolis, MN, USA). All samples were assayed in duplicate (SpectraMax Plus 384 Microplate Reader, Sunnyvale, CA, USA) (3).

2.6. Statistical analysis

Results were expressed as averages with standard deviations. A Shapiro–Wilk test was used to check the homogeneity of groups. A Friedman test was used to assess the effect of medicines on VEGF levels, and a one-way analysis of variance was used to compare the distribution of PDGF-AB results. Statistical differences between groups were accepted for $P < 0.05$ (StatGraphics Plus, Manugistics Group, Rockland, MD, USA).

3. Results

After exposure to PRPs with each dose of the medicines, the measured growth factor levels were similar among groups ($P > 0.05$) (Figures 2 and 3). PDGF-AB and VEGF serum levels correlated significantly ($P < 0.05$) with each other when all PDGF-AB and VEGF data from whole samples were pooled (Figure 4).

4. Discussion

There is no clear evidence whether NSAIDs affect platelet activity in PRP injections. The research question of this study was whether reversible and irreversible COX inhibitors would decrease PDGF and VEGF serum levels after PRP solutions were exposed to medicines.

Our results showed that adding diclofenac or meloxicam in vitro with the dosages used in this study did not change PDGF-AB and VEGF serum levels in the PRP solution. Although ASA is known to irreversibly inhibit platelet aggregation, adding ASA to the PRP solution also did not change the amount of growth factors.

Increased cellular proliferation and vascularity are the most important properties that PRP promotes in the healing of musculoskeletal tissues, positively contributing to the overall repair process. PDGF-AB plays a major role in the chemotactic activity of PRP, along with other PDGF ligands (17). It is also a potent mitogen for connective tissue cells and enhances fibroblast proliferation. VEGF is a powerful stimulator of angiogenesis, building new vasculature to bring additional extrinsic cells, nutrients, and growth factors to injured areas (18,19). Therefore, we specifically analyzed these two growth factors (PDGF-AB and VEGF).

Previous studies have assessed the antiaggregant effects of diclofenac, meloxicam, and ASA in vitro. Yokoyama et al. noted a 20% inhibition of platelet aggregation by adding diclofenac and meloxicam. In the same study, the inhibition rate of platelet aggregation was 60% to 80% with ASA (16). In another study, a different dosage of diclofenac (0.1–0.8 µg mL^{-1}) and ASA (100 µm) were tested (14). Diclofenac resulted in a dose-dependent increase in the inhibition of platelet aggregation, and the inhibition rate was much higher for ASA. We expected that NSAIDs and ASA would suppress platelet secretion and growth factor

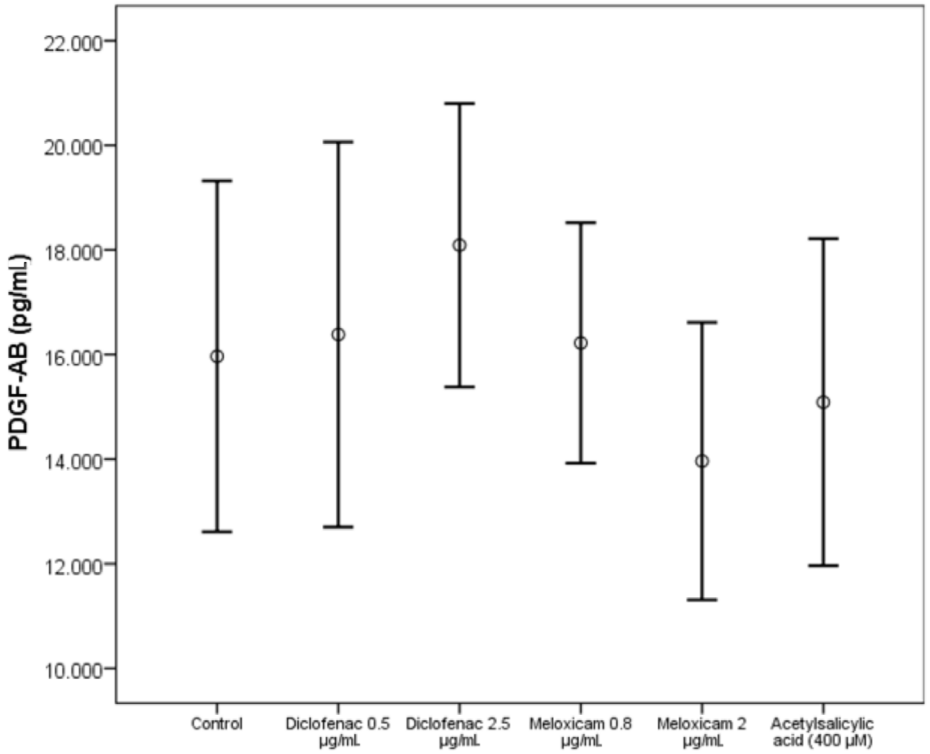

Figure 2. Effects of diclofenac, meloxicam, and acetylsalicylic acid on PDGF-AB levels for all groups (n = 20). The error bar represents the 95% confidence interval for PDGF-AB. There were no significant differences among groups (P > 0.05).

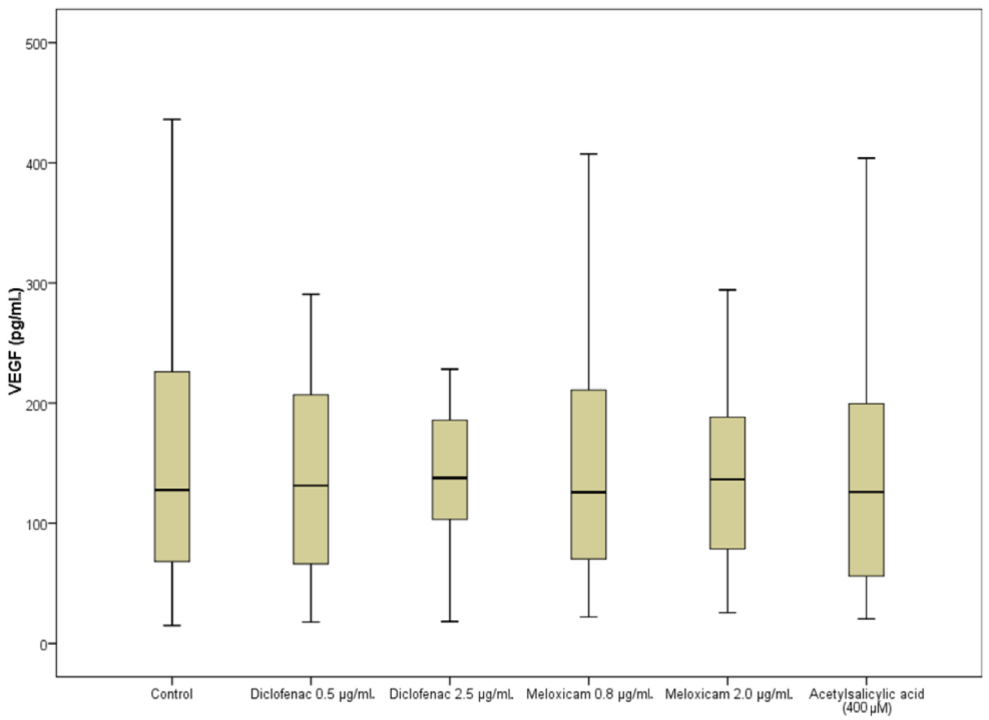

Figure 3. Effects of diclofenac, meloxicam, and acetylsalicylic acid on VEGF levels for all groups (n = 20). The box plot represents the median line across the box and 25th and 75th percentiles. There were no significant differences among groups (P > 0.05).

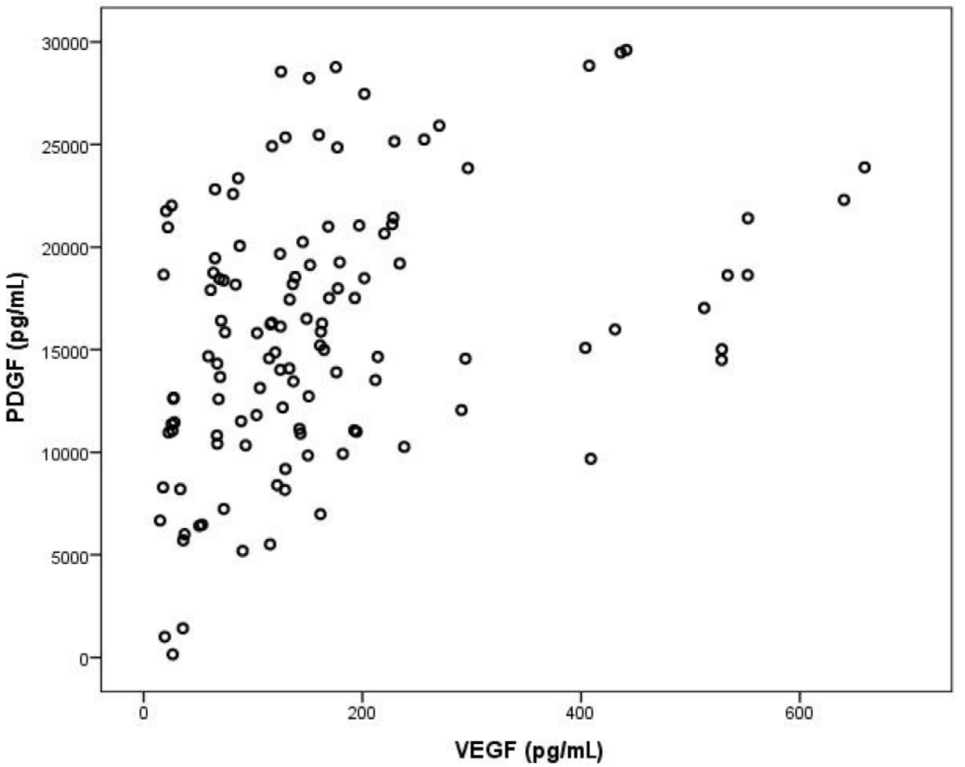

Figure 4. PDGF-AB and VEGF levels of whole PRP samples are correlated (P = 0.0001, r = 0.4).

levels; however, such a result was not obtained. Adding calcium to a PRP solution initiates the coagulation cascade and, as a result, thrombin is formed (3). Thrombin binds to protease-activated receptors (PAR-1) and initiates platelet activation through the phospholipase C pathway. When activated, platelets secrete their granule contents and release growth factors (20,21). Findings in this study could be explained by the highly stimulating effect of thrombin on platelet secretion, which may hinder the antiaggregant effects of NSAIDs and ASA.

Recently, two studies conducted by Anitua et al. evaluated the serum growth factor levels (including PDGF-AB and VEGF) and the proliferation capabilities of PRPs in cell cultures obtained from chronic ASA users (22,23). Similar to our findings, there were no significant differences for the amounts of growth factors and proliferation rates compared with the control groups.

Our study has shown that the growth factor levels of the samples were not correlated with the number of platelets. These findings support the result of Weibrich et al., who evaluated 115 PRP samples and could not find any relation between the platelet counts and the levels of growth factor (24). These data support the notion that platelet counts in a PRP solution may not be the only method to estimate platelet secretion quality.

PDGF-AB and VEGF are found in alpha granules and secreted with the other growth factors described above. Weibrich et al. (24) showed that there is a correlation between PDGF-AB and TGF-β1 ($r_S = 0.78$). Therefore, they concluded that PDGF-AB serum levels could be used in estimating TGF-β1 serum levels. To support that notion, we found a significant correlation (P = 0.0001, r = 0.4) between the serum levels of PDGF-AB and VEGF when all 6 groups were included in tests measuring the relationship of these growth factors.

The study has some limitations. First, these medications were tested only in vitro. Thus, we do not know if diclofenac, meloxicam, or ASA would have any effect on the levels of growth factors in vivo. Second, we only evaluated the levels of PDGF-AB and VEGF. Therefore, we do not know if other growth factors stored in platelets could be affected. In order to eliminate the amount of growth factors coming from leukocytes, we used a leukocyte-reduced PRP preparation technique defined by Anitua for minimizing the leukocyte effect (3,25). The number of leukocytes in our PRP solutions was higher (1620 ± 771.57 μL^{-1}) than what Anitua reported before (3). Nonetheless, the previous literature has shown that leukocytes have a poor growth factor content (26). Therefore, we do not think that the number of leukocytes in our study affected our results.

According to our study, diclofenac, meloxicam, and ASA did not affect PDGF-AB and VEGF levels of PRP in vitro. However, we cannot extrapolate from the information gained here for clinical practice. Studies that obtain PRPs from donors who are prescribed NSAIDs need to examine their effects on PRP secretion in vivo.

Acknowledgments
The authors would like to thank Professor Mutlu Hayran for contributing statistical analysis. The Hacettepe University Scientific Research Department funded this study (08.01.2014/16969557-15).

References

1. Anitua E, Andía I, Sanchez M, Azofra J, del Mar Zalduendo M, de la Fuente M, Nurden P, Nurden AT. Autologous preparations rich in growth factors promote proliferation and induce VEGF and HGF production by human tendon cells in culture. J Orthop Res 2005; 23: 281-286.
2. Marques LF, Stessuk T, Camargo IC, Sabeh Junior N, dos Santos L, Riberio-Paes JT. Platelet-rich plasma (PRP): methodological aspects and clinical applications. Platelets 2015; 26: 101-113.
3. Boswell SG, Cole BJ, Sundman EA, Karas V, Fortier LA. Platelet-rich plasma: a milieu of bioactive factors. Arthroscopy 2012; 28: 429-439.
4. Engebretsen L, Steffen K, Alsousou J, Anitua E, Bachl N, Devilee R, Everts P, Hamilton B, Huard J, Jenoure P et al. IOC consensus paper on the use of platelet-rich plasma in sports medicine. Brit J Sport Med 2010; 44: 1072-1081.
5. Bausset O, Magalon J, Giraudo L, Louis ML, Serratrice N, Frere C, Magalon G, Dignat-George F, Sabatier F. Impact of local anaesthetics and needle calibers used for painless PRP injections on platelet functionality. Muscles Ligaments Tendons J 2014; 4: 18-23.
6. Carofino B, Chowaniec DM, McCarthy MB, Bradley JP, Delaronde S, Beitzel K, Cote MP, Arciero RA, Mazzocca AD. Corticosteroids and local anesthetics decrease positive effects of platelet-rich plasma: an in vitro study on human tendon cells. Arthroscopy 2012; 28: 711-719.
7. Holmes N, Cronholm PF, Duffy AJ, Webner D. Nonsteroidal anti-inflammatory drug use in collegiate football players. Clin J Sport Med 2013; 23: 283-286.
8. Warner DC, Schnepf G, Barrett MS, Dian D, Swigonski NL. Prevalence, attitudes, and behaviors related to the use of non-steroidal anti-inflammatory drugs (NSAIDs) in student athletes. J Adolesc Health 2002; 303: 150-153.
9. Colville-Nash PR, Willoughby DA. Growth factors in angiogenesis: current interest and therapeutic potential. Mol Med Today 1997; 3: 14-23.
10. Foster TE, Puskas BL, Mandelbaum BR, Gerhardt MB, Rodeo SA. Platelet-rich plasma: from basic science to clinical applications. Am J Sports Med 2009; 37: 2259-2272.
11. Anitua E, Zalduendo MM, Alkhraisat MH, Orive G. Release kinetics of platelet-derived and plasma-derived growth factors from autologous plasma rich in growth factors. Ann Anat 2013; 195: 461-466.
12. Blaicher AM, Landsteiner HT, Zwerina J, Leitgeb U, Volf I, Hoerauf K. Effect of non-selective, non-steroidal anti-inflammatory drugs and cyclo-oxygenase-2 selective inhibitors on the PFA-100 closure time. Anaesthesia 2004; 59: 1100-1103.
13. Bauer KA, Gerson W, Wright C, Wang J, McNicol E, Lanier RK, Kramer W, Carr DB. Platelet function following administration of a novel formulation of intravenous diclofenac sodium versus active comparators: a randomized, single dose, crossover study in healthy male volunteers. J Clin Anesth 2010; 22: 510-518.
14. Munsterhjelm E, Niemi TT, Ylikorkala O, Silvanto M, Rosenberg PH. Characterization of inhibition of platelet function by paracetamol and its interaction with diclofenac in vitro. Acta Anaesthesiol Scand 2005; 49: 840-846.
15. Mazzeo D, Panina-Bordignon P, Recalde H, Sinigaglia F, D'Ambrosio D. Decreased IL-12 production and Th1 cell development by acetylsalicylic acid-mediated inhibition of NF-kappa B. Eur J Immunol 1998; 28: 3205-3213.
16. Yokoyama H, Ito N, Soeda S, Ozaki M, Suzuki Y, Watanabe M, Kashiwakura E, Kawada T, Ikeda N, Tokuoka K et al. Influence of non-steroidal anti-inflammatory drugs on antiplatelet effect of aspirin. J Clin Pharm Ther 2013; 38: 12-15.
17. Colciago A, Celotti F, Casati L, Giancola R, Castano SM, Antonini G, Sacchi MC, Negri-Cesi P. In vitro effects of PDGF isoforms (AA, BB, AB and CC) on migration and proliferation of SaOS-2 osteoblasts and on migration of human osteoblasts. Int J Biomed Sci 2009; 5: 380-389.
18. Cochran DL, Wozney JM. Biological mediators for periodontal regeneration. Periodontol 2000; 19: 40-58.
19. Gruber R, Karreth F, Kandler B, Fuerst G, Rot A, Fischer MB, Watzek G. Platelet-released supernatants increase migration and proliferation, and decrease osteogenic differentiation of bone marrow-derived mesenchymal progenitor cells under in vitro conditions. Platelets 2004; 15: 29-35.
20. Strukova SM. Thrombin as a regulator of inflammation and reparative processes in tissues. Biochemistry-Moscow+ 2001; 66: 8-18.
21. Bahou WF. Thrombin receptors. In: Michelson A, editor. Platelets. 2nd ed. Amsterdam, the Netherlands: Academic Press/Elsevier; 2007. pp. 45-73.
22. Anitua E, Troya M, Zalduendo MM, Orive G. The effect of different drugs on the preparation and biological outcomes of plasma rich in growth factors. Ann Anat 2014; 196: 423-429.

23. Anitua E, Troya M, Zalduendo MM, Orive G. Effects of anti-aggregant, anti-inflammatory and anti-coagulant drug consumption on the preparation and therapeutic potential of plasma rich in growth factors (PRGF). Growth Factors 2015; 33: 57-64.

24. Weibrich G, Kleis WK, Hafner G, Hitzler WE. Growth factor levels in platelet-rich plasma and correlations with donor age, sex and platelet count. J Cranio Maxill Surg 2002; 30: 97-102.

25. Ehrenfest DMD, Rasmusson L, Albrektsson T. Classification of platelet concentrates: from pure platelet-rich plasma (P-PRP) to leukocyte- and platelet-rich fibrin (L-PRF). Trends Biotechnol 2009; 27: 158-67.

26. Maloney JP, Ambruso DR, Voelkel NF, Silliman CC. Platelet vascular endothelial growth factor is a potential mediator of transfusion-related acute lung injury. J Pulm Respir Med 2014; 4: 1000212.