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Short Communication

A potential treatment for erectile dysfunction: Effect of platelet-rich plasma administration on axon and collagen regeneration in cavernous nerve injury

Jufriady Ismy[1,2*], Said A. Khalilullah[1,2], Reza Maulana[1,2] and Furqan Hidayatullah[1,2]

[1]Department of Urology, Faculty of Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia; [2]Department of Urology, Faculty of Medicine, Dr. Zainoel Abidin Hospital, Banda Aceh, Indonesia

*Corresponding author: jufriadyismy@usk.ac.id

Abstract

Recent studies highlighted the role of platelet-rich plasma (PRP) in progenitor cell homing, migration, and nerve cell regeneration while also inhibiting fibrosis and apoptosis in cavernous nerve injury (CNI). The aim of this study was to investigate the effect of PRP administration on axon and collagen regeneration in CNI. A true experimental study using a post-test-only control group design was conducted. Twenty-five male Wistar rats (*Rattus norvegicus*), weighing 200–300 grams, were divided into five groups: two control groups (sham procedure and negative control), and three experimental groups receiving local PRP, intraperitoneal PRP, and a combination of local and intraperitoneal PRP. The cavernous nerve was injured with a hemostasis clamp for one minute before 200 µL of 200 PRP was injected locally, intraperitoneally, or both, depending on the group. After four weeks, the rats were euthanized, tissue segments (2 mm) from each cavernous nerve and mid-penis were collected and analyzed for collagen density, axon diameter, and number of myelinated axons. Our study found that collagen growth was slower in CNI group without PRP (sham procedure) compared to all PRP groups (local, intraperitoneal, and combination). The intraperitoneal PRP group had the highest collagen density at 5.62 µm; however, no significant difference was observed in collagen density among all groups (p=0.056). Similar axon diameter was found across the groups, with no statistically significant difference observed (p=0.856). In the number of myelinated axons, a significant difference was found among all groups with significantly more axons in local PRP and combined local and intraperitoneal PRP groups compared to others (p=0.026). In conclusion, PRP administration improved the number of myelinated axons in CNI, suggesting PRP role in CNI regeneration and the potential for an innovative approach to treating erectile dysfunction associated with CNI.

Keywords: Platelet-rich plasma, cavernous nerve injury, collagen, axon, erectile dysfunction

Introduction

Erectile dysfunction, the inability to initiate or sustain an erection, is projected to affect over 300 million men globally by 2025–incidence rates vary from 3% to 76.5%, with post-urological procedure rates exceeding 85% [1]. Cavernous nerve injury (CNI) is a leading cause of erectile dysfunction following urological procedures [1,2]. Neurogenic erectile dysfunction occurs when nerve signals fail to reach the corpus cavernosum, primarily due to peripheral lesions such as CNI

Received: May 19, 2024 - Accepted: August 1, 2024 - Published online: August 5, 2024

from pelvic surgery or trauma rather than central lesions such as multiple sclerosis or spinal cord injury [3,4]. CNI, especially from radical prostatectomy, is the leading cause of iatrogenic erectile dysfunction [5,6]. While phosphodiesterase 5 inhibitors are a standard erectile dysfunction treatment, it is less effective for nerve injury-related erectile dysfunction [5]. A recent meta-analysis found phosphodiesterase 5 inhibitors do not significantly improve spontaneous erectile function post-surgery [5,6]. With no surgical method to fully prevent CNI and the limited effectiveness of penile rehabilitation, understanding the molecular causes of erectile dysfunction from nerve injury and developing new therapeutic approaches to restore erectile function is essential.

Platelet-rich plasma (PRP) accelerates soft tissue and bone regeneration by promoting cell growth (mitogenesis) and vascular ingrowth (angiogenesis) [7,8]. Recent studies highlighted PRP role in progenitor cell homing, migration, and nerve cell regeneration, while also inhibiting fibrosis and apoptosis in injured cavernous nerves [9,10]. Tai *et al*. found that PRP has improved erectile function in diabetic-erectile dysfunction rats and models with CNI [11]. The aim of this study was to investigate the effect of PRP administration on axonal and collagen regeneration in CNI.

Methods

Study design and setting

A true experimental study using a post-test-only control group design was conducted at Laboratory of Experimental Animal Study at Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia, in 2023. The study involved twenty-five male Wistar rats (*Rattus norvegicus*) to investigate the effect of PRC administration on collagen density, axon diameter, and number of myelinated axons in CNI model. This study was prepared in accordance with animal research-reporting of in vivo experiments (ARRIVE) guideline [12]. An untreated control group (C) was used as a negative control. Another control group (CC) served a sham procedure, which underwent a sham surgery comprising the crushing of the dorsal nerve in rats but did not receive any further therapy. In addition, this study consisted of three experimental groups, of which all animals in these groups had bilateral CNI and treated with 200 µL of local PRP to the corpus cavernosum (LP), 200 µL of PRP intraperitoneally (IP) or combination of those LP and IP injections. After four weeks, collagen density, axon diameter, and number of myelinated axons of cavernous nerve and mid-penis were measured.

Sample calculation, randomization, and allocation

Sample size calculation was based on the Federer formula, determined by the number of experimental groups and the number of experimental attempts, resulting in a minimum of four rats per group. To anticipate dropouts, the sample size was increased by 10%, resulting in five rats included in each group. Each rat was assigned an identification number, and the animals were randomized into groups. Twenty-five healthy male Wistar rats (*R. norvegicus*), aged 5–10 weeks and weighing 200–300 grams, were included in this study. Animals with the presence of structural or functional abnormality and any sign of infection were excluded. The animals were divided into five groups: two control groups (sham procedure (CC) and negative control (C)) and three experimental groups receiving local PRP (LP), intraperitoneal PRP (IP), and combined local and intraperitoneal PRP (LIP).

Animal preparation

Rats were caged in groups of five in well-shaded, quiet rooms with a stable environment. The rats were provided with the same type and amount of food, and trays beneath each cage collected urine and feces, which were cleaned daily. Ventilated cages were maintained at a temperature of 25–27°C and a humidity level of 50–60%. A 12-hour light-dark cycle was implemented, with lights turning on at 5:30 AM. Rats were fed formulated pellet feed twice daily, amounting to 10% of their body weight. Clean water was supplied *ad libitum* through specialized bottles, and the acclimatization period lasted for seven days.

Platelet-rich plasma preparation

Ten healthy male Wistar rats (*R. norvegicus*), separate from control and experimental groups, were anesthetized with ether, and 2 mL of blood was collected from the retroorbital plexus into a tube with 0.3 mL of 3.2% sodium citrate anticoagulant. The blood was centrifuged twice: first at 1600 rpm for 10 mins, then at 2000 rpm for 10 mins. Platelets were counted with a hematology analyzer and stored at -80°C. PRP was activated just before use with $CaCl_2$ (0.2 mL PRP with 0.2 mL of 10% $CaCl_2$).

Experimental procedure of cavernous nerve injury model and intervention

Rats were anesthetized with intraperitoneal sodium pentobarbital (40 mg/kg). Abdominal incisions were made to identify the prostate gland, major pelvic ganglion, and cavernous nerves. The cavernous nerve was injured with a hemostasis clamp for one min. A total of 200 µL PRP was then injected locally into the corpus cavernous, intraperitoneally, or both, depending on the group. After four weeks, the rats were euthanized with 800 mg/kg intraperitoneal of sodium pentobarbital. Tissue segments (2 mm) from each cavernous nerve and mid-penis were collected, stained with 1% luxol fast blue, and analyzed for collagen density, axon diameter, and number of myelinated axons. All surgical procedures were conducted by the same individual, a professional veterinarian with more than five years of experience.

Outcome measurements

Collagen density was scored as follows: (0) no growth, (1) minimal growth (>10 µm), (2) moderate growth (5–10 µm), and (3) significant growth (<5 µm). Axon diameter was measured using an image viewer program modified from an ocular micrometer method. Images were captured with an OPTILAB microscope camera across five fields of vision. Axon and fiber diameter were measured by determining the longest axis and then the longest perpendicular line, as explained [13]. The number of myelinated axons was counted in five fields of view (400× magnification) across top, bottom, left, right, and center regions, each 2.73 mm^2. Results are expressed as the number of axons per 2.73 mm^2.

Statistical analysis

Continuous data were presented as mean and standard deviation, and categorical data were presented as percentages. The Shapiro-Wilk test was utilized to assess data normality. One-way analysis of variance (ANOVA) test was used for normally distributed data and Kruskal-Wallis test was used for non-normally distributed data. Post hoc tests were conducted to identify specific differences between group means when one-way ANOVA test showed significant results. The SPSS version 25.0 software (IBM SPSS, Chicago, IL, USA) was employed for data analysis, with $p<0.05$ considered statistical significance.

Results

Collagen density

In the present study, collagen density was not normally distributed. Collagen growth was slower in CNI group without PRP (CC) compared to PRP groups (LP, IP, and LIP). The combined local and intraperitoneal PRP group had the highest collagen density at 5.62 µm. The Kruskal-Wallis test showed no significant difference in collagen density among all groups ($p=0.056$) (**Table 1**).

Table 1. Comparison of collagen density among study groups

Group	Mean±SD (µm)	*p*-value
Control (C)	5.82±1.27	0.056
Sham procedure (CC)	8.99±2.12	
Local PRP (LP)	7.20±2.32	
Intraperitoneal PRP (IP)	5.81±3.21	
Combined local and intraperitoneal PRP (LIP)	5.62±2.42	

Diameter of axon

Histology data on axon diameter followed a normal distribution. Our data indicated that the axon diameter across had no statistically significant difference was found among the groups (p=0.856) (**Table 2**).

Table 2. Comparison of axon diameter among study groups

Group	Mean±SD (µm)	p-value
Control (C)	3.74±0.25	0.856
Sham procedure (CC)	3.78±0.33	
Local PRP (LP)	3.60±0.22	
Intraperitoneal PRP (IP)	3.85±0.47	
Combined local and intraperitoneal PRP (LIP)	3.67±0.54	

Number of myelinated axons

Mean values of myelinated axon numbers showed a normal distribution. One-way ANOVA test indicated a significant difference among all groups (p=0.026) (**Table 3**). A post-hoc analysis found that the local PRP group and the combined local and intraperitoneal PRP group had significantly more axons than the sham procedure group (p=0.012 and p=0.002, respectively) (**Table 4**).

Table 3. Comparison of the number of myelinated axons among study groups

Group	Mean±SD (/2.73 mm²)	p-value
Control (C)	12.64±2.59	0.026
Sham procedure (CC)	9.52±1.01	
Local PRP (LP)	13.84±2.29	
Intraperitoneal PRP (IP)	12.60±2.40	
Combined local and intraperitoneal PRP (LIP)	15.12±3.50	

Table 4. Results of post-hoc test comparing the number of myelinated axons between study groups

Group	Mean±SD (/2.73 mm²)	p-value				
		C	CC	LP	IP	LIP
Control (C)	12.64±2.59		1.000	0.455	0.064	0.131
Sham procedure (CC)	9.52±1.01			0.012	0.980	0.002
Local PRP (LP)	13.84±2.29				0.440	0.425
Intraperitoneal PRP (IP)	12.60±2.40					0.125
Combined local and intraperitoneal PRP (LIP)	15.12±3.50					

Discussion

Emerging treatments for erectile dysfunction aim at regenerating damaged erectile tissue, offering potential cures rather than mere symptom management [14]. Ideal regenerative models facilitate sustained erectile function by regulating growth factors and promoting nerve and muscle cell repair [15]. A prior review has examined regenerative treatments for erectile dysfunction, including PRP, amniotic fluid matrices, low-intensity extracorporeal shockwave therapy, and stem cell therapy (SCT). The review suggests that PRP treatment for ED is safe, as human studies have shown a minimal likelihood of negative outcomes. Nevertheless, this review also determined that although regenerative medicine appears promising at first glance, the available data is insufficient to endorse any of these procedures in the field of urology [14]. PRP, derived from whole blood, contains high levels of platelet growth factors that attract cells to damaged sites, stimulate division, and encourage angiogenesis [16]. PRP administration aids nerve regeneration by leveraging platelet growth factors to enhance the process [17].

The present study indicated PRP administration increased the number of myelinated axons in local and combined intraperitoneal and local PRP groups compared to the control group. However, no difference was found in collagen density (p=0.056) and axon diameter (p=0.856) among groups, possibly due to insufficient PRP administration duration to induce molecular differences. A study observed a significant elevation of alpha-smooth muscle actin (α-SMA) levels and the number of myelinated axons in rats treated with PRP compared to untreated rats after

nine weeks [11]. In contrast, a study utilizing PRP in rats after cavernous nerve-sparing prostatectomy after the 4th week resulted in reduced α-SMA levels compared to sham procedure [18]. α-SMA is crucial for collagen synthesis, regulating the transformation of collagen type 1 to collagen type 3 in myofibroblasts [19]. Since the present study focuses on histopathological changes, a longer duration may be necessary for more reliable data.

Prior studies have yielded inconclusive results regarding the effect of PRP on peripheral nerve diameter [20,21]. A study found no increase in the diameter of the sciatic nerve following injury with PRP treatment; however, the myelinated nerve fiber density was increased after PRP administration, contrasting with findings on functional recovery [21]. A previous study also showed an increase in NADPH-diaphorase-positive nerve fibers in dorsal nerves, suggesting PRP may enhance nerve healing post-injury [20]. The methodological approach in the present study differed from other studies as the present study assessed histopathological analysis at an earlier stage, contrasting with studies [20,21] that typically wait at least twelve weeks or longer before analysis.

PRP boosts Schwann cell proliferation and circulation, aiding in the synthesis of collagen for nerve tissue regeneration [22]. Bioactive proteins in PRP kickstart and regulate peripheral neuron repair, including transforming growth factor beta (TGF-β), platelet-derived growth factor (PDGF), and insulin-like growth factor 1 (IGF), potentially enhancing nerve regeneration [23]. Schwann cells and neurons both produce PDGF receptor, which stimulates and protects Schwann cells [21]. TGFβ-1 plays a role in Schwann cell proliferation and differentiation of nerve growth factors [24]. Injection site for PRP administration remains a topic of interest, with Lichtenfels *et al*. showed no significant changes in axon diameter or myelin sheath thickness in rat sciatic nerve tissue treated with PRP, platelet-rich fibrin (PRF), and control injections [21]. However, PRP and PRF have shown beneficial effects on functional nerve recovery [21].

The present study observed a significant increase in the number of myelinated axons, consistent with prior studies [25,26]. A study reported substantial damage to myelinated axons in the sciatic nerve after PRP injection compared to controls, with added curcumin enhancing PRP effectiveness. The benefit was notable at four weeks and more pronounced at twelve weeks [26]. Another study supported the findings in the present study, highlighting PRP's superior effects on nerve gap reconstruction compared to control [25].

The present study indicated that early-stage PRP administration may increase myelinated axon numbers in nerve injuries but not axon diameter or collagen density. However, axon diameter and collagen density may not align post-PRP injection – PRP's efficacy may vary with time, given the present study focuses on the early post-injury phase. In the present study, PRP showed promise as a novel therapy for erectile dysfunction associated with CNI. However, the present study's limitation lies in observing histopathological changes without accompanying molecular changes, hindering definitive mechanism determination. Future studies may benefit from extending the treatment duration beyond the initial phase of nerve injury.

Conclusion

PRP administration improved the number of myelinated axons in CNI, suggesting PRP's significant role in CNI regeneration. This indicates the potential for innovative approaches to treating erectile dysfunction associated with CNI.

Ethics approval

The protocol of the present study was reviewed and approved by Ethical Committee of Clinical Animal Research, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh, Aceh, Indonesia (Approval number: 098/EA/FK/2022).

Acknowledgments

The authors would like to thank the staff of the Laboratory of Experimental Animal Study, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh, Aceh, Indonesia, for helping this study.

Competing interests

All the authors declare that there are no conflicts of interest.

Funding

This study received no external funding.

Underlying data

Derived data supporting the findings of this study are available from the corresponding author on request.

How to cite

Ismy J, Khalilullah SA, Maulana R, Hidayatullah F. A potential treatment for erectile dysfunction: Effect of platelet-rich plasma administration on axon and collagen regeneration in cavernous nerve injury. Narra J 2024; 4 (2): e880 - http://doi.org/10.52225/narra.v4i2.880.

References

1. Kessler A, Sollie S, Challacombe B, *et al.* The global prevalence of erectile dysfunction: A review. BJU Int 2019;124(4):587-599.
2. Jiang N, Wu C, Zhou X, *et al.* Cavernous nerve injury resulted erectile dysfunction and regeneration. J Immunol Res 2021;2021:1-10.
3. Song G, Hu P, Song J, *et al.* Molecular pathogenesis and treatment of cavernous nerve injury-induced erectile dysfunction: A narrative review. Front Physiol 2022;13:1029650.
4. Thomas C, Konstantinidis C. Neurogenic erectile dysfunction. Where do we stand? Medicines 2021;8(1):3.
5. Liu C, Lopez DS, Chen M, *et al.* Penile rehabilitation therapy following radical prostatectomy: A meta-analysis. J Sex Med 2017;14(12):1496-1503.
6. Limoncin E, Gravina GL, Corona G, *et al.* Erectile function recovery in men treated with phosphodiesterase type 5 inhibitor administration after bilateral nerve-sparing radical prostatectomy: A systematic review of placebo-controlled randomized trials with trial sequential analysis. Andrology 2017;5(5):863-872.
7. Anastasiadis E, Ahmed R, Khoja AK, *et al.* Erectile dysfunction: Is platelet-rich plasma the new frontier for treatment in patients with erectile dysfunction? A review of the existing evidence. Front Reprod Health 2022;4:944765.
8. Marx RE. Platelet-rich plasma (PRP): What is PRP and what is not PRP? Implant Dent 2001;10(4):225-228.
9. Liu MC, Chang ML, Wang YC, *et al.* Revisiting the regenerative therapeutic advances towards erectile dysfunction. Cells 2020;9(5):1250.
10. Epifanova MV, Gvasalia BR, Durashov MA, *et al.* Platelet-rich plasma therapy for male sexual dysfunction: Myth or reality? Sex Med Rev 2020;8(1):106-113.
11. Tai H, Tsai W, Chang M, *et al.* Intracavernous injection of platelet-rich plasma reverses erectile dysfunction of chronic cavernous nerve degeneration through reduction of prostate hyperplasia evidence from an aging-induced erectile dysfunction rat model. FASEB J 2023;37(4):e22826.
12. Percie du Sert N, Hurst V, Ahluwalia A, *et al.* The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. BMC Vet Res 2020;16(1):242.
13. Zhang X, Liquan H, Jing Y, *et al.* Rat model of erectile disfunction caused by cavernous nerve ablation. Chin Med J (Engl) 2002;115(8):1179-1182.
14. Campbell JD, Milenkovic U, Usta MF, *et al.* The good, bad, and the ugly of regenerative therapies for erectile dysfunction. Transl Androl Urol 2020;9(S2):S252-S261.
15. Hakim L, Van der Aa F, Bivalacqua TJ, *et al.* Emerging tools for erectile dysfunction: A role for regenerative medicine. Nat Rev Urol 2012;9(9):520-536.
16. Alves R, Grimalt R. A review of platelet-rich plasma: History, biology, mechanism of action, and classification. Skin Appendage Disord 2018;4(1):18-24.
17. Marx RE. Platelet-rich plasma: Evidence to support its use. J Oral Maxillofac Surg 2004;62(4):489-496.
18. Liao CH, Chang CJ, Chen KC, *et al.* Effects of platelet-rich plasma glue placement at the prostatectomy site on erectile function restoration and cavernous nerve preservation in a nerve-sparing prostatectomy rat model. Biomed Pharmacother 2023;161:114499.
19. Shinde AV, Humeres C, Frangogiannis NG. The role of α-smooth muscle actin in fibroblast-mediated matrix contraction and remodeling. Biochim Biophys Acta Mol Basis Dis 2017;1863(1):298-309.
20. Ding XG, Li SW, Zheng XM, *et al.* The effect of platelet-rich plasma on cavernous nerve regeneration in a rat model. Asian J Androl 2009;11(2):215-221.

21. Lichtenfels M, Colomé L, Sebben AD, *et al.* Effect of platelet rich plasma and platelet rich fibrin on sciatic nerve regeneration in a rat model. Microsurgery 2013;33(5):383-390.
22. Ye F, Li H, Qiao G, *et al.* Platelet-rich plasma gel in combination with Schwann cells for repair of sciatic nerve injury. Neural Regen Res 2012;7(29):2286-2292.
23. Savignat M, De-Doncker L, Vodouhe C, *et al.* Rat nerve regeneration with the use of a polymeric membrane loaded with NGF. J Dent Res 2007;86(11):1051-1056.
24. Ardhani R, Susilowati R, Ana ID. Functional recovery of axonal injury induced by gelatin-hydrogel film and PRP: An initial study in rats. J Biomed Sci Eng 2015;8(3):160-169.
25. Teymur H, Tiftikcioglu YO, Cavusoglu T, *et al.* Effect of platelet-rich plasma on reconstruction with nerve autografts. Kaohsiung J Med Sci 2017;33(2):69-77.
26. Zavala A, Martinez PC, Gutierrez GG, *et al.* The combined use of curcumin and platelet-rich plasma enhances axonal regeneration in acute nerve injuries: An experimental study in a rat model. J Hand Microsurg 2023;15(1):31-36.