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Axonal Supercharging with Reverse End-to-Side Nerve Transfer in Delayed Peripheral NerveRepair: Its Impact in SV2B mRNA Expression in Rat Sciatic Nerve Injury Model

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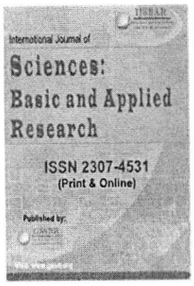

Axonal Supercharging with Reverse End-to-Side Nerve Transfer in Delayed Peripheral Nerve Repair: Its Impact in SV2B mRNA Expression in Rat Sciatic Nerve Injury Model

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Abstract

To investigate the role of reverse end-to-side nerve transfer in delayed repair of peripheral nerve injury, a rat sciatic nerve injury model was used. The dynamic of SV2B mRNA expression was investigated.

Sixteen Wistar rats were divided into four groups (four rats in each group). In Group I, the right tibial nerve was ligated 1 cm proximal to sciatic trifurcation, and the peroneal nerve was ligated distally at its entrance to peroneal tunnel. Two weeks later, the resulting neuroma was excised and the tibial nerve was repaired in end-to-end (ETE) fashion. The peroneal nerve was transferred to the distal stump of the tibial nerve in a reverse end-to-side fashion (RETS / axonal supercharging). In Group II, similar procedure to create the sciatic nerve injury was performed. Two weeks later, the tibial nerve was repaired in ETE fashion. No axonal supercharging procedure was added. In Group II, the sciatic nerve was exposed, and the wound was closed again (sham surgery / positive control). In Group IV, the sciatic nerve was injured in similar fashion, and never repaired (negative control). SV2B mRNA was measured from venous blood, taken at baseline, prior to nerve repair, and at the end of study (ten weeks after repair).

Results from the test showed that the expression of SV2B mRNA, which represents the formation of neuromuscular junction, indicated that recovery of the denervated muscles was promoted by axonal

supercharging (RETS transfer), and the result was better than conventional repair alone.

In conclusion, axonal supercharging (RETS transfer) may be useful in delayed peripheral nerve repair for nerve injuries-in-continuity.

Keywords: axonal supercharging; RETS transfer; SV2B; axon augmentation

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1. Introduction

Nerve injuries-in-continuity still posed a challenge with regards to its surgical management and functional outcome [1]. Surgical options in this situation include 1) neurolysis, 2) excision of traumatic neuroma and repair with direct ETE repair, with or without nerve graft, and 3) nerve transfer using standard ETE technique. Benefit of neurolysis alone is unclear [1]. Intraoperative electrophysiology test documenting Nerve Action Potential (NAP) across the injured segment may help substantially in intraoperative decision-making process. However, this tool is not readily available in many hospitals. Excision of traumatic neuroma and direct repair with or without graft still considered as the standard treatment for nerve injuries without evidence of NAP across the injured segment. Quite recently, nerve transfer has gained popularity as an addition in the armament of peripheral nerve surgeon dealing with difficult cases of nerve injuries, such as brachial plexus injuries [2].

Nerve transfer, essentially, is the transfer of expendable and healthy foreign nerve to denervated recipient nerve, which considered more important functionally. The donor nerve chosen is usually motor nerve from muscle with redundant innervation, or from non-dominant muscle, in which case the sacrifice of that nerve will not cause significant deficit to the patient. Nerve transfer offers several benefits compared to conventional nerve repair, such as the proximity of nerve repair to the target organ. This, of course, significantly reduced the time needed for regenerating axons to reach its target muscle, limiting the adverse effect of prolonged denervation [2-4]. Nerve transfer also allows the surgeon to simply choose which muscle to innervate and which donor to use, rather than doing complex nerve exploration and electrophysiology test. The drawback is, cortical adaptation is required to overcome the barrier of different nerve functionality, e.g. the use of intercostal nerves to reinnervate musculocutaneous nerve [2]. In such case, patients have to relearned to use their respiratory nerve to flexed the elbow. In time, they will be able to separate the functionality of the two.

Studies have shown that nerve transfer has given comparable, and in some cases, better outcome to conventional direct nerve repair [5-9]. However, nerve transfer procedure in a sense, completely disregard the potential of nerve regeneration of the injured nerve. Therefore, this procedure may not be ideal for nerve injuries-in-continuity with residual nerve functions, or when spontaneous regeneration is expected. In which, many of such cases failed to achieve meaningful functional recovery.

Isaacs et al first described reverse end-to-side nerve transfer in rat model[1]. They found RETS transfer can achieve comparable functional outcome with conventional ETE repair [1, 10]. Fujiwara et al first coined the term axonal supercharging for RETS transfer [11]. Since then, several studies has been published showing good result of RETS transfer / axonal supercharging for acute nerve repair [12-14]. However, there has not been a

study of RETS transfer for delayed nerve repair. It has been shown that even delayed nerve repair as early as 2 weeks significantly decreased muscle mass and muscle force after 6 months of recovery period [15].

This study aims to investigate the role of axonal supercharging in delayed nerve repair. First step, we tried to study the dynamic of SV2B mRNA expression following nerve injury and repair. SV2B is an isoform of SV2, which is a glycosylated synaptic vesicle membrane protein [16]. SV2 is known to participate in the regulation of calcium-mediated synaptic transmission and to play a role in vesicle trafficking by binding to other cell surface proteins [16]. Wang et al has shown that SV2B is expressed at the pre-synaptic terminal of rat neuromuscular junctions (NMJs) [17]. Therefore, the expression of SV2B, and lack thereof may reflect integrity of NMJs.

We hypothesized that axonal supercharging or axonal augmentation will increase the number of regenerative axons growing into the distal stump of recipient nerve, and in turn forming more NMJs with target muscles, and this will be reflected in the expression of mRNA SV2B.

2. Material and Methods

2.1. Animal model

Experiment was performed in 8-10 weeks old Wistar rats (*Rattus norvegicus*), weighted 200-250 gr. The rats were obtained from Molecular Biology and Immunology Laboratory, Hasanuddin University, Makassar, Indonesia. The University Committee on the Use and Care of Animals approved and monitored the experimental protocol. Rats were housed in a restricted-access facility, given food and water ad libitum, and exposed to a 12-hour light-dark cycle. For all surgical procedures, rats were anesthetized with an intraperitoneal injection of ketamine and subcutaneous injection of lidocaine 2%. Surgical procedures were conducted under aseptic conditions.

2.2. Experimental design

Sixteen Wistar rats were randomly allocated into four groups (four rats in each group). In Group I, the axonal supercharging group (AS+), the right tibial nerve was ligated 1 cm proximal to sciatic trifurcation, and the peroneal nerve was ligated distally at its entrance to peroneal tunnel. Two weeks later, the resulting neuroma was excised and the tibial nerve was repaired in end-to-end (ETE) fashion. An epineurial window was created on the side of the distal stump of tibial nerve, approximately 5 mm from the sciatic trifurcation, and then the peroneal nerve was transected and transferred in a reverse end-to-side fashion (RETS / axonal supercharging). In Group II, control group (AS-), similar procedure to create the sciatic nerve injury was performed. Two weeks later, the tibial nerve was repaired in ETE fashion. No axonal supercharging procedure was added. In Group III, the sciatic nerve was exposed, and the wound was closed again without further manipulation to the nerves (sham surgery / positive control). In Group IV, the sciatic nerve was injured in similar fashion, and was not repaired (negative control).

2.3. SV2B mRNA

SV2B mRNA was measured from venous blood, taken at the tail lateral vein. Samples were collected in three time points during the experiment: 1) baseline, 2) prior to nerve repair, and 3) at the end of study (ten weeks after repair). SV2B mRNA in blood was measured using Real Time PCR technique. RT-PCR was performed in a standard fashion as described previously [18]. The reactions and runs for all samples were performed three times.

2.4. Statistical analysis

All values are presented as a mean ± standard deviation. Means between each time points within a group were compared with paired t test. Means between each group were compared with One-Way Anova and LSD test. Alpha was set at 0.05 for all statistical comparisons.

3. Results and Discussion

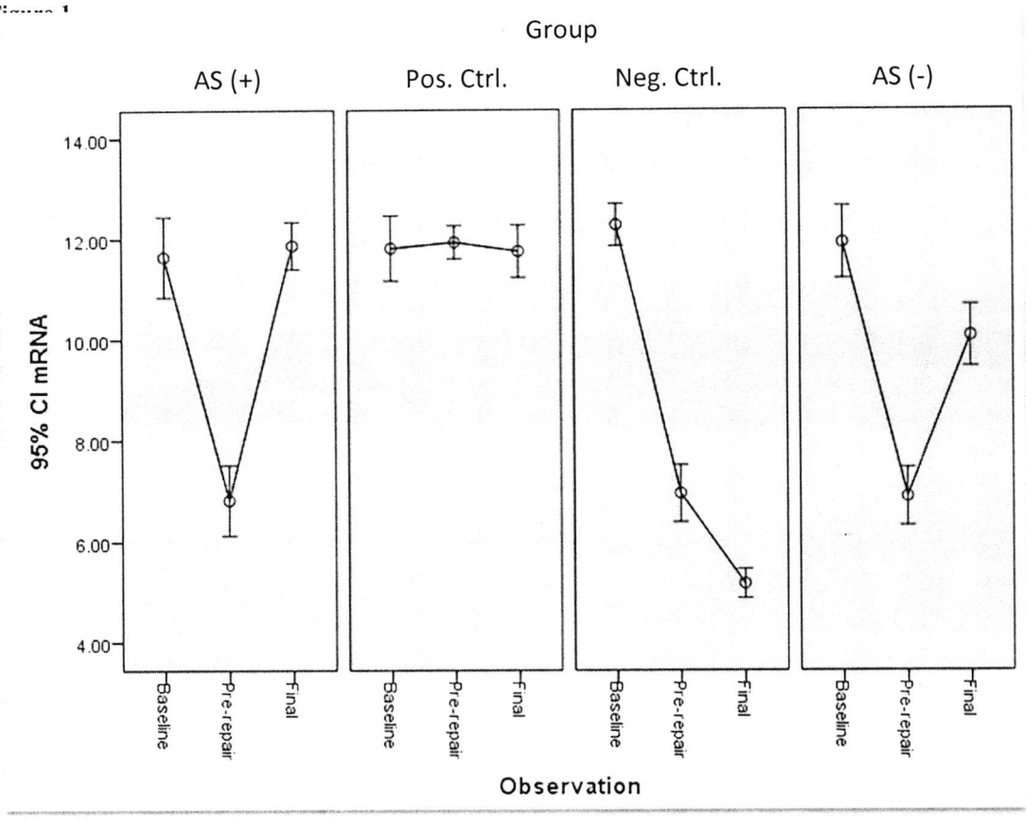
To investigate the effect of axonal supercharging on the expression of SV2B mRNA, group means were compared between each time points and between each group. The result is summarized in table 1 below.

Table 1. Dynamics of SV2B mRNA

Group	Mean±SD mRNA SV2B			p*
	Baseline**	Pre-repair**	Final**	
I. AS(+); (n=4)	11.64±0.51[a]	6.82±0.44[b]	11.86±0.30[a]	<0.001
II. SA(-); (n=4)	11.92±0.46[a]	6.88±0.36[b]	10.06±0.38[c]	<0.001
III. Pos. Control; (n=4)	11.81±0.41[a]	11.93±0.21[a]	11.75±0.33[a]	1.000
IV. Neg. Control; (n=4)	12.27±0.26[a]	6.94±0.35[b]	5.17±0.18[b]	<0.001

*Paired t test; **One-Way Anova + LSD test; similarly coded superscript in the same rows or column indicates non-significant difference ($p>0.05$); different codes, indicate statistically significant difference ($p<0.05$).

Table 1 indicates that there were significant changes of SV2B mRNA expression between each time point in each group, except in the positive control group (III). In baseline, there was no significant difference between each group ($p>0.05$). Prior to repair, the mRNA expression decreased significantly ($P<0.05$), except in the positive control group. Between the other three groups, there was no significant difference noted ($P>0.05$). At the end of the study (10 weeks after repair), the mRNA expression in axonal supercharging group returned to baseline level; in group II, the level increased but did not reach baseline level; in group III, mRNA expression remained stable, while in group IV, mRNA expression continued to fall off.



Error bar showing dynamics of SV2B mRNA expression based on group

Figure 1 clearly shows the dynamics of SV2B mRNA expression in each group at each time points. Here again we can see that in the axonal supercharging group (AS +), the mRNA expression increased and returned to baseline at ten weeks after repair. In the positive control group, the mRNA expression remained stable. In the negative control group, mRNA level continued to decline, and in the conventional repair group (ETE without axonal supercharging), mRNA expression increased following repair, but did not reach baseline at week ten.

4. Discussion

These data supported our hypothesis that axonal supercharging technique or reverse end-to-side nerve transfer can increase the number of regenerating axons growing into the distal stump of recipient nerve, reflected by the increase of mRNA SV2B (a marker of NMJs) following repair. Not only it successfully reinnervate the muscle, it also provide a more robust innervation compared to conventional repair (ETE alone). This may positively impact the functional outcome in delayed nerve repair. The following step would be to correlate this finding with the histopathological features of the RETS transfer, functional recovery of the affected nerve, and perhaps with a longer delay in repair, and a longer observation.

5. Conclusion

The results of this study suggest that axonal supercharging technique or reverse end-to-side nerve transfer is an effective method to promote nerve regeneration in delayed nerve repair. It effectively augments the number of regenerating axons without sacrificing the native axons of the injured nerve. Its clinical application in nerve injuries-in-continuity may proof beneficial, but it remains to be investigated.

Conflicts of Interest Disclosure

The authors report no conflict of interest. The authors have no personal, financial, or institutional interest in any of the drugs, materials or devices used in the article.

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