

Is Antiplatelet Therapy Contraindicated After Platelet-Rich Plasma Treatment?

A Narrative Review

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Background: Antiplatelet therapies are often withheld before and after platelet-rich plasma product (PRPP) administration due to theoretical concerns that therapies that inhibit the function of platelets would inhibit the effects of PRPP.

Purpose/Hypothesis: The purpose of this study was to evaluate the effect that antiplatelet therapies have on the ability of PRPP to stimulate wound healing and tissue regeneration. Our hypothesis was that antiplatelet therapies would have highly heterogeneous effects on the biological activity of PRPP.

Study Design: Narrative review.

Methods: The Medline database was searched via PubMed to identify all studies related to PRPP and antiplatelet therapies, yielding 1417 publications. After the search was confined to articles published after 1995, there were 901 articles remaining. All abstracts were then screened to identify animal or human clinical studies that focused on growth factor or inflammatory cytokine production or treatment outcomes. We limited our analysis to studies reporting on orthopaedic pathologies and in vitro studies of antiplatelet therapies. Ultimately, 12 articles fit the search criteria.

Results: The majority of studies reported on the use of nonsteroidal anti-inflammatory drugs as antiplatelet therapy. The majority of studies were in vitro analyses of growth factors, inflammatory cytokines, or cell viability, whereas 1 study examined clinical outcomes in an animal model. None of the studies investigated clinical outcomes in humans. All of the studies showed no effect or mixed effects of antiplatelet therapies on PRPP efficacy. One study showed PRPP recovery to baseline function after a 1-week washout period.

Conclusion: The literature did not provide support for the common clinical practice of withholding antiplatelet therapies in patients being treated with PRPP.

Keywords: NSAIDs; biological healing enhancement; platelet-rich plasma; growth factors/healing enhancement

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Platelet-rich plasma products (PRPPs) are therapeutics used to enhance tissue regeneration. The use of these products began >20 years ago in oral and maxillofacial fields.³ Efficacy of these modalities has been shown in many patient populations, including cardiac surgery,⁵³ dentistry,²⁶ and orthopaedics.⁴⁹

PRPP encompasses 4 autologous formulations: platelet-rich plasma (PRP), platelet-rich fibrin, plasma rich in growth factors (PRGF), and platelet-rich plasma gel. Each formulation has a specific preparation protocol, and uses vary by specialty.^{4,18,49,71} The formulations are similar in that they contain concentrations of platelets, growth factors, inflammatory cytokines, and other anabolic molecules that are higher than are corresponding concentrations in whole blood. Red blood cells are typically eliminated, but different centrifugation protocols may either exclude or concentrate leukocytes, producing either leukocyte-rich PRP (LR-PRP) or leukocyte-poor PRP.⁴² Recent classification systems also consider the use of PRPP activation,

including which molecule is used to activate the biological substance.^{17,44} Wide variability is found in the final concentrations of the numerous anabolic and catabolic proteins in the different formulations, resulting in differences in biological activity. Consequently, there is controversy over which preparation is most efficacious in treating symptoms or stimulating tissue regeneration.

Despite the frequent use of PRPPs, their mechanism of action is not well understood. It is thought that the benefits stem from the abundance of platelet-derived anabolic factors, inflammatory cytokines, anti-inflammatory mediators, vasoactive amines, and numerous other signaling molecules found in α -granules and dense granules.¹ Important factors released by α -granules include insulin-like growth factor 1, transforming growth factor β (TGF- β), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), and basic fibroblast growth factor (FGF).⁴⁹ Dense granules release serotonin, adenosine, dopamine, calcium, histamine, adenosine diphosphate (ADP), adenosine triphosphate, and catecholamines.⁴⁹ In addition, platelet activation leads to the release of inflammatory mediators, which include platelet factor 4, interleukin (IL) 1 β , and platelet basic protein.¹ In sum, these factors may influence cell chemotaxis, cell migration, mitosis, extracellular matrix production, and angiogenesis, leading to cell maturation, differentiation, and eventual tissue repair.⁴⁹

In the setting of orthopaedic surgery, PRPPs have been used to treat various pathologies. Clinical trials have shown PRP is a promising treatment for lateral epicondylitis^{16,19,30,34,46,55,67} and osteoarthritis.^{24,29,33,35,54} Patients with these conditions often take medications to treat their pain, most commonly nonsteroidal anti-inflammatory drugs (NSAIDs). Current clinical practice includes the recommendation to withhold these medications before PRPP therapy. These recommendations are largely based on known inhibitory effects of NSAIDs on platelets. However, such recommendations are based on minimal clinical and laboratory data. Given how commonly these medications are prescribed, clarification is needed regarding whether and to what extent the antiplatelet agents taken by patients at the time of blood donation for PRP preparation adversely affect the efficacy of autologous platelet delivery products. Only with this information can rational and evidence-based recommendations be made concerning the use of antiplatelet agents in PRPP donors.

Considerable work has been done to examine the effect of antiplatelet therapy on the function of platelets. Much of this research has used only PRP as a modality to isolate platelets and has mainly focused on the aggregation of platelets *in vitro* as a measure of platelet function. In this literature review, we evaluate the effect that antiplatelet therapies have on the ability of PRP to stimulate wound healing and tissue regeneration. Our hypothesis was that antiplatelet therapies would have highly heterogeneous effects on the biological activity of PRPP. The literature

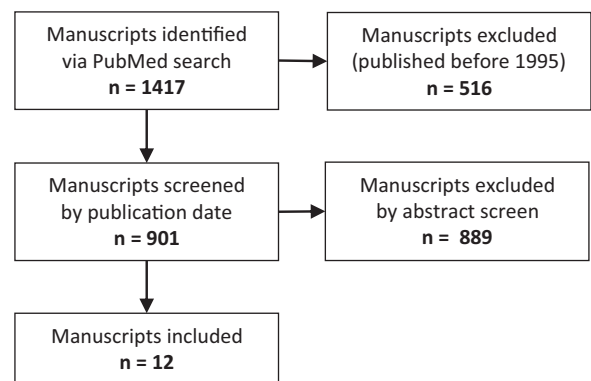


Figure 1. Flow diagram of selection process for studies included in this review.

search included all types of PRPP preparations and focused on *in vitro* studies that have examined differential growth factor and inflammatory marker expression as well as *in vivo* studies.

METHODS

The MEDLINE database via PubMed (<https://www.ncbi.nlm.nih.gov/pubmed>) was searched using the following search criteria: (“Platelet Aggregation Inhibitors”[Medical Subject Headings (MeSH)] OR PRASUGREL[limited to title or abstract (tiab)] OR “Antiplatelet”[tiab] OR “Platelet Aggregation Inhibitors”[tiab] OR “Platelet Antagonists”[tiab] OR CANGRELOR[tiab] OR TICAGRELOR[tiab] OR TIROFIBAN[tiab] OR EPTIFIBATIDE[tiab] OR CLOPIDOGREL[tiab] OR ABCIXIMAB[tiab] OR CILOSTAZOL[tiab] OR DIPYRIDAMOLE[tiab] OR aspirin [tiab]) OR (“Anti-Inflammatory Agents, Non-Steroidal”[MeSH] OR “Non-steroidal anti-inflammatory drugs”[tiab] OR “Non-steroidal anti-inflammatory drug”[tiab] OR “Non-steroidal anti-inflammatory agent”[tiab] OR “Non-steroidal anti-inflammatory agents”[tiab] OR NSAID*[tiab] OR “Anti-Inflammatory Analgesics”[tiab] OR “Aspirin-Like Agents”[tiab])) AND (“Platelet-Rich Plasma”[MeSH] OR “platelet rich plasma”[tiab]).

This search yielded 1417 articles. When the search was restricted to publications after 1995 (when the use of PRPP for enhanced wound healing in patients generally began), the number of articles was reduced to 901 (Figure 1).

Inclusion criteria were as follows: all animal and human studies of the effect of antiplatelet therapy on the efficacy of PRPP for tissue regeneration, focusing on the production of anabolic factors, inflammatory and anti-inflammatory mediators, and clinical outcomes. Exclusion criteria were studies including patients <18 years old, any study assessing how platelet function is affected in patient populations other than healthy participants or those with musculoskeletal diseases, and any studies that added antiplatelet agents *in vitro*. After elimination by these criteria, 12 studies remained (Table 1).

TABLE 1
Summary of the 12 Studies Included in This Review^a

Lead Author (Year)	Outcome ^b	PRPP Formulation	Conclusions	Effect of NSAIDs on PRPP
Anitua (2015) ⁵	Anabolic factors, cell proliferation, and migration	PRGF	PRGF release of PDGF-AB, TGF- β 1, VEGF, and HGF was not affected by drug consumption. Cell proliferation and migration were not affected.	No effect
Anitua (2014) ⁶	Anabolic factors, cell proliferation, and migration	PRGF	PRGF release of VEGF, PDGF-AB, and IGF-1 was not affected by drug consumption. Cell proliferation was not affected by drug consumption, and cell migration was significantly increased in the experimental groups. There was no effect on extracellular matrix proteins secreted by gingival fibroblasts.	No effect
Beitzel (2013) ¹⁰	Cell viability	PRP	Chondrocyte viability was not affected by ketorolac use. Tenocyte viability trended lower with ketorolac use but was not completely inhibited compared with control.	Mixed effect
Jayaram (2019) ³²	Anabolic factors	LR-PRP	ASA use inhibited AA-mediated release of VEGF, TGF- β 1, and PDGF-AB. ASA had no effect on thrombin-mediated release of VEGF and TGF- β 1 and only partially inhibited PDGF-AB.	Mixed effect
Ludwig (2017) ⁴⁰	Anabolic factors	PRP	COX-2 inhibitors did not impair platelet activation, growth factor release, or TXB2 production when activated by human- γ -thrombin.	No effect
Mannava (2019) ⁴¹	Anabolic factors	LR-PRP	Naproxen use diminished PDGF-AA, PDGF-AB, and IL-6 but did not affect TNF- α , IL-1 β , IL-8, VEGF, and FGF-2. All factors that were diminished returned to normal after a 1-wk washout period.	Mixed effect
Meadows (2017) ⁴⁵	Energy to failure	PRP	NSAID use after rotator cuff surgery did not affect PRP efficacy.	No effect
Schippinger (2015) ⁶⁰	Anabolic factors	PRP	NSAIDs inhibited platelet aggregation when activated by AA but not TRAP-6, ADP, or collagen.	Mixed effect
Smith (2007) ⁶³	Anabolic factors	PRP	No evidence was found of decreased anabolic factor production with the use of ASA or ASA + clopidogrel.	No effect
Tian (2019) ⁶⁸	Anabolic factors	PRP	GDF-11 was significantly decreased in the group taking antiplatelet medications, whereas FGF and PDGF-AA were significantly increased in this group. Six other anabolic factors studied were not significantly different between groups.	Mixed effect
Utku (2017) ⁷⁰	Anabolic factors	PRP	Meloxicam, diclofenac, and ASA use did not affect PDGF-AB or VEGF production.	No effect
Velier (2018) ⁷¹	Anabolic factors	PRP	No difference was seen in thrombin production and platelet activation in response to TRAP-6, but there was significantly decreased ADP-induced platelet activation.	Mixed effect

^aAA, arachidonic acid; ADP, adenosine diphosphate; ASA, aspirin; COX-2, cyclooxygenase 2; FGF, basic fibroblast growth factor; GDF-11, growth differentiation factor 11; HGF, hepatocyte growth factor; IGF-1, insulin-like growth factor 1; IL, interleukin; LR, leukocyte-rich; NSAID, nonsteroidal anti-inflammatory drug; PDGF, platelet-derived growth factor; PRGF, platelet-rich growth factor; PRP, platelet-rich plasma; PRPP, platelet-rich plasma product; TGF- β 1, transforming growth factor β -1; TNF- α , tumor necrosis factor α ; TRAP-6, thrombin receptor-activated protein 6; TXB2, thromboxane B2; VEGF, vascular endothelial growth factor.

^bThe term *anabolic factors* means that studies attempted to measure growth factors, inflammatory cytokines, and other molecules believed to stimulate platelet activation and wound healing.

RESULTS

In Vitro Analyses

A large body of research, mainly in the field of hematology, has established the effects and mechanisms of action of antiplatelet drugs. Many of these studies have used PRP in order to measure the ability of platelets to aggregate as an indicator of platelet function. More recent studies have examined these questions relevant to musculoskeletal conditions. Schippinger et al⁶⁰ showed that patients who received daily NSAIDs produced PRP that exhibited significantly inhibited platelet aggregation as measured using

light transmission aggregometry. Importantly, this result was found when their PRP was activated via arachidonic acid (AA) but not via collagen, ADP, or thrombin receptor-activated protein 6 (TRAP-6).

The orthopaedic literature has also examined the effects of commonly used anti-inflammatory medications and PRP. In one study, levels of VEGF and PDGF-AB in PRP were measured in healthy volunteers treated using diclofenac, meloxicam, or aspirin (ASA) (each medication at 2 doses) and compared with levels in healthy volunteers taking no medications. The investigators found no differences in levels of growth factors in the experimental groups compared with in controls.⁷⁰ Further evidence to support the

lack of effect of cyclooxygenase (COX) 2 inhibition on PRP protein release came from a study in dogs. Ludwig et al⁴⁰ showed no statistically significant decreases in TGF- β 1, PDGF-BB, or thromboxane B2 release in PRP in dogs given carprofen compared with in controls when activated in vitro via human- γ -thrombin.

Other studies examining NSAIDs and PRP have found mixed results. In 2019, Mannava et al⁴¹ investigated the influence of naproxen on expression of growth factors (PDGF-AA, PDGF-AB, VEGF, and FGF-2) and inflammatory cytokines (tumor necrosis factor α [TNF- α], IL-1 β , IL-6, and IL-8). Patients taking naproxen showed significant decreases in PRP expression of PDGF-AA, PDGF-AB, and IL-6 but no decrease in TNF- α , IL-1 β , IL-8, VEGF, and FGF-2. However, PDGF-AA, PDGF-AB, and IL-6 showed recovery back to baseline after a 1-week washout period.

Tian et al⁶⁸ published a similar study in 2019 that examined the effect of antiplatelet medications on 9 anabolic factors after activation via thrombin and calcium. Only 1 anabolic factor (growth differentiation factor 11) was significantly lower in the group taking antiplatelet therapies compared with in a control group of the same age range. In addition, 2 anabolic factors (FGF-2 and PDGF-AA) were significantly higher in the antiplatelet therapy group. Of note, the antiplatelet therapies used were not specified.

A study of 12 healthy volunteers showed that 81 mg of ASA daily led to significantly inhibited AA-mediated release of VEGF, PDGF-AB, and TGF- β 1 in LR-PRP.³² However, ASA did not affect thrombin-mediated release of VEGF and TGF- β 1 and only partially inhibited PDGF-AB.

Another field driving research in PRPP is cardiac surgery. The effect of antiplatelet therapy is important in this patient population, where these agents are often required to manage underlying vascular disease. One group investigated cardiac surgery patients receiving ASA, ASA plus clopidogrel, or no antiplatelet therapy.⁶³ The investigators found a slight decrease in measured PDGF-BB and TGF- β 1 in PRP from patients in the 2 experimental groups as compared with control, but it was statistically insignificant.

The use of anticoagulants has also been reviewed. One study examined the effects of anticoagulants and antiplatelet therapy on platelet-rich plasma gel.⁷¹ The investigators reported no difference between antiplatelet/coagulation therapy and control groups in terms of basal expression of P-selectin, autologous thrombin production, and platelet aggregation in response to TRAP-6. P-selectin, a transmembrane protein found on α -granules translocated to the cell membrane during degranulation, is used as a biomarker of platelet activation.⁶⁵ Patients taking anticoagulants/platelet therapies had a significantly lower ADP-induced P-selectin expression. However, an important limitation of this study was the comparison of healthy, young volunteers (control) with older patients with comorbidities taking anticoagulants or antiplatelet therapies.⁷¹

One study examined the effects of ASA, acenocoumarol (vitamin K antagonist), and glucosamine sulfate on PRGF.⁶ Platelet aggregation, activation, and anabolic factor production were not significantly affected by any of the drugs. No differences were found between groups in proliferation

and migration of gingival fibroblasts after the addition of PRGF in cell culture. The investigators also measured the synthesis of different growth factors and extracellular matrix proteins by gingival fibroblasts, including hepatocyte growth factor, insulin-like growth factor 1, VEGF, and procollagen type 1, and found no statistically significant differences.

The same group conducted a similar experiment in tendon fibroblasts.⁵ The investigators enrolled patients treated using ASA, acenocoumarol, glucosamine sulfate, or glucosamine sulfate plus chondroitin sulfate and isolated PRGF. The investigators found that platelet activation and the platelet content of PDGF-AB, TGF- β 1, VEGF, and hepatocyte growth factor in PRGF did not differ between the experimental groups and the control group. They also found no differences between experimental and control groups in terms of cell migration and proliferation of tendon fibroblasts after treatment using PRGF. However, significantly lower expression of VEGF, hyaluronic acid, and fibronectin was noted in tendon fibroblasts treated using PRGF from the group taking acenocoumarol. This difference was not present for the ASA, glucosamine sulfate, or glucosamine sulfate plus chondroitin sulfate groups.

Beitzel et al¹⁰ examined the viability of chondrocytes and tenocytes after treatment using PRP. The investigators studied chondrocyte and tenocyte viability when treated in culture using PRP with and without ketorolac. They found that chondrocyte viability at 120 hours after treatment was similar between the 2 groups, whereas tenocyte viability trended lower for the PRP plus ketorolac group. Of note, the tenocyte cell count in the PRP plus ketorolac group was greater than that in the control group and the ketorolac group.

In Vivo, Human Clinical Studies

The basic laboratory studies described above examined whether antiplatelet therapies could affect platelet release of various anabolic factors. However, determining whether those effects translate into significant differences in clinical outcomes will be critical to formulating recommendations for patient care. To the best of our knowledge, no clinical studies in humans have been conducted to explore the effects of antiplatelet therapy on PRPP efficacy, and only anecdotal evidence has been published in this regard.

In Vivo, Animal Clinical Studies

The in vivo effects of antiplatelet therapy on PRPP were evaluated in 1 animal study. Meadows et al⁴⁵ tested the biomechanical properties of rat supraspinatus tendons in animals that were treated using PRP after supraspinatus tendon detachment and repair then randomized to a diet with or without the addition of indomethacin and in a comparison control group treated using saline. Biomechanical testing 3 weeks after tendon repair and PRP treatment demonstrated no difference in energy to failure between the PRP and the PRP plus indomethacin groups.

DISCUSSION

Over the past few decades, autologous blood products have been used by physicians as modalities to treat symptoms, improve wound healing, and possibly stimulate tissue regeneration. Theoretical concern has arisen as to whether therapies that inhibit the function of platelets will inhibit the effects of PRPP. Current practice by many physicians prescribing NSAIDs and other medications with antiplatelet activity is to withhold these medications before and after PRPP administration. The major finding of this review is that the literature provides minimal support for the conclusion that antiplatelet therapies are contraindicated in patients being treated using PRPP.

Our purpose was to perform a comprehensive literature review of the effect of antiplatelet therapies on biological activity and clinical efficacy of PRPP. We focused on growth factor and inflammatory cytokine production as well as clinical outcomes in humans and animals to evaluate whether the clinical practice of withholding antiplatelet medications is supported by evidence. The vast majority of studies have focused on NSAIDs, which is the focus of this discussion.

The general consensus among practicing physicians is to withhold NSAIDs before administration of PRPP, which is based on the extensive *in vitro* research studying platelet activation and thrombus formation. These data have shown decreased platelet aggregation and thrombus formation in the presence of NSAIDs⁵⁹ and are supported by platelet physiologic characteristics. NSAIDs inhibit COX-1-induced conversion of AA into thromboxane A₂ (TXA₂), a potent platelet activator. TXA₂ is important in the continuation of platelet activation during thrombus formation and the mediation of platelet aggregation via fibrinogen- α II β 3 bridges among adjacent platelets.³¹ Without efficient formation of TXA₂, the ability to maintain thrombi is diminished.

The data presented in this review do not support an inhibitory effect of antiplatelet medications on the biological activity of PRPP. We posit that this discrepancy could be due to at least 3 possible reasons, which are discussed in the remainder of this review: (1) Antiplatelet therapies block only 1 pathway (AA/COX-1) in a pathophysiologically complex system of platelet activation; (2) the extravascular environment into which PRP is introduced for treatment (eg, connective tissue) activates platelets *in vivo* via COX-1-independent mechanisms; and (3) inhibition of the COX-1 pathway by NSAIDs diverts AA metabolism away from COX-1-mediated TXA₂ production to increased activity of the alternative pathway, 12-lipoxygenase, the products of which may stimulate tissue regeneration.

Platelet Activation Pathways Have Significant Overlap

Platelet activation can occur via multiple overlapping pathways. One of these is the AA/COX-1 pathway. When this pathway is inhibited by NSAIDs, platelets can still be activated via other pathways, particularly when the platelets are stimulated by more potent agonists such as thrombin or

collagen, which are more likely to be encountered *in vivo* by platelets within the tissues into which they are injected.

If degranulation (which releases anabolic factors necessary for tissue regeneration) plays an important role in the regenerative effects of PRPP, then the key concepts to understand entail the pathways that (1) stimulate granule secretion (primary activation) and (2) are stimulated by factors produced after primary activation (secondary activation). Under normal circumstances, platelets circulate in blood in a quiescent state that is maintained by the antithrombotic properties of intact vascular endothelial cells that line the intimal surfaces of all vessels. When local vascular injury occurs, a breach in the continuity of the endothelial monolayer exposes subendothelial substances in vascular connective tissue, the most important of which is collagen. Collagen-bound von Willebrand factor stimulates platelet adhesion, the process of platelet-endothelial interaction *in vivo*, and primary platelet activation in both COX-1-dependent and -independent ways.^{20-22,25,38,39} Collagen itself can bind to platelets and stimulate activation without requiring the formation of TXA₂ via COX-1.^{20,23} NSAIDs inhibit a downstream step in activation of platelets via collagen, thus only partially inhibiting primary platelet activation.

Platelets are also activated directly via inflammatory cytokines. Damage-associated molecular patterns are molecules produced by host tissue in response to tissue damage. They activate platelets due to the presence of toll-like receptors on platelet membranes^{2,8,14,62,77} and can be activated by oxidized lipids,¹¹ oxidative stress,⁵² and high mobility group box 1, a DNA-binding protein released by macrophages and monocytes during inflammation⁴⁷ and released from platelet granules.⁷² NSAIDs do not affect the release of high mobility group box 1 from platelet granules, which may play a role in mitigating any inhibitory effect on platelets in PRPP.

Furthermore, secondary platelet activation is an important mechanism by which platelet activation signals are amplified and sustained. TXA₂ is unique in that it stimulates secondary platelet activation after *de novo* synthesis from free AA in the cytoplasm rather than after release from platelet granules.^{9,20,76} Although inhibition of COX-1 significantly decreases TXA₂ production, platelet granules release a large variety of molecules, many of which stimulate platelet activation (eg, ADP, serotonin, von Willebrand factor, fibrinogen, coagulation factors).²⁸ Inhibiting TXA₂ production blocks one of the secondary platelet activation mechanisms and likely does not lead to major effects on biological activity of PRPP.

Thrombin is considered to be one of the strongest activators of platelets and participates in their sustained activation. It is the final product of the coagulation cascade and catalyzes the formation of the fibrin mesh network that stabilizes clots. Thrombin also activates platelets via pathways that are independent of COX-1, described elsewhere by Estevez and Du.²⁰

Thrombin's independence from COX-1 has been supported by recent studies. Jayaram et al³² showed that ASA significantly inhibited AA-mediated release of VEGF, PDGF-AB, and TGF- β 1 in LR-PRP but had no effect on

thrombin-mediated release of VEGF and TGF- β 1 and only partially inhibited the release of PDGF-AB. Schipinger et al⁶⁰ showed that orthopaedic patients taking NSAIDs postoperatively showed no difference in PRP platelet aggregation compared with healthy controls when activated via TRAP-6. In contrast, platelet aggregation was abolished when activated via AA in that study.

Outside-in signaling also acts via COX 1—*independent* mechanisms. Outside-in signaling is the process by which platelets aggregate, when linker molecules (eg, fibrinogen) connect integrins (most commonly α IIB β 3) of adjacent platelets. These linker molecules that bind to integrins also trigger cytoplasmic signaling, activation of platelets, and thrombus formation.^{37,61} These pathways were reviewed elsewhere²⁰ and are thought to be COX-1 independent.

All of the data presented above may seem to contradict our understanding of why NSAIDs are effective at treating thrombosis. The explanation may require a more nuanced understanding of platelet activation. Platelet activation to achieve tissue regeneration may not be affected by antiplatelet drugs to the same extent as is thrombus formation. The ability of platelets to form patent and functional thrombi is a complex process that relies on the simultaneous activation of multiple transmembrane receptors by multiple signaling molecules: ADP, 5-hydroxytryptamine, TXA2, collagen, and others.^{13,50,51} Thrombus formation is likely to be inhibited more easily because it requires many concomitant activation signals to maintain. Tissue regeneration may not require this simultaneous stimulation.

It is clear that platelets mediate other processes in addition to hemostasis, including tissue regeneration. The mechanisms of platelet activation and inhibition are also much more complex than originally thought. Inhibitors of COX-1 may be quite sufficient to provide thromboprophylaxis for arterial vascular events but may not completely affect pathways of platelet activation that promote tissue regeneration.

Intravascular Versus Extravascular Platelet Environment

Platelets exist in homeostatic balance within the vasculature. A healthy endothelial cell layer prevents thrombosis through the release of inhibitory mediators from the intact endothelium,²⁷ such as nitric oxide, carbon monoxide, prostacyclin, endothelial ecto-ADPase (CD39/nucleoside triphosphate diphosphohydrolase), and glycocalyx.

When platelets leave the vasculature, the endothelial factors that inhibit platelet activation are no longer present, leading to a shift from homeostasis toward activation. The lack of environmental inhibitory signals in musculoskeletal tissues, such as the joint space, around tendon, or other locations where PRPP is injected, likely contributes to platelet activation in these tissues. It is well established that collagen can activate platelets, and collagen is abundant in all connective tissues that are commonly treated using PRPP. The combination of a lack of intravascular inhibitory signals and additional

excitatory signals in musculoskeletal tissues may lead to a strong level of platelet activation that overcomes any inhibitory effects of antiplatelet therapies used by patients.

COX-1 Inhibition May Lead to Tissue Regeneration via the Lipoxygenase Pathway

Lipoxygenases (LOXs) catalyze the oxygenation of unsaturated fatty acids, such as AA.⁵⁶ The isoform highly expressed in platelets is 12-LOX.¹² 12-LOX is analogous to COX-1 in that it converts AA into an intracellularly active product, 12-hydroxy-5,8,10,14-eicosatetraenoic acid (12-HETE) (the analogous product for COX-1 would be TXA2).⁶⁹ Inhibition of the COX-1 pathway may lead to the availability of a higher concentration of AA to be converted into 12-HETE.

Studies to date have suggested that 12-HETE plays a role in platelet activation. The first evidence of this came from a clinical study in which patients with decreased 12-LOX activity had a higher incidence of bleeding complications.⁵⁸ Animal studies have also supported the conclusion that 12-LOX plays an important role in platelet activation.⁷³⁻⁷⁵ The proaggregatory effect of 12-HETE is due to its ability to activate platelet Nicotinamide adenine dinucleotide phosphate oxidase to generate reactive oxygen species (a platelet activator)⁴⁸ and its role in protease activated receptor-4 and Glycoprotein VI-mediated signaling and subsequent dense granule degranulation.⁷³

The important implication for use of PRP in musculoskeletal tissues is that 12-HETE has been shown to play an important role in tissue regeneration and wound repair. For example, Tang et al⁶⁶ showed that exogenous 12(S)-HETE promoted wound repair of endothelial cells, likely due in part to augmenting DNA synthesis. In addition, a select 12-HETE inhibitor diminished normal endothelial cell growth *in vitro*, suggesting that 12-HETE is part of an endogenous physiologic tissue repair response.⁶⁶ 12-HETEs have also been shown to play an important role in inflammation and wound repair of the skin and ocular surface.^{7,15,57} These studies highlight the complexity of tissue regeneration pathways and the need for more research in this area. Further exploration of these may explain, in part, the lack of complete inhibition that NSAIDs have on PRPP growth factor and cytokine production.

Other Considerations

It is possible that PRPP augments tissue regeneration via mechanisms other than the production of anti-inflammatory and immunomodulatory mediators. An important mechanism may be the ability of platelets to attract connective tissue progenitor or stem cells to the site of injury. Platelets express stromal cell—derived factor 1, which recruits CD34+ hematopoietic stem cells as well as release factors that promote their differentiation into endothelial cells.^{36,43,64} Augmentation of endothelial repair likely plays a role in regeneration of bone and soft connective tissues.

Limitations

The method of the narrative review is limited because we did not follow the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines for systematic reviews, which means we did not assess for study quality or heterogeneity, we had no protocol for assessment of studies, and we used only 1 medical literature database. The review is also limited by the fact that it includes a small set of heterogeneous studies primarily examining in vitro analyses of anabolic factor production. There is a paucity of in vivo studies that have evaluated the effect of antiplatelet therapies on platelet aggregation, thrombus formation, and production of anabolic signaling molecules. Further study is clearly required to understand whether and how the lack of inhibition of PRPP by antiplatelet agents in vitro translates to the clinical setting. Future research should be conducted in humans following Minimum Information for Studies Reporting on Biologics guidelines with robust analysis of outcomes, including evaluation of pain reduction, structural changes on imaging, and functional outcomes.

CONCLUSION

The complex biological process of platelet function makes it difficult to draw definitive conclusions about the role of antiplatelet therapies on biological activity and ultimate efficacy of PRPP. However, the available basic science and limited clinical data do not support the current widespread practice of withholding antiplatelet therapies before or during PRPP administration. Further rigorous research is required to conclusively understand how such data translate to clinical outcomes.

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REFERENCES

- Andia I, Maffulli N. Platelet-rich plasma for managing pain and inflammation in osteoarthritis. *Nat Rev Rheumatol*. 2013;9(12):721-730.
- Andonegui G, Kerfoot SM, McNagny K, Ebbert KV, Patel KD, Kubes P. Platelets express functional Toll-like receptor-4. *Blood*. 2005;106(7):2417-2423.
- Anitua E. Plasma rich in growth factors: preliminary results of use in the preparation of future sites for implants. *Int J Oral Maxillofac Implants*. 1999;14(4):529-535.
- Anitua E, Sánchez M, Orive G, Andia I. The potential impact of the preparation rich in growth factors (PRGF) in different medical fields. *Biomaterials*. 2007;28(31):4551-4560.
- Anitua E, Troya M, Zalduendo M, 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(1):57-64.
- Anitua E, Troya M, Zalduendo M, Orive G. The effect of different drugs on the preparation and biological outcomes of plasma rich in growth factors. *Ann Anat*. 2014;196(6):423-429.
- Ashkar S, Mesentsev A, Zhang WX, Mastuyugin V, Dunn MW, Laniado-Schwartzman M. Retinoic acid induces corneal epithelial CYP4B1 gene expression and stimulates the synthesis of inflammatory 12-hydroxyeicosanoids. *J Ocul Pharmacol Ther*. 2004;20(1):65-74.
- Aslam R, Speck ER, Kim M, et al. Platelet toll-like receptor expression modulates lipopolysaccharide-induced thrombocytopenia and tumor necrosis factor-alpha production in vivo. *Blood*. 2006;107(2):637-641.
- Becker RC, Sexton T, Smyth SS. Translational implications of platelets as vascular first responders. *Circ Res*. 2018;122(3):506-522.
- Beitzel K, McCarthy MB, Cote MP, et al. The effect of ketorolac tromethamine, methylprednisolone, and platelet-rich plasma on human chondrocyte and tenocyte viability. *Arthroscopy*. 2013;29(7):1164-1174.
- Biswas S, Xin L, Panigrahi S, et al. Novel phosphatidylethanolamine derivatives accumulate in circulation in hyperlipidemic ApoE^{-/-} mice and activate platelets via TLR2. *Blood*. 2016;127(21):2618-2629.
- Burkhart JM, Vaudel M, Gambaryan S, et al. The first comprehensive and quantitative analysis of human platelet protein composition allows the comparative analysis of structural and functional pathways. *Blood*. 2012;120(15):e73-e82.
- Cho MJ, Liu J, Pestina TI, et al. The roles of alpha IIb beta 3-mediated outside-in signal transduction, thromboxane A2, and adenosine diphosphate in collagen-induced platelet aggregation. *Blood*. 2003;101(7):2646-2651.
- Cognasse F, Hamzeh H, Chavarin P, Acquart S, Genin C, Garraud O. Evidence of toll-like receptor molecules on human platelets. *Immunol Cell Biol*. 2005;83(2):196-198.
- Conners MS, Urbano F, Vafeas C, Stoltz RA, Dunn MW, Schwartzman ML. Alkali burn-induced synthesis of inflammatory eicosanoids in rabbit corneal epithelium. *Invest Ophthalmol Vis Sci*. 1997;38(10):1963-1971.
- Creaney L, Wallace A, Curtis M, Connell D. Growth factor-based therapies provide additional benefit beyond physical therapy in resistant elbow tendinopathy: a prospective, single-blind, randomised trial of autologous blood injections versus platelet-rich plasma injections. *Br J Sports Med*. 2011;45(12):966-971.
- DeLong JM, Russell RP, Mazzocca AD. Platelet-rich plasma: the PAW classification system. *Arthroscopy*. 2012;28(7):998-1009.
- Dohan Ehrenfest DM, 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(3):158-167.
- Edwards SG, Calandruccio JH. Autologous blood injections for refractory lateral epicondylitis. *J Hand Surg Am*. 2003;28(2):272-278.
- Estevez B, Du X. New concepts and mechanisms of platelet activation signaling. *Physiology (Bethesda)*. 2017;32(2):162-177.
- Estevez B, Kim K, Delaney MK, et al. Signaling-mediated cooperativity between glycoprotein Ib-IX and protease-activated receptors in thrombin-induced platelet activation. *Blood*. 2016;127(5):626-636.
- Estevez B, Stojanovic-Terpo A, Delaney MK, et al. LIM kinase-1 selectively promotes glycoprotein Ib-IX-mediated TXA2 synthesis, platelet activation, and thrombosis. *Blood*. 2013;121(22):4586-4594.
- Ezumi Y, Shindoh K, Tsuji M, Takayama H. Physical and functional association of the Src family kinases Fyn and Lyn with the collagen receptor glycoprotein VI-Fc receptor gamma chain complex on human platelets. *J Exp Med*. 1998;188(2):267-276.
- Filardo G, Kon E, Buda R, et al. Platelet-rich plasma intra-articular knee injections for the treatment of degenerative cartilage lesions and osteoarthritis. *Knee Surg Sports Traumatol Arthrosc*. 2011;19(4):528-535.
- Garcia A, Quinton TM, Dorsam RT, Kunapuli SP. Src family kinase-mediated and Erk-mediated thromboxane A2 generation are essential for VWF/GPIIb-induced fibrinogen receptor activation in human platelets. *Blood*. 2005;106(10):3410-3414.
- Giudice A, Esposito M, Bannardo F, Brancaccio Y, Buti J, Fortunato L. Dental extractions for patients on oral antiplatelet: a within-person randomised controlled trial comparing haemostatic plugs, advanced-platelet-rich fibrin (A-PRF+) plugs, leukocyte- and platelet-rich fibrin (L-PRF) plugs and suturing alone. *Int J Oral Implantol*. 2019;12(1):77-87.

27. Golebiewska EM, Poole AW. Platelet secretion: from haemostasis to wound healing and beyond. *Blood Rev.* 2015;29(3):153-162.
28. Golebiewska EM, Poole AW. Secrets of platelet exocytosis—what do we really know about platelet secretion mechanisms? *Br J Haematol.* 2013;165(2):204-216.
29. Görmeli G, Görmeli CA, Ataoglu B, Çolak C, Aslantürk O, Ertem K. Multiple PRP injections are more effective than single injections and hyaluronic acid in knees with early osteoarthritis: a randomized, double-blind, placebo-controlled trial. *Knee Surg Sports Traumatol Arthrosc.* 2017;25(3):958-965.
30. Gosens T, Peerbooms JC, van Laar W, den Ouden BL. Ongoing positive effect of platelet-rich plasma versus corticosteroid injection in lateral epicondylitis: a double-blind randomized controlled trial with 2-year follow-up. *Am J Sports Med.* 2011;39(6):1200-1208.
31. Jackson SP. The growing complexity of platelet aggregation. *Blood.* 2007;109(12):5087-5095.
32. Jayaram P, Yeh P, Patel SJ, et al. Effects of aspirin on growth factor release from freshly isolated leukocyte-rich platelet-rich plasma in healthy men: a prospective fixed-sequence controlled laboratory study. *Am J Sports Med.* 2019;47(5):1223-1229.
33. Kon E, Buda R, Filardo G, et al. Platelet-rich plasma: intra-articular knee injections produced favorable results on degenerative cartilage lesions. *Knee Surg Sports Traumatol Arthrosc.* 2010;18(4):472-479.
34. Krogh TP, Fredberg U, Stengaard-Pedersen K, Christensen R, Jensen P, Ellingsen T. Treatment of lateral epicondylitis with platelet-rich plasma, glucocorticoid, or saline: a randomized, double-blind, placebo-controlled trial. *Am J Sports Med.* 2013;41(3):625-635.
35. Lai LP, Stitik TP, Foye PM, Georgy JS, Patibanda V, Chen B. Use of platelet-rich plasma in intra-articular knee injections for osteoarthritis: a systematic review. *PM R.* 2015;7(6):637-648.
36. Langer H, May AE, Daub K, et al. Adherent platelets recruit and induce differentiation of murine embryonic endothelial progenitor cells to mature endothelial cells in vitro. *Circ Res.* 2006;98(2):e2-e10.
37. Li Z, Delaney MK, O'Brien KA, Du X. Signaling during platelet adhesion and activation. *Arterioscler Thromb Vasc Biol.* 2010;30(12):2341-2349.
38. Li Z, Xi X, Du X. A mitogen-activated protein kinase-dependent signaling pathway in the activation of platelet integrin alpha IIb beta3. *J Biol Chem.* 2001;276(45):42226-42232.
39. Li Z, Zhang G, Feil R, Han J, Du X. Sequential activation of p38 and ERK pathways by cGMP-dependent protein kinase leading to activation of the platelet integrin alpha IIb beta3. *Blood.* 2006;107(3):965-972.
40. Ludwig HC, Birdwhistell KE, Brainard BM, Franklin SP. Use of a cyclooxygenase-2 inhibitor does not inhibit platelet activation or growth factor release from platelet-rich plasma. *Am J Sports Med.* 2017;45(14):3351-3357.
41. Mannava S, Whitney KE, Kennedy MI, et al. The influence of naproxen on biological factors in leukocyte-rich platelet-rich plasma: a prospective comparative study. *Arthroscopy.* 2019;35(1):201-210.
42. Mariani E, Pulsatelli L. Platelet concentrates in musculoskeletal medicine. *Int J Mol Sci.* 2020;21(4):1328.
43. Massberg S, Konrad I, Schürzinger K, et al. Platelets secrete stromal cell-derived factor 1alpha and recruit bone marrow-derived progenitor cells to arterial thrombi in vivo. *J Exp Med.* 2006;203(5):1221-1233.
44. Mautner K, Malanga GA, Smith J, et al. A call for a standard classification system for future biologic research: the rationale for new PRP nomenclature. *PM R.* 2015;7(4)(suppl):S53-S59.
45. Meadows MC, Levy DM, Ferry CM, Gardner TR, Teratani T, Ahmad CS. Effects of platelet-rich plasma and indomethacin on biomechanics of rotator cuff repair. *Am J Orthop (Belle Mead NJ).* 2017;46(5):e336-e343.
46. Mishra AK, Skrepnik NV, Edwards SG, et al. Efficacy of platelet-rich plasma for chronic tennis elbow: a double-blind, prospective, multicenter, randomized controlled trial of 230 patients. *Am J Sports Med.* 2014;42(2):463-471.
47. Müller S, Ronfani L, Bianchi ME. Regulated expression and subcellular localization of HMGB1, a chromatin protein with a cytokine function. *J Intern Med.* 2004;255(3):332-343.
48. Nardi M, Feinmark SJ, Hu L, Li Z, Karpatkin S. Complement-independent Ab-induced peroxide lysis of platelets requires 12-lipoxygenase and a platelet NADPH oxidase pathway. *J Clin Invest.* 2004;113(7):973-980.
49. Nguyen RT, Borg-Stein J, McInnis K. Applications of platelet-rich plasma in musculoskeletal and sports medicine: an evidence-based approach. *PM R.* 2011;3(3):226-250.
50. Offermanns S. Activation of platelet function through G protein-coupled receptors. *Circ Res.* 2006;99(12):1293-1304.
51. Offermanns S, Toombs CF, Hu YH, Simon MI. Defective platelet activation in G alpha(q)-deficient mice. *Nature.* 1997;389(6647):183-186.
52. Panigrahi S, Ma Y, Hong L, et al. Engagement of platelet toll-like receptor 9 by novel endogenous ligands promotes platelet hyperactivity and thrombosis. *Circ Res.* 2013;112(1):103-112.
53. Patel AN, Selzman CH, Kumpati GS, McKellar SH, Bull DA. Evaluation of autologous platelet rich plasma for cardiac surgery: outcome analysis of 2000 patients. *J Cardiothorac Surg.* 2016;11(1):62.
54. Patel S, Dhillon MS, Aggarwal S, Marwaha N, Jain A. Treatment with platelet-rich plasma is more effective than placebo for knee osteoarthritis: a prospective, double-blind, randomized trial. *Am J Sports Med.* 2013;41(2):356-364.
55. Peerbooms JC, Sluimer J, Bruijn DJ, Gosens T. Positive effect of an autologous platelet concentrate in lateral epicondylitis in a double-blind randomized controlled trial: platelet-rich plasma versus corticosteroid injection with a 1-year follow-up. *Am J Sports Med.* 2010;38(2):255-262.
56. Rådmark O, Samuelsson B. 5-lipoxygenase: mechanisms of regulation. *J Lipid Res.* 2009;50(suppl):S40-S45.
57. Ruzicka T. The role of the epidermal 12-hydroxyeicosatetraenoic acid receptor in the skin. *Eicosanoids.* 1992;5(suppl):S63-S65.
58. Schafer AI. Deficiency of platelet lipoxygenase activity in myeloproliferative disorders. *N Engl J Med.* 1982;306(7):381-386.
59. Scharf RE. Drugs that affect platelet function. *Semin Thromb Hemost.* 2012;38(8):865-883.
60. Schippinger G, Prüller F, Divjak M, et al. Autologous platelet-rich plasma preparations: influence of nonsteroidal anti-inflammatory drugs on platelet function. *Orthop J Sports Med.* 2015;3(6):2325967115588896.
61. Shen B, Delaney MK, Du X. Inside-out, outside-in, and inside-outside-in: G protein signaling in integrin-mediated cell adhesion, spreading, and retraction. *Curr Opin Cell Biol.* 2012;24(5):600-606.
62. Shiraki R, Inoue N, Kawasaki S, et al. Expression of toll-like receptors on human platelets. *Thromb Res.* 2004;113(6):379-385.
63. Smith CW, Binford RS, Holt DW, Webb DP. Quality assessment of platelet rich plasma during anti-platelet therapy. *Perfusion.* 2007;22(1):41-50.
64. Stellos K, Langer H, Daub K, et al. Platelet-derived stromal cell-derived factor-1 regulates adhesion and promotes differentiation of human CD34+ cells to endothelial progenitor cells. *Circulation.* 2008;117(2):206-215.
65. Stenberg PE, McEver RP, Shuman MA, Jacques YV, Bainton DF. A platelet alpha-granule membrane protein (GMP-140) is expressed on the plasma membrane after activation. *J Cell Biol.* 1985;101(3):880-886.
66. Tang DG, Renaud C, Stojakovic S, Diglio CA, Porter A, Honn KV. 12(S)-HETE is a mitogenic factor for microvascular endothelial cells: its potential role in angiogenesis. *Biochem Biophys Res Commun.* 1995;211(2):462-468.
67. Thanasis C, Papadimitriou G, Charalambidis C, Paraskevopoulos I, Papanikolaou A. Platelet-rich plasma versus autologous whole blood for the treatment of chronic lateral elbow epicondylitis: a randomized controlled clinical trial. *Am J Sports Med.* 2011;39(10):2130-2134.
68. Tian J, Lei XX, Xuan L, Tang JB, Cheng B. The effects of aging, diabetes mellitus, and antiplatelet drugs on growth factors and anti-aging proteins in platelet-rich plasma. *Platelets.* 2019;30(6):773-792.
69. Tournodot BE, Holinstat M. Targeting 12-lipoxygenase as a potential novel antiplatelet therapy. *Trends Pharmacol Sci.* 2017;38(11):1006-1015.

70. Utku B, Dönmez G, Erişgen G, et al. Meloxicam and diclofenac do not change VEGF and PDGF-AB serum levels of platelet-rich plasma. *Turk J Med Sci.* 2017;47(2):570-576.
71. Velier M, Magalon J, Daumas A, et al. Production of platelet-rich plasma gel from elderly patients under antithrombotic drugs: perspectives in chronic wounds care. *Platelets.* 2018;29(5):496-503.
72. Vogel S, Bodenstern R, Chen Q, et al. Platelet-derived HMGB1 is a critical mediator of thrombosis. *J Clin Invest.* 2015;125(12):4638-4654.
73. Yeung J, Apopa PL, Vesce J, et al. 12-lipoxygenase activity plays an important role in PAR4 and GPVI-mediated platelet reactivity. *Thromb Haemost.* 2013;110(3):569-581.
74. Yeung J, Tourdot BE, Adili R, et al. 12(S)-HETE, a 12-lipoxygenase oxylipin of dihomo- γ -linolenic acid, inhibits thrombosis via G α s signaling in platelets. *Arterioscler Thromb Vasc Biol.* 2016;36(10):2068-2077.
75. Yeung J, Tourdot BE, Fernandez-Perez P, et al. Platelet 12-LOX is essential for Fc γ RIIIa-mediated platelet activation. *Blood.* 2014;124(14):2271-2279.
76. Yun SH, Sim EH, Goh RY, Park JI, Han JY. Platelet activation: the mechanisms and potential biomarkers. *Biomed Res Int.* 2016;2016:9060143.
77. Zhang G, Han J, Welch EJ, et al. Lipopolysaccharide stimulates platelet secretion and potentiates platelet aggregation via TLR4/MyD88 and the cGMP-dependent protein kinase pathway. *J Immunol.* 2009;182(12):7997-8004.