online © ML Comm
www.jkns.or.kr http://dx.doi.org/10.3340/jkns.2015.58.6.504
Print ISSN 2005-3711 On-line ISSN 1598-7876
J Korean Neurosurg Soc 58 (6) : 504-507, 2015

Laboratory Investigation

Histopathological Effects of Tissue Adhesives on Experimental Peripheral Nerve Transection Model in Rats

Idiris Altun, M.D.,[1] Harun Çıralık, M.D.[2]
Departments of Neurosurgery,[1] Pathology,[2] Kahramanmaras Sutcu Imam University Medical Faculty, Kahramanmaras, Turkey

Objective : Our aim was to evaluate the histopathological effects of tissue adhesives on peripheral nerve regeneration after experimental sciatic nerve transection in rats and to search whether these tissue adhesives may possess a therapeutic potential in peripheral nerve injuries.
Methods : This experimental study was performed using 42 female Wistar-Albino rats distributed in 6 groups subsequent to transection of right sciatic nerves. Group I underwent external circumferential neurolysis; Group II received suture repair; Group III had local polymeric hydrogel based tissue adhesive administration; Group IV received suture repair and polymeric hydrogel based tissue adhesive application together; Group V had gelatin based tissue adhesive application and Group VI had suture repair and gelatin based tissue adhesive together. After a 6-week follow-up period, biopsies were obtained from site of neural injury and groups were compared with respect to histopathological scoring based on inflammatory, degenerative, necrotic and fibrotic changes.
Results : There were remarkable differences between control group and study groups with respect to inflammation (p=0.001), degeneration (p=0.002), necrosis (p=0.007), fibrosis (p<0.001) and vascularity (p=0.001). Histopathological scores were similar between study groups and the only noteworthy difference was that Group V displayed a lower score for necrosis and higher score in terms of vascularization.
Conclusion : Our results imply that tissue adhesives can be useful in repair of peripheral nerve injuries by decreasing the surgical trauma and shortening the duration of intervention. Results with gelatin based tissue adhesive are especially promising since more intense vascularity was observed in tissue after application. However, trials on larger series with longer durations of follow-up are essential for reaching more reliable conclusions.

Key Words : Peripheral nerve · Injury · Regeneration · Sciatic nerve · Tissue adhesive.

INTRODUCTION

The peripheral nervous system is capable of regeneration, but a residual functional loss linked with extension and severity of injury as well as the configuration of nerve stumps and efficacy of surgical repair procedure[11,15]. A tension free repair is the main goal during repair and of the repair procedures such as end-to-end neurorrhaphy, nerve grafting and tubulization repair, end-to-end neurorrhaphy is associated with the most satisfactory outcomes[5]. Nerve grafts are used in cases with extensive tissue loss that makes direct repair impossible. Tubulization consists of introduction and fastening of sectioned nerve stumps into a tubular prosthesis sometimes in conjunction with regenerative factors.[5]

Connection of the injured nerves by suturing can affect the alignment of nerve fascicles and postoperative morbidities such as neuroma or granumola may be seen[5]. Peripheral nerve repair for complete section injuries mostly necessitate reconstructive methods that include sutures. However, sutures do not seal the nerve and cannot prevent the oozing of intraneural fluids from the regenerating nerve[1]. Moreover, suture repair may detrimental scarring that interfeers with healing and functional recovery. In order to eliminate these drawbacks, biocompatible glues and tissue adhesives have been introduced for repair of peripheral nerves[1,16]. These substances are practical and safe alternatives that reduce the duration of surgical procedure and diminish the need for sutures. It has been reported that fibrin glue can be used effectively for repairing damaged nerves and is not linked with increased likelihood of inflammation or necrosis[1,15]. Félix et al.[5] reported that use of fibrin glue could be an option for especially reconstruction of small sized nerves.

Bioglue® is a gelatin based tissue adhesive. It is a commercially available sealant (Cryolife, Atlanta, GA, USA) used as as a hemostatic adjunct for cardiac and vascular surgery that is composed of glutaraldehyde 10% and 45% albumin[3]. DuraSeal®

• Received : July 7, 2015 • Revised : September 1, 2015 • Accepted : September 14, 2015
• Address for reprints : Idiris Altun, M.D.
Department of Neurosurgery, Kahramanmaras Sutcu Imam University Medical Faculty, Kahramanmaras 46100, Turkey
Tel : +90-3442803398, Fax : +90-3442803409, E-mail : idrisaltun46@hotmail.com

(Covidien, Waltham, MA, USA) is a polymeric hydrogel based synthetic product utilized for enhancing the watertight repair of dural defects after cranial and spinal surgery[6]. It is self-polymerizing hydrogel sealant that forms a firm layer within seconds after application[6].

To the best of our knowledge, roles of gelatin based and polymeric hydrogel based tissue adhesives in the peripheral nerve injury model have not been studied in the literature yet. The aim of the present study was to assess the histopathological effects of gelatin based and polymeric hydrogel based tissue adhesives on regeneration after experimental peripheral nerve transection in experimental rat model.

MATERIALS AND METHODS

Experimental design

This experimental study was carried out after the approval of the Institutional Animal Care and Use Committee of our institution (Date : 12.12.2013/ No : 47). Maximum effort was spent for minimizing the suffering of animals and reduction of the number of animals used. Animals were kept at constant temperature (20−22°C) and humidity (50−60%) with a diurnal cycle consisting of 12-hour light and dark periods. Access to food and water was allowed ad libitum and all procedures were performed with adherence to the guidelines of the National Institute of Health for the care and use of laboratory animals (NIH Publication No. 8023, revised 1978).

This experimental study was performed using 42 female Wistar-Albino rats (average weight : 200−250 g) in 6 groups with respect to the procedure applied after transection of right sciatic nerves. Group I underwent only external circumferential neurolysis; Group II received suture repair with 4 sutures at 3, 6, 9 and 12 o'clock; Group III had local polymeric hydrogel based tissue adhesive administration; Group IV received suture repair (2 sutures at 6 and 12 o'clock) and polymeric hydrogel based tissue adhesive application together; Group V had gelatin based tissue adhesive and Group VI had suture repair (2 sutures at 6 and 12 o'clock) and gelatin based tissue adhesive together. After a 6-week follow-up period, biopsies were obtained from site of neural injury and groups were compared with respect to histopathological scoring based on inflammatory, degenerative and fibrotic changes.

Rats were anesthesized using intraperitoneal injection of 15 mg/kg of xylazine and 100 mg/kg of ketamine, respectively (Bayer AG, Leverkusen, Germany). Non-absorbable 10/0 Ethilon sutures (Ethicon Inc., Somerville, NJ, USA) were used in epineural plane to unite the transected nerve ends. Either 4 or 2 sutures were used as for repair of nerve transection in Groups II, IV, and VI.

BioGlue® was injected at a dose of 2 mL on the distal and proximal tips of transected nerves after apposition and end-to-end contact was established. Site of application was maintained as dry as possible to provide proper contact and waiting period without wiping or suctioning was 2 minutes for setting BioGlue®.

DuraSeal® (Covidien, Waltham, MA, USA) was injected at a dose of 2 mL on the distal and proximal tips of transected nerves after apposition and end-to-end contact was established. Site of application was maintained as dry as possible to provide proper contact and waiting period without wiping or suctioning was 2 minutes for setting DuraSeal®.

All surgical procedures were performed by the same surgeon (IA) using the same equipment under anesthesia with a premixed solution containing ketamine and xylazine. Identification of the sciatic nerve was made at the mid-level of right thigh. Avulsion via full-thickness incision of sciatic nerve with a no. 15 scalpel was performed at the location 1 cm from the sciatic notch. Following the reapposition of the muscular and cutaneous layers in anatomical planes, the wound was closed with fine sutures. In the control group, following the identification of right sciatic nerve as described above, circumferential neurolysis was performed without any additional interventions.

Histopathological examination

Neural tissue biopsies taken from the site of biopsy were kept in a solution containing 10% formalin for 24 hours. Subsequent to prepareation with ethanol and xylene, tissue was put in paraffin and slicing was made with thickness of 4 mm. After staining with hematoxylin-eosin, histopathological examination was accomplished by 2 experienced histopathologists blinded to data of groups using a ligth microscope (Zeiss, Oberkochen, Germany). Scoring was made for inflammation, fibrosis, degeneration, necrosis and vascularity as described by Orhan et al.[10,12] Thus, scoring for was made from 0 to 4 points : 0, 0% none; 1, 0−22% (mild); 2, 23−44% (moderate); 3, 44−66% (moderate to severe); 4, ≥67% (severe). All examinations were performed by the 2 experienced histopathologists using the same light microscope (Zeiss, Oberkochen, Germany).

Statistical analysis

Data was analyzed using IBM Statistical Package for Social Sciences version 20 (SPSS Inc., Chicago, IL, USA). Differences between histopathological scores of groups were tested with Kruskal-Wallis test.

RESULTS

Comparison of groups with respect to median scores of histopathological parameters including inflammation, degeneration, necrosis, fibrosis and vascularization is shown in Fig. 1.

Kruskal-Wallis test yielded that there was remarkable difference between groups in terms of inflammation (p=0.001), degeneration (p=0.002), necrosis (p=0.007), fibrosis (p<0.001) and vascularization (p<0.001). The difference regarding inflammation, degeneration and fibrosis ensources from the control group. Therefore, analysis performed after excluding the control

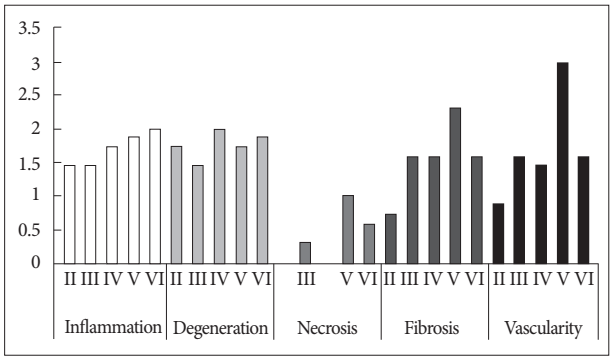

Fig. 1. Comparison of groups with respect to their mean scores for inflammation, degeneration, necrosis, fibrosis and vascularity.

Table 1. Homogeneous groups for necrosis

Groups	Homogeneous groups		*p*-value
	1	2	
Group II	*		
Group III	*		
Group IV	*		0.017*
Group V		*	
Group VI	*	*	

Statistically significant

Table 2. Homogeneous groups for vascularization

Groups	Homogeneous groups		*p*-value
	1	2	
Group I	*		
Group II	*		
Group III	*		0.001*
Group IV		*	
Group V	*		

Statistically significant

group revealed that groups were similar in terms of inflammation (p=0.602), degeneration (p=0.684) and fibrosis whereas there was remarkable difference with regard to necrosis (p=0.017) and vascularity (p=0.001).

Homogeneous groups formed for necrosis and vascularity are presented in Table 1, 2. It turned out that Group V was remarkably different from Groups II, III, and IV regarding necrosis. With respect to vascularity, Group V was significantly different from Groups II, III, IV, and VI. No statistically significant difference was detected between Groups Groups II, III, IV, and V with respect to necrosis and vascularity.

DISCUSSION

The objective for implementation of this experimental trial was to determine the histopathological effects of gelatin based and polymeric hydrogel based tissue adhesives on regeneration after peripheral nerve transection. Our results yielded that tissue adhesives can be useful for enhancement of regeneration process after sciatic nerve transaction on experimental rat model.

Peripheral nerves are vulnerable to many types of injuries such as avulsion, compression, or crush. Despite the recent advances in healthcare, substantial rates of functional and morphologic morbidity could not be reduced. Peripheral nerves are able to regenerate if Schwan cells can appropriately get in contact with the distal nerve segment. However, if structural integrity is not restored as required, functional recovery is inevitable[17].

In the literature, role of fibrin glue as an alternative to reconnect peripheral nerves has been extensively studied[9,14]. However, reports on the use of fibrin glue in peripheral nerve surgery are controversial and experimental studies on nerve repair with different materials is scarce[4,8].

Gelatin based tissue adhesive has made a significant impact on the repair of acute dissections and has the potential for significantly wider applicability[3]. In parallel to our findings, its safety and efficacy have been documented in a recent study by Sener et al.[13]. Some studies have indicated that gelatin based tissue adhesive can cause local toxicity and extensive scarring[7]. However, we noted no necrosis and vascularity was found to be improved with application of gelatin based tissue adhesive.

Polymeric hydrogel based tissue adhesive is shown to be safe and effective sealant that provides strong tissue adherence that can withstand irrigation and gentle suction without dislodgement[2,6]. No adverse events have been reported with polymeric hydrogel based tissue adhesive after cerebrospinal fluid leak repair[2,6]. After these reports, the use of polymeric hydrogel based tissue adhesive was extended as an adjunct to suture closure of an iatrogenic dural tear[2]. However, one of the drawbacks of using polymeric hydrogel based tissue adhesive is its potential to swell up to 50% and slow absorption rate[6]. To the best of knowledge, this study is the first trial that tests the histopathological effects of polymeric hydrogel based tissue adhesive on regeneration of peripheral nerves.

Results of the current study imply that tissue adhesives can be viable alternatives to suture and they may allow nerve repair without any secondary damage due to needle and suture trauma. Advantages offered by use of tissue adhesives include biocompatibility, reduction of surgery time and inflammatory reaction. Absence of necrosis and increased vascularity in biopises received from animals receiving gelatin based tissue adhesive remind that this material can be useful for repair purpose. Whether they can be used as alternatives to conventional suture technique necessitates further investigations.

Limitations of the current study must be mentioned. First, there has not been evaluation of functional recovery. Second, extrapolation of our results to human procedures call for evolution of data with further experimental and clinical trials. Moreover, environmental and technical factors may affect the results.

CONCLUSION

To conclude, we found that both gelatin based and polymeric hydrogel based tissue adhesives, can be useful in repair of pe-

ripheral nerve injuries by decreasing the surgical trauma and shortening the duration of intervention. Results with gelatin based tissue adhesive are especially promising since more intense vascularity was observed in tissue after application. However, trials on larger series with longer durations of follow-up and evaluation of functional recovery are essential for reaching more reliable conclusions.

References

1. Barton MJ, Morley JW, Stoodley MA, Lauto A, Mahns DA : Nerve repair : toward a sutureless approach. **Neurosurg Rev 37** : 585-595, 2014
2. Boogaarts JD, Grotenhuis JA, Bartels RH, Beems T : Use of a novel absorbable hydrogel for augmentation of dural repair : results of a preliminary clinical study. **Neurosurgery 57 (1 Suppl)** : 146-151; discussion 146-151, 2005
3. Chao HH, Torchiana DF : BioGlue : albumin/glutaraldehyde sealant in cardiac surgery. **J Card Surg 18** : 500-503, 2003
4. Cruz NI, Debs N, Fiol RE : Evaluation of fibrin glue in rat sciatic nerve repairs. **Plast Reconstr Surg 78** : 369-373, 1986
5. Félix SP, Pereira Lopes FR, Marques SA, Martinez AM : Comparison between suture and fibrin glue on repair by direct coaptation or tubulization of injured mouse sciatic nerve. **Microsurgery 33** : 468-477, 2013
6. Lee SH, Park CW, Lee SG, Kim WK : Postoperative cervical cord compression induced by hydrogel dural sealant (DuraSeal®). **Korean J Spine 10** : 44-46, 2013
7. Lemaire SA, Ochoa LN, Conklin LD, Schmittling ZC, Undar A, Clubb FJ Jr, et al. : Nerve and conduction tissue injury caused by contact with Bio Glue. **J Surg Res 143** : 286-293, 2007
8. Maragh H, Meyer BS, Davenport D, Gould JD, Terzis JK : Morphofunctional evaluation of fibrin glue versus microsuture nerve repairs. **J Reconstr Microsurg 6** : 331-337, 1990
9. Narakas A : The use of fibrin glue in repair of peripheral nerves. **Orthop Clin North Am 19** : 187-199, 1988
10. Orhan Z, Cam K, Alper M, Ozturan K : The effects of extracorporeal shock waves on the rat Achilles tendon : is there a critical dose for tissue injury? **Arch Orthop Trauma Surg 124** : 631-635, 2004
11. P M, S H, M D, M S : Advances of peripheral nerve repair techniques to improve hand function : a systematic review of literature. **Open Orthop J 6 :** 60-68, 2012
12. Renno WM, Al-Maghrebi M, Alshammari A, George P : (-)-Epigallocatechin-3-gallate (EGCG) attenuates peripheral nerve degeneration in rat sciatic nerve crush injury. **Neurochem Int 62** : 221-231, 2013
13. Sener I, Bereket C, Arslan G, Özkan N, Özdemir M, Mercan U, et al. : The effect of hemostatic agents and tissue adhesive on injured peripheral nerve healing in rats - Part I. Electrophysiological study. **Adv Clin Exp Med 24** : 23-29, 2015
14. Smahel J, Meyer VE, Bachem U : Glueing of peripheral nerves with fibrin : experimental studies. **J Reconstr Microsurg 3** : 211-220, 1987
15. Webber C, Zochodne D : The nerve regenerative microenvironment : early behavior and partnership of axons and Schwann cells. **Exp Neurol 223** : 51-59, 2010
16. Yeh DL, Bushley DM, Kim T : Treatment of traumatic LASIK flap dislocation and epithelial ingrowth with fibrin glue. **Am J Ophthalmol 141** : 960-962, 2006
17. Yin ZS, Zhang H, Bo W, Gao W : Erythropoietin promotes functional recovery and enhances nerve regeneration after peripheral nerve injury in rats. **AJNR Am J Neuroradiol 31** : 509-515, 2010