Review

Murine models of human neuropathic pain

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A B S T R A C T

Neuropathic pain refers to pain that originates from pathology of the nervous system. Diabetes, infection (herpes zoster), nerve compression, nerve trauma, and autoimmune diseases are examples of diseases that may cause neuropathic pain. Unfortunately no satisfactory treatment is yet available for this type of pain. This consideration has led to an explosion of interest for the underlying mechanisms, accompanied by a growing number of animal models. In recent years, most of the neuropathic pain models initially developed in the rat have been translated to mice in order to exploit the resource represented by genetically modified mice. Obviously the most useful animal models of pain would be ones in which the etiology of the pain is endogenous and not induced by the experimenters: together with the classic models based on peripheral nerve ligation, in the last years other techniques are being developed that mimic more closely clinical pain syndromes, often by attempting to induce the disease associated to neuropathic pain. Although several variables must be taken into account when using animal models for mimicking clinical neuropathic pain, the huge number of models that are now reproducible and well characterized should help to reach important goals in the comprehension of mechanisms and to discover novel therapeutic target for this disease.

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1. Introduction

A definition of neuropathic pain useful for both clinical and research purposes is that recently developed by Treede et al. [1]: “pain arising as a direct consequence of a lesion or disease affecting the somatosensory system”. Neuropathic pain can be divided into peripheral or central based on the anatomic location of the lesion or the disease: peripheral nervous system (PNS, e.g. peripheral nerves, dorsal root ganglia (DRG), and dorsal roots) and central nervous system (CNS e.g. spinal cord and thalamus). These injuries arise from diabetic neuropathy, viral infections (Herpes virus, HIV), major surgeries or trauma (amputation, thoracotomy, entrapment or compression), spinal cord injury, and stroke. Examples of neuropathic pain include carpal tunnel syndrome, trigeminal neuralgia, post herpetic neuralgia, radiculopathy, phantom limb pain, complex regional pain syndromes and the various peripheral neuropathies, such as those deriving from chemotherapy. Sensory loss and spontaneous pain together with a sensory gain, such as mechanical allodynia (pain resulting from stimuli that are normally innocuous) are distinct symptoms of neuropathic pain, although this pain is also characterized by heat and mechanical hyperalgesia (increased pain responses to thermal and mechanical stimuli), all of which affect adversely the quality of patients’ daily life [1,2]. Neuropathic pain is a common clinical problem affecting millions people in the USA and Europe, and it has become a major problem since unfortunately it tends to be long-lasting (neuropathic pain often lasts years or even indefinitely) and difficult to manage due to the poor efficacies and severe well-known adverse side effects associated with the current conventional antinociceptive treatments [3]. The search for new drug molecules to alleviate this intractable pain is priority nowadays, and elucidating the molecular mechanisms of neuropathic pain is an important prerequisite for the rational development of novel analgesic drugs for the therapy of this chronic pain.

Neuropathic pain arises from both PNS and CNS causes and many etiologists have been recognized in the human: a partial list is given in Table 1 [4]. Unfortunately the existence of different pathological conditions leading to the development of neuropathic pain makes more difficult the identification of a simple and reliable animal model and explains the huge numbers of models present in the literature.

2. Murine models of neuropathic pain

Animal research must always be evaluated by three general criteria: the generation of knowledge, the ability of the study to be reproduced, the relevance of the study and the predictive validity of clinical pain states. Despite the controversy on whether data from animal models can be applied to humans, this research serves as a valuable source of information in many medical areas. Animal models provide pivotal systems for preclinical studies of neuropathic pain and serve as an experimental basis for mechanistic investigations and testing new therapeutic interventions. Experiments featuring
behavioural measurements of neuropathic pain in animals are becoming more common over time in published papers. Non-human animals cannot self-report, but their behaviours in response to noxious stimuli can be reliably and objectively scored.

Most of animal models of neuropathic pain were generated starting from the late 1980, using rat as preferred species. More recently pain models originally developed in rats have been transposed for use in mice; a strong motive for the use of mice is the availability of genetically characterized or manipulated inbred strains, particularly transgenic mouse lines in which specific proteins or signal transduction component have been altered throughout genetic knockout technology. Although transgenic technologies has transformed basic pain research, allowing the role of individual proteins in pain to be studied even in the absence of selective ligands or antibodies, the complexity of the chronic pain phenomenon has made it difficult to assess the true value of these advances.

In addition, in pain studies more than in other animal models of disease, particular care has to be given to the strains used, since a strong influence of genetic background on pain sensitivity exists.

A common pitfall of all rodent models of neuropathic pain is the inappropriateness of the outcome measures utilized. In fact they focus on stimulus-evoked pain and hyperreflexia at a particular moment in time, whereas a high proportion of patients with neuropathic pain have ongoing, spontaneous pain and sensory loss. The recognition of spontaneous pain in experimental animals is particularly difficult. Weight loss, sleep disturbances, reduced movement, spontaneous paw lifting, scratching or shaking have all been accepted to reflect spontaneous pain [2]. Awareness is leading to the use of more complex measures of integrated pain behaviour including evaluation of neuropathic pain comorbidities, such as the presence of affective component of persistent pain [5], frequently observed in patients. However while this more comprehensive paradigm to evaluate neuropathic pain is increasingly applied to the rat model, this aspect has not been yet taken into enough consideration in the more recently developed murine models.

The numerous models of neuropathic pain in mice can be classified in many different ways. In the present review we divide them into five gross categories:

- central pain models,
- peripheral nerve injury models,
- models of disease-induced neuropathic pain
- iatrogenic (drug-induced) neuropathic pain, and
- inherited neuropathies.

3. Central pain models

These models mimic neuropathic pain resulting from CNS pathologies. Central pain syndromes represent a form of neuropathic pain that is associated with lesions of the brain or the spinal cord after a stroke or other traumatic injury. Stroke is the leading cause of disability in the industrialized world and it is estimated that up to 8% of stroke victims suffer from some form of central post-stroke pain.

3.1. Thalamic syndrome

Thalamic syndrome is a form of central pain that typically results from stroke in the thalamus and is characterized by spontaneous pain, attacks of allodynia, and dysesthesia. The lack of suitable thalamic syndrome models has hampered research into the dysregulated perception of pain resulting from stroke in the thalamus. Therefore, the development and characterization of a rodent model of thalamic syndrome was the first step to discover the underlying mechanisms of this disease and possible therapeutics. Very recently a rat model of this syndrome was developed based on a small hemorrhagic stroke lesion induced by collagenase injection in the ventral posterolateral nucleus of the rat thalamus [6]. Animals displayed hyperesthesia in response to mechanical pinch stimulation, with sensitivity localized in the hind limb and increased thermal sensitivity. This novel model has not been developed in mice yet.

3.2. Spinal cord injury (SCI)

SCI occurs in most countries at an annual rate of 20–40 persons per millions. Following mechanical injury to the spinal cord, a wave of secondary pathological changes occurs and amplifies the extent of the initial damage. Apoptosis is critical in triggering collateral damage after primary injury in SCI. Spontaneous and evoked pain are frequently sequelae of traumatic or ischemic SCI. Several models of central pathologies causing neuropathic pain were raised mainly in rats and mostly based on SCI caused by contusion or weight dropping, spinal cord compression, excitatory neurotoxins, photochemically induced ischemia, spinal cord hemisection, crushing of spinal cord. All these models were adapted for mice [7,8]. As described above, the development of reliable models of neurotrauma in mice provides great promise for evaluating overexpression or inactivation of a gene on lesion pathophysiology and functional outcome.

3.2.1. Contusive models

The spinal cord contusion is the oldest and most widely used model and has been recently employed also in mice; in addition to motor dysfunction, this injury elicits sensory dysfunction including neuropathic pain, such as tactile allodynia and thermal hyperalgesia [9-11]. Several techniques can be applied in order to mimic SCI. Some examples are dorsal column crush surgery [7]; spinal cord completely transected intervertebrally with microscissors inserted between the 9th and 10th thoracic vertebrae [12]; laminectomy at T9 or T5–T6 and compression with a vascular clip (clip compression: simple, reliable and inexpensive model) [13,14].

3.2.2. Excitotoxic models

Intraspinal or intrathecally injection of some excitotoxins, such as quisqualic acid or other excitatory aminoacids (glutamate, N-methylaspartate, kainic acid) have been reported to produce long lasting spontaneous pain, mechanical allodynia and thermal hyperalgesia also in mice [15].

3.2.3. Photochemical model

Over the past two decades, the photochemical model of SCI, developed by Watson et al. [16] has been one of the most reliable and reproducible graded experimental models of SCI in rats [17] widely used in neurotrauma research. This model consists in an intravenous injection of the photosensitising dye, Rose Bengal, and an irradiation of the translucent dorsal surface of the T9 vertebral lamina with a 560-nm wavelength-light for 3–8 min (the beam of a xenon lamp, conveyed by fiber optics). This irradiation induced excitation of the injected dye in the spinal cord microvasculature. The resultant photochemical reaction led to vascular stasis and ischemia. The model is successfully used now also in mice where it was confirmed to
be a reproducible and reliable method of producing controlled, graded ischemic SCI [18].

4. Peripheral nerve injury models

The majority of neuropathic pain models use nerve injury to induce hyperalgesia and allodynia in rodents, which are similar to the symptoms of neuropathic pain in humans. Numerous models have been developed in rodents that generate a mixture of intact and injured fibres within a peripheral nerve, usually the sciatic, for ease of access and its relatively large size. Neuropathic pain behaviour is thought to be attributable to altered properties in the intact rather than the damaged fibres.

Numerous types of nerve lesions have been employed including transection, resection, crush, complete or partial tight ligation, cryoneurolysis, and loose ligation with inflammatory materials. These variations in nerve lesions may be categorized by criteria as follows: the onset, acute or progressive; completeness, entire vs. partial nerve involvement; duration, permanent or regenerative; the injury's inflammatory potential, immune cells recruitment at the site of injury either spontaneous or induced by exogenous stimulus (chronic gut). The type of nerve lesion can clearly influence the character of the ensuing neuropathic behaviours [19].

4.1. Complete lesion

4.1.1. Sciatic nerve (neuroma model)

Phantom limb pain occurs in about 80% of amputees [20], but its intensity, frequency and duration varies widely, even for similar lesions [21]. Following complete nerve transection of the sciatic nerve of rats and mice, a neuroma develops at the proximal nerve stump, consisting of regenerative nerve sprouting in all directions. This lesion results in immediate and irreversible interruption of electrical nerve conduction, followed by Wallerian degeneration of the axons distal to the lesion and sprouting of the proximal axonal stumps in an attempt to regenerate the nerve fiber. Complete lesions (total nerve transaction and ligation) of the sciatic nerve simulating the clinical conditions of amputation are not practical for behavioural studies since they produce motor weakness and, until sufficient regenerations occurs (3–4 weeks), preclude most evoked response testing. In this model self-attack and mutilation of the denervated leg were observed; the label “autotomy” was given to this behaviour, a term commonly used in studies where a form of excessive self-care or self-grooming occurs that results in bite wounds and eventually in the self-amputation of digits. Although autotomy is not a normal response by humans to total limb denervation or chronic pain, it occurs in species ranging from rodents to primates. There have been some controversies on whether or not autotomy behaviour is a sign of spontaneous pain. Similar behaviours can be induced in animals by skin irritations or skin diseases assumed to induce itching. It has been argued that the absence of sensory feedback from the denervated leg may result in the self attack because the animal does not recognize the anesthetic limb as part of its body and therefore autotomy should be considered a consequence of the presence of a numb insensate yet painless limb [22,23]. However there are many reasons for believing that autotomy in rodent reflects ongoing dysesthesia or pain rather than simple numbness. Autotomy responds to drugs known to be effective in treatment of neuropathic pain including local anaesthetics, opiates, benzodiazepines, tricyclic antidepressants and sympathetic agents [24], while drugs such as non-steroidal antiinflammatory drugs that are minimally effective against neuropathic pain in the clinic do not affect autotomy [25]. It has been clearly shown that rendering a rodent limb numb by prolonged period with local anesthetic block does not trigger autotomy [25]. Moreover this response has been recently reported also in other models of neuropathic pain such as the Chung model (see paragraph 4.2.3) [25]. Therefore it is widely assumed now that excessive grooming and autotomy following a nerve lesion reflect chronic spontaneous neuropathic pain [2,26–28]. Autotomy levels vary greatly among different inbred, outbred and selected lines of mice and rats under identical conditions [29–31] demonstrating that genetic factors play a major role in autotomy in these rodents. Identifying a gene having a major effect on autotomy may provide targets for the development of novel analgesics for neuropathic pain in humans [24].

4.1.2. Brachial plexus avulsion in mice

It has been reported that in humans, the brachial plexus avulsion (BPA) induces a constant crushing and intermittent shooting pain that remains without an adequate treatment [32]. This lesion may lead to pathological plasticity of the CNS that is associated with altered pain sensations [33]. The main characteristics of BPA are the rapid onset of pain (an effect which occurs immediately after the trauma) and the long-lasting development of neuropathy, which may be evidenced distant from the site of the lesion, either on the ipsilateral or contralateral side [34,35].

A model of this neuropathic pain was originally described for rats [35] and it was recently adapted to mice, where this avulsion induced long-lasting mechanical and thermal hypernociception [36]. The avulsion of brachial plexus in mice was not associated to autotomy, a process that is frequently reported in several models of neuropathic pain involving the transection of peripheral nerves. Also relevant in this model is the fact that hypernociceptive responses observed following the BPA persisted for a long period and that this effect could be observed at distant sites from the injury, similarly to what happens in the human.

4.2. Partial nerve lesions

Various partial nerve lesions have been developed experimentally to study neuropathic pain. Such models include chronic constriction injury of the sciatic nerve (CCI) [37]; partial sciatic nerve ligation (PSL) [38] and spinal nerve ligation (SNL L5/L6, Chung model) [39,40], which represent three of the best-characterized rodent models of peripheral neuropathy (Fig. 1). These classic models produce similar pain behaviours with some variation in the magnitude [41,42].

4.2.1. Sciatic nerve chronic constriction injury

Sciatic nerve CCI resembles human neuropathy resulting from trauma of peripheral nerves, with some functional preservation of the innervation (entrapment or compression). The model of CCI is one of the most commonly used models because it is reliable and easily reproducible, and due to the presence of the constrictive...
ligatures, it combines nerve compression with Wallerian degeneration and an epineurial inflammatory lesion [37,43,44]. This model in mice is produced by three loose ligatures that are tied around the common sciatic nerve exposed at the level of the mid-thigh, proximal to the trifurcation of the nerve [45]. This procedure results in intraneural oedema, which strangles the nerve, effectively axotomizing many but not all of the nerve axons [46-51]. It results in the development of hyperalgesia to thermal stimulus, as indicated and measured by reduced paw withdrawal latency to heat source. Allodynia to mechanical stimuli also occurs. Moreover in some mice strains autotomy was also observed after CCI, suggesting the spontaneous pain presence.

4.2.2. Partial sciatic nerve injury (PNI)

In contrast with CCI model that consists in the ligation of the total sciatic nerve, Seltzer et al. [38] developed in rat a partial sciatic nerve injury that consists in the tight ligation of only 1/3 to 1/2 of the common sciatic diameter. Malmberg and Basbaum [52] have reported the same mouse model of partial nerve injury (PNI) that has been largely employed to study mechanisms involved in neuropathic pain and new therapeutic treatments [53-56].

Spared nerve injury (SNI) was more recently induced in mice in accordance to procedure described by Decosterd and Woolf [57] in rats, with minor modifications [58,59]. The three peripheral branches (sural, common peroneal, and tibial nerves) of the sciatic nerve were exposed without stretching the nerve structures. Both tibial and common peroneal nerves were ligated and transected together. Some authors developed a mouse model of partial sciatic nerve injury following the ligation only of the common peroneal nerve [6,60] or only of the tibial nerve [59] and have showed the mechanical allodynia development, beginning 3 days after surgery and lasting 4 weeks. In the whole, mice subjected to PSI showed motor paralysis immediately after surgery but recovered in a few days, so the PSI models with less severe motor paralysis are likely to be more useful than CCI.

4.2.3. Spinal nerve ligation (SNL)

Another experimental mononeuropathy model simulating human causalgia was developed by Kim and Chung [39]: it consists of ligating unilaterally and tightly spinal nerves L5 and/or L6 of rodents at a location distal to the dorsal root ganglia and results in behavioural signs that are similar to the symptoms of human neuropathic pain, namely increased sensitivity to heat and painful responses to otherwise non-painful mechanical stimuli [30]. Allodynia and hyperalgesia develops quickly after ligation and last for at least 4 months. Although there are behavioural signs of spontaneous pain (guarding, licking, and lifting of ipsilateral hind paw), autotomy is absent in the spinal nerve ligation. Compared to CCI and PSI, the spinal nerve ligation induces a more extensive and relevant damage and has the advantage of having separate injured and intact spinal segments.

On the other hand, SNL requires the most extensive surgical procedures of the three models. There have been only a limited number of investigations of L5/L6 SNL, probably due to the relatively difficult surgical operation. However, Kiso et al. [61] have recently developed an L5/L6 spinal nerve ligation model for neuropathic pain also in mice that showed mechanical allodynia beginning on day 1 and lasting for at least 2 months following surgery. They have validated this model with a pharmacological approach and with the analysis of the gene expression profiling in dorsal root ganglia.

Ligation of L4 or L5 spinal nerve was also reported. Ligation of L4 spinal nerve is not a useful pain model, since it has an abundance of motor fibers and therefore causes severe motor deficits that interfere with behavioural tests [39]. The L5 ligation model has not been fully characterized yet but induces long lasting hyperalgesia and mechanical allodynia. Since single L5 nerve ligation is much easier to perform than L5/L6 ligation, this model provides a useful option especially in studies involving mice [15,62-64].

4.2.4. Mouse model of neuropathic pain following photochemically induced ischemia in the sciatic nerve

The ischemia was induced by unilateral irradiation of the sciatic nerve with an argon ion laser after intravenous administration of photosensitizing dye, erytrosin B. The partial nerve injury resulted in a prolonged significant decrease in withdrawal threshold of the hindpaws to mechanical stimulation with von Frey hairs as well as prolonged increased responsiveness to cold and heat stimulation. This model is characterized by ischemic origin with minimal mechanical trauma, unlike the models of partial nerve injury, which are primary based on mechanical injury, thus the nerve injury produced is more consistent and reproducible [65]. Moreover it is also important to note that ischemia constitutes an important factor in human peripheral nerve injury and neuropathy in conditions such as carpal tunnel syndrome and diabetic neuropathy.

4.2.5. Polyethylene cuff

A recent murine model of neuropathic pain was developed by Benbouzid et al. [66]. Neuropathy was induced by inserting a polyethylene cuff around the main branch of the right sciatic nerve: it induces a long-lasting mechanical allodynia (2 months) and hyperalgesia to a hot thermal stimulus lasting 3 weeks [67].

4.2.6. Partial injury of the nerve supplying the tail

Back et al. [68] induced peripheral neuropathy in mice by a partial injury of the nerve supplying the tail. The left superior caudal trunk was exposed and transected at the level between the S3 and S4 spinal nerves, and piece of the trunk, about 1 mm, was removed from the proximal end. This surgery eliminated the S1–S3 spinal nerve innervation of the tail via the superior caudal trunk. Partial injury of the nerve supplying the tail produced a robust and consistent increase of mechanical, cold and warm sensitivities (mechanical and thermal allodynia) in the tail. Differently from other mouse models of partial injury of the nerves supplying the hind paw, where the deformity of the foot following the nerve injury makes it difficult to conduct the behavioural tests for neuropathic pain and impossible to perform blind behavioural tests, this tail model can avoid these problems. The application of both mechanical and thermal stimuli to the tail is straightforward and in addition the lack of deformity in the tail after the nerve injury easily allows blind behavioural tests. Surgical procedures for this model are so simple that mice can be easily used; in fact although some of the previous techniques developed for rats can be applied to the mouse, they are quite invasive approaches in the smaller rodent.

4.2.7. Partial saphenous nerve injury in mouse

Recently a partial injury of the saphenous nerve (a purely sensory nerve) was described in the rat, resulting in mechanical allodynia and cold and heat hyperalgesia [69]. This lesion has several advantages over sciatic injury, including improved animal welfare because no motor damage occurs, ease of surgical access and thus a reduction in surgical trauma, and that is highly amenable to pharmacological intervention. Although the mouse saphenous nerve is fine and friable, this model was translated into the mouse and demonstrated that it can be used for the study of primary afferent properties. It has advantages over others for several reasons: it is surgically simple to perform, produces clear, reproducible neuronal damage but also leaves intact neurons available for electrophysiological study and improves animal welfare owing to lack of motor nerve damage. Interestingly while rats and mice show comparable behavioural phenotypes after similar peripheral injuries in the many models reported above, significant differences were observed with the saphenous lesion: mice did not develop hypersensitivity to thermal...
responses after partial saphenous nerve injury, whereas rats showed both heat hyperalgesia and cold allodynia. In contrast the changes in mechanical responses with respect to both magnitude and duration were similar in rats and mice [70].

4.2.8. Mouse model of trigeminal neuralgia

Orofacial pain disorders encompass a wide range of conditions including trigeminal neuralgia, temporomandibular joint disorders, periodontal pain, and atypical face pain. Trigeminal neuralgia is a form of neuropathic pain characterized by severe lancinating pain in orofacial regions innervated by the trigeminal nerve. Most cases of trigeminal neuralgia are caused by sensory nerve root compression. For the trigeminal neuralgia Vos et al. [71] developed a model in rat due to the CCI of infraorbital trigeminal branch with loose ligatures. Only one readily performed mouse model of trigeminal pain was published. Xu et al. [72] have adapted the partial sciatic nerve ligation model of Seltzer et al. [24] to the infraorbital branch of the trigeminal nerve of mice. This model is a combination of the partial nerve ligation (dental maxillary nerve fibers and medial infraorbital nerve fibers are not injured) and spared nerve injury (trigeminal mandibular and ophthalmic branches are intact) models of chronic pain. This partial trigeminal injury in mice effectively mimics many of the qualities of human trigeminal pain syndromes and induces a transient change of grooming time and mechanical allodynia lasting for more than 3 weeks as well as many persistent anatomical changes.

5. Central and peripheral neuropathic pain models: injection of TNF-alpha

Recently it has emerged that proinflammatory cytokines play an important role in the onset and maintenance of neuropathic pain. Tumour necrosis factor alpha (TNF), interleukin-1 and interleukin-6 are among the most involved cytokines [50,51,73]. Consistently intraperitoneal or epineurial as well as intrathecal injection of TNF can elicit neuropathic pain-like behaviours, suggesting that the prototypic pro-inflammatory cytokine can act at several levels of the neuraxis to modulate pain processing [74,75]. Gao et al. [62] and Narita et al. [76] showed that also an application of TNF via lumbar puncture to mouse spinal cord caused the development of mechanical allodynia and heat hyperalgesia. These novel modalities of inducing painful symptoms can be particularly useful to test innovative drug treatment targeting the immune/proinflammatory component of neuropathic pain.

6. Murine models of the disease-induced neuropathic pain

In order to have models that more directly mimic prevalent clinical pain syndromes, researchers have attempted to induce the disease that will thereafter cause the neuropathic damage. These diseases include painful peripheral diabetic neuropathy, post-herpetic neuralgia, cancer-associated neuropathic pain, multiple sclerosis neuropathic pain, complex regional pain syndrome type I, HIV (and antiretroviral)-induced painful neuropathy.

6.1. Multiple sclerosis

Central neuropathic pain is a common but little understood symptom associated with the autoimmune disease multiple sclerosis (MS). The experimental autoimmune encephalomyelitis (EAE) and Theiler’s murine encephalomyelitis virus (TMEV) models in mice represent the two most common animal models of MS and their use has led to a greater understanding of MS and the development of clinical therapies. EAE is normally induced in mice by immunization with myelin oligodendrocyte glycoprotein and shares many features of the pathology seen in MS patients, including widespread CNS inflammation, demyelination and locomotion impairments. Mice with this form of EAE develop a robust tactile allodynia early in the disease process prior to any overt neurological deficits [77,78] similar to some patients with MS [79-81]. The viral model of MS is represented by mice infected with TMEV. Intracerebral inoculation of mice with TMEV induces a biphasic CNS disease characterized by inflammatory infiltration, demyelination, remyelination, and axonal damage that correlate with neurological disability. In these animals mechanical allodynia and thermal hyperalgesia are always observed [82].

6.2. Postherpetic peripheral neuropathic pain model

Postherpetic neuralgia is a persistent chronic burning pain often associated with allodynia and/or hypesthesia that may occur as a sequela of reactivation of latent herpes-zoster virus in dorsal root ganglia. While the disease is invariably painful in its acute phase during the skin rash, the incidence of postherpetic neuralgia over months or years after the disease depends on several risk factors, including the age of patient, the intensity of shingle eruption as well as the area of the affected skin [83]. It is clear now that many of the DRG neurons affected by the virus degenerate, most probably by the mechanism of apoptosis, as has been discussed for virtually all virus diseases involving the nervous system [84]. Due to the species specificity of varicella zoster virus, the identification of rodent models has been particularly difficult. A rat model of varicella zoster virus-associated pain has been developed some years ago by Fleetwood-Walker et al. [85] and recently refined [5]. While no mouse model of varicella zoster virus-induced pain has ever been described, a few papers report that the inoculation of mice with herpes simplex virus type-1 (HSV-1) causes herpes zoster-like skin lesions and pain-related responses (tactile allodynia and mechanical hyperalgesia) from 5 days after the inoculation up to 40 days [86,87]. However due to the fact that HSV-1 is not the relevant virus in the human, they cannot be considered appropriate models for clinical post-herpetic neuropathic pain.

6.3. HIV-associated sensory neuropathy

Human immunodeficient virus (HIV)-associated sensory neuropathy (HIV-SN) is the most common neurological complication of HIV infections, symptomatically affecting over one third of patients with HIV. The symptoms of HIV-sensory neuropathy are dominated by neuropathic pain, which is often excruciating. The pain is usually most severe on the soles of the feet, making walking difficult. There are presently no effective therapies for HIV-sensory neuropathy, and moreover until recently there has been no robust animal model of HIV-SN in which candidate therapeutic agents could be tested. In the last years two different models of HIV-SN have been published in the mouse and in the rat. A recent study of Keswani et al. [88] developed such a model consisting in transgenic mice expressing the HIV coat protein gp120 under a GFAP promoter, characterized by distal sensory axonal degeneration as seen in HIV patients with early stages of HIV-SN.

A reliable model of HIV neuropathy has been described, only in rat, by the group of Wallace and Rice, based on the contact between the HIV envelope protein gp120 and the rodent sciatic nerve [89,90]. The study of HIV-induced neuropathy is even more difficult since a toxic neuropathy is precipitated also by the use of nucleoside reverse transcriptase inhibitors normally part of antiretroviral therapy. Hopefully these models should be helpful for the study of both HIV and antiretroviral precipitated neuropathy.

6.4. Diabetic peripheral neuropathic pain model

Distal symmetric sensory neuropathy is a common complication of diabetes. Diabetic patients can suffer from either a painless syndrome, with loss of sensations to touch, pain or temperature, or a painful disorder characterized by spontaneous pain, mechanical hyperalgesia
and allodynia, depending on the type of nerve fibers being affected. Experimental models of the disease exhibit responses similar to those present in patients [91-93], including spontaneous pain [94], decrease in mechanical nociceptive thresholds [95] and/or hypoalgesia characterized by decreased responses to mechanical and thermal stimuli. In particular short-term diabetes has been shown to be associated with thermal and mechanical hyperalgesia, whereas long-term diabetes is associated with thermal and mechanical hypoalgesia [96,97]. A few murine diabetic models are available either genetically determined or drug-induced. NOD (non-obese diabetic) mice [98,99] develop spontaneous diabetes. Spontaneous autoimmune diabetes in NOD mice involves a long-term inflammatory process that closely resembles the human type 1 diabetes [100]. NOD mice develop inflammation of pancreatic islets at 3 weeks of age, but do not begin to develop diabetes until 10 weeks later, while they develop a significant time-dependent hyperalgesia, which does not correlate with the increase in the plasma glucose concentration but appears very early and is significant at young age (8–10 weeks), so preceding the hyperglycaemic state of mice. Similarly, also in human it is known that diabetic complications, including hyperalgesia, start to develop during the early inflammatory stage of the disease, even before establishing the hyperglycaemia and/or the glucosuria necessary for diabetes diagnosis [101]. Insulin resistant ob/ob and db/db mice [102-104] develop obesity and type 2 diabetes as a result of spontaneous genetic mutations that cause a decrease in functional leptin (ob/ob) or its receptor (db/db). In the 11-week-old leptin-deficient ob/ob mice, [105,106] deficits in motor nerve and hind limb digital sensory nerve conduction velocity are present together with a remarkable loss of intraepidermal nerve fibers and tactile allodynia. Recently other mouse model is explored for its contribution to diabetes research: C57BL/6j mouse that develops obesity and type 2 diabetes when fed a high fat diet [107]. The presence of altered thermal pain sensitivity has been reported also in a complex double transgenic mouse model of type 1 diabetes (TCR-SF/Ins-HAmouse) [108] that becomes hyperglycaemic spontaneously by 4–6 weeks of age [109]. This model parallels human diabetes in that it is immune-mediated, affects both genders equally and results from selective destruction of islet beta cells. However the most commonly used model for the study of this pain remains that of streptozotocin (STZ) or alloxan-induced diabetic neuropathy. STZ and alloxan kill insulin-secreting islet Langerhans pancreatic cells and produces the rapid development of hyperglycaemia [110]. In mice, single or repeated i.p. injection of STZ induces long lasting thermal and mechanical hyperalgesia and cold, thermal and mechanical allodynia [55,111]. In contrast to the STZ rat model, mice rendered diabetic with STZ and studied over sufficient periods of time (e.g., 4–8 months), do exhibit more robust features of neurodegeneration [112]. Interestingly insulin treatment abolishes pain and hyperalgesia [113]. We must underline that there are many features and underlying mechanisms that differ from human disease: this model produces severe distress to the animal with deterioration of general conditions and it was difficult to interpret data or obtain clear pain scores; moreover STZ has toxic effects on cells other than islet beta cells that might confound results making it important to use other models of diabetes.

7. Cancer-related neuropathic pain

Cancer-induced neuropathic pain may result from compression of nerve, plexus, or roots, or direct infiltration by the growing tumour, or secondarily from changes in the neuronal milieu resulting from cancer growth or from the resulting inflammatory response such as tissue pH (acidosis), release of tumour algogens or circulating chemokines and cytokines [114]. Moreover it is increasingly recognized that cancer-related neuropathic pain can also arise as a consequence of cancer-directed therapy, such as surgery, radiotherapy and chemotherapy [115].

7.1. Models of cancer-related neuropathic pain

Several murine models of direct inoculation of compatible murine cancer cells have been developed, such as squamous cell carcinoma into the hind paw and hepatocellular carcinoma cells into thigh muscle [116], which result in spontaneous pain behaviour, heat hyperalgesia and mechanical allodynia over varying time courses. Early onset pain behaviour was noted 1–3 days after inoculation of squamous cell carcinoma cells into the mouse hind paw. There was no sign of tumour infiltration into the nerve, although plantar nerves were clearly encapsulated by tumour cells, producing substantial compression of nerves and a degree of nerve damage. However over 60% of mice died within 16 days post-surgery due to lung metastasis [117]. A different modality is the direct inoculation of tumour cells close to the nerve. Meth-A sarcoma cells injected around mouse sciatic nerve resulted in the growth of a tumour mass surrounding the nerve with a maximum pain behaviours by day 21 post-inoculation, when histological signs of nerve damage were identified [118].

8. Chemotherapy-induced peripheral neuropathy

Peripheral neuropathy is a very frequent and severe side effect of chemotherapy and is often the limiting factor for achieving effective doses. Neurotoxicity is particular problematic for vincristine, platinum compounds, and taxols. Chemotherapy-induced neuropathy may continue after the cessation of therapy. When administered to mice, these chemicals also produce neuropathy which may be used to study causes, prevention and treatment of their neurotoxicity. Several studies have attempted to model chemotherapy induced peripheral neuropathy. Recently three mice models have been reported.

8.1. Vincristine-induced peripheral neuropathy model

Vincristine, a vincristine, prevents tumour cell replication via inhibition of tubulin polymerisation and is currently in wide clinical use as a treatment for leukaemia and lymphoma. However it shows the serious adverse effect of neuropathic pain, which limits its clinical use. Experimental models of vincristine-induced peripheral neuropathy have been established also in mice. Repeated injections of the chemotherapeutic agent produced dose-dependent mechanical allodynia in two strains of mice (ICR and BALB/c), which was observed even after cessation of vincristine administration [119,120].

8.2. Paclitaxel (taxol)-induced peripheral neuropathy model

Paclitaxel is an antineoplastic agent derived from the Pacific yew Taxus brevifolia and is used to treat a variety of cancers, including ovarian and breast tumours and non-small cell lung cancer. Taxol binds to tubulin, at a site different from that used by the vincristine alkaloids and blocks deassembly of microtubules, followed by an arrest of mitosis. Its effectiveness is limited by the development of severe painful peripheral neuropathy that is dose-dependent.

A number of mouse models of taxol-induced neuropathic pain have been reported. Both allodynia and thermal hyperalgesia can be detected in taxol-treated mice. Hidaka et al. [121] demonstrated that paclitaxel intraperitoneal administration at a single dose of 10 mg/kg or more in mice induces painful peripheral neuropathy, which is similar to the muscle pain in humans after this chemotherapeutic treatment. Painful peripheral neuropathy began after 24 h and lasted for 72 h: these findings suggest a recovery in functions of impaired sensory neurons. So, this animal model presented transient neuropathic pain with quick onset and the mice recovered within a few days. Furthermore, these time courses were similar to those of human muscle pain after administration of paclitaxel at a dose of 180 mg/m² in cancer patients [122,123].
Also Matsumoto et al. [124] found that a single paclitaxel intraperitoneal treatment of mice (4 mg/kg) led to a decrease in both thermal and mechanical nociceptive threshold as well as A-fiber specific hypersensitization. Paclitaxel-treated cancer patients support these results, since they also show A/β fiber hypersensitivity [125].

Several other mouse models presenting paclitaxel-induced neuropathy have been reported, but in these models only repeated administration of high dose paclitaxel induced pathological changes such as axonal degeneration and neuropathy lasting for a long time, sometimes with no recovery [126,127]. In these studies the paclitaxel administration in mice has been performed multiple times on consecutive / alternative days or in a single but very high dose.

8.3. Cisplatin-induced peripheral neuropathy

Cisplatin is used to treat several cancers; it induces polymyoneuropathy that is dose- and treatment duration-dependent and can last for over 10 years. Repeated daily i.p. injections of cisplatin produce in mice mechanical allodynia and hyperalgesia. So these models have been used to test a number of experimental treatments that may be useful to alleviate cisplatin neuropathy [128]. The pronounced neurotoxicity of this drug is supposed to be due to cisplatin-induced DNA adduct made of intrastrand cross-links. Interestingly, Dzagidze et al. [129] demonstrated an accelerated accumulation of unrepairred intrastrand cross-links in DRG neuronal cells of mice with dysfunctional nucleotide excision repair. In such animals this phenomenon coincided with an earlier onset of peripheral polineuropathy-like functional disturbance of their sensory nervous system. This new model accentuates the crucial role of effectual DNA repair capacity in the target cells for the individual risk of therapy-induced polineuropathy.

9. Inherited-induced neuropathies

The normal function of peripheral nerves requires myelination of axons by Schwann cells. These highly specialized glial cells form myelin sheaths that facilitate the rapid impulse propagation along the axon. In recent years, numerous membrane proteins that are localized to myelin and axon-glial junctions have been molecularly cloned and functionally characterized [130,131]. Many of these proteins could also be associated with inherited myelin diseases and neuropathies in humans. Recent progress in human genetics and neurobiology has led to the identification of various mutations in particular myelin genes as the cause for many of the known inherited demyelinating peripheral neuropathies. Hereditary motor and sensory neuropathies, defined by single gene mutations, constitute a heterogeneous group of diseases, referred to as Charcot-Marie-Tooth (CMT) disease by geneticists. Mutations in three distinct myelin genes PMP22, P0, and connexin 32 cause the three major demyelinating subtypes of Charcot-Marie-Tooth disease, CMT1A, CMT1B and CMTX, respectively. With the identification of these responsible disease genes significant progress has been made in understanding CMT disease mechanisms. The main clinical feature of all forms of CMT is the symmetric and distally pronounced muscle weakness of the lower limb, and to a lesser extent of the upper limb, which slowly progresses proximally. Initially, the muscle atrophy affects the distal leg and intrinsic foot muscles, causing steppage gait and foot deformities. Sensory deficits are a common feature but less prominent [132]. Although positive sensory symptoms are classically considered a hallmark of acquired neuropathies, sensory nerve involvement in CMT is well demonstrated on neurophysiological and pathological grounds and some observations suggest that clinical sensory manifestations are common in CMT. There are some papers which evaluate the presence of neuropathic pain in CMT patients. Carter et al. [133] report a clinical study according to which neuropathic pain is a significant problem for many people with CMT, using a validated measurement tool, the neuropathic pain scale, and concluded that symptoms reported by CMT patients are similar to those reported by patients with neuropathic pain. According to these authors, the frequency and intensity of pain reported in CMT is comparable in many ways to post-herpetic neuralgia, diabetic neuropathy and peripheral nerve injury. Since pain is susceptible to treatment, it warrants evaluation in CMT patients and it should be considered as a relevant symptom. Also Gemignani et al. [134] reported positive sensory symptoms in 58% of CMT patients, including neuropathic pain. Recently, Houlden et al. [135] reported the results of a study on the hereditary sensory and autonomic neuropathy type I (HSAN I), that is the most frequent type of hereditary neuropathy that primarily affects sensory neurons. These authors showed that patients presented positive symptoms such as limb pain, positive sensory symptoms of shooting and burning, and paraesthesia. Also, Padua et al. [136] following to a multicenter multidimensional study underlined that pain should be considered as a relevant symptom in CMT patients.

9.1. Mouse models of inherited neuropathies

There are a few mouse models of inherited neuropathy. A particular advantage of inherited neuropathy animal models is that the onset and progress of the disease can be much better characterised than in human patients, who are usually investigated only when symptoms have already developed [137]. However these models are not fully useful for the detection of the presence of pain, since the severe compromission of motor fibers due to the pathology does not allow the evaluation of the classical neuropathic pain symptoms, such as allodynia and hyperalgesia. In these models it should be particularly important the application of integrated procedures for the evaluation of pain-associated behaviour.

9.1.1. Spontaneous inherited neuropathy

When PMP22 was identified as a myelin gene [138] a search for possible spontaneous mutations in mice was performed. Two well-known mutants affected by Schwann cell-mediated neuropathies [139], the Trembler (Tr) and Trembler-j (Tr-j) mouse, have been found to carry particular mutations in the PMP22 gene [140,141]. The Tr mouse is associated with more severe pathological alterations as compared with the Tr-j mutant. The spontaneous mouse mutants Tr and Tr-j both carry point mutations in the PMP22 gene [140,141] and share pathological features with CMT1A [142]. Indeed, the same mutation has been identified in mouse and in one human CMT1A family [143]. However, the subcellular consequences of PMP22 misfolding in Tr mice and PMP22 overexpression in the majority of CMT1A cases are different [144].

9.1.2. Engineered inherited neuropathies

Overexpression of wild-type PMP22 can only be modeled by transgenic extracopies of the wild-type gene. PMP22 transgenic mice, with PMP22 overexpression, exhibit clinical abnormalities, such as severe dysmyelinated phenotype, reduced nerve conduction velocity and lower grip strength, that mimic findings in CMT1A patients and demonstrates the variability of disease expression as a function of increased gene dosage [145-150]. An interesting “inducible” model of CMT1A has also been generated in which PMP22 overexpression was achieved under cell-type-specific control of a tetracyclin-activable transactivator [151]. PMP22-deficient knock-out mice have been also generated by homologous recombination [152,153]; peripheral nerves of homozygous null mutants were severely affected, in that they showed focal hypermyelination; heterozygous mice have a phenotype much milder than the homozygous mice, demonstrating that PMP22 is a dosage-sensitive myelin component.

Also mutations encoding for the myelin components P0 and connexin 32 are clinically relevant, since they were found in the majority of patients with inherited demyelinating neuropathies [154].
Mice expressing half of the normal dose of P0 (P0+/- mice) or Mice deficient in the gene for Cx32 show pathological alterations in peripheral nerves comparable to those seen in the CMTX patients [137].

10. Conclusion

Important advances have been achieved in the study of neuropathic pain with the use of the many animal models that are becoming increasingly available in the mouse. Whereas the most used models still remain those based on nerve ligation, or great importance is the fact that in the last years other techniques are being developed that mimic more closely clinical pain syndromes, often by attempting to induce the disease or the genetic mutation associated to neuropathic pain. Moreover it is now clear that other important variables such as sex, age and strain should be more carefully taken into consideration when performing the experimental studies. However, by widening the number of animal model of neuropathic pain, new challenges have emerged. Although neuropathic pain with various aetiologies appears to lead to similar behavioural end points, it is becoming clear that they could respond differentially to pharmacological agents, suggesting that distinct mechanisms may underlie specific neuropathic states deriving from different pathological conditions. Particularly interesting is the observation that in most, if not all, the experimental models reported, a relevant role in the development and maintenance of neuropathic pain is played not only by sensitive and motor neurons, but also by immunocompetent cells both resident in the peripheral and central nervous system, such as Schwann cells and microglia, and recruited from blood. A comparative study of the molecular pathways at various levels in different neuropathies will help to finally translate basic science knowledge into the development of new clinically effective treatments.

References

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