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● PERSPECTIVES

Clinical strategies to enhance nerve regeneration

The slow rate of nerve regeneration after injury or reconstruction remains a clinical problem because it prohibits the timely reinnervation of distant target muscles before the irreversible degeneration of the neuromuscular junction and breakdown of muscle tissue. As such, high (proximal) nerve injuries result in the incomplete recovery of motor function and poor functional outcomes despite current and timely surgical management. Experimentally, several strategies have been shown to enhance nerve regeneration and improve functional recovery in animal models but translation to clinical practice has not been realized. Two potential treatments, tacrolimus (immunosuppressant) and electrical stimulation are commonly used for other reconstructive indications and as such, are both readily available clinically. There is some evidence, which will be reviewed in subsequent sections, that these approaches may also be useful in enhancing neuronal regeneration.

Tacrolimus: Tacrolimus (Prograf, FK506) is a hydrophobic macrolide isolated from *Streptomyces tsukubaensis* and has well established immunomodulatory and anti-inflammatory properties. It is approved for the prophylaxis of transplant allograft rejection, primarily affects T cell function by binding to FK binding proteins (FKBP), and mediates immunosuppression by inhibiting calcineurin, a calcium- and calmodulin-dependent phosphatase. The drug's immunosuppressive effects are mediated largely through FKBP12 which is involved in intracellular calcium flux and cell cycle regulation. However, its neuroregenerative properties are also related to its receptor FKBP52, a heat-shock protein (HSP-59) as well as FKBP 12. Therefore, the potential exists to optimize the neurologic effect independently of the immunosuppressive properties (Fonofaos and Terzis, 2013).

Experimentally, tacrolimus has been shown to have numerous neuroprotective and neuroregenerative effects in multiple models of nerve injury. The benefits of tacrolimus therapy after nerve injury has been shown to include 1) faster onset of functional recovery, 2) enhancing regeneration in rodent models of axonotmetic and neurotmetic injury, 3) reducing the time period of denervation and its associated negative effects (muscle atrophy and loss of motor endplates), and 4) accelerating collateral axonal sprouting (Fonofaos and Terzis, 2013). The acceleration of nerve regeneration has been quantified in rodent models where tacrolimus has been shown to double the number of axons that regenerate following a nerve injury.

and significantly increase myelin thickness in a model of chronic axotomy (Fonofaos and Terzis, 2013).

Because the neuroregenerative and immunosuppressive effects appear to act through different mechanisms, a low sub-immunosuppressive dose of tacrolimus that can still speed the rate of nerve regeneration without inducing immunosuppression has been demonstrated. In the rat model, doses of tacrolimus insufficient to permit survival of skin allografts with full major histocompatibility complex disparity still accelerated nerve regeneration after nerve injury and repair (Fonofaos and Terzis, 2013). Dosages used in the rodent model are very different from those used clinically and therefore cannot be extrapolated to the human model. These were defined by their effects on immunosuppression as measured by tissue allograft survival or loss, and nerve regeneration.

Adverse effects of tacrolimus: The primary morbidity of tacrolimus stems from the lifelong general immunosuppression that is required for the survival of transplanted organs and tissues. Permanent immunosuppression increases the risk of infection, fracture, drug toxicity including hypertension and nephrotoxicity, malignancy, and metabolic derangement such as hyperlipidemia and diabetes mellitus (Fonofaos and Terzis, 2013). Its use for nonvital reconstructive purposes where the primary focus is the restoration of function and form rather than the treatment of a life-threatening condition remains controversial given these serious potential side effects. In support of broadening its application, a growing literature base exists on the temporary use of tacrolimus for reconstructive allografts that are ultimately incorporated by host tissue (Mackinnon et al., 2001), and its more recent application for diseases whose treatment do not require transplantation. The latter especially to date includes multiple phase II and III clinical trials, dose-ranging studies, and several smaller open-label trials, which collectively have helped to define the actual incidence of adverse events when used only temporarily at lower doses (Yocum et al., 2004).

Unconventional clinical applications: There have been a small reported number of bone, tendon, and nerve allografts in which there is ingrowth of host tissue and once healed, immunosuppression can be stopped. Adverse sequelae related to standard maintenance doses of immunosuppressive medications were not seen when used for a relatively short time, usually 1-2 years or less (Mackinnon et al., 2001). These cases support the safety of short-term and monitored use of standard maintenance single-drug immunosuppressive therapy. In the only reported case of tacrolimus therapy after upper arm replantation, the authors noted "exceptional" results with clinical and electro-



muscles.

There is also a substantial and growing literature based on the use of tacrolimus for diseases that do not involve transplantation. The most extensive and informative data come from its application for the treatment of rheumatoid arthritis. Since 2002 there have been 2 phase II clinical trials (USA and Japan), one phase III clinical trial (USA), at least 2 open-label studies (USA and Japan) and other small and large series ($n > 200$). Other systemic applications have also included the treatment of myasthenia gravis, ulcerative colitis, Crohn's disease, juvenile dermatomyositis, systemic lupus erythematosus, and ocular disease. Collectively these have involved study enrollment in the range of 2000 patients or more and have included reports focused on the correlation of drug safety with pharmacologic dosing.

The largest patient-safety study was an open label study undertaken to determine the long-term safety of tacrolimus monotherapy for the treatment of rheumatoid arthritis (Yocum et al., 2004). A total of 651 patients received tacrolimus 3 mg/d for ≥ 6 months, 497 were treated ≥ 12 months, and 54 received 18 months of treatment. The median trough tacrolimus levels were 2–3 ng/mL and the level did not accumulate over the course of the study. The incidence of adverse events previously identified as safety concerns in transplant studies were notably lower than transplant patients, including hypertension (9.2% vs. 38–50%), tremor (10.5% vs. 48–56%), diabetes (< 5% vs. 24%), and increased creatinine (7.4% vs. 24–45%). Furthermore, multiple other side effects such as insomnia, paresthesias, oliguria, hyper or hypokalemia, hyperglycemia, hypomagnesemia, hypophosphatemia, anemia, and peripheral edema that have a reported incidence of at least 15% in liver and/or kidney transplant patients, occurred in less than 5% of patients in this study. This has been attributed to the lower dose used to treat rheumatoid arthritis (3 mg/d) as compared to the prevention of transplant rejection (0.1–0.2 mg/kg per day). In addition, no increase but an actual decrease in the initial incidence of adverse effects was seen with longer duration of treatment in rheumatoid arthritis patients (Yocum et al., 2004).

The general consensus collectively reached based on these trials has been that tacrolimus is effective for rheumatoid arthritis and other autoimmune diseases, and at low doses is safe and generally well tolerated with a low incidence of serious adverse events. The reported effects of tacrolimus vary but at best, it has been shown to double the number of axons that regenerate following a nerve injury, increase the number of myelinated axons by 40%, and reduce by half the time to neurological recovery (Fonofaos and Terzis, 2013). These effects may be clinically significant and further study is warranted.

the concept of endogenous electrical fields in injured and regenerating tissues. Epithelial tissues form barriers and transport ions to generate transepithelial potentials in many tissue types. Although these electric voltage gradients are normally very small (hundreds to thousands mV/mm) in healthy tissue, they increase in the wounded state. Epithelial barriers are breached and the transepithelial potential differences are short-circuited to form an electric current that flows towards the compromised epithelium and establish laterally oriented electrical fields, which are readily measurable during wound healing (Kloth, 2005). These wound electrical fields are thought to be the result of passive leaking of ions through wound tissues, and play a role in the control and integration of multiple cell behaviors such as cellular proliferation, oriented cell division, directed epithelial cell migration, and directed nerve sprouting (Wang and Zhao, 2010). Applied electrical fields attempt to mimic endogenous electrical fields and have been shown experimentally to direct and accelerate cell migration, regulate cell proliferation, direct the orientation of cell division, affect cell shape and orientation (Nuccitelli, 1988), direct vascular endothelial cell differentiation and angiogenesis, and direct nerve growth and neuronal migration (Robinson and Cormie, 2008).

Electrical stimulation (ES) of muscle to lessen denervation atrophy after peripheral nerve injury has been long studied. The recent application of ES to transected nerves to promote axonal outgrowth provides a potential therapeutic modality to improve motor reinnervation. ES has been demonstrated experimentally to accelerate axonal sprouting, enhance recovery of twitch force, tetanic tension, and muscle action potential in the soleus muscle, and significantly improve recovery of toe spread reflex. More recent studies have also shown ES to significantly increase the number of motoneurons that regenerate by accelerating the sprouting of axons across the nerve repair site (Brushart et al., 2002), significantly increase the proportion of motor and sensory neurons that regenerate into their appropriate pathways to minimize axonal 'misdirection' that can compromise outcome, and improve axonal regeneration over long gap distances that prohibit spontaneous regeneration. Reconstruction of nerve gaps of up to 10–15 mm in the rodent model with nerve grafts, artificial conduits or scaffolds has been shown to benefit from short-term ES with a high rate of connectivity of regenerating axons to their target muscle, and accelerated nerve regeneration and functional recovery (Finkelstein et al., 1993).

At the molecular level, ES has been found to upregulate neurotrophic factor and trkB receptor expression in motoneurons, increase immunoreactivity of trkB receptor and brain-derived neurotrophic factor (BDNF) in sensory neurons, and increase the levels of multiple neural trophic factors including BDNF in additional cell types.



ic factor (GDNF), neurotrophin-3 and pleiotrophin in distal nerves, as well as BDNF and GDNF levels in distal muscles. Following transection and repair, ES also upregulates α -tubulin, a regeneration associated gene that is upregulated by increased BDNF expression, and downregulates neurofilament. This genetic profile, which is further enhanced by ES, is usually observed after peripheral nerve injury to allow for more rapid transport of tubulin and faster axon elongation (Hoffman and Lasek, 1980).

Clinical applications: ES has been used in various ways clinically to improve functional outcome after spinal and neuromuscular injury. Human clinical trials of applied direct current electrical fields have been in progress to treat spinal cord injuries (Hamid and Hayek, 2008). Such applications are based on experimental findings that applied DC electrical fields promote spinal cord repair by stimulating and directing axonal regeneration. The EU Project RISE was initiated in 2001 to develop examination methods and devices for evaluation of ES training effects in humans with denervated lower limbs after spinal cord injury. Approximately 1 year of ES produced a distinct increase in the cross-sectional area of the quadriceps and hamstring muscles, both visible and measurable. The excitability of the stimulated denervated muscles also increased significantly during this time with tetanic contractions being very weak initially but increasing to outputs up to 5–20 Nm after several months. There has also been a phase I clinical trial of oscillating field stimulation in acute spinal cord injured patients. An oscillating field stimulation device was surgically implanted above and below the level of injury for 15 weeks. Significant neurological improvement was noted in motor function based on the American Spinal Injury Association (ASIA) score and sensory function by electrophysiological studies (somatosensory evoked potential, SSEP) in 9 of 10 patients with the one patient lost to follow-up. All patients also reported improvement in proprioceptive and exteroceptive sensations. Most recently, ES was demonstrated to be beneficial after carpal tunnel decompression (Gordon, et al. 2010). In these patients, low frequency ES accelerated regeneration and target reinnervation by increasing the number of motor units reinnervated, and sensory nerve conduction studies significantly improved earlier than controls.

Because ES is already in clinical use for various applications including fracture healing and bony nonunions, and non-healing skin wounds, the equipment is readily available and the cost for use is low. It is non-invasive so it can be administered by a member of the surgical team or a technician, and side effects are minimal as long as standard precautions are taken.

could potentially improve functional outcomes after nerve injury especially for critical cases in which the level of injury is high and the distance to reach the target muscles is long and may otherwise be prohibitive. Both are commonly available at many medical institutions and application would be straightforward. We believe these are promising strategies that warrant further investigation in appropriately selected patients.

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