Nerve regeneration over a 25 mm gap in rat sciatic nerves using tubes containing blood vessels: the possibility of clinical application

R. Kakinoki, N. Nishijima, Y. Ueba, M. Oka, T. Yamamuro, T. Nakamura

Department of Orthopaedic Surgery, Faculty of Medicine, Kyoto University, Kyoto, Japan

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Summary. *This study was undertaken to investigate the effect of including vessels in a tube used to promote nerve regeneration across a gap. A tube containing sural vessels was designed in a rat model and interposed between the proximal and distal stumps of a divided sciatic nerve, leaving a 25 mm gap. At 12 weeks, a few myelinated axons were seen at the most distal parts of regenerated nerves in 6 out of 10 rats, none of which evoked action potentials in the tibialis anterior muscle, but by 24 weeks all the rats had developed neural tissue in the tubes, which evoked action potentials in the muscle. The vessels within the tube enhanced nerve regeneration and its distance up to 25 mm. This type of vessel-containing tube would be useful for the repair of divided human peripheral nerves with long gaps, almost equivalent to or slightly longer than the maximum length over which nerve fibres can regenerate through a unvascularised unmodified tube.*

Résumé. *Plusieurs auteurs ont rapporté que la repousse maximum des axones du nerf sciatique de rat, dans un tube, est d'environ 10 mm. La présente étude a été réalisée pour confirmer que la mise en place d'un tube dans un vaisseau permet aux fibres nerveuses d'atteindre une régénération de plus de 10 mm par cette technique de tubulation. Chez un rat, une veine surale contenant un tube a été utilisée et interposée entre les moignons sectionnés d'un nerf sciatique avec un espace de 25 mm. A la 12^{ème} semaine, quelques axones myélinisés ont été observés à la partie la plus distale des nerfs régé-*

nérés chez 6 rats sur 10. Aucun de ceux-ci n'a pu donner de potentiels d'action dans les muscles tibiaux antérieurs. Après 24 semaines, tous les rats ont développé une repousse neuronale dans les tubes, qui ont donné des potentiels d'action dans les muscles tibiaux antérieurs. Cette chambre intravasculaire augmente la régénération des nerfs ainsi que la distance de régénération jusqu'à 25 mm pour un nerf sciatique de rat. Un vaisseau contenant un tube est considéré comme étant cliniquement, utile pour la réparation de la section de nerf périphérique avec un espace important, qui sont équivalents ou légèrement supérieurs, au maximum de longueur dont les nerfs peuvent repousser dans des tubes nonvascularisés classiques.

Introduction

The repair of divided peripheral nerves with gaps between the stumps presents a surgical problem. Autogenous nerve grafting is widely accepted, but there are drawbacks such as the defect created at the donor site, the limits of length and thickness of the graft, and the possibility of misdirection of the axons. Furthermore, the results are not always satisfactory [22, 23].

Another method has been practised since the beginning of the 19th century [4, 5, 7, 26]. Axons can grow across a gap through a tube-like material (tubulation) which does not produce the defects associated with nerve grafting. Nerve fibres which have regenerated through tubulation have been

Fig. 1A–C. Diagrams showing the operative procedures involving the preparation of a silicone rubber tube for a 25 mm gap. *SUV*, sural vessels; *SUN*, sural nerve; *SN*, sciatic nerve; *PN*, peroneal nerve; *TN*, tibial nerve, and *F*, monitor flap

reported to show several specific qualities for regeneration, such as tissue [12], topographic [20] and motor-sensory [1, 2] specificities. Tubulation may also reduce the possibility of axon misdirection.

There is a limit to the distance across which nerves can regenerate in unmodified empty tubes which is reported to be about 10 mm for rat sciatic nerves [9, 10, 11]. The axons of these nerves can

Fig. 2. The sural vessels elevated at the popliteal fossa. *SUV*, sural vessels; *PN*, peroneal nerve; *TN*, tibial nerve

Fig. 3. Photograph taken just before wound closure of a vessel-containing tube with a 25 mm gap. *Arrows* show the sural vessels inserted into a 28 mm silicone rubber tube

extend across a gap of 15 mm to 20 mm using tubes containing dialysed plasma [13], collagen-glycosaminoglycan polysaccharide matrix [32], laminin-containing gel [17, 18] or collagen matrix [17].

We directed our attention to the value of vessels in a tube designed to contain sural vessels and which would encompass a 25 mm gap in sciatic nerves in the rat, and studied whether axons could regenerate through the tube for this distance. We also discuss the possibility of using such a tube in patients.

Materials and methods

Twenty adult male Sprague-Dawley rats (15–18 weeks old, weighing 330–365 g) were used. After anaesthesia by an intraperitoneal injection of pentobarbital sodium (Nembutal, 40 mg/kg body weight), the left leg was shaved and prepared for operation. The sural neurovascular bundle was exposed

Fig. 4. The appearance of a nerve which has regenerated through a vessel-containing tube with a 25 mm gap at 24 weeks. *Sp*, section of the proximal part; *Sm*, the middle part; *Sd*, the distal part

Fig. 5A, B. Light micrographs of transverse sections of the most distal part of a nerve which has regenerated through a vessel-containing tube with a 25 mm gap, 24 weeks after the operation. **A** An arrow indicates the remnants of the necrotic vessels, $\times 400$. **B** $\times 800$, the bar at the bottom right corner = 10 μ m

from the popliteal fossa to the ankle, and the nerve was separated from its accompanying vessels and removed completely (Fig. 1a). A 10 $\times$ 5 mm myocutaneous flap vascularised by the sural vessels was harvested at the posterior surface of

the ankle and elevated from the lower leg (Fig. 2). The left sciatic nerve was then exposed from piriformis to the popliteal fossa through a gluteal muscle-splitting approach, and a 20 mm segment removed (Fig. 2B). The myocutaneous flap with its pedicle was mobilised up to the thigh. The sural vessels were inserted into the silicone tube through a preformed longitudinal slit. The epineurium of the proximal and distal sciatic stumps were sutured to the 28 mm silicone rubber tube leaving a gap of 25 mm between the stumps using a 10 – 0 monofilament nylon (Fig. 1C). The slit was sealed with a small amount of silicone rubber (Fig. 3). The myocutaneous flap was sutured to the buttock and the skin closed. The flap was used to monitor the patency of the sural vessels in the tube, and its colour was checked every day.

Electrophysiological study

Twelve and 24 weeks after operation, the animals were anaesthetised, the left sciatic nerve exposed distal to piriformis and stimulated with a bipolar electrode. A pair of needle electrodes was inserted into the tibialis anterior muscle and the presence of an evoked action potential recorded.

Histological study

The tube and adjacent nerve were removed together and fixed in 2.5% (v/v) glutaraldehyde, postfixed with 2% (v/v) osmic acid and embedded in epoxy resin. Transverse sections, 1–2 μ m thick, were taken from the most proximal part (*Sp*), the middle part (*Sm*) and the most distal part (*Sd*) of each regenerated nerve. Each was stained with 0.5% (w/v) toluidine blue and examined by light microscopy.

Results

No rat died during operation or in the follow up period. Two developed necrosis of the monitor flaps and were excluded. Ten and 8 rats were sacrificed at 12 and 24 weeks, respectively.

At 12 weeks, neural tissue had developed in every silicone rubber tube (Fig. 4) and the vessels were patent in all 10 rats. Myelinated axons were present in the proximal and middle portions in every rat, and in the distal part in 6 out of 10. No action potentials were evoked in the tibialis anterior muscle of any rat because the numbers of axons which had regenerated in the 6 rats were too small.

At 24 weeks, the 8 rats examined had developed neural tissue in the tubes, and action potentials were evoked in their tibialis anterior muscles. Myelinated axons were present in every part of each regenerated nerve in every rat (Fig. 5). In 6 of the 8, the vessels had become necrotic, although the monitor flaps were viable.

Discussion

The present study has demonstrated that axons are able to regenerate across a 25 mm gap and re-innervate the tibialis anterior muscle 6 months after operation using a silicone rubber tube containing vessels. It is not possible to exceed this distance in our model because the maximum length of the sural vessels is about 30 mm. Mackinnon and Dellon investigated spontaneous nerve regeneration in rats, and the mean distance across which axons regenerated was 23.7 ± 6.4 mm at 5 months, after removal of a 4.5 cm segment of sciatic nerve [11]. The nerves had regenerated in a tube-like space surrounded by well vascularised muscle fascia. Vascularity enabled axons to extend up to 45 mm.

Vascularity is one factor which is critical for determining the distance of axon regeneration in tubes. Comparing the results of a vessel-containing tube with a 25 mm gap with a 10 mm gap in a similar tube [6], the number and mean diameter of axons in the 25 mm gap were 1500 and $1.9 \mu\text{m}$ at 24 weeks compared with 8000 and $2.4 \mu\text{m}$ in the 10 mm gap. This is due to the decrease of neurotropism from the distal nerve stumps which decreases as the distance between the stumps increases [10, 19, 21, 24]. Neurotropism is weaker in tubes with a 25 mm gap than in those with a 10 mm gap. Vessels within a tube enable axons to regenerate for a longer distance, but cannot increase their number or diameter of the axons [6].

Tubes containing vessels are considered to be useful clinically for the repair of divided nerves. As peripheral nerves usually accompany blood vessels forming neurovascular bundles, it is not difficult to include vessels in the tubulation, even in the presence of scar tissue [25].

Regeneration is quicker in tubes containing blood vessels; re-innervation can be achieved earlier than through unvascularised tubes [6, 8], and the axons will extend further. The distance will vary in different species [13], and the length across which axons can extend is 10 mm in rat sciatic nerves [9, 10, 11]. Mackinnon et al. reported the successful repair of 3–5 cm gaps in primates [13, 16]. Other studies have shown that satisfactory results follow repair of human digital nerve gaps of less than 3 cm using unvascularised tubes, such as autogenous veins or biodegradable PGA (polyglycolic acid) tubes [3, 15, 23, 14, 28]. A gap of 25 mm in rats corresponds to a longer distance in man, and so gaps in nerves could be bridged over a longer distance using tubes containing vessels.

Tang et al. used autogenous veins as nerve conduits clinically and reported success with gaps of 0.5–3 cm in digital nerves, and of 2.5–4.5 cm in ulnar nerves, but failed with a 5 cm gap in a median nerve [25]. The critical length with this method was considered to be 3 cm for digital nerves and 4.5 cm for trunk nerves. A tube containing vessels should allow axons to bridge a gap of more than 5 cm in human digital nerves, but the number of axons will decrease as the gap increases. These tubes should therefore be used in man for repair of peripheral nerves with gaps less than, or slightly longer, than the critical length for axon regeneration in unvascularised tubes, the aim being to accelerate the rate rather than improving the distance of axon regeneration.

In the present study, the inserted vessels were patent in every rat at 12 weeks, but they had become necrotic in 75% at 24 weeks, although there was good nerve regeneration within the tubes. The blood supply of the neural tissue gradually switched from the previously inserted vessels to capillaries which spread from both stumps [6]. The monitor flap survived with its blood supply from the inserted vessels for up to 12 weeks, although it began to be nourished by collateral vessels from the surrounding tissue by 24 weeks. When this type of tube is used in man, the blood flow in the included vessels will not be reduced because peripheral vessels connect with other vessels to form a vascular network.

We have just begun to repair divided human digital nerves using vessel-containing tubes. Recovery of the repaired nerves has been satisfactory, although the longest follow up is less than 6 months.

We have used silicone tubes to act as a barrier for immigrating scar tissue into the lumen of tubes, for the emigration of neurochemical factors secreted from nerve stumps, and as a space for forming fibrin matrix which would act as a scaffold for nerve growth [29, 30]. Biodegradable or biological tubes should be used clinically because silicone rubber tubes have to be removed after several months, since they might affect joint movement and induce synovitis.

A longer follow-up and improvement of the material used for the tubes is needed. Nevertheless, we conclude that vessel-containing tubes can accelerate nerve regeneration and extend its distance. Clinically this can be useful for repairing divided nerves with gaps which are almost the same, or slightly longer, than the length across which regeneration can occur in unvascularised unmodified tubes.

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