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Submission date: 21-Apr-2023 12:16PM (UTC+0800)
Submission ID: 2070998809
File name: 3119-3124.pdf (221.33K)
Word count: 3355
Character count: 17498

DOI : 10.35124/bca.2020.20.S1.3119

Biochem. Cell. Arch. Vol. 20, Supplement 1, pp. 3119-3124, 2020 | www.connectjournals.com/bca | *ISSN 0972-5075*

INDUCING SCHWANN CELLS INCREASE USING FREEZE-DRIED PLATELET-RICH PLASMA FOR CHRONIC CONSTRICTION NERVE INJURY IN RAT MODEL

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(Received 22 March 2020, Revised 7 May 2020, Accepted 11 May 2020)

ABSTRACT : Neuropathic pain is a chronic pain condition and it refers to all pain initiated or caused by primary lesions or transient dysfunction or disorders of the peripheral or central nervous system (CNS). Neuropathic pain is caused by damage or injury to nerves that transfer information to the brain and spinal cord from the skin, muscles and other body parts. Platelet concentrates such as Platelet-Rich Plasma (PRP) have been used for tissue regeneration because it contains high Growth Factor. PRP was tested for nerve injury to examine the potential of PRP in nerve repair improvement. The purpose of this study was to prove the PRP role in increasing the number of Schwann cells for nerve repair. This study used three-month-old *Rattus novergicus* experiment animals. The experimental animals were randomly divided into seven groups, with six rats per group. On the day 14 and 21 post-treatment, the rats were sacrificed. Schwann cells were calculated using Hematoxylin Eosin staining. The results were analyzed using ANOVA statistical tests and Independent T-test. On day 21, rats treated with single-dose Platelet-Rich Plasma in the treatment group demonstrated increased Schwann cell growth $p<0.05$ (0.000) in comparison to the ligation group. However, it was not significantly different in comparison to the single PRP treatment on day 14 with $p>0.05$ (0.219). Platelet-Rich Plasma treatment can increase the number of Schwann cells for neuroregeneration. Administering a single dose of Platelet-Rich Plasma for 21 days was the most effective treatment for neuroregeneration.

Key words : Schwann cell, platelet rich plasma, nerve, neuroregeneration.

INTRODUCTION

Orofacial pain includes many disorders, including temporomandibular disorders (TMD), trigeminal neuralgia, headaches and myofascial pain; and it occurs in 23% of the populationand 7–11% of them are chronic pain (Krzyzanowska *et al*, 2012; Benoliel *et al*, 2015). Chronic pain associated with damage to the nerve tissue, disrupting pain modulation in the nerve and is often referred to as neuropathic pain (Damasceno *et al*, 2016; Tender *et al*, 2013). It was estimated that 37.6 million people suffered from neuropathic pain in 2005, and the prevalence increased to 39.1 million in 2011. Some neuropathic pains in orofacial are trigeminal neuralgia, glossopharyngeal neuralgia, postherpetic neuralgia, and peripheral neuropathic (due to malignancy and diabetes mellitus) (Wang *et al*, 2014). Other neuropathic pains in orofacial are stomadynia (burning mouth syndrome), phantom tooth pain (atypical odontology) and traumatic nerve injuries (Benoliel *et al*, 2015). Inflammatory components may present in neuropathic pain; therefore, effective management requires several types of medication.

Neuroregeneration is needed for neuropathic pain therapy as it may occur due to nerve tissue damage. Neuroregeneration or repair of nervous tissue means regrowth or recovery of nervous tissue, cells, or products of cells (Winias S et al, 2020). Schwann cells play a critical role in nerve regeneration. Nerves in the peripheral nervous system (PNS) consist of many axons myelinated by Schwann cells. In the event of nerve damage, Schwann cells help by phagocytizing their axons and guiding them into the regeneration target.

Pharmacotherapy for neuropathic pain has limitations, and it usually utilizes NSAIDs and opioids in symptomatic therapy to reduce pain (Jaggi *et al*, 2011). Therefore, new neuropathic therapies to regenerate damaged nervous tissue are in need. One of the neuropathic treatments is Platelet-Rich Plasma (PRP) therapy. PRP does not only stop the degeneration process of necrotic tissue, but it also improves regeneration (Maghsoudi *et al*, 2015; Kon *et al*, 2010).

METHODS

The study has been approved by the Ethics Commission of Faculty of Dental Medicine, Universitas Airlangga, No. 068/HRECC.FODM/VI/2017. This was an in-vivo experimental laboratory study with a post-test only control group design. There were 7 treatment groups, and each group was treated and named with group Normal, group A (14 days with repeat PRP dose), group B (14 days with single PRP dose), group C (21 days with repeat PRP dose), group D (21 days with single PRP dose), group ligation 14 (14 days ligation) and group ligation 21 (21 days ligation). The instruments of the study were 4000 rpm centrifuge, -800° C freezer, ocular micrometer, magnetic stirrer, UV sterilizer clean bench, freeze dryer, and microscope.

The ingredients of the study were Formaldehyde 10%, Dextrose citrate acid 9%, EDTA Vacutainer 5 ml, ketamine xylazine, silk thread 3/0, osmium tetraoxide, and Hematoxylin Eosin.

Preparation of experimental animals

The experimental animals were randomized and divided into 7 groups: group Normal, group A (14 days with repeat PRP dose), group B (14 days with single PRP dose), group C (21 days with repeat PRP dose), group D (21 days with single PRP dose), group ligation 14 (14 days ligation) and group ligation 21 (21 days ligation). The animals were adapted to the environment by feeding basal rations for seven days.

Methods of Preparation of Allogeneic Platelet-Rich Plasma (PRP)

Blood was obtained from ten Wistar rat hearts using spoit with anticoagulant dextrose citrate acid to prevent coagulation. The blood was then stored in sterile tubes and centrifuged at 4000 rpm for 10 minutes to separate red blood cells and plasma. Plasma at the upper tube was later moved to another tube using a dysposible syringe. It was then centrifuged again at 4000 rpm for 10 minutes to separate the platelet-rich plasma (1/3 of the lower tube) and platelet-less plasma (2/3 at the upper tube). The 1/3 plasma at the bottom of the tube is the platelet-rich plasma (PRP).

Platelet concentrates were dissolved in PBS, collected, incubated at room temperature (300°C), centrifuged to remove clots, calibrated, and frozen at -800°C for subsequent use. The PRP was dissolved in PBS with a 1:1 ratio before use. The PRP was freeze-dried and dissolved in CMCNa 2% with a 1:1 ratio before use in the experimental animals.

Experimental animal treatment

Aseptic survival surgery was performed using povidone-iodine in the abdominal area to expose the nerve fibers. These sciatic nerve fibers were then tied loosely with 4 ligatures 1 mm apart using 3/0 non-absorbable black-silk. Nervous tissue damage occurred after 2x24 hours. The injured nervous area was then ready to be treated. The PRP was applied using 27 gauge insulin syringe in 0.1 ml dose. Once finished, the wound was then sutured. On day 14 and 21, the rats in each group were asphyxiated using ketamine, and the number of Schwann cells as nerve regeneration indicator was calculated.

Methods of histopathological preparation

The tissue was cut 5 mm distally to the injury and washed with a phosphate buffer pH 7.4 three times. The tissue was put into 2% glutaraldehyde solution under fixed conditions for 3 hours. It was then transferred to a phosphate buffer solution with a pH of 3 and stored at 40°C.

The tissue was then dehydrated, cleared, impregnated, embedded, cut and colored. Hematoxylin-eosin staining was utilized to observe the osteoblasts.

Data analysis

The data were analyzed with SPSS 18 using the ANOVA parametric test and the Independent T-test.

RESULTS

Schwann cells are cells around axons, have a single nucleus, and form myelin sheaths. They were observed using a light microscope at 400x magnification. The Schwann cell distribution of 14 days treatment is presented in Fig. 1. Schwan cells were mostly observed in the normal group. There were more inflammation cells in group A and ligation 14 than group B. The connective tissue was more visible in treatment group B and normal group than group A and ligation 14.

The number of Schwann cells after single and repeat PRP treatment for 21 days is observable on the transversal cut of the HE stained sciatic nerve in group C and D. Fig. 1 shows that there were more Schwan cells in the normal group than in group B, C and ligation 21. There was more inflammatory cell distribution such as lymphocytes, neutrophils, fibroblasts in group C. Several Schwan cells, inflammatory cells, fibroblasts and endoneurial connective tissue were observed in group D. Inflammatory cells, and vacuolar degeneration was observed in group ligation 21, indicating tissue damage.

The highest and lowest number of myelin was observed in group D and A, respectively. The myelin of

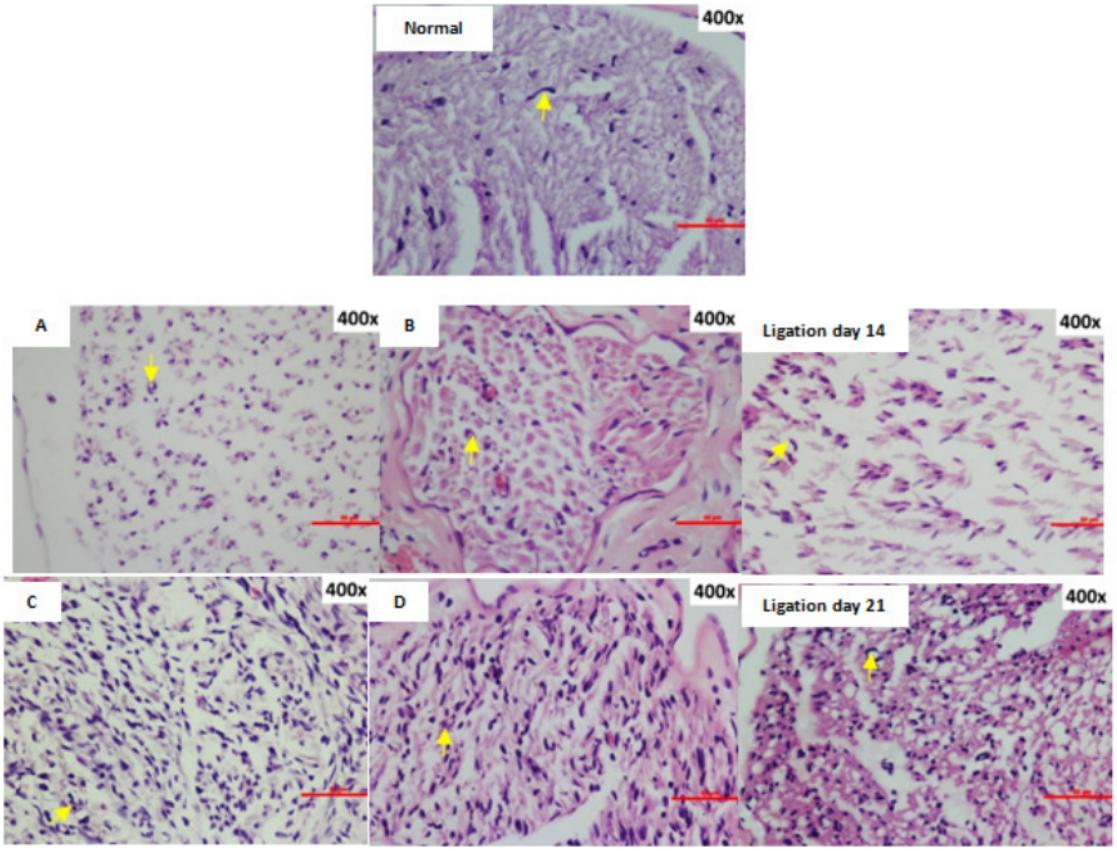

Fig. 1 : Cross-sectional view of the hematoxylin-eosin stained sciatic nerves were observable at 100x and 400x magnification in Normal, A (14 days of repeat PRP dose), B (14 days of single PRP dose), Ligation 14 (nerve ligation for 14 days), C (21 days of repeat PRP dose), D (21 days of single PRP dose), and Ligation 21 (nerve ligation for 21 days).

Table 1 : Table of Schwann cell mean for each treatment group.

Group	Mean	SD
Normal	83	12
A	23	6
B	46	8
C	56	12
D	61	8
Day ligation 14	40	10
Day ligation 21	29	8

group ligation 14 was thicker than the group A. The highest number of myelin of all groups was observed in the normal group.

The result of statistical analysis was tested using the Kolmogorov-Smirnov test and it obtained Sig (2-tailed) value of 0.686> 0.05, confirming that the data were normally distributed. The homogeneity test using Lavene's test obtained sig.> 0.05, confirming that the data were homogeneous and validating the use of the Independent T-test for parametric tests. The different tests using the

Table 2 : The significance value of the ANOVA post hoc Bonferroni test.

A	B	0.002*
	Ligation 14	0.067
	Normal	0.000*
B	A	0.002*
	Ligation 14	1,000
	Normal	0.000*
C	D	1,000
	Ligation 21	0.000*
	Normal	0.001*
D	C	1,000
	Ligation 21	0.000*
	Normal	0.007

ANOVA revealed that group A was significantly different from group B with Sig. 0.002 <0.05 and group C was not significantly different from group D as the Sig. 1,000 >

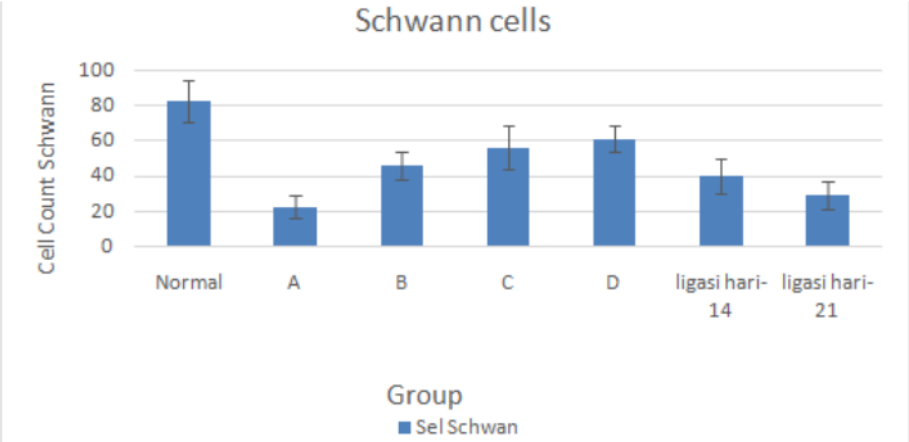

Fig. 2 : Mean of Schwann cell for each treatment group.

0.005. Independent t-test between the same treatment groups (14 days and 21 days) revealed that group A was significantly different from group C with Sig. 0.000 <0.05, and group B was not significantly different from group D with Sig. 0.219 <0.05.

DISCUSSION

The material was allogenic PRP obtained from Wistar rats and freeze-dried for easy storage and flexible application time. Freeze-Dried Platelet-Rich Plasma (FD-PRP) is sterilized using UV light, and it can be stored for one month, while gamma-ray sterilization can extend the storage time to five years.The allogeneic PRP was expected to be replicable on humans, human allogeneic PRP. Although several studies suggested autologous PRP in consideration of the risk of infection and hypersensitivity, other studies suggested the opposite for immunocompromised conditions, especially in thrombocytopenia or inadequate platelet counts. In such case, allogeneic PRP can be the option (Burnouf *et al*, 2016). Research by Rachmawati, Astuti, Purwati (2015) found that Freeze-Dried Allogeneic PRP has a similar effect with Fresh Autologous PRP. FD-PRP also did not cause an immunological hypersensitivity response in rabbit, as evidenced by no increase in humoral immunity (immunoglobulin M/Ig M) after injection of liquid FD-PRP in liquid form. FD-PRP improves TGF â1 function as Fresh PRP does. Freeze-Dried (FD) processes at dry -83°C temperature for 24 hours suggested Host/Human Protein Antigen (HPA) deactivation on the platelet membranes.

Nerve injury in experimental animals as a neuropathic pain model of the sciatic nerve was ligation induced (Jaggi *et al*, 2011; Zhu *et al*, 2014; Dubovy, 2009). These treatments cause damage to the peripheral nervous tissue. The degeneration process at the nerve endings occurs immediately after the peripheral nerve injured. Histological changes mainly involve the physical fragmentation of axons and myelin. Neurotubules and neurofilament separated and the shape of the axons become irregular. The continuity of the axons disappeared, the conduction of impulses did not occur, and myelin disintegration started.

Neuroregeneration or repair of nervous tissue means regrowth or recovery of nervous tissue, cells, or products of cells. The axon ends appear from the proximal end and progresses to its distal. Its development is regulated by chemotactic factors secreted by Schwann cells (Patel *et al*, 2017; Cobianchi *et al*, 2013). The nerve regeneration parameter was Schwann cells. To achieve complete nerve recovery after an injury, the nerve undergoes three main processes of neuroregeneration, namely: Wallerian degeneration (myelin debris and axons clearance on the distal side), axon regeneration and target organ reinnervation. Schwann cells increase in all neuroregeneration stages, especially in the first two stages, namely during inflammation (in Wallerian degeneration) and axon regeneration (the band of Büngner formation).

The highest mean of Schwann cells and the closest to the normal nerve was observed in group D, while the lowest mean was observed in group A. The number of Schwann cells in group D was the same as the normal group and group C and was significantly different from the group ligation group 21. Growth factors present in Platelet-Rich Plasma increase the Schwann cells proliferation and differentiation of several neurotrophic factors that can accelerate nerve regeneration (Vokurka *et al*, 2016). Repeat dose caused recurring inflammation, thereby slowing the nerve recovery as observed in group

C. The neutrophils present in PRP contain more than 40 hydrolytic enzymes and toxic molecules in their granules, making them oxidants such as superoxide anions, hydrogen peroxides, and hypocaloric acids. These toxic molecules can cause tissue damage that disturbs nerve regeneration. CMC as a scaffold PRP carrier maintains the PRP position on damaged peripheral nerve, making the growth factors in the PRP available on the wound site sustainably (Chen and Fan, 2007).

Schwann cell is the main mediator that triggers many events in Wallerian degeneration and changes in protein expression at the injured site, which is the key to axon regeneration. In the absence of axonal contact, Schwann cells change to non-myelinating phenotype – which decreases the expression regulation of some proteins such as PMP22, Krox-2052, P0, and connexin-3253. The synthesis is captured, and differentiation is promoted by the production of C-jun54 and then neurotrophic factors such as NGF and CNTF are produced. Together, these factors contribute to the formation of new Schwann cell groups under the protection of endogenous compounds such as erythropoietin. It was then followed by the release of mitogen from the proximal stump of neurons such as ATP and neuregulin, which together and with the help of acetylcholine, produce new Schwann cells with myelinating phenotype (Menorca *et al*, 2015).

Platelet-Rich Plasma can increase the Schwann cell proliferation, Schwann cell migration, and the synthesis of extracellular matrices such as collagen that have benefits in nerve regeneration. The recovery of peripheral nerves is initiated and controlled by bioactive proteins found in platelets and plasma (Savignat *et al*, 2008). Increased bioactive proteins such as TGF-α, PDGF, IGF act as catalysts to accelerate peripheral nerve regeneration. Schwann cells and neurons express PDGF receptors and PDGF functions as mitogen and defense factor (survival) for Schwann cells. The TGFα-1 bioactive protein plays a role in Schwann cell proliferation and the differentiation of several neurotrophic factors (Ardhani *et al*, 2015). Thus, the study concluded that a single dose FD-PRP administration in chronic constriction nerve injury could increase the number of Schwan cells on day 21.

ACKNOWLEDGEMENT

This research is financially supported by Ministry of Research, Technology & Higher Education of Indonesia [grant numbers 6/E/KPT/2019].

REFERENCES

Ardhani R, Susilowati R and Ana I D (2015) Functional Recovery of Axonal Injury Induced by Gelatin-Hydrogel Film and PRP: An Initial Study in Rats. *J. Biomedical Sci. Engineer.* **8**, 160-169

Benoliel R, Ananthan S, Zagury J G, Khan J and Eliav E (2015) Orofacial Pain. In : *Burket's Oral Medicine*. (eds. Glick M and Feagan Chair W), People's Medical Publishing House, 309-321.

Burnouf Koh Strunk Schallmoser (2016) Human platelet lysate: replacing fetal bovine serum as a gold standard for human cell propagation?. *Biomaterials* **76**, 371-387.

Chen, H and Fan, M (2007)Chitosan/Carboxymethyl cellulose polyelectrolyte complex scaffolds for pulp cells regeneration. *J. Bioact. Compat. Polym.* **22**, 475–491.

Cobianchi S, Casals-Diaz L, Jaramillo J and Navarro X (2013) Differential effects of activity dependent treatments on axonal regeneration and neuropathic pain after peripheral nerve injury. *Exp. Neurol.* **240**, 157–167.

Damasceno Marina B M V, José De Maria A De Melo Júnior, Sacha Aubrey A R Santos, Luana T M Melo, Laura Hévila I Leite, Antonio E. Vieira-Neto, Renato De A Moreira, Ana Cristina De O Monteiro-Moreira and Adriana R Campos (2016) Frutalin Reduces Acute and Neuropathic Nociceptive Behaviours in Rodent Models of Orofacial Pain. *Chemico-Biological Interactions* **256**, 9–15.

Dubovy P (2009) Experimental models of peripheral neuropathic pain based on traumatic nerve injuries – An anatomical perspective. *Ann. Anat. Anatomischer Anzeiger* **191**, 248–259.

Jaggi A S, Jain V and Singh N (2011) Animal Models of Neuropathic Pain. *Fundam. Clin. Pharmacol.* **25**, 1–28.

Kon E, Filardo G, Delcogliano M, Fini M, Salamanna F, Giavaresi G, Marin I and Marcacci M (2010) Platelet autologous growth factors decrease the osteochondral regeneration capability of a collagen-hydroxyapatite scaffold in a sheep model. *BMC Musculoskelet. Disord.* **11**, 220.

Krzyzanowska A and Avendan C (2012) Behavioral testing in rodent models of orofacial neuropathic and inflammatory pain. *Brain and Behaviour* **2**(5), 678-697.

Maghsoudi O, Beheshtiha S H S, Abarkar M and Anvar S A (2015) Standardization and Modification Techniques of Platelet- Rich Plasma (PRP) Preparation in Rabbit. *Int. Clin. Pathol. J.* **4**, 1–5.

Menorca Ron M G, Fussell Theron S and Elfar John C (2015) Peripheral Nerve Trauma: Mechanisms of Injury and Recovery **29**(3), 317–330.

Patel A K, Park K K and Hackam A S (2017) Wnt Signaling Promotes Axonal Regeneration Following Optic Nerve Injury in the Mouse. *Neuroscience* **343**, 372–383.

Rachmawati Astuti Purwat (2015) Research Article: The Effect of Allogenic Freeze Dried Platelet- Rich Plasma in Immunological Responses of Rabbits. *Sch Acad. J. Biosci.* **3**(4), 325-327.

Savignat M, Voduhe C, Ackerman A, Haikel Y, Lavalle P and Libersa P (2008) Evaluation of early nerve regeneration using a polymericmembrane functionalized with nerve growth factor after a crush lesion of rat mental nerve. *J Oral Maxillofac Surg.* **66**, 711-717.

Tender G C, Li Y Y and Cui J G (2013) The role of nerve growth factor in neuropathic pain inhibition produced by resiniferatoxin treatment in the dorsal root ganglia. *Neurosurgery* **73**, 158–165.

Tuomi H and Best T M (2003) The inflammatory response: friend or enemy to muscle tissue*? Br J Sports Med.* **37**(4), 284-286.

Vokurka J, Gopfert E, Blahutkova M, Buchalova E and Faldyna M (2016) Concentrations of growth factors in platelet-rich plasma

and platelet-rich fibrin in a rabbit model. *Vet. Med. (Praha)* **61**, 567–570.

Wang D, Couture R and Hong Y (2014) Activated microglia in the spinal cord underlies diabetic neuropathic pain. *Eur. J. Pharmacol.* **728**, 59–66.

Winias S, Radithia D, Parmadiati AE, Soebadi B, Ernawati DS, Hadi P, Soesilowati R (2020) Allogenous Freeze-Dried Platelet-Rich Plasma Promotes Peripheral Myelin Repair in Chronic Constriction Nerve Injury. *Syst.Rev. Pharm.* **11** (6), 254-258

Zhu Q, Sun Y, Zhu J, Fang T, Zhang W and Li J X (2014) Antinociceptive effects of sinomenine in rat model of neuropathic pain. *Sci Rep*. **1**(4), 6–10.

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