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Review Article

Stem cell therapy for neuropathic pain treatment

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Abstract

Pain initiated or caused by a primary lesion or dysfunction in the nervous system is defined as neuropathic pain.

About 75 -150 million people in the United States are suffering for chronic pain disorder. Neuropathic pain has a great impact on the human wellbeing. It is very debilitating and often has an associated degree of depression that contributes to decreasing the quality of life. Moreover, the management of chronic pain is costly to the health care system. Pain is a national healthcare priority in US: the United States Congress has declared the present decade (2001-2010) as the "Decade of Pain Control and Research".

Neuropathic pain is a very complex disease, involving several molecular pathways. Due to its individual character, its treatment is extremely difficult. Current available drugs are usually not acting on the several mechanisms underlying the generation and propagation of pain.

Nowadays, pain research is focusing on newer molecular ways, such as stem cell therapy, gene therapy, and viral vectors for delivery of biologic anti-nociceptive molecules. These methods could provide a new therapeutic approach to neuropathic pain relief.

Key words; neuropathic pain, stem cell therapy, gene therapy, virus vector

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Pathophysiology of Neuropathic Pain

Neuropathic pain is defined as pain initiated or caused by a primary lesion or dysfunction in the nervous system [1, 2], and several clinical symptoms are associated with it [3]. Most common are hyperalgesia (an increased response to a stimulus which is normally painful; patients with hyperalgesia perceive pain spontaneously) and allodynia (pain as a result of a stimulus which does not provoke pain; patients with allodynia do not feel constant pain, in fact in the absence of a stimulus there is no pain) [4]. Neuropathic pain can be triggered by central or peripheral nerve injury. Changes in the spinal cord or in the peripheral nerve, but also in the brain, have been reported, although these molecular alterations are still far to be clarified. Nociceptive signalling terminates in the spinal cord, the first centre involved in the controlling and processing of pain transmission. Indeed, in the dorsal horn of the spinal cord nociceptive afferent fibers terminate where the nociceptive neurons are located in the superficial lamina I (marginal layer) and in the lamina II (substantia gelatinosa). Interactions between nociceptive and non-nociceptive afferent pathways control the transmission of nociceptive information to higher centres in the brain [5].

Due to nociceptive input, such as peripheral nerve injury, the spinal cord anatomical structure is subjected to a re-organization. Indeed, the myelinated primary afferent fibers sprout into lamina II of the dorsal horn, establishing synaptic contacts with second-order neurons. In this way, they help to conduct the allodynic transmission [6].

Another change is a phenomena called "wind-up", a condition of central sensitization resulted from severe and persistent injury. In this condition, C-fibres are frequently sped on, releasing glutamate, and the response of the neurons of the dorsal horn spinal cord progressively increases [7, 8].

Glutamate is the major nociceptive excitatory neurotransmitter released from A-delta and C-fibres. Once released, glutamate is able to evoke fast synaptic potentials in dorsal horn neurons by activating the pre- and post-synaptic glutamate receptors. Among them, the ionotropic NMDA receptor is most involved in the events correlated with nociception [9], and with the maintenance of central sensitization and hyperexcitability of dorsal horn neurons. Activation of NMDA receptors increases the concentration of the calcium ion by the indirect activation of protein kinase C [10].

In the brain, the insular cortex is directly involved in the pain modulation. In this area, anti-nociceptive response is increased by the GABA neurotransmission [11]. In particular, there is evidence that GABAa receptors modulate the nociceptive threshold affecting the noradrenergic bulbo-spinal projections from the insular cortex to the locus coeruleus, and GABAb receptors modulate the projections from cortex to amygdala [11].

Is the neuropathic pain a complete disease and not only the result of an other syndrome or injury? Interesting, newer molecular studies support this idea. Changes in DNA expression in the neuropathic pain syndrome have been observed. In response to peripheral noxious stimuli, dorsal horn neurons over-express the immediate early genes encoding transcription factors, such as c-jun and c-fos. These genes could be involved in cell death induction via a long-lasting cascade of transcriptional processes [12]. Indeed, the apoptotic genes mRNA expression levels of the bcl-2 cell death-associated family in the lumbar dorsal horn of the spinal cord of neuropathic rats are modified by peripheral nerve injury [13].

Following nerve injury, the afferent neurons (injured sensory neurons and their uninjured neighbours) close to the site of the injury increase their level of firing. This massive activity is called ectopic discharge, and it has also been proven in humans with neuropathic pain [14]. Altered expression of several types of sodium channels is responsible for the ectopic

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firing after nerve injury, such as the voltage-gated sodium channels [15, 16]. The mechanisms responsible for the changes in the channel expression are not yet clear. Involvement of the neurotrophin (such as NGF, GDNF) supply has been suggested as a possibility [17].

The calcium channels may also contribute to the induction of hyperalgesia and allodynia [18].

After peripheral nerve injury, sprouting of collateral fibres from sensory axons in the skin into denervated areas has been observed [19, 20]. Neurotrophic factors and several cytokines, such as interleukin-1 (IL-1) and tumour necrosis factor-alpha (TNF-alpha), may be involved in the sprouting formation and in pathophysiology of neuropathic pain [21, 22].

Classical pharmacological treatment

Pain has a very complex nature. Nowadays there are not drugs for the neuropathic pain treatment acting in a complete and definitive way.

Currently, lidocaine, lamotrigine, acetaminophen, dextromethorphan, carbamazepine, gabapentin, valproic acid, opioid analgesics, tramadol hydrochloride, and tricyclic antidepressants are used for the classical pharmacological treatment of neuropathic pain.

Clinical research is studying new direct-acting compounds to sodium and calcium channels since the ability of these channels to contribute to the development of neuronal hyperexcitability and the production of pain-associated behaviour. Lidocaine, a sodium channel blocker, is effective in the pain relief [23], however, the available blockers are not specific between the several types of sodium channels. A private company is developing a new sodium channel blocker, Ralfinamide, for the potential treatment of neuropathic pain [24, 25].

Specific antagonists for the neuronal calcium channel are able to reduce heat hyperalgesia and mechanical allodynia in a pain model, the chronic constriction injury of the sciatic nerve, if administered locally on the site of nerve injury [17]. More interesting, reduction of neuropathic pain associated with spinal cord injury in humans has been shown with intrathecal ziconotide, a marine-derived peptide [26].

Calcium flux is decreased by activation of the cannabinoid receptor subtype 1. The synthetic cannabinoid CB1 receptor agonist Win 55,212-2 decreases neuropathic pain behaviour, such as thermal hyperalgesia and mechanical allodynia [27].

As mentioned above, the main nociceptive neurotransmitter is glutamate. Inflammation and central sensitization are also controlled by NMDA glutamate receptors. NMDA receptor antagonists are able to attenuate neuropathic pain. Indeed, the NMDA receptor antagonist MK-801 has a potent anti-nociceptive effect [28, 29, 30], but due to its high toxic properties and low safety margins it is not available for clinical use on human patients. Nevertheless, amantadine, dextromethorphan, ketamine, and memantine are commercially available NMDA-receptor antagonists. The opioids methadone, dextropropoxyphene and ketobemidone are also NMDA-antagonists, as well as the triciclic antidepressant amitriptiline [31, 32]. NMDA-receptor antagonists in combination with opioids might represent a new class of analgesic and might have potential as a co-analgesic; NMDA-receptor antagonists help to enhance development of tolerance to opioid analgesics [33].

In pain transmission, glutamate activates also group I metabotropic receptors (mGluRs). Peripheral and central mGluR5 receptors are responsible of the nociceptive transmission observed during post-operative pain [34]. MPEP, the potent and selective antagonist for metabotropic glutamate receptor subtype 5 (mGlu5), is able to prevent the development of thermal hyperalgesia, transiently reduce mechanical hyperalgesia in neuropathic rats, and prevent the over-expression of pro-apoptotic genes in dorsal horn spinal cord

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neurons [3]. This subtype of metabotropic glutamate receptors could represent the prototype of new potential drugs in pain treatment; however, due to the complex role of glutamate in the nervous central system, blockade of glutamate receptors is associated with several side effects.

The typical mu-opioid analgesics, such as morphine, can be relatively ineffective in treating neuropathic pain since different opioids can produce analgesia by affecting different pain pathways [35].

Likely, the optimal classical drugs in the treatment of neuropathic pain are the anticonvulsant gabapentin, and its successor pregabalin [36, 37, 38, 39]. They are able to decrease the hyperexcitability of dorsal horn neurons induced by tissue injury, but their mechanism of action is still unclear. Interesting, they have only an effect in a condition of sensitization of a nociceptive pathway.

Molecular methods for neuropathic pain treatment

Newer molecular methods, such as gene therapy and viral vector for the delivery of biologic anti-nociceptive molecules, could represent a novel therapeutic approach to the neuropathic pain treatment [40].

Following peripheral nerve injury, spinal re-organization and changes in the excitatory or inhibitory pathways controlling neuropathic pain development are correlated with altered gene expression. Novel molecular pharmacological strategy is directed toward the control of the gene up- or down-regulation. Antisense knock-down strategy could represent a novel approach to the neuropathic pain therapy in the nearest future. As next step, antisense research has to elucidate the pharmacodynamics, pharmacokinetics and distributions of antisense oligonucleotides.

Among the genes showing altered expression in neuropathic pain, several sodium and calcium channels contribute to the hyper-responsiveness of dorsal horn sensory neurons and to hyperalgesia and allodynia [16, 41, 42, 43, 44, 45, 46]. Gene silencing by the use of antisense oligonucleotides, a novel molecular pharmacological approach, causes a decrease in pain-related behaviour.

Nicotinic receptors, P2X receptors, 5-HT1A receptors, NMDA glutamate receptors and opioid receptors have been successfully used as target for antisense knock-downing strategy, showing a decrease in nociceptive behaviour [47, 48, 49, 50, 51, 52].

Immediate early genes, such as c-fos, are over-expressed in dorsal horn neurons of the spinal cord after peripheral nerve injury. Also in this case, intrathecal administration into the lumbar region L1-L5 of c-fos antisense oligonucleotides has shown a role played by the c-fos gene in neuropathic pain [53].

Viral vector technology to delivery anti-nociceptive molecules could represent a novel therapeutic strategy. Dorsal root ganglion neurons transduced with replication-incompetent herpes simplex virus (HSV-) based vector, encoding the GAD67 isoform of human glutamic acid decarboxylase, are able to produce GAD and release GABA, reducing neuropathic pain following a spinal cord injury [54]. Constitutive GABA expression via recombinant adeno-associated virus producing GAD65 attenuates neuropathic pain [55]. It has been demonstrated that virus encoding human pre-proenkephalin (hPPE) are able to decrease the activation-levels of nociceptors by capsaicin treatment in mice and macaques [56].

Coupling antisense knock-down and viral vector technology is showing promising results. Virus delivering antisense cDNA versus calcitonin gene-related peptide precursor (ACGRP) decreases C-fiber hyperalgesia due to the application of capsaicin on the skin in mice [56].

Potentially, all the molecules, such as neurotrophines, having nociceptive effects could be delivered by adenovirus. Candidate gene products include directly analgesic

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molecules, as well as molecules that are able to interfere with pain-associated biochemical changes in pain pathways. Recombinant adenovirus encoding NT-3, BDNF, GDNF, or

Semaphorin3A into animal models of neuropathy showed good results for neuropathic pain relief [57, 58, 59, 60, 61, 62]. Intrathecal delivery of the adenovirus-mediated IL-2 gene has a relatively long anti-nociceptive effect [63].

Non-invasive gene delivery systems could be usefully used for targeting peripheral nervous system pathologies. Subcutaneous peripheral injection of plasmid DNA complexed with a non-viral cationized gelatin (CG) vector led to transgene expression in rat lumbar dorsal root ganglia [64].

Stem cell therapy

Nowadays, stem cell therapy represents the great promise for the future of molecular medicine. Several diseases can be slowed or even blocked by stem cell transplantation. Stem cells could be neuroprotective in a variety of nervous system injury models. As neurodegenerative disease, also neuropathic pain undergoes to stem cell therapy [40], even if the state of the art is still poor of basic and clinical research.

Marrow mononuclear cells containing mixed stem cell populations have been intravenous used in neuropathic rats showing recovery from pain [65].

Stem cell implantation could be a possible solution for spinal cord injury. Stem cells have the ability to incorporate into spinal cord, differentiate, and to improve locomotor recovery [66].

Despite ethical problems, it has been demonstrated that human embryonic neural stem cells can promote functional corticospinal axons regeneration and synapse reformation in the injured spinal cord of rats. The action is mainly through the nutritional effect of the stem cells on the spinal cord. Transplanted cells were found to migrate into the lesion, but not scatter along the route of axon grows. The cells differentiated into astrocytes or oligodendrocytes, but not into the neurons after transplantation [67].

Spinal progenitor cells intrathecally transplanted in neuropathic rats are able to alleviate neuropathic pain [68]. Murine neural stem cells (NSCs) homografted onto the injured spinal cord improved motor behaviour [69].

How do stem cells work? Stem cells transplanted following spinal cord injury are able to reduce allodynia and improve functional recovery if they produce more oligodendrocytes than astrocytes [70]. Serotonergic neural precursor cell grafts are able to reduce hyperexcitability caused by spinal injury [71]. Neuropathic pain causes a decrease in the number and activity of GABAergic neurons, the spinal progenitor cells show glutamic acid decarboxylase immunocompetence, in this way they can supply the decreased GABA profile [70, 72].

Is the stem cell differentiation the key for the pain care? Or do they provide several molecules with analgesic action? Indeed, using of genetically engineered stem cells expressing anti-nociceptive molecules or trophic factors seems to be an useful tool in neuropathic pain relief. Stem cells could be used as biologic "minipumps" to chronically deliver anti-nociceptive molecules close to the pain processing centers or the sites of injury [73, 74].

Besides genetic engineering, stem cells applied to the site of the injury could provide trophic factors directly in situ, by this way acting as anti-nociceptive drug.

Among the stem cell population, mesenchymal stem cells (MSCs) rise probably best potential good results in pain-care research. These cells are a population of progenitor cells of mesodermal origin found in the bone marrow of adults, giving rise to skeletal muscle cells, blood, fat, vascular and

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urogenital systems, and to connective tissues throughout the body [75, 76]. MSCs show a high expansion potential, genetic stability, stable phenotype, can be easily collected and shipped from the laboratory to the bedside and are compatible with different delivery methods and formulations [77]. In addition, MSCs have two other extraordinary characteristics: they are able to migrate to sites of tissue injury and have strong immunosuppressive properties that can be exploited for successful autologous as well as heterologous transplantations [78]. Besides, MSCs are capable of differentiating into neurons and astrocytes in vitro and in vivo [79]. Recently, MSC injection has shown good results for amyotrophic lateral sclerosis treatment in human [80]. They are able to improve neurological deficits and to promote neuronal networks with functional synaptic transmission when transplanted into animal models of neurological disorders [81].

MSCs have been observed to migrate to the injured tissues and mediate functional recovery following brain, spinal cord and peripheral nerve lesions, suggesting that MSCs could modulate pain generation after sciatic nerve constriction [82], although the underlying mechanisms by which MSCs exert their actions on pain behavior is still to be clarified.

We are currently studying the use of human mesenchymal stem cells (hMSCs) for neuropathic pain treatment in rodents. hMSCs micro-injected into specific nuclei involved in pain processing were able to completely abolish pain-like behaviour in neuropathic mice (Siniscalco, 2007, unpublished data).

Recently, Dr Stephen Richardson of the University of Manchester has developed, under patent, a cell-based tissue engineering approach to regenerate the intervertebral disc at the affected level in the low back pain (www.ls.manchester.ac.uk/ukctr). This is achieved through the combination of the patients' own mesenchymal stem cells and a naturally occurring collagen gel that can be implanted through a minimally-invasive surgical technique. Hopefully, once implanted the differentiated MSCs would produce a new tissue with the same properties as the original and would both treat the underlying cause of the disease and remove the painful symptoms.

Conclusions

Neuropathic pain has a great impact on the quality of life, reducing human wellbeing. Management of chronic pain is very costly to the health care system. Since 75-150 million people in the United States have a chronic pain disorder [40]. The United States Congress has declared the present decade (2001-2010) as the "Decade of Pain Control and Research", making pain a national healthcare priority.

Neuropathic pain involves several molecular pathways and is a very complex disease. It has an individual character, making its treatment extremely difficult. Currently, available treatments address the pain-symptoms using a combination of painkillers. None of these is ideal as they only treat the symptoms and temporal pain properties, not the cause, and are of limited long-term success.

Novel molecular methods, such as antisense strategy, gene therapy, and virus therapy, are acting on the several mechanisms underlying the generation and propagation of pain. More recently, preliminary clinical evidence suggests that stem cell therapy could provide best results, this strategy could be the definitive pain-relief drug for the next future.

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