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

Burkay UTKU1,*, Gürhan DÖNMEZ2, Gülriz ERİŞGEN3, Şenay AKIN4
Haydar Ali DEMİREL2,4, Feza KORKUSUZ2, Mahmut Nedim DORAL2,5

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

* Correspondence: burkay.utku@gmail.com

1. Introduction
Platelet-derived growth factor (PDGF), endothelial growth factor (EGF), insulin-like growth factor (IGF-I), transforming growth factor-β-1 (TGF-β-1), 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 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

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-1), 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:
**Table.** Preparation of samples for each group. The final volume was 550 µL for each suspension.

<table>
<thead>
<tr>
<th>Groups</th>
<th>PRP (µL)</th>
<th>Medicine (µL)</th>
<th>Saline (µL)</th>
<th>Final volume (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>500</td>
<td>---</td>
<td>50.0</td>
<td>550</td>
</tr>
<tr>
<td>Diclofenac 0.5 µg/mL</td>
<td>500</td>
<td>5.5</td>
<td>44.5</td>
<td>550</td>
</tr>
<tr>
<td>Diclofenac 2.5 µg/mL</td>
<td>500</td>
<td>27.5</td>
<td>22.5</td>
<td>550</td>
</tr>
<tr>
<td>Meloxicam 0.8 µg/mL</td>
<td>500</td>
<td>11.0</td>
<td>39.0</td>
<td>550</td>
</tr>
<tr>
<td>Meloxicam 2.0 µg/mL</td>
<td>500</td>
<td>27.5</td>
<td>22.5</td>
<td>550</td>
</tr>
<tr>
<td>Acetylsalicylic acid (450 µm)</td>
<td>500</td>
<td>50.0</td>
<td>---</td>
<td>550</td>
</tr>
</tbody>
</table>
2.3. Platelet-rich plasma preparation

We briefly collected 20 mL samples of venous blood using 21 gauge butterfly needles (Dahlhausen Scap 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 vacuum following at least 3 h of fasting. Blood samples were allowed to retract for 20 to 30 min and were then centrifuged at 1000 × g for 10 min (Hettich Mikro 200R Centrifuges, Tuttingen, Germany). Supernatants were stored at –20 °C for subsequent ELISA analysis.

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, Tuttingen, 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).
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).
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
10. Foster TE, Puskas BL, Mandelbaum BR, Gerhardt MB, Foster TE, Puskas BL, Mandelbaum BR, Gerhardt MB, Foster TE, Puskas BL, Mandelbaum BR, Gerhardt MB, Foster TE, Puskas BL, Mandelbaum BR, Gerhardt MB, Foster TE, Puskas BL, Mandelbaum BR, Gerhardt MB, Foster TE, Puskas BL, Mandelbaum BR, Gerhardt MB.

