Minireview

Evolving techniques for reducing phantom limb pain

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Impact statement

It is essential to develop novel techniques for reducing phantom limb pain (PLP) because the pain has a lifelong negative impact on more than two million people in the United States of America alone. Studies aimed at understanding the causes of PLP and techniques that might reduce it are presented. New evidence is reviewed indicating that the application of platelet-rich plasma (PRP) reduces/eliminates chronic neuropathic pain, which suggests it may also reduce/eliminate PLP. These studies may help those working on PLP and pain, in general, considering new ways to develop novel techniques for suppressing pain.

Abstract

At least two million people in the United States of America live with lost limbs, and the number is expected to double by 2050, although the incidence of amputations is significantly greater in other parts of the world. Within days to weeks of the amputation, up to 90% of these individuals develop neuropathic pain, presenting as phantom limb pain (PLP). The pain level increases significantly within one year and remains chronic and severe for about 10%. Amputation-induced changes are considered to underlie the causation of PLP. Techniques applied to the central nervous system (CNS) and peripheral nervous system (PNS) are designed to reverse amputation-induced changes, thereby reducing/eliminating PLP. The primary treatment for PLP is the administration of pharmacological agents, some of which are considered but provide no more than short-term pain relief. Alternative techniques are also discussed, which provide only short-term pain relief. Changes induced by various cells and the factors they release are required to change neurons and their environment to reduce/eliminate PLP. It is concluded that novel techniques that utilize autologous platelet-rich plasma (PRP) may provide long-term PLP reduction/elimination.

Keywords: Amputation-induced pain, amputations, chronic pain, pain elimination, platelet-rich plasma, PRP, PLP

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Introduction

In the United States of America, more than two million people presently live with an amputation.[1] This number increases annually by more than 185,000 and is expected to double by 2050.[1] Of these, 67% are lower extremity amputations, and 17% are bilateral lower limb amputations (Amputee Coalition Organization). Although the level of phantom limb pain (PLP) may decrease or PLP may disappear, some studies report no change in its frequency or severity over time, resulting in many individuals suffering lifelong pain.[2]

PLP prevalence rates of residual limb pain vary widely, from 10% to 76%. However, PLP rates are reported as high as 90%.[3] Up to 25% of these suffer severe chronic PLP,[4] with 30–40% of individuals finding their PLP moderately or severely limiting or bothersome.[5] Making PLP one of the most challenging pain problems to resolve is that no treatment provides long-term relief.[6] Therefore, a better understanding of neurological changes following an amputation is needed to develop techniques to reverse these changes, leading to permanent PLP reduction/elimination.

PLP is considered a complex pain state believed to be caused by central nervous system (CNS)[7] and peripheral nervous system (PNS)[8] changes. While the mechanisms underlying PLP are unclear, they appear to involve hyperactivated neurons that may or may not be associated with reorganized somatosensory processing pathways and neural circuits in the CNS. Current theories about what underlies PLP and approaches developed for its treatment are examined.

When considering how to reduce PLP, it is essential to consider whether some of its underlying causes are associated with non-painful phantom limb sensations (npPLSs). This is because, while both may be associated with the spontaneous electrical activity of large sensory fibers (touch) and small C (pain), they may differ in the relative ratios of which fibers are spontaneously electrically active.

Are there predictors of PLP development?

It has been suggested that preamputation pain and early PLP intensity are not good predictors of the development of chronic PLP.[9] However, other studies find it a good predictor of half the variance in the incidence and severity of postamputation PLP.[10] However, no evidence indicates that preamputation pain is a predictor of persistent PLP, although

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some differences associated with preamputation pain and PLP may be due to differences in patients, the cause of the limb loss, and other factors such as the patient's psychological state. For example, people with PLP are reported to have less anxiety and depression than those with other types of pain, such as lower back pain.[10]

Potential underlying causes of PLP

Cortical reorganization

One hypothesis is that PLP results from a combination of amputation-induced changes in nociceptive neurons, activity in both the PNS and CNS, a maladaptive cortical reorganization of brain and spinal cord neural circuits,[11] impairment of intracortical inhibitory mechanisms, enhancement of the excitability of corticospinal neurons, and an imbalance between inhibitory and excitatory amino acids.[12] It is also hypothesized that a higher level of PLP is associated with the increasing area of cortical reorganization.[13]

These hypotheses imply that PLP results from both top-down and bottom-up causes. Top-down pain modulation is associated with painful sensations maintained by the CNS that are affected by memories, attention, and emotional state.[14] Bottom-up pain mechanisms are associated with peripheral nerve injury triggering chronic aberrant inputs that induce changes in the CNS.[15] Thus, peripheral nerve damage causes the loss of sensory nerve input to the CNS leading to changes in both the ascending and descending signals from the sensorimotor body representation. These changes result in reduced pain thresholds, supraspinal and central plasticity, and peripheral/spinal dysfunction.[16] Thus, limb amputation results in the loss of cortical motor representation of the missing limb, although the limb's sensory representation does not disappear, which allows and causes patients to "feel" their phantom limbs.[17]

Top-down theory

The top-down theory for the origin of PLP postulates that it is triggered by the loss of cortical sensory input and by maladaptive cortical plasticity and remapping.[18] This involves the deafferented cortical regions becoming innervated by axons of neurons from the adjacent primary somatosensory and motor cortex representing other body parts.[19] This results in altering local neurons' properties, the development of new neural circuits, and changes to the existing brain and spinal cord neural circuits.[11] PLP is also proposed to result from the impairment of intracortical inhibitory mechanisms, enhancement of the excitability of corticospinal neurons, and an imbalance between inhibitory and excitatory amino acids (gamma-aminobutyric acid and glutamate).[12] These changes are visible with neuroimaging,[20] which shows afferent brain areas becoming larger.[21] Due to these neural circuitry changes, when an individual makes imagined movements of the phantom extremity, brain activity is triggered in cortical regions corresponding to both the lost extremity and adjacent body parts. It is further postulated that the increasing size of the area of cortical reorganization is associated with a higher intensity of PLP.[13] This is proposed to result from the impairment of intracortical inhibitory mechanisms, an enhancement in the excitability of corticospinal neurons, and an imbalance between inhibitory and excitatory amino acids (gamma-aminobutyric acid and glutamate).[12]

Chronic PLP is reported to correlate with the maintained representation of the missing hand in the primary sensorimotor missing hand cortex.[22] Thus, amputees suffering from severe chronic PLP have greater electrical activity in the primary sensorimotor missing hand cortex during phantom hand movements.[22] However, the correlation between the chronic PLP and the missing hand representation is not explained by the experience of the chronic non-painful phantom sensations or the compensatory usage of the residual arm.[22] These results support a positive relationship between persistent peripheral inputs from the missing hand and chronic PLP.[22]

However, some studies find no statistical relationship between the extent of cortical reorganization and the development of, or level of, PLP and phantom sensations.[23] Further, functional magnetic resonance imaging (fMRI) and functional diffusion tensor imaging find no significant relationship between pain and cortical reorganization because PLP develops even without changes in cortical representations.[24] This apparent conflict may be best explained by the cortical representation of the missing limb and neighboring body parts normally overlapping, so invasion and preservation can coexist.[25] Thus, apparent boundary changes may be due to the unmasking of existing innervation areas. These results also suggest that, despite the persistence of CNS reorganization, it may be possible to eliminate PLP and that the development of PLP can be blocked or existing PLP can be reduced/eliminated by applying techniques to peripheral nerves only.[24]

Other observations raise questions about the origins of PLP. For example, what underlies the changes in PLP that occur immediately to several years after an amputation[26] if it is a direct consequence of rapid cortical reorganization? How can complex regional pain syndrome (CRPS)[27] and carpal tunnel syndrome[28] be associated with somatosensory cortical reorganization but not result in site-associated pain, as seen with PLP? How is it that during the slow loss of innervation and limb due to leprosy, PLP onset appears more correlated with the loss of sensory-motor input than limb presence?[29] Thus, although many studies suggest a correlation between deafferentation, PLP, and cortical reorganization, the evidence supporting this relationship is not strong.[24] Therefore, additional questions must be addressed: (1) What are the relative influences of peripheral versus central mechanisms on the development and maintenance of PLP? (2) Are cortical changes causally related to the development of PLP or merely associated with PLP? (3) Do CNS presentational changes induce perceptional changes to stimuli? In addition, these questions open the possibility that, despite the persistence of CNS reorganization, the development of PLP can be prevented or existing PLP can be reduced/eliminated by applying techniques only to peripheral nerves or the brain.[24]

Bottom-up theory

The bottom-up theory of PLP involves several different arguments on how to reduce PLP. One is the observation

that increasing prosthesis ownership, but not the frequency of use, is associated with reduced PLP.[30] The mechanism of action is not known. However, potential explanations for reduced PLP include (1) prosthesis-induced non-specific activation of sensory nerves in the extremity stump,[31] (2) greater confidence in walking and upper limb use, or (3) psychological changes, including self-confidence and reduced anxiety provided by the prosthesis, which induce CNS changes.

One concept is that the development of PLP is evoked by pressure on the amputation stump inducing spontaneous ectopic electrical activity from axons associated with neuromas.[32] However, PLP exists without neuromas.[33] In this case, PLP is attributed to the spontaneous electrical activity of dorsal root ganglion (DRG) neurons, which exhibit more significant numbers of spontaneous action potentials than are induced by neuromas.[34] The application of lidocaine to amputees, either intrathecally or to the surface of DRG neurons, to block action potential conduction suggests the potential contribution of DRG neuron electrical activity to PLP.[32] This was reported to induce rapid and reversible PLP elimination and npPLSs.[32] However, the validity of the results has been questioned, and the study has not been repeated. However, another study tested the efficacy of a single Botox injection or a combination of lidocaine/methylprednisolone (Depo-medrol) into the most tender region of a residual limb or into neuromas. It found that both induced an immediate and long-lasting (6 months) reduction in residual limb pain but had no effect on PLP, with Botox inducing more pain suppression than lidocaine/Depo-medrol[35]

Abnormal spontaneous ectopic electrical activity of hyperexcitability nociceptive neurons

Part of the bottom-up theory proposes that PLP results from excessive input to the cortex due to the ectopic electrical activity of axotomized primary afferent DRG neurons that innervate the limb.[15] This electrical activity develops because nerve injury induces the abnormal accumulation of voltage-gated sodium channels (Na(v) 1.8 and Na(v) 1.7) in DRG nociceptive neuron soma.[36] Expression of these channels results in the neurons becoming abnormally hyperexcitable and exhibiting spontaneous ectopic electrical activity,[37] which is pronounced in the dorsal horn.[38] This high level of spontaneous electrical activity is represented as nociceptive activity in the somatosensory cortex.

An additional change induced by axotomy is that DRG nociceptive neurons become excessively responsive to endogenous pain-producing substances, such as the pro-inflammatory cytokine tumor necrosis factor (TNF)-α, interleukins (ILs), complement components, adenosine triphosphate (ATP), and chemokines. For example, TNF-α, released from activated Schwann cells and glia, contributes to the pathogenesis of neuropathic pain[39] by sensitizing primary afferent neurons by increasing their Na(v) channel currents.[40] This raises the question of whether pain can be blocked by techniques that provide a long-term blockade of nociceptive neuron electrical activity.

Reducing PLP

Drug administration

The first technique applied to treat PLP is the administration of pharmacological agents aimed at treating the symptoms, not the causes of the pain. Pharmacological treatments involve drugs such as non-steroidal anti-inflammatory drugs (NSAIDs), tricyclic antidepressants, anticonvulsants, and antiepileptics, followed by weak opioids and strong opioids.[41] However, it has been suggested that opioids may act by reducing activity in the somatosensory cortex and cortical reorganization.[42] Antidepressants act primarily by inhibiting serotonin–norepinephrine uptake, sodium channel blockade, and *N*-methyl-D-aspartate (NMDA) receptor antagonism.[43] Although effective for various neuropathic pain conditions, it is not very effective against PLP.[44] Opioids (levorphanol, oxycodone, methadone, and morphine) (oral and intravenous) decrease pain without causing the loss of proprioception, touch, or consciousness and effectively reduce cortical reorganization, apparently disrupting one mechanism underlying PLP. However, they are associated with more side effects than tricyclic antidepressants and gabapentin.[45]

Although gabapentin is effective in adults,[46] young adults, and children, its efficacy is variable.[47] For pediatric amputation patients, the administration of gabapentin starting four days before an amputation results in lower postoperative PLP than in control patients.[48] When effective, it has fewer side effects in adults than other opioids.[49] However, although neuromodulators such as gabapentin may reduce[48] or have no effect on PLP,[50] several meta-analyses found they did not provide a more significant benefit than other medical treatments.[51]

Other PLP treatments involve acetaminophen and NSAIDs, which are the most typically administrated medicine.[52] Although these drugs may provide analgesia, they are not effective for some patients, while some patients may develop tolerance or paradoxical pain, and others suffer adverse side effects that preclude their use. Therefore, pharmacological agents prescribed to treat the pain associated with PLP provide only minimal to moderate benefits.[46]

Non-analgesic agents, such as botulinum neurotoxins (BoNTs), do not reduce PLP compared with lidocaine/methylprednisolone.[46] NMDA receptor antagonists ketamine and dextromethorphan, but not memantine, have analgesic effects, but their adverse events are serious.[46] A single application of a capsaicin 8% patch reduces spontaneous amputation stump pain, PLP, and evoked stump pain.[53] fMRI studies show that capsaicin-induced pain reduction is associated with the restoration of the normal innervation of the cerebral cortex.[53]

The PLP of some patients can be reduced by infusing the brachial plexus with mepivacaine combined with epinephrine.[54] The prolonged infusion of a high concentration of local anesthetic (ropivacaine) solution onto the perineural nerve also provides long-acting (12 months) PLP relief.[55] While these results may suggest PLP is not solely of peripheral origin, it may be that spontaneous electrical activity is still occurring at locations not affected by the anesthetic or that different types or extents of PNS injury induce different

amounts of cortical reorganization, which are not affected by peripheral blocking of electrical activity.

Visual feedback training

Mirror therapy, also called graded motor imagery (GMI), is a non-pharmacological technique for attempting to reduce an amputee's complex pain and perceptions. It involves training the brain not to focus on pain by using real or imagined imagery. The underlying concept is that imagining the missing painful part of one's body gives the illusion that it is moving and thus effectively makes it pain-free by training the brain to stop creating pain.

Although studies find that mirror therapy significantly reduces PLP, the level of evidence is insufficient to rely on its efficacy in reducing pain.[51] In addition, any apparent effect decreases when a patient has a telescoped limb,[56] although virtual reality training appears effective with telescoping limbs.[57] However, the efficacy of mirror therapy appears to be increased when followed by augmented reality mirror therapy or sensory-motor exercises of the intact limb without a mirror, followed by self-delivered exercises.[56] Another study found that the efficacy of mirror therapy increases when combined with transcranial direct current stimulation (tDCS).[58] Similarly, the intensity of PLP is reduced by 32% by applying multimodal sensory-motor training of phantom limb movements involving visual and tactile feedback elicited by evoked stump muscle activity.[59]

GMI is suggested to be more effective in reducing PLP than physical therapy.[60] In one study asking the opinion of PLP pain experts, more than 50% suggested that in clinical practice, cognitive behavioral therapy and virtual reality training are effective in reducing PLP despite the lack of scientific evidence to support their ranking.[61] This raises the question of whether the efficacy of mirror therapy depends on other factors associated with the patient, such as stress or depression.

Brain stimulation to alter CNS neural circuits

The findings indicate that amputations and the development of PLP are associated with PNS and CNS changes. This has led to different types of non-invasive neuromodulatory treatments. These aim to re-alter the CNS neural reorganization to reduce PLP[62] or activate descending inhibitory pathways to the thalamus, which would modulate the ascending nociceptive signals leading to reduced PLP.[63] An alternative action might be to induce the release of endogenous opioids,[64] increase or decrease neurotransmitter release, and block the receptors for opioids and neurotransmitters at the stimulation sites in the spinal cord, brainstem, and brain.[65]

Pulsed radiofrequency ablation (PRFA) is a variation of conventional continuous radiofrequency (CRF). It provides 80% relief from PLP for at least six months.[66] The advantage of PRFA is that it avoids the danger of destroying tissue and other negative and painful consequences associated with CRF.

Repetitive transcranial magnetic stimulation (rTMS), tDCS, and PRFA are alternative stimulatory techniques. The pain relief provided by rTMS-induced brain stimulation is reported to be transient[67] and prolonged.[63] Studies also show that the reliability and degree of pain relief are increased based on the stimulation site, such as by applying high-frequency rTMS over the contralateral motor cortex (M1) and applying low-frequency rTMS over the unaffected hemisphere.[68]

Further, although the efficacy of rTMS is improved by optimizing the frequency of rTMS stimulation,[69] additional studies are required to determine the best frequency parameters for applying rTMS.[63] Nevertheless, the efficacy of TMS is variable, partly due to patient variability in terms of sensitivity to stimulation,[70] and the influence of stimulation is not consistently better than controls.[71]

A single session of tDCS significantly reduces PLP, with the effect lasting at least one week.[72] The pain relief is associated with reduced S1/M1 activity in the cortical region representing the missing appendage.[72] However, multiple stimulation sessions promote greater and long-lasting PLP reduction, while sustained stimulation induces sustained PLP relief.[73] These data support the hypothesis that PLP is associated with increased S1/M1 activity and that reduced PLP after electrical stimulation is significantly correlated with reduced S1/M1 activity in the missing hand cortex, and in turn, that increased S1/M1 cortical activity underlies PLP.[22] Further, this supports the hypothesis that electrical stimulation of this region leads to the restoration of intracortical inhibitory processes[74] or indirectly affects pain-modulating structures such as the thalamus.[75]

Although studies report that electrical brain stimulation that activates descending inhibitory pathways induces significant short-term PLP suppression,[63] there is limited high-quality evidence supporting this efficacy.[76] A more effective approach appears to be applying brain stimulation to increase the electrical activity of the limb's S1/M1 cortical region of the amputated part while the patient performs phantom hand movements.[22] This induces significantly long-lasting PLP reduction[72] by restoring intracortical inhibitory processes[74] or by indirectly acting on pain-modulating structures, such as the thalamus.[75] The technique's efficacy may be improved by better localization of the stimulation site, applying high-frequency rTMS over the contralateral motor cortex (M1), applying low-frequency rTMS over the unaffected hemisphere,[68] and optimizing the stimulation rTMS frequency.[69] However, a more effective way to reduce PLP is by modulating DRG neuron electrical activity[77] and radiofrequency stimulation.[78]

While these results are very promising, many questions must still be addressed. For example, why are combined tDCS and mirror therapy reported not to improve outcomes[79] but to induce strong, long-lasting effects?[80] It is also essential to determine how the outcomes are influenced by the stimulus intensity[81] and how the efficacy of stimulation in reducing PLP may be altered depending on an individual's psychological status, such as when they are suffering from depression and anxiety.[63] The interpretation of these data is further complicated by the findings of an fMRI study showing that PLP intensity is not associated with postamputation significantly increased S1/M1 activity or shifted motor cortex representation.[82]

Peripheral nerve electrical stimulation as a substitute for lost sensory input

PLP and the high levels of mental and physical fatigue suffered by amputees using a prosthesis and their reduced

confidence and speed of walking are considered to result from the lack of sensory information about motion and interactions with the ground results.[83] One hypothesis is that somatosensory feedback through a prosthesis may reduce PLP while increasing the functionality of the limb with a prosthesis. Prostheses have been tested that provide electrocutaneous feedback to a patient's thigh whenever the foot and toes of the prosthesis touch the ground. This results in reduced PLP after two weeks of training and is associated with increased functional use of the prosthesis, including walking longer distances, more stable walking, improved posture, and increased patient satisfaction with prosthesis use.[84] It has also been found that electrical stimulation of the tibial nerve via intraneural stimulation electrodes[85] provides tactile information to amputees. This stimulation increases walking speed and self-reported confidence while simultaneously decreasing mental and physical fatigue.[86]

While biomimetic electrical stimulation frequency modulation is perceived as a more natural sensation, amplitude modulation results in better task performance,[87] and combining frequency and amplitude neuromodulation improves functional accuracy and gross manual dexterity while simultaneously reducing the development of phantom limb sensations, such as telescoping.[87] This is important because, clinically, telescoping limbs are negatively associated with positive clinical outcomes following PLP interventions that benefit amputees with non-telescoped limbs.[88] However, efforts are still necessary to identify an encoding strategy that elicits natural and effective perceptions for prosthesis control.

Transcutaneous electrical nerve stimulation (TENS) is another technique tested for its ability to reduce PLP and reverse the loss of afferent input to the cortex. TENS,[89] direct stimulation of the nerve stumps of the limb manifesting PLP,[90] reduces the pain levels, although long-term pain relief has not been achieved.

TENS potentially acts by blocking the direct or indirect activation of afferent C fibers. It may also act by reversing/modifying amputation-induced CNS remapping.[91] In contrast to the limited effect of cortical stimulation in reducing pain, it is more effective to modulate DRG neurons' electrical activity by electrically stimulating them directly.[77] Selective radiofrequency stimulation of individual DRG neurons results in 60–90% pain relief in areas innervated by the stimulated neurons.[78] However, a meta-analysis indicates that the evidence from most studies is of very low quality and does not provide confidence for the efficacy of TENS.[92]

These data indicate that peripheral nerve electrical activity underlies some component of PLP.[34] However, blocking peripheral nerve activity does not reduce PLP in all patients.[54] This indicates that some of the origins of PLP may involve issues associated with amputation-induced CNS changes.

Surgical removal of neuromas

Neuromas are a major PNS trigger for the development of PLP.[93] Symptomatic neuromas are associated with 4.2% of patients with chronic PLP.[94] The development of painful neuromas in amputees results in the reduced use of prosthetics and the increased administration of pharmacotherapies leading to unacceptable side effects of tricyclic antidepressants and long-term narcotics, psycho-social impairment, and the diagnosis of stigmatizing chronic pain.[95] To avoid these consequences and to reduce chronic neuropathic pain, surgery is extensively used to remove painful neuromas.[96] However, although the pain is reduced in 30–50% of patients, persistent pain returns to 42% within one year, which may be less intense, at its original level, or more severe than before the resection.[97] Neuroma reoccurrence is associated with redeveloping symptomatic and asymptomatic neuromas.[98]

Blocking neuroma formation—conduits

Clinically onset of PLP can be delayed,[99] and neuropathic pain in rats is significantly reduced[100] if, at the time of amputation, the exposed nerve stumps are secured in an empty collagen tube or the nerve is secured inside an epineurial graft.[101] However, as with all other techniques, these wraps provide only temporary pain relief due to slowing neuroma reformation and pain redevelopment.[99]

Targeted muscle reinnervation

One approach increasingly used to reduce or delay the formation of painful neuromas is implanting nerve stumps into various tissue target tissues. The most effective target is a denervated muscle, a technique called targeted muscle reinnervation (TMR)[102] or regenerative peripheral nerve interface (RPNI).[103] The concept is that the axons will reinnervate muscle fibers leading to the cessation of pain. The initial applications of TMR involved implanting nerve stumps into intact and innervated muscles. However, such applications have poor reliability and limited efficacy because most of the target muscle fibers are innervated and incapable of being innervated.[104] Significantly more successful pain reduction is achieved by implanting the nerve into a denervated muscle[97] because the muscle fibers can be reinnervated.[104] However, TMR is significantly more effective in reducing pain, even for amputees, when the denervated muscle target is vascularized.[105]

The efficacy of TMR in reducing the incidence of platelet-rich plasma (PRP) development[102] and the level of PLP[103] is significantly greater when applied at the time of amputation than when delayed. However, TMR applied three weeks after a nerve injury when PLP has already developed is effective in reducing PLP.[106] TMR is more effective than neuromodulator medications in reducing PLP.[107] Thus, it appears that TMR is effective by providing axons a target to innervate and, in effect, restoring physiological continuity and function[97] while preventing neuroma formation[108] and preventing cortical reorganization.[109] However, it is still cautioned that because some PLP is of central origin, TMR may not be as reliable as desired.[105]

Pro-inflammatory mediators and the reduction of PLP

All patients with chronic PLP, CRPS, and chronic neuropathic pain have elevated levels of pro-inflammatory mediators and low levels of anti-inflammatory mediators.[110,111] This suggests that PLP is associated with a chronic pro-inflammatory environment.[110] This concept is supported by the observation that following peripheral nerve injury in

various animal models, there is a proliferation of microglia in DRG and the dorsal horn, and the sensitization of spinal cord dorsal horn neurons.[112] Those microglia release the pro-inflammatory cytokines IL-1 and TNF, and there is the development of inflammation and increased levels of neuropathic pain.[36] This, in turn, suggests that, conversely, by administering steroids and NSAIDs, it should be possible to eliminate chronic inflammation and reduce pain.[113] However, they are no more effective than an anesthetic nerve blockade,[114] and their efficacy is short-lived.[115] Further, a recent clinical study found that the administration of anti-inflammatory drugs prolongs, rather than shortens, the process of pain elimination and that pain is more rapidly resolved by promoting inflammation.[116]

Chronic inflammation stimulates neutrophil production and increases neutrophil numbers in the blood.[117] Focal injury-induced inflammation induces macrophage recruitment,[118] where the macrophages release the chemokine IL-8,[119] which, in turn, recruits the up-regulated circulating neutrophils.[120] These neutrophils are critical for modulating and resolving inflammation, wound healing, and tissue repair.[121] Their up-regulating pro-inflammatory gene expression enhances inflammation, which reduces pain.[116] This role is confirmed by a study showing that the development of chronic pain is prevented by neutrophil activation causing an acute inflammatory response.[116]

Part of the influence of neutrophils is by releasing TGF-β.[122] This can exert a potent anti-inflammatory influence by transforming a wound site from a pro- to an anti-inflammatory site.[123] This can be by the short-term action of TGF-β1 activating RhoA, while long-term exposure inactivates RhoA. This is because brief TGF-β1 treatment stimulates macrophage invasion and the induction of further inflammation, while longer exposure suppresses inflammation by inhibiting lipopolysaccharide (LPS)-induced macrophage chemotaxis[124] and thereby preventing their release of the pro-inflammatory mediators TNF-α, IL-1α, and IL-1β,[125] thereby reducing the induction of inflammation.[124] Thus, physiologically, enhancing inflammation leads to an ultimate reduction in inflammation.

A similar reduction in inflammation can also be induced by administering anti-inflammatory cytokines such as IL-4, IL-10, IL-11, IL-13, and TGF-β.[126] The application of IL-10 and TGF-β to an inflammation-associated pain site reduces the inflammation and produces immediate pain relief.[127] IL-10 and TGF-β are uniquely suited for this role because they down-regulate the expression and production of pro-inflammatory mediators, including IL-1β, IL-2, IL-12, IL18, interferon (IFN)-γ, and TNF-α.[128] In addition, they reduce inflammation by blocking the receptors for the pro-inflammatory cytokines IL-1, IL-6, IL-8, IL18, TNF-α, chemotactic cytokines motif ligand (CCL): CCL2, CCL3, CCL7, and chemokine (C-X-C motif) ligand 10.[129] IL-10 also reduces inflammation by up-regulating the release of endogenous anti-inflammatory cytokines[130] while inducing the expression and release of the anti-inflammatory mediator IL-1 receptor agonist 6 (IL-1ra6), which blocks IL-1β-mediated pain.[131] Thus, eliminating chronic inflammation may facilitate eliminating chronic neuropathic pain.[132] As mentioned previously, administering IL-10 and IL-4 blocks PLP by down-regulating the synthesis of the Na(v) channels, which silences chronically electrically active nociceptive.[133] However, pain suppression is not long-lasting because IL-10 has a short half-life.[127]

Inflammation-associated pain can also be rapidly reduced by applying IL-10 to an inflammatory site.[127] This reduces pain by up-regulating the expression of both the IL-1ra and TNF-α receptors, which decreases the availability of pro-inflammatory cytokine proteins,[134] and by blocking adenosine-induced neutrophil release of oxygen radicals.[135] However, an alternative approach in rats is intrathecal administration of viral gene therapy of plasmid DNA encoding for IL-10.[136] This continuous administration of IL-10, and continuous suppression of the pro-inflammatory mediators IL-1 and TNF, reduces rat pain behavior,[137] although the relief is only short-lived.[138] However, non-viral IL-10-induced gene therapy provides long-term relief.[139] Unfortunately, such gene therapy cannot presently be applied clinically, although its application may eventually be useful. Thus, IL-10 intrathecal infusion reduces pain[136] by exerting interrelated anti-inflammatory and analgesic influences.[140]

PRP

As mentioned, clinically[99] and in a rat model,[100] PLP is temporarily reduced by securing nerve stumps in an empty collagen conduit or wrapping the nerve stump in an epineurial graft.[101] This raises the question of whether this pain reduction can be made complete and permanent by elaborating on these techniques by adding multiple factors to conduits.

While applying IL-10 and gene therapy to induce IL-10 production reduces pain, the efficacy is short-lived. However, this reduction becomes long-term when LI-10 is combined with other factors. Thus, the analgesic influences of IL-10 and IL-4 are greater than biologics, which inhibit only single pro-inflammatory mediators. This is because both IL-4 and IL-10 block the production and release of multiple pro-inflammatory mediators, including chemokines, proteases, cytokines, and reactive oxygen species (ROS). The increased efficacy is by them acting through different mechanisms, with IL-4 inhibiting glial cell proliferation[141] and increasing the degradation of pro-inflammatory cytokine mRNA, while IL-10 primarily inhibits transcription.[142] Further, although IL-4 and IL-10 separately reduce pain, each exerts only limited analgesia,[143] and when combined, the increased level of analgesia is not of clinical significance.[144] This is partly because they are relatively small proteins that are cleared rapidly. However, when IL-10 and IL-4 are combined into a single IL4-10 fusion protein, a larger molecule is created, which has a longer time of bioactivity.[143] Thus, in two animal models, multiple intrathecal injections of IL4-10 result in the complete and permanent reduction of persistent inflammatory hyperalgesia.[143] Although the IL-4-10 fusion protein has not been tested clinically, these data suggest that combining IL-10 with other factors should induce a significant and possibly permanent reduction/elimination in chronic neuropathic pain.

Clinically testing the efficacy of multiple factors, such as cytokines, is extremely difficult due to the Food and Drug Administration (FDA) regulations. However, a readily available source of a complex physiological cocktail of potentially effective factors is platelets, which appear to contain and

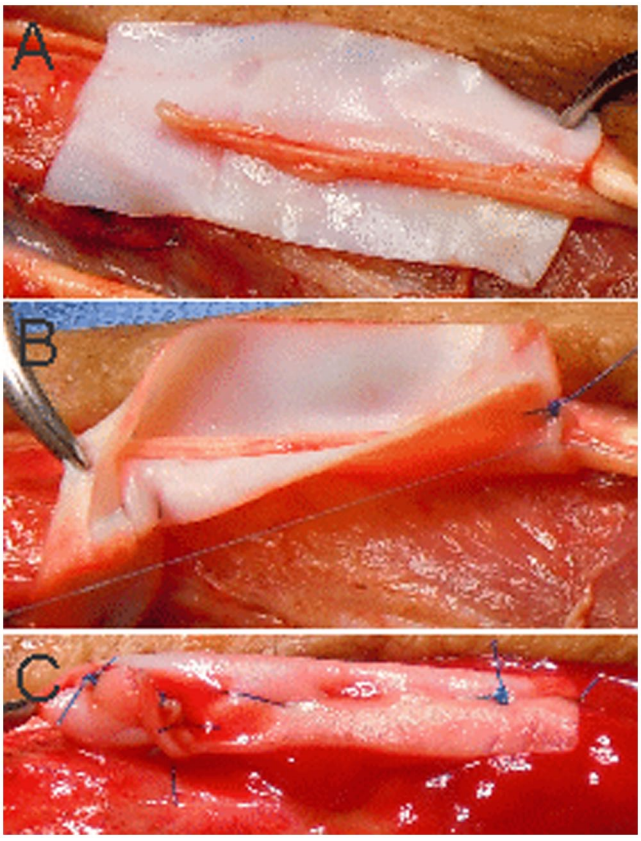

Figure 1. Technique applied to a patient one year after nerve trauma suffering from chronic excruciating neuropathic pain. (A) Refreshed central nerve stump laid on a collagen sheet. (B) Sewing the collagen sheet into a closed-ended tube. (C) Completed closed-ended collagen tube filled with autologous PRP.

release all the factors required to trigger all the cellular and molecular changes necessary for inducing long-term pain elimination.[145] Thus, PRP-released factors should induce a permanent transition of nerve injury sites from chronic pro-inflammatory to permanent anti-inflammatory.[146] This hypothesis is supported by clinical studies showing that chronic neuropathic pain is reduced by injecting PRP under a nerve perineurium,[147] directly into a digital nerve,[148] and when applied to the median nerve at the proximal edge of the carpal tunnel.[149]

A case study reported that inserting the stump of a nerve evoking chronic neuropathic pain into a PRP-filled collagen tube eliminates the pain permanently (Figures 1 and 2).[150] The pain began to decrease rapidly during the first two weeks, was eliminated within two months, and did not return during 1–12 years of follow-up.[151] Although these data do not directly address whether the application of PRP to nerve stumps of amputees might prevent the development of or the reduction/elimination of existing PLP, they suggest it will be effective and should be tested.

What underlies the efficacy of PRP?

The efficacy of PRP is ascribed to platelet releasing the major anti-inflammatory cytokines, including IL-4, IL-10, IL-11, IL-13, and TGF-β1[152]; hepatocyte growth factor (HGF); and the anti-inflammatory mediator IL-1ra6.[153] Platelet-released HGF exerts an anti-inflammatory action by preventing

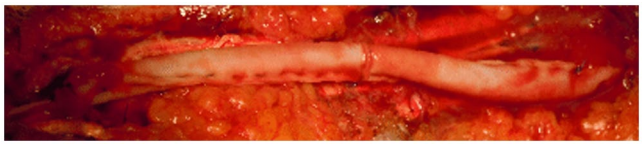

Figure 2. Repair of a nerve evoking chronic neuropathic pain with a 16-cm nerve gap. The gap was bridged with a sensory nerve graft within a PRP-filled collagen tube. The pain was permanently eliminated.

monocyte-like cell chemotaxis, such as of inflammatory T cells and macrophages, and suppressing the expression of both regulated upon activation, normal T cell expressed and secreted and monocyte chemoattractant protein.[154]

The high concentration of platelets released TGF-β1[155] which inhibits monocyte TNF-α expression and release[156] while promoting further TGF-β1 expression.[157] It also suppresses pro-inflammatory cytokine production by inhibiting macrophage and Th1 cell activity by blocking the actions of IL-1, IL-2, IL-6, and TNF-α.[133] TGF-β1 also reduces pain by exerting a prolonged anti-inflammatory effect on microglia/macrophages,[158] inhibiting the production of microglia ROS during their activation or reactivation[159] and activating antioxidant response elements (AREs).[160] In addition, platelet-released factors convert macrophages from a pro- to an anti-inflammatory phenotype[146] while blocking macrophage production of NO,[152] which is involved in the final common neuropathic pain pathway.[161]

Platelet-released IL-10 reduces inflammation and pain by down-regulating the expression of genes for pro-inflammatory cytokines,[128] blocking the receptors for the pro-inflammatory cytokines,[129] up-regulating the release of endogenous anti-inflammatory cytokines,[130] and inducing the expression and release of the anti-inflammatory mediator IL-1ra6.[131] However, its efficacy is short-lived.[127] While in rats, short-term pain relief is induced by the viral induction of IL-10,[138] long-term pain reduction is achieved by intrathecal administration of non-viral IL-10-induced gene therapy,[139] which reduces the expression of the pro-inflammatory mediators IL-1 and TNF.[137] While promising, such gene therapy is not yet permitted clinically. However, although the analgesic efficacy of IL-10 alone is generally short-lived, long-term pain reduction/elimination may be possible when it acts synergistically with other factors. Thus, platelets provide a potentially ideal physiological cocktail of such factors.[162]

While the application of PRP induces the long-term transition of chronic pro-inflammatory sites to permanent anti-inflammatory sites,[133] platelet-released factors also trigger all the cellular and molecular changes required to induce that wound healing and long-term pain elimination.[145] These capabilities suggest that applying PRP may induce long-term reduction/elimination of PLP via the synergistic actions of platelet-released factors.[145] Although studies are required to test the efficacy of PRP in reducing/eliminating PLP, the long-term pain relief provided by PRP suggests it is via the sequential/simultaneous actions of multiple platelet-released factors.[145]

Conclusions

Following amputations, up to 90% of amputees suffer chronic PLP. Although various techniques induce a short-term PLP

reduction, none induce a long-term effect, and therefore, novel effective techniques are required. This review discusses the efficacies and limitations of techniques presently used to reduce PLP. It concludes with a discussion of studies showing that novel application techniques of PRP can induce the permanent reduction/elimination of chronic neuropathic pain and may induce similar influences on PLP.

AUTHORS' CONTRIBUTIONS

DPK conceptualized and wrote the article.

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