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Advances in our understanding of central nerve regeneration: is a cure for spinal cord injury now in sight?

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Traumatic damage to the spinal cord is truly one of the most devastating injuries to afflict the human body. Whether from the blade of a sword or the shrapnel of an explosion, from the jolt of a horse or the impact of a high speed collision, spinal cord injuries have occurred throughout history and will no doubt continue to do so with increasing frequency as the pace of human life quickens. Yet while modern medicine has provided effective treatments for many of the seemingly incurable physical ailments of the past, physicians in the hospitals of today are as powerless to cure spinal cord injury as the apothecaries of ancient times, and patients must come to terms with the fact that they face a lifetime of complete paralysis and loss of sensation below the affected site.

While a solution to the seemingly impossible task of repairing the damaged spinal cord has eluded scientists thus far, significant progress has undoubtedly been made. Advancements in our understanding of molecular neuroscience, genetics, immunology and microsurgical grafting techniques over the last century have coalesced to create a realistic prospect that a cure is one day achievable, and spinal cord research is now one of the most rapidly evolving and exciting areas of medical research. In this essay, I will outline the key landmarks in our understanding of spinal cord injury across the ages, before focusing upon how fascinating insights into molecular biology over the past thirty years have been directly translated into novel strategies for promoting nerve regeneration, thus changing the idea of a cure from a fantasy to a possibility.

Recognising the implications of spinal cord injury

‘The frog instantly dies when the spinal cord is pierced; and previous to this it lived without head, without heart or any bowels or intestines or skin; and here therefore it would seem lies the foundation of movement and life’. Leonardo da Vinci.
The devastating impact of injury to the spinal cord has inspired physicians throughout history to gain a deeper understanding of its physiological role and the mechanism preventing its functional recovery after transection. The earliest recorded description of spinal cord injury is found within the Edwin Smith papyrus of Ancient Egypt (c. 2500 BC), the most authoritative medical manuscript of the time. Patients suffering from a fracture or dislocation of the neck vertebrae are described with the cardinal signs of spinal cord transection (one patient, for example, said to be ‘unconscious of his two arms and two legs’ and ‘dripping from his member without knowing it’), followed by the damning declaration that should one encounter such a person ‘thou shouldst say concerning him...that this is “an ailment not to be treated”’. Two millennia later, by the time of the Ancient Greeks, little had changed in this perception of spinal cord injury as a lethal affliction: in Homer’s Odyssey, when the character Elpenor fell from a roof and broke his neck, it is written simply that ‘his soul went to Hades’. The renowned Greek physician Hippocrates (460-377 BC) also confirmed the futility of treatment in the case of spinal injury with concomitant paralysis, seeking merely to treat uncomplicated fractures of the spine by placing them under mechanical traction.

Through selective lesioning experiments on animals, Galen (150 BC) and later Aulus Cornelius Celsus (30 BC) were the first to recognise the importance not simply of the spinal column but of the cord in particular- but even until the Medieval period still frustratingly little could be offered to affected patients. Guy de Chauliac (1300-1368), considered by many to be one of the fathers of modern surgery, writes emphatically: “One should not labor to cure the paralysis of spinal cord injury”. Significant improvements in spinal orthopaedic surgery and supportive nursing care were made over the 17th and 18th centuries, but compared to other areas of science which were progressing rapidly at this time, the medical management of spinal cord injury had advanced astonishingly little.

However, with the turn of the 20th century and the widespread emergence of the disciplines of histopathology and molecular biology, hope was enlightened by the rational approach to scientific investigation that was being adopted, and it is from this point that the prospect of a cure for spinal cord injury began to take shape in earnest.
Understanding what prevents spinal cord regeneration

The first step in the search for a method to promote nerve regeneration after injury is to understand what prevents this process from occurring under normal conditions. Seeking to answer this very question, Ramon y Cajal published in 1928 his seminal two-volume work summarising numerous experiments on peripheral and central nerve injuries in a range of animals. Using novel staining techniques, he demonstrated for the first time that, following traumatic injury to the central nervous system (CNS), axon sprouting yielded only abortive clubs of growth with no long regeneration: whereas dorsal root axons extended in the peripheral nervous system, most were unable to penetrate the dorsal root entry zone, suggesting that the CNS environment itself is non-permissive for growth. Tello (a researcher in Cajal’s lab) further supported this proposition when he demonstrated that CNS axons are capable of extending normally in peripheral nerve grafts, growing healthily through or besides the bands of Bungner in a similar fashion to normal peripheral nerve regeneration. This key insight by Cajal and his colleagues paved the way for future questioning about the critical inhibitory role of CNS glial cells in nerve regeneration.

Confirming the guilt of the glia: from Cajal to Schwab

CNS injury evokes an extensive cellular reaction due to disruption of the blood brain barrier and the release of inflammatory mediators from damaged cells, perpetuated by the subsequent recruitment of cytokine-producing leukocytes and microglia: the ensuing gliosis appears to be critical in inhibiting regeneration. Some 50 years after Cajal, Schwab et al (1985) provided the first compelling evidence that the CNS glial cells oligodendrocytes carry major axon-inhibitory signals. They showed that cultured neurones from the dorsal root ganglion (which normally serves as an interface between the central and peripheral systems) extend across Schwann cells but not across oligodendrocytes; three years later, Caroni and Schwab demonstrated that myelin extracted directly from peripheral nerves is growth-permissive whereas CNS myelin is not. Now that the key role of oligodendrocytes in preventing spinal cord regeneration had been established, the search was on to find the precise molecules to blame.
The next major breakthrough came when Schwab and colleagues identified a protein from CNS myelin that strongly inhibited neurite growth, IN-250, now known as Nogo-A. Implanting an anti-Nogo-A producing hybridoma into the brain of young adult rats with spinal cord transection permitted corticospinal tract regeneration up to 11mm (Schnell and Schwab, 1990) and, moreover, antibody therapy also produced functional recovery, with treated rats displaying improved performance of skilled motor tasks (Bregman et al, 1995). The prospect for using therapeutic anti-Nogo-A antibodies in a clinical setting became clear when regeneration occurred following administration directly into the cerebrospinal fluid via an intrathecal lumbar catheter (Liebscher et al, 2005), and work on adult macaque monkeys further confirmed the efficacy of Nogo-A antibody treatment in primates (Freund et al, 2006).

Thus, the exciting discovery of Nogo-A as a key mediator in the prevention of CNS nerve regeneration is a result of gradual advances in knowledge since the pioneering work of Cajal, and scientists around the world are now holding their breath for the emergence of a humanised monoclonal antibody against Nogo which is currently completing clinical trials in a large multi-centre study across Europe.

**The emergence of genetics as an invaluable research tool**

Recent advances in genetic manipulation have enabled the identification of other myelin-associated inhibitory molecules such as myelin-associated glycoprotein (MAG), as well as allowing rigorous assessment to be made of their true involvement in axon inhibition: as is the case with all scientific discovery, an increase in knowledge sometimes serves only to increase confusion as to how a system functions.

Using the technique of genetic knockout, Li et al (1996) found that MAG knockout mice had both longer and an increased number of regenerating axons while Bartsch et al (1995) found no difference between knockout and control mice. Interestingly, Nogo, MAG and oligodendrocyte myelin glycoprotein (Omgp) were found to all act through a single Nogo receptor (Fournier et al, 2001) composed of three subunits (NgR1 + p75NTR +LINGO). However, genetic deletion of the Nogo receptor failed to reduce neurite inhibition in vivo (Zheng et al, 2005) and, moreover, it seemed that
p75 is actually essential for neuronal cell survival and functional recovery following spinal cord injury! (Chu et al, 2007). While LINGO-1 antagonism promotes functional recovery and axonal sprouting (Ji et al, 2006), the fact that it is expressed prior to NgR1 (Llorens et al, 2008) suggested that it too may have other as yet unknown functions, and thus attempting to inhibit its expression (especially in younger SCI patients) may have severe developmental consequences.

Thus, in addition to giving us novel therapeutic targets, research has begun to flag up key pathways whose disruption by a well-intentioned physician may cause more harm than good. As spinal cord research progresses in the future, we would do well to remember the near misses of the past.

**Seeking to boost intrinsic axon regrowth: two decades of progress**

In addition to blocking inhibitory surfaces, axon regeneration may also be augmented using neurotrophic factors such as NT-3, NGF or GDNF. The first insight that adult CNS neurones may have a reduced intrinsic growth capacity (and therefore that extrinsic growth factor administration may be beneficial in promoting regeneration) came from the work of Li and Raisman (1993), who showed that embryonic neurones transplanted into an adult spinal cord are able to extend their axons normally. Four years later, Grill et al (1997) demonstrated that NGF-mediated axonal growth is comparable after acute or chronic spinal injury and thus, unlike the strategies already discussed, the efficacy of neurotrophins in treating spinal cord injury may persist for an extended period of time after the initial injury, raising the hope that therapy could be offered regardless of when spinal cord damage occurred.

More recently, the use of neurotrophins has been combined with other techniques to provide novel strategies for promoting regeneration. Since Xu et al (1995) demonstrated that Schwann-cell seeded guidance channels grafted into the transected adult spinal cord serve to promote regeneration, work on neurotrophins has largely run parallel to but not in conjunction with this grafting approach. However, the two areas were recently united when it was shown by Golden et al (2007) that Schwann cells transduced ex vivo using viral vectors encoding the bifunctional neurotrophin
molecule D15A (that mimics both NT-3 and BDNF) increase the number of axons at a contusion site significantly. Moreover, utilising the effect of preconditioning (transplanting from a chronically compressed sciatic nerve graft) also increases effectiveness (Dinh et al, 2007). This increasingly holistic approach to treating spinal cord injury, using several strategies at once, makes the prospect of a cure more likely than ever before. Recent years have also seen the development of novel methods for practically implementing grafting, for example through the use of a biodegradable poly-beta-hydroxylate scaffold seeded with Schwann cells (Novikova et al, 2008).

In addition to degenerating myelin, another important source of inhibition is the glial scar which forms after CNS injury (Rudge and Silver, 1990): astrocytes in the injured area become hypertrophic and adopt a reactive phenotype, releasing axon-inhibitory chondroitin sulphate proteoglycans (CSPGs), in particular neurocan. In 2002, Moon et al showed that chondroitinase injection into the injured brain can temporarily restore axon regeneration, confirmed by Bradbury et al later that year who showed that motor function improved significantly post-infusion. However, as chondroitinase appears to function by inducing sprouting and not long-distance regeneration, its efficacy in human subjects where the absolute distance of regeneration required is far greater than in rodents remains to be seen. Another consideration for the practical therapeutic use of chondroitinase ABC is that, as for Nogo inhibition, a therapeutic time window exists for its administration (Garcia-Alias et al, 2007). Moreover, other factors appear to be beneficial in reinforcing its regeneration-promoting effect: for example, concomitant administration of lithium chloride causes a 42% increase in the regeneration of rubrospinal tract neurones compared to only a 20% increase upon administration of chondroitinase alone (Yick et al, 2004).

Progress in developing novel therapies for spinal cord injury is thus likely to depend not only upon finding novel strategies but upon maximising the effectiveness of those strategies that already exist. As Bilroth said, “Only the man who is familiar with the art and science of the past is competent to aid in its progress in the future”. In the field of spinal cord regeneration, this could not be more true.
Future directions: OECs and the stem cell phenomenon

One problem associated with the use of Schwann cells for nerve grafting is that they tend to elicit a hypertrophic astrocyte response. Olfactory ensheathing cells (OECs) which (unlike Schwann cells) disperse well into CNS tissue and generate astrocyte-like cells which are thought to actually facilitate integration have emerged as more promising candidates for grafting, and studies by Ramon-Cueto and Nieto-Sampedro have suggested that OEC grafting could promote both dorsal root growth past the DREZ and corticospinal tract repair, thus leading to functional recovery.

OECs contribute to the glia limitans of the olfactory bulb in a cooperative manner with astrocytes, in contrast to the PNS/ CNS boundary anywhere else in the body which is formed purely by astrocytes. Understanding their interaction with the reactive astrocytes found at the CNS is critical if we wish to understand their role more clearly; it seems that, in contrast to Schwann cells, they have the capacity to make the glial scar more growth-permissive. Improving OEC effectiveness may also be achieved by transfecting them with viral vectors encoding neurotrophins such as BDNF and developing biocompatible bridging channels (Deumans et al, 2006). Interestingly, the outgrowth-promoting activity of SPARC (secreted protein acidic and rich in cysteines), one of the factors enriched in OEC-conditioned media, is dependent on the synergistic presence of Schwann cells, highlighting again the need not only to consider therapies such as OEC grafting in isolation, but in the context of their interactions with other cell types present at the site of SCI. When Fouad et al (2005) employed a triple combination therapy combining Schwann cells, OECs and chondroitinase, this was found to deliver better results than grafting alone.

In addition to the use of OECs to facilitate spinal nerve regrowth, the discovery of stem cells has truly revolutionised our approach towards regeneration. If an effective technique can be refined for controlling the cell type into which pluripotent stem cells differentiate (Salewski et al, 2010), the potential implications for spinal cord regeneration are exciting indeed. The task of refining stem cell therapy is, however, a daunting one, and I believe that tackling this challenge will be the main drive of spinal cord research in upcoming years.
Hope of a cure?

While we have undoubtedly made significant progress in our understanding of spinal cord injury over the last thirty years, our lack of understanding is being continually manifested by the diversity of outcomes in experimental studies. The different conditions and increased scale under which regeneration must operate in humans compared to rodent models also presents a significant challenge in translating experimental studies to clinical therapies. The use of combination therapies, maximising the efficacy of individual treatments and establishing an effective therapeutic window for intervention are likely to be critical in the development of strategies to treat this devastating condition; whether we can progress beyond axon regeneration to long-lasting functional repair, and beyond treatment to a complete cure, remains to be seen. However, what is certain is that the prospect of repairing the damaged spinal cord is no longer seen as an impossible task but as one that is entirely worth pursuing, and this in itself is a remarkable achievement and the greatest cause for hope.

Key References


Ramon y Cajal (1913). Degeneration and Regeneration in the Nervous System.
