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Chapter 4

Nerve Root Reimplantation in Brachial Plexus Injuries

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http://dx.doi.org/10.5772/intechopen.82431

Abstract

Nerve root avulsion is the most severe form of brachial or lumbosacral plexus injury. Spontaneous recovery is extremely rare, and when all the nerve roots of the affected plexus are avulsed, the therapeutic options are very limited. Nerve root reimplantation has been attempted since the 1990s, first in experimental animal models and afterwards in human beings. Currently, only partial recovery of the proximal limb muscles has been achieved. New therapeutic strategies have been developed to improve motor neuron survival and axonal regeneration, with promising results. Neurotrophic factors and some drugs have been used successfully to improve the regenerating ability, but long-term studies in humans are needed to develop complete recovery of the affected limb.

Keywords: brachial plexus injury, nerve root avulsion, nerve root reimplantation, motor neuron death, muscle atrophy, neurotrophic factor, axonal regeneration, motor and sensory recovery

1. Introduction

A common event in brachial plexus (BP) injury is nerve root avulsion (NRA) in which the nerve rootlets (NRts) are torn from the spinal cord (SC) [1–3]. Once avulsed, the NRts retract towards the nerve root (NR) sleeve [4]. The most common cause is traumatic NR stretching due to road accidents or parturitions [3, 5]. These injuries can also happen but are much rarer at the lumbosacral plexus [6]. The ventral rootlets (motor) are weaker and thus get injured more often and more seriously than their posterior counterparts [7].

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Soon after avulsion anterior horn motor neurons (MN) and sensory neurons at the dorsal root ganglion (DRG) undergo apoptosis [8–17]. Inside the avulsed NR itself, there is a Wallerian degeneration with axonal and myelin loss [18]. The muscles, devoid of nervous impulses, undergo atrophy and fibrous transformation [19, 20]. At the SC, the neurons suffer loss of synapses with destruction of previous neuronal networks and creation of new anomalous ones that will lead to abnormal nerve impulses which might induce chronic neuropathic pain [21–24].

After complete NRA, spontaneous regeneration is impossible [9]. In case of a single NRA, recovery coming from nearby healthy ones can be expected in neonates but not in adult patients [25]. Ventral root surgical reimplantation has been attempted both in experimental animals and in human beings with partial recovery [26, 27].

Axonal regeneration is stronger in direct ventral NR reimplantation [26, 28]. This is rarely possible [4, 7, 29, 30], so peripheral nerve grafts (NGs) are used to cover the gap between the SC and the remains of the avulsed NR [31–33]. These NGs are usually taken from a peripheral sensory nerve (medial antebrachial cutaneous, radial cutaneous, and saphenous), which is not the ideal situation as motor nerve regeneration is worse if sensory nerves are used as donors compared to mixed or pure motor nerves [34–36]. Acellular conduits have also been used, but the regeneration does not grow further than 2 cm [37, 38].

1.1. Historical background

Surgical repair of spinal NRs after traumatic avulsion in live human beings was considered technically impossible until the pioneering work of Carlstedt et al. [39]. The first studies were done in rats [40], then in cats [41] and finally in primates [42, 43], before attempting NR reimplantation in humans [44]. Initially, the efforts were directed at repairing the ventral rootlets (motor), but in adult human beings, it provided only mild improvement in shoulder and elbow movements [45]. In children, some hand movement was recovered but with limited function [29]. In addition, it was found that the number of surviving MNs and the number of axons that regenerated after NR reimplantation had a direct relationship with the final functional recovery [7, 30]. Ever since, many research groups have focussed on understanding the underlying pathophysiology and to find surgical strategies and drugs that can enhance regenerating capacities.

2. Pathophysiology

The interface between the central and peripheral nervous systems is known as the transitional zone (TZ) [46], and the regenerating capacities are influenced by both of them. The first is rich in astrocytes that create channels through which motor fibers pass [15]. The latter has Schwann cells that secrete neurotrophic factors (NFs) with higher regeneration abilities [47].

NRA disconnects the transverse arch that exists at each spinal level between the posterior horn sensory, the lateral horn autonomic and anterior horn neurons [23] as well as disconnection of

the DRG neurons from the bulbar and thalamic sensory nuclei [48]. NRA also induces loss of synapses and dendritic arborisation, fiber degeneration, neuronal death, posterior spinal column degeneration and glial proliferation [23, 48]. The synaptic and neuronal changes in the posterior horn produce neuropathic pain [24, 48, 49].

NRA is followed by an intense inflammatory SC reaction [50] with microglia, macrophage and glial proliferations [51]. At the TZ a dense scar tissue and a neuroma from the avulsed MN develop [15, 46, 52–55]. In the normal situation, the central nervous system is rich in astrocytes that create channels through which the nerve fibers pass [15]. After NRA, astrocytes proliferate and rearrange, blocking those channels and making it difficult for the regenerating nerve fibers to grow [15, 46, 56]. Axonal and dendrite regeneration is inhibited by the secretion of some substances by the astrocytes (chondroitin sulphate proteoglycans or CSPGs) [57–59] and oligodendrocytes (myelin protein [60–62] and semaphorin-3 [63]). Additionally, the glia secrete neurotoxic products like glutamate [15] and free radicals [64] that induce massive neuronal death among motor [8], sympathetic [12], parasympathetic [12] and posterior horn sensory neurons [17].

About 80% of the MNs die in the following weeks [13, 65, 66], but this death does not happen immediately after NRA [13, 67, 68]. Instead, there is a 12-day period in which different treatment strategies can reduce this MN loss [65, 69]. The chemical compounds that counteract the glutamate toxic effects can reduce the MN loss by 70%, provided that they are administered in the first 2 weeks after the NRA [16, 65, 69].

The closer the axonal injury to the neuronal body [55], the smaller the regenerating capacity of the axon and the higher the chance that the neuron will die. Four millimeters is the minimum amount of peripheral nerve that should remain to avoid MN death [70].

The surviving MNs develop axonal sprouts within 1 month after the NRA [41], but to achieve a successful regeneration, the axons must cross the gliotic TZ, grow inside the distal peripheral nerves, and reach the motor end plates [71]. The long distance to cover is a big impediment to a successful functional recovery [72, 73]. By the time the muscles get reinnervated, they are atrophic and with fibrotic changes, particularly the most distal ones [74]. The regeneration is not privative to the axon, and the dendrites can also regenerate as axon, creating what has been called a dendraxon. These also have the capacity to grow into the peripheral nerves and reinnervate muscles [75, 76].

Although the MN regenerating axon has a chance to cross the anterior SC white matter to reach its surface and then attempt to grow in a possible reimplanted NR [77, 78] for the DRG growing axon, the same is almost impossible as they have to cross a very hostile and gliotic posterior SC Dorsal Root Entry Zone (DREZ) [79–81].

In the human being, the avulsion damages more frequently the ventral NRts as they are more fragile than their posterior counterparts [15].

NRA creates four problems that have to be addressed to achieve a successful repair. First, if the axon is torn closer than 4 mm to the cell body, motor and preganglionic parasympathetic neurons undergo apoptosis [10–13, 23, 67, 68, 70, 82–84]. Second, muscles are fibrotic by the

time the regenerating axonal sprouts reach the motor end plates [72, 73]. In rats, functional recovery is seen only in cervical but not in lumbosacral avulsion models as the distance to cover is much shorter for the cervical NRs [9, 40, 85–87], and in any case only proximal limb muscle recovery is seen [86–89]. Third, the regenerating fibers may reach the wrong target due to misrouting [53], and in the absence of NG or conduit, the regenerating axons will grow along the surface of the SC [27, 43, 53, 83, 87]. The misrouting is responsible for simultaneous contractures in agonist and antagonist muscles leading to ineffective limb movements [30]. Fourth, there is severe muscular atrophy due to lack of use [74]. Hence, for a successful clinical result, MN survival must be improved, axonal regeneration has to be enhanced and accelerated, misrouting should be minimized and muscle atrophy should be prevented [15, 72].

Although the MN cell body can regenerate and grow a new axon after this is torn [69, 90], many MNs apoptose [13, 65, 69], and only 80% of the surviving MNs do finally project a regenerating axon in the reimplanted ventral root or NG [26, 27, 31, 86]. Reimplantation of avulsed NRs either directly or by means of a peripheral NG helps to reduce the number of MNs undergoing apoptosis, probably because of local NF production [69, 77, 89, 91–93]. Exogenous NFs can be administered to enhance the regenerating capacity of cells [47, 94, 95].

Historically, the first attempts were directed at motor recovery with ventral rootlet reimplantantion [96], but recently sensory recovery has been proved possible by reimplanting dorsal rootlets [97]. The results of dorsal rootlet repair are dismal because the SC glial proliferation creates barriers that prevent the regenerating DRG axons from reaching the posterior SC horn [81]. The lack of sensory recovery induces chronic neuropathic pain [49, 98], and the lack of proprioception causes limb clumsiness [30]. This has been partially avoided by direct implantation of the dorsal rootlets or their NGs' extensions inside the posterior horn itself rather than on the surface of the SC [81, 99]. The repair of both motor and sensory NRts leads to better functional results with more accurate movements and less muscular synkinesis [100]. Functional MRI studies have corroborated affected limb sensory cortex function recovery in the area corresponding to the reimplanted NR [100].

The **timing of NR reimplantation** is crucial, as a longer waiting period will correlate with a greater amount of MNs undergoing apoptosis [20, 27, 91, 93, 101–103]. The percentage of dead MNs increases from 20% by 10–12 days post-avulsion [13, 65, 69] to 50% by 4 weeks [104, 105], 85% by 6 weeks [106] and 90% by 20 weeks [27, 83, 93, 107]. Early NR reimplantation seems to have neuroprotective effects [27, 83, 89, 93, 108, 109], but some MN loss will happen even if repair occurs immediately after avulsion [93, 101]. In animal models, NRA followed by immediate reimplantation in the same surgical procedure minimizes MN apoptosis and achieves muscle reinnervation with some limited functional recovery, which is better in the brachial plexus than in the lumbosacral plexus [27, 69, 83, 110]. Ideally, the surgical repair must be performed no later than 10 days post-injury [65] as a delay over 2 weeks will lead to poor clinical results [20, 26, 27]. In clinical practice, patients suffering from brachial or lumbosacral plexus avulsions often experience other concomitant injuries, sometimes quite serious, that force delaying NR repair [111]. Another common scenario is that the precise diagnosis takes weeks or even months [3]. In any case, in human beings NRA repair has to occur no later than 1 month after the injury to allow any motor function recovery [45, 74, 97, 100]. NGs are almost

always needed as torn NRts retract and undergo fibrosis with time, making direct reimplantation to the SC impossible unless the repair is done just a few days after the injury [74]. This is a further difficulty as regeneration is worse with NGs than with direct NRt reimplantation [26].

3. Pharmacological aids to enhance regeneration after nerve root reimplantation

Several pharmacological aids have been introduced to improve MN survival and axonal regeneration after anterior spinal NRt reimplantation. They can be classified into NFs, drugs and cell-derived products (**Table 1**).

NF administration improves MN survival as well as synaptic and axonal regrowth [87, 112–115] improving the NR reimplantation results. NFs enhance Schwann cell migration, axonal regeneration and myelination [8, 16, 69, 93, 105, 116–120] and delay MN apoptosis—by 6 weeks 80–90% of them are still alive [8, 69, 116, 118–121]. To be maximally effective, they must be administered locally at the SC-NR interface within the first 3 days and no later than 2 weeks post-avulsion [20, 87, 93, 116]. NFs ought to be applied with Gelfoam or fibrin glue to avoid dilution in the CSF [72], but free intrathecal application by means of an injecting pump is not recommended [122]. Their short half-life limits their use, particularly because NFs have to be applied directly to a surgically exposed SC [123]. Although NFs increase MN survival and axonal regeneration, their effect on muscle recovery and final functional results is very limited [4, 7, 18, 20, 27, 37, 93, 105]. It has been observed that in areas where the concentration of NFs is high, the regenerating axons get trapped and do not grow to reach their final distal targets [18, 102]. Some have cautioned against the possible adverse effects of using NFs in human clinical practice [124]. The currently used NFs are brain-derived neurotrophic factor (BDNF) [115], glial-derived neurotrophic factor (GDNF) [8, 18, 20, 37, 102, 105, 125], ciliary neurotrophic factor (CNTF) [87] and intracellular sigma peptide (ISP) [126]. GDNF shows the strongest action and a single direct application to the SC are enough, provided that they are applied within the first 2 weeks after NRA [18, 20, 37, 102, 116, 127]. GDNF delays MN cell death for 6 weeks, therefore broadening the window for avulsed NR reimplantation [20]. Similarly, the intracellular sigma peptide (ISP) blocks astrocytic inhibitory action, thus facilitating axonal regeneration [126].

Moreover, the distance to cover by the regenerating axons from the SC avulsion site to the muscular end plates is so long that by the time the axons reach their destination, the muscles are atrophic and fibrotic [20, 128]. To avoid and delay this muscle atrophy as much as possible, several strategies have been attempted: manipulating the molecular pathways involved in muscle atrophy [129–131], nerve transfers from neighboring functioning nerves [132–136], direct electrical stimulation of the affected muscles [137–139] and neuronal transplantation inside the denervated muscle [20, 140–142]. In rats, the combination of GDNF at the SC-NR injury site and embryonic spinal foetal neuron transplant inside the target muscles provided the best possible functional result [20]. These embryonic neurons reinnervate the muscle end plates just after the injury, preventing muscle atrophy while the regenerating axons arrived

Agent	Group	Mechanism of action	Administration route	Motoneuron survival post-injury	Axonal regeneration	Observation	Applied to	Current human clinical use
Brain-derived-neurotrophic-factor (BDNF)	NF	Reverses cholinergic transmitter-related enzyme deficiency	Intrathecal	Motoneuron survival 53% by 16 weeks	Abundant regenerating fibers reaching cord-avulsed root interface	Active against many neurodegenerative disorders	Rat	None
Glial-derived-neurotrophic-factor (GDNF)	NF	⇑ Survival of dopaminergic neurons	Direct administration on spinal cord	Completely prevents motoneuron loss at 16 weeks post-avulsion	⇑ Axonal regeneration and coiling and regeneration Schwann cells	Strongest NF. ⇑ Effect combined with Riluzole Administration before 2 week post-avulsion	Rat	None
Ciliary NF (CNTF)	NF	Activates motor neuron signal transducer and transcription 3 activator(STAT3)	Direct administration on spinal cord	Motoneuron survival 23 ± 4.3% by 3 weeks post-avulsion	⇑ Axon regeneration across interface spinal cord/ nerve root	Conjugation it with transferrin prolongs its action	Rabbit	None
Intracellular sigma peptide (ISP)	NF	⇓ Inhibition of astrocyte secreted chon-droitin sulfate proteoglycans	Subcutaneous injection	Motoneuron survival 61.2% at 12 weeks post-avulsion	⇑ Amount and size of regenerated axons	Act as synapse organizing agent	Rat	None
Resveratrol	Drug	Topoisomerase II inhibitor	Added to nerve graft culture	Motoneuron survival 69% at 8 weeks post-avulsion	⇑ Axonal regeneration, Schwann cell migration and myelination	Only tried on autologous nerve graft cultures	Rat	Cancer, Chronic diseases, Aging
Riluzole	Drug	Inhibitor presynaptic glutamate release	Orally	Motoneuron survival 70% by 5 weeks post-avulsion	⇑ Myelinated axons in re-implanted nerve root. ⇓ Sensory hypersensitivity and allodynia	Administration before 2 week after injury. Maximum effect combined with GDNF	Rat	Amyotrophic lateral sclerosis, Nervous Depression, Spinal Cord Injury

Agent	Group	Mechanism of action	Administration route	Motoneuron survival post-injury	Axonal regeneration	Observation	Applied to	Current human clinical use
Lithium	Drug	⇑ Endogenous BDNF secretion	Orally	Motoneuron survival 69% by 12 weeks post-avulsion	⇑ Myelinated axons inside re-implanted nerve root	Helps prevent muscle atrophy	Rat	Bipolar disorder
Minocycline	Tetracyclyne derivative	Inhibits glial proliferation. Strong anti-inflammatory effect	Orally	Motoneuron survival 48±7% at 5 weeks. Autonomic neurons ∅ effect	Improves axonal sprouting and migration	Neurotoxic at high doses. Prevents and reverses hypersensitivity	Rat, mice	Bacterial infections, Stroke
Recombinant erythropoietin	Drug	Counteracts glutamate's cytotoxic effect	Subcutaneously	Motoneuron survival 51.7 ± 0.8% at 12 days post-avulsion	Suppresses microglia proliferation. Protects axon regeneration	Induces a pro-thrombotic state. Neuroprotective effect NOT long-lasting	Rat	Anemia
FK506-tacrolimus	Drug	Immunosuppression. Target heat shock protein 90	Sublingual	Motoneuron survival not reported. Used ONLY in dorsal nerve root repair	⇑ Regenerating axons penetrating and reaching the posterior horn	Immunosuppression. Long-term administration needed	Rat	Organ transplant immunosuppression
Geldamycin	Ansamycin antibiotic	On heat shock protein 90. NOT immunosuppression	Parenteral injection	⇑ Survival dorsal ganglion neuron. Motoneuron not studied	Accelerates axonal regeneration	No immunosuppression. Toxic at high doses	Rat	Cancer
Acamprosate	Drug	⇓ Synaptic glutamate	Orally	Associated with ribavirin ⇑	Associated with ribavirin accelerates	Side effects if ethanol consumption	Rat	Alcoholism

http://dx.doi.org/10.5772/intechopen.82431

Agent	Group	Mechanism of action	Administration route	Motoneuron survival post-injury	Axonal regeneration	Observation	Applied to	Current human clinical use
				motoneuron survival by 64.62% at 1 week	axonal regeneration >4 weeks			
Ribavirin	Drug	Synthetic guanosine antiviral properties	Orally	Associated with acamprosate ⇑ motoneuron survival by 64.62% at 1 week	Associated with Acamprosate accelerates axonal regeneration >4 weeks	Can induce anemia	Rat	Hepatitis virus C
N-acetyl cysteine	Drug	Stabilizes oxidative metabolism	Orally	Neuron survival 26% motor, 95% sensory	Facilitates axonal regeneration	Vitamin C counteracts side effects	Rat	Mucolytic
Glatiramer	Drug	Immunomodulator	Subcutaneously	⇑ Motoneuron survival but NOT quantified	Reduction in astrocyte proliferation	⇑ Risk of infection and malignancy	Rat	Multiple sclerosis

Table 1. NFs (neurotrophic factors) and drugs used in nerve root reimplantation with their effects.

[20]. However, when the regenerating axons reached the muscular end plates, they had to compete with the already existing axons coming from the locally injected embryonic foetal neurons [20, 140, 143, 144].

Some **drugs** have been administered **to minimize MN apoptosis and improve NR regeneration**: resveratrol (3,4′,5-trihydroxystilbene) [145], riluzole (2-amino-6-trifluoromethoxybenzothiazole) [8, 69, 121], lithium [146, 147], minocycline [119], recombinant erythropoietin [118], FK506-tacrolimus [148–151], geldanamycin [152, 153], acamprosate [67, 154], ribavirin [154], N-acetyl cysteine [155] and glatiramer [156]. Some researchers have administered combinations such as acamprosate and ribavirin [154] or riluzole and GDNF [8]. The main advantage of acamprosate, ribavirin, and riluzole is that they can be administered orally [67, 154, 157].

Resveratrol has been added to the autologous NG culture for a week in the rat experimental C_6 NRA and reimplantation model [145], finding that it improves axonal regeneration, Schwann cell migration and myelination and MN survival—69% surviving 8 weeks after NR repair.

In experimental brachial plexus avulsion (BPA) rat models, **riluzole** has been proved to improve MN survival, prolonging the time period at which reimplantation can be successful [65, 69, 101, 121]. If administered within 2 weeks post-avulsion, riluzole helps to keep 70% of the MNs [65, 69, 121] alive and minimizes the sensory hypersensitivity and allodynia [119]. Its maximum effect is achieved when combined with GDNF [8], and it can be administered orally [157].

In rat, experimental avulsion models and at doses used in the treatment of mood disorders, **lithium** improves neuronal survival, axonal regeneration and myelination, allowing an earlier and better functional recovery [146, 147]. One of its mechanisms of action is by increasing endogenous BDNF secretion [158]. Its effect on growing axon myelination starts 4 weeks post-NR reimplantation, reaching its pinnacle at 6 weeks and slowing down by 12 weeks [146].

Minocycline is a tetracycline derivative that inhibits glial proliferation [159]—a barrier against axonal and dendrite growth [160]—and decreases neuronal [161] and oligodendrocyte cell loss [120, 162, 163]. Minocycline can cross the blood–brain barrier and has anti-inflammatory properties [120]. In rats, it has been administered intraperitoneally and intrathecally, with better results through the latter route [106]. At low doses, minocycline has neuroprotective properties, but at high concentrations it is neurotoxic [164], among other reasons, because glial proliferation and Wallerian degeneration are a *sine qua non* for nerve regeneration [106].

Recombinant erythropoietin injected subcutaneously once a day for 3 days has shown neuroprotective properties in a rat NRA experimental model [118]. These neuroprotective properties are short lasting but can help to delay motor neuron apoptosis after NRA, increasing the period in which a NR reimplantation can be undertaken [118]. Recombinant erythropoietin seems to counteract the cytotoxic effect of glutamate, block free radicals, increase the release of neurotransmitters and decrease microglial activation [165]. The positive effects of recombinant erythropoietin are maximal when its administration is started within 96 hours (4 days) after NRA and reimplantation [118]. The side effects related with the administration of

this drug—increase in erythrocyte production and a prothrombotic state—are not problematic because this drug is only administered for 3 days [118]. Perhaps administering this drug for a longer period of time could provide additional neuroprotective effects, but 3 days are enough to prolong the period in which a successful NR reimplantation can be performed [118].

FK506-tacrolimus improved the amount of regenerating posterior NR axons penetrating the SC and reaching the posterior horn [151].

Acamprosate is a taurine analogue used to prevent relapse in alcoholic patients that acts as neuroprotective and accelerates axonal regeneration [154, 166].

Ribavirin is a nucleoside antimetabolite antiviral agent that blocks nucleic acid synthesis that is administered together with acamprosate to encourage axonal regeneration [154].

N-Acetyl cysteine administered intraperitoneally and intrathecally in rats enhances the rate of MN survival and facilitates regeneration in case of NR reimplantation [155].

Glatiramer is a polymer of L-alanine, L-glutamic acid, L-lysine and L-tyrosine that structurally resembles the myelin basic protein and that when administered daily reduces the gliosis and the avulsed MN synaptic stripping [156].

To summarize, in NRA reimplantation GDGF applied directly to the anterior SC—to the point where the motor rootlets go out—associated with oral riluzole provides the highest rate of MN survival and axonal regeneration [8]. For the dorsal root, CNTF [87] applied directly to the section of the posterior SC where the sensory rootlets get in combined with oral N-acetyl cysteine [155] allows maximal sensory neuron survival. Other agents could be added, such as oral minocycline [106, 120], tacrolimus [151] or recombinant erythropoietin [118, 165]to reduce the reactive glial proliferation that impairs the axonal regeneration. ISP should be administered subcutaneously to minimize astrocyte inhibition of axonal regeneration [126, 167]. The data are summarized in **Table 1**.

Another strategy has been to apply **pluripotent cells** at the SC avulsion site to improve MN survival and axonal regeneration. These have been particularly useful in minimizing neuronal apoptosis. Among them are induced pluripotent stem cells (iPSC) [143], mesenchymal stem cells (MSCs) [168–170], olfactory ensheathing glial cells (OECs) [85, 171], bone marrow stem cells (BMC) [172], human fibroblast growth factor 2 (FGG2) [95], neuroectodermal stem cells (ESC) [143], murine neural crest stem cells (MNCSC) [173], embryonic stem cell-derived neuron precursors (ESCDNP) [173] and neural progenitor cells (NPC) [140, 141, 168, 174]. The human embryonic stem cells overexpressing human fibroblast growth factor 2 (FGG2) applied at the injury site improved MN survival and reduced the glial reactivity, thus improving the regenerating capacities [95]. However, it has unknown effectivity, only shown in animal experimental studies, and its application in the human being creates ethical issues.

Some researchers have found in vivo that a week time gap between NG harvest and its subsequent use in nerve repair improves the regenerating capacities [175] by increasing the number of Schwann cells and macrophages inside the NG [145, 176, 177] as well as by inducing the local GDNF release [145, 178, 179]. This is another possibility but difficult to use in clinical practice.

A word of caution is to be said about the **materials used to glue the peripheral NGs to the SC**. Only Tisseel® causes no long-term histological reaction [180, 181], while other preparations available in the market (BioGlue®, Adherus®) induce local fibrous reaction with SC adherences and at times neurological sequelae [181]. BioGlue® when applied close or in contact with nervous tissues can create serious damages [182]. In rats, some researchers have used snake (*Crotalus durissus terrificus*) venom-derived fibrin glue and reported excellent results [183, 184]. In clinical practice, fibrin glue from human origin is usually used [15, 30, 33, 45, 185].

On the other hand, conduits can be used to substitute autologous NGs. They have been extensively tried in peripheral nerve repairs [186, 187], but in NR reimplantation the data available are more limited [188, 189]. In peripheral nerve repair, these conduits have proved useful up to distances of 70 mm in length [37, 38, 190]. Certainly, the central-peripheral nervous tissue interface is a place in which autologous NFs provided by the autologous NGs play a pivotal role in regeneration of the reimplanted NR [69, 77, 89, 91–93]. Some researchers have tried nerve conduits enriched with BDNF that have had a good result in a rabbit experimental model [191]. In human clinical practice, there are currently no published reports [45, 74].

However, the applicability of all these studies is limited since they were generated with experimental animal models and with reimplantation immediately following the avulsion. On top of that, the regenerating capacities of the human nervous system are much less than that observed in research animals (the rat especially [73]), and the reimplantation of an avulsed NR has to be delayed weeks or even months until the patient is stabilized from other traumatic lesions and when an adequate diagnosis and treatment strategy are well defined [111].

4. Surgical technique of human NR reimplantation

Surgical techniques can be useful, particularly in complete BPA and with a delay between the injury and the surgical repair of no longer than 4 weeks [45]. Some significant problems are that MN apoptosis is greater as the time goes by [20, 27, 91, 93, 101–103] and that by 4 weeks, there is a dense scar around the BP as well as the avulsed NRs and in their intervertebral foramina that hinders any surgical manoeuvres [45, 74].

The surgical approaches described can be summarized into posterior subscapular [192], lateral [193], anterolateral [194, 195] and single-stage combined anterior (first) and posterior (second) [33].

4.1. Posterior subscapular approach

With the patient in the prone position, a longitudinal incision is made halfway between the spine and the scapula [39, 192, 196]. The trapezius muscle is sectioned transversally in the direction of its fibers. The rhomboid major and minor muscles are also divided following the direction of their fibers. The T_1 transverse process is identified and removed with the aid of a drill. A section of the first rib is also removed. A laminectomy and facetectomy are needed to

access the spinal canal. The dura is opened and the dentate ligaments sectioned to rotate the SC to reach the implantation site of the ventral roots. As no access to the anterior structures is possible, another anterior approach to the BP is needed to identify and mobilize it and to pass the NGs from one surgical field to the other [7]. Depending on the degree of bone removal, a posterior cervical fusion might be required. This approach only allows access to the avulsed NRs that lie inside the spinal canal or outside it but very close to the foramina [39]. Only one case was reported in 1995 [39], which did not spark much interest within the BP surgical community. Currently this technique is not used for NR reimplantation.

4.2. Lateral approach

This has been well described in the publications of Carlstedt and co-authors [7, 44, 45, 193]. The patient is placed on the lateral decubitus position with the affected arm at the highest position and slightly rotated outwards with the hand in supination. The head is supported in a Mayfield head clamp (Integra LifeSciences, Austin, Texas, USA) and, slightly laterally, bent towards the healthy side. The idea is not only to allow surgical access to the whole BP but also to the possible donor sensory nerves (median antebrachial cutaneous and radial sensory nerves). The ipsilateral lower limb saphenous nerve can also be accessed with ease. The surgical table is placed in a 15% head-up position to reduce venous bleeding. A skin incision is performed from the mastoid to the clavicle following the posterior border of the sternocleidomastoid muscle [7, 44, 45],or by incising from the sternocleidomastoid muscle-clavicular incision and running parallel to the clavicle about 2 cm above it in the direction of the C_7 spinous process [193]. After dissecting the platysma and sternocleidomastoid muscles, the spinal accessory and cervical plexus nerves are identified and referenced with loops. Care has to be taken not to damage the spinal accessory nerve at the junction between the upper and middle-third sternocleidomastoid muscle posterior border. After careful subcutaneous fat dissection, the transverse processes of the cervical vertebrae can be felt deep to the sternocleidomastoid muscle with the tip of the finger. The scalene muscles anterior, middle and posterior as well as the levator scapula muscle are identified. Next, the transverse cervical artery and vein are isolated and referenced. It is best not to sacrifice them as they can be used in the future to vascularise a possible gracilis muscle graft [197]. The BP is fully exposed and the avulsed NRs identified. The avulsed NRs are trimmed until normal-appearing nervous tissue is seen. Many surgeons remove the dorsal root including its ganglion [15, 45]. Unless the NR reimplantation is attempted in the first 2 weeks post-avulsion injury, the BP retracts distally and undergoes fibrotic changes adhering to the nearby structures [1, 26, 33, 198, 199], so the BP has to be completely freed to be able to move it upwards. This maneuver can be troublesome at times due to dense fibrotic tissue, particularly when surgical reimplantation has been delayed over 4 weeks [15, 45]. When this is not possible or the BP cannot regain its former position in contralateral C_7 NR transfer, some have shortened the humerus shaft by 4 cm [198]. The alternative is to use long autologous NGs that cover the gap between the SC and the NR remnants [15, 26, 45, 109].

The C_5-T_1 NR foramina and zygapophyseal joints are approached between the elevator scapula and the middle and posterior scalene muscles. Then the *longissimus* muscle is split longitudinally to expose the spine. The *multifidus* muscles are detached from the zygapophyseal joints

and laminae. The transverse processes and the anterior and posterior tubercles are exposed by removing all the muscles attaching to them. These bone structures plus a section of the lateral mass are removed and a C5-C7 hemilaminectomy performed. The removed bone pieces are saved for later use.

Care must be taken with the vertebral artery, as it does not need to be mobilized. As most of the lateral mass, the disc and the contralateral facet joints are spared; the procedure usually does not induce spine instability. The avulsed NRs can be identified by pseudomeningoceles. The C_5–C_7 foramina are exposed with ease, while the C_8 and T_1 are much more difficult, and some surgeons refuse to do it to concentrate in repairing only the C_5–C_7 NRs, even if the lower ones are also damaged [45]. This is important because no improvement can be expected in roots that have never been reimplanted and explains one of the reasons why the distal muscles of the hand are seldom reinnervated [15, 45]. Some researchers have proven in rat experimental studies that a single reimplanted NR can attract regenerating axonal sprouts from nearby levels [200].

The dura mater is exposed and opened longitudinally and the dentate ligaments sectioned. Intraoperative neurophysiological monitoring is recommended particularly on rotating the SC and when performing the longitudinal myelotomy and inserting the NGs inside it [45].

4.2.1. Ventral root repair

The SC is rotated, pulling from the dentate ligaments to expose its anterior aspect. Serial 2–3 mm-long stab incisions are done at the same place where the anterior NRs formerly stood. Peripheral nerve sensory NGs (medial antebrachial cutaneous nerve, superficial radial nerve, saphenous nerve) are introduced 1 mm inside the SC tissue [201] and secured with Tisseel fibrin glue (Immuno AG, Vienna, Austria). The distal stumps of these NGs are sutured with the corresponding avulsed NR remnant. The dura mater is repaired with a dural substitute and the suture reinforced with fibrin glue to prevent CSF leaks.

Some anatomical studies have found that the best spot where to insert the NGs in the SC is where the anterior NRs formerly stood and not in the lateral SC side [201]. This latter place is technically easier and achieves some regeneration by lateral MN axon sprouting, but the results are inadequate [201]. As the NG implantation inside the SC will cause a further damage to it [26], suturing the NGs to the SC pial surface in an experimental avulsion model has been tried, finding that it allows adequate MN survival and axonal regeneration [27]. This ventral root pial reimplantation is not only less risky but technically easier [26, 33].

4.2.2. Dorsal rootlet repair

This was first reported in 1997 in an experimental rat NRA model [202]. Peripheral NGs were used to cover the gap between the remaining dorsal NR and the SC. A DREZ longitudinal myelotomy was performed to insert the NGs 2 mm inside the posterior horn. Some regeneration was seen with peroxidase staining [202]. The addition of olfactory ensheathing cells at the DREZ in 2003 did not improve the results [171]. In 2004, Tang et al. [188] also in rats used bioresorbable nerve conduits to repair a 6 mm dorsal NR gap, showing signs of recovery. This

repair was enhanced by injecting a viral vector inside the DRG [203]. In 2017, Konig et al. [173] reported the application of murine neural crest stem cells and embryonic stem cell-derived neuron precursors at the DREZ in an experimental rat cervical dorsal NRA showing differentiation into neurons and their migration, transforming into interneurons and facilitating the creation of synapsis with the regenerating axons coming from the reimplanted dorsal NR.

In humans, dorsal rootlet repair has been recently attempted by Carlstedt et al. [97]. As they noticed the extreme difficulty for the growing axons coming from the DRG to cross the glial scar at the surface of the posterior horn, they sectioned the avulsed NR distal to the DRG and sutured the peripheral sensory stump to the posterior horn by means of NGs introduced in the SC through a longitudinal myelotomy. The rationale was to get some sensory recovery from the growing axons of the posterior horn neurons that are expected to grow distally inside the implanted NG [99]. As the neuronal bodies of the DRG are removed, the regeneration has to depend on the neuronal plasticity of neurons coming from the posterior horn that have to stretch their axons to reach the skin though the NGs and peripheral nerves. The results are poor [99, 100], but it is the first strategy that has provided some success in humans. This is not ideal as sensation could be recovered if the dorsal rootlets were replaced by NGs and the tip of those grafts inserted inside the posterior SC horn through a longitudinal myelotomy while maintaining the neuronal bodies that lie at the DRG. This technique proved effective in rats [202], but no attempts in humans have been found in the literature. To improve the results, CNTF [87] should apply locally to the posterior SC at the DREZ associated with N-acetyl cysteine [155] *orally* to allow maximal sensory neuron survival. Oral minocycline [106, 120], oral tacrolimus [151] or subcutaneous recombinant erythropoietin [118, 165] could be also administered to reduce the reactive glial proliferation that acts as a barrier against dorsal root axonal regeneration.

4.2.3. Wound closure

The dura mater is closed with a dural substitute and reinforced with fibrin glue to prevent CSF leaks. The morcellized bone obtained from the transverse processes and lateral masses supplemented together with demineralized bone matrix is laid on the cervical spinal column defect to enhance bone fusion. A lumbar drain is inserted and kept for 5 days to prevent CSF leaks.

Postoperatively, patients are kept with a sling for 6 weeks before starting any passive movements, to prevent NG dislodgement [45]. Cervical X-rays are taken every 3 months for a year to detect any possible instability that might require a cervical fusion.

The most important disadvantage of this approach is that it entails extensive muscular damage, particularly at the scalene muscles [33]. The most significant advantage is that the NGs needed for the repair are the shortest of all the NRA reimplantation approaches [45, 193].

4.3. Anterolateral approach

It is first described by George et al. for the treatment of cervical spinal spondylosis and tumors [204, 205]. This approach is much more direct but demands a partial multilevel oblique partial

corpectomy of the affected levels that can be C_4 to T_1 when the whole BP is involved. This involves an extensive anterior cervical fusion, not optimal for younger individuals due to its possible long-term consequences [206]. The anterolateral approach provides good access to the BP and ventral NR, but the dorsal NR cannot be reimplanted [195]. This approach has been reported in research animals—cats [207, 208]—in ten cadavers and four clinical cases [194], but no long-term clinical results have been reported.

4.4. Single-stage combined anterior (first) and posterior (second) approach

The antecedent of this approach is the **two-stage combined approach posterior (first) and anterior (some days later)** [185]. In the first stage, the cervical spinal canal was approached with the patient prone. A C_4–T_1 laminectomy with medial-third facetectomy was performed and the SC inspected after longitudinal dural opening. The dentate ligaments were sectioned and SC rotated and inspected looking for avulsed NR. In case the avulsed NRts were inside the dura mater, they were reimplanted where they formerly stood. Both ventral and dorsal NRs were reimplanted. When the NRs were outside the spinal canal, NGs were inserted and sutured to the SC tissue through small myelotomies and their distal end tunneled through the paraspinal muscles and placed in the supraclavicular area with two metallic hemoclips to facilitate their identification in the future. The dura mater was sutured and sealed with fibrin glue. A posterior mass cervical fusion was performed to prevent postoperative kyphotic deformities. Some days later the patient was taken back to the operating room and in the supine position the BP identified and isolated in the supraclavicular region. The NG distal ends were localized through the hemoclips with X-ray guidance and sutured to the corresponding BP cords. Apart from the original report [116], no further publications on this seem to exist.

The **single-stage combined anterior (first) and posterior (second) approach** was reported by Amr el al. in 2009 [33]. The patient is placed in the lateral decubitus position and the skin sterilized front and back of neck and chest as well as the whole affected upper limb and both lower limbs. Then the patient is rotated backwards and placed supine. In this position a traditional BP exploration is done through a transverse supraclavicular incision. If needed, a second incision perpendicular to it can be done following the delto-pectoral groove. This allows exploration of the infra-clavicular BP, particularly when it has migrated distally. Once the whole BP is dissected free and the damages evaluated, several peripheral sensory NGs are obtained from the affected upper limb and both lower limbs. These grafts are sutured to the cords of the avulsed NR.

Next, the patient is placed again in the lateral position. Through a posterior midline incision from occiput to T_2, the whole cervical spine is exposed. The spinal muscles are detached from the spinous process and separated laterally. A laminectomy and partial medial facetectomy C_4–T_1 are performed on the affected side. The dura is opened through a longitudinal incision and the dentate ligaments sectioned. The NG that had been previously sutured to the BP cords in an end-to-side versus end-to-end technique [33, 209] is passed subcutaneously from the anterior surgical field to the laminectomy area. These NG needs to be long enough to cover the distance between the SC and the BP. Then the proximal ends of the NGs are sutured subpially

in a longitudinal fashion, parallel to the side where the ventral roots stood. No SC incisions are performed. The proximal ends of the NGs are sutured intradurally to C_4 above and to T_1 below. In the only publication that we have found, the dorsal NRs are not repaired [33]. The dura is closed with interrupted stitches reinforced with fibrin glue. No cervical fusion is applied.

The advantage of this double approach is that it is more conservative to the muscles. The disadvantage is that long NGs are needed, making the distance between the motoneuron and the muscular end plates still larger. To the best of our knowledge, there is only a single publication attesting the validity of this technique [33]. It is of particular interest that ventral NR regeneration can be achieved by laying the NGs subpially at the SC without having to insert them inside the SC tissue through myelotomies [33].

5. Clinical results in human beings

Some clinical studies have reported definitive although limited motor and sensory improvements particularly in the proximal limb areas after NR reimplantation in complete BPAs [15, 30, 32, 33, 45, 185]. The best motor recovery was seen at the deltoid, pectoralis, infraspinatus, biceps and triceps muscles [15, 30, 45, 185, 209]. One patient showed signs of partial recovery of the flexor digitorum superficialis and another of the first dorsal interosseous muscle [45]. A functional recovery of the hand has only been reported in a 9-year-old child with a complete BPA [29]. Hand intrinsic muscle motor grade 2 recovery was reported by Amr et al. [33]. The best sensory improvement was patent at dermatomes C_5, C_6 and T_1, particularly at C_5 [33, 45]. One of the reasons by which only proximal muscles show signs of reinnervation in the work of Kachramanoglou et al. is because only the C_5–C_7 NRs are reimplanted as C_8 and T_1 are more technically demanding and they were reluctant to risk neurological complications on handling the SC at these levels [45]. This could also be the reason by which Amr et al. [33] report hand intrinsic muscle grade 2 motor recovery, as they did repair the C_8 and T_1 roots. Another extremely important reason is that when the regenerating axons reach the distal limb muscles, they are already atrophied and fibrotic [72, 73]. The C_5 and T_1 sensory recovery can in part be due to overlapping sensory covering from nearby dermatomes (C_4 for C_5 and T_2 for T_1) [32, 45].

6. Conclusions

NRA keeps being in an area in which improvement is desperately needed, particularly in complete BPAs in which not many alternatives are possible. As clinical results in humans keep being dismal, further research is needed. The administration of drugs, preferably orally, has to be pursued to find a combination of them that helps to achieve a successful limb recovery. NR reimplantation has to be undertaken as soon as the patients' clinical condition allows it. Ventral NRt implantation provides better results than its posterior counterparts.

Abbreviations

BDNF	brain-derived neurotrophic factor
BMC	bone marrow stem cells
BP	brachial plexus
BPA	brachial plexus avulsion
CNF	ciliary neurotrophic factor
GDNF	glial-derived neurotrophic factor
iPSC	induced pluripotent stem cells
ISP	intracellular sigma peptide
MN	motor neuron
MSCs	mesenchymal stem cells
NF	neurotrophic factor
NGs	nerve grafts
NPC	neural progenitor cells
NR	nerve root
NRA	nerve root avulsion
NRts	nerve rootlets
NSC	neuroectodermal stem cells
OECs	olfactory ensheathing glial cells
SC	spinal cord

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