PERIPHERAL NERVE REPAIR USING MUSCLE AUTOGRAFTS

RECOVERY OF TRANSMISSION IN PRIMATES

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Skeletal muscle grafts, when thawed after freezing, can be used to repair peripheral nerves. This method was used after transection of the median nerve in the upper arm in marmosets. Examination at 28 days showed total denervation of flexor carpi radialis; at 150 days electrophysiological evidence of recovery of nerve conduction across the graft and of muscle activation was seen. Sections at this time showed nerve fibres and new functional neuromuscular junctions in the muscle. It is concluded that effective reinnervation of target muscles is possible after peripheral nerve repair using skeletal muscle autografts.

Earlier work has shown that if skeletal muscle is frozen in liquid nitrogen and then thawed in an hypo-osmotic environment it may be used successfully to repair peripheral nerves with deficiencies requiring grafts (Glasby et al. 1986 a,b,c,d; Davies et al. 1986). Freezing and thawing was suggested by Fawcett and Keynes (1986) to produce fragmentation of the graft but if the muscle is harvested in such a way that a covering of epimysium is present on one of its faces, then satisfactory grafts can be obtained and this freeze-thawing method of preparation is faster and more appropriate to the operating theatre than more lengthy chemical procedures.

Orientation of the fibres within the graft affects the rate of growth of the regenerating fibres, at least in the early phase of regeneration. Thus coaxially aligned, treated grafts permit a faster growth rate of the nerve than other graft orientations or untreated muscle (Glasby et al. 1986 b; Davies et al. 1987). It is not possible to conclude from any of the existing studies to what extent the absolute length of the graft determines the final outcome of regeneration; this is the subject of a separate study in our laboratory. In the studies of Fawcett and Keynes (1986) and Glasby et al. (1986 a,b,c) rats were used and the graft lengths were 0.5 cm and either 1.0 cm or 2.0 cm respectively. Fawcett and Keynes have also reported successful grafting in the rabbit using 4.0 cm grafts and Glasby et al. (1986 d) have used 3 cm grafts in the marmoset with recovery of function.

While all of this evidence offers considerable encouragement in the use of muscle grafts for peripheral nerve repair, none of the studies reported so far has considered whether nerves regenerating through treated muscle grafts are able to establish functional connections with their target organs. The present study seeks to investigate this point in relation to motor function by examining the degeneration and subsequent regeneration of functional motor endplates in a specific target muscle. Whereas it would seem highly unlikely that exact rewiring would take place since it does not do so in any known form of nerve repair (Seddon 1975), imprecise reinnervation with functional neuromuscular transmission is still of enormous advantage in rehabilitation following peripheral nerve injuries. The time-course of events is, of course, critical and successful recovery depends on reinnervation having been established before muscle degeneration has produced irreversible loss of function. In any study the animal species is critical and the commonly used laboratory rat often produces results which are not reproducible in higher animals. The ultimate test is the human. Results obtained in the non-human primate lend much greater weight to the extension of an idea from the laboratory to clinical practice. The study of Glasby et al. (1986 d) used the marmoset to examine regeneration through muscle grafts.

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and the present study goes on to consider events taking place at the target organs of motor nerves in the same species and after similar treatment.

METHODS

Four marmosets (*Callithrix jaccus*) were used for the experiments. These animals had been bred in a closed colony maintained along the lines recommended for laboratory primates (Poole 1987). No particular pre-operative treatment was necessary other than routine fasting. Anaesthesia was induced and maintained with intramuscular “Saffan” (Glaxovet; Glaxo Laboratories, UK) at a dose of 12 mg/kg. The right arm was shaved and prepared for surgery using Hibitane in alcohol; full aseptic precautions were maintained throughout. The skin was incised longitudinally over the medial border of biceps and extended as a sigmoid crease incision across the anteceubital fossa. Pronator teres was used to furnish the graft as this muscle has a parallel arrangement of its fibres. An appropriate segment was excised and the muscle repaired with several interrupted sutures of 6/0 Vicryl (Ethicon, UK).

Minor modifications have been made to the technique of graft preparation used previously (Glasby et al. 1986 d). Although we have experienced few instances of graft fragmentation this phenomenon was described by Fawcett and Keynes (1986) in their freeze-thawed grafts. It is a problem which might be expected to occur more often where longer grafts are used. Pinning out of the graft under tension during freezing did not appear to improve its performance. Work currently in progress in our laboratory suggests that as long as disruption of the contents of the basement membrane tubes has taken place, clearing out of these tubes is not a prerequisite of graft recolonisation by nerves. It is only necessary, therefore, to produce disruption of the sarcoplasm. The graft was excised retaining its epimysial covering on one of its faces. It was placed without tension on a piece of filter-paper and then immersed in liquid nitrogen until thermal equilibrium was obtained. Subsequent thawing in sterile distilled water caused the graft to swell somewhat at right angles to its long axis but this subsided to produce an intact and easily handled graft of approximately its original dimensions if left for about five minutes on a saline-dampened swab. In clinical practice this time might be used for preparation of the implantation site.

The technique for insertion of the 2 cm graft was identical to that described by Glasby et al. (1986 d) except that in the present study the median nerve was used exclusively as the implantation site. The graft was inserted into a gap in the main trunk of the nerve so that its distal end lay 0.5 cm above the upper border of the cubital fossa. After the graft was inserted, electrodes were placed on either side of it to establish that nerve conduction had been lost.

A single marmoset was anaesthetised at 28 days after operation and flexor carpi radialis removed for histology. At this time it would be expected that the original motor endplates but not the muscle fibres would have degenerated. Neither propagated action potentials nor muscle activation were recordable at this time.

The remaining three animals were anaesthetised at 150 days and the median nerve dissected out from its lateral and medial heads to its entry into the carpal tunnel. A bipolar stimulating electrode was placed around the nerve proximal to the graft and connected to a Grass “S8” stimulator (Grass Instruments, Massachusetts, USA). A bipolar recording electrode was placed around the nerve at the most distant site that was accessible; the distance between the stimulating and recording electrodes was measured. The recording electrode for compound extracellular action potentials was connected to a DAM 80 AC differential amplifier (World Precision Instruments Inc., New Haven, Connecticut, USA) with the gain set at 1000, the low frequency cut-off at 300 Hz and the high frequency cut-off at 10 kHz. The output of this amplifier was sent to a Tektronix 5A26 dual differential DC coupled amplifier and Tektronix 5113 Dual beam storage oscilloscope with 5B12 dual time base (Tektronix Inc. Oregon, USA). Electromyograms were recorded from flexor carpi radialis using a unipolar needle electrode fed directly to an AC coupled (second) 5A26 differential amplifier for recording against a common earth.

When electrophysiological assessment was complete flexor carpi radialis along with its nerve and tendons was removed and fixed for 24 hours in neutral buffered formalin. The corresponding muscle from the unoperated side was also removed and treated in a similar manner. After fixation, 40 micron longitudinal frozen sections were cut serially from each muscle. Free floating sections were stained using a combined two-stage staining technique for acetylcholinesterase activity (Gschmeissner et al. 1987), at neuromuscular junctions and for nerve fibres. The method is a modification of the cholinesterase techniques described by Koelle and Friedenwald (1949) and Namba, Nakamura and Grob (1967) for staining of the junctional enzyme systems. The nerve fibres are stained using a modification of the Schofield (1960) variant of Bielschowsky’s silver stain for nerve fibres (Bielschowsky 1904; Cook 1974).

Sections were dehydrated in alcohol after staining and mounted in DPX. They were viewed using a Zeiss (Oberkochen) photomicroscope and photographed with Kodak Ektachrome 50 and Ilford FP4 film.

RESULTS

Twenty-eight days after operation all the animals showed signs typical of a median nerve palsy. Electrophysiological examination of a single animal at this stage showed complete loss of both electrical activity in flexor carpi
Fig. 1

Light photomicrograph (x 200) of a 40 micron frozen section of flexor carpi radialis, taken from the marmoset 28 days after implantation of a 2 cm coaxially aligned freeze-thawed skeletal muscle autograft into the median nerve above the cubital fossa. The section was stained using the combined silver cholinesterase method for nerve fibres and localised cholinesterase activity. Note that at this time the muscle fibres, (m), appear normal but there is a complete absence of motor end plates.

Fig. 2

Combined trace of propagated extracellular compound action potential (A), and electromyogram (B), recorded across the graft and from flexor carpi radialis respectively, 150 days after graft implantation. The first peak of the action potential corresponds to a population of fibres having a mean conduction velocity of 10.65 m/sec.
Light photomicrograph (x 560) of a 40 micron frozen section of flexor carpi radialis, taken from the normal, unoperated arm of a marmoset 150 days after implantation of a 2 cm coaxially aligned freeze-thawed skeletal muscle autograft into the median nerve above the cubital fossa in the contralateral arm. The section was stained using the combined silver cholinesterase method for nerve fibres and localised cholinesterase activity. Note the presence of a typical neuromuscular junction comprising muscle fibres (m), and nerve terminals (n). Localised cholinesterase activity (c), is revealed at the motor endplate regions implying a functioning synapse.

A similar section to that in Figure 3 but taken from flexor carpi radialis of the arm which had received a muscle graft into the median nerve 150 days previously. The newly regenerated neuromuscular junction does not appear different from the control, and shows all of the same features.
REFERENCES

GRAFT

CHOLINESTERASE

SERIAL

FIBRES

NEUROMUSCULAR

J.

DISCUSSION

The present experiments show that nerve fibres which have regenerated through a freeze-thawed skeletal muscle autograft are capable of forming new functional connections with skeletal muscles provided that the latter have not degenerated in the time taken for the growing axons to reach them. This observation strongly supports our previous suggestion that in situations where a larger graft is required than can be obtained by sacrifice of an expendable nerve, treated skeletal muscle autografts offer an acceptable solution. The use of a non-human primate as an experimental model further reinforces the applicability of the technique to its use in human reconstructive procedures.

At 150 days, although many endings appeared to have features consistent with those described by Bowden and Duchen (1976) and Gwyn and Aitken (1966), some had clearly not completed the regenerative process and did not yet show cholinesterase activity. One hundred and fifty days is a relatively early time at which to consider regeneration in the human and this may well be the case in other primates. The varied appearances of the endings suggests that this may represent an early stage after the arrival of the regenerating nerve fibres at the target muscle. Our earlier study (Glasby et al. 1986d), showed that even at six months after operation myelination fell considerably short of control values and it is reasonable to suppose that maturation continues for some time after connections have been established. The recorded action potential shown in Fig. 2 has an initial peak corresponding to a population of fibres with a mean conduction velocity of 10.65 m/sec. This relatively slow velocity of conduction probably corresponds with the incomplete myelination recorded in our previous study. Cragg and Thomas (1964), have suggested that after nerve crush or transection, maximum conduction velocity at one year has only returned to 75% of control values.

Our results suggest that functional reinnervation of target muscles is possible following peripheral nerve repair with treated muscle autografts in the non-human primate. Furthermore, connections are established, though maturation is incomplete within the time period prior to irreversible muscle atrophy. The use of treated skeletal muscle autografts may have significant implications in the repair of motor nerves in human patients.

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