REPAIR OF CERVICAL NERVE ROOTS
PROXIMAL TO THE ROOT GANGLIA

AN EXPERIMENTAL STUDY IN SHEEP

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An experimental model was established to investigate the possibility of repairing cervical nerve roots damaged above the dorsal root ganglion, as occurs in traction injuries of the brachial plexus. In four sheep the C6 root was divided and repaired within the dura using freeze-thawed muscle grafts.

Recovery was assessed after eight months by electrophysiology and histology. Action potentials were recorded distal to the grafts in all four sheep, indicating regeneration of motor fibres. Histological examination showed regenerated fibres in the ventral roots below the grafts in all cases. These fibres could be traced distally to the brachial plexus. There was no evidence of recovery of dorsal roots.

Traction injuries to the brachial plexus have devastating effects on the function of the upper limb. Where damage is confined to the nerves distal to their dorsal root ganglia some recovery may occur spontaneously or after surgical repair. In recent years surgical exploration of the brachial plexus soon after injury with excision and grafting of damaged areas has become more common (Jamieson and Bonney 1979). A nerve damaged above its dorsal root ganglion, however, is not thought to be amenable to repair (Bonney 1991; Millesi 1991).

Sunderland (1974) extensively reviewed the mechanism of avulsion of cervical nerve roots. The rootlets within the dura are the weakest part of the nerve root and rupture is likely to occur at this level when traction is applied. The manner in which the dura ensheathes and is attached to the spinal nerve is such, however, that lateral traction on the nerve is transmitted to the dural sac and thereby offers some protection. In addition, the C5 and C6 roots have strong fibrous attachments to their respective transverse processes which make avulsion of these roots less common. Sunderland proposed two mechanisms of root avulsion: a peripheral mechanism from lateral traction on the brachial plexus and a central mechanism in which an abnormal movement of the cervical spine causes stretching and rupture of the nerve roots within the dura. The former is associated with a dural tear and additional infraganglionic damage whereas the latter is not. It has also been reported that ventral roots are slightly more susceptible to traction injury than dorsal roots (Carlstedt et al 1989).

As mentioned above the prevailing view among clinicians is that root avulsions cannot be repaired. There is, however, some experimental evidence to suggest that nerve roots do have some regenerative potential. In the earliest reference available, Kilvington (1907) stated that useful regeneration can take place in the ventral but not in the dorsal roots. Although the experimental basis for his opinion is not clear, it has prevailed in the literature ever since (Tower 1943; Moyer and Kimmel 1948; Christophersen and Wintsch 1977). The cell bodies of the motor axons in the ventral roots are in the anterior horn of the spinal cord. A cut ventral root may therefore be expected to behave similarly to a more distally divided peripheral nerve. The dorsal roots above the dorsal root ganglion have an uncertain regenerative potential since it is doubtful whether they can make functional reconnections in the spinal cord.

In the present study a model was used to investigate the possibility of repairing damaged cervical nerve roots above the dorsal root ganglion. A large animal makes the operation technically feasible and simulates the human situation as closely as possible. The sheep was selected as previous experience has been gained with this species.

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and it was necessary to create a lesion which would not cause a serious neurological deficit. The available anatomical information (Dyce, Sack and Wensing 1987) suggested that division of C6 (the highest root contributing to the sheep brachial plexus) would cause only minor proximal limb weakness.

Previously, in a number of experimental models, it has been shown that freeze-thawed coaxially aligned skeletal muscle autografts are a satisfactory method of repair of short defects in peripheral nerves (Glasby et al 1986a,b; Glasby et al 1990). Experiments on human digital nerves have produced promising results (Norris et al 1988; Pereira et al 1992), and the technique has been found to be of value in the treatment of leprosy (Pereira et al 1991). The grafts provide a temporary matrix of orientated basement membrane to guide regenerating axons to the distal stump (Glasby 1990). The technique does not require the sacrifice of another nerve for grafting and muscle for the graft can often be obtained through the same incision and interposed into the nerve defects using microsurgical suturing and/or fibrin glue. It was therefore thought that this would be a convenient method for root repair in our experimental model.

MATERIAL AND METHODS

We used four sheep anaesthetised with intravenous ketamine 5 mg/kg. Endotracheal intubation was carried out immediately and anaesthesia was maintained by inhalation of a 1:1 mixture of oxygen and nitrous oxide supplemented with halothane 0.75% to 2.5%. Intermittent positive-pressure ventilation was used and the arterial blood gases were monitored to keep the partial pressure of carbon dioxide low and hence reduce bleeding. A neuromuscular blocking agent (pancuronium) was given to prevent unwanted muscular contraction while the laminae were being removed and the nerve roots handled. Neostigmine was sometimes required to reverse the neuromuscular blockade. Saliva and ruminal fluid losses were replaced with intravenous Ringer lactate solution at 500 ml/hour.

All surgery was carried out in an operating theatre under fully sterile conditions. The animals were placed prone and the head securely fixed in a Mayfield neurosurgical head holder.

The laminae of C5 and C6 were exposed on one side through an 8 cm longitudinal midline incision. A hemilaminectomy was performed at the level of the C5 to C6 space to expose the dura over the origin of the C6 root; it was then opened longitudinally under an operating microscope (Wild, Heerbrug M650). The C6 root, consisting of a number of small rootlets, was identified and divided close to the spinal cord.

A piece of muscle was obtained from the erector spinae, frozen in liquid nitrogen, and thawed in distilled water as previously described (Glasby et al 1986a,b). A small piece, with fibres longitudinally aligned, was then cut to size, inserted into the gap in the root, and secured in place with fibrin glue (Tissel: Immuno Ltd, Seven-oaks, England) surrounding the nerve-graft junctions but not interposed between them. The dura was patched with Surgicel (Ethicon, Edinburgh, Scotland) which was also glued into place. The wound was then closed and the animal was allowed to recover from the anaesthetic. As expected, only minor proximal weakness of the forelimb was noted after the operation.

After eight months electrophysiological and histological examinations were carried out. The sheep were anaesthetised as before and a C5 to C6 laminectomy was performed. The incision was then extended laterally on both sides with resection of the facet joints and division of the paravertebral muscles to expose both C6 roots from the spinal cord to the brachial plexus.

A unipolar needle electrode insulated except for the last 0.5 mm was placed in the spinal cord with its tip in the anterior horn. Short high-voltage (0.1 ms, 10V) square-wave stimuli were applied from a signal generator and isolated power source (Dagan Omnipulse 9200: Dagan Corporation, Minnesota, USA). The brachial plexus branch of the C6 root was divided as far distally as possible and placed over a 0.3 mm palladium wire bipolar recording electrode connected to a Neurolog NL104A differential a.c. amplifier (Digitimer Ltd, Welwyn Garden City, England) and filter NL125. The output was fed to the Y-input of a digital oscilloscope. Recordings of the action potential in the root were made first from the normal side and then from the grafterd side. When the dorsal roots were divided the action potential was unchanged, indicating that only motor neurones of the anterior horn were being stimulated. The signal averaging facility averaged 16 action potentials in each case. The distance between the cathodes and the time to the first peak of the action potential were measured to calculate the conduction velocity. The C6 root was then removed and specimens for histology were obtained in all cases from: A, the graft; B, the ventral root above the dorsal root ganglion; C, the dorsal root above the dorsal root ganglion; D, the dorsal root ganglion; E, the complete root before the branching of the posterior primary ramus; F, the anterior primary ramus; and G, the brachial plexus root (Fig. 1).

The specimens were processed as described by Gschmeissner, Gattuso and Glasby (1990) to produce 1 μm thick resin-embedded transverse sections for light microscopy. Sections were examined for overall appearance. Morphometric analysis, including axon and fibre diameter and the number of myelinated fibres per mm², was carried out on the ventral roots above the dorsal root ganglion using a Vids III computerised image-analysis system (Analytical Measuring Systems Ltd, Cambridge, England). A sample of 200 fibres was measured from each section. Statistical analysis was carried out using ‘Lotus Symphony’ (Lotus Corporation, Massachusetts, USA).
Diagram to show the position of the graft in the C6 root and the sites A to G (see text) from which the specimens were taken for histology at the time of assessment.

Typical action potential recorded from normal and repaired C6 roots while stimulating the anterior horn of the spinal cord. Note the longer latency in the repaired root.

Figure 3a - Photomicrograph of a transverse section of a normal ventral root above the level of the dorsal root ganglion (paraphenylenediamine stain × 600). Figure 3b - A transverse section of a normal dorsal root at the same magnification (paraphenylenediamine stain × 600).

Photomicrograph of a section of a ventral root below a graft (level B) showing small-diameter regenerated fibres (paraphenylenediamine stain × 600).

The distribution of the diameters of myelinated nerve fibres in normal and repaired ventral roots. A sample of 200 fibres was recorded.
RESULTS

The sheep showed a gradual improvement in the proximal forelimb weakness which had started immediately after operation.

Action potentials were recorded distally to the graft in all four sheep indicating recovery of motor fibres (Fig. 2). The mean conduction velocity in the grafted roots was 35.4 ± 1.9 m/s which was significantly less than the normal of 59.1 ± 3.4 m/s (p < 0.001).

Photomicrographs of sections of normal ventral and dorsal roots are shown in Figure 3. The normal ventral root had more fibres of large diameter while the dorsal root had a wider range of fibre sizes. In all the sheep the ventral roots distal to the grafts (level B) contained regenerated fibres (Fig. 4). The mean number of fibres in the grafted and normal roots was similar, 4920 ± 242 fibres/mm² and 3880 ± 51 fibres/mm² respectively. The mean diameter of myelinated fibres in the grafted roots was 5.26 μm, less than that of normal ventral roots (11.98 μm).

The normal ventral roots had two peaks of fibre distribution representing the alpha and gamma motor neurones (Fig. 5). In the regenerated roots there was only one peak of smaller diameter fibres but some larger fibres, about 10 μm in diameter, were present. It has been suggested that the presence of such large fibres is evidence that functional connections have been made (Carlstedt et al 1986). The regenerated motor fibres could be traced distally at levels E, F, and G. Careful comparison of the normal and grafted sides showed that areas of normal large-diameter motor fibres were occupied by smaller-diameter regenerated fibres (Fig. 6).

The fibres of the dorsal root have their cell bodies below the graft in the dorsal root ganglion. Above the dorsal root ganglion the grafted dorsal roots (level C) had few fibres (Fig. 7) suggesting that considerable retrograde degeneration of the central processes of the sensory neurones (axons) had occurred with little regeneration. The dorsal root ganglion appeared normal and there were many normal sensory fibres (dendrites) in the root at all levels below this (levels E, F and G). The graft itself contained fibres grouped into minifascicles typical of the appearance of freeze-thawed muscle grafts populated by regenerating nerve fibres.

DISCUSSION

These experiments have established a satisfactory model for the study of the regeneration of cervical nerve roots. Section of the C6 root produces a slight neurological deficit which is acceptable within the legal limitations for animal experimentation. The electrophysiological and histological results clearly show that there was regeneration in the ventral roots after repair, similar to that seen in a peripheral nerve. Death of the entire cell is believed to be more likely if damage to the axon occurs close to the cell body, but many cells did survive and their fibres regenerated. The smaller fibre diameter, and hence the lower conduction velocity, are both typical of peripheral nerve regeneration.

There seems to be no theoretical reason why such regenerated motor fibres should not make functional
connections similar to those after more peripheral nerve injuries. We cannot be sure in this experiment, however, that the functional improvement observed in animals was due to reinnervation of muscles. Since few muscles are innervated exclusively by one root, improved function could result from intramuscular branching of nerve fibres from another root or from hypertrophy of muscles fibres innervated by another root. This limitation of the experiment stems from the requirement to produce only a slight neurological deficit. It may be possible in the future to modify the model to show functional reinnervation of muscles.

Our results agree with those of some other workers. Carlstedt et al (1986) showed, in the rat, regeneration of axons into ventral roots reimplanted into the spinal cord after avulsion and Cullheim et al (1989) demonstrated reinnervation of muscles in a similar experiment in cats. Carlstedt et al (1989) also investigated the regeneration of damaged dorsal roots and their results support the classical view that axons cannot re-enter the spinal cord in adult animals, although reinnervation of the cord from dorsal roots may be possible in neonates.

The freeze-thawed muscle grafts used here supported the regeneration of fibres in ventral roots as they do more distally in the peripheral nervous system. Other methods of repair may also be effective but at this site the combination of freeze-thawed muscle grafts and fibrin glue certainly has technical advantages.

The possibility of repairing ventral roots above the dorsal root ganglia has considerable clinical potential in the management of brachial plexus injuries. In our experiments the roots were divided rather than avulsed, but, as has been mentioned above, Carlstedt et al (1986, 1989) obtained similar results after root avulsion. Most benefit from repair might be expected in cases in which the upper roots had been avulsed, causing loss of shoulder and elbow movements but leaving sensation and motor function in the hand intact. Proximal limb muscles can function well without sensory innervation, and the distance which regenerating axons have to grow to reach these muscles is short enough to permit reinnervation before irreversible atrophy has occurred. Such a repair, however, would require a cervical laminectomy as well as exposure of the brachial plexus and the morbidity of this would have to be taken into account. It may also be necessary to consider whether the whole root should be repaired so that the regenerating fibres are distributed widely to all the muscles innervated or whether the fibres should be 'directed' by nerve grafts to restore particular movements, for instance to the musculocutaneous nerve for elbow flexion.

In conclusion, nerve regeneration can follow repair of damaged ventral roots in sheep. This finding deserves further investigation to define the type of nerve-root injuries which may benefit from intraspinal repair in man.

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