Tobacco use and neurogenesis: A theoretical review of pathophysiological mechanism affecting the outcome of peripheral nerve regeneration

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ABSTRACT

Peripheral nerve injury often requires medical intervention. Unfortunately, many patients never have a full recovery, despite a multi-disciplinary approach, including operative intervention and physical and/or occupational therapy. Outcomes are multifactorial, but are largely affected by the original injury severity, and patient comorbidities. A alcoholism, diabetes mellitus and ageing may detrimentally affect the outcomes of nerve injury; however little is known about tobacco's potential impact on nerve regeneration. Tobacco has known immunomodulatory effects, which suggests that it might affect peripheral nerve regeneration and functional recovery following injury. This review characterizes the effects of tobacco use on the complex cellular and chemokine interactions in peripheral nerve regeneration.

1. Introduction

The majority of peripheral nerve injuries occur in the upper limb secondary to trauma, most frequently in young adults and military personnel.¹ Injury severity, surgical repair technique, and patient factors can affect nerve regeneration capacity. Even with prompt repair, factors such as ageing, alcoholism, diabetes mellitus, size of the nerve gap, neuroma, or scar tissue formation can impede healing.^{2–6} Current evidence demonstrates that the immune system contributes to regeneration of damaged tissues (e.g. cardiac, liver, musculoskeletal, central and peripheral nervous system) by cellular recruitment and modulation of resident progenitor cells through cytokines, growth factors, and clearance of debris.^{7,8} Of interest, cigarette smoke contains approximately 7000 compounds, of which 250 are absorbed into the blood stream.⁹ These, essentially cause immunomodulation, slower cell division, DNA damage, and chronic tissue hypoxia through carbon monoxide, vasoconstriction and atherosclerosis.^{10,11} Clinically, tobacco smokers demonstrate delayed bone and wound healing,¹² and increased morbidity and mortality rates.^{9,13} Few studies have evaluated the association between tobacco and peripheral nerve regeneration after injury. Tobacco consumption has been implicated as a risk factor for peripheral nerve injury in the perioperative period.¹⁴ Some

investigators have shown that smoking increases the risk of diabetic peripheral neuropathy in diabetic patients,^{15,16} and others have demonstrated in rats that chronic nicotine exposure produces hypersensitivity and alters cytokine production following sciatic nerve injury.¹⁷ Given the pervasive impact of tobacco consumption systematically on all body systems, and the resulting significant public health implications of tobacco abuse, and the paucity of clinical studies in peripheral nerve regeneration in patients with tobacco consumption, we chose to review the current state of literature of the molecular mechanisms involved in nerve injury regeneration under tobacco's immunomodulatory effect. This review article aims to expose chronic tobacco use as a potential deleterious factor in peripheral nerve regeneration after injury at a cellular and molecular level.

2. Nerve injury

Nerve injury classification is based on injury severity. In 1943, Seddon defined nerve lesions by the nerve structures affected: neuropraxia (myelin injury only), axonotmesis (axonal with endoneurial or perineurial injury), and neurotmesis (i.e., complete injury to myelin, axon, and epineurium).²⁰ Soon after, Sunderland expanded this classification with a grading system for clinical and prognostic utility.²¹

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Grade I corresponded to neuropraxia, grade II through IV to axonotmesis, and grade V to neurotmesis.²² Usually, grades I and II recover completely, grade III recover partially, and grades IV and V require surgical intervention. Peripheral nerve injury can result from excessive traction, compression, crushing, lacerations secondary to penetrating trauma or adjacent bone fracture, and gunshot wounds, among others.¹ The surgeon relies on clinical examination and considers surgical exploration on a case-by-case basis, keeping in mind that a prompt repair generally leads to better outcomes.²³ Some advocate the use of MRI to aid in distinguishing the different Sunderland grades and in devising treatment strategies.²⁴ On the other hand, historically, nerve conduction studies (NCS) and electromyograms (EMG) have been extremely useful for the evaluation of nerve lesion extent and distribution, but may take up to nine days after injury to differentiate between a neuropraxia versus axonotmesis or neurotmesis.^{25,26} Lack of clinical improvement or persistent conduction velocity abnormalities three to six months after injury warrants surgical exploration and repair. However, generally, delayed intervention decreases the chance of regeneration and functional recovery.²⁷ Usually, repairs done immediately after injury have better results, but progressively worse results if done at 3, 6, 9, or ≥ 12 months.²⁸

Direct surgical repair with epineural microsutures remains the gold standard for neurotmesis in the setting of complete nerve injury. The primary principle of nerve repair is to guide regenerating axons through the distal nerve and potentially allow reinnervation of the target organs. Autologous or allogenic nerve grafts, or nerve conduits may be used if there is an excessive gap or tension between nerve ends. Autologous grafting is the first line option for gaps > 3 cm, proximal nerve lesions, and motor nerves.²⁹ The primary principle of nerve repair is to guide regenerating axons through the distal nerve and potentially allow reinnervation of the target organs.

There are many factors that contribute to full nerve recovery. Treatment plans are generally multi-disciplinary, involving operative intervention, and pre- and post-operative physical/occupation therapy. There are many mechanical and physical characteristics of the injury or lesion itself that can affect treatment outcomes. The gap between the proximal and distal stumps, distance from the lesion site to the cell body, the time since injury, and age, and presence of intact endoneurial tube, are generally considered. Additional important patient factors that have been studied include alcoholism, diabetes, nutrition and tobacco use. These factors have general systemic effects and have been found to have cellular and molecular mechanisms directly involved in nerve injury regeneration with immunomodulatory effects.^{2–6}

2.1. Cellular and molecular mediators of nerve regeneration

End-organ reinnervation after nerve injury may occur in two ways: collateral branching or axonal regeneration. Collateral branching prevails in injuries affecting approximately 30% of the peripheral nerve axons, while axonal regeneration takes place when at least 90% of the axons are affected.^{30,31} Peripheral nerve repair occurs in a stepwise fashion: Wallerian degeneration (WD), axonal regeneration and, lastly, end-organ reinnervation.³¹

After injury, axonal degeneration occurs proximally and distally to the zone of trauma. This usually extends proximally to the nearest node of Ranvier. However, if the injury is close enough to the cell body, apoptosis may occur.^{29,31} Distally, axonal degeneration will progress outside the zone of trauma. Within the first 48 h WD will progress to the motor or sensory end-organ receptor.

Nerve degeneration is an intrinsic cytokine-mediated process led by Schwann cells (SC) and macrophages (M). Following injury, SC lose contact with axons and shift from a myelinating to a non-myelinating phenotype through a C-Jun signaling process. Neurotrophic factors such as nerve growth factor are upregulated.³¹ Schwann cells proliferate and begin to phagocytose axonal debris and myelin, leaving empty endoneurial tubes (Fig. 1).²⁹ Additionally, they recruit and

Figure 1. Normal nerve anatomy.

activate monocytes and M through secretion of monocyte chemoattractant protein-1 (MCP-1), interleukin 1 α and β (IL-1 α/β), and tumor necrosis factor- α (TNF- α).^{18,34,35}

The M are highly plastic and can perform in different ways as dictated by their microenvironment.³² In nerve regeneration, M are polarized in two complementary subtypes: M1 (pro-inflammatory) and M2 (anti-inflammatory).⁸ The activation of M1 is promoted by lipopolysaccharide (LPS) or T-helper lymphocyte (Th) type 1 cytokines such as interferon- γ (IFN- γ); while M2 activation is promoted by Th2 cytokines, including interleukins (IL) IL-4, IL-10, and IL-13, amongst others.⁸ IL-13 plays a key role in programming Th2 response and M2 activity.³³ Wallerian degeneration occurs in an inflammatory environment triggered by SC, fibroblasts, and resident immune cells and is maintained by recruited M.²⁹

The blood-nerve barrier is degraded by matrix-metalloproteinase-9 (MMP-9), produced by activated SC and M, and allows influx and efflux of blood factors and cells to facilitate tissue repair in the first 2 weeks.^{35,36} Additionally, tumor necrosis factor- α (TNF- α) and IL1 α induce fibroblasts to produce IL-6 and granulocyte-macrophage colony-stimulating factor (GM-CSF), which enhance immune cell chemotaxis.³⁵ This dynamic secretory sequence of inflammatory cytokines and growth factors rapidly rise and are crucial for M recruitment.

Infiltration of M at the site of injury starts at 1–3 days, and peaks at 7 days post-injury. M1 predominate for the first 3–4 days,¹⁹ which produce TNF- α , IL-1 α/β , and IL-6 among others; and between 7 and 14 days post-injury, M2 predominate and mainly secrete IL-6, IL-10, and transforming growth factor-beta (TGF- β).^{8,37} These cellular populations are intertwined in a complex, and only partially understood, network repertory of cytokines and growth factors.³⁵

The myelin debris at nerve injury sites blocks axonal regrowth, and its clearance is initially performed by SC and then M1, which increases the inflammatory response during the first week after injury. Defects in debris clearance prevent effective regeneration.⁷ Following debris clearance, there is an upregulation of collagen VI and apolipoprotein-E which, coupled with myelin phagocytosis, polarizes M1 towards M2.^{4,19} The M2 population attenuates the inflammatory response and in conjunction with SC promote axonal regeneration.³⁵ Conversely, premature activation of M2 may disrupt efficient tissue healing due to incomplete debris clearance.⁷ Studies show that this M1/M2 homeostatic balance is necessary for efficient nerve regeneration following debris clearance, as seen in other tissues.^{7,43}

Axonal regeneration occurs once WD is concluded.³¹ Axonal regrowth begins at the adjacent Node of Ranvier and will progress towards the end-organ.²⁹ After myelin debris clearance occurs, the SC proliferate into the remaining endoneurial tubes of extracellular matrix

(ECM), forming hollow tubes called Büngner bands, that guide and allow the growth of the regenerating axon.⁴⁴ If the endoneurial tube is not populated by the growing axon, it shrinks within four months. Neuroma formation can occur in the absence of intact endoneurial tubes.^{30,31} Therefore, the more intact or preserved these endoneurial tubes are, the greater potential for functional recovery.

A growth cone is formed at the proximal nerve stump, which samples the cytokines and growth factors in the microenvironment through filopodia and by contact-mediated or chemical neurotrophism to progress toward the endoneurial tube.^{45,46} Both neurotrophic and neurite-outgrowth promoting factors are upregulated and support axonal growth and cell body survival. Examples of these are: nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), ciliary neurotrophic factor (CNTF), neurotrophins (NTs), the TGF- β family, glial cell line-derived neurotrophic factor (GDNF), fibronectin, and laminin, amongst others.^{31,47} IL-1 induces SC to upregulate NGF secretion and NGF-receptor, which not only potentiates proliferation of SC, but also provides guidance to the outgrowing axon.^{30,31,48} The rate of axonal regeneration varies from 1 to 3 mm/day, with an expected increased likelihood of end-organ reinnervation when the endoneurial tube is present. The outgrowing axon produces adenosine triphosphate (ATP) and acetylcholine, which direct SC towards a myelinating phenotype. Re-myelination follows, allowing for maturation of the regenerated axon, and eventually, functional recovery.^{31,49}

3. Tobacco dysregulation

In a systematic review of prognostic factors following digital nerve repair by Kim et al., smoking was clearly indicated as a negative predictor of nerve recovery.⁵⁰ While this effect is well accepted in clinical practice, few molecular mechanisms have been developed or accepted to explain the observation. Given the state of the current body of literature, we are unable to definitively describe how smoking tobacco affects peripheral nerve regeneration on a molecular basis. Rather, our main objective is to propose links between accepted pathways of peripheral nerve healing with known molecular effects of cigarette smoking to postulate a basis for molecular dysregulation in peripheral nerve regeneration amongst smokers. From this groundwork, we aim to develop more robust molecular relationships and identify potential therapeutic targets for peripheral nerve regeneration in smokers.

3.1. Immune dysfunction

Cigarette smoking primarily suppresses Th1 activity and facilitates a Th2 mediated response, which ultimately favors M2 activity. Predilection for a Th2 response is facilitated through IL-4, IL-5, IL-6, IL-10, and IL-13, and cigarette smoking has been associated with elevated levels of IL-6, IL-10, and IL-13.^{35,38,40–42} Studies comparing cytokines and lymphocyte phenotype populations from plasma between smokers and non-smokers showed that T-cell response skewed towards Th2, with increased production of IL-13 in smokers and no difference in INF- γ .^{33,51,52} Additionally, Yuan et al. proved *in vitro* polarization of alveolar, peritoneal, and bone marrow derived macrophages and *in vivo* polarization of mouse macrophages towards M2 when exposed to cigarette smoke. The exposed group had higher IL-10, IL-6, TGF- β 1, and TGF- β 2 levels, and lower TNF- α and IL-12 levels.⁵³ This milieu of cytokines would be expected to have an anti-inflammatory effect resulting in attenuation of the immune response, limited debris clearance and scar formation at the nerve injury site.

The Janus kinase (JAK)/signal transducer and activator of transcription (STAT) signaling pathways, particularly JAK2/STAT3, are important due to their involvement in the polarization of macrophages towards M2. Interestingly, in this same study Yuan et al. found that this signaling pathway was enhanced by cigarette smoking through phosphorylation. The cytokines IL-1 β , IL-4, IL-6, IL-10 and IL-13 activate this pathway, thus STAT3 is regarded as one of the primary signaling

pathways in mediating M2 polarization.⁵³ STAT3 activation increases IL-6 expression, which in turn may lead to STAT3 phosphorylation, resulting in a positive feedback loop and attenuation of the inflammatory response.⁵⁴ The Nuclear factor- κ B (NF- κ B) transcription factor, c-Jun N-terminal kinase (JNK) and c-Jun signaling pathways are involved in initiation of the M1 response.⁵⁵ However, these are susceptible to alkylation by acrolein, one of many reactive electrophiles found in tobacco smoke, and a suppressor of macrophage activation.⁵⁶ In a mouse model study by Hristova et al.,⁵⁷ *in vivo* alveolar macrophages and *in vitro* bone marrow derived macrophages exposed to tobacco smoke resulted in selective suppression of the M1 response to LPS and INF- γ . They found that acute transient exposure (< 2 hours) of JNK2 cysteine alkylation and previously described alkylation of NF- κ B⁵⁸ are responsible for truncating M1 activation; and sustained M2 promotion is probably associated with activation of NF-E2-related factor (Nrf2) and induction of anti-inflammatory cytokines.⁵⁷

3.2. Cytokine dysregulation

The main cytokines involved in peripheral nerve regeneration include TNF- α , IL-1, IL-6, IL-10, and TGF- β .³⁵ In a recent study investigating baseline inflammatory biomarkers in 39 healthy smokers versus a sample of 101 healthy non-smokers, baseline levels of TNF- α , IL-6, and IL-10 were similar.⁵⁹ Previous studies found pro-inflammatory cytokines, such as TNF- α , to be elevated in smokers and demonstrated a dose-dependent response.⁶⁰ Increased expression of TNF- α following nerve injury is necessary to promote inflammation and axonal regrowth,⁶¹ however, the isolated effect of constitutively elevated TNF- α on peripheral nerve regeneration has yet to be studied.

Similar to TNF- α , IL-1 levels have been shown to be elevated in smokers in response to exposure to ultrafine particles (UFP) produced in combustion of tobacco.⁶² In the event of peripheral nerve injury, SC that lose contact with axons upregulate IL-1 production. Increased concentration of IL-1 following peripheral nerve injury is necessary to promote WD and an initial inflammatory response, followed by modulation of anti-inflammatory cytokines.³⁵ In a murine study, sciatic nerve regeneration was significantly worse in IL-1 β knockout mice compared to wildtype; however, IL-1 β levels were observed to peak within 3 days and return to baseline in 14 days.⁶³ While no studies have been conducted to elucidate the effect of elevated IL-1 β on peripheral nerve regeneration, it is plausible that constitutively elevated levels would promulgate the inflammatory phase and hinder axonal regeneration following adequate debris clearance.

Baseline levels of TGF- β 1 in healthy smokers is not well established in the current literature. One study by Moghddam et al., reported depressed levels of TGF- β 1 following long bone fracture in smokers compared to controls.⁶⁴ Additionally, an *in vitro* study where mesenchymal stem cells were exposed to tobacco smoke concentrates demonstrated depressed TGF- β signaling.⁶⁵ Following peripheral nerve injury, TGF- β 1 is normally elevated and has been found to be critical in attenuating inflammation and promoting proliferation of non-myelinating phenotype SC.⁶⁶ Reduction of TGF- β 1 following peripheral nerve injury has been shown to significantly hinder axonal regeneration in a rat knockdown study, whereas axonal regeneration is significantly supported by SC treated with TGF- β 1, even in chronic injury cases.^{67,68} Furthermore, in a murine model study by Kritis et al., TGF- β 1 demonstrated a biphasic response following sciatic nerve injury. Levels were only increased 12 h and 1 month after injury, which alludes to an early mobilization phase, followed by a late myelination phase coordinated by TGF- β 1.⁶⁹ It is highly plausible that this orchestrated effort is not conserved in smokers with constitutively elevated levels of TGF- β 1, contributing to poor nerve regeneration. Overall, it is the cytokine balance that may be altered by tobacco compounds, thus affecting nerve recovery (Table 1).

Table 1

Prominent cytokines involved in peripheral nerve regeneration and proposed effects on function in the presence of cigarette smoke. Note: SC, Schwann Cells; M, Macrophages; TNF- α , tumor necrosis factor alpha; IL, interleukin; TGF, transforming growth factor; NGF, nerve growth factor.

Cytokine	Source	Function	Proposed Effect of Tobacco on Cytokine Function
TNF- α ^{35,38}	SC, M1, Mast Cells	<ul style="list-style-type: none"> - M recruitment - MMP-9 secretion by SC and M - Decreases SC proliferation - Increase IL-6 production - Pain modulation - Induce fibroblasts to produce NGF 	<ul style="list-style-type: none"> - ↓ Cytokine concentration - Reduced M at injury site - Delayed myelin clearance - Decreased axonal growth
IL-1 α ^{35,39}	SC	<ul style="list-style-type: none"> - Induce fibroblasts to produce IL-6, GM-CSF, NGF 	<ul style="list-style-type: none"> - ↓ Cytokine concentration - Reduced chemotaxis - Decreased axonal growth
IL-1 β ^{18,35,39,40}	SC, M1	<ul style="list-style-type: none"> - M recruitment - Promote myelin phagocytosis - Pain modulation - Induce fibroblasts and SC to produce NGF 	<ul style="list-style-type: none"> - ↓ Cytokine concentration - Reduced M at injury site - Delayed myelin clearance - Decreased axonal growth
IL-6 ^{18,35,38,40,41}	SC, M1, M2, Fibroblasts	<ul style="list-style-type: none"> - M recruitment - Enhances T-cell activation - Neurotrophic for cholinergic and dopaminergic neurons - Pain modulation - Promotes axon regeneration - Promotes NGF synthesis in neurons 	<ul style="list-style-type: none"> - ↑ Cytokine concentration - Increased neuropathic pain - Decreased axonal growth - Increased M2 polarization
IL-10 ^{35,42}	M2, monocytes, T -cells, B-cells, fibroblasts, epithelial cells, mast cells	<ul style="list-style-type: none"> - Limits secretion of inflammatory cytokines - Deactivates M1 - Decreases scar formation at site of injury - Control M polarization 	<ul style="list-style-type: none"> - ↑ Cytokine concentration - Decreased persistence of M1 activity - Reduced myelin clearance
TGF- β ³⁵	SC, M2	<ul style="list-style-type: none"> - Maintenance of non-myelinating SC phenotype inhibiting SC myelination - Modulates NGF to increase neuronal survival - Downregulates non-myelinating SC once nerve regeneration occurs 	<ul style="list-style-type: none"> - ↑ Cytokine concentration - Increased thrombospondin-1 (TSP-1) activating additional TGF-β (positive feedback loop) - Suppresses immune response - Increased scar formation - Increased decorin concentration

3.3. Nicotine in peripheral nerve regeneration

Of the numerous components in cigarette smoke, nicotine is considered the major addictive component. It has been largely implicated in delayed wound healing and fracture healing as a consequence of chalone production and subsequent adrenergic mediated vasoconstriction.^{70,71} With regards to nerve injury, nicotinic mediated vasoconstriction does not seem to be implicated in poor nerve regeneration. Rather, nicotine has been investigated as a potential neuroprotectant and enhancer of axonal regeneration. In a murine study by Wang et al., nicotine was found to interact with the α 7nAChR resulting in decreased levels of the pro-inflammatory cytokines TNF- α and IL-1 β .⁷² Although TNF- α and IL-1 β are critical mediators of WD, persistent inflammation following nerve damage can result in secondary injury.⁷⁰ Additionally, arachidonic acid increases acutely following nerve injury and is associated with neuronal apoptosis. Neurons cultured with nicotine expressed increased levels of NGF and the corresponding tyrosine kinase receptor, Tropomyosin receptor kinase A (TrkA), which reduced neuronal apoptosis following exposure to arachidonic acid.⁷³ Similar anti-inflammatory effects of nicotine exposure are observed following injury to various tissue types, including adipose, pulmonary, renal and hepatic.^{74–77}

4. Conclusion

Peripheral nerve injury regeneration is a complex and often tenuous process that requires careful modulation of a host of immune cells and cytokines to effectuate adequate healing and restoration of function. The process is marked by an early inflammatory phase for debris clearance carried out by Th1 and M1, followed by an anti-inflammatory shift to Th2 and M2 to facilitate axonal regeneration. Cigarette smoke has been shown to alter both the innate and adaptive immune responses and overall attenuate inflammation, an effect that limits debris clearance and promotes scar formation at a peripheral nerve injury site. This

predilection for an anti-inflammatory state are corroborated by animal models and *in vitro* studies investigating the effects cigarette smoke has on a number of cytokines that are essential in peripheral nerve healing. In particular, a decrease in TNF- α and IL-1 which normally promote inflammation and an increase in IL-6, IL-10, and TGF- β , which normally reduce inflammation have been shown. Alteration of the inflammatory response, vasoconstriction, and relative hypoxia as a result of cigarette smoke exposure suggest delayed healing following peripheral nerve injuries in smokers; however, these molecular and cellular processes have not been investigated in humans. As such, smoking cessation is recommended to mitigate the potential risks and complications following peripheral nerve injury, but additional interventions to promote or restore a native healing response remain to be desired. These important topics warrant further investigation.

Disclosures

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Declaration of competing interest

None.

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