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Review

Predictive validity of pharmacologic interventions in animal models of neuropathic pain

Per Hartvig Honoré*, Anna Basnet, Pernille Kristensen, Lene Munkholm Andersen, Signe Neustrup, Pia Møllgaard, Laila Eljaja, Ole J. Bjerrum

Department of Pharmacology and Pharmacotherapy, Faculty of Pharmaceutical Sciences, University of Copenhagen, Universitetsparken 2, DK-2100 Copenhagen, Denmark

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ABSTRACT

Introduction: The pathophysiologic and neurochemical characteristics of neuropathic pain must be considered in the search for new treatment targets. Breakthroughs in the understanding of the structural and biochemical changes in neuropathy have opened up possibilities to explore new treatment paradigms. However, long term sequels from the damage are still difficult to treat.
Aim of the study: To examine the validity of pharmacological treatments in humans and animals for neuropathic pain.
Method: An overview from the literature and own experiences of pharmacological treatments employed to interfere in pain behavior in different animal models was performed.
Results: The treatment principles tested in animal models of neuropathic pain may have predictive validity for treatment of human neuropathies. Opioids, neurotransmitter blockers, drugs interfering with the prostaglandin syntheses as well as voltage gated sodium channel blockers and calcium channel blockers are treatment principles having efficacy and similar potency in humans and in animals. Alternative targets have been identified and have shown promising results in the validated animal models. Modulators of the glutamate system with an increased expression of glutamate re-uptake transporters, inhibition of pain promoters as nitric oxide and prostaglandins need further exploration. Modulation of cytokines and neurotrophins in neuropathic pain implies new targets for study. Further, a combination of different analgesic treatments may as well improve management of neuropathic pain, changing the benefit/risk ratio.
Implications: Not surprisingly most pharmacologic principles that are tested in animal models of neuropathic pain are also found to be active in humans. Whereas many candidate drugs that were promising in animal models of neuropathic pain turned out not to be effective or too toxic in humans, animal models for neuropathic pain are still the best tools available to learn more about mechanisms of neuropathic pain. Better understanding of pathogenesis is the most hopeful approach to improve treatment of neuropathic pain.

Contents

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Abbreviations: AMPA, α-amino-3-hydroxi-5-metylisoxazol-4-propanoic acid; ATP, adenosine triphosphate; BDNF, brain-derived neurotrophic factor; CCI, chronic constriction injury; COX, cyclooxygenase; EAAT-2, excitatory amino acid transporter type 2; GABA, γ-amino butyric acid; GFAP, glial fibrillary acidic protein; IGF, insulin growth factor; NGF, nerve growth factor; NMDA, N-methyl-D-aspartic acid; NO, nitric oxide; NR2B, NMDA receptor type 2B; NSAID, non-steroidal anti-inflammatory drug; $p75^{NTR}$, p75 neurotrophin receptor; SNI, spared nerve injury; SNRI, serotonin and noradrenaline reuptake inhibitor; SNL, spinal nerve ligation; SNT, spinal nerve transection; SSRI, selective serotonin reuptake inhibitor; TCA, tricyclic antidepressant; TNF-α, tumour necrosis factor α; TREK-1, potassium channel subfamily K member 2 (KCNK 2); Trk, tyrosine kinase receptor; TRPV1, transient receptor potential vanilloid 1.

* Corresponding author. Tel.: +45 35 33 65 75; fax: +45 35 33 60 20.
E-mail address: peh@farma.ku.dk (P.H. Honoré).

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1. Introduction

Animal disease models allow test of pharmacological substances and might be predictive for the outcome in humans. The validity of an applied model can be divided into three categories: construct, face and predictive validity. In animal models of pain, *construct validity* is present when etiology and the underlying mechanism for pain are similar in humans and the animal. *Predictive validity* are shown as pharmacological treatments are acting in an analogous way in humans and animals, e.g. to be sensitive to effective drugs but fail to respond to ineffective drugs. The animal model also need to simulate pain conditions in humans, based on as many symptoms and signs for pain as possible, thus ensuring *face validity* [1]. All three categories need to be considered when evaluating the validity of an animal model.

This present text aims to describe pathophysiologic changes as well as the development and maintenance of neuropathic pain, giving targets for therapy as shown in the spared nerve injury (SNI) and spared nerve ligation (SNL), or the similar spared nerve transection (SNT) models in rats and mice. The pharmacologic treatments are reviewed and discussed in light of clinic possibility and feasibility.

2. Nerve tract pathophysiology indicates targets for pharmacologic interventions

The condition of neuropathic pain cannot always be explained by a single etiology or specific lesion, but multiple mechanisms may contribute, as well as a complex interaction between damaged and non-damaged neurons that might account for the pain signal [2,3]. Furthermore, the condition is complicated by the various distinct cellular changes in the peripheral and central nervous system following a specific injury.

2.1. Peripheral changes following nerve damage

Inflammatory and damaged cells in the peripheral nervous system following a lesion or a disease play an important role in the development of neuropathic pain. Cells release their intracellular content in consequence of an injury in the peripheral nervous system, which in turn sensitize nociceptors to further stimulation [4]. In addition, a lesion in the peripheral nervous system triggers changes in the number and location of ion channels, especially sodium channels on the damaged C-fibres as well as TREK-1 and TRPV1 channels causing heat hypersensitivity [5]. These channels will accumulate along the primary afferent fibres and in the dorsal root ganglion, resulting in a lowered threshold and an increased spontaneous firing, termed *ectopic discharges* [3,4,6,7].

Normally, adjacent afferent fibres have no contact and thereby no impact on the activity of each other. However, after nerve injury, chemical or electrical connections between injured and uninjured nerve fibres may form, known as "cross talk" or *ephaptic conduction*. Through this connection, the properties of the uninjured afferent fibres are altered and non-painful stimuli may cause excitation of normally "silent" nociceptors [3,7].

2.2. Central nervous system responses

Next to the changes in the periphery, continued nociceptor input into the dorsal horn of the spinal cord increases the responsiveness to incoming stimuli and contributes to plasticity changes in the central nervous system. A major process in the *central sensitization* is manifested as increased excitability, initiated and maintained by the sensitized primary afferent fibres. These fibres sensitize the spinal cord by presynaptic release of tachykinins (substance P and neurokinin A) and neurotransmitters (glutamate, calcitonin gene-related peptide and GABA) [3,4].

Glutamate acts on AMPA receptors, while the tachykinins bind to neurokinin receptors on the postsynaptic membrane. The binding of substance P to its receptors triggers the release of intracellular calcium, thereby increasing the neuronal excitability and facilitating up-regulation of another kind of ionotropic glutamate receptor; the NMDA receptor [4,8]. Under normal circumstances, glutamate has no effect on NMDA receptors because the receptor channels are blocked by magnesium ions at resting membrane potentials. However, during central sensitization, the increased action potentials remove the magnesium ions, resulting in further influx of calcium ions into the cell. The increased intracellular calcium contributes to maintenance of the central sensitization, due to its action as secondary messenger. This activates protein kinase C, leading to phosphorylation of the NMDA receptor that leaves the receptor in an open state, due to continuous removal of the magnesium ions [8,9]. The central sensitization may manifest in three ways: the threshold to noxious stimuli is lowered, the response to stimuli increased and the area available to receive stimuli enlarged which is evidenced as *disinhibition* in the spinal dorsal horn and *descending facilitation* from the brainstem and various plastic changes in the pain processing areas of the brainstem and the cerebral cortex.

In conclusion, the pain transmission system involves a number of actors both peripherally and in the central nervous system. These include besides signal disinhibition in the spinal dorsal horn, descending facilitation from the brainstem, plastic changes in the pain processing areas of the brainstem and the cerebral cortex. The exact role of the various functional mechanisms is still not completely understood and their interactions must be further elucidated. Nevertheless, increased knowledge in this complex interplay will be of importance for finding and selection of new targets to modulate pain signaling and also to interact with the neuronal plasticity caused in neuropathic pain.

Furthermore, profound changes in microglia and astrocytes may occur in the spinal cord microenvironment in neuropathic pain in form of low grade inflammation in response to signals from released glutamate; brain derived neutrophic factor (BDNF), substance P, adenosine triphosphate (ATP), chemokines and peptides [10,11,92]. A pronounced activation of glial fibrillary acidic protein (GFAP) immunoreactivity which indicates astrocyte activity and an exhibited large leakage of albumin are observed [12].

3. Treatment options

A lot of information has been collected in recent years on pathophysiologic changes following nerve injury in animal models. These changes are a result of inflammation, derangement as well as plasticity of the nervous system to adapt to the new situation and altered signaling. The immediate result of these alterations is that normal analgesic treatment paradigms often fail. On the other hand, the changes will also open up for new targets for treatment aside from the use of traditional analgesic drugs as well as support for reparation principles in deranged nerve tracts.

Pharmacologic treatment are frequently used in the management of neuropathic pain and the algorithms are mainly based on five drug classes [13,14],

- *sodium channels blockers* attenuating the pain signal,
- *calcium channel blockers* diminishing calcium influx into pain signaling cells mediated through the $\alpha_2\delta$-subunit site,
- *opioids* strengthening the response of enkephalins/endorphins to inhibit pain signaling in the spinal dorsal horn,
- *neurotransmitter modulators* enhancing serotonin and/or noradrenaline, acetylcholine or GABA availability in the pain inhibitory descending pathways, or
- *interference in the prostaglandin cascade*.

There are a few new alternative drug classes that recently have come into focus, for example drugs with inhibitory action on cytokines and drugs interfering with the neurotrophins [15]. However, drugs with other pharmacological targets and interactions in the nervous tracts have also been tested with ambiguous results in animals, such as the importance of sortilin [15,16] and antagonists for the vanilloid TRPV1-receptor [17]. A careful validation of those principles showing predictive validity would open up for necessary alternatives in the drug armamentarium in neuropathic pain.

4. Pharmacologic interventions for the management of neuropathic pain

4.1. Voltage-gated sodium channel blockers

Voltage-gated sodium channels are multi-subunit protein complexes composed of a large transmembrane pore-forming and voltage-sensitive α-subunit and two smaller β-subunits. These channels are essential for the generation and propagation of action potentials and play a central role in primary afferent ectopic discharges that originate from the site of injury or the dorsal root ganglia. Changes in expression of Na_v1 subtypes are thought to contribute to neuropathic pain processes [18].

Blockers of the voltage-gated sodium channels belong to drug classes used for epilepsy (e.g. phenytoin, carbamazepine and lamotrigine); arytmia (e.g. mexiletine); local anesthesia (e.g. lidocain and bupivacain), but is also operant for tricyclic antidepressive drugs. The pain relief following oral or intraperitoneal administration of lamotrigine and mexiletine has been demonstrated in both the SNL and SNI model [18–20]. Lamotrigin inhibits the release of excitatory amino acids as well, while carbamazepine works by blocking sodium channels [21].

4.2. Voltage-gated calcium channel blockers

Calcium channels reflect and control the influx of calcium ions to the cell and neuronal voltage-gated calcium channels contain three subunits: (1) the α_1 channel-forming subunit, (2) the intracellular β-subunit and (3) the $\alpha_2\delta$-subunit that consist of two disulfide-linked polypeptides (α_2 and δ) [22]. The nervous system expresses at least six different calcium channels, referred to as the L, N, P, Q, R and T types according to the type of α_1 subunit. Except for the T-type, all are high-threshold channels [8]. The voltage-gated N-calcium channels, located at the presynaptic neurons, are considered important in the central sensitization and play a role in neuropathic pain transduction [23]. Studies in rats have shown that voltage-gated N-calcium channels are up-regulated after peripheral nerve injury [22].

The primary afferent fibres play a crucial role in pain processing and these fibres have been shown to express a mixed population of voltage-gated calcium channels, consisting of a number of different subtypes of α_1 (N-, L-, P/Q and R-type poreforming subunits) associated with β and $\alpha_2\delta$-1 subunits (at least three subunits) [23]. There is evidence for a strong association between high affinity binding of the originally antiepileptic drug gabapentin to the $\alpha_2\delta$ subunit of voltage-gated calcium channels and its anti-hypersensitive properties in a streptozocin animal model of neuropathic pain [24]. Moreover, recent studies suggest that activation of the descending noradrenergic pathway is one of the pivotal mechanisms of gabapentin analgesia. Orally administrated gabapentin has been shown to induce spinal release of noradrenaline, which in turn activates spinal cholinergic circuits resulting in increased release of acetylcholine. This cascade suppresses the activation of spinal nociceptive neurons [25–28]. Pregabalin is thought to act by the same mechanisms [29].

Dose–response relationships have been established for gabapentin for various routes of administration in the SNL as well as the SNI model [28,30–34]. Obvious signs of sedation with a decrease in locomotor activity have been observed in doses above 100 mg/kg [33].

4.3. Opioids

The endogenous opioid peptide-containing neurons have a pronounced importance in pain inhibition and has been found represented in the regions involved in the nociceptive response, the thalamus, periaqueductal grey, limbic system, cortex and in the spinal cord. Similarly, the autonomic nervous system centers have been shown to be innervated by central and peripheral opioid neurons. It must be anticipated that the opioid peptides play a central role in the pain transmission and inhibited signaling in neuropathic pain states.

Numerous studies in various animal models of peripheral or central nerve injury suggest that opioids are effective in alleviating neuropathic pain behaviors. However, inconsistencies are apparent. While systemic administration of morphine attenuates

hypersensitivity in the SNL and the SNI models [35–39], intrathecal morphine is apparently ineffective [38–42]. The selected doses must be considered as variances explaining the negative outcome.

Codeine and methadone have been tested in the SNI model, without showing pain relieving effects for the doses tested (codeine 30 mg/kg and methadone >1 mg/kg administered subcutaneously to rats) [39].

4.4. Neurotransmitter modulators

In the pain inhibitory system interactions by endogenous transmitters take place in the interneurons of e.g. the spinal opioid system. Drugs may increase the availability of serotonin, noradrenaline, acetylcholine and also GABA, thereby promoting pain inhibition via different mechanisms. Efforts have also been devoted to modulate the pain signaling transferred by excitatory amino acids.

4.4.1. Serotonin and/or noradrenaline reuptake inhibitors

Most drugs with antidepressant indication are blocking the reuptake of serotonin and/or noradrenaline, and for some of the substances also dopamine, from the synaptic cleft thereby enhancing the action on the postsynaptic receptors.

The working action of these drugs is to increase the concentration of neurotransmitters in the synapse in the descending inhibitory pain pathways in the spinal cord. The TCA drugs have also a pronounced inhibitory action on sodium channels, which is much larger than for the other types of antidepressants (SSRI and SNRI).

TCA (e.g. nor- and amitriptyline), SSRI (e.g. citalopram and fluoxetine) and SNRI (e.g. duloxetine and venlafaxine) have been tested in the SNL and SNI models, respectively [17,32–34,43,44]. Of SNRI drugs, duloxteine had analgesic effect in both SNL-operated rats and mice [17,43].

4.4.2. Cholinesterase inhibitors

The cholinesterase inhibitors are thought to act predominantly in the central nervous system. The drugs block the acetyl- and/or butyrylcholinesterase activity, leading to increased levels of acetylcholine to stimulate the muscarinic receptors [45]. Studies have shown that oral administration of the selective acetylcholinesterase inhibitor donepezil to rats increases the brain content of acetylcholine to a higher extent than in plasma [46,47], where the inhibition of acetylcholinesterase is weak [48]. A 14-fold higher dose was needed to inhibit the acetylcholinesterase in plasma compared to that in the brain due to its high permeability into the brain [46,47]. Previous studies have reported that donepezil treatment does not lead to desensitization of muscarinic receptor-coupled G-proteins in the brain or the spinal cord, suggesting that donepezil may not cause tolerance with subsequent need for dose escalation [49]. Although promising effect in animal models [33,34,49], no cholinesterase inhibitor have so far been tested for the indication of clinical neuropathic pain.

4.4.3. GABA agonists and benzodiazepines

Central disinhibition is a process with selective loss of inhibitory interneurons releasing the transmitter GABA, which gives the central neurons possibility to fire spontaneously in response to stimuli from the periphery [3,7,8]. Some of these changes are located in the noradrenergic and/or serotonergic descending pathways and loss of inhibitory GABA-releasing interneurons results in a suppressed descending inhibition of pain [9,50]. Application of the GABA agonist gaboxadol acting directly on the GABA binding sites exhibited antinoceptive effect in SNI rats [93]. The sedative effect of gaboxadol observed at higher concentrations makes the therapeutic window very narrow but the pain relieving effect may be used as an added value for neuropathic pain when using gaboxadol as a hypnotic drug.

The benzodiazepines are working as agonists on the inhibitory GABA receptor but the pain inhibition is very minute of these drugs [93]. In fact, intrathecal administration of the peripheral benzodiazepine agonist PK-11195 showed significant effect in SNL mice [51].

4.4.4. NMDA- and AMPA-receptor antagonists and glutamate reuptake stimulators

Glutamate is the primary excitatory neurotransmitter in CNS and participates in the transmission of nociceptive information together with other transmitters modulating the signal. The action of glutamate released into the synaptic cleft is terminated by uptake of glutamate into both neuronal and astroglia tissue by specific glutamate transporters [52]. In neuropathic pain, the expression of these spinal transporters is down-regulated, resulting in enhanced concentration of glutamate and increased nociception [53].

NMDA antagonists acting in particular on NR2B-containing NMDA receptors have found value in different pain states [54]. Preclinical studies have shown that agents that are selective for receptors that include the NR2B subunit have a substantially better profile for treating neuropathic pain than current NMDA antagonists; some emerging clinical evidence supports this view [55].

In the SNI model, the NMDA receptor antagonist MK-801 showed no effect in the doses tested, whereas the AMPA antagonist NS-1209 showed significant effect in dose of 6 mg/kg [31]. The possibility for increasing the glutamate transporter expression in CNS represents a new option to treat neuropathic pain [56]. Since glutamate is part of the pathogenesis in the development of neuropathic pain, reduction of its concentration may have a preventive effect [53]. Several β-lactam antibiotics have been found to up-regulate the expression of the glutamate transporter EAAT-2, where ceftriaxone seemed to be most potent [57]. Reduced nociception following administration of ceftriaxone has recently been demonstrated in SNL rats [58].

4.5. Interference in the prostaglandin cascade

4.5.1. Prostaglandin synthesis inhibitors

The prostaglandins are together with formed NO working as a pain signaling enhancer of the peripheral nerve signaling to the spinal cord and the brain. Peripheral injection of nonselective and selective COX inhibitors attenuate neuropathic pain following partial sciatic nerve transection [59], indicating that pro-inflammatory prostaglandins are involved in the development of neuropathic pain.

The NSAIDs ibuprofen, diclofenac and celecoxib have been tested in the SNL model. Ibuprofen and celecoxib showed significant pain relief in SNL-operated mice, while there were no effect of diclofenac [20]. The difference in effect is not understood although ibuprofen is a COX-1 and COX-2 inhibitor, whereas celecoxib has a pronounced inhibitory action on COX-2.

The steroids have a more potent anti-inflammatory action than the NSAIDs and are frequently used to diminish clinical inflammation after nerve damage.

4.5.2. Nitric oxide synthase inhibitors

NO has a dual role in relation to nociception; a small increase of NO reduces nociception while a large increase results in hyperalgesia. Up-regulation of neuronal NO synthase has been shown following ligation of spinal nerves in the SNL model, causing injuries to the cord (distorted neurons, membrane disruption and myelin vesiculation). These injuries were significantly reduced if rats were pre-treated with the NO synthase inhibitor L-NAME and may therefore furnish a new alternative in the treatment of

neuropathic pain to minimize and/or prevent neurodegeneration [60]. Also, an antiserum for the opioid dynorphin A attenuated this neuronal up-regulation and induced neuroprotection. This indicates a close relation between opioid active peptide dynorphin A and the regulation of NO synthase [61].

4.6. Cytokines

Following damage of a nerve, the distal stump of injured axons will undergo *Wallerian degeneration*, i.e. breakdown of myelin sheaths, recruitment of inflammatory cells from the circulation and over-production of growth factors and pro-inflammatory cytokines or mediators. Some pro-inflammatory cytokines and mediators do not only promote the regeneration of injured axons, but also activate and sensitize nociceptors [62]. Thus, there is a role for central interleukin-1β and TNF-α in the development and maintenance of neuropathic pain through induction of a proinflammatory cytokine cascade [63]. TNF-α also reduced the up-regulation of neuronal NO synthase induced by neuropathy, imparting a regulatory role of NO on TNF-α expression [64].

4.7. Neurotrophins

Neurotrophins are structurally and functionally related proteins known as trophic factors or growth factors. The neurothrophins exert a wide range of effects in the peripheral and central nervous system. They are involved in development, survival and maintenance of vertebra neurons, in addition to inducing apoptosis and synaptic modulations. Therefore, this class of endogenous substances opens up possibilities for use in neuropathies to understand illicit growth after trauma and also to inhibit neurodegeneration. In mammals, four types of neurothrophins have been characterized: NGF, BDNF, neurotrophin-3 and neurotrophin-4/5. In some tissues NGF and pro-BDNF induce cleavage, raising the possibility that the uncleaved forms may show distinct biological activity [65–69].

Two classes of receptors mediate the effects of neurotrophins; the tyrosine kinase receptors (TrkA, TrkB and TrkC) and the $p75^{NTR}$. TrkB binds BDNF and neurotrophin-4/5 [65,67,68]. The anti-apoptosis function of BDNF is mediated through high-affinity TrkB receptors, while $p75^{NTR}$ binds pro-BDNF with high affinity, and its ability to induce apoptosis requires interaction with sortilin as a co-receptor [15,69,70].

4.7.1. Nerve growth factor

NGF is discovered as a trophic molecule essential for the survival and maturation of developing neurons in nervous system [71,72]. More recently, a considerable body of evidence implicates endogenous NGF in conditions in which pain is a prominent feature. NGF expression is increased following nerve injury. Production of NGF by fibroblasts, Schwann cells and macrophages, is triggered by cytokines released from endogenous or exogenous phagocytes [73]. Increased NGF mRNA and protein in the lumbar dorsal root ganglia have been demonstrated after injury of the sciatic nerve in rats [74] and it is up-regulated in Schwann cells surrounding the dorsal root ganglia for at least two months after nerve injury [75]. Anti-NGF treatment in sciatic neuropathy resulted in complete block of thermal hypoalgesia and mechanoallodynia in the CCI model, but only limited effect in the SNL [76]. The differences were ascribed differences in adrenergic nerve sprouting after injury.

4.7.2. Brain-derived neurotrophic factor and insulin growth factor

BDNF is the most functionally diverse member of the neurotrophin family [67]. BDNF plays a central role in neuronal development, physiology and pathology [15,77]. It is now established that BDNF appears essential to molecular mechanisms of synaptic plasticity, aside from its importance in neuronal differentiation and apoptosis. Basic activity-related changes in the central nervous system are thought to depend on BDNF modifications of synaptic transmission [15]. BDNF is thought to be present in the mammalian nervous system in a pro-form (pro-BDNF) produced and released by neurons as well as activated microglia [15,67]. More recent literature on neurotrophins states that pro-BDNF and BDNF display opposite effects on neural cell proliferation and apoptosis.

BDNF and IGF reduce edema as well as the up-regulation of neuronal NO synthase following trauma of the spinal cord. This indicates that IGF may as well have a role and that both neutrophins may attenuate cellular stress response [78–80].

5. Discussion and future perspectives

There are interesting new pharmacological alternatives in other pain relieving mechanism on the scene which probably will show predictive validity as seen from animal studies. Cholinesterase inhibitors have shown positive results in animal studies and are hoped to show a correspondence in neuropathic pain in patients [33,34,49,81]. Another interesting principle with positive results in animals is the compounds increasing the EAAT-2 expression of glutamate [57,58,82]. This is a new mechanism of action which may have a long duration and do not necessitate daily treatment. The effect on protection and restoration of nerve damage of growth hormones as BDNF also needs further investigation [15]. Immunologic principles as cytokine release and TNF-α damage are highlighted to combat the damage insulted by the nerve injury but also reparation supported by neurotrophins plays a role.

Treatment of neuropathic pain states in the clinic is a challenge. No method seems to afford analgesia to a majority of the patients although many treatment principles are used. Analgesia may be, apart from the pharmacological interventions discussed, accomplished by invasive methods such as central nerve blockades, nerve stimulation techniques and manipulation of signal transduction. Animal models might be of value in the effort to mimic the clinical conditions. The animal models are used in drug development for neuropathic pain with a high presumption of being predictive for use in patients. Possibly the results from animal models might give an exaggeration of the analgesic response. In the animals, allodynia or hyperalgesia have usually developed and further the doses used are not correctly scaled from animal to man and back. Studies in animals are performed for short time intervals and the acute and particularly long term side effects developing in man are poorly highlighted and difficult to abstract in the animals. The models are also poor regarding observation of pharmacological effects on spontaneous, ongoing pain [1,83,84]. Either the models do not express spontaneous pain or there are no measures to observe [85].

The limited success with single pharmacological agents due to incomplete efficacy and dose-limiting adverse effects has made combination therapy a common approach to improve treatment outcome in the clinic [86]. Combination therapy is, however, not recommended as a first choice in the treatment algorithm, due to lack of sufficient evidence of optimal drug combinations and doses [13,14].

The rationale behind drug combinations is that multiple mechanisms generate neuropathic pain and it is unlikely that a single drug effectively will relieve these various pain mechanisms. Combinations of various drugs acting through different mechanisms may instead give rise to more satisfactory outcome. Thus the benefits may include greater efficacy, lower doses and fewer adverse effects [86]. A clinically useful combination does not necessarily need to produce synergistic analgesia as an additive effect may also be useful. However, the adverse effects of the combination must be of the same or lower magnitude than for monotherapy [86–88]. Choice of

drugs must be based on pain components and origin as well as a diagnosis of mechanisms of different pain entities in order to select an optimal drug combination. Doses must be slowly titrated since synergistic effects and adverse effects may occur.

Combination therapy is not without limitations because of problems such as adverse drug reactions, pharmacokinetic interactions, increased risk of medication errors and creating hospitalization, and patient noncompliance [86]. Due to the lack of evidence for combination therapy, more preclinical and clinical studies are needed in order to evaluate the effect, safety and cost-benefit of any given drug combination [86,89].

6. Conclusions

The neuropathic pain models in the rat and mouse provide adequate specific symptom models for neuropathic pain [90,91]. The nerve damage and the following change in nerve function, signaling alteration and plasticity changes in various transmitter systems are well highlighted in the models. Not surprisingly most pharmacologic principles that are tested in animal models of neuropathic pain are also found to be active in humans. Candidate drugs have also been promising in animal models of neuropathic pain, but did not turn out to be effective or too toxic in humans. The models might not always show predictive validity, but currently they represent the best tool available. The validity improves if the models are combined with a functional understanding of the involved chemical pathways. There are still pharmacologic entities tested in animal models showing significant effects in the doses used that in patients have various efficacy. It is advised that complete preclinical dosing studies are performed not to rule out any analgesic action. High doses in the animal might be effective but prevents its use in humans due to severe adverse effects or toxicity. Recent neurochemistry studies in nerve injured animals advise new treatment alternatives. A new road might be a combination of different working principles for neuropathic pain based on different mechanisms of action, not similar adverse events, in order to enhance efficacy but also as an effort to enhance duration of effect.

Conflict of interest

All authors declare that they have no conflicts of interest.

References

[1] Blackburn-Munro G. Pain-like behaviours in animals – how human are they? Trends Pharmacol Sci 2004;25:299–305.
[2] Scholz J, Woolf CJ. Can we conquer pain? Nat Neurosci 2002;5(Suppl.):1062–7.
[3] Baron R. Mechanisms of disease: neuropathic pain—a clinical perspective. Nat Clin Pract Neurol 2006;2:95–106.
[4] Pasero C. Pathophysiology of neuropathic pain. Pain Manag Nurs 2004;5:3–8.
[5] Alloui A, Zimmermann K, Mamet J, Duprat F, Noel J, Chemin J, Guy N, Blondeau N, Voielly N, Rubat-Coudert C, Borsotto M, Romey G, Heurteaux C, Reeh P, Eschalier A, Lazdunski M. TREK-1, a K+ channel involved in polymodal pain perception. EMBO J 2006;25:2368–76.
[6] Costigan M, Woolf CJ. Pain: molecular mechanisms. J Pain 2000;1:35–44.
[7] Bridges D, Thompson SW, Rice AS. Mechanisms of neuropathic pain. Br J Anaesth 2001;87:12–26.
[8] Beydoun A, Backonja MM. Mechanistic stratification of antineuralgic agents. J Pain Symptom Manage 2003;25:S18–30.
[9] Woolf CJ, Mannion RJ. Neuropathic pain: aetiology, symptoms, mechanisms, and management. Lancet 1999;353:1959–64.
[10] Honore P, Rogers SD, Schwei MJ, Salak-Johnson JL, Luger NM, Sabino MC, Clohisy DR, Mantyh PW. Murine models of inflammatory, neuropathic and cancer pain each generates a unique set of neurochemical changes in the spinal cord and sensory neurons. Neuroscience 2000;98:585–98.
[11] Ren K, Dubner R. Neuron-glia crosstalk gets serious: role in pain hypersensitivity. Curr Opin Anaesthesiol 2008;21:570–9.
[12] Gordh T, Sharma HS. Chronic spinal nerve ligation induces microvascular permeability disturbances, astrocytic reaction, and structural changes in the rat spinal cord. Acta Neurochir Suppl 2006;96:335–40.
[13] Attal N, Cruccu G, Haanpaa M, Hansson P, Jensen TS, Nurmikko T, Sampaio C, Sindrup S, Wiffen P. EFNS guidelines on pharmacological treatment of neuropathic pain. Eur J Neurol 2006;13:1153–69.
[14] Dworkin RH, O'Connor AB, Backonja M, Farrar JT, Finnerup NB, Jensen TS, Kalso EA, Loeser JD, Miaskowski C, Nurmikko TJ, Portenoy RK, Rice AS, Stacey BR, Treede RD, Turk DC, Wallace MS. Pharmacologic management of neuropathic pain: evidence-based recommendations. Pain 2007;132:237–51.
[15] Teng HK, Teng KK, Lee R, Wright S, Tevar S, Almeida RD, Kermani P, Torkin R, Chen ZY, Lee FS, Kraemer RT, Nykjaer A, Hempstead BL. ProBDNF induces neuronal apoptosis via activation of a receptor complex of p75NTR and sortilin. J Neurosci 2005;25:5455–63.
[16] Nykjaer A, Lee R, Teng KK, Jansen P, Madsen P, Nielsen MS, Jacobson C, Kliemannel M, Schwarz E, Willnow TE, Hempstead BL, Petersen CM. Sortilin is essential for proNGF-induced neuronal cell death. Nature 2004;427:843–8.
[17] Joshi SK, Hernandez G, Mikusa JP, Zhu CZ, Zhong C, Salyers A, Wismer CT, Chandran P, Decker MW, Honore P. Comparison of antinociceptive actions of standard analgesics in attenuating capsaicin and nerve-injury-induced mechanical hypersensitivity. Neuroscience 2006;143:587–96.
[18] Lindia JA, Kohler MG, Martin WJ, Abbadie C. Relationship between sodium channel NaV1.3 expression and neuropathic pain behavior in rats. Pain 2005;117:145–53.
[19] Erichsen HK, Hao JX, Xu XJ, Blackburn-Munro G. A comparison of the antinociceptive effects of voltage-activated Na+ channel blockers in two rat models of neuropathic pain. Eur J Pharmacol 2003;458:275–82.
[20] Kiso T, Watabiki T, Tsukamoto M, Okabe M, Kagami M, Nishimura K, Aoki T, Matsuoka N. Pharmacological characterization and gene expression profiling of an L5/L6 spinal nerve ligation model for neuropathic pain in mice. Neuroscience 2008;153:492–500.
[21] Jensen TS, Finnerup NB. Management of neuropathic pain. Curr Opin Support Palliat Care 2007;1:126–31.
[22] Luo ZD, Chaplan SR, Higuera ES, Sorkin LS, Stauderman KA, Williams ME, Yaksh TL. Upregulation of dorsal root ganglion (alpha)2(delta) calcium channel subunit and its correlation with allodynia in spinal nerve-injured rats. J Neurosci 2001;21:1868–75.
[23] Sutton KG, Martin DJ, Pinnock RD, Lee K, Scott RH. Gabapentin inhibits high-threshold calcium channel currents in cultured rat dorsal root ganglion neurones. Br J Pharmacol 2002;135:257–65.
[24] Field MJ, Hughes J, Singh L. Further evidence for the role of the alpha(2)delta subunit of voltage dependent calcium channels in models of neuropathic pain. Br J Pharmacol 2000;131:282–6.
[25] Takasu K, Honda M, Ono H, Tanabe M. Spinal alpha(2)-adrenergic and muscarinic receptors and the NO release cascade mediate supraspinally produced effectiveness of gabapentin at decreasing mechanical hypersensitivity in mice after partial nerve injury. Br J Pharmacol 2006;148:233–44.
[26] Takeuchi Y, Takasu K, Honda M, Ono H, Tanabe M. Neurochemical evidence that supraspinally administered gabapentin activates the descending noradrenergic system after peripheral nerve injury. Eur J Pharmacol 2007;556:69–74.
[27] Hayashida K, DeGoes S, Curry R, Eisenach JC. Gabapentin activates spinal noradrenergic activity in rats and humans and reduces hypersensitivity after surgery. Anesthesiology 2007;106:557–62.
[28] Hayashida K, Parker R, Eisenach JC. Oral gabapentin activates spinal cholinergic circuits to reduce hypersensitivity after peripheral nerve injury and interacts synergistically with oral donepezil. Anesthesiology 2007;106:1213–9.
[29] Tanabe M, Takasu K, Kasuya N, Shimizu S, Honda M, Ono H. Role of descending noradrenergic system and spinal alpha2-adrenergic receptors in the effects of gabapentin on thermal and mechanical nociception after partial nerve injury in the mouse. Br J Pharmacol 2005;144:703–14.
[30] Pan HL, Eisenach JC, Chen SR. Gabapentin suppresses ectopic nerve discharges and reverses allodynia in neuropathic rats. J Pharmacol Exp Ther 1999;288:1026–30.
[31] Erichsen HK, Blackburn-Munro G. Pharmacological characterisation of the spared nerve injury model of neuropathic pain. Pain 2002;98:151–61.
[32] Rode F, Brolos T, Blackburn-Munro G, Bjerrum OJ. Venlafaxine compromises the antinociceptive actions of gabapentin in rat models of neuropathic and persistent pain. Psychopharmacology (Berl) 2006;187:364–75.
[33] Folkesson A, Honore PH, Bjerrum OJ. Co-administered gabapentin and venlafaxine in nerve injured rats: Effect on mechanical hypersensitivity, motor function and pharmacokinetics. Scand J Pain 2010;1:91–7.
[34] Folkesson A, Honore PH, Andersen LM, Kristensen P, Bjerrum OJ. Low dose of donepezil improves gabapentin analgesia in the rat spared nerve injury model of neuropathic pain: single and multiple dosing studies. J Neural Transm 2010;117:1377–85.
[35] Lee SH, Kayser V, Desmeules J, Guilbaud G. Differential action of morphine and various opioid agonists on thermal allodynia and hyperalgesia in mononeuropathic rats. Pain 1994;57:233–40.
[36] Backonja MM, Miletic G, Miletic V. The effect of continuous morphine analgesia on chronic thermal hyperalgesia due to sciatic constriction injury in rats. Neurosci Lett 1995;196:61–4.
[37] Kayser V, Lee SH, Guilbaud G. Evidence for a peripheral component in the enhanced antinociceptive effect of a low dose of systemic morphine in rats with peripheral mononeuropathy. Neuroscience 1995;64:537–45.
[38] Lee YW, Chaplan SR, Yaksh TL. Systemic and supraspinal, but not spinal, opiates suppress allodynia in a rat neuropathic pain model. Neurosci Lett 1995;199:111–4.

[39] Erichsen HK, Hao JX, Xu XJ, Blackburn-Munro G. Comparative actions of the opioid analgesics morphine, methadone and codeine in rat models of peripheral and central neuropathic pain. Pain 2005;116:347–58.
[40] Ossipov MH, Lopez Y, Nichols ML, Bian D, Porreca F. Inhibition by spinal morphine of the tail-flick response is attenuated in rats with nerve ligation injury. Neurosci Lett 1995;199:83–6.
[41] Mao J, Price DD, Mayer DJ. Experimental mononeuropathy reduces the antinociceptive effects of morphine: implications for common intracellular mechanisms involved in morphine tolerance and neuropathic pain. Pain 1995;61:353–64.
[42] Bian D, Ossipov MH, Ibrahim M, Raffa RB, Tallarida RJ, Malan TP, Lai J, Porreca F. Loss of antiallodynic and antinociceptive spinal/supraspinal morphine synergy in nerve-injured rats: restoration by MK-801 or dynorphin antiserum. Brain Res 1999;831:55–63.
[43] Iyengar S, Webster AA, Hemrick-Luecke SK, Xu JY, Simmons RM. Efficacy of duloxetine, a potent and balanced serotonin-norepinephrine reuptake inhibitor in persistent pain models in rats. J Pharmacol Exp Ther 2004;311:576–84.
[44] Hayashida K, Eisenach JC. Multiplicative interactions to enhance gabapentin to treat neuropathic pain. Eur J Pharmacol 2008;598:21–6.
[45] Liston DR, Nielsen JA, Villalobos A, Chapin D, Jones SB, Hubbard ST, Shalaby IA, Ramirez A, Nason D, White WF. Pharmacology of selective acetylcholinesterase inhibitors: implications for use in Alzheimer's disease. Eur J Pharmacol 2004;486:9–17.
[46] Kosasa T, Kuriya Y, Matsui K, Yamanishi Y. Effect of donepezil hydrochloride (E2020) on basal concentration of extracellular acetylcholine in the hippocampus of rats. Eur J Pharmacol 1999;380:101–7.
[47] Kosasa T, Kuriya Y, Yamanishi Y. Effect of donepezil hydrochloride (E2020) on extracellular acetylcholine concentration in the cerebral cortex of rats. Jpn J Pharmacol 1999;81:216–22.
[48] Sugimoto H, Ogura H, Arai Y, Limura Y, Yamanishi Y. Research and development of donepezil hydrochloride, a new type of acetylcholinesterase inhibitor. Jpn J Pharmacol 2002;89:7–20.
[49] Clayton BA, Hayashida K, Childers SR, Xiao R, Eisenach JC. Oral donepezil reduces hypersensitivity after nerve injury by a spinal muscarinic receptor mechanism. Anesthesiology 2007;106:1019–25.
[50] Millan MJ. Descending control of pain. Prog Neurobiol 2002;66:355–474.
[51] Kondo D, Saegusa H, Yabe R, Takasaki I, Kurihara T, Zong S, Tanabe T. Peripheral-type benzodiazepine receptor antagonist is effective in relieving neuropathic pain in mice. J Pharmacol Sci 2009;110:55–63.
[52] Bunch L, Erichsen MN, Jensen AA. Excitatory amino acid transporters as potential drug targets. Expert Opin Ther Targets 2009;13:719–31.
[53] Sung B, Lim G, Mao J. Altered expression and uptake activity of spinal glutamate transporters after nerve injury contribute to the pathogenesis of neuropathic pain in rats. J Neurosci 2003;23:2899–910.
[54] Wu LJ, Zhuo M. Targeting the NMDA receptor subunit NR2B for the treatment of neuropathic pain. Neurotherapeutics 2009;6:693–702.
[55] Chizh BA, Headley PM. NMDA antagonists and neuropathic pain—multiple drug targets and multiple uses. Curr Pharm Des 2005;11:2977–94.
[56] Rothstein JD, Patel S, Regan MR, Haenggeli C, Huang YH, Bergles DE, Jin L, Dykes Hoberg M, Vidensky S, Chung DS, Toan SV, Bruijn LI, Su ZZ, Gupta P, Fisher PB. Beta-lactam antibiotics offer neuroprotection by increasing glutamate transporter expression. Nature 2005;433:73–7.
[57] Hu Y, Li W, Lu L, Cai J, Xian X, Zhang M, Li Q, Li L. An anti-nociceptive role for ceftriaxone in chronic neuropathic pain in rats. Pain 2010;148:284–301.
[58] Eljaja L, Bjerrum OJ, Honore PH, Abrahamsen B. Effects of the excitatory amino acid transporter subtype 2 (EAAT-2) inducer ceftriaxone on different pain modalities in rat. Scand J Pain 2011;2:132–6.
[59] Syriatowicz JP, Hu D, Walker JS, Tracey DJ. Hyperalgesia due to nerve injury: role of prostaglandins. Neuroscience 1999;94:587–94.
[60] Gordh T, Sharma HS, Alm P, Westman J. Spinal nerve lesion induces upregulation of neuronal nitric oxide synthase in the spinal cord. An immunohistochemical investigation in the rat. Amino Acids 1998;14:105–12.
[61] Winkler T, Sharma HS, Gordh T, Badgaiyan RD, Stalberg E, Westman J. Topical application of dynorphin A (1–17) antiserum attenuates trauma induced alterations in spinal cord evoked potentials, microvascular permeability disturbances, edema formation and cell injury: an experimental study in the rat using electrophysiological and morphological approaches. Amino Acids 2002;23:273–81.
[62] Ma W, Eisenach JC. Morphological and pharmacological evidence for the role of peripheral prostaglandins in the pathogenesis of neuropathic pain. Eur J Neurosci 2002;15:1037–47.
[63] Sweitzer S, Martin D, DeLeo JA. Intrathecal interleukin-1 receptor antagonist in combination with soluble tumor necrosis factor receptor exhibits an anti-allodynic action in a rat model of neuropathic pain. Neuroscience 2001;103:529–39.
[64] Sharma HS, Winkler T, Stalberg E, Gordh T, Alm P, Westman J. Topical application of TNF-alpha antiserum attenuates spinal cord trauma induced edema formation, microvascular permeability disturbances and cell injury in the rat. Acta Neurochir Suppl 2003;86:407–13.
[65] Barker PA. p75NTR is positively promiscuous: novel partners and new insights. Neuron 2004;42:529–33.
[66] Reichardt LF. Neurotrophin-regulated signalling pathways. Philos Trans R Soc Lond B Biol Sci 2006;361:1545–64.
[67] Jansen P, Giehl K, Nyengaard JR, Teng K, Lioubinski O, Sjoegaard SS, Breiderhoff T, Gotthardt M, Lin F, Eilers A, Petersen CM, Lewin GR, Hempstead BL, Willnow TE, Nykjaer A. Roles for the pro-neurotrophin receptor sortilin in neuronal development, aging and brain injury. Nat Neurosci 2007;10:1449–57.
[68] Willnow TE, Petersen CM, Nykjaer A. VPS10P-domain receptors – regulators of neuronal viability and function. Nat Rev Neurosci 2008;9:899–909.
[69] Fauchais AL, Lalloue F, Lise MC, Boumediene A, Preud'homme JL, Vidal E, Jauberteau MO. Role of endogenous brain-derived neurotrophic factor and sortilin in B cell survival. J Immunol 2008;181:3027–38.
[70] Lee R, Kermani P, Teng KK, Hempstead BL. Regulation of cell survival by secreted proneurotrophins. Science 2001;294:1945–8.
[71] Levi-Montalcini R. The nerve growth factor 35 years later. Science 1987;237:1154–62.
[72] Crowley C, Spencer SD, Nishimura MC, Chen KS, Pitts-Meek S, Armanini MP, Ling LH, McMahon SB, Shelton DL, Levinson AD. Mice lacking nerve growth factor display perinatal loss of sensory and sympathetic neurons yet develop basal forebrain cholinergic neurons. Cell 1994;76:1001–11.
[73] Heumann R, Korsching S, Bandtlow C, Thoenen H. Changes of nerve growth factor synthesis in nonneuronal cells in response to sciatic nerve transection. J Cell Biol 1987;104:1623–31.
[74] Wells MR, Vaidya U, Schwartz JP. Bilateral phasic increases in dorsal root ganglia nerve growth factor synthesis after unilateral sciatic nerve crush. Exp Brain Res 1994;101:53–8.
[75] Zhou XF, Deng YS, Chie E, Xue Q, Zhong JH, McLachlan EM, Rush RA, Xian CJ. Satellite-cell-derived nerve growth factor and neurotrophin-3 are involved in noradrenergic sprouting in the dorsal root ganglia following peripheral nerve injury in the rat. Eur J Neurosci 1999;11:1711–22.
[76] Ramer MS, Bisby MA. Adrenergic innervation of rat sensory ganglia following proximal or distal painful sciatic neuropathy: distinct mechanisms revealed by anti-NGF treatment. Eur J Neurosci 1999;11:837–46.
[77] Binder DK, Scharfman HE. Brain-derived neurotrophic factor. Growth Factors 2004;22:123–31.
[78] Sharma HS, Nyberg F, Gordh T, Alm P, Westman J. Topical application of insulin like growth factor-1 reduces edema and upregulation of neuronal nitric oxide synthase following trauma to the rat spinal cord. Acta Neurochir Suppl 1997;70:130–3.
[79] Sharma HS, Nyberg F, Westman J, Alm P, Gordh T, Lindholm D. Brain derived neurotrophic factor and insulin like growth factor-1 attenuate upregulation of nitric oxide synthase and cell injury following trauma to the spinal cord. An immunohistochemical study in the rat. Amino Acids 1998;14:121–9.
[80] Sharma HS, Westman J, Gordh T, Alm P. Topical application of brain derived neurotrophic factor influences upregulation of constitutive isoform of heme oxygenase in the spinal cord following trauma an experimental study using immunohistochemistry in the rat. Acta Neurochir Suppl 2000;76:365–9.
[81] Poyhia R, Xu M, Kontinen VK, Paananen S, Kalso E. Systemic physostigmine shows antiallodynic effects in neuropathic rats. Anesth Analg 1999;89:428–33.
[82] Ramos KM, Lewis MT, Morgan KN, Crysdale NY, Kroll JL, Taylor FR, Harrison JA, Sloane EM, Maier SF, Watkins LR. Spinal upregulation of glutamate transporter GLT-1 by ceftriaxone: therapeutic efficacy in a range of experimental nervous system disorders. Neuroscience 2010;169:1888–900.
[83] Mogil JS, Crager SE. What should we be measuring in behavioral studies of chronic pain in animals? Pain 2004;112:12–5.
[84] Vierck CJ, Hansson PT, Yezierski RP. Clinical and pre-clinical pain assessment: are we measuring the same thing? Pain 2008;135:7–10.
[85] Mogil JS, Graham AC, Ritchie J, Hughes SF, Austin JS, Schorscher-Petcu A, Langford DJ, Bennett GJ. Hypolocomotion, asymmetrically directed behaviors (licking, lifting, flinching, and shaking) and dynamic weight bearing (gait) changes are not measures of neuropathic pain in mice. Mol Pain 2010;6:34.
[86] Gilron I, Max MB. Combination pharmacotherapy for neuropathic pain: current evidence and future directions. Expert Rev Neurother 2005;5:823–30.
[87] Black DR, Sang CN. Advances and limitations in the evaluation of analgesic combination therapy. Neurology 2005;65:S3–6.
[88] Davis MP. What is new in neuropathic pain? Support Care Cancer 2007;15:363–72.
[89] Sindrup SH, Otto M, Finnerup NB, Jensen TS. Antidepressants in the treatment of neuropathic pain. Basic Clin Pharmacol Toxicol 2005;96:399–409.
[90] Kim KJ, Yoon YW, Chung JM. Comparison of three rodent neuropathic pain models. Exp Brain Res 1997;113:200–6.
[91] Dowdall T, Robinson I, Meert TF. Comparison of five different rat models of peripheral nerve injury. Pharmacol Biochem Behav 2005;80:93–108.
[92] Hansson E. Longterm pain, neuroinflammation and glial activation. Scand J Pain 2010;1:67–72.
[93] Rode F, Jensen DG, Blackburn-Munro G, Bjerrum OJ. Centrally-mediated antinociceptive actions of $GABA_A$ receptor agonists in the rat spared nerve injury model of neuropathic pain. Eur J Pharmacol 2005;516:131–8.