

Corticosteroids and Local Anesthetics Decrease Positive Effects of Platelet-Rich Plasma: An In Vitro Study on Human Tendon Cells

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Purpose: To determine the effects of mixing anesthetics or corticosteroids with platelet-rich plasma (PRP) on human tenocytes in vitro. **Methods:** Two separate protocols (double spin and single spin) were used to obtain homologous PRP from the blood of 8 healthy volunteers. Discarded tendon acquired during biceps tenodesis served as tendon specimens for all experiments. After cell isolation, tenocytes were treated in culture with PRP alone or in combination with corticosteroids and/or anesthetics. Fetal bovine serum in concentrations of 2% and 10% served as controls. Cell exposure times of 5, 10, and 30 minutes were used. Radioactive thymidine and luminescence assays were obtained to examine cell proliferation and viability. **Results:** The presence of lidocaine, bupivacaine, or methylprednisolone resulted in significantly less proliferation than the negative 2% fetal bovine serum control ($P < .05$). When we compared groups, both lidocaine and bupivacaine had a greater inhibitory effect than methylprednisolone ($P < .05$). At all time points, viability was significantly decreased in the presence of lidocaine, bupivacaine, or methylprednisolone compared with the negative control ($P < .05$). **Conclusions:** The addition of either anesthetics or corticosteroids to PRP resulted in statistically significant decreases in tenocyte proliferation and cell viability. These results suggest that incorporation of anesthetics or corticosteroids, either alone or in combination, with PRP injection may compromise the potentially beneficial in vitro effects of isolated PRP on tendon cells and compromise cell viability at the site of tendon injury. **Clinical Relevance:** Anesthetics or corticosteroids either alone or in combination should be used carefully to preserve the proposed positive effects of PRP in the treatment of tendon injury.

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Conservative treatment of various tendinopathies has historically involved local injections of corticosteroids or anesthetics.¹ The results of these treatments are variable, with both positive and negative effects observed.^{1,2} Recent orthopaedic research has focused on platelet-rich plasma (PRP) for its potential use in the treatment of various tendon injuries and disorders.³⁻¹⁰ "PRP" is a generic term that refers to any sample of autologous plasma with platelet concentrations above baseline blood values.¹¹ PRP treatment is primarily intended to enhance the healing process through the secretion of various growth factors.⁵

Literature regarding the clinical efficacy of PRP treatment for tendon injuries is variable, with both positive and negative effects being reported in se-

lected studies.^{4,6,12-17} Some of the recent literature has failed to show significant benefit with the application of PRP during surgical tendon repair.¹²⁻¹⁴ Conversely, Mishra et al.^{6,15} reported favorable results for PRP when compared with corticosteroids or local anesthetics for the treatment of epicondylitis. Peerbooms et al.¹⁶ reported similar findings in a double-blind randomized trial comparing PRP application with corticosteroids. Applications of PRP for the treatment of plantar fasciitis have also shown positive results.^{4,17}

The emergence of PRP as a treatment option for tendon-related injury has led to clinicians providing injections of platelet preparations in isolation and in combination with commonly used pharmaceuticals. The injection of PRP has been anecdotally noted to be painful, prompting clinicians to add local anesthetics. PRP may enhance efficacy and improve treatment outcome. Furthermore, a combination of PRP and corticosteroids may reduce the harmful effects of corticosteroid injections.² This potential effect has been reported by Wong et al.,¹⁸ who described platelet-derived growth factor (PDGF) as a protective agent for the negative effects of dexamethasone on cultured human tenocytes in an in vitro model.

To elucidate the effects of such combinations of anesthetics and steroids with PRP, laboratory investigations conducted in a clinically applicable manner are needed. Such investigations would require application of PRP to be matched to the donor tissue, to meet the criteria of autologous treatment. This can be challenging because it would require coordination of intraoperative tissue harvesting, time needed for cell isolation/culture, and fresh plasma preparations at the time of application. As a result, previous investigators have used homologous PRP in comparable experimental designs.¹⁸⁻²² As in these studies, the term "PRP" in our study refers to homologous platelet concentrates. In addition, in vitro tenocyte models have been used to study the dose effects of pharmaceuticals either in isolation or in combination.¹⁸ The utilization of human tendon cells compared with animal tendon cells in in vitro models is considered the "gold standard" for laboratory investigation.²³

The purpose of this study was to determine in vitro whether the addition of anesthetics or corticosteroids, either alone or in combination, alters the effects of PRP on human tenocytes. Our null hypothesis was that the addition of an anesthetic or corticosteroid to 2 different types of PRP products would have no detrimental effects on tenocyte proliferation or viability.

METHODS

Plasma Concentrates

Venous blood was collected from 8 healthy volunteers (2 women and 6 men) with a mean age of 31.7 ± 11.1 years (Institutional Review Board No. 10-204-2). To obtain PRP, 2 separate isolation protocols were used: single spin (PRP_{SS}) and double spin (PRP_{DS}). These protocols were selected to reflect current clinical practice.³

PRP_{SS}: The Arthrex Double Syringe (Arthrex, Naples, FL) was used for production of Autologous Conditioned Plasma (ACP; Arthrex). The Double Syringe was filled with 10 mL of blood to produce approximately 3 mL of PRP_{SS}. The syringe was centrifuged with 1,500 rpm for 5 minutes. This separated the blood into 2 layers: plasma and the red and white blood cell layer containing erythrocytes and leukocytes. The plasma containing the platelets was then isolated with the inner syringe.

PRP_{DS}: A literature-based double-spin method was used to fractionate whole blood.²⁰ After a first centrifugation of 1,500 rpm for 5 minutes, the plasma layer was drawn up and was centrifuged a second time (20 minutes at 6,300 rpm). Finally, half of the superficial plasma layer was removed, and the platelet pellet was suspended in the remaining half of the plasma volume.

Corticosteroids and Local Anesthetics

Substances were selected with concentrations reflecting our clinical practice: lidocaine (1%), bupivacaine (0.5%), and methylprednisolone (40 mg/mL). Recognizing that surrounding body fluids quickly dilute substances after an injection, diluted concentrations were used in an effort to replicate conditions experienced by tenocytes in vivo.²⁴ In a 1-mL well, the following dilutions were used: 0.05 mL of lidocaine, 0.05 mL of bupivacaine, and 0.01 mL of methylprednisolone.

Isolation of Tenocytes

Discarded biceps tendons were taken during biceps tenodesis from 4 different male donors (mean age, 46.8 ± 4.2 years), and cells were isolated according to previously described techniques (Institutional Review Board No. 07-224).²⁵ Tendons were cut into $3 \times 3 \times 3$ -mm pieces and placed into 2% collagenase. The resulting tenocyte cell suspension was filtered to remove debris and cultured in Dulbecco's modified Eagle's medium (Invitrogen, Carlsbad, CA) containing

10% fetal bovine serum (FBS) (Atlanta Biologicals, Atlanta, GA).²⁵ All experiments were conducted using cells within the first and second passages. Cells for each experiment were taken from individual donors, thus representing a separate sample for each experiment. All experiments were repeated 4 times, each with a different donor.

Tenocyte Culture Experiments

Before the application of any treatment, tenocytes were plated at a density of 10,000 cells per well. To determine the effects and interaction of the different treatments, several experimental groups were required. A control was composed of isolated treatment with saline solution for identical application times and then the treatment. To provide sufficient nourishment to support cell viability without stimulating proliferation, 2% FBS was selected for the proliferation experiment as an additional negative control. Two percent FBS keeps the cells in a very low proliferative state and has no negative effects on the cells. For a positive control, 10% FBS was chosen because it is known to simulate cellular proliferation.^{26,27} Additional groups consisted of lidocaine (1%), bupivacaine (0.5%), and methylprednisolone (40 mg/mL) and combinations of

lidocaine with methylprednisolone, as well as bupivacaine with methylprednisolone. For the experimental groups, tenocytes were treated in culture with each PRP preparation (PRP_{DS} and PRP_{SS}) with or without the addition of a local anesthetic or steroid. This resulted in experimental combinations that included the same combinations used in the control groups with the addition of each PRP product. The complete experimental setup is depicted in Fig 1.

Analysis of Cell Proliferation

A radioactive thymidine assay was used to measure tenocyte proliferation. Cells were plated and tenocytes were treated (30 minutes) with saline solution, both PRPs, lidocaine, bupivacaine, methylprednisolone, or a combination thereof as described earlier (Fig 1). After 4 days of culture, proliferating cells were labeled with radioactive thymidine by adding 5.0 μCi [³H] Thymidine/ml to each well for 24 hours.²⁷ After labeling of cells with radioactive thymidine, the fibrin clot commonly found was removed from each well containing PRP to avoid assay interference and false-positive results. The radioactive thymidine was incorporated into the DNA of dividing cells, and therefore an increase in radioactivity above the negative control

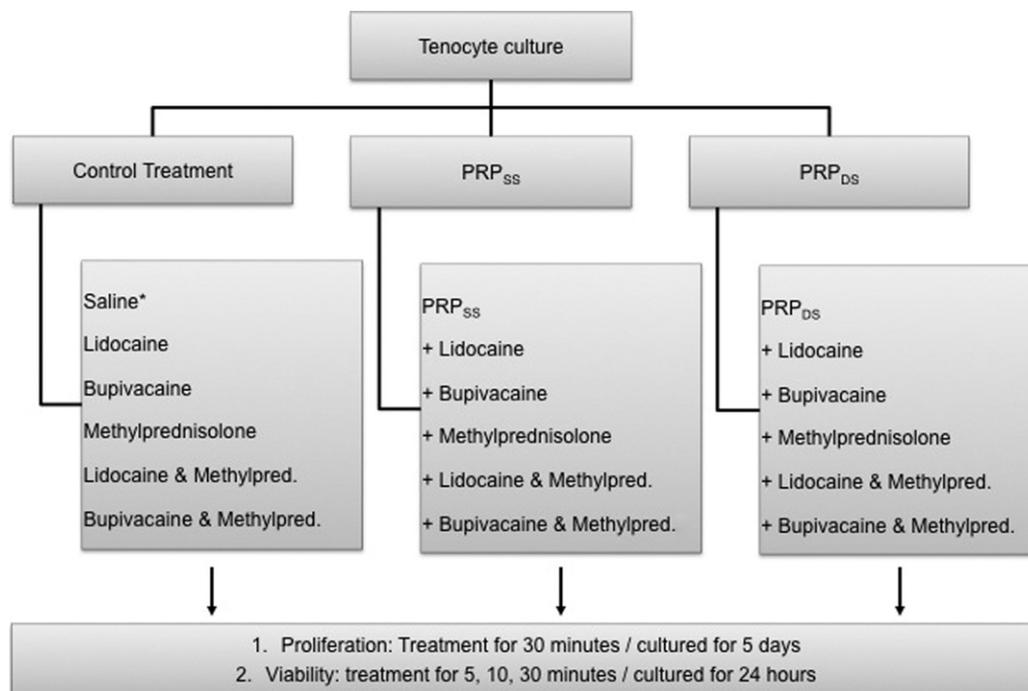


FIGURE 1. Flowchart showing experimental setup and treatment groups. Asterisk, as controls for the proliferation experiment, tenocytes were plated in media containing 2% and 10% FBS; cells were plated in media containing 10% FBS as controls for the viability experiment. (Methylpred, methylprednisolone.)

directly correlates to cellular proliferation.²⁷ To remove unbound thymidine, cells were washed twice with 10% trichloroacetic acid and then 0.5-mol/L sodium hydroxide was added to the cells to release the nuclear-bound radioactive compound.²⁷ The number of proliferating cells was assayed by measuring the number of disintegrations per minute (dpm) with a scintillation counter. Only cells that had incorporated the radioactive thymidine were counted. Each well was measured in triplicate and reported as the mean to reduce the amount of variability.

Analysis of Cell Viability

Tenocytes were plated at a density of 15,000 cells per well and cultured for 24 hours in 10% FBS before experimentation.²⁸ Cells were plated at a higher density for this experiment to facilitate the luminescence measurements described later. After 24 hours, the 10% FBS was removed and the tenocytes were treated with PRP, lidocaine, bupivacaine, methylprednisolone, or a combination thereof at the same concentrations that were tested in the tenocyte proliferation assay. Groups consisted of lidocaine (1%), bupivacaine (0.5%), and methylprednisolone (40 mg/mL) and combinations of lidocaine with methylprednisolone, as well as bupivacaine with methylprednisolone. For the experimental groups, tenocytes were treated in culture with each PRP preparation (PRP_{DS} and PRP_{SS}) with or without the addition of a local anesthetic or steroid. The cells were exposed for 5, 10, and 30 minutes. These short time points were selected because pilot experiments using identical cell cultures showed that longer exposures (5 days) resulted in 100% tenocyte death. Exposures of 1 and 4 hours still showed cell death of approximately 90%. In addition, little *in vivo* data exist on the local distribution and absorption of local anesthetics. Other authors have also stated a rapid solution of PRP soon after injection (minutes).²⁰ However, a recent magnetic resonance imaging study examining the local distribution of anesthetics in oral surgery observed a clear reduction after 60 minutes and maximal final absorption times of 120 minutes.²⁴

After the exposure period, the experimental media were removed and replaced with 10% FBS for a 24-hour recovery prior to performing the viability assay. Cell viability was measured with a luminescence assay (CellTiter-Glo; Promega, Madison, WI). This assay produces a measurable luminescent signal, which is directly related to the amount of adenosine triphosphate present in the well, and is proportional to the number of viable cells. Each well was measured in

triplicate and reported as the mean to reduce the amount of variability. The number of viable tenocytes was determined by creating a standard curve with known concentrations (R^2 range, 0.9023 to 0.9568). Luminescence values were transformed to obtain the number of viable cells. Cell toxicity was determined to be any number less than the number obtained from cells grown in the negative control (2% FBS).

Statistical Analysis

The Kolmogorov-Smirnov test was performed for each experiment, and the mean skewness and kurtosis were calculated and divided by the standard deviation to identify non-normal distributions. One-way analysis of variance was used to compare group means for experiments with normally distributed data followed by Bonferroni post hoc tests for experiments with a statistically significant difference in means. The Kruskal-Wallis test was used to compare group means for experiments that had a non-normal data distribution even after appropriate data transformations had been performed. For the Kruskal-Wallis tests, the Conover method was used to calculate the P value for the critical difference of the mean ranks. This allowed control for family-wise error. Experiments with a statistically significant difference in means were followed by post hoc tests. A P value (α level) of $< .05$ was used to determine statistical significance. All statistical analyses were performed with SPSS software (IBM, Armonk, NY).

RESULTS

Plasma Concentrates

The preparation of platelets increased the platelet number in the PRP_{SS} group on average to 2.6 times the baseline concentration of whole blood, whereas the platelet number in the PRP_{DS} group was on average 3.3 times the concentration of whole blood. White blood cell concentrations in both the PRP_{SS} group and the PRP_{DS} group were decreased at least 10-fold in comparison with the concentration in whole blood samples (Table 1).

Analysis of Cell Proliferation

There were several significant findings among both the control and experimental groups, as shown in Fig 2. With regard to the control groups (no PRP added), significant increases were observed in tenocyte proliferation in the 10% FBS ($1,379 \pm 543$ dpm) in com-

TABLE 1. Mean Concentrations of Blood Components (Platelets, Red Blood Cells, and White Blood Cells) in Native Blood and According to Separation Method

Product	Platelets ($10^3/\mu\text{L}$)		Red Blood Cells ($10^6/\mu\text{L}$)		White Blood Cells ($10^3/\mu\text{L}$)	
	Mean	SD	Mean	SD	Mean	SD
Blood	142.7	41.7	4.1	0.3	5.7	1.5
PRP _{SS}	361.5	90.3	0.02	0.01	0.6	0.14
PRP _{DS}	447.7	85.9	0.02	0.02	0.17	0.11

parison with the 2% FBS group (601 ± 141 dpm) ($P < .05$). The application of lidocaine, bupivacaine, or methylprednisolone each resulted in significantly less proliferation when compared with the 2% FBS control ($P < .05$). When we compared lidocaine and bupivacaine, both resulted in significantly less tenocyte proliferation than methylprednisolone ($P < .05$). There was no significant difference between the lidocaine and bupivacaine groups (Fig 2).

With regard to the experimental groups, both preparations of PRP (PRP_{SS} and PRP_{DS}) stimulated tenocyte proliferation and each produced significantly greater proliferation than both the negative

control (2% FBS) and positive control (10% FBS) (Fig 2). There was no significant difference between the PRP_{SS} and PRP_{DS} groups.

All combinations of PRP with the addition of lidocaine, bupivacaine, and methylprednisolone to the media either in isolation or in combination resulted in significantly less tenocyte proliferation ($P < .05$).

PRP_{SS} and methylprednisolone added to negative 2% FBS control media ($1,457 \pm 475$ dpm) resulted in greater tenocyte proliferation when compared with PRP_{SS} and lidocaine (188 ± 84 dpm) or PRP_{SS} and bupivacaine (215 ± 72 dpm) ($P < .05$). PRP_{DS} and methylprednisolone added to negative 2% FBS con-

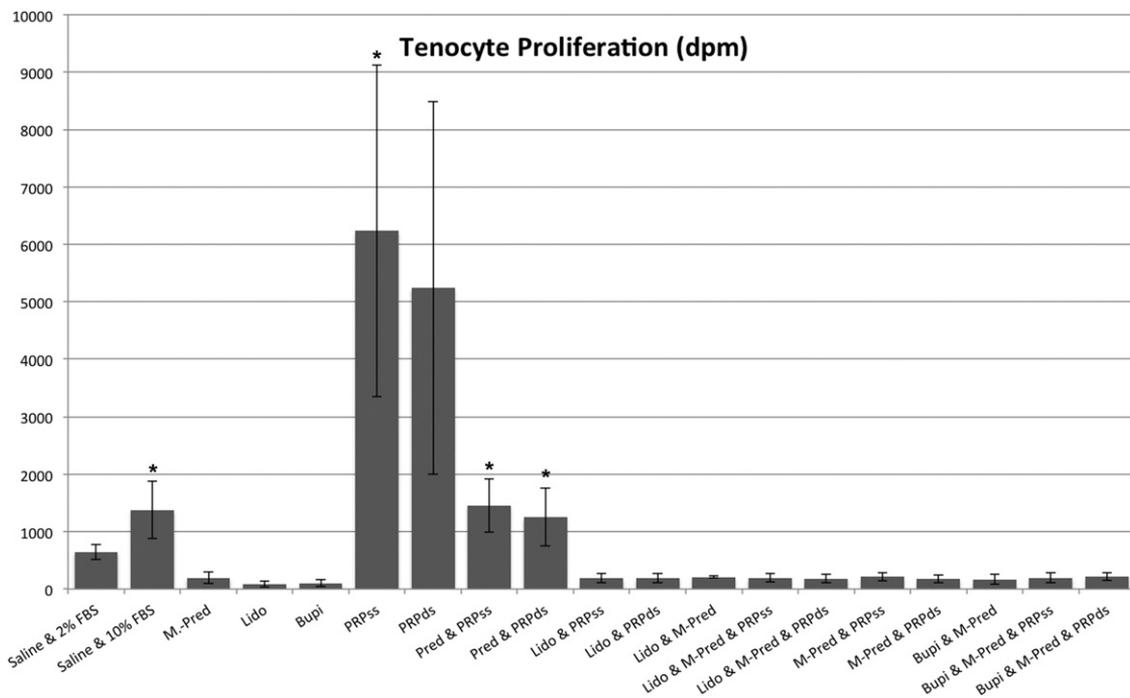


FIGURE 2. Graph showing tenocyte proliferation 5 days after treatment for 30 minutes. It should be noted that treatment with both PRPs increased proliferation significantly and the combination of both PRPs and methylprednisolone showed results comparable to the control groups. Adding local anesthetics decreased tenocyte proliferation significantly for all isolated and combined groups. $*P < .05$. (M-Pred, methylprednisolone; Lido, lidocaine; Bupi, bupivacaine.)

trol media also showed significant increases in proliferation when compared with PRP_{DS} and lidocaine or PRP_{DS} and bupivacaine ($P < .05$). All 4 combinations of the PRPs and anesthetics (PRP_{DS} and lidocaine, PRP_{DS} and bupivacaine, PRP_{SS} and lidocaine, and PRP_{SS} and bupivacaine) resulted in significant decreases in proliferation when compared with the negative 2% FBS control ($P < .05$) (Fig 2).

Analysis of Cell Viability

Cell viability, as detected by luminescence, is shown in Fig 2. After 10 and 30 minutes, viability was significantly decreased in the presence of lidocaine ($P < .001$ and $P = .047$, respectively), bupivacaine ($P < .001$ and $P = .042$, respectively), or methylprednisolone ($P < .001$ and $P < .001$, respectively) compared with saline solution. There were no significant differences in cell viability after 5 minutes of treatment when the anesthetics or methylprednisolone was added to PRP (PRP_{SS} or PRP_{DS}). At 10 and 30 minutes, the addition of methylprednisolone ($P = .05$ for PRP_{SS} and $P = .013$ for PRP_{DS}) or bupivacaine ($P = .001$ for PRP_{SS} and $P = .009$ for PRP_{DS}) to either PRP (PRP_{SS} or PRP_{DS}) resulted in significantly decreased viability compared with PRP (PRP_{SS} or PRP_{DS}) alone. The addition of lidocaine to both PRP preparations did not significantly decrease viability at these time periods (Table 2).

DISCUSSION

This study was designed to provide in vitro data to clinicians who are interested in combining PRP with anesthetics or corticosteroids. With regard to our hypothesis, tenocyte proliferation and viability were increased when PRP_{SS} and PRP_{DS} preparations were added to the tenocyte culture. Conversely, tenocyte proliferation and viability were substantially decreased when an-

esthetics and corticosteroids were added. Combined treatment, using PRP preparations and anesthetics or corticoids, showed a decreased reaction compared with the isolated PRP treatment; however, it was still increased compared with the isolated treatment with anesthetics, corticoids, or the combination of anesthetics and corticoids.

Two different PRP preparations, single spin (PRP_{SS}) and double spin (PRP_{DS}), were used in this study to control for effects possibly correlated to the production process of the PRP. For additions to PRP, we used commonly known and frequently used products (lidocaine, bupivacaine, and methylprednisolone).^{2,29,30} Lidocaine and bupivacaine are both representatives of amino amide-type local anesthetics; lidocaine has a quicker onset but a shorter duration of action than bupivacaine.³⁰ Methylprednisolone is a commonly used synthetic glucocorticoid to decrease inflammatory reactions.^{29,30} Although various products exist for treatment of tendon injuries, these products appear to be representative of our intended pharmacologic groups and are routinely used by the senior author in his practice.^{30,31}

With regard to model selection, human tenocytes (proximal long head of the biceps tendon) were used in an effort to provide in vitro results that were meaningful to clinicians using PRP as a treatment for tendon-related injury. Such 2-dimensional cell cultures allow for a very controlled evaluation of the treatment effects of pharmaceuticals in a uniform environment.¹⁹⁻²¹ However, it should be regarded that cultured tenocytes from different origins (e.g., biceps and Achilles tendon) may react differently according to their harvesting site.¹⁸

Previous investigators have used similar in vitro models to examine the effects of PRP on human tenocytes. Anitua et al.^{7,19} showed proliferation of tenocytes when cultured in PRP. De Mos et al.²⁰ found

TABLE 2. Results of Viability Experiment According to Treatment and Time of Exposure to Treatment

Addition	Exposure Time (min)	Saline Solution	Lidocaine	Bupivacaine	Methylprednisolone	Lidocaine and Methylprednisolone	Bupivacaine and Methylprednisolone
None	5	11,331.7 ± 3,619.9	4,446.9 ± 520.2	2,278.2 ± 448.8	5,550.1 ± 1,450.3	1,889.6 ± 761.3	2,761.1 ± 625.5
	10	17,299.2 ± 2,283.5	3,153.0 ± 423.9	1,632.4 ± 870.0	3,350.1 ± 932.1	2,010.1 ± 324.5	1,258.1 ± 290.0
	30	7,640.8 ± 2,089.5	1,221.2 ± 879.7	882.8 ± 144.2	3,533.0 ± 1,007.2	1,087.1 ± 334.2	1,850.2 ± 600.6
PRP _{SS}	5	14,389.0 ± 1,603.2	16,010.4 ± 1,802.1	17,310.6 ± 1,400.5	12,137.1 ± 4,338.9	9,317.4 ± 1,249.1	14,765.9 ± 1,739.8
	10	12,152.2 ± 2,615.2	16,010.1 ± 1,801.3	6,993.8 ± 481.1	8,042.3 ± 2,992.0	6,888.6 ± 836.8	8,845.2 ± 1,012.2
	30	8,315.8 ± 622.6	16,010.8 ± 1,804.9	9,875.0 ± 812.9	4,799.4 ± 1,112.9	6,552.2 ± 318.2	9,374.6 ± 989.5
PRP _{DS}	5	18,375.0 ± 1,959.5	12,674.7 ± 791.1	17,373.2 ± 1,237.5	14,020.3 ± 3,415.8	12,235.7 ± 1,076.7	15,023.6 ± 2,187.2
	10	13,229.5 ± 2,338.2	13,306.0 ± 1,290.0	8,044.7 ± 3,308.1	2,992.0 ± 2,337.6	7,578.1 ± 370.2	9,168.2 ± 1,123.0
	30	9,046.1 ± 1,772.6	10,474.4 ± 627.5	10,363.5 ± 895.1	6,051.8 ± 859.1	9,123.2 ± 343.9	9,529.9 ± 720.0

NOTE. Data are presented as mean ± SD.

that PRP stimulates increased total collagen production, cell proliferation, and expression of endogenous growth factors. Similar to these previous studies, tenocytes treated in our study with PRP from either PRP_{SS} or PRP_{DS} preparation resulted in statistically significant increases in tenocyte proliferation ($P < .05$) compared with the controls.

Conversely, the isolated treatment with lidocaine and bupivacaine resulted in significant decreases in cell proliferation when compared with the controls ($P < .05$) or cells only treated with PRP_{SS} and PRP_{DS}. The observed inhibitory effect was greater with the application of the anesthetics. These results are consistent with those of Scherb et al.,³² who also reported decreased human tenocyte proliferation and extracellular matrix production after treatment with bupivacaine, as well as Fedder et al.,³³ who showed negative effects of treatment with lidocaine, bupivacaine, or ropivacaine on fibroblasts. Adverse effects of anesthetics on a cellular level are not clearly understood. Local anesthetic solutions are generally prepared at a pH of 5.0 to 6.0, which may be detrimental to treated cells due to the acidity alone.²⁹

The reduction in tenocyte proliferation and viability observed after exposure to the methylprednisolone is also consistent with findings of other investigators who reported decreases in tenocyte proliferation, viability, and collagen synthesis and increases in markers of apoptosis.^{18,22,34,35} These effects of glucocorticoids may be mediated through glucocorticoid receptors in the cytoplasm of the tenocytes.³⁶ Reports also exist on a decrease in proteoglycan production of tenocytes, an important component of the extracellular matrix, after treatment with corticoids.²² These findings may help explain previous reports of in vivo complications after local corticosteroid application including tendon rupture after corticoid injections.^{37,38}

Considering the individual effects of anesthetics and corticoids, PRP preparations are yet not entirely understood; it is difficult to anticipate the exact mechanism for the improved results when combining the PRPs and the pharmaceuticals.^{18,21} One explanation for the positive effect of combining PRP solutions with local anesthetics may be seen in a buffering effect of the PRP solution, because amino amide solutions are slightly acidic. Despite significant decreases, cells treated with PRP and methylprednisolone showed greater amounts of proliferation when compared with cells treated with methylprednisolone alone. Wong et al.¹⁸ reported a comparable

preventive effect using a specific platelet-correlated growth factor (PDGF) as a protective agent for the negative effects of corticosteroid treatment on tenocytes. Besides the positive effects of PDGF on viability and synthesis of tenocytes treated with corticoids, Zargar Baboldashti et al.²¹ recently showed protective effects on tenocytes with the addition of PRP to corticoids and antibiotics.¹⁸ The authors of that study discussed a protective effect of the PRP's growth factors on the upregulation of stress response transcription factors, a cellular reaction attributed to corticoid treatment.²¹ Such possible interactions of intracellular messenger signaling warrant evaluation in future studies. Tenocytes are important for production and maintenance of the tendon's extracellular matrix. Negative effects on tenocyte viability and proliferation, and therefore decreases in matrix production, should be minimized to preserve tendon structure during treatment of tendon injuries.

There are several limitations of this study. The in vitro behavior of biceps tendon cells may not mimic the in vivo environment. Attempts were made to reproduce the complex in vivo conditions with our cell model; however, we were only able to estimate the concentrations found in or around various tendons of the human body. This is primarily because of the wide variation of injection methods and doses used in a clinical setting.^{1,2,5,30,38} When considering evidence of a dose-dependent effect, along with the possibility that the concentrations used were different from the unknown in vivo environment, the consistency in proportions permits conclusions regarding the relative effects of the investigated combinations.¹⁸ The heterogeneous nature of the blood and tissue samples may have impacted the study. Because the culturing and separation of the tenocyte culture required several days and the possible storage time for PRP products is unknown, different donors had to be used to match the needs for a controlled and structured experiment. This situation raises concern over a potential graft-versus-host reaction from incidental exposure of white blood cells from one individual on the tendon to a different individual. This potential effect was likely minimized because up to 98% of white blood cells were eliminated with the PRP_{SS} and PRP_{DS} preparations. However, multiple previous investigators have used homologous PRP in comparable experimental designs.¹⁸⁻²² Exposure times of 5, 10, and 30 minutes were selected based on pilot data and to simulate a high metabolic in vivo environment (product's elimination half-life, dilution, and so on) where body fluids can rapidly dilute injected substances.^{20,24,29} This type of environment was believed to

be representative of common areas of tendon injury at the time of injection.

CONCLUSIONS

The addition of either anesthetics or corticosteroids to PRP resulted in statistically significant decreases in tenocyte proliferation and cell viability. These results suggest that incorporation of anesthetics or corticosteroids, either alone or in combination, with PRP injection may compromise the potentially beneficial in vitro effects of isolated PRP on tendon cells and compromise cell viability at the site of tendon injury.

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