The Influence of Naproxen on Biological Factors in Leukocyte-Rich Platelet-Rich Plasma: A Prospective Comparative Study

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Purpose: To quantify and compare normative catabolic and anabolic factor concentrations in leukocyte-rich platelet-rich plasma (LR-PRP) at various time points, including baseline, 1 week after initiating naproxen use, and after a 1-week washout period. **Methods:** Asymptomatic healthy donors aged between 18 and 70 years were recruited (average age, 36.6 years; range, 25-64 years). Subjects were excluded from the study if they were actively taking any prescribed medications or nonsteroidal anti-inflammatory drugs (NSAIDs) or if they had any of the following at present or previously: blood or immunosuppression disorders, cancer, osteonecrosis, rheumatoid arthritis, avascular necrosis, NSAID intolerance, gastrointestinal or peptic ulcer disease, or kidney dysfunction. The anabolic factors vascular endothelial growth factor, fibroblast growth factor 2, platelet-derived growth factor AB (PDGF-AB), and platelet-derived growth factor AA (PDGF-AA) and the catabolic factors interleukin (IL) 1β, IL-6, IL-8, and tumor necrosis factor α in LR-PRP were measured. Peripheral blood was drawn at 3 time points: baseline, after 1 week of naproxen use, and after a 1-week washout period. **Results:** The angiogenic factors PDGF-AA (44% decrease in median) and PDGF-AB (47% decrease) significantly declined from baseline ($P < .05$) after 1 week of naproxen use. There was a significant recovery ($P < .05$) of PDGF-AA (94% increase) and PDGF-AB (153% increase) levels after the 1-week washout period, with a return to baseline levels. The catabolic factor IL-6 also had a significant decline from baseline (77% decrease in median, $P < .05$) after 1 week of naproxen use. After a 1-week washout period, the IL-6 level was similar to the baseline level (130% increase, $P < .05$). **Conclusions:** Naproxen use diminished several biological factors in LR-PRP; however, a 1-week washout period was sufficient for the recovery of PDGF-AA, PDGF-AB, and IL-6 to return to baseline levels. Tumor necrosis factor α, IL-1β, IL-8, vascular endothelial growth factor, and fibroblast growth factor 2 did not show differences between the 3 time points of data collection. Discontinuing NSAIDs for a minimum of 1 week before LR-PRP treatment may improve certain biological factor levels. **Level of Evidence:** Level II, prospective comparative study.

Musculoskeletal (MSK) disorders are one of the leading causes of physical disability worldwide, and the treatment and prevention of MSK disorders can alleviate substantial indirect and direct health care costs.[1] Leukocyte-rich platelet-rich plasma (LR-PRP) has been used to treat the injured MSK system by initiating the healing and regeneration processes.[1-4] LR-PRP is composed of elevated numbers of leukocytes and platelets that infiltrate the site of injury where inflammatory factors (i.e., tumor necrosis factor α [TNF-α], interleukin [IL] 1β, IL-6, and IL-8) and angiogenic factors (i.e., vascular endothelial growth factor [VEGF], platelet-derived growth factor [PDGF], and insulin growth factor [IGF]) are released into the extracellular space.[5-8] Growth factors and other cytokines in platelet concentrates have shown a temporal effect on various MSK conditions but are highly variable in concentration depending on the preparation methodology and donor demographic characteristics. Age and sex contribute to the biological variances of

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The authors report the following potential conflict of interest or sources of funding: J.H. receives royalties from Cook Myosite. R.F.L. receives support from Arthrex, Ossur, and Smith & Nephew. Full ICMJE author disclosure forms are available for this article online, as supplementary material.

Received February 21, 2018; accepted July 12, 2018.

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0749-8063/18241/$36.00

https://doi.org/10.1016/j.arthro.2018.07.030

cytokines and chemokines in platelet-rich plasma (PRP) and may have a significant role in tissue healing responses and outcomes.[9,10] Evanson et al.[9] found that female persons had elevated concentrations of endothelial growth factor, hepatocyte growth factor, IGF, and PDGF-BB compared with male persons and that levels of endothelial growth factor, IGF-1, PDGF-AB, PDGF-BB, and transforming growth factor β1 were significantly higher in young donors. Studies reporting on significant associations between growth factors and age or sex might also indicate that biological variability exists not only among individual donors but also as a result of variation in specific preparation processes.

Many patients with MSK pain often choose pharmaceutical agents, such as nonsteroidal anti-inflammatory drugs (NSAIDs), as the first line of treatment to alleviate pain and reduce inflammation. However, NSAIDs can deter platelet function and potentially obviate growth factor levels and cytokine signaling.[11,12] The mechanism of action of NSAIDs is to inhibit platelet activation by irreversibly binding to cyclooxygenase (COX) enzymes and to influence the arachidonic acid pathway.[13,14] Enzyme inhibition can cause platelet dysfunction through the platelets' 7- to 10-day life span.[15-17] Nonselective NSAIDs, such as naproxen, alter platelet function by prolonging bleeding time, slowing platelet aggregation and activation, and reducing thromboxane levels.[13,14,18] NSAIDs are known to impair function of platelets and biological constituents in whole blood by selectively inhibiting cytokine production,[19] such as PDGF[20]; fibroblast growth factor 2 (FGF-2)[21]; VEGF[22,23]; IL-1β, IL-6, and IL-8[24-27]; and enhancing TNF-α.[28] However, limited data exist regarding the molecular influence of NSAIDs on PRP.[29-31] In addition, there is little evidence to guide physicians as to when it is beneficial to harvest and administer PRP therapy in patients who use NSAIDs.[32]

A 1-week washout period was chosen because this is typically the amount of time we ask our surgical patients to refrain from NSAID use before surgical intervention. Suggested washout periods are not directly related to the NSAIDs' half-life (3-4 days) but are recommended to allow targeted prostaglandins to replenish at the tissue level, therefore preventing long-term inhibition of COX-1 isoenzymes. The simplest model to observe the effect of naproxen on angiogenic and proinflammatory factors in LR-PRP was to evaluate normative values of biological factors in healthy donors.

The purpose of this study was to quantify and compare normative catabolic and anabolic factor concentrations in LR-PRP at various time points, including baseline, 1 week after initiating naproxen use, and after a 1-week washout period. Our primary hypothesis was that naproxen use would alter both the catabolic and anabolic biological factors in LR-PRP. Our secondary hypothesis was that discontinuing naproxen use for 1 week would be sufficient to return LR-PRP's biological constituents to baseline.

Methods

Donor Enrollment and Study Design

After institutional review board approval (Vail Health Institutional Review Board, protocol 2017-07), asymptomatic healthy donors aged between 18 and 70 years were recruited through flyers posted in our clinic and hospital; interested applicants were screened and enrolled in the study. Previously established STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines for the design and implementation of cohort or group studies were followed.[18] Subjects were excluded from the study if they had any of the following at present or previously: blood or immunosuppression disorders, cancer, osteonecrosis, rheumatoid arthritis, avascular necrosis, NSAID intolerance, gastrointestinal or peptic ulcer disease, or kidney dysfunction. Subjects were also excluded if they were actively taking any prescribed medications or NSAIDs. Donor demographic data including sex, age, height, weight, and body mass index (BMI) were collected from each study participant.

The participants underwent a standard blood draw procedure to extract 30 mL of peripheral blood once a week for 3 weeks (total blood volume collected, 90 mL). The first blood draw was performed to obtain a baseline complete blood count (CBC) and cytokine and chemokine measurements from the LR-PRP. The data for CBC and quantitative measurements of biological factors were taken at all time points. After the first blood draw, all participants were instructed to take oral tablets of naproxen (220 mg per tablet; total, 440 mg) twice a day for 1 week and to document their use. This specific dosage was chosen because it represents the recommended over-the-counter package instruction dosage for this medication. After 1 week of naproxen treatment, the second blood draw was performed, and the participants were then instructed to discontinue all use of NSAIDs. The participants returned for a third blood draw after a 1-week washout period. The 1-week washout period was chosen because this is typically the amount of time we ask our surgical patients to refrain from NSAID use before surgical intervention.

Preparation, Processing, and Characteristics of PRP

A certified phlebotomist cleansed the venipuncture site with an alcohol swab and drew 30 mL of peripheral blood into a syringe prefilled with 5 mL of anticoagulant citrate dextrose (Fenwal Laboratories, Lake Zurich, IL). The syringe was capped and taken by a technician to conduct automated hematologic analysis using the

Cell-Dyn Ruby system (Abbott, Abbott Park, IL) followed by immediate processing of LR-PRP.

A previously described standard protocol was used for the LR-PRP preparation.[33] In brief, 30 mL of peripheral blood was drawn by standard technique. The whole blood sample was taken to a laboratory for processing. Then, a whole blood sample was used to obtain a CBC from the hematology analyzer. The automated hematology analyzer quantified platelets, leukocytes, and 5 differentials including neutrophils, lymphocytes, monocytes, eosinophils, and basophils in $\times 10^3$ K/μL, as well as erythrocytes in $\times 10^6$ K/μL.

Manual centrifugation and extraction techniques were used to concentrate peripheral blood into LR-PRP. The remaining whole blood was transferred into 2 sterile 50-mL conical tubes under a biosafety hood. The whole blood was then placed into a Sorvall ST 8 benchtop centrifuge (Thermo Fisher Scientific, Waltham, MA) for 10 minutes at 2,400 revolutions per minute (rpm). After the completion of the first soft spin centrifugation, a pipette was used to extract the excess platelet-poor plasma; this was placed into a separate 15-mL conical tube. Then, the remaining leukocyte blood cell layer ("buffy coat layer") and the top fraction of the erythrocyte blood cell layer were consolidated into one 15-mL conical tube and centrifuged at 3,600 rpm for 6 minutes. The final step included the final extraction of platelet-poor plasma and resuspension of the buffy and erythrocyte layer to produce 1 to 2 mL of LR-PRP. Hematologic analysis was conducted on the LR-PRP to quantify the platelet fold difference from baseline measurements. The LR-PRP samples were immediately assayed and analyzed using a MagPix multiplex instrument (Luminex, Austin, TX) after processing completion. Data are reported as median and range because these are nonparametric data; these methods are described later.

Quantification of PRP Composition

Luminex multiplex immunoassays (EMD Millipore, Billerica, MA) were used to measure the levels of angiogenic, anabolic, and proinflammatory factors, including VEGF, PDGF-AB, PDGF-BB, FGF-2, IL-1β, IL-6, IL-8, and TNF-α (Table 1). The following factors were assessed based on previous studies that showed the effect of NSAIDs on growth factor, cytokine, and chemokine expression. Specific immunoassay kits (EMD Millipore) used a human cytokine-chemokine magnetic bead panel.

Table 1. Factor Names and Classification

Factor Name	Classification
Interleukin 1β (IL-1β)	Catabolic
Interleukin 6 (IL-6)	
Interleukin 8 (IL-8)	
Tumor necrosis factor α (TNF-α)	
Vascular endothelial growth factor (VEGF)	Anabolic
Fibroblast growth factor 2 (FGF-2)	
Platelet-derived growth factor AB (PDGF-AB)	
Platelet-derived growth factor AA (PDGF-AA)	

The manufacturer's standard protocol for the MagPix multiplex instrument was used based on previously published literature.[34] All reagents were prepared and stored according to the manufacturer's instructions. Background, standards, and controls were added in duplicate to the appropriate wells with serum matrix solution. Unknown samples were added in duplicate along with premixed cytokine and chemokine antibody—immobilized magnetic beads. The plate was sealed and covered with foil during incubation with agitation at 600 rpm. By use of a handheld magnet, the plate was washed 2 times using the 1× wash buffer provided. Detection antibodies were added to the plate and incubated at room temperature for 1 hour at 600 rpm. Streptavidin-phycoerythrin solution was added and incubated at room temperature for 30 minutes at 600 rpm. After 2 plate washes, MagPix drive fluid was added to resuspend the beads at 300 rpm for 5 minutes. Finally, the plate was analyzed in the MagPix multiplex instrument using xPonent software (EMD Millipore), which creates a standard curve for each respective analyte using a 5-parameter logistic curve—fitting method with the median fluorescent intensity. Cytokine and chemokine concentrations in the unknown samples were calculated based on the previously described method.

Statistical Analysis

We analyzed a subset of 8 biological factors chosen a priori that were hypothesized to be affected by naproxen: VEGF, PDGF-AA, PDGF-AB, FGF-2, TNF-α, IL-1β, IL-6, and IL-8. We selected these biologically active factors to study based on previously published reports on the primary biologically constituent components of LR-PRP.[35] Medians and ranges were used to summarize measurements stratified by time point because the data were nonparametric.

A priori power and sample size calculations were made based on growth factors and other cytokine and chemokine concentrations from a simplifying assumption of matched-pair *t* testing. Our group previously conducted a pilot study between PDGFs in whole blood and LR-PRP. It was hypothesized that a similar effect would be observed in healthy donor LR-PRP growth factors and other chemokines and cytokines. On the basis of the assumption of 2-tailed testing and an α of .05, 14 subjects were sufficient to detect an effect size (d) of 0.8 with 80% power. To address the primary hypothesis, we used the Friedman test to identify statistically significant differences among the baseline, naproxen, and washout time points while accounting

Table 2. Complete Blood Count Results Quantified by Composition of WB and LR-PRP Content Measured Before Naproxen Administration, After 1 Week of Naproxen Use, and After Washout (1-Week Discontinuation of Naproxen Use)

Time	PLT, K/μL		WBC, K/μL	
	WB	LR-PRP	WB	LR-PRP
Before naproxen use	199 (124-300)	1,246 (320-2,686)	6 (3.6-8.3)	24 (6.0-66.3)
After 1 wk of naproxen use	191 (116-247)	1,537 (649-2,870)	5 (4.0-8.9)	37 (11.7-19.7)
After washout (1-wk discontinuation of naproxen use)	186 (111-247)	1,407 (475-2,723)	5 (3.8-7.7)	42 (21.3-96.5)

NOTE. Data are presented as mean (range).
K/μL, thousands per cubic milliliter; LR-PRP, leukocyte-rich platelet-rich plasma; PLT, platelets; WB, whole blood; WBC, white blood count.

for the repeated-measures design of this study. When the Friedman test was significant, we made pair-wise comparisons among the 3 groups using the Fisher least significant difference. R statistical software (R Foundation for Statistical Computing, Vienna, Austria) was used for all analyses. The figures were made using Prism software (GraphPad Software, La Jolla, CA).

Results

Donor Demographic Characteristics

A total of 19 healthy volunteers were enrolled in the study. Of these volunteers, 3 had their samples clot and declined participation with a repeated blood draw during the first week of the study. A total of 16 participants (8 women and 8 men), with a mean age of 36.6 years (range, 25-64 years), met the inclusion criteria and voluntarily participated in the study to its completion. The average height of the study participants was 1.74 m (range, 1.52-1.9 m). The average weight was 75.7 kg (range, 61.2-99.7 kg). The average BMI of the study participants was 24.4 (range, 20.8-32.8). All donors documented daily use of naproxen as instructed. Of the remaining 16 study subjects, 1 became sick with a viral illness during the first week of the study and could not be considered a healthy donor,

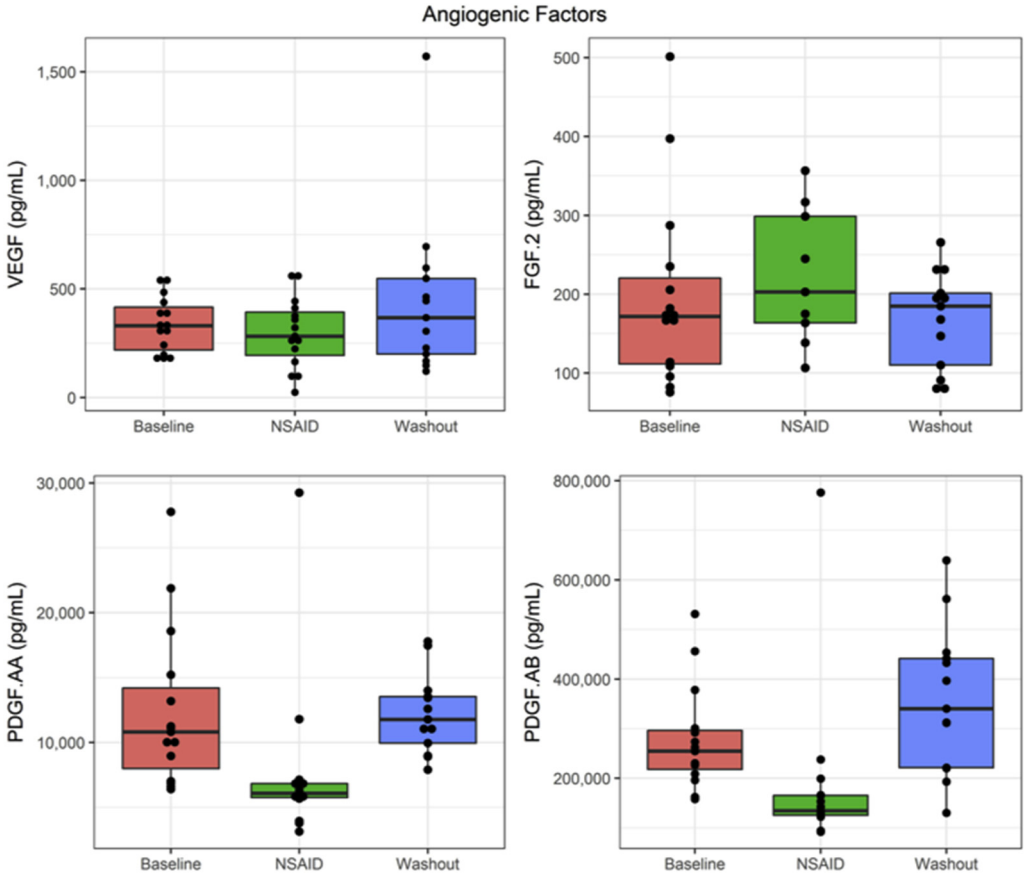

Fig 1. Correlation between age and fibroblast growth factor 2 (FGF-2), tumor necrosis factor α (TNF-α), and interleukin 1β (IL-1β) in leukocyte-rich platelet-rich plasma from healthy donors at baseline. There was a significant negative correlation ($P < .05$) between age and the baseline composition of leukocyte-rich platelet-rich plasma with the anabolic factor FGF-2 ($\rho = -0.583$, $P = .018$) and the catabolic factors TNF-α ($\rho = -0.617$, $P = .011$) and IL-1β ($\rho = -0.532$, $P = .034$).

so the subject's data were excluded from the analysis. Thus the final analysis included 15 study subjects (7 women and 8 men) for all time points.

Characteristics of PRP

The whole blood and LR-PRP CBC results from each time point are presented in Table 2. There was substantial biological variability in LR-PRP between subjects based on the initial analysis of the baseline data. For this reason, a repeated-measures analysis was undertaken for data analysis as described previously in the Methods section.

Among the biological factors tested, there was a significant negative association between age and the baseline composition of LR-PRP with the anabolic factor FGF-2 ($\rho = -0.583$, $P = .018$) and the catabolic factors TNF-α ($\rho = -0.617$, $P = .011$) and IL-1β ($\rho = -0.532$, $P = .034$) (Fig 1). Among the biological factors tested, there was a significant negative association between BMI and the baseline levels of VEGF-A ($\rho = -0.724$, $P = .002$), FGF-2 ($\rho = -0.722$, $P = .002$), and IL-6 ($\rho = -0.595$, $P = .015$) (Fig 2). Baseline levels did not significantly differ between men and women for any of the anabolic or catabolic factors measured (all $P > .05$).

LR-PRP Characteristic Variations With Naproxen and After the Washout Period

Anabolic Factors. The effects of naproxen on anabolic factors in LR-PRP were assessed, and several significant findings regarding growth factor levels were observed (Table 3). Levels of PDGF-AA (baseline median, 10,811 pg/mL; post-naproxen median, 6,069 pg/mL) and PDGF-AB (baseline median, 254,847 pg/mL; post-naproxen median, 134,683 pg/mL) showed significant declines (44% and 47%, respectively; $P < .05$) from baseline after 1 week of naproxen use. There was a significant recovery of PDGF-AA (baseline median, 10,811 pg/mL; post-naproxen median, 6,069 pg/mL; post-washout median, 11,773 pg/mL) and PDGF-AB (baseline median, 254,847 pg/mL; post-naproxen median, 134,683 pg/mL; post-washout median, 340,250 pg/mL) levels after a 1-week washout of naproxen (94% and 153%, respectively; $P < .05$), with a return to levels similar to baseline

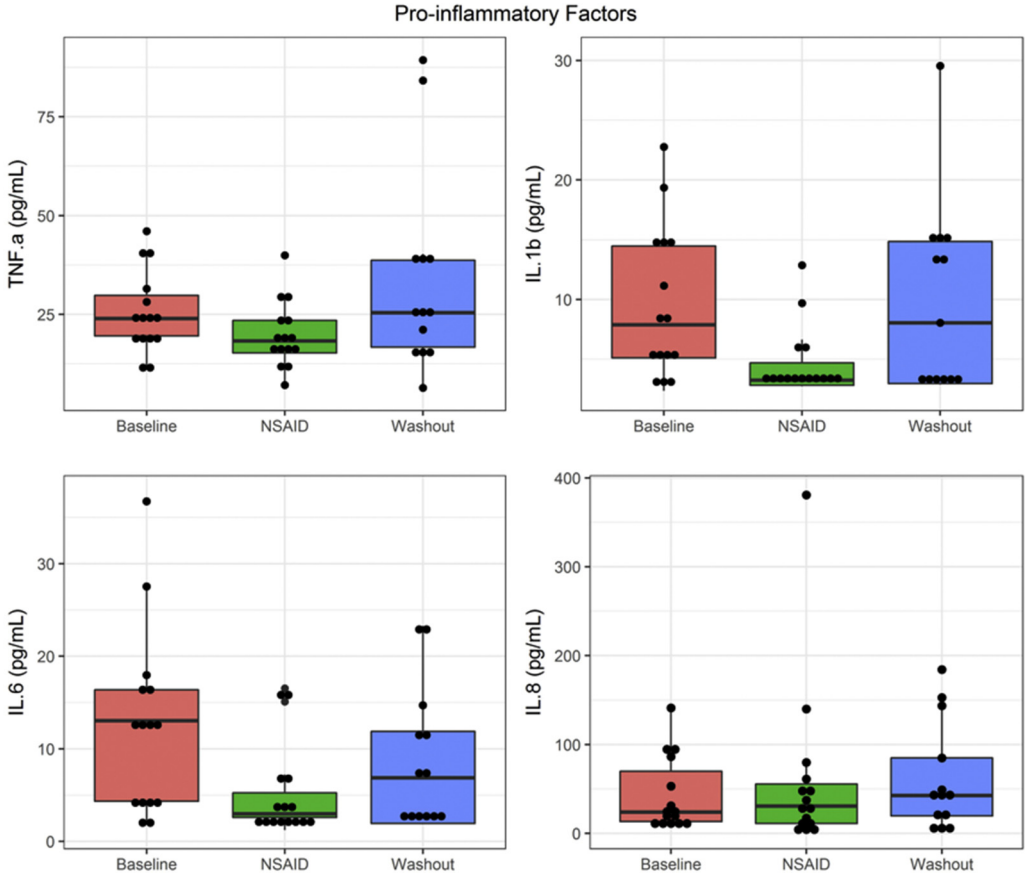

Fig 2. Correlation between body mass index (BMI) and vascular endothelial growth factor (VEGF), fibroblast growth factor 2 (FGF-2), and interleukin 6 (IL-6) in leukocyte-rich platelet-rich plasma from healthy donors at baseline. There was a significant negative correlation ($P < .05$) between BMI and the baseline levels of VEGF ($\rho = -0.724$, $P = .002$), FGF-2 ($\rho = -0.722$, $P = .002$), and IL-6 ($\rho = -0.595$, $P = .015$).

Table 3. Median and Mean for Each Factor Measured Before Naproxen Administration, After 1 Week of Naproxen Use, and After Washout (1-Week Discontinuation of Naproxen Use)

	Before Naproxen Administration	After 1 Week of Naproxen Use	After Washout (1-wk Discontinuation of Naproxen Use)
VEGF, pg/mL			
Median (minimum-maximum)	330.9 (173.8-546.9)	281.9 (23.2-563.9)	367.9 (120.5-1,572)
Mean ± SD	336.2 ± 126.1	296.2 ± 161	450.3 ± 384.1
FGF-2, pg/mL			
Median (minimum-maximum)	172 (76-501.2)	202.9 (106.7-356.8)	184.9 (79.2-265.9)
Mean ± SD	197.8 ± 119	222.7 ± 86.4	167.9 ± 61.6
PDGF-AA, pg/mL			
Median (minimum-maximum)	10,811 (6,393-27,777)	6,069 (3,124-29,255)	11,773 (7,881-17,786)
Mean ± SD	12,372 ± 6,163	7,646 ± 6,293	12,177 ± 3,077
PDGF-AB, pg/mL			
Median (minimum-maximum)	254,847 (157,821-531,142)	134,683 (91,966-776,318)	340,250 (130,170-639,436)
Mean ± SD	277,269 ± 104,915	186,208 ± 167,514	358,081 ± 148,717
TNF-α, pg/mL			
Median (minimum-maximum)	23.9 (11.3-46)	18.3 (7.1-39.9)	25.4 (6.4-89.4)
Mean ± SD	25.6 ± 10.3	19.8 ± 8.3	34 ± 25.5
IL-1β, pg/mL			
Median (minimum-maximum)	7.9 (2.4-22.8)	3.3 (2.7-12.9)	8.1 (2.9-29.5)
Mean ± SD	9.8 ± 6.2	4.6 ± 3	9.9 ± 8
IL-6, pg/mL			
Median (minimum-maximum)	13.1 (1.8-36.7)	3 (1.2-16.5)	6.9 (1.9-23.3)
Mean ± SD	12.5 ± 9.9	5 ± 4.7	8.6 ± 7.7
IL-8, pg/mL			
Median (minimum-maximum)	24 (9-141.1)	30.9 (2.8-380.8)	42.7 (3.7-184.5)
Mean ± SD	43.2 ± 41.3	60.5 ± 95.7	61.7 ± 61

FGF-2, fibroblast growth factor 2; IL, interleukin; PDGF, platelet-derived growth factor; SD, standard deviation; TNF-α, tumor necrosis factor α; VEGF, vascular endothelial growth factor.

measurements. There was no difference between baseline and washout levels of PDGF-AA or PDGF-AB (Fig 3). VEGF and FGF-2 levels were not significantly different ($P > .05$) between the 3 time points.

Catabolic Factors. The effects of naproxen on catabolic factors in LR-PRP were assessed, and one significant finding was observed (Table 3). The proinflammatory factor IL-6 (baseline median, 13.1 pg/mL; post-naproxen median, 3 pg/mL) showed a significant decline from baseline (77%, $P < .05$) after 1 week of naproxen use. There was a significant recovery (130%, $P < .05$) of IL-6 levels after the 1-week washout period, with a return to levels similar to baseline measurements (baseline median, 13.1 pg/mL; post-naproxen median, 3.0 pg/mL; post-washout median, 6.9 pg/mL). We found no significant difference in the levels of IL-6 between baseline and the 1-week washout period (Fig 4). TNF-α, IL-1β, and IL-8 levels were not significantly different ($P > .05$) between the 3 time points.

Discussion

The most important finding of this study was that after 1 week of naproxen use in healthy donors, the anabolic factors PDGF-AA and PDGF-AB, as well as the catabolic factor IL-6, showed diminished levels in LR-PRP. After a 1-week washout period, PDGF-AA, PDGF-AB, and IL-6 had returned to baseline levels. Previous studies have examined the effects of naproxen on growth factors and found that VEGF and PDGF, as well as other anabolic factors, were impaired.[13,14] It is interesting that we did not find a significant difference in VEGF levels or IL-8, IL-1β, TNF-α, and FGF-2 levels between the time points. Despite previous reports that have shown NSAIDs' inhibitory effect on angiogenic and proinflammatory factors, the dose, duration of treatment, and type of NSAID must be taken into account to understand the role of COX inhibitors regarding individual factors.

Our results suggest that 1 week of naproxen consumption significantly affects the angiogenic factors PDGF-AA, PDGF-AB, and IL-6 within LR-PRP. PDGF isoforms A and B are potent mitogens that promote angiogenesis and muscle growth through the activation of intrinsic pathways.[36,37] The proinflammatory factor IL-6 is also an important mediator during the inflammatory and angiogenic phases of tissue healing. Our results showed that PDGF-AA, PDGF-AB, and IL-6 levels normalized after a 1-week washout period in healthy donors, who were not long-term NSAID users. Although inhibitory effects of naproxen on PDGF and IL-6 factors in LR-PRP were observed, it is important to mention that these findings do not reflect changes in

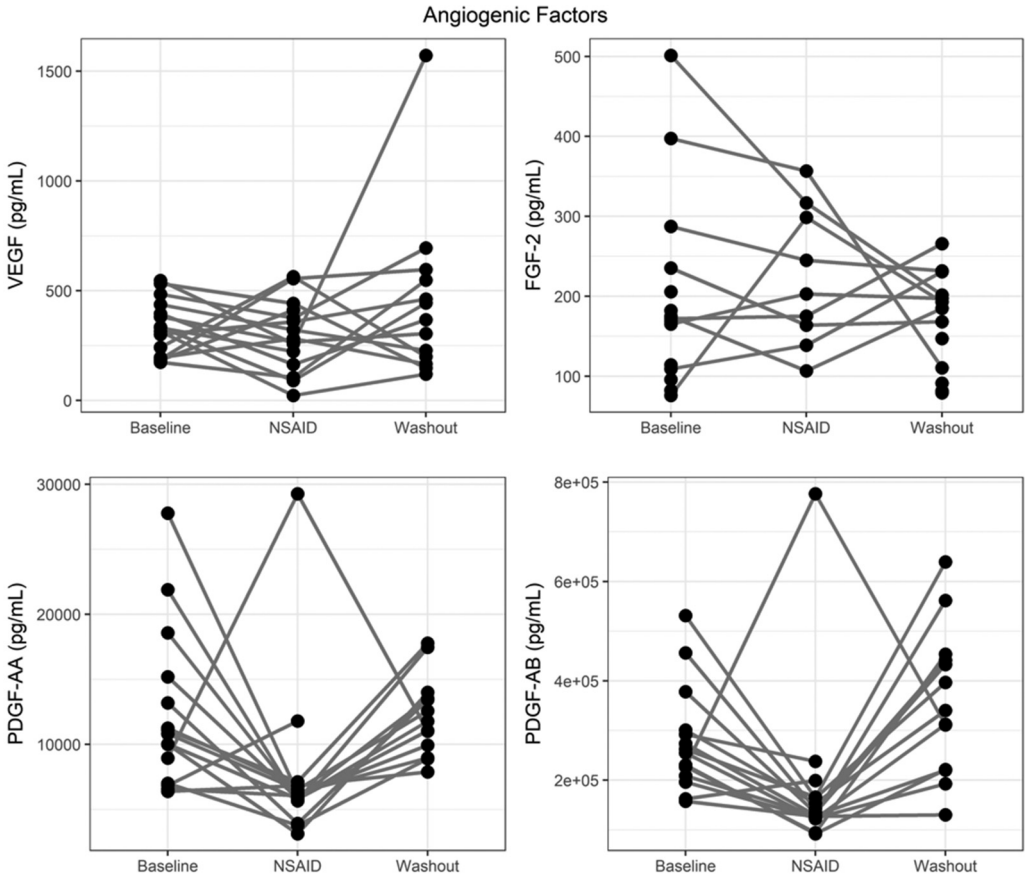

Fig 3. Line plots showing effect of naproxen use and 1-week washout period on 4 anabolic factors. The black dots represent individual subject factor measurements, and the gray lines connect measurements from the same subject at different time points. After 1 week of naproxen treatment, there was a significant decline ($P < .05$) in platelet-derived growth factor (PDGF) AA and PDGF-AB levels, with a significant increase ($P < .05$) after a 1-week washout period (median PDGF-AA levels of 10,811 pg/mL [range (minimum-maximum), 6,393-27,777 pg/mL] at baseline, 6,069 pg/mL [range, 3,124-29,255 pg/mL] after 1 week of naproxen, and 11,773 pg/mL [range, 7,881-17,786 pg/mL] after washout; median PDGF-AB levels of 254,847 pg/mL [range, 157,821-531,142 pg/mL], 134,683 pg/mL [range, 91,966-776,318 pg/mL], and 340,250 pg/mL [range, 130,170-639,436 pg/mL], respectively). There was no significant difference between the baseline and washout values for vascular endothelial growth factor (VEGF) or fibroblast growth factor 2 (FGF-2). Levels of VEGF (median of 330.9 pg/mL [range, 173.8-546.9 pg/mL] at baseline, 281.9 pg/mL [range, 23.2-563.9 pg/mL] after 1 week of naproxen, and 367.9 pg/mL [range, 120.5-1,572 pg/mL] after washout) and FGF-2 (median of 172 pg/mL [range, 76-501.2 pg/mL], 202.9 pg/mL [range, 106.7-356.8 pg/mL], and 184.9 pg/mL [range, 79.2-265.9 pg/mL], respectively) were not significantly different ($P > .05$) between the 3 time points. (NSAID, nonsteroidal anti-inflammatory drug.)

LR-PRP's biological activity or therapeutic efficacy. Owing to the lack of clinical evidence showing the negative effects of naproxen on outcomes after LR-PRP treatment, a greater emphasis is being placed on the elucidation of specific clinical responses after PRP treatment with concurrent use of NSAIDs for specific MSK conditions. However, it is first necessary to determine the effect of naproxen on LR-PRP's biological activity in vitro.

We found inherent biological variability in platelet and growth factor concentrations among healthy individuals, which highlights the importance of PRP characterization. Other studies have similarly reported on the biological variability of PRP composition.[38,39] In this regard, different methods of preparation, activation, and application might affect platelet and ultimately growth factor quantity and quality.[40-42] In addition, the effect of patient demographic characteristics and medications on the biological composition of PRP is not well understood. There does seem to be a negative correlation, defined by 2 factors being inversely associated—that is, when one factor increases, the other decreases—between age, as well as BMI, and certain growth factors, cytokines, and biologically active molecules. Increasing age and BMI seem to negatively influence or diminish levels of biologically active molecules that comprise PRP. A better understanding of the biological constituents of PRP combined with biological characterization will aid in a more scientifically based application to various disease states.[39,43-47]

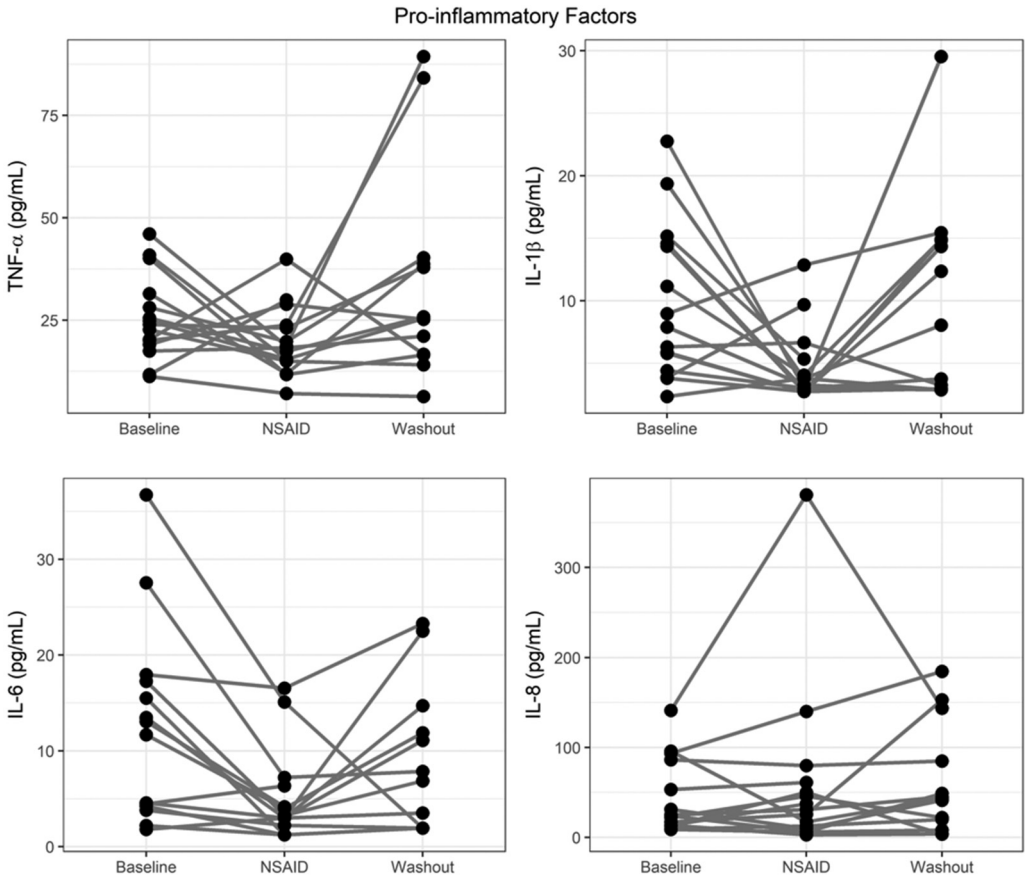

Fig 4. Line plots showing effect of naproxen use and 1-week washout period on 4 catabolic factors. The black dots represent individual subject factor measurements, and the gray lines connect measurements from the same subject at different time points. After 1 week of naproxen treatment, there was a significant decline ($P < .05$) in interleukin (IL) 6 levels, with a significant increase ($P < .05$) after a 1-week washout period (median of 13.1 pg/mL [range (minimum-maximum), 1.8-36.7 pg/mL] at baseline, 3 pg/mL [range, 1.2-16.5 pg/mL] after 1 week of naproxen, and 6.9 pg/mL [range, 1.9-23.3 pg/mL] after washout). There was no significant difference between the baseline and washout values for IL-8, tumor necrosis factor α (TNF-α), or IL-1β. Levels of TNF-α (median of 23.9 pg/mL [range, 11.3-46 pg/mL] at baseline, 18.3 pg/mL [range, 7.1-39.9 pg/mL] after 1 week of naproxen, and 25.4 pg/mL [range, 6.4-89.4 pg/mL] after washout), IL-1β (median of 7.9 pg/mL [range, 2.4-22.8 pg/mL], 3.3 pg/mL [range, 2.7-12.9 pg/mL], and 8.1 pg/mL [range, 2.9-29.5 pg/mL], respectively), and IL-8 (median of 24 pg/mL [range, 9-141.1 pg/mL], 30.9 pg/mL [range, 2.8-380.8 pg/mL], and 42.7 pg/mL [range, 3.7-184.5 pg/mL], respectively) were not significantly different ($P > .05$) between the 3 time points. (NSAID, nonsteroidal anti-inflammatory drug.)

Furthermore, characterizing the biological profiles of PRP will allow a more targeted scientific approach to study the mechanistic effects of PRP on various tissue types.[48-50]

Considering the wide use of LR-PRP in the fields of orthopaedics and sports medicine, there are few foundational studies that have attempted to understand the biological profile variances.[39,40,43-47,51] In addition, there is little evidence to guide physicians as to when it is beneficial to administer LR-PRP therapy in patients concurrently using NSAIDs.[14,18,39,52] Orally administered NSAIDs are frequently used as a nonsurgical treatment modality to reduce subsequent pain and swelling to improve functional ability in acute and chronic MSK disorders.[18] NSAID platelet dysfunction is irreversible and will prolong its effect through a platelet's life span and potentially obviate growth factor levels and cytokine signaling.[13,14,51] Our study shows that the use of naproxen influences both catabolic and anabolic factors associated with neovascularization in LR-PRP, which is used to treat a variety of MSK conditions. The anabolic and angiogenic factors VEGF, FGF-2, PDGF-AA, and PDGF-AB and the proinflammatory factors IL-6, IL-8, TNF-α, and IL-1β were identified in this study because of their roles in neovascularization.

The results of this study provide a conceptual basis for understanding the effects of naproxen, a commonly used NSAID, on LR-PRP profiles in healthy donors. We recommend that follow-up studies compare biological profiles in healthy donors and patients who are long-term NSAID users; compare baseline whole blood and final product measurements with those in patients with

an underlying pathology, with correlations to patient demographic characteristics; and compare the effects of COX-1 and COX-2 inhibitors on angiogenic and proinflammatory concentrations in LR-PRP. This study quantified several cytokines and chemokines, as well as other biologically active factors, but the clinical relevance of those factors at the levels reported is not known.

Limitations

There were some limitations to this study. Long-term NSAID use may result in longer dysfunction of platelets and megakaryocytes, as well as other hematogenous tissue. Regarding the study protocols, only 1 processing method was used for preparation of PRP, which could potentially alter the biological variability among donors. On the basis of the final sample size of 15 donors, this study is not powered to rule out subtle effects (effect size [d] < 0.8) that may exist in association with NSAID use. In addition, the pilot data that we used to inform our power and sample size calculation did not provide a high level of effect size precision. Finally, only 1 NSAID, naproxen, was tested; it is possible that other NSAIDs could likewise change the effects of growth factor, chemokine, or cytokine expression on LR-PRP.

Conclusions

Naproxen use diminished several biological factors in LR-PRP; however, a 1-week washout period was sufficient for the recovery of PDGF-AA, PDGF-AB, and IL-6 to return to baseline levels. TNF-α, IL-1β, IL-8, VEGF, and FGF-2 did not show differences between the 3 time points of data collection. Discontinuing NSAIDs for a minimum of 1 week before LR-PRP treatment may improve certain biological factor levels.

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