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Review

Improving peripheral nerve regeneration: From molecular mechanisms to potential therapeutic targets ☆

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ABSTRACT

Peripheral nerve injury is common especially among young individuals. Although injured neurons have the ability to regenerate, the rate is slow and functional outcomes are often poor. Several potential therapeutic agents have shown considerable promise for improving the survival and regenerative capacity of injured neurons. These agents are reviewed within the context of their molecular mechanisms. The PI3K/Akt and Ras/ERK signaling cascades play a key role in neuronal survival. A number of agents that target these pathways, including erythropoietin, tacrolimus, acetyl-L-carnitine, n-acetylcysteine and geldanamycin have been shown to be effective. Trk receptor signaling events that up-regulate cAMP play an important role in enhancing the rate of axonal outgrowth. Agents that target this pathway including rolipram, testosterone, fasudil, ibuprofen and chondroitinase ABC hold considerable promise for human application. A tantalizing prospect is to combine different molecular targeting strategies in complementary pathways to optimize their therapeutic effects. Although further study is needed prior to human trials, these modalities could open a new horizon in the clinical arena that has so far been elusive.

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Introduction

Peripheral nerve injury is common and afflicts individuals from all walks of life. Many of these patients exhibit incomplete recovery, with the ensuing disability resulting in substantial societal and personal costs (Evans-Jones et al., 2003; Noble et al., 1998). Although injured peripheral nerves can regenerate, axon regeneration proceeds slowly at rates of only 1–3 mm/day (Gutmann et al., 1942; Sunderland, 1947). The regenerative capacity of axons and the growth support of Schwann cells (SCs) decline with time and distance from injury, so regenerating axons must race against the clock in order to achieve a useful functional outcome (Fu and Gordon, 1995a,b). In a patient with a brachial plexus injury at the shoulder level, the injured neurons must regenerate their lost axons over a distance of as much as a meter to reinnervate the denervated muscles and sensory organs of the hand, a feat that can take several years. Within this long period of time, the chronically denervated SCs progressively lose their growth supportive phenotype, becoming atrophic and unable to support regeneration (Bunge, 1993; Chen et al., 2005; Hall, 1999; Sulaiman and Gordon, 2000; You et al., 1997) and muscles may have atrophied beyond repair. Similarly, prolonged periods of disconnection of the neurons and the proximal axonal stump from the end target, referred to as chronic axotomy, also render them less effective in regeneration (Fu and Gordon, 1995a). As a result, it becomes unlikely that the patient will ever regain hand function or sensation. This problem is compounded in patients with polytrauma where life-threatening injuries and complications often delay surgical repair by weeks and sometimes months. Development of strategies to enhance peripheral nerve regeneration is critical if surgical repair of injured nerves is to have more positive functional outcomes.

Advances in molecular biology have significantly improved our understanding of the mechanisms of peripheral nerve regeneration. Targeting specific steps in the molecular pathways may allow pharmacologic enhancement of regeneration, potentially leading to better functional recovery after injury. The purpose of this review is to examine the pharmacologic interventions studied to date in animal and human models of peripheral nerve injury. This is not intended to be an exhaustive review of the molecular mechanisms of nerve regeneration, but rather to highlight key components of the signaling pathways as they pertain to the therapeutic agents that are currently under investigation.

Brief overview of mechanisms of peripheral nerve regeneration

After nerve injury, the isolated axons in the denervated distal nerve stump undergo Wallerian degeneration (WD) (Stoll et al., 2002). The removal of myelin and myelin-associated glycoproteins (MAGs) is essential for axon regeneration to proceed after nerve injury. Myelin breakdown is triggered within hours after injury by phospholipases expressed in the SCs through the action of lysophosphatidylcholine(LPC) (Gaudet et al., 2011). SCs are initially the only cells responsible for releasing cytokines. That in turn plays a role in phagocytosis and recruiting macrophages into the distal nerve stump (Avellino et al., 2004; Bendszus and Stoll, 2003). A complex interaction exists between macrophages, fibroblasts, SCs, and endothelial and mast cells expressing inflammatory and anti-inflammatory cytokines during WD (Gaudet et al., 2011). The mechanisms that terminate the inflammatory response are not well understood. Macrophages switch to an anti-inflammatory phenotype with expression of anti-inflammatory cytokines such as IL-10 (Jander et al., 1996; Ydens et al., 2012).

Within 2 days of injury, SCs in the isolated nerve stump dedifferentiate from their myelinating form to a growth-permissive form (Arthur-Farraj et al., 2012). In response to a number of SC-derived mitogens including neurotrophins (Stewart et al., 1991), axon-derived calcitonin gene-related peptide (CGRP) (Toth et al., 2009), neuregulin (Carroll et al., 1997) and macrophage-derived molecules such as IL-1α/β (Cheng et al., 1995), SCs proliferate and line the endoneurial tubes, forming the Bands of Büngner to support and guide regenerating axons (Bungner, 1891). The process is relatively slow with initial disarray of the SCs in the extracellular matrix of the injury site (Witzel et al., 2005). This delay together with remaining chondroitin sulfate (Groves et al., 2005) and inhibition by MAGs (Cai et al., 2001) accounts for the initial abortive sprouting of nerves from the proximal stump and for the phenomenon known as staggered axon regeneration (Al Majed et al., 2000). The denervated SCs up-regulate several growth factors within the first 1 to 2 weeks (Boyd and Gordon, 2003; Mi et al., 2007). These include those specific for the SCs in endoneurial tubes where motor and sensory nerve fibers formerly resided (Brushart et al., 2013; Hoke, 2006; Hoke et al., 2006; Mi et al., 2007). The relationship between axons and SCs is an intimate one. Even when axons grow into a space that does not contain pre-existing SCs, such as a gap between the proximal and distal stumps of a transected nerve, the axons grow out in close approximation to accompanying SCs (Webber and Zochodne, 2010). The processes of WD, SC proliferation and activation are vital first steps toward preparing peripheral nerves for subsequent regeneration.

A cascade of cell body changes unfolds after a nerve injury, converting the neuron from a maintenance/transmitting mode to a regeneration mode (Fu and Gordon, 1997). Axotomized neurons up-regulate regeneration associated genes (RAGs) including those that express tubulin, actin, and growth-associated protein 43 (GAP43) at the regenerating nerve front where the growth cone forms (Igarashi et al., 1995; Strittmatter et al., 1994; Tetzlaff et al., 1988). The growth cone relies on the upregulation of tubulin in the core of the growth cone and the trailing axon, and F-actin to form the filopodia. The growth cones continually sample their environment through extension, retraction and turning of filopodia (Shim and Ming, 2010). Growth cone receptors include tropomyosin related kinases (Trks) that serve as cognate receptors for specific neurotrophins and the common neurotrophin receptor, p75 that is expressed widely on axons (Boyd and Gordon, 2003). Correctly targeted growth of regenerating axons is important to direct axons into appropriate pathways. This process is somewhat random but there is preferential reinnervation of motor pathways by motor axons (Al Majed et al., 2000; Brushart, 1988; Mesulam and Brushart, 1979). The repertoire of molecules that influence directionality and turning of adult axons into correct trajectories is largely unknown but the specific growth factors expressed by motor and sensory SCs appear to be critical (Gordon, 2014; Hoke, 2006; Hoke et al., 2006; Webber et al., 2008).

Molecular pathways that promote survival and regeneration

The major molecular pathways that have substantial bearing on axon growth form a conceptual framework for the discussion of the pharmacological agents that exert their influence on peripheral nerve regeneration.

PI3K/Akt signaling

The PI3K (phosphatidylinositol-3 kinase)–Akt signaling cascade is a key survival pathway that mediates trophic factor support of neurons and blocks apoptosis (Dudek et al., 1997). It is important in promoting not only survival but also growth, differentiation and directional signaling (Boyd and Gordon, 2003; Kaplan and Miller, 2000).

Nerve growth factor (NGF) binding to the Trk receptor promotes the activation of PI3K through the adaptor proteins Gab-1 and Ras which allow PI3K to phosphorylate and activate downstream molecules, Akt (Kaplan and Miller, 2000) and ERK-1/2, respectively (Fig. 1). Activated Akt participates in several signaling cascades (Dudek et al., 1997; Hetman et al., 1999). Akt reduces the expression of the Fas ligand (FasL) via inhibition of the pro-apoptotic transcription factorsForkhead and Bad (an inhibitor of Bcl-2 anti-apoptotic protein) (Vanhaesebroeck and Alessi, 2000). Akt phosphorylates and inactivates glycogen synthase kinase 3β (GSK-3β) located at the growth cone, thereby, via disinhibition, setting free the machinery for cytoskeletal assembly and neurite