

# Effects of Antiplatelet and Nonsteroidal Anti-inflammatory Medications on Platelet-Rich Plasma

## A Systematic Review

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**Background:** Platelet-rich plasma (PRP) has wide applications in orthopaedic care. Its beneficial effects are attributed to the growth factor profile from the platelet secretome. In theory, these effects would be diminished by medications that inhibit platelet activation and/or the subsequent release of growth factors.

**Purpose:** To determine whether commonly used antiplatelets, nonsteroidal anti-inflammatory drugs (NSAIDs), or anticoagulant medications affect platelet growth factor release in PRP.

**Study Design:** Systematic review; Level of evidence, 2.

**Method:** A systematic review of the literature related to antiplatelet, anti-inflammatory, and anticoagulant drugs was performed following the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines. We used the Downs and Black objective quality scoring system. The literature search consisted of PubMed and Cochrane Library databases. Search terms consisted of 1 item selected from “platelet-rich plasma,” “platelet-derived growth factor,” and “platelet-rich plasma AND growth factor” combined with 1 item from “antiplatelet,” “aspirin,” “anticoagulant,” and “NSAID.” Only studies published within the past 25 years were included.

**Results:** A total of 15 studies met the inclusion criteria: 7 studies detected no significant decrease in growth factors or mitogenesis, whereas 6 detected a decrease with antiplatelet agents, 1 detected mixed results with an antiplatelet agent, and 1 had mixed results with an antiplatelet agent/vasodilator. In terms of PRP activation, all 3 studies assessing collagen, the 2 studies analyzing adenosine diphosphate alone, and the 1 study investigating arachidonic acid found a decrease in growth factor concentration.

**Conclusion:** Antiplatelet medications may decrease the growth factor release profile in a cyclooxygenase 1– and cyclooxygenase 2–dependent manner. Eight of 15 studies found a decrease in growth factors or mitogenesis. However, more studies are needed to comprehensively understand antiplatelet effects on the PRP secretome.

**Keywords:** platelet-rich plasma; aspirin; growth factor; nonsteroidal anti-inflammatory; cyclooxygenase inhibitor; platelet

Originally used in oromaxillofacial surgery, platelet-rich plasma (PRP) is currently used to treat many different conditions, ranging from sports injuries to androgenic alopecia.<sup>17,21</sup> This biologic therapy is thought to promote healing by delivering the concentrated growth factors to damaged tissues<sup>20</sup> and augment the natural healing process with mitogenesis or cellular proliferation through mitosis, chemotaxis, and other cellular processes.<sup>20</sup> In order to release these restorative molecules, platelets must be activated. This complex process begins with binding of myriad agonists to platelet G-protein-coupled receptors (GPCRs) or immunoreceptor

tyrosine-based activation motif complexes.<sup>35</sup> These lead to signaling cascades and platelet responses, including regulating surface receptors and releasing growth factors into the environment through degranulation.

There are various medications that can inhibit the aforementioned processes. For example, nonsteroidal anti-inflammatory drugs (NSAIDs) are thought to inhibit growth factor release by competitively and irreversibly inhibiting cyclooxygenase (COX) 1 and 2. More specifically, this is thought to occur through the inability of arachidonic acid (AA) to allow downstream thromboxane-A<sub>2</sub> (TxA<sub>2</sub>) binding to TxA<sub>2</sub> receptor to allow for platelet activation. Consequently, many physicians recommend cessation of antiplatelet and anti-inflammatory drugs before initiating PRP therapy.

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TABLE 1  
PubMed and Cochrane Database Search Results<sup>a</sup>

	Search Terms	Platelet-Rich Plasma	Platelet-Derived Growth Factor	Platelet-Rich Plasma and Growth Factor
PubMed database	Antiplatelet	390	70	9
	Aspirin	306	39	22
	Anticoagulant	360	2447	55
	NSAID	130	210	8
Cochrane database	Antiplatelet	34	11	0
	Aspirin	53	12	1
	Anticoagulant	29	14	6
	NSAID	17	5	7

<sup>a</sup>NSAID, nonsteroidal anti-inflammatory drug.

A variety of activating agents can stimulate platelets from thrombin (TBN) to epinephrine.<sup>35</sup> The exact mechanism of platelet activation is complex and beyond the scope of this article. Therefore, this study will focus more on the effect of antiplatelet medications on PRP.<sup>9,25</sup> To date, there have been no systematic reviews on the effects of antiplatelet medications and anti-inflammatory drugs on PRP yield and growth factor profile. Considering the inhibitory effect of these medications on platelet function, it is reasonable to hypothesize a reduction in the growth factor expression that is dependent on the activating agent. The purpose of this review was to determine whether commonly used antiplatelets, NSAIDs, or anticoagulant medications affect platelet growth factor release in PRP. It was anticipated that these findings might lead to recommendations in administering clinical PRP in the setting of NSAID, antiplatelet, or anticoagulant medication use.

## METHODS

The literature search was in accordance with PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines and consisted of PubMed and Cochrane Library database inquiries for the effects of antiplatelet, anticoagulant, and anti-inflammatory drugs on PRP growth factor release. The initial search strategy used combinations of 2 keywords, 1 item selected from “platelet-rich plasma,” “platelet-derived growth factor,” and “platelet-rich plasma AND growth factor” combined with 1 item from “antiplatelet,” “aspirin,” “anticoagulant,” and “NSAID.” This method yielded 12 different combinations of inquiries per database (Table 1). The selection process,

including inclusion/exclusion criteria, is illustrated in Figure 1. The initial search had a return of 4235 results, of which 323 were duplicates. Only studies published within the past 25 years were included. Both human and animal studies were included as well as in vivo or in vitro studies. We excluded articles in which the outcomes did not measure the growth factor levels and where the interventions only focused on PRP activations and not on the different medications or metabolites. Review articles and case reports were excluded. Two additional articles that fit the inclusion criteria were included at the later stages of the manuscript writing.

A total of 15 studies met the aforementioned inclusion criteria. Of the 15 studies included, 12 (80%) analyzed PRP,<sup>11</sup> 1 (6.7%) examined plasma,<sup>11</sup> and 2 (13.33%) examined platelets directly.<sup>26,36</sup> Of the studies that analyzed PRP, 2 used calcium chloride (CaCl<sub>2</sub>),<sup>1,2</sup> 5 used TBN,<sup>12,18,23,29,34</sup> 3 used collagen,<sup>14,16,30</sup> 2 used adenosine diphosphate (ADP),<sup>14,32</sup> 1 used AA,<sup>12</sup> and 1 assessed both TBN and calcium gluconate as activating agents.<sup>27</sup>

All studies were evaluated using a modified 27-item Downs and Black checklist.<sup>7</sup> We elected to use a simple 0 or 1 score for the power analysis, with papers receiving 1 point if they included power calculations showing sufficient sample size.

Data concerning changes in growth factors and/or mitogenesis, platelet activation, statistical significance, model type, activating factor, and type of medication were extracted by 1 author (C.F.) and recorded in a table. Results and statistical analysis were retrieved directly from each study. Next, factors that required methodological quality analysis for the Downs and Black checklist were extracted.

## RESULTS

### Quality Assessment

The manuscript scores ranged from 13 to 25 of 28 points using our modified Downs and Black checklist (Table 2). Downs and Black scores were given the corresponding quality levels as previously reported by Hooper et al<sup>10</sup>: 26 to 28 (excellent), 20 to 25 (good), 15 to 19 (fair), and <15 (poor). Five of 15 studies were in the poor category, 6 of 15 were in the fair category, and 4 of 15 were in the good category. As expected, the clinical trials scored the highest. Most papers scored well (7 of 10 or higher) in the reporting and external validity segments; however, most papers scored low in the internal validity ratings, as only 1 was randomized or blinded. Moreover, none had calculated predetermined sample sizes to achieve

<sup>||</sup>References 1, 2, 12, 14, 16, 18, 23, 27, 29, 30, 32, 34.

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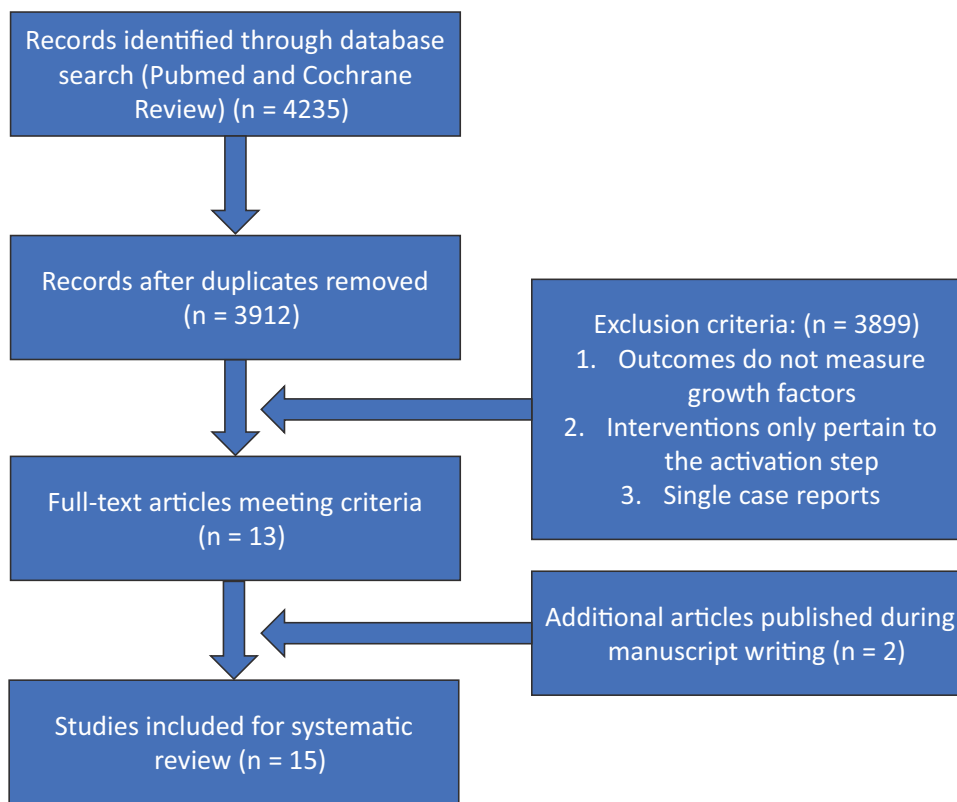
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**Figure 1.** Workflow of PubMed and Cochrane Review database query following PRISMA (Preferred Reporting Items for Systematic Meta-Analyses) guidelines.

**TABLE 2**  
Downs and Black<sup>7</sup> Quality Assessment of Individual Studies

Study	Downs and Black Score	Study	Downs and Black Score
Anitua et al <sup>2</sup>	13	Takehara et al <sup>26</sup>	14
Anitua et al <sup>1</sup>	13	Tian et al <sup>27</sup>	20
Jagroop et al <sup>11</sup>	19	Vissinger et al <sup>30</sup>	15
Jayaram et al <sup>12</sup>	16	Vissinger et al <sup>29</sup>	15
Kariyazono et al <sup>14</sup>	14	Wilson et al <sup>32</sup>	25
Lanas et al <sup>16</sup>	17	Yazawa et al <sup>34</sup>	14
Ludwig et al <sup>18</sup>	18	Zhao et al <sup>36</sup>	22
Smith et al <sup>23</sup>	20		

**TABLE 3**  
Level of Evidence of Individual Studies

Study	Level of Evidence	Study	Level of Evidence
Anitua et al <sup>2</sup>	2	Takehara et al <sup>26</sup>	2
Anitua et al <sup>1</sup>	2	Tian et al <sup>27</sup>	3
Jagroop et al <sup>11</sup>	1	Vissinger et al <sup>30</sup>	1
Jayaram et al <sup>12</sup>	2	Vissinger et al <sup>29</sup>	1
Kariyazono et al <sup>14</sup>	2	Wilson et al <sup>32</sup>	1
Lanas et al <sup>16</sup>	2	Yazawa et al <sup>34</sup>	1
Ludwig et al <sup>18</sup>	2	Zhao et al <sup>36</sup>	1
Smith et al <sup>23</sup>	2		

adequate power or had recruitment methods that represented the target population. Owing to these limitations, most studies had a level of evidence of 2 (Table 3).<sup>3</sup>

### Effects of Antiplatelet Medications With CaCl<sub>2</sub>-Dependent Activation on Growth Factor Profile

Only 2 studies<sup>1,2</sup> utilized CaCl<sub>2</sub> as an activating agent, both of which were in vivo studies in humans (Table 4). Both papers were written by Anitua et al<sup>1,2</sup> and had a similar study design comparing the growth factor production and clotting times of participants on medications with unmedicated controls. Both

papers found no significant influences on growth factor expressions for the groups assessed.

In their 2014 publication, Anitua and colleagues<sup>2</sup> measured platelet-derived growth factor (PDGF) AB, vascular endothelial growth factor (VEGF), insulin-like growth factor 1 (IGF-1), clotting time, and fibroblast proliferation in elderly (>60 years old) controls (no medications) against patients using chronic 100 mg/d aspirin (acetylsalicylic acid [ASA]); varying doses of acenocoumarol; a vitamin K antagonist; or 1500 mg/d glucosamine sulfate, a cartilage precursor often taken as an over-the-counter supplement.<sup>2</sup> No significant differences were detected among clot formation

TABLE 4  
Summary of Individual Studies<sup>a</sup>

Author	Activating Agent	Model	N	Intervention	Growth Factors Assessed	Decrease in Factors?
Anitua et al <sup>2</sup>	CaCl <sub>2</sub>	Human, in vivo	12	ASA, acenocoumarol, glucosamine	PDGF-AB, VEGF, IGF-1, mitogenesis	No
Anitua et al <sup>1</sup>	CaCl <sub>2</sub>	Human, in vivo	20	ASA, acenocoumarol, glucosamine, glucosamine + chondroitin	HGF, PDGF-AB, TGF-β1, VEGF	No
Yazawa et al <sup>34</sup>	TBN	Human, in vitro	5	PGE1, PGE1 + ASA + apyrase	PDGF-AB, TGF-β1	No
Smith et al <sup>23</sup>	TBN	Human, in vivo	18	ASA, ASA + clopidogrel	PDGF-BB, TGF-β1	No
Vissinger et al <sup>29</sup>	TBN	Human, in vivo	12	Dipyridamole	PDGF	No
Ludwig et al <sup>18</sup>	TBN	Dog, in vivo	10	Carprofen	TGF-β1, PDGF-BB	No
Jayaram et al <sup>12</sup>	TBN, AA, none	Human, in vivo	12	ASA	VEGF, PDGF-AB, TGF-β1	With TBN, ASA slightly attenuated the release of PDGF-AB alone. With AA, ASA significantly inhibited the release of all 3 growth factors.
Kariyazono et al <sup>14</sup>	Collagen, ADP, AA	Human, in vitro	8	ASA, cilostazol, ramatroban	TGF-β1	Yes with ASA, cilostazol, ramatroban
Lanas et al <sup>16</sup>	Collagen	Human, in vivo and fibroblast in vitro	5	ASA	Mitogenesis	Yes
Vissinger et al <sup>30</sup>	Collagen	Human, in vivo	12	ASA	PDGF	Yes
Wilson et al <sup>32</sup>	ADP	Human, in vivo and rat, in vitro	50	ASA + placebo, ASA + clopidogrel	PDGF	Yes with clopidogrel + ASA but not ASA alone
Tian et al <sup>27</sup>	TBN and calcium gluconate	Human, in vivo	44	Either ASA or clopidogrel	EGF, FGF-2, IGF-1, VEGF-A, GDF-11, PDGF-AB/BB, PDGF-AA	Yes, FGF-2, PDGF-AA, and GDF-11 in the CVD and antiplatelet group compared with healthy controls. Growth factors were also diminished with age and diagnosis of DM.
Takehara et al <sup>26</sup>	None	Human, in vivo and fibroblast in vitro	3	Dipyridamole, ASA, trapidil, ticlopidine	Mitogenesis, PDGF	Yes with dipyridamole
Zhao et al <sup>36</sup>	None	Human, in vivo	22	ASA, clopidogrel, dipyridamole, ASA + clopidogrel, ASA + dipyridamole, clopidogrel + dipyridamole, ASA + clopidogrel + dipyridamole	PDGF	Yes with ASA in healthy volunteers but not in patients with stroke
Jagroop et al <sup>11</sup>	None	Human, in vivo	20	Clopidogrel or ASA then clopidogrel + ASA	PDGF-AB	No

<sup>a</sup>AA, arachidonic acid; ADP, adenosine diphosphate; ASA, acetylsalicylic acid; CVD, cardiovascular disease; DM, diabetes mellitus; EGF, epidermal growth factor; FGF-2, fibroblast growth factor 2; GDF-11, growth differentiation factor 11; HGF, hepatocyte growth factor; IGF-1, insulin-like growth factor 1; PDGF, platelet-derived growth factor; PGE1, prostaglandin E1; TBN, thrombin; TGF-β1, transforming growth factor beta1; VEGF, vascular endothelial growth factor.

time, amount of growth factor content, or cell proliferation rate of human gingival fibroblasts for any of the groups.<sup>2</sup>

Anitua and colleagues<sup>1</sup> in 2015 conducted a similar study design analyzing different growth factors: hepatocyte

growth factor (HGF), PDGF-AB, transforming growth factor beta (TGF-β1), and VEGF. Patients either had consumed 100 mg/d ASA, acenocoumarol, 1500 mg/d glucosamine sulfate, or 1500 mg/d glucosamine sulfate with 400 mg/d

chondroitin (another constituent of cartilage taken as an over-the-counter supplement) or had consumed no medications for at least 1 year.<sup>1</sup> There was a significant increase in time to achieve clot formation in the acenocoumarol group (a coumarin derivative sometimes used as an anticoagulant) compared with controls ( $P < .05$ ) but no significant change in platelet activation, tendon fibroblast proliferation, or growth factor content (PDGF-AB, TGF- $\beta$ 1, VEGF, and HGF) of platelet-rich in growth factors when compared with the control group. Of note, cell treatment in the acenocoumarol group led to a significant decrease in fibroblast protein secretion of VEGF as well as the extracellular matrix proteins, hyaluronic acid, and fibronectin compared with the control group, which the authors attributed to matrix metalloproteinases ( $P < .05$ ).<sup>1</sup>

### Effects of Antiplatelet Medications With TBN-Dependent Activation on Growth Factor Profile

We identified 5 studies<sup>12,18,23,29,34</sup> that assessed growth factors in platelets activated with TBN, 1 of which found a decrease in 1 of several growth factors (Table 4). Yazawa et al<sup>34</sup> measured the effects of in vitro addition of prostaglandin E1 (PGE1) (a vasodilator and antiplatelet endogenous molecule) only, PGE1 with ASA and apyrase (an antiplatelet enzyme), or nothing (control group) on PRP blood samples (administered before the second centrifugation) drawn from 5 healthy human participants. After preparation, the authors measured PDGF-AB and TGF- $\beta$ 1 concentrations in whole blood and PRP with or without antiplatelet substances in 3 ways: direct measurement, Nonidet-P40-treated measurement, and TBN-treated measurement. Focusing on PRP that was activated with TBN, the mean PDGF was measured at 30,554 pg/mL in the control group, 120,188 pg/mL in the PGE1 only group, and 122,379 in the PGE1 + ASA + apyrase group. They found that PDGFs were concentrated to a mean of  $>400\%$  in the samples with antiplatelet substances as compared with the samples without antiplatelet substances.<sup>34</sup>

Smith et al<sup>23</sup> assessed the effect of 80 mg/d ASA, 80 mg/d ASA + 75 mg/d clopidogrel (an antiplatelet medication), and a control group (no known antiplatelet therapy regimen) on PRP produced in patients undergoing cardiac surgery. They found an insignificant decrease in the amount of TGF- $\beta$ 1 ( $P = .26$ ) with ASA + clopidogrel compared with controls and no significant difference in platelet degranulation or PDGF-BB or TGF- $\beta$ 1 in any group.

Vissinger et al<sup>29</sup> investigated the effects of dipyridamole, a molecule with vasodilator and antiplatelet properties, in healthy volunteers before and after administering 100 mg dipyridamole 3 times a day for 3 days. They found no significant difference in platelet count or platelet content of PDGF, serum PDGF concentration, or PRP PDGF concentration before and after receiving the medication.

Ludwig et al<sup>18</sup> analyzed the growth factor levels in 10 dogs at baseline and after a 7-day or 11-day course of 4.4 mg/kg carprofen (an NSAID with more COX-2 inhibition). The PRP for all groups was divided into 4 aliquots: 2 nonactivated and

2 activated with human gamma thrombin (HGT). There were no statistically significant ( $P < .05$ ) effects of the COX-2 inhibitor on the percentage of platelets positive for CD62P (a granule protein that appears on the platelet's outer surface upon fusion of the granule with the plasma membrane during platelet activation and release of  $\alpha$  granule contents) or on concentrations of TGF- $\beta$ 1 or PDGF-BB. It was noted that all HGT-activated aliquots had a significantly higher platelet expression of CD62P and canine activated platelet-1 (CAP1) and released significantly higher concentrations of TGF- $\beta$ 1 and PDGF-BB than did platelets that were not activated.<sup>18</sup>

Jayaram et al<sup>12</sup> analyzed leukocyte-rich PRP (LR-PRP) in 12 healthy human male participants before and after 14 days of 81 mg/d ASA. The PRP samples were collected and aliquoted into 3 groups: nonactivated, AA activated, and TBN activated, and immediately after activation TGF- $\beta$ 1, VEGF, and PDGF-AB were measured with enzyme-linked immunosorbent assays. Subsequently, the 12 participants took 81 mg aspirin daily for 14 days, followed by having a repeat collection of whole blood and PRP with real-time analysis. The authors found that TBN-activated PRP aliquots had a significant increase in PDGF-AB and VEGF release but not TGF- $\beta$ 1 release compared with nonactivated controls. Aspirin intake significantly reduced the amount of PDGF-AB release but not the VEGF release in the LR-PRP samples activated by TBN.

### Effects of Antiplatelet Medications With Collagen-Dependent Activation on Growth Factor Profile

We found 3 papers<sup>14,16,30</sup> that investigated the effects of anti-inflammatory drugs and antiplatelets on PRP using collagen for activation (Table 4). All 3 studies found a decrease in the growth factor production or mitogenesis associated with ASA use.

Kariyazono et al<sup>14</sup> incubated PRP from healthy volunteers with 100  $\mu$ mol ASA, 10  $\mu$ mol cilostazol (a phosphodiesterase-3 inhibitor), or 1  $\mu$ mol ramatroban (a thromboxane receptor antagonist) before stimulating the samples using ADP, collagen, and AA. Aliquots were then mixed with ASA, cilostazol, or ramatroban in vitro, and changes in TGF- $\beta$ 1 and platelet markers were measured. The authors found that all 3 medications significantly decreased TGF- $\beta$ 1 when stimulated by any of the 3 activating agents compared with vehicle control. When the samples were stimulated by collagen, TGF- $\beta$  levels were decreased by roughly 80% using ASA, 30% using cilostazol, and 60% using ramatroban compared with vehicle ( $P < .05$ ).

Lanas et al<sup>16</sup> measured fibroblast mitogenesis in cells obtained from 5 patients before and after ingestion of various doses of ASA (160 mg, 320 mg, 640 mg, and 960 mg). The authors found that the mitogenic activity in collagen-stimulated PRP as measured by <sup>3</sup>H-thymidine incorporation was reduced on average by  $71.6\% \pm 2.5\%$  ( $P = .006$ ) using ASA and was dose dependent. Furthermore, the serum derived from collagen-stimulated PRP after ASA use

was less potent in promoting fibroblast growth than the serum before ASA use ( $P = .01$ ).

Last, Vissinger et al<sup>30</sup> studied 12 healthy male volunteers and measured the values of platelet factors in human serum and in PRP stimulated with submaximal levels of collagen before and 12 hours after 1 dose of 300 mg ASA. With regard to the PRP, while there was no difference in platelet count in the PRP before and after ASA use, there was a significant decrease in concentrations of PDGF ( $P < .01$ ) after ASA use.

### Effects of Antiplatelet Medications With ADP-Dependent Activation on Growth Factor Profile

There were 2 studies<sup>14,32</sup> that used ADP as an activating agent (Table 4). Both detected a negative effect on growth factors.

Wilson et al<sup>32</sup> administered patients already using chronic 75 mg/d ASA with either a placebo or a 300-mg clopidogrel loading dose at least 12 hours before undergoing percutaneous transluminal angioplasty (PTA). Venous blood samples were taken at baseline, 1 hour pre-PTA, 1 hour post-PTA, 24 hours post-PTA, and 30 days post-PTA. In the aspirin and placebo groups, no significant differences in PDGF levels were seen pre-PTA or at any measured post-PTA time points compared with baseline. There was a significant decrease in PDGF ( $P = .004$ ) in the clopidogrel group pre-PTA but no significant changes post-PTA at any of the measured time points.

In the previously described manuscript, Kariyazono et al<sup>14</sup> found a decrease in TGF- $\beta$ 1 among other factors when collagen-activated PRP was incubated with ASA, cilostazol, and ramatroban. They had similar results using ADP to activate platelets. TGF- $\beta$ 1 was decreased by roughly 30% for each of the 3 medication groups ( $P < .01$ ).

### Effects of Antiplatelet Medications With AA-Dependent Activation on Growth Factor Profile

Two studies<sup>12,14</sup> investigated the effect of ASA on AA-activated PRP (Table 4). Both studies, which have been previously mentioned, noted decreases in growth factor levels after aspirin administration.

Jayaram et al<sup>12</sup> detected a significant decrease in all 3 growth factors tested. The authors found that TBN-activated PRP aliquots had a significant increase in PDGF-AB and VEGF release compared with nonactivated controls, but aspirin intake only partially reduced the amount of PDGF-AB release in the LR-PRP samples activated by TBN. VEGF, TGF- $\beta$ 1, and PDGF-AB releases were increased in the AA-activated groups compared with nonactivated controls without ASA, and all 3 growth factor releases were significantly diminished after 14 days of ASA use. Similarly, Kariyazono et al<sup>14</sup> found that ASA, cilostazol, and ramatroban all greatly decreased TGF- $\beta$ 1 in PRP activated by AA use. The results are in line with the effect on PRP activated by collagen.

### Effects of Antiplatelet Medications With Combined Activating Agents on Growth Factor Profile

Tian et al,<sup>27</sup> using a combination of activating agents (Table 4), analyzed several growth factors and aging biomarkers in 4 groups: healthy and <45-year-old individuals, healthy and >45-year-old individuals, >45-year-old individuals with diabetes, and >45-year-old individuals with cardiovascular disease already taking both ASA and clopidogrel for at least 3 months. The authors then used both TBN and calcium gluconate together as activating agents for PRP and assessed different growth factor levels. They found that growth factors generally decreased with age, the presence of diabetes, and the use of antiplatelet medications. Specifically, fibroblast growth factor 2 (FGF-2), PDGF-AA, and growth differentiation factor (GDF-11) but not epidermal growth factor, VEGF-A, IGF-1, or PDGF-AB/BB were lower in the antiplatelet group than the healthy group of the same age ( $P < .05$ ). There were no significant differences in growth factor levels between the diabetes and cardiovascular disease groups.

### Effects of Antiplatelet Medications Without Activation on Growth Factor Profile

Four studies<sup>11,12,26,36</sup> assessed the effects of antiplatelet medications on growth factor profile without the use of activating agents and had varying results (Table 4). Two studies detected a decrease in PDGF, whereas the other 2 studies found no effect on growth factors.

Takehara et al<sup>26</sup> measured the mitogenic activity of skin fibroblasts, illustrating the biological activity of PDGF, incubated in the serum of 3 healthy volunteers who took 225 mg/d dipyridamole, 3g/d ASA, 300 mg/d trapidil, or 300 mg/d ticlopidine. Serum was taken before drug administration, 2 hours after the first drug administration, and 2 hours after the third day of medication administration. The authors found that dipyridamole decreased mitogenesis in fibroblasts by 65% after 1 dose and by 78% after 3 days in 1 patient. Aspirin, trapidil, or ticlopidine did not alter the mitogenic activities in the serum.

Zhao et al<sup>36</sup> gave healthy volunteers and patients with previous ischemic stroke alternating combinations of 75 mg/d ASA, 75 mg/d clopidogrel, and 200 mg twice a day dipyridamole in a randomized multiway trial for 2 weeks. ASA significantly decreased PDGF in healthy volunteers but not in the patients with stroke. There were no significant PDGF-level changes in either the volunteer or patient groups with clopidogrel only, dipyridamole only, or any 2 or 3 drug combinations of the 3 medications.

Jagroop et al<sup>11</sup> administered ASA and clopidogrel to patients with intermittent claudication. Patients initially received 75 mg/d of either drug as monotherapy for 8 days then received them in combination for 8 more days. Blood samples were drawn on days 8 and 16. Although the investigators utilized PRP, they only used it to study aggregation. PDGF-AB was measured in the serum, and no significant change was detected in either group.

Jayaram et al<sup>12</sup> included a PRP aliquot without activation. These samples yielded significantly lower levels of growth factors than the activated aliquots, with low amounts of VEGF or PDGF-AB release and undetectable levels of TGF- $\beta$ 1 release. Nonetheless, ASA had no significant effect on any of the growth factor release amounts in nonactivated PRP.

## DISCUSSION

In this review, we analyzed the effects of common medications on the growth factor profile in PRP and further delineated the effects of activating agents. The Downs and Black objective quality scoring system was used to evaluate the methodological quality because it has been validated for both randomized and nonrandomized studies.

Overall, 8<sup>12,14,16,26,27,30,32,36</sup> of 15 studies found a decrease in growth factors or mitogenesis. Two studies<sup>1,2</sup> using CaCl<sub>2</sub> alone and 4<sup>18,23,29,34</sup> of 5 studies investigating TBN alone for PRP activation found no effect on growth factor production or mitogenesis with antiplatelet or anti-inflammatory medications. The fifth study, conducted by Jayaram et al,<sup>12</sup> found a partial inhibition. Meanwhile, all 3 studies<sup>14,16,30</sup> using collagen, 2 studies<sup>14,32</sup> using ADP alone, and the 1 study<sup>14</sup> utilizing AA for PRP activation found a decrease in growth factor concentration with antiplatelet or anti-inflammatory medications. Of the studies that did not use activating factors, 2<sup>26,36</sup> had mixed results, and 2<sup>11,12</sup> found no significant decrease on growth factor levels in PRP using antiplatelet or anti-inflammatory medications.

Aspirin is thought to exert its antiplatelet effect through irreversibly inhibiting COX-1 and COX-2 and attenuating the generation of downstream proaggregation factors.<sup>31</sup> This inhibits AA conversion to prostaglandin (PG) G<sub>2</sub> and PGH<sub>2</sub>, further preventing the synthesis of other prostaglandins and TxA<sub>2</sub> that activate platelets. Under normal conditions, TxA<sub>2</sub> would bind its receptor, leading to a protein kinase C (PKC)-mediated signal cascade through Rho and/or phospholipase C (PLC). This results in shape change, platelet activation, and GPIIb/IIIa translocation for aggregation.<sup>24</sup> Aspirin and other COX inhibitors are capable of inhibiting this positive feedback system, although they differ in reversibility and COX isomer selectivity. The other medications found to negatively affect growth factor production include cilostazol, clopidogrel with aspirin, dipyridamole, and ramatroban. Although each of these substances was only validated by a single study, it is possible that these compounds may alter PRP efficacy.

A variety of activating agents are being used to stimulate platelets in the preparation of PRP to elicit maximum growth factor release. CaCl<sub>2</sub> is thought to exert its effect by directly activating platelets.<sup>28</sup> Toyoda et al<sup>28</sup> were unable to detect a Ca<sup>2+</sup>-sensing receptor and postulated that calcium may enter the cell through a leak pathway and directly activate cellular machinery, resulting in TxA<sub>2</sub> synthesis and granule secretion. Cavallo et al<sup>5</sup> found that it induced a more progressive release of growth factors than TBN or collagen. This may explain the resilience of CaCl<sub>2</sub>-activated PRP to ASA in the 2 studies.<sup>1,2</sup>

TBN primarily induces platelet activation through protease-activated receptor (PAR) 1.<sup>4</sup> It is thought to cleave the receptor's N-terminal exodomain, yielding a tethered ligand, which then binds the receptor. G-proteins are then activated, resulting in downstream PLC and PKC activation. Other complexes are involved, such as the activators PAR4 and glycoprotein (GP) Iba and the possible inhibitor GPV, which may check GPIba until it is cleaved. The activation of PLC and PKC by the G-proteins may offer a mechanism of platelet activation that is not dependent on TxA<sub>2</sub> from COX-1 and COX-2, explaining the relatively robust growth factor release when ASA is introduced.

Collagen type I was found to be a weaker platelet activator than CaCl<sub>2</sub> or TBN, with lower levels of growth factors and no clot formation.<sup>5</sup> It is believed to activate platelets through a 2-step mechanism.<sup>15</sup> Collagen may initially bind GPIa/IIa to hold the platelet in place and GPVI to activate the platelet. This is based on the finding that platelets with GPIa/IIa but not GPVI only elicit a partial response to TBN, activating tyrosine kinase cellular sarcoma (c-src), but not protein-tyrosine kinase p72syk (P72<sub>syk</sub>), phospholipase C $\gamma$ 2 (PLC $\gamma$ 2), or focal adhesion kinase (P125<sub>fak</sub>). PLC then cleaves phosphatidylinositol-4,5-bisphosphate to diacylglycerol and inositol 1,4,5-trisphosphate, which go on to activate PKC and release Ca<sup>2+</sup>.<sup>22</sup> Cho et al<sup>6</sup> hypothesized that there are 2 different pathways, one with high levels of collagen causing aggregation in platelets via integrin  $\alpha$ IIB $\beta$ 3 through a mechanism independent of PLC or TxA<sub>2</sub> and another that responds to lower levels and requires secreted products, such as thromboxane.

ADP mediates platelet activation through binding the GPCRs P2Y<sub>1</sub> and P2Y<sub>12</sub>, both of which are required for complete activation.<sup>33</sup> P2Y<sub>1</sub> is coupled to a G<sub>q</sub> protein that activates PLC, resulting in downstream effects, such as inducing platelet shape change and intracellular Ca<sup>2+</sup> release and platelet aggregation.<sup>13</sup> P2Y<sub>12</sub> is coupled to a G<sub>i</sub> protein that inhibits adenylate cyclase and subsequently impairs cAMP production, which normally impairs aggregation.<sup>8</sup> This may be related to the decrease in lectin-like oxidized LDL receptor-1 (LOX-1) associated with ASA.<sup>19</sup> Considering that LOX-1 is important for ADP-mediated platelet integrin activation, possibly mediated by PKC, it is conceivable that ASA may inhibit ADP-induced platelet activation through impairing LOX-1.

Last, AA is an upstream molecule that provides prostaglandins after getting cleaved by COX enzymes. Given that ASA directly inhibits COX-1 and COX-2, it offers a reasonable explanation for the significant attenuation of growth factor release in the 1 study using AA.<sup>12</sup>

It appears that the type of activating agent used in PRP is crucial and may allow PRP's therapeutic activity to be maintained despite anti-inflammatory drug and/or antiplatelet use. An evidence-based support for activating agents that maintain PRP's therapeutic activity will not only provide patients the opportunity to avoid choosing between maintaining their therapeutic regimen and utilizing PRP, but also allow providers and patients to have a more educated discussion on the risk-benefit analysis of holding antiplatelet and anti-inflammatory drugs before initiating PRP therapy.

## Limitations

This study has several limitations. First, criteria were defined using terms that are commonly reported in PRP activation; however, PRP terminology is variable, which could have resulted in the exclusion of important studies. Moreover, many of the studies were level 2, marked by nonrandomized design and small sample sizes. Results from these may not be representative of the clinically relevant population. The studies in this review primarily assessed human PRP, however their characteristics of growth factor release were limited by ex vivo activation and non-freshly isolated PRP.

## CONCLUSION

As the use of PRP and orthobiologics becomes more prevalent in clinical practice, taking into account the effects of using antiplatelets and anti-inflammatory drugs on PRP becomes more crucial. In this systematic review, we focused on the effect of using anti-inflammatory and antiplatelet medications with and without activating agents on the production of platelet growth factors. While the results were not definitive, it appeared that the use of CaCl<sub>2</sub> or TBN alone as activation agents for PRP was, in general, not significantly affected by antiplatelets, and the use of collagen, ADP, and AA as activation agents showed a reduction in growth factors. There are limited high-quality data on the subject at this time, and future clinical research is warranted.

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