Chapter

Effects of Timing of Nerve Injury and Repair in Neonatal and Adult Brachial Plexus Injury Models

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Abstract

Brachial plexus Injury causes severe and long-term upper limb deficits at any age. The outcome from current reconstructive options depends on the severity of nerve injury and timing of intervention. This chapter summarises the differing biological responses to nerve injury that occur during neonatal, young adult and mature adult life. The central and peripheral reactions to nerve injury, the effects of timing of repair on both motor and sensory neuronal survival and basic science evidence to support early intervention are discussed.

Keywords: brachial plexus, nerve injury, neuronal survival

1. Introduction

Brachial plexus injuries (BPI) in both adults and children have serious and lifelong consequences. Fixed prognostic indicators for recovery relate to age at the time of the injury, co-morbidities and the severity and extent of the injury. Variable prognostic indictors include strategies for surgical or medical intervention and the timing of such interventions. Thus, knowledge of the best timing for intervention is critically one of the only parameters we can assess, evaluate, and optimise.

All nerve injuries, regardless of age, have both central and peripheral effects, however, these effects vary between neonates and adults. In neonates the central effects in the spinal cord after unrepaired nerve injury are early and profound with rapid cell loss while in adults the loss of motor and sensory neurons is a slower process over weeks and months and often depends on the distance from the injury site to the parent cell bodies and the type of traumatic nerve injury. Laboratory evidence to evaluate the consequences of both nerve injury and repair has demonstrated the importance in understanding the true extent of the injury including the effects over time and the benefit of early nerve repair in both adult and neonatal models.

2. Neonatal brachial plexus injury

Neonatal brachial plexus injury occurs at the time of birth and affects between 0.41–1.5 per 1000 live births per year. About half of these cases have a residual deficit at 6 months of age and a 25% of the cases at 12 months. Clinical examination

is the current standard for assessment using either the return of elbow flexion as described by Tassin and Gilbert (1984) or the Toronto Scoring system (2002) popularised by Curtis and Clarke. Objective imaging and electrophysiological evidence to support the clinical finding is often undertaken but controversy remains about the role of these techniques in treatment decision making.

Operative exploration and reconstruction are the current gold standard in injury evaluation and intraoperative frozen sections, electrophysiology and visual nerve assessment are utilised to decide the severity of injury and the reconstructive algorithm. Even with complex peripheral nerve reconstruction these cases have long-term disability with ensuing social, psychological and economic sequelae.

2.1 Central effects of injury and early repair in experimental in neonatal BPI model

Retrograde degeneration of the sensory neurons in the dorsal root ganglia (DRG) and motor neurons in the spinal cord has been demonstrated in experimental neonatal brachial plexus injury models with activation of neuro inflammation and reaction of glial cells. Rapid loss of motor neurons with only 9% surviving in the anterior horn of the spinal cord at 28 days in a neonatal BPI model suggests a truly devastating reaction to nerve injury in this immature nervous system [1]. Loss of this volume of neurons so early suggests permanent long-term effects are predictable even with intervention. Timeline analysis of this early loss over 28 days post injury in one day old neonatal BPI model illustrates the loss of motor neurons apparent from day 2 after injury and continuing rapidly to fewer than 10% surviving motor neurons at 28 days from injury [1].

The methods for injury in these models have been transection and crush due to the difficulties in producing a reliable and reproducible traction model in this experimental population (weight at surgery 6–8 grammes). Clinical evidence would suggest that a traction injury would be less uniform and the possibility of associated preganglionic injury including avulsion of ventral and dorsal roots and direct spinal cord injury infers this to be an optimistic view of neuronal survival in this age group.

Other central changes to the spinal cord architecture including increased inflammatory reaction, activation of astrocytes and microglial cells and loss of synaptic boutons and dendritic branches in the anterior horn of the spinal cord.

Regarding age at the time of injury, more extensive neurological loss has been demonstrated in a sciatic nerve injury model at the age of 3–7-day old neonates when compared with 30-day old, matched neonates illustrating the fragility of the immature nervous system. In contrast to adults, distal peripheral nerve injury in neonates also results in spinal cord motor neuron loss [2, 3].

Sensory neurons are even more vulnerable to injury. Retrograde cell loss in the DRG occurs rapidly with both proximal and distal peripheral nerve injuries in neonatal experimental models [4–6].

Studies demonstrating this extensive cell death also suggest that plasticity in the spinal cord may play a role with adjacent nerve root zones compensating for the injury zone. Experiments focused on the role of C7 after unrepaired C5/C6 injury demonstrated a four -fold increase in the C7 contribution to biceps innervation [7].

Early BPI nerve repair at 1 day following injury in newborn rodents reduces the degree of retrograde motor neuron degradation with preservation of up to 20% of ventral horn motorneurons at 28 days This is a doubling of the number of preserved neurons compared with those without repair. Simultaneous decrease in activity of microglial cells and macrophages in the ventral horn supports the influence of repair on the rate and extent of neuroinflammation [1]. This has

important implications for neuronal regeneration and the number of axons reaching distal targets for reinnervation.

2.2 Target organ changes following injury and repair in neonatal BPI model

Studies exploring the mechanism of elbow contracture in a neonatal model have highlighted the direct effects of muscle denervation on muscle tightness and the lack of fibrosis concluding that elongated sarcomeres secondary to denervation is key to elbow contracture. The underlying mechanism is likely one of increased protease activity. This mechanism of elongated denervated sarcomere length is unique to the neonatal period.

Groups with no recovery of elbow flexion had significantly more severe elbow contractures than those with even partial recovery of elbow bending. When considering the axonal counts, experimental evidence supports the inverse relationship between the number of axons and the severity of the contracture with no evidence of elbow contracture in groups that has elbow flexion motor recovery [8]. This theory can be extended to shoulder contracture but experimental modelling and the number of muscles around the shoulder girdle make this more challenging to reproduce [9].

Differential effects on fast and slow muscle fibres following denervation and reinnervation in neonatal sciatic nerve injury has also been studied by Lowrie and Vbrova [10]. In newborns both fast and slow muscle development was impaired. In contrast, they demonstrated that in slightly older (6 days of age) age groups permanent changes only occurred in fast muscle with extensive fibre loss. Slow muscle fibres suffered temporary atrophy followed by recovery once reinnervated [10].

2.3 Pharmacological salvage

Neuronal rescue via pharmacological manipulation has been trialled in neonatal models. P7C3, a novel class of aminopropyl carbazoles, has demonstrable influence on neuronal survival, regenerative potential of both motor and sensory neurons with ensuing end organ functional benefit. High dose N- acetylcysteine (750 mg/kg twice daily) has been shown in one neonatal study to increase motor neuronal survival in crush and transection models in this age group. There was no effect demonstrated on sensory neurons survival in this study [11].

Previous studies have also demonstrated that neurotrophic factors can provide neuroprotection for axotomised neonatal neurons following different types of nerve injury [2, 3, 6].

2.4 Summary and translational value

Early and rapidly progressive proximal changes in the spinal cord and DRG, combined with loss of the distal neurological architecture and the degenerative changes in the sensory and motor target organs, implies that the chance for recovery without early intervention to reinnervate the limb in global injuries seems bleak. The value of very early repair is supported by evidence that repair promotes motor neurons rescue in the anterior horn of the spinal cord. This, combined with the evidence that reinnervated muscle has the potential to adjust the elongated muscle sarcomeres associated with joint contractures in these newborn models, would encourage further translational research of the effects after early nerve repair.

Translating this experimental work to clinical practice is challenging, not only as current surgical decision making relies on clinical evaluation and recovery over a period of time but also the potential for increased anaesthetic risk in the

newborn compared to the older infant. Early objective assessments that do not rely on prolonged observational recovery would be immensely helpful with this patient population. Imaging such as diffusion techniques or improved MRI that would allow earlier decision making for surgery might encourage the advent of very early surgery with the potential for preservation of motor and sensory neurons and better distal reinnervation of end organs. Previous experimental findings have shown that MRI can be used for assessment of retrograde degeneration in the dorsal root ganglia and spinal cord after peripheral nerve and spinal roots injury [12, 13].

3. Adult distal peripheral nerve injury

Peripheral nerve injuries to the upper limb in adults are common occurring in over 3% of trauma cases. They are more common in younger age groups and males predominate. Forearm and wrist injuries are associated with lack of full hand and upper limb functional recovery [14]. Clinical evidence supports work disability in cases of poor sensory and motor recovery. Proximal injury and type of work affect the delay in return to work [15]. Hand therapy can positively influence the outcome. Combined forearm injuries of median and ulnar nerves produce more severe deficits and as they are usually associated with injured tendons and/ or vessel injury that require more complex assessment, intervention, and rehabilitation. Most of these injuries affect the median (50%) and ulnar nerves (44%) far more frequently than the radial nerve (20%) [16]. Socioecomonic costing are considerable including care delivery, loss of income, sick days, and long-term permanent disability. Long-tern cost analysis suggests that up to 30% of patients with peripheral nerve injury have permanent disability, receiving financial compensation.

Understanding the complexity of the injury and delivering the best treatment is imperative to return to work and functional activities in this population.

Classification systems for nerve injury have been well described by Seddon and Sunderland [17, 18]. The former by way of neurapraxia, axonotmesis and neurotmesis is favoured by neurophysiologist. Neurapraxia refers to a conduction block affecting both motor and sensory nerve fibres but without Wallerian degeneration distally so the chance of functional recovery in meaningful time is high. In contrast, in axonotmesis the nerve axons and myelin sheath is injured by traction or crush but the surrounding neural sheaths are preserved. Theoretically the axons should be able to regenerate along the remaining endoneurial tubes. In neurotmesis, the axons surrounding stroma are transected or scarred, thus, unless there is intervention to overcome this gap the nerve will not recover independently. In a cohort of closed crush and traction injuries the continuity of the anatomical nerve can be difficult to confirm without surgical intervention. This suggests an overlap between these diagnostic categories.

Sunderland's classification system is more elaborate with 5 degrees of injury depending on a pathological assessment of the nerve ie. requiring histological analysis. Pathological finding ranges from myelin injury or ischaemia to axon loss and disruption of the neural sheaths surrounding the axons, fascicles or nerves. McKinnon modified this classification system to add a sixth degree describing a mixed lesion with both axon loss and conduction block occurring simultaneously within a nerve but affecting different nerve fibres [19].

Radiological assessment of nerve injury along with neurophysiological assessment in these mixed closed injuries can be challenging and surgical exploration and visualisation is often best practice.

Interventions within surgery are limited to repair, reconstruction, and repositioning of the injured nerve. Methods of nerve coaptation are varied but

evidence supports simple microsurgical epineural coaptation with fascicular alignment. Historically, considerable emphasis has been placed on assessment of motor recovery as an outcome for nerve injury, repair, and reconstruction. Recent and historic papers have demonstrated that 90% of axons within peripheral nerves are afferent fibres suggesting a need to shift focus from efferent to afferent fibres to explore potential avenues of improved outcomes in nerve injury and repair [20].

3.1 Central effects of injury and early repair in experimental animal models

Experimental evidence has demonstrated the retrograde cell death in the dorsal root ganglion (DRG) of small diameter afferent neurons. Laboratory evidence comparing cutaneous afferent, muscular afferents and motor neurons demonstrated a greater sensitivity to injury and more profound retrograde loss in the cutaneous afferent fibres in an adult model [21, 22]. In this experimental model up to 50% of DRG neurons projecting to the mainly cutaneous sural nerve were lost after 8–24 weeks following distal sciatic nerve transection while counts of DRG neurons innervating sensory targets in the gastrocnemius muscle did not demonstrate any neuronal loss in the same timeline. Following immediate repair there was an increased survival of up to 30% of cutaneous afferent fibres with associated promotion of nerve regeneration. The significance of the distance of the injury from the cell body in influencing the extent of retrograde cell loss is important. Distal sciatic nerve injury yields sensory cell loss in the DRG over time but it does not result in detectable retrograde motor neuron loss in the anterior horn of the spinal cord in experimental mature adult animal models [22–24]. Both primary repair and peripheral nerve grafts improved the survival of sensory DRG neurons, however only about 50–60% of sensory and motor neurons regenerated into the distal nerve stump [22].

3.2 Target organ changes following distal nerve injury and repair

Both prolonged axotomy and prolonged denervation affect functional long-term recovery after delayed nerve repair. It is well known that nerve injury results in muscle denervation and subsequent atrophy [25]. The force and speed of muscle contraction is reduced along with a conversion to slow twitch muscle fibre type.

Degeneration of the distal nerve stump with associated Schwann cell death and fibrosis limits the nerve regeneration and subsequent recovery. Experimental work has shown that equal numbers of axons are present in the centre of nerve grafts after early (before 1 months) and late (after 3 months) reconstruction [26].

However, it is the number of axons projecting into the distal stump which decreases as time passes beyond one month after repair. Regarding the muscle fibre recovery, slow fibres predominate after denervation in the animal model gastrocnemius muscle after sciatic nerve injury and repair. This suggests they are more robust, earlier to reinnervate or preferentially reinnervated. Neuromuscular junctions are also affected by denervation. Poor reinnervation after delayed repair (greater than 3 months from injury) is supported by the presence of increased levels of embryonic specific gamma-nAChR and increased expression of MuSk (muscle specific tyrosine kinase), a coordinator of neuromuscular junction differentiation. These changes to the muscle fibre type and the neuromuscular junction in the reinnervated muscle have phenotypic consequences with changes to muscle characteristics and performance. This evidence supports the earlier repair of distal peripheral nerve injuries with better preservation of the distal stump and potential for end organ recovery.

3.3 Pharmacological salvage

The sensory neuronal retrograde death in the DRG following axonal injury or transection with effects on mitochondrial function and apoptosis following reactive oxygen species led to the laboratory trials of antioxidants such as N-acetylcysteine (NAC) in order to combat these changes. N acetylcysteine is a thiol containing compound that had antioxidant properties along with inhibition of proliferation and stimulation of transcriptase and enhancing intracelluar glutathione levels. Intrathecal administration of NAC combined with nerve grafting in adult distal peripheral nerve injury experimental models provides additive protective effects with increased survival (up to 90%) of DRG sensory neurons [22]. However, NAC did not affect the number of myelinated axons in the nerve graft or in the distal nerve stump, nor did it have any growth promoting effect on the spinal motoneurons in this model [22].

3.4 Translational implications

Nerve injuries in the forearm and hand are relatively common. Long-term cutaneous sensory deficit after nerve injury are often present at follow up assessment but recovery is often focused on recovery of the motor deficit. Perhaps this is due to the lack of sensory outcome assessment availability and the relative ease of movement evaluation. However, sensory cutaneous and muscle afferents are critical for all fluid and coordinated movements upper limb and hand movements. Loss of cutaneous sensation leads to a "blind hand" with frequent injury and accident. Earlier repair and agents such as NAC combined with focused specialist hand therapies for sensory and motor re-education may improve these outcomes in the clinical setting based on these experimental studies.

4. Preganglionic and postganglionic BPI in adults

Adult brachial plexus injuries occur in 1% of polytrauma cases. They are devastating life changing injuries with serious health consequences - physical, psychological, social and economic. The most common aetiology is direct collision road traffic accidents involving young male motorcyclists. Over 50% of these injuries result in complete brachial plexus injuries with permanent limb paralysis and neuropathic pain. Socioeconomic costs are high in this working population and some studies quote up to 84% partial or permanent physical disability after motorcycle accidents [27].

In this polytrauma population injuries associated with trauma brachial plexus include cervical spinal injuries, lung perforation, rib fractures, head and trunk injuries and injuries to the vasculature and bony skeleton of the upper limb and scapulothoracic association. These nerve injuries are often predominantly avulsion injuries from the spinal cord, but there remains a cohort of extraforaminal traction -rupture injuries that merit evaluation for regenerative and reconstructive potential.

Assessment of nerve injury with radiological imaging and electrophysiological investigation can be useful in identifying associated injuries that will affect prognosis, for example vascular injury or severe scapulothoracic dissociation. Improvement in MRI imaging including the advent of Diffusion Tensor Imaging to assess continuity between the spinal nerve rootlets and the spinal cord and DRG is promising. DTI also has the potential to assess nerve function and health which may provide information on the severity of nerve injury, the extent of regeneration and the recovery before the nerve reaches the sensory or motor end organ [28, 29]. Newer MRI techniques along with 3 T as opposed to 1.5 T scanning yields clearer and more defined images of the cord- rootlet junction. However

even with these advanced imaging systems there are still gaps in the information acquired. Acquisition of good quality T1 images remains challenging and overall sensitivity and specificity of MRI is not 100% leaving doubt about abandoning nerve exploration when there is even the slightest possibility of nerve continuity available for reconstruction [30–34].

Clinical studies assessing the effect of presurgical delay on the outcome in upper trunk brachial plexus reconstruction in adults support a better functional outcome in cases treated within 2 months of injury. In those cases, treated later than 2 months from injury there was no significant difference in the pre and postoperative elbow flexion grade using the Medical Research Council Motor Grading System [35]. This supports relatively early surgery for brachial plexus reconstruction in adults.

4.1 Central effects of pre- and postganglionic plexus injury and repair

It has been demonstrated that pre- and postganglionic experimental BPI injuries in adult animals can induce significant degeneration among sensory neurons in the DRGs and motor neurons in the spinal cord [36–38].

Experimental work evaluating the neuroprotective and growth-promoting effects of early and delayed nerve grafting if the 7th cervical root has demonstrated a difference in early (4 weeks) and late (8 weeks) repair groups. In the timeline of cell loss assessment at 4 week did not demonstrate obvious sensory or motor cell loss but at 8 and 16 weeks both motor and sensory cell loss was apparent. In the anterior horn the motor loss increased from 15–29% between 8 and 16 weeks and in the DRG the sensory neuron loss increased from 32–50%. Both early repair and delayed repair were effective in preventing retrograde degeneration of motoneurons but of course the repairs at 8 weeks have far fewer neurons remaining in any case [37]. Neither early nor late repair were able to rescue sensory neurons but again the population was higher in the early repair group. In both groups the proportion of regenerating neurons remained constant. Thus, although delayed nerve repair is neuroprotective it will only protect those remaining neurons. This evidence supports the proposal that early nerve repair is optimal to promote best motor and sensory recovery and even this is at considerable loss of both motor and sensory neurons. Avulsion of the ventral root in the adult lumbar cord yields 40–80% neuronal cell death at 2–4 weeks from injury [39–41]. This, combined with the technical difficulties of any type of root reimplantation due to the disruption to normal architecture, have forced the field of reconstruction surgery after avulsion injury nearer the end organ in nerve transfers from adjacent functioning motor or sensory nerves.

Experimental evaluation of primary and secondary changes visible on MRI imaging of the spinal ventral root after transection and avulsion of a lumbar plexus model has also been completed [12]. MRI and histological analysis of the ventral horn demonstrated differences in the volume of the ventral horn on MRI with avulsion injury which is not apparent with transection injury. Histological analysis of these imaged ventral horns revealed severe loss of neurons, dendrites, axons, and synapses with increases in microglial cells and astrocytes. It is suggested that the loss of neuronal cells may contribute to the change in MRI signal leading to the changes in the images acquired.

4.2 Pharmacological intervention and salvage

Experimental evidence exists that supports that the use of NAC can prevent active death after proximal sensory nerve injury [42]. Suggested pathways include mitochondrial pathway blockade to inhibit caspase cascade. In ventral horn motor neurons NAC is also protective (>90%) with dose dependent effects. In avulsion

injuries the response to NAC was diluted to 70% suggesting the immediate necrosis of neuronal cells at the time of injury to be a significant factor in cell loss. Regarding delivery methods intrathecal was more effective than intraperitoneal but the later is more practical and clinically relevant suggesting systemic therapy would be effective. Such therapies are already used for acetaminophen overdose and toxicity [43]. Timing of intervention is important considering the very early and rapid loss of both sensory and slightly less rapid loss of motor neurons with proximal transection or avulsion injuries. In addition, earlier studies also demonstrated the efficacy of prolonged intrathecal treatment with various neurotrophic factors to prevent retrograde cells death in both the DRGs and spinal cord [40, 44]. However, significant side effect on the rate of axon regeneration and normal synaptic composition could significantly limit their potential clinical application [41, 45].

5. Summary

Central and peripheral effects of nerve injuries in neonates, young adults and mature adults have been studied experimentally. Loss of neurons is seen in all age groups, but early rapid loss is particularly apparent in neonates and in sensory neurons in all age groups. Early repair has neuroprotective properties, but cell loss is still significant even with almost immediate intervention. These experimental studies support nerve repair as soon as is safely possible given the other associated injuries in both adult and neonatal populations. Proximal nerve injuries such as brachial plexus injuries are particularly damaging to the motor and sensory nerve pool. Avulsion injuries in adults have similar devastating levels of neuronal cell loss when compared with neonatal transection injuries. However, early repair is technically very challenging is avulsion injuries due to the associated necrosis of the supporting ventral horn cell architecture. Pharmacological antioxidant therapies have been neuroprotective in adult studies and may be particularly relevant to sensory neuronal support.

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