The Effect of Omental Vascularization on Peripheral Nerve Healing (Experimental Study)

Mustafa ÖZBEK Necmettin KUTLU Murat EMİROĞLU Erdem YORMUK PERİ FERİK SİNİR İYİLEŞMESİNDE OMENTAL VASKÜLARİZASYONUN ETKİSİ (DENEYSEL ÇALIŞMA)

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SUMMARY

To observe the effects of omental revascularization in neuroraphies, sciatic nerves of 16 dogs were mobilised and cut. Then primary neuroraphy was perfonned by microsurgical methods. After the neuroraphy, greater omentum was prepared by median laparotomy which was later passed through a tunnel formed in the inguinal region by preserving its vascular supply and adapted and wrapped around the zone of neurorarphy like a sandwich. As the control group, the sciatic nerves of the other legs of the same dogs were severed and primary neuroraphy was done without applying the omental flap.

In the morphometric and histological examination of the biopsy specimens which was done in the 4th, 8th, 16th and 24th weeks, the amount of the regenerated axons were compared and intraneural connective tissue increase in different groups was examined. In the revascularized neuroraphies there was a significant increase in the number of large axons (more than 5 milimicrons) when compared with the control group.

As a result; it has been observed that the disrupted segmental vascular structure of the peripheral nerves could be supplied by the omental flap and this application has a positive effect on the peripheral nerve healing.

KeyWords: Peripheral nerve healing - omental vascularization.

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ÖZET

Nörorafdcrde revaskülarizasvonu omentumla sağlamak ve sinir iyileşmesindeki etkilerini incelemek amacıyla 16 köpekte siyatik sinir mobilize edilip kesildikten sonra mikrocerrahi vöntemleri nörorafderi gerçekleştirilmiştir. Aynca, median laparatomiyle serbestleştirilen omentum majus, vasküler beslenmesi konınarak kasık bölgesinde hazırlanan tünelden geçirilip nörorafi bölgesine sandviç şeklinde sarılarak adapte edilmistir. Kontrol grubu olarak, diğer bacak sinirine sadece primer nörorafi uygulanmıştır.

inceleme dönemi olarak seçilen 4, 8 16, 24. haftalarda yapılan biyopsilerin morfomelrik-histolojik tetkiklerinde rejenere akson sayılan karşılaştmlmış, aynca intranöral bağ dokusu artımının farklı gruplardaki değişimi incelenrevaskülarize mistir. Omcnlumla nörorafdcrde, ilk inceleme dönemlerinde büyük caplı (5 milimikrondan büvük) akson rejenerasyonunun kontrol grubuna oranla çok daha fazla olduğu saptanmıştır.

Sonuç olarak; periferik sinirin bozuan segmental vasküler yapısının omentum flebi kullanmak suretiyle yeniden sağlanabildiği ve bu uygulamanın akson rejenerasyonunu etkileyerek sinir iyileşmesine pozitif yönde katkıda bulunduğu gösterilmiştir.

Anahtar Kelimeler: Periferik sinir iyileşmesi omental vaskülarizasyon.

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A great deal of experimental and clinical studies have been carried out in the restoration of complex funcitons of peripheral nerves for many-years.

The first primary neuroraphy was reported by LANGLEY and HASHIMOTO in 1917 (24). On the other hand, first attempts for neural grafting were done by BIELCHOWSKY and UNGER in 1918 (10,16).

SUNDERLAND has studied the nerve vascularization in 1945 and has observed that the vascular supply to the peripheral nerves was segmental. Furthermore, he reported the vasculature passing through thin, membranous connective tissue and thus, he was the first to define the mesoneurium (10).

The presence of lymphatics in the epineural tissue being together with the blood vesels was shown by MILLESI in 1983. However, he also added that those lymphatics could not reach to the interfascicular region (12,16).

After the operation microscope was routinely put into use in peripheral nerve surgery a complete mastery of the anatomy has been gained. On the other hand, the physiologic and histological studies carried out have proved the importance of the vascular supply for the successful axonal regeneration. The aim of the experimental research presented is to study the effects of the omental revascularization on the peripheral nerve healing.

We have begun this experimental research from the point to maintain revascularization for accelerating the axonal regeneration (nerve healing) in peripheral nerves with disrupted vascularization because of such causes like traumas, nerve graft applications, long preparations and freeing from peripheral soft tissues and compartment syndromes. In this study, we have transposed the omentum by preserving its vascular pedicle, but in clinical applicatioon free microvascular omental transpositions were thought to be more suitable.

We have chosen the omentum majus because of its rich vascular anatomical structure and the characteristics of the serous membrane with its selective permeability which overlies it, the peritoneum. The peritoneum which covers the lymphatic and vascular tissues provides an equilibrium

between the secretion and resorption thus affects the healing in abdominal pathologies (4,9,11,20). On the other hand, the cellular elements of the healing process, the fibroblasts, macrophages can easily reach the neuroraphy site through the rich lymphatic and vascular network provided by the omentum and thus cause a rapid and effective healing of peripheral nerves (2,11).

MATERIALS AND METHOD

As the experimental animal we have used 16 dogs from different sexes and races weighed between 10-12 kgs and were previously made sure that they were free of any disease. In addition, a pilot research was carried out in 2 rats and 1 rabbit so as to observe the mobility and availability of the omentum majus.

The operations were made under the magnification provided by x4 operation loops, x30 Zeiss and x40 Week operation microscopes. For the histological examination Haematoxylene Eosine, Mason Trichromc and Van Giesson nerve stains were used. The statistical evaluation of the histopathological parameters was made using the IBMPC type computer by the SYSTAT and HPG programmes.

At the operative stage, pentobarbital was given to dogs intravenously and entubated. After the necessary preparation of operative field, with a vertical incision on the lateral femoral area, the sciatic nerve was reached transmuscularly. The nerve was freed 6-8 cms and neurolysis was performed. After the free segment was severed right in the middle, primary neuroraphy was performed by the epiperineural technique and using 10/0 Polyamid suture material. Following the neuroraphy, greater omentum was prepared by median laparatomy which was then passed through a tunnel arranged in the inguinal region by preserving its vascular supply and adapted and wrapped around the zone of neuroraphy like a sandwich (Fig. 1). As the control group, the sciatic nerves of the other legs of the same dogs were severed and primary neuroraphy was done without applying the omental flap. The same procedure was performed in all of the 16 animals. According to the experimental planning, 16 dogs were divided in 4 groups (4 dogs each group) then the animals were reoperated for

having biopsies from the distal ends of the neuroraphies and were sacrificed. These procedures were performed for each group separately; first group at the 4 th, second group at the 8th, third group at the 16th and the last group at the 24th week respectively (Table 1).

The histological examination of the biopsy specimens were carried out (o make the numeric comparisons of the regenerated, myelinated axons at the distal segment of the anastomosis and to

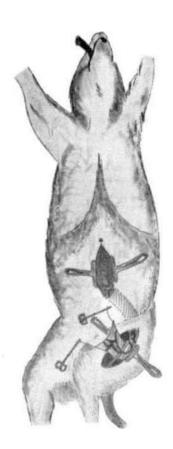


Fig. 1. Schematic view of the omentum before being wrapped around the site of neuroraphy.

Table 1. Experimental Planning

Group	No.of	Neuroraphy		
No.	animals			
1	4	Right femoral		
1	4	*		
3	4 "			
4	4 "			

identify the effect of omental revascularization to the amount of the intraneural connective tissue increase.

The criterion for the comparison of the results was the evaluation of the average number of the regenerated axons per square milimetre of the randomly chosen four different zones in the histological specimens prepared from the cross-sections of the distal anastomosis site. Here, the axons with a diameter greater than 5 milimicrons were considered parametrically significant (Fig. 2).

At x40 magnification, 6.3 objective area was accepted to be approximately equal to 1 square milimetre and in order to verify the axons with a diameter greater than 5 milimicrons, the diameter of leukocytes which is 6 milimicrons was considered as a standard parameter. The statistical evaluation of the number of regenerated axons was made by computers and the comparisons of the average values obtained at a different period were graphically illustrated (Table II, III, IV, V).

RESULTS

The postoperative evaluations were made according to the macroscopic and histologic-morphometric parameters.

In the control group, which we have performed only neurolysis and primary neuroraphy, excessive intraneural connective tissue proliferation and perineural adhesions were observed histologically, especially at the 8th and 16th weeks. On the other hand; we have noticed that, this increase in connective tissue proliferation was fewer in the animals which we have performed neuroraphy and omental revascularization. In addition to these findings, newly formed collagen fibres at the neuroraphies of the control group were immature, but were mature at the experimental group. Omental

Neuroraphy+	Time of having	
omental flap	biopsy	
Left femoral	4. week	
"	8. week	
**	16. week	
"	24. week	

revascularized neuroraphies have showed collagen proliferation at the 4th, 8th and 24th weeks.

At all the histological evaluation stages (especially 4th and 8th weeks) we have found out more regenerated axons in the omental revascularized nuroraphies (Table II, III, IV, V).

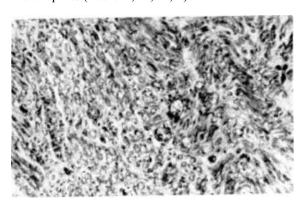
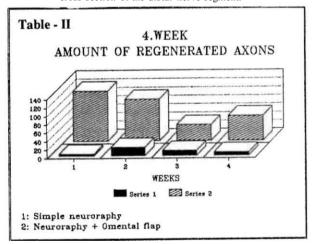
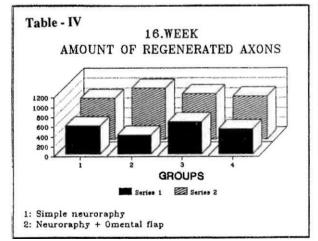


Fig. 2, Photographic view of the axonal regeneration on the cross-section of the distal nerve segment.



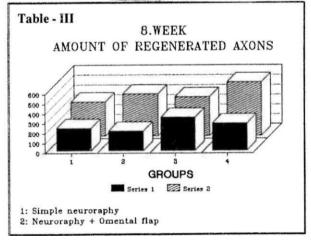


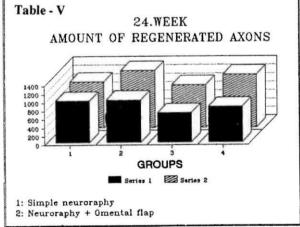
We have compared the results that we obtained from the experimental studies with the control groups and came to these conclusions:

- 1) In all stages of the experimental planning, especially in the 4th and 8th weeks the amount of regenerated axons is greater.
- 2) The results of morphometric and histological parameters obtained have been very close to the ones obtained from the similar or even more complicated experimental studies such as the vascular nerve grafting procedures.
- 3) The intraneural immature collagen has been observed minimally in the later periods of (he sludy, thus the fibrosis can be prevented and secondary nerve disruption can be avoided.

DISCUSSION

In the experimental researches concerning the peripheral nerve repairs; rats, rabbits, cats and dogs have been used as the experimental animals because their anatomy of the peripheral nerves is similar to humans (9,16,17,21).





Since it has got a gross anatomic structure and it tolerates the anaesthesia and the operations much easier we have preferred the dog in our study.

Especially, during the last 5 years, many experimental studies have been carried out for successful restoration of the peripheral nerves with disrupted segmental vascular supply (3,5,6,8,19,22)

Actually, there are many examples of the microsurgical repair of this vascular structure. We can even see that there have been successful experimental vascular nerve grafting procedures (8,7,18,23). However we believe that our method which provides revascularization using omental flap is applicable where vasanervorum anastomosis is not possible as an aid for revascularization.

One of the major reasons of failure in the repair of peripheral nerves is the tension at the neuroraphy site (10,11,12,13). On the other hand, in such cases as neurolysis or nerve grafting, although the nutritional arteries are out of function, the intraneural vascular network can maintain a restricted amount of vascular supply, However, if the neuroraphy has been performed under tension then this will not be possible also. Furthermore, the experimental studies have shown that the extent of neurolysis without disturbing vascular supply is 6-8 cms. totally in both directions, otherwise the chance of success will decrease (1,10,16,17). Whereas in a primary neuroraphy to be done without any tension, the maximal defect should be no more than 1-1.5 cm. (13,14). In our method, we provide external revascularization to the nerve by

the omental flap. With the help of this procedure, longer nerve segments which were free from their vascular supplies can be able to survive. This shows us the increase in axonal regeneration rate.

The principal aim of all the stated techniques is to avoid the increase of the intraneural and perineural connective tissue which prevent the success of neuroraphy (7).

During the nerve regeneration the axonal buds normally advance 1-3 mms. daily. This is largely affected by increase of the connective tissue at the anastomosis site (1,10,15,17). In the neuroraphies which the vascularization was enhanced by our technique, the rapid axonal regeneration inhibits the connective tissue proliferation at the anastomosis site held in optimal conditions. Compratively high amount of regenerated axons at the histological specimens also support this idea.

At the end of certain periods stated in the experimental planning, especially at 8th and 16th weeks, in the control group, where only neurolysis and neuroraphy was performed, there were perineural adhesions and a great increase in the amount of connective tissue in 80% of the dogs. On the other hand, in the group where omental revascularization was performed, especially in the 16th and 24th weeks, this increase was minimal (Table VI-a,b and VII).

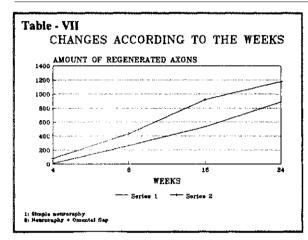
As a result; by the help of this method, axonal regeneration rate after neuroraphies and nerve grafting successful. Modifying this method in humans by free omental transfers will be an aid in the future for surgeons dealing with nerve surgery.

Table VI. The Mean Cantitative Values of Great Axons With Diameters Bigger Than 5 Milimicrons (± Standard deviation)

WEEKS	1. Group	2. Group	3. Groi-p	4. Group
4. week	4.5	20	12	8
	\pm (2.598)	\pm (6.702)	$\pm (5.196)$	\pm (1.225)
8. week	230	198	340	280
	$\pm(31.401)$	$\pm(14.440)$	$\pm(24.729)$	$\pm(43.766)$
16. week	580	390	660	510
	\pm (37.676)	\pm (15.050)	$\pm(42.113)$	$\pm (19.170)$
24. week	990	1010	700	850
	\pm (81.814)	\pm (59.687)	± (97.990)	$\pm(24.423)$

WEEKS	1. Group	2. Group	3. Group	4. Group
4. week	120	100	38.5	60
	\pm (9.618)	\pm (12.708)	$\pm (8.529)$	$\pm (7.648)$
8. week	350	430	400	550
	\pm (34.125)	\pm (8.515)	$\pm(24.259)$	$\pm(32.787)$
16. week	836	1040	927	877
	$\pm(21.691)$	$\pm(42.714)$	$\pm (59.068)$	$\pm (4.473)$
24 week	1089	1356	1010	1256
	\pm (29.925)	\pm (35.228)	$\pm (28.142)$	$\pm (97.026)$

b) Neuroraphy + Omental flap



REFERENCES

- Berger A, Meissl G, Samii M: Experiméntele erfahrungen mit Kollagenfolien über Nerven anastomosen. Acta Neurochirurgica Vienna, 23:141,1970.
- Bruneiii G: Neurolysis and free microvascular omentum transfer in the treatment of postactinic palsies of the brachial plexus. <u>Int.Surg.65</u> (6): 515,1980.
- Cruz NI: Evaluation of fibrin glue in rat sciatic nerve repairs. Plast. Reconstr. Surg. 78 (3): 370,1985.
- Goldsmith HS: Salvage of endstage ischemic extremities by intact omentum. Surgery. 88(5): 732,1977.
- Gu YD, Wu MM, Zheng YL, Xu YN: Arterialized venous free sural nerve grafting. Ann. Plast. Surg. 15 (4): 332,1985.
- Koshima I, Harii K: Experimental study of vascularized nerve grafts: Morphometric study of axonal regeneration of nerves transplanted into silicon tubes). Ann. Plast. Surg. 14 (3): 235,1985.
- Koshima I, Harii K: Experimental study of vascularized nerve grafts: Multifactorial analysis of axonal regeneration of nerves transplanted into acute burn wounds. J.Hand Surg. 10 (1): 64,1985.
- McCullough CJ, Gagey O, Higginson DW, Sandin BM, Crow JC, Sebille A1: Axon regeneration and vascularization of nerve grafts: An experimental study. J.Hand Surg. (Br.),9(3):323,1984.
- Micheau P, Moreau JP, Chavoin JP, Chiotasso P: Epiploon et revascularisation etude experimentale chez le chien perspectives cliniques (Revascularization using the greater omentum, Results of an experimental study in dogs, clinical perspectives). J.Chir (Paris) 118(3): 197,1981.

- Michon J, Moberg E: Traumatic Nerve Lesions of the Upper Limb. Chuchill Livingstone. Edinburgh, 1975.
- Miller ME, Christensen GC, Evans HE: Anatomy of the dog. W.B. Saunders Company, Philadelphia, 1968.
- Millesi H: Wiederherstellung, durchtrennter Peripherer Nerven und Nerventransplantation. Münch. Med. Vohenschr. 111:2669.1969.
- Millesi H, Berger A, Meissl G: Experimentele Untersuchung zur Heilung durchtrennter peripherer Nerven. Chir. Plastica 10174,1972.
- Mitchell J, Stauber V, Anderson PN, Mayor D: Axonal regeneration through a peripheral nerve implanted into a brain cavity. Acta Neuropathol. (Berlin) 67(3-4): 235,1985.
- Molander H, Engkvist O, Haeglund J, Olsson Y, Torebjoerk E: Nerve repair usin polyglactin tube and nerve grafts (An experimental study in the rabbit). Biomaterials 4(4): 276,1983.
- Nigst H, Buck-Gramco D, Millesi H: Handchirurgie (II). George Thieme Verlag Stuttgart 30:1-67,1983.
- Peacock E, Jr: Wound Repair. W.B. SAunders, Philadelphia, 10: 363,1984.
- Pho RW: Histological studies of vascularised nerve graft and conventional nerve graft. J.Hand Surg. (Br.) 10 (1): 45, 1985.
- Radek A, Chomiczewihi K: A possibility to utilize morphometric examinations in microangiographic evaluation of revascularization of places of the peripheral nerve anastomosis in rabbit in various anastomosing methods. Folia Morpol. (Warsz., Poland) 40 (2): 123,1981.
- Schultz CB: THc mechanism controlling migration of the omentum. Surg. Gynec. Obstet. 50: 541,1930.
- Taxi J: Etude au microscobe electronique de la degenerescence Vallerienne des fibers nerveuses amyeliniques. C.R-Acad. Sei. (Paris) 248:2796,1959.
- Towsend PL, Taylor GI: Vascularized nerve grats using composite arterialized neuro-venous systems. BrJ.Plast.Surg. 37(1): 1,1984.
- Yamazaki Y, Noma H: Effect of the perineural vascular net on experimental grafting on the inferior alveolar nerve. Oral Maxillofac. Surg. 41 (4): 219,1983.
- Yormuk E, Özbek M: Mikrovasküler Cerrahi ve Replantasyon. A.Ü.T.F. Plast ve Rekonst. Cer. Geliştirme Derneği Yayınlan, 1987.

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