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Lacosamide has protective disease modifying properties in experimental vincristine neuropathy

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Abstract

Lacosamide has protective disease modifying properties in experimental vincristine neuropathy

Pain and paresthesias are the most common symptoms of chemotherapy-induced painful neuropathy (CIPN). Current treatment and preventive strategies of CIPN are ineffective, and the neuropathy may lead to discontinuation of anti-tumor therapy. Here experimental vincristine-induced neuropathy in rats was used to evaluate the disease-modifying potential of lacosamide using a sustained release formulation and the acute treatment effects of a rapid release formulation. Pain behavior was assessed by withdrawal responses to von Frey hairs, acetone drops, the Randall-Selitto device, and to radiant heat. Preventive lacosamide treatment (30 mg/kg subcutaneously b.i.d. for 17 days) was well tolerated, and pharmacokinetic analysis revealed a peak plasma concentration 2 hours post-injection with a plasma half-life of approximately 3 hours. Rats treated with lacosamide, in contrast to vehicle-treated rats, did not develop vincristine-induced cold allodynia. A protective disease modifying potency of lacosamide could thus be demonstrated in an animal model of CIPN. Lacosamide may be a promising candidate for preventive treatment of CIPN in patients receiving chemotherapy with vinca alkaloids or platinum drugs.

Abbreviations

AAN	American Academy of Neurology
ANOVA	analysis of variance
AWMF	Arbeitsgemeinschaft der Wissenschaftlichen Medizinischen Fachgesellschaften
b.i.d.	twice daily
CaV2.2	calcium channel
CIPN	chemotherapy-induced painful neuropathy
CFA	Freund's Complete Adjuvant
Cmax	maximal concentration
C_{12}	concentration at 12 hours
CNS	central nervous system
CRMP-2	collapsin-response mediator protein 2
DRG	Dorsal root ganglia
err prob	probability of error
g	gram
h	hours
HIV	human immunodeficiency virus
ION	infraorbital nerve injury-induced pain
i.p.	intraperitoneal injection
kg	kilogram
L5	lumbal segment 5
MIA	monosodium iodoacetate-induced arthritic pain
mg	milligram
min	minutes
mm	millimeter
msec	milliseconds
n	number
n.t.	not tested
PNS	peripheral nervous system
rpm	rounds per minute

s	seconds
SCI	spinal cord injury-induced pain
s.c.	subcutane
S.E.M.	standard error of the means
Ser	serin
Sema3A	semaphorin 3A
STZ	streptocotozin-induced diabetic neuropathic pain
TNF	tumor necrosis factor α
VGSCs	voltage-gated sodium cannels
vin	vincristine

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1. Introduction

Pain is the leading reason for patients seeking medical care (Zagari et al. 1996) and is one of the most disabling, burdensome, and costly conditions. Given the aging population there is a clear and imminent need for valid health care economic evaluation data to establish health care funding priorities, including for chronic pain treatment modalities (Davis et al., 2011). According to the International Pain Society, pain management is inadequate in most of the world because there are major deficits in knowledge on the part of the health care professionals regarding the mechanisms and management of pain.

Neuropathic pain compared to nociceptive or inflammatory pain is not protective, but maladaptive, resulting from abnormal functioning of the nervous system. It is not a symptom of some disorder but rather a disease state of the nervous system, which can occur after damage to the nervous system (Backonja and Woolf 2010).
Pain and loss of function are intimately associated with the reaction of the nervous system to neural damage, and both provide important diagnostic clues that such damage has occurred. Peripheral neuropathic pain results from lesions to the peripheral nervous system (PNS) caused by mechanical trauma, metabolic diseases, neurotoxic chemicals, infection, or tumor invasion and involves multiple pathophysiological changes both within the PNS and in the CNS (Dworkin et al. 2003, Woolf & Mannion 1999). Central neuropathic pain most commonly results from spinal cord injury, stroke, or multiple sclerosis (Ducreux et al. 2006). The conventional approach to neuropathic pain has been to classify and treat it on the basis of the underlying disease (Dworkin et al. 2007). However, such an etiological approach does not capture the essential feature of neuropathic pain, which is the manifestation of maladaptive plasticity in the nervous system. The primary disease and the neural damage it causes are only the initiators of a cascade of changes that lead to and sustain neuropathic pain. Although treatment targeted at the primary pathology is

obviously essential, understanding the mechanisms responsible for the maladaptive plasticity offers specific therapeutic opportunities to prevent the development of neuropathic hypersensitivity and normalize function in established neuropathic pain (Costigan et al., 2009).

Many rodent models of neuropathic pain have been developed. Some have been designed to mimic human diseases, others to explore pathophysiological mechanisms in the nervous system, and some as a convenient means to screen for putative analgesics (Table 1. Costigan et al., 2009).

Model	Nature of injury	Extent of neural damage and lesion site	Clinical correlate
Sciatic nerve transection (Wall et al. 1979)	Transection and ligation of sciatic nerve	~60% of DRG cells; mid nerve.	Nerve trauma, iatrogenic nerve injury
Partial sciatic nerve ligation (Seltzer et al. 1990)	Partial ligation of sciatic nerve	~30% of DRG cells; mid nerve; intact axons interacting with Schwann cells	Partial peripheral nerve injury
Spinal nerve ligation (Kim & Chung 1992)	Ligation of the L5 and L6 spinal nerves	~100% DRG cells; proximal nerve; intact axons interacting with Schwann cells	Proximal peripheral nerve damage, e.g., after disc prolaps
Spared nerve injury (Decosterd & Woolf 2000)	Ligation and transection of two of three distal sciatic nerve branches	~40% of DRG cells; distal nerve.	Partial peripheral nerve damage
Chronic constriction injury (Bennett & Xie 1988)	Loose ligature of the sciatic nerve with chromic gut suture	Mainly myelinated axons, <30% of DRG cells; mid nerve; intact axons interacting with Schwann cells	Nerve entrapment, e.g., carpal tunnel syndrome
Sciatic inflammatory neuropathy (Chacur et al. 2001)	Perineural injection of immune activator (zymosan or CFA)	No axonal loss; secondary DRG cell damage; mid nerve	Peripheral neuritis
Peripheral nerve demyelination (Wallace et al. 2003)	Immune- or toxin-mediated demyelination	Minimal axon loss; secondary DRG cell damage; mid nerve	Demyelination, e.g., diabetic neuropathies
Diabetic neuropathy (Sullivan et al. 2008)	Streptozotocin, diet, genetic models	Primarily distal axon loss; systemic injury of the PNS; intact axons interacting with Schwann cells	Diabetic neuropathy
Viral neuropathy (Wallace et al. 2007)	Herpes simplex virus, varizella zoster virus, HIV (gp120)	Distal axon damage; DRG cell damage; distal nerve; intact axons interacting with Schwann cells	Zoster-associated pain, postherpetic neuralgia HIV-associated neuropathy
Drug-induced neuropathy (Peltier & Russell 2002)	Vincristine, paclitaxel, cisplatin	Distal axon loss; DRG cell damage; systemic injury of the PNS; intact axons interacting with Schwann cells	Polyneuropathy caused by tumor chemotherapy

Table 1. Animal models of neuropathic pain (Costigan et al., 2009)

The aim of this study was to analyze the disease modifying potency of lacosamide (Vimpat®), a new antiepileptic drug, in a rodent neuropathic pain model. Up to date there is no disease modifying drug for pain treatment available only symptoms are treated. Lacosamide, R- 2-acetamido-N-benzyl-3-methoxypropionamide, SPM 927, formerly called harkoseride) is a functionalized amino acid, approved in the USA and in Europe as adjunctive therapy for partial-onset seizures. Lacosamide was further evaluated in patients with diabetic neuropathic pain, fibromyalgia and for migraine prophylaxis (Bialer et al., 2009). Its analgesic efficacy was shown in several short- and long-term Phase II/III clinical trials in humans with diabetic neuropathic pain (Rauck et al., 2007; Wymer et al., 2009, Ziegler et al., 2010).

Lacosamide has demonstrated antinociceptive potential as shown in many experimental animal pain models that reflect distinct types and symptoms of neuropathic as well as chronic inflammatory pain (Beyreuther et al., 2007a-e, 2006). For example anti-hyperalgesic efficacy of lacosamide was shown in a rat model for muscle pain induced by TNF (tumor necrosis factor α) (Beyreuther et al., 2007d) as well as in the monosodium iodoacetate rat model for osteoarthritis pain (Beyreuther et al., 2007e). Additionally antinociceptive efficacy of lacosamide could be demonstrated in a rat model for painful diabetic neuropathy showing stronger antinociceptive activity than lamotrigine, levetiracetam, pregabalin and venlafaxine (Beyreuther et al., 2006, Figure 1). As expected, lacosamide had no acute antinociceptive effects in nondiabetic animals indicating specific antihyperalgesic and antiallodynic effects under conditions of diabetic neuropathic pain. Lacosamide was effective in animal models for central neuropathic pain, the infra-orbital nerve and the spinal cord injury model suggesting usefulness as an analgesic for treating central and trigeminal neuropathic pain (Hao et al. 2006).

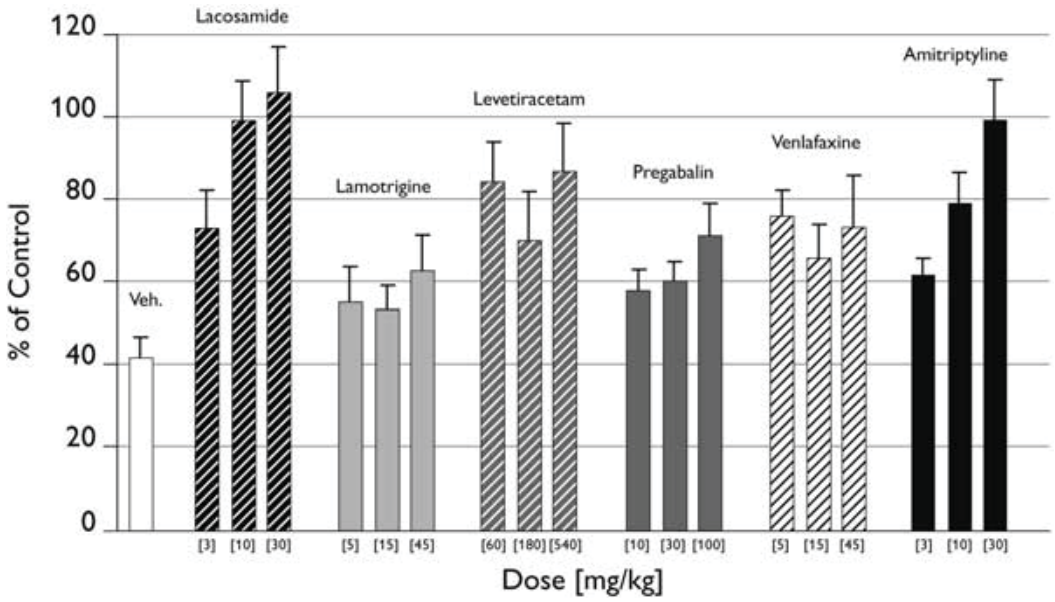


Figure 1. Lacosamide was tested in the streptozotocin rat model of diabetic neuropathic pain in comparison to drugs that are commonly used in the treatment of diabetic neuropathic pain, that is, antidepressants and anticonvulsants (Beyreuther et al. 2006). Diabetes was induced by intravenous injections of streptozotocin in the left saphena magna. Blood glucose levels were checked before each phase of behavioral testing. Tests for allodynia (warm plate at 38°C) were performed on day 10 after the induction of diabetes with streptozotocin. In diabetic rats, lacosamide attenuated warm (3, 10, 30 mg/kg, i.p.) allodynia. Morphine (3 mg/kg) showed similar efficacy on allodynia (data not shown). Amitriptyline (10 mg/kg), venlafaxine (15 mg/kg), levetiracetam (180 mg/kg), and pregabalin (100 mg/kg) exhibited significant effects on thermal allodynia. Only treatment with amitriptyline (30 mg/kg, i.p.) produced full reversal of thermal allodynia comparable to lacosamide. Lamotrigine (45 mg/kg, i.p.) had no effect on behavioral readouts.

In summary, lacosamide has broad analgesic activity in multiple animal models for chronic pain (Table 2). The effects were mostly observed at doses ranging from 3 to 30 mg/kg, i.p., which with respect to drug exposure correspond to the clinically tested doses of 200 mg, 400 mg, and 600 mg b.i.d. (Beyreuther et al., 2007).

	Tail-flick	Formalin	Carrageenan	CFA	STZ	MIA	SCI	ION
Thermal allodynia	n.t.	n.t.	n.t.	n.t.	+	n.t.	+	n.t.
Mechanical allodynia	n.t.	n.t.	+	n.t.	+	+	+	+
Thermal hyperalgesia	n.t.	n.t.	+	n.t.	+	n.t.	+	n.t.
Mechanical hyperalgesia	n.t.	n.t.	+	+	+	+	+	n.t.
Others pain endpoints	0	+	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
Edema	n.t.	n.t.	0	n.t.	n.t.	n.t.	n.t.	n.t.

Table 2. n.t., not tested; + activity, 0 no activity. CFA, complete Freund's adjuvant test; STZ, streptocotozin-induced diabetic neuropathic pain; MIA, monosodium iodoacetate-induced arthritic pain; SCI, spinal cord injury-induced pain; ION, infraorbital nerve injury-induced pain (Beyreuther et al., 2007).

Recent results suggest that lacosamide has a new mode of action underlying its anticonvulsant and analgesic activity. It was found in electrophysiological experiments in neuroblastoma cells that lacosamide selectively enhances slow inactivation of voltage-gated sodium channels without affecting fast inactivation. In contrast to other means of sodium channel modulation the selective enhancement of slow inactivation results in a more favorable safety profile by attenuating pathophysiological neuronal hyperexcitation while leaving physiological activity intact. Most other anti-epileptic drugs, affecting voltage-gated sodium channels fast inactivation, lead to side effects reflecting those of general anaesthetics (Bee and Dickenson, 2009; Errington et al., 2008; Sheets et al., 2008, Figure 2).

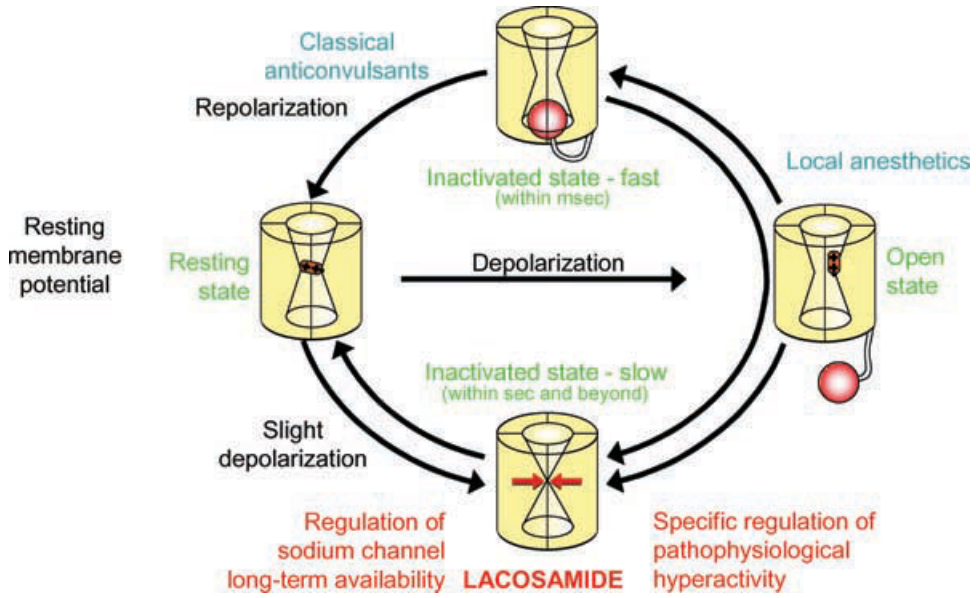


Figure 2. Physiology of voltage-gated sodium channels. Depending on the membrane potential and the neuronal activity voltage-gated sodium channels are in different states. At the resting potential sodium channels are closed and can be opened by depolarization of the membrane potential allowing the flux of sodium ions into the cell. Within a few milliseconds the channels close from the inside of the neuron and go into the fast inactivated state from which they cannot be activated. When the membrane potential returns to its baseline the sodium channel goes back to its resting state. Under conditions of slight prolonged depolarization or repetitive neuronal activity the sodium channel can go into the slow inactivated state by closing the pore from the inside. This process happens on a second-to-minute time scale. Drugs can either block the open channel (e.g., local anaesthetics), or enhance fast inactivation (classical anticonvulsants) or enhance slow inactivation (lacosamide) (Beyreuther et al., 2007).

Furthermore, employing proteomic affinity-labeling techniques, collapsin-response mediator protein 2 (CRMP-2 alias DRP-2, DPYSL2, CRMP2) was identified as a binding partner (Beyreuther et al., 2007). It was postulated that CRMP-2 levels affect the actions of lacosamide on voltage-gated sodium cannels (VGSCs). CRMP-2 labeling by lacosamide analogs was competitively displaced by excess lacosamide in rat brain lysates. *In silico* docking was performed to identify putative binding sites in CRMP-2 that may modulate the effects of lacosamide on VGSCs. Those studies identified five cavities in CRMP-2 that could accommodate lacosamide (Figure 3). Electrophysiological characterization of lacosamide binding sites on CRMP-2 key residues were identified that coordinate lacosamide binding thus making it more effective on its primary clinical target (Wang et al., 2010).

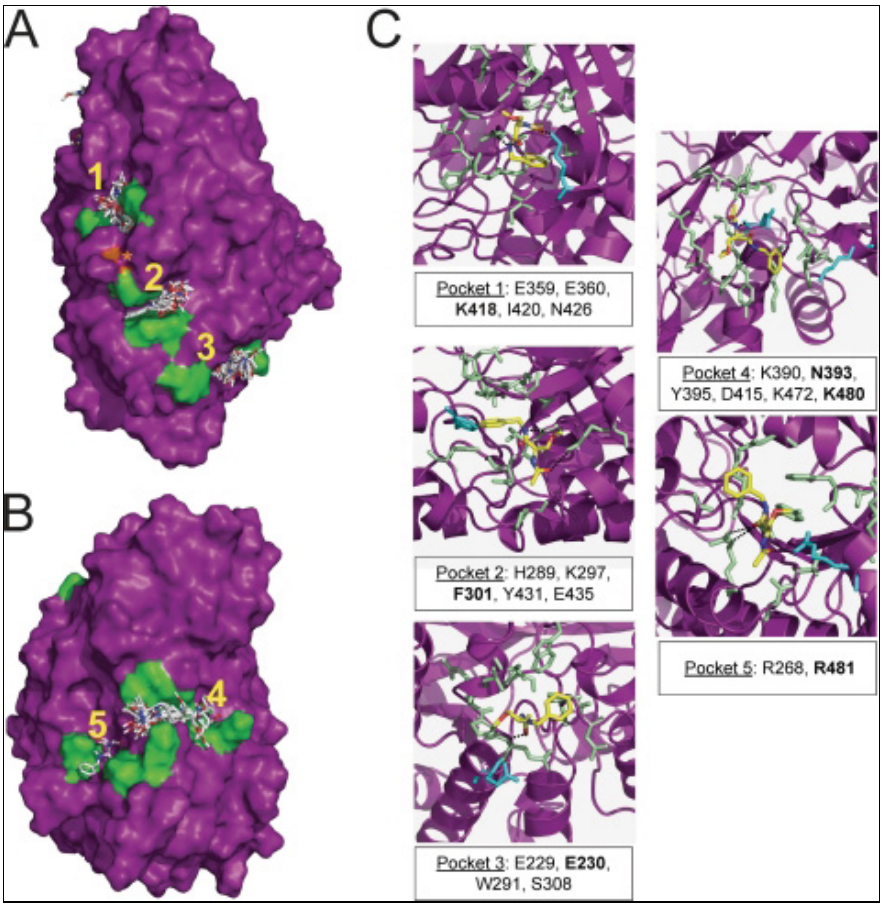


Figure 3. *In silico* docking identifies five putative (*R*)-lacosamide binding pockets on CRMP-2. *A* and *B*, surface representations of the three-dimensional structure of the CRMP-2 monomer (PDB code: 2GSE) with the locations of the putative binding pockets (*1–5*) highlighted in *green*. One or more lacosamides bound to the five binding cavities within CRMP-2 are indicated. Lacosamide is shown in *capped-sticks representation*. Lacosamide is color-coded according to atom types (C, N, and O in *white*, *blue*, and *red*, respectively). The structure in *B* is rotated ~180° relative to that in *A. C*, an *enlarged view* of each of the five binding pockets. *C atoms* are shown in *yellow*. Only the predominant conformational state of lacosamide is shown in each pocket. The *asterisk* denotes the position of amino acid Ser-421 (*orange* residue) that is adjacent, but not predicted to be within coordinating range, of putative binding pocket 2. The residues coordinating lacosamide binding are indicated in *single amino acid letter code*. Residues in *bold* (shown in *blue*) indicate positions that were mutated to alanine. Hydrogen bonds are shown with *dashed lines* (Wang et al., 2010).

CRMP-2 was first identified by Goshima and colleagues in chick dorsal root ganglia (DRG) cultures as a signal transducer responsible for axon growth cone retraction evoked by negative guidance signals in the semaphorin 3A (Sema3A) pathway of the developing nervous system (Goshima et al., 1995). Subsequent work largely performed by the Strittmatter, Kaibuchi, Goshima, and Charrier groups demonstrated that CRMP-2 serves functions as a dynamic structure regulator of post-mitotic mammalian neurons as well, and clarified several mechanisms of this function (Charrier et al., 2003; Cole et al., 2004; Deo et al., 2004; Inagaki et al., 2001; Yoshimura et al., 2005). CRMP-2 acts largely, but not solely, by binding and stabilizing tubulin at the plus end of microtubules (Figure 4) thus promoting axon extension (Hensley et al., 2010; Quach et al., 2004). Thus, over-expressing CRMP-2 in neuro-culture causes general increase of neurite length, and can result in supernumerary axons. Since chronic and neuropathic pain conditions as well as epilepsy are associated with abnormal neurite sprouting the functional antagonism of lacosamide could explain some of its utility against disease symptoms.

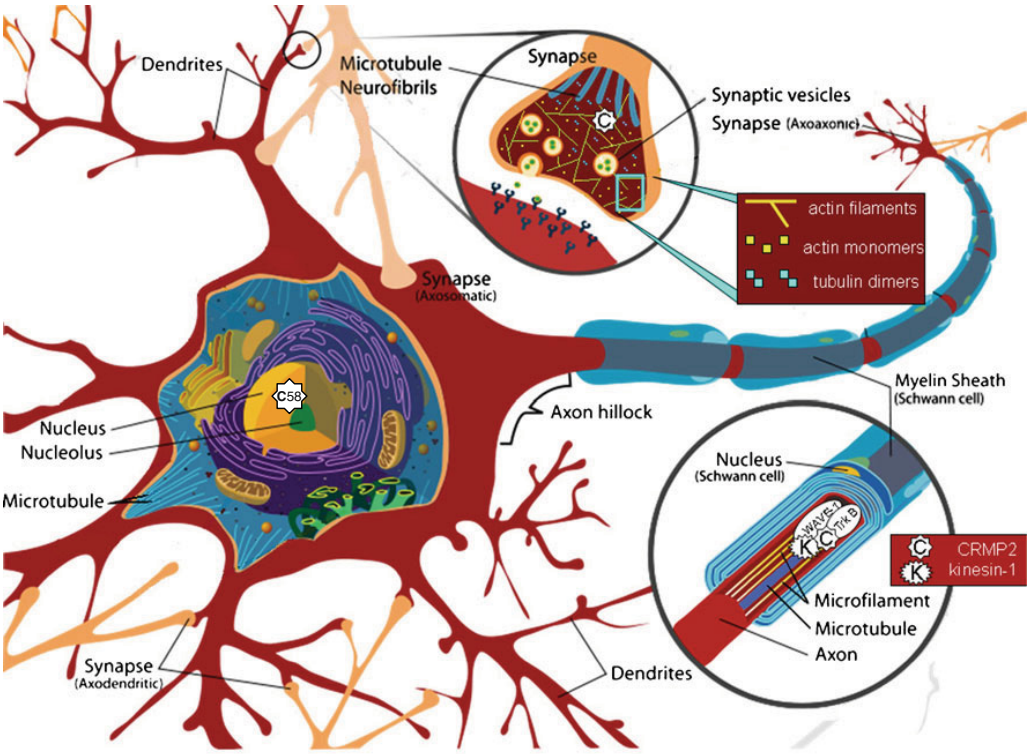


Figure 4. Postulated mechanisms by which CRMP-2 affects neural structure. The cytoskeletal structure of a neuron is determined by tubulin-based microtubule networks that provide rigidity inside axons, by actin-based microfilament networks that provide flexibility near curvilinear branch points and synapses, and by intermediate (neuro) filaments that set axon diameter. Full-length CRMP-2 promotes neurite growth through mechanisms involving all three types of cytoskeletal networks. Additionally, a 58 kDa CRMP-2 proteolytic fragment (C58 in this figure) translocates to the nucleus under certain stress conditions and functions to inhibit neurite outgrowth (Hensley et al., 2010).

The consequences of the interaction between lacosamide and CRMP-2 are not fully understood, and might include facilitation of the drug's action on sodium channels (Wang et al., 2010). Recent findings suggest that the biochemical interaction between CRMP-2 and CaV2.2 is required for proper channel trafficking and function (Brittain et al., 2009, 2011, Figure 5).

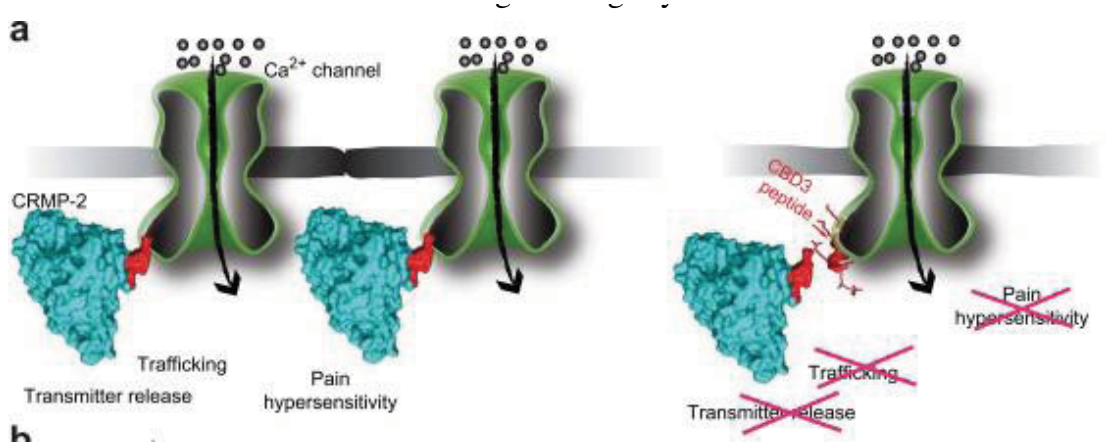


Figure 5. The designed specific binding protein CBD3 allows suppression of pain hypersensitivity without directly blocking CaV2.2, but rather by inhibiting the binding of a regulator of CaV2.2 function, CRMP-2. Thus, these findings represent a novel approach potentially useful in managing clinical pain (Brittain et al. 2011).

Chemotherapy-induced painful neuropathy (CIPN) is still a common side effect of anti-tumor treatment with vinca alkaloids, platinum drugs, taxanes, and other chemotherapeutic drugs affecting up to 30% - 40% of patients (Kaley and Deangelis, 2009; Wolf et al., 2008). Symptoms usually start when chemotherapy is ongoing and tend to improve after completing therapy. However, in 25% - 30% of patients, pain or unpleasant paresthesias remain or even increase after terminating the chemotherapeutic treatment (Verstappen et al., 2005). Vincristine is one of the most common anti-cancer drugs applied for the treatment of many types of cancers such as leukemias, lymphomas, and sarcomas, and entails the side effect of a dose- and duration-dependent

peripheral neuropathy (Dougherty et al., 2007; Postma et al., 1993). There is up to date no effective prevention or treatment for CIPN, such that many patients are forced to reduce or discontinue their potentially highly effective and potentially curative anti-tumor neurotoxic drugs (Kaley and Deangelis, 2009; Wolf et al., 2008). Even symptomatic treatment of pain in CIPN may be difficult. Recently, the tricyclic antidepressants nortriptyline and amitriptyline (Hammack et al., 2002; Kautio et al., 2008) as well as the anti-convulsive agents gabapentin and lamotrigine (Rao et al., 2008, 2007) were shown to be ineffective in placebo-controlled, double blind phase III studies.
The anti-tumor action of vincristine is due to its binding to β-tubulin, which leads to disorganization of the axonal microtubule cytoskeleton. This effect underlies its anti-mitotic property and also in part explains the neurotoxic effect (Tanner et al., 1998). In contradistinction to the treatment of rheumatic pain with disease modifying anti-rheumatic drugs only the symptoms of chemotherapy-induced neuropathic pain might be treated. The influence of vincristine on axonal microtubule organization makes this pain model especially interesting to analyze the disease modifying potential of lacosamide regarding its CRMP-2 mode of action.

Since the antinociceptive effects of lacosamide were demonstrated in several studies in the past (Beyreuther et al., 2007a) this study was performed to evaluate the disease modifying and preventive effect of lacosamide in a rat model of vincristine-induced painful neuropathy. To evaluate the modification of disease development it was necessary to use a chronic treatment protocol. The plasma half-life of an immediate release formulation of lacosamide differs substantially between rodents (~1 h) and humans (~11 h). It was not feasible to inject the animals several times in 24 h to keep the plasma levels at an effective concentration. Therefore it was necessary to identify a sustained release formulation of lacosamide for these experiments. The best formulation identified was an oily suspension, which was well tolerated. Nevertheless pharmacokinetic analysis at the end of the study of a group of satellite animals showed that lacosamide was detectable in the rats during the whole treatment

period but did not keep a steady plasma level (Geis et al., 2011).

Before beginning this study a pilot study was performed to identify the best behavioral readouts with significant outcome after vincristine treatment. The final experiments where conducted with 0.1 mg/kg vincristine intraperitoneally. As behavioral readouts the paw pressure test (mechanical hyperalgesia), the von Frey test (tactile allodynia) and the acetone drop test (cold allodynia) where chosen. Additionally body weight, food intake and locomotor activity were measured reflecting the physical condition of the rats. As a control experiment naïve rats were chronically treated with lacosamide (Figure 6).
In a parallel study neuropathy was assessed using electrophysiological recordings from the sciatic nerve of the tested rats (Geis et al., 2011).

2. Material and Methods

2.1 Animals

32 adult male Sprague Dawley rats with a body weight of 250 g - 300 g (Charles River, Sulzfeld, Germany) were used. Animals were group-housed (5 animals per cage) and maintained in a room with controlled temperature (21ºC - 22ºC) and a reversed light-dark cycle (12 h / 12 h) with food and water available ad libitum. All experiments were conducted according to Bavarian state regulations for animal experimentation, were approved by Bavarian State authorities and followed the guidelines in the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978). All efforts were made to minimize animal suffering and to reduce the number of animals used. The study has been performed in accordance with the ARRIVE guidelines for reporting animal research and the criteria for good laboratory practice (Kilkenny et al., 2010; Macleod et al., 2009). The investigators performing behavioral tests were blinded to the treatment of the rats.

2.2 Vincristine intoxication, lacosamide treatment and experimental groups

Rats were intraperitoneally (i.p.) injected with vincristine or vehicle according to a published protocol (Weng et al., 2003). I.p. bolus injections of vincristine sulfate (Hexal, Holzkirchen, Germany; dissolved in sterile saline) were performed daily, from day 1 to 5, from day 8 to 12, and on days 15 and 16. Before treatment and on day 17, animals were submitted to behavioral tests (Figure 6). Vincristine was used in a concentration of 0.1 mg/kg. Tolerability of chronic treatment with lacosamide (lacosamide in oily suspension: 1.1% miglyoleimwitor solution, sustained release; dose 30 mg/kg, s.c. 30 mg/ml; n = 8) was tested compared to naïve animals (n = 4) for a treatment period of 16 days with injections twice daily.

Vincristine-intoxicated rats (0.1 mg/kg) were randomly distributed into two experimental groups (group size n = 8). Randomization was done by an independent person not involved in drug administration and group size was determined with an a-priori power analysis (α (err prob) = 0.05, power (1-β err prob) = 0,8 and biological relevant difference of 1.5). 1) Vehicle control (vincristine / vehicle, 1.1% miglyoleimwitor solution, dose 30 mg/kg, s.c. 30 mg/ml b.i.d.), 2) Chronic lacosamide treatment (vincristine / lacosamide in oily suspension; dose 30 mg/kg, s.c. 30 mg/ml b.i.d.). Lacosamide and the vehicle were provided by Schwarz Biosciences GmbH (Monheim, Germany). Treatment with vehicle or lacosamide started one day before the first vincristine dose (day-1) and continued throughout vincristine application until day 16 (day 16 only morning injection). Additionally, an acute treatment with 10 mg/kg i.p. lacosamide in aqueous solution was performed on day 18, and pain behavior was assessed 2 h post application (Figure 6).

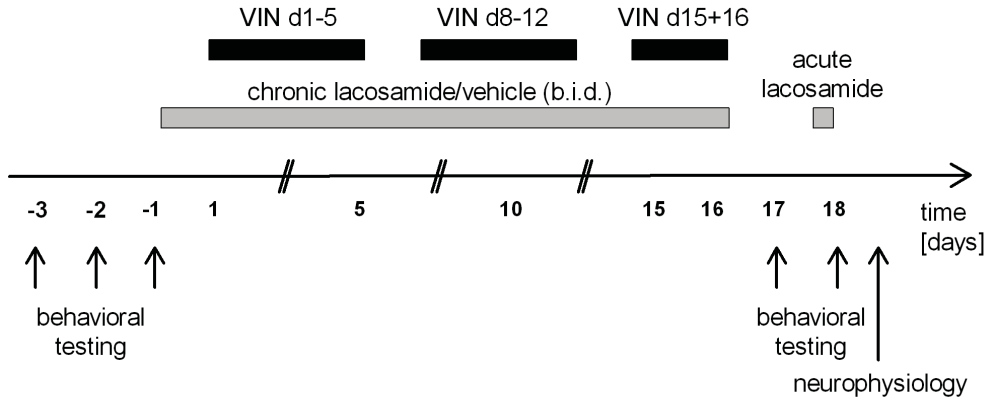


Figure 6. Experimental protocol: chronic lacosamide / vehicle treatment (30 mg/kg, extended release formulation, b.i.d., under dorsal skin) was administered from day-1 until day 16 (only morning injection), vincristine treatment was given (0.1 mg/kg) once daily. Behavioral testing was done before starting lacosamide injections and on day 17. Another single injection of lacosamide (aqueous solution) was applied on day 18 followed by behavioral testing 2 h later.

2.3 Behavioral testing, body weight and food intake

Behavioral tests were performed on three days before vincristine treatment to obtain baseline values and on day 17. For all tests, rats were adapted to the testing environment for at least 1 h before testing. The investigator was unaware of the treatment assignment.
For assessment of body weight and food intake, animals and rat food pellets were weighed daily between day -3 and 18 as a parameter for wellbeing and stress.

2.4 Assessment of cold thermal allodynia (acetone drop test)

The acetone test was performed according to Choi et al. (Choi et al., 1994). Rats were individually placed on an elevated metallic wire mesh floor in polyethylene cages. A drop of acetone was formed at the end of a polyethylene tube with a tip diameter of 0.8 mm connected with a syringe and gently applied at the plantar aspect of the hind paw. A drop of water from a similar tube with a temperature of 37°C serves as control. A response to acetone is defined as sharp withdrawal of the hind paw lasting >1 s. The paw elevation time was measured with a digital stopwatch from the onset of the paw withdrawal until the paw was rested again for at least 2 s. The acetone was applied three times on each paw and the mean of 3 trials was calculated. Repetitive testing was performed with an interval of at least 5 min for the same paw and of at least 1 min for the contra-lateral paw. Rats were tested on day -3, -2, -1 for baseline, on day 17 after lacosamide chronic treatment, and on day 18 after acute lacosamide treatment in aqueous solution.

2.5 Assessment of tactile allodynia (von Frey hair withdrawal thresholds)

Rats were individually placed on an elevated metallic wire mesh floor in polyethylene cages. The von Frey filaments (Stoelting, USA) were inserted through the mesh floor and applied to the plantar surface of the hind paw. The force required to bend the hairs ranged from 0.03 g to 8.5 g. Hairs were applied

six times each on the basis of the up-and-down method as described previously (Chaplan et al., 1994). The time interval between two trials was at least 1 min on the same paw and at least 30 s on the alternate paw. A brisk withdrawal of the paw was considered as a positive response. The 50% withdrawal threshold (i.e., force of the von Frey hair to which an animal reacts in 50% of the presentations) was recorded.

2.6 Assessment of mechanical hyperalgesia (paw pressure, Randall-Selitto test)

The test was performed as described previously (Beyreuther et al., 2007c). The nociceptive flexion reflex is quantified using the Randall-Selitto paw pressure device (Ugo Basile, Italy), which applies a linearly increasing mechanical force to the dorsum of the rat's hind paw. The mechanical nociceptive threshold is defined as the force in grams at which the rat withdraws its paw. The cut off pressure was set to 250 g.

2.7 Motor performance

Rats were trained on an accelerating RotaRod (TSE Systems, Bad Homburg, Germany) starting with 5 rpm and acceleration of 10 rpm during 100 s. For each testing procedure 5 runs per animal were performed and the mean of the 3 best runs were taken for further analysis. The time interval between two trials was at least 1 min. Rats were tested on day -3, -2, -1 for baseline and on day 17 after treatment. No significant effect could be observed in the vincristine / vehicle group, therefore the test was not included in the final assay.

2.8 Data analysis

The behavior data are presented as ± standard error of the means (S.E.M.). The behavioral data were statistically analyzed by the two-way analysis of variance (ANOVA) followed by post-hoc analysis (Dunnett's test) to compare groups of behavioral data in each individual time points.

3. Results

3.1 Chronic lacosamide treatment in naïve animals

Lacosamide or vehicle were injected twice daily s.c. in naïve animals for 17 days (beginning from day -1) and behavioral tests were performed on day 17. Lacosamide and vehicle oily solution were well tolerated and no relevant side effects were noticed. No significant differences could be observed between the two experimental groups on body weight and behavioral tests (Figure 7). Neurophysiological measurements of lacosamide treated animals were normal (Geis et al., 2011).

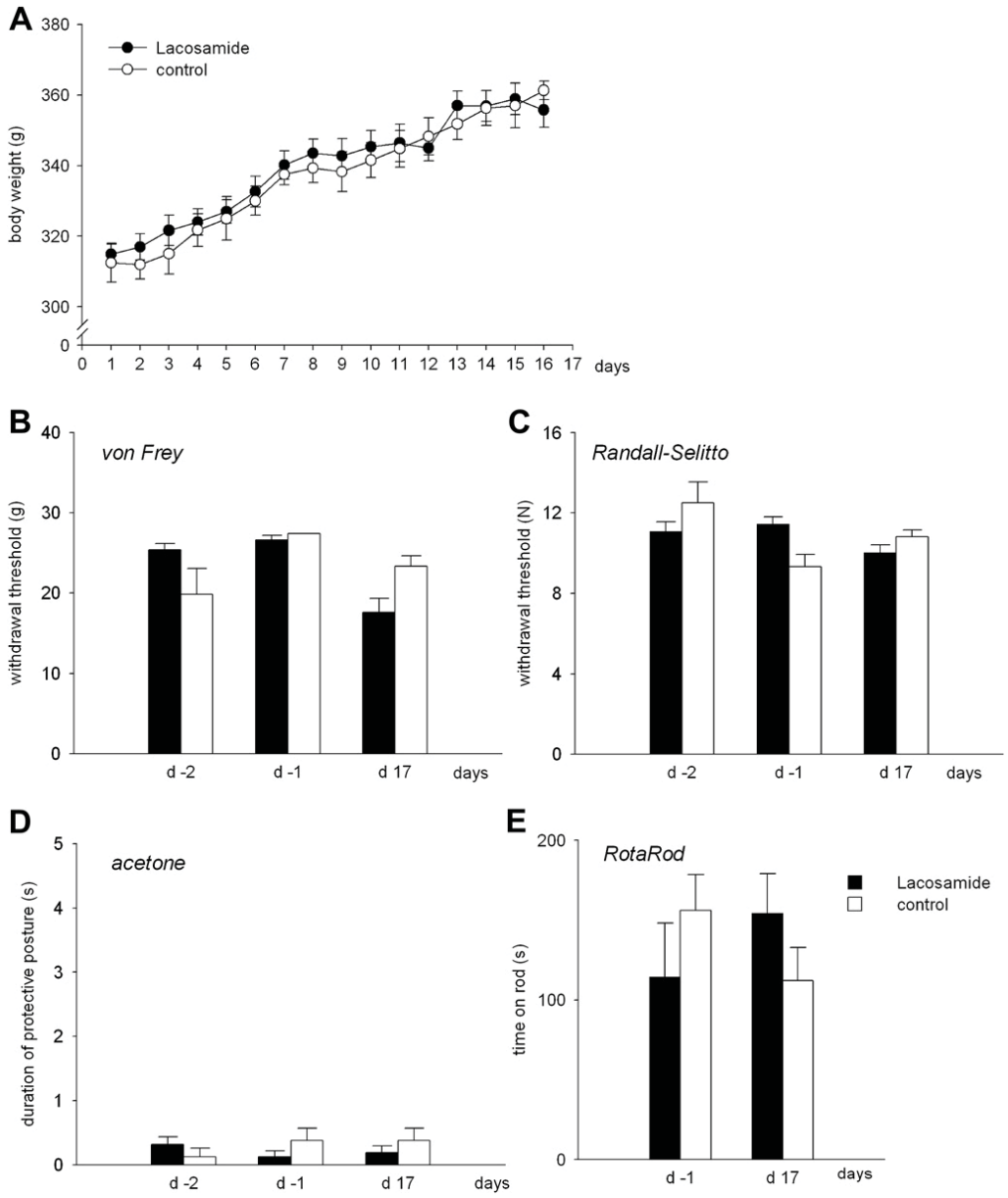
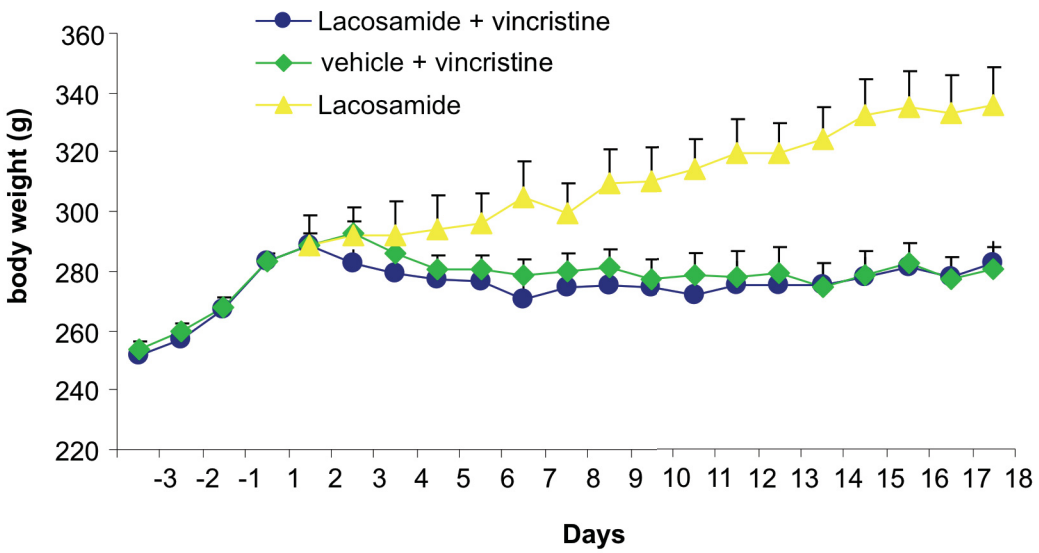


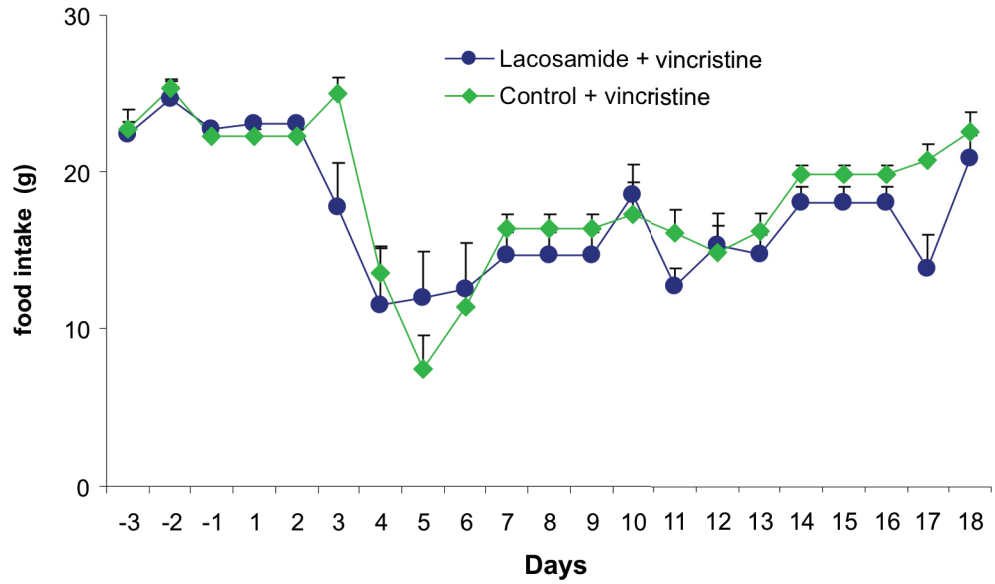
Figure 7. Lacosamide treatment (30 mg/kg, subcutaneous injections b.i.d.) in naïve animals had no influence on body weight (A). No significant effects were observed in the von Frey test (B), in the Randall-Selitto test (C), and in the acetone drop test (D). Also, locomotor activity on an accelerating rotarod was unaffected (E).

3.2 Chronic lacosamide treatment did not reverse vincristine-induced reduction of body weight and food intake

Vincristine / vehicle injected animals did not gain body weight during the 18 day period, whereas rats treated with lacosamide only (30 mg/kg, s.c, b.i.d., no vincristine treatment) gained 15% in the same period. Accordingly, vincristine treatment led to reduced food intake compared to baseline values. Rats administered vincristine and lacosamide did not differ from vincristine / vehicle injected rats regarding their body weight development and food intake (Figure 8).

A





B

Figure 8. Effects of chronic lacosamide, vincristine and vehicle, and combined vincristine and lacosamide treatment on body weight (A) and food intake (B). Lacosamide did not influence the body weight or food intake of vincristine-intoxicated rats.

3.3 Chronic lacosamide treatment prevents vincristine-induced cold allodynia

Vincristine / vehicle injected rats had a significantly prolonged duration of protective posture after acetone application, reflecting cold allodynia (comparison of baseline values and values on day 17). In vincristine / lacosamide treated rats, the duration of protective posture on day 17, was not different from baseline (Figure 9). On day 17 lacosamide was not applied, therefore the protection from vincristine-induced cold allodynia is not due to acute treatment but to chronic treatment during the days before.

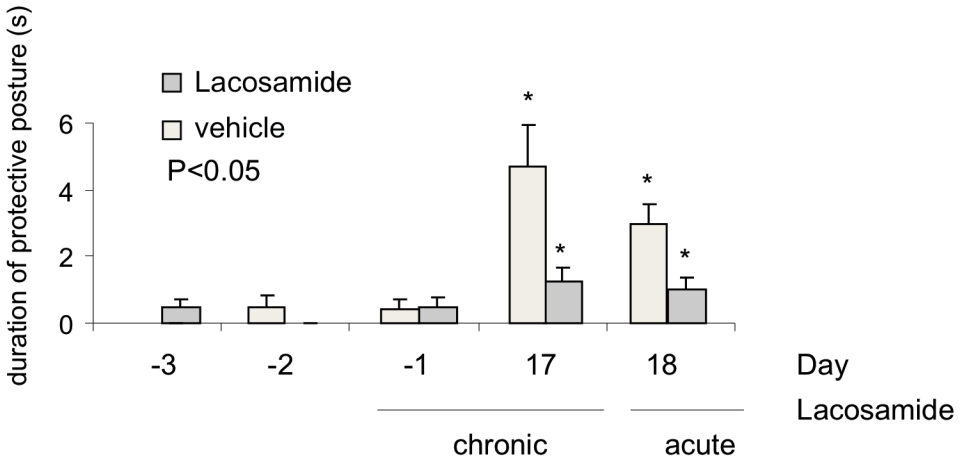


Figure 9. Chronic lacosamide treatment (30 mg/kg s.c. b.i.d. sustained suspension) prevents significantly the development of paw cold allodynia. The duration of protective posture was reduced on day 17 in the acetone drop test after 16 days of lacosamide application as well as 2 h after acute lacosamide treatment (10 mg/kg i.p., aqueous solution) on day 18. *p < 0.05 baseline vs. vehicle injected vincristine-treated group; *p < 0.05 lacosamide vs. vehicle injected vincristine-treated group.

3.4 Chronic lacosamide treatment had no effect on tactile allodynia

Vincristine / vehicle treated rats developed mechanical allodynia as indicated by a reduction of paw withdrawal threshold to 46% of baseline values in the von Frey test. Preventive administration of lacosamide did not alter withdrawal thresholds to von Frey hairs on day 17. A single dose of aqueous lacosamide (10 mg/kg, i.p.) on day 18 led to a significant reduction of mechanical allodynia in the rats pretreated with lacosamide until day 16, but not in the vincristine / vehicle group (Figure 10).

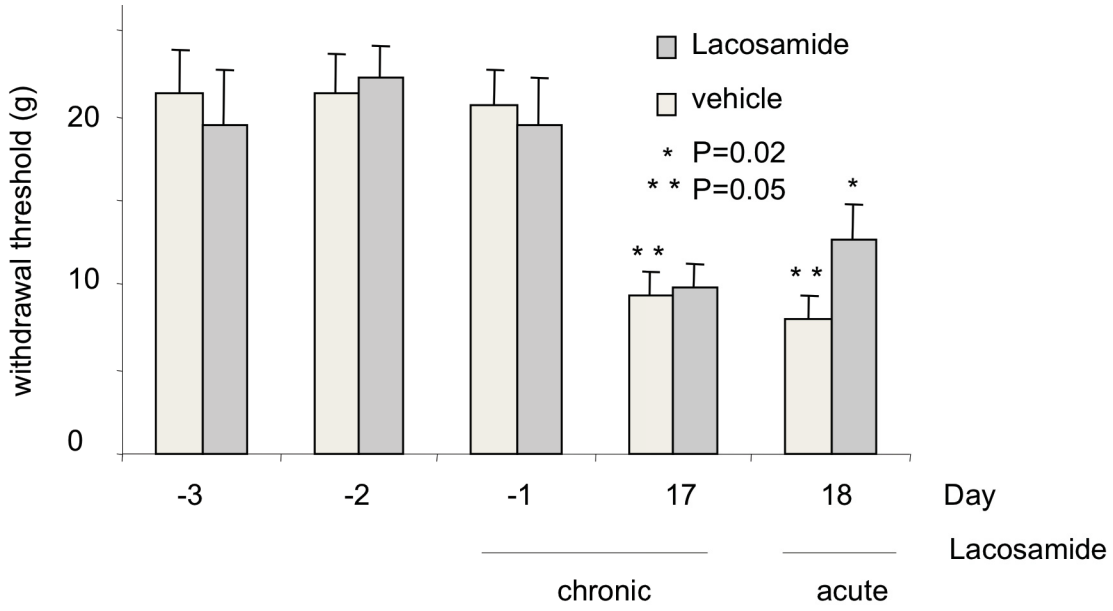


Figure 10. Acute (10 mg/kg i.p. aqueous solution), but not chronic (30 mg/kg s.c. b.i.d. sustained suspension) lacosamide treatment prevents mechanical allodynia. The withdrawal threshold is reduced after vincristine intoxication (*). Mechanical allodynia is reduced 2 h after acute lacosamide treatment on day 18. **p < 0.05 baseline vs. vehicle injected vincristine-treated group; *p < 0.02 lacosamide vs. vehicle injected vincristine-treated group.

3.5 Chronic lacosamide treatment had no effect on mechanical hyperalgesia

No significant reduction of mechanical hypersensitivity to pressure in the Randall-Selitto test could be observed either in the vincristine / vehicle or the vincristine / lacosamide group (Figure 11).

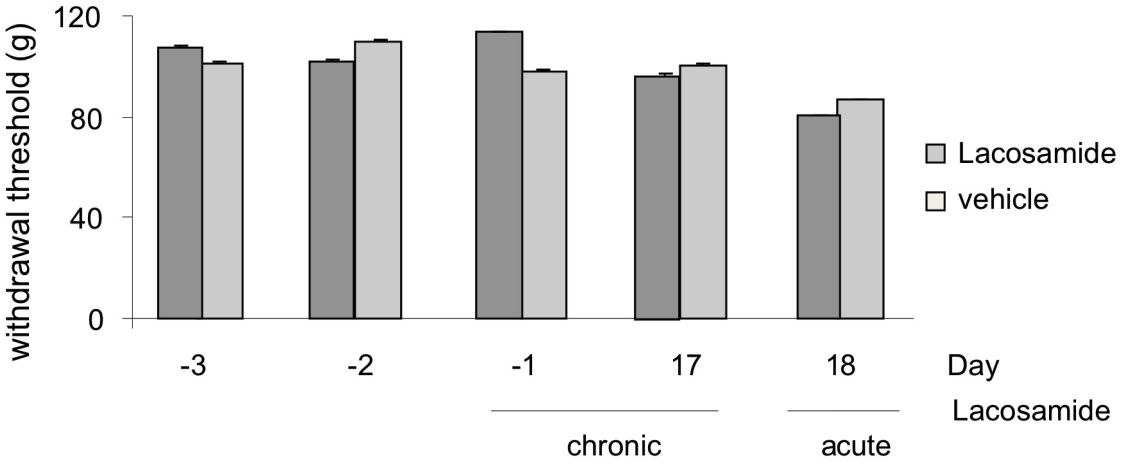


Figure 11. Effects of chronic (30 mg/kg, s.c.) and acute lacosamide (10 mg/kg, i.p.) treatment on mechanical hyperalgesia (paw pressure test, Randall-Selitto) in vincristine treated rats. The mean withdrawal threshold was not reduced after vincristine treatment (dark grey bars). Values are mean ±S.E.M., n=7-8 rats per group.

4. Discussion

Existing therapies for neuropathic pain are far from effective for the majority of patients. For the newer drugs with specific labels for neuropathic pain, for example pregabalin and duloxetine, only one in four patients finds relief, and that relief is generally only a 50% reduction in pain (Finnerup et al. 2005). There are several new targets and new technologies in the pipeline to address this unmet medical need. However, a review of recent clinical trials suggests that treatments in clinical practice offer only 30 to 40% of patients 40 to 50% pain relief. Furthermore, all current treatments for neuropathic pain are symptomatic rather than disease modifying or curative (Backonja et al., 2006).
Chemotherapy-induced neuropathic pain usually gradually improves after the treatment is stopped or the dose is reduced; occasionally, neuropathic pain becomes chronic. Until now, there is no substance proven to be effective in prevention or causative treatment of CIPN. There were first encouraging results of small case series testing potentially preventive substances in CIPN, e.g. magnesium, calcium, vitamin E, glutamine, venlafaxine and others, but either controlled randomized studies are missing or gave negative results (Argyriou et al., 2006; Durand et al., 2003; Savarese et al., 2003), or the substances reduced the anti-tumor-effect of the chemotherapeutic agent (Hochster et al., 2007). Also, many substances failed in effective symptomatic treatment of existing CIPN, e.g. tricyclic antidepressants (Kautio et al., 2008) or anticonvulsants such as gabapentin (Rao et al., 2007) or lamotrigine (Rao et al., 2008).

Here, the disease modifying potential of the new anti-epileptic drug lacosamide was tested in a novel formulation for sustained release in a rat model of vincristine-induced painful neuropathy.
As expected, chronic vincristine intoxication led to a dose-dependent neuropathy with increased mechanical and cold allodynia but no thermal hyperalgesia, confirming previous findings (Nozaki-Taguchi et al., 2001; Weng et al., 2003). Knowing that orofacial pain (particularly jaw pain) is the most

common manifestation, with multiple sites affected in the distribution of the trigeminal and glossopharyngeal nerves in patients treated with vincristine it would be of interest in future to develop a more specific and maybe clinical more relevant animal model.
Electrophysiological recordings showed that the protocol of vincristine intoxication used here resulted preferentially in a mild form of peripheral neuropathy of large nerve fibers: amplitudes of the muscle compound action potential and the nerve conduction velocity were in similar ranges as control values in this study and comparable to previous reports (Ja'afer et al., 2006). The observed pain behavior induced by vincristine intoxication is likely to result from damage of the small cutaneous nerve endings and is not fully measurable by conventional neurographic recordings.

Chronic lacosamide treatment with s.c. injection of the sustained release, oily suspension b.i.d. was well tolerated. Rats pre-treated with lacosamide had a significant reduction of cold allodynia in the acetone test to almost baseline levels. Blood levels of lacosamide were negligible at the time of testing, thus this was not a direct analgesic effect of the drug, indicating a protective effect of lacosamide in this model of vincristine neuropathy. Additionally, acute treatment with lacosamide in an aqueous solution induced a significant reduction of mechanical allodynia, consistent with previous results (Beyreuther et al., 2007a). Chronic lacosamide treatment showed only a trend to reverse the small deficits of muscle compound action potential and the nerve conduction velocity. However, lacosamide treatment led to a significant effect on delayed F-wave latency. F-waves are the most sensitive electrophysiological readout parameters for slight damage in nerve conduction due to peripheral neuropathy, since the nerve action potential has to pass nearly the whole peripheral nerve twice. Thus, in addition of the effect on fibers conveying cold sensation (A-delta fibers), there may be a protective effect of chronic lacosamide application on the mild neuropathy of larger nerve fibers induced by vincristine intoxication. Pain behavior due to vincristine intoxication is associated with dysfunction and increase in spontaneous discharges of A-fibers and C-fibers (Dougherty et al.,

2007; Xiao and Bennett, 2008), the presumed electrophysiological correlate of hyper responsiveness to sensory stimuli in vincristine-induced CIPN. Furthermore, after-discharges and abnormal ‘wind-up’ to electrical stimuli in spinal cord neurons suggest a central sensitization after repeated vincristine treatment that may contribute to the spontaneous pain and hyperalgesia seen in patients (Weng et al., 2003).

The aim of using the oily suspension formulation of lacosamide was to reach a steady concentration level of lacosamide during the chronic treatment of 16 days b.i.d. This is also closer to the clinical situation since in human plasma half-life of lacosamide is around 12 h (Doty et al., 2007). Chronically administered lacosamide was detectable during the 12 h before the second daily injection was applied. Nevertheless the concentration decreased during time from Cmax of 10.1 mg/mL at 2 h to C_{12} of 5.1 mg/mL. Thus, the positive effect seen on cold allodynia after chronic lacosamide treatment might be improved by further optimized slow release formulations.

Lacosamide is a drug with a new mechanism of action, distinct from other anti-epileptic drugs. First, lacosamide enhances the slow inactivation of voltage-gated sodium channels, a mechanism that targets specifically pathophysiological hyperactivity (Errington et al., 2008; Sheets et al., 2008). Second, lacosamide may have neuroprotective and anti-apoptotic effects to excitotoxicity in-vitro and in-vivo possibly by interaction with the CRMP-2 (Wang et al., 2011). These properties of lacosamide on hyper-excitability and exaggerated responsiveness of sensory neurons to external stimuli, especially in A-delta and C-fibers, and the potential neuroprotective features may contribute to the beneficial disease modifying and treatment effects shown here in the vincristine-induced neuropathic pain model.

Lacosamide is currently used as adjunctive therapy in patients with focal epilepsy who did not respond to other anti-epileptic drugs. One clinical advantage of lacosamide is a low potential to cause sedation. No dose reduction is necessary in the elderly, no dosage adjustment in patients with

moderate hepatic or renal impairment. Clinical studies to date have not identified any major influences of lacosamide on the plasma concentration of concomitantly administered drugs (Fattore and Perucca 2011). These drug qualities are essential in pain therapy as well. Phase III trials showed positive outcome for monotherapy in patients with painful diabetic neuropathy (Shaibani et al., 2009; Wymer et al., 2009).

The results presented here and in other preclinical and clinical studies should encourage evaluation of lacosamide for preventive or symptomatic treatment in CIPN and other neuropathic pain patients. Nevertheless the guidelines of The American Academy of Neurology (AAN) and the European guidelines do not recommend the use of lacosamide for the treatment of neuropathic pain: The following observations were made: Pregabalin (300 to 600 mg daily), Gabapentin, 900 to 3600 mg daily, Sodium valproate, 500 to 1200 mg daily, Amitriptyline, 25 to 100 mg daily, Duloxetine, 60 to 120 mg daily, Venlafaxine, 75 to 225 mg daily, Dextromethorphan, 400 mg daily, Morphine sulphate, titrated to 120 mg daily, Oxycodone, mean 37 mg daily, maximum 120 mg daily, Tramadol, 210 mg daily, Capsaicin, 0.075 percent four times daily, Isosorbide dinitrate spray and percutaneous electrical nerve stimulation for three to four weeks were regarded as effective. A Lidocaine patch was regarded as possibly effective. Treatments regarded as probably not effective by the AAN were: oxcarbazepine, lamotrigine, lacosamide, clonidine, pentoxifylline, mexiletine, magnetic field treatment, low-intensity laser therapy, and Reiki therapy. (AAN Guidelines 2011).

Treatment recommendation by the AWMF are for post herpetic neuralgia: Amitriptylin, Nortriptylin, Gabapentin, Pregabalin, Lidocain-Pflaster, Desipramin, Tramadol ret., Morphin ret., Oxycodon, Capsaicin-Salbe; for polyneuropathy: Amitriptylin, Nortriptylin, Venlafaxin, Duloxetin, Gabapentin, Pregabalin, Tramadol, Oxycodon,, Desipramin, Maprotilin, Carbamazepin, Capsaicin-Salbe; posttraumatic neuralgia: Amitriptylin, Capsaicin-Salbe; phantom pain: Gabapentin, Tramadol, Morphin; HIV-induced neuropathy: Gabapentin,

Lamotrigin; stroke: Amitriptylin, Lamotrigin; Spinal cord lesion: Gabapentin, Pregabalin, Lamotrigin; multiple sklerosis: Tetrahydrocannabinol (AWMF-Leitlinien-Register Nr. 030/114).

Lacosamide, developed for the indications pain and epilepsy with several positive preclinical and clinical study results in both indications, is only approved for the treatment of epilepsy (McCleane 2010). How can that be?

Experiments on pain using human subjects are practically challenging, fundamentally (and perhaps inescapably) subjective, and ethically self-limiting, and thus laboratory animal models of pain are widely used (Table 1). However, pain studies using nonhuman animals pose their own significant challenges and ethical constraints. Moreover, a vigorous debate as to the value of currently popular animal models has emerged among pain researchers. Frustration is mounting over the limited success of the field in translating the veritable explosion of basic scientific data collected over the past few decades using animal models into truly new, effective and safe clinical analgesics.

Although the animal models collectively have great utility in exploring the maladaptive plasticity induced by neural damage, they are generally less useful as direct surrogates of pain phenotypes in patients and, by themselves, not always good predictors of the involvement of particular targets or processes in human neuropathic pain. How distinct forms of neural damage activate different sets of changes in the nociceptive system, particularly over a time course that is relevant to the transition from acute to chronic pain, and how these changes engage different outcome measures need to be carefully explored. Reflexive changes in the thresholds to defined stimuli, complex behaviors that capture sensory and mood disturbances, and alterations in operant behavior or choice paradigms that may reflect spontaneous pain also need to be investigated further. One still does not have enough insight into which specifically pain related mechanisms in the nervous system are responsible for behavioral outcome measures in animals. Because subjective symptoms cannot be evaluated, the representation of neuropathic pain in animal models is

necessarily incomplete and the human experience of pain too complex to be fully reproduced (Costigan et al., 2010).

Failures have been related to both adverse side effects and lack of efficacy in humans of drugs that seemed to be safe and effective in animal models. It should be noted that the blame for a failed clinical trial should not automatically be shouldered by the animal model. Poor clinical trial design or implementation and the lack of sufficiently sensitive toxicity screens for important side effects may also play their part (Mogil 2009). Having chosen a patient cohort, what outcome measures in humans should be used? Global pain measures, whether categorical or visual analog, are very crude instruments. One needs to be more sophisticated about the information one gathers from patients at all phases of research and treatment. One of the key problems is that some patients respond to some treatments and some don't, and there is no capacity at present to identify the responders during early drug development. If a trial fails, is it really the drug that failed or the trial that failed to rise to the complexity of the problem of neuropathic pain (Backonja and Woolf 2010).

Chronic pain accompanies a variety of diseases and occurs across the entire life span making treatment difficult. Individuals with multiple chronic conditions have been found to have higher health care expenditures, including some avoidable medical care costs (Wolff et al., 2002). To improve the research and management of pain, greater understanding is needed on the heterogeneity of patients with pain, yet epidemiological and clinical research traditionally imposes constraints that prevent us from assessing the holistic view of a patient with pain. Either studies look broadly at the severity or impact of pain in global terms or, if a condition is examined, pain related to other conditions is often excluded (eg, all chronic pain) (Davis et al., 2011).

Several studies have identified the strong association between the presence of chronic pain and mental health conditions, such as depression (Arnow et al., 2009; Arnow et al., 2006; Bair et al., 2004; Bair et al., 2003), anxiety (Sareen et al. 2005), or mental health in general (Gerdle et al., 2004). Depression has

been found, in numerous studies, to co-occur with spinal cord injury and many other conditions, including osteoarthritis and diabetic neuropathy (Craig et al., 2009).
Pain has also been associated with sleep disorders (Ohayon 2005) and complex chronic illnesses such as heart failure and diabetes (Butchart et al., 2009). Neuropathic pain conditions have also been found to occur with sleep disturbances, as well as depression and anxiety (Nicholson & Verma 2004). Less is known about the rates of multiple pain diagnoses. The presence of pain is believed to cause individuals to be more susceptible to pain in other areas. This may be explained by central sensitization theory, which purports cells of neurons change following a prolonged pain stimulus. These changes increase the responsiveness of the neurons and lower pain thresholds following repeated stimulation (Woolf 2007).

Many patients who obtain pain relief from trials considered a success still have enough pain after the treatment to enroll in more trials. Currently, there is no answer to the question of whether a drug has failed in a clinical trial or whether the trial has failed to rise to the complexity of neuropathic pain. To be able to exploit such findings in the future, one needs a coordinated bench-to-bedside approach to identify pain mechanisms while at the same time advancing treatment options. It has to be recognized that basic and clinical research endeavors cannot be separate (Backonja and Woolf 2010). The recent publication of McCleane (McCleane 2010) summarizes the development of lacosamide and discusses its potential for neuropathic pain treatment. Promising experience with ‘off-label’ use in patients with lumbar and cervical radiculopathy is described.

5. References

AWMF-Leitlinien-Register, Nr. 030/114, Entwicklungsstufe: 1 Leitlinien für Diagnostik und Therapie in der Neurologie; Therapie neuropathischer Schmerzen, 4. überarbeitete Auflage 2008, S. 654 ff, ISBN 978-3-13-132414-6; Georg Thieme Verlag Stuttgart)

Argyriou, A.A., Chroni, E., Koutras, A., Iconomou, G., Papapetropoulos, S., Polychronopoulos, P., Kalofonos, H.P., Preventing paclitaxel-induced peripheral neuropathy: a phase II trial of vitamin E supplementation. J. Pain Symptom Manage 2006. 32, 237e244.

Arnow, B.A., Hunkeler, E.M., Blasey, C.M., et al. Comorbid depression, chronic pain, and disability in primary care. Psychosom. Med. 2006; 68(2): 262–268.

Arnow, B.A., Blasey, C.M., Lee, J., et al. Relationships among depression, chronic pain, chronic disabling pain, and medical costs. Psychiatr. Serv. 2009; 60(3): 344–350.

Backonja, M.M., Irving, G.A., Argoff, C. Rational multidrug therapy in the treatment of neuropathic pain. Curr Pain Headache Rep 2006; 10: 34 –38.

Backonja, M.M., Woolf, C.J. Future directions in neuropathic pain therapy: closing the translational loop. Oncologist 2010; 15 Suppl 2: 24-29.

Bialer, M., Johannessen, S.I., Levy, R.H., et. al. Progress report on new antiepileptic drugs: a summery of the ninth Eilat Conference. Epilepsy Res. 2009; 83: 1-43

Bair, M.J., Robinson, R.L., Eckert, G.J., Stang, P.E., Croghan, T.W., Kroenke, K. Impact of pain on depression treatment response in primary care. Psychosom. Med. 2004; 66(1): 17–22.

Bair, M.J., Robinson, R.L., Katon, W., Kroenke, K. Depression and pain comorbidity: a literature review. Arch. Intern. Med. 2003; 163(20): 2433–2445.

Bee, L.A., Dickenson, A.H. Effects of lacosamide, a novel sodium channel modulator, on dorsal horn neuronal responses in a rat model of neuropathy. Neuropharmacology 2009; 57, 472e479.

Bennett, G.J., Xie, Y.K. A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. Pain. 1988 Apr.; 33(1): 87-107.

Beyreuther, B.K., Callizot, N., Brot, M.D., Feldman, R., Bain, S.C., Stohr, T. Antinociceptive efficacy of lacosamide in rat models for tumor- and chemotherapy-induced cancer pain. Eur. J. Pharmacol. 2007a, 565, 98e104.

Beyreuther, B.K., Freitag, J., Heers, C., Krebsfanger, N., Scharfenecker, U., Stohr, T. Lacosamide: a review of preclinical properties. CNS Drug Rev. 2007b, 13, 21e42.

Beyreuther, B.K., Geis, C., Stohr, T., Sommer, C. Antihyperalgesic efficacy of lacosamide in a rat model for muscle pain induced by TNF. Neuropharmacology 2007c, 52, 1312e1317.

Beyreuther, B.K., Geis, C., Stöhr, T., Sommer, C. Antihyperalgesic efficacy of lacosamide in a rat model for muscle pain induced by TNF. Neuropharmacology. 2007d Apr; 52(5): 1312-1317.

Beyreuther B, Callizot N, Stöhr T. Antinociceptive efficacy of lacosamide in the monosodium iodoacetate rat model for osteoarthritis pain. Arthritis Res Ther. 2007e; 9(1)

Beyreuther B, Callizot N, Stöhr T. Antinociceptive efficacy of lacosamide in a rat model for painful diabetic neuropathy. Eur J Pharmacol. 2006 Jun 6; 539(1-2): 64-70.

Bril, V., England, J., Franklin, G.M., Backonja, M., Cohen, J., Del Toro, D., Feldman, E., Iverson, D.J., Perkins, B., Russell, J.W., Zochodne, D. American Academy of Neurology; American Association of Neuromuscular and Electrodiagnostic Medicine; Guidelines — The American Academy of Neurology (AAN) performed a systematic review and published guidelines in 2011 for the treatment of painful diabetic neuropathy. Evidence-based guideline: Treatment of painful diabetic neuropathy: report of the American Academy of Neurology, the American Association of Neuromuscular and Electrodiagnostic Medicine, and the American Academy of Physical Medicine and Rehabilitation. Neurology. 2011 May 17; 76(20): 1758-1765.

Brittain JM, Piekarz AD, Wang Y, Kondo T, Cummins TR, Khanna R. An atypical role for collapsin response mediator protein 2 (CRMP-2) in neurotransmitter release via interaction with presynaptic voltage-gated calcium channels. J Biol Chem. 2009 Nov. 6; 284(45): 31375-31390

Brittain, J.M., Duarte, D.B., Wilson, S.M., Zhu, W., Ballard, C., Johnson, P.L., Liu, N., Xiong, W., Ripsch, M.S., Wang, Y., Fehrenbacher, J.C., Fitz, S.D., Khanna, M., Park, C.K., Schmutzler, B.S., Cheon, B.M., Due, M.R., Brustovetsky, T., Ashpole, N.M., Hudmon, A., Meroueh, S.O., Hingtgen, C.M., Brustovetsky, N., Ji, R.R., Hurley, J.H., Jin, X., Shekhar, A., Xu, X.M., Oxford, G.S., Vasko, M.R., White, F.A., Khanna, R. Suppression of inflammatory and neuropathic pain by uncoupling CRMP-2 from the presynaptic Ca^{2+} channel complex. Nat Med. 2011 Jun. 5; 17(7): 822-829.

Butchart, A., Kerr, E.A., Heisler, M., Piette, J.D., Krein, S.L. Experience and management of chronic pain among patients with other complex chronic conditions. Clin J Pain. 2009; 25(4): 293–298.

Chacur, M., Milligan, E.D., Gazda, L.S., Armstrong, C., Wang, H., Tracey, K.J., Maier, S.F., Watkins, L.R. A new model of sciatic inflammatory neuritis (SIN): induction of unilateral and bilateral mechanical allodynia following acute unilateral peri-sciatic immune activation in rats. Pain. 2001 Dec.; 94(3): 231-244.

Chaplan, S.R., Bach, F.W., Pogrel, J.W., Chung, J.M., Yaksh, T.L. Quantitative assessment of tactile allodynia in the rat paw. J. Neurosci. Methods 1994, 53, 55e63.

Charrier, E., Reibel, S., Rogemond, V., Aguera, M., Thomasset, N., Honnorat, J. Collapsin response mediator proteins (CRMPs): involvement in nervous system development and adult neurodegenerative disorders. Mol Neurobiol. 2003 Aug.; 28(1): 51-64. Review.

Choi, Y., Yoon, Y.W., Na, H.S., Kim, S.H., Chung, J.M. Behavioral signs of ongoing pain and cold allodynia in a rat model of neuropathic pain. Pain 1994, 59, 369e376.

Cole, A.R., Knebel, A., Morrice, N.A., Robertson, L.A., Irving, A.J., Connolly, C.N., Sutherland, C. GSK-3 phosphorylation of the Alzheimer epitope within

collapsin response mediator proteins regulates axon elongation in primary neurons. J Biol Chem. 2004 Nov. 26; 279(48): 50176-50180.

Costigan, M., Scholz, J., Woolf, C.J. Neuropathic Pain: A Maladaptive Response of the Nervous System to Damage, Annu Rev Neurosci. 2009; 32: 1–32.

Craig, A.,Tran, Y., Middleton, J. Psychological morbidity and spinal cord injury: a systematic review. Spinal Cord. 2009; 47(2):108–114.

Davis, J.A., Robinson, R.L., Le, T.K., Xie, J. Incidence and impact of pain conditions and comorbid illnesses. J Pain Res. 2011; 4: 331–345.

Decosterd, I., Woolf, C.J. Spared nerve injury: an animal model of persistent peripheral neuropathic pain. Pain. 2000 Aug.; 87(2): 149-158.

Deo, R.C., Schmidt, E.F., Elhabazi, A., Togashi, H., Burley, S.K., Strittmatter, S.M. Structural bases for CRMP function in plexin-dependent semaphorin3A signaling. EMBO J. 2004 Jan. 14; 23(1): 9-22.

Doty, P., Rudd, G.D., Stoehr, T., Thomas, D. Lacosamide. Neurotherapeutics 2007, 4, 145e148.

Dougherty, P.M., Cata, J.P., Burton, A.W., Vu, K., Weng, H.R. Dysfunction in multiple primary afferent fiber subtypes revealed by quantitative sensory testing in patients with chronic vincristine-induced pain. J. Pain Symptom Manage 2007 33, 166e179.

Ducreux, D., Attal, N., Parker, F., Bouhassira, D. Mechanisms of central neuropathic pain: a combined psychophysical and fMRI study in syringomyelia. Brain. 2006 Apr.; 129(Pt 4): 963-976.

Durand, J.P., Brezault, C., Goldwasser, F. Protection against oxaliplatin acute neurosensory toxicity by venlafaxine. Anticancer Drugs 2003, 14, 423e425.

Dworkin, R.H., O'Connor, A.B., Backonja, M., Farrar, J.T., Finnerup, N.B., Jensen, T.S., Kalso, E.A., Loeser, J.D., Miaskowski, C., Nurmikko, T.J., Portenoy, R.K., Rice, A.S., Stacey, B.R., Treede, R.D., Turk, D.C., Wallace, M.S. Pharmacologic management of neuropathic pain: evidence-based recommendations. Pain 2007 Dec. 5; 132(3): 237-251.

Dworkin, R.H., Backonja, M., Rowbotham, M.C., Allen, R.R., Argoff, C.R., Bennett, G.J., Bushnell, M.C., Farrar, J.T., Galer, B.S., Haythornthwaite, J.A., Hewitt, D.J., Loeser, J.D., Max, M.B., Saltarelli, M., Schmader, K.E., Stein, C., Thompson, D., Turk, D.C., Wallace, M.S., Watkins, L.R., Weinstein, S.M.

Advances in neuropathic pain: diagnosis, mechanisms, and treatment recommendations. Arch Neurol. 2003 Nov.; 60(11): 1524-1534. Review.

Errington, A.C., Stohr, T., Heers, C., Lees, G. The investigational anticonvulsant lacosamide selectively enhances slow inactivation of voltage-gated sodium channels. Mol. Pharmacol. 2008, 73, 157e169.

Fattore, C., Perucca, E. Novel medications for epilepsy. Drugs. 2011 Nov. 12; 71(16): 2151-2178.

Finnerup, N.B., Otto, M., McQuay, H.J. et al. Algorithm for neuropathic pain treatment: An evidence based proposal. Pain 2005; 118: 289 –305.

Geis, C., Beyreuther, B.K., Stöhr, T., Sommer, C., Lacosamide has protective disease modifying properties in experimental vincristine neuropathy. Neuropharmacology 2011, 61(4): 600-607.

Gerdle, B., Bjork, J., Henriksson, C., Bengtsson, A. Prevalence of current and chronic pain and their influences upon work and health care-seeking: a population study. J Rheumatol. 2004; 31(7): 1399–1406.

Goshima, Y., Nakamura, F., Strittmatter, P., Strittmatter, S.M. Collapsin-induced growth cone collapse mediated by an intracellular protein related to UNC-33. Nature. 1995 Aug. 10; 376(6540): 509-154.

Hammack, J.E., Michalak, J.C., Loprinzi, C.L., Sloan, J.A., Novotny, P.J., Soori, G.S., Tirona, M.T., Rowland Jr., K.M., Stella, P.J., Johnson, J.A., 2002. Phase III evaluation of nortriptyline for alleviation of symptoms of cis-platinum-induced peripheral neuropathy. Pain 98, 195e203.

Hao, J.X., Stöhr, T., Selve, N., Wiesenfeld-Hallin, Z., Xu, X.J. Lacosamide, a new anti-epileptic, alleviates neuropathic pain-like behaviors in rat models of spinal cord or trigeminal nerve injury. Eur J Pharmacol. 2006 Dec. 28; 553(1-3): 135-140.

Hensley, K., Christov, A., Kamat, S., Zhang, X.C., Jackson, K.W., Snow, S., Post, J. Proteomic identification of binding partners for the brain metabolite lanthionine ketimine (LK) and documentation of LK effects on microglia and motoneuron cell cultures. J Neurosci. 2010 Feb. 24; 30(8): 2979-2988.

Hochster, H.S., Grothey, A., Childs, B.H. Use of calcium and magnesium salts to reduce oxaliplatin-related neurotoxicity. J. Clin. Oncol. 2007, 25, 4028e4029.

Inagaki, N., Chihara, K., Arimura, N., Ménager, C., Kawano, Y., Matsuo, N., Nishimura, T., Amano, M., Kaibuchi, K. CRMP-2 induces axons in cultured hippocampal neurons. Nat Neurosci. 2001 Aug.; 4(8): 781-782.

Ja'afer, F.M., Hamdan, F.B., Mohammed, F.H. Vincristine-induced neuropathy in rat: electrophysiological and histological study. Exp. Brain Res. 2006, 173, 334e345.

Kaley, T.J., Deangelis, L.M. Therapy of chemotherapy-induced peripheral neuropathy. Br. J. Haematol. 2009, 145, 3e14.

Kautio, A.L., Haanpaa, M., Saarto, T., Kalso, E. Amitriptyline in the treatment of chemotherapy-induced neuropathic symptoms. J. Pain Symptom Manage. 2008, 35, 31e39.

Kilkenny, C., Browne, W.J., Cuthill, I.C., Emerson, M., Altman, D.G. Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. PLoS Biol 2010, 8, e1000412.

Kim, S.H., Chung, J.M. An experimental model for peripheral neuropathy produced by segmental spinal nerve ligation in the rat. Pain. 1992 Sep.; 50(3): 355-363.

Macleod, M.R., Fisher, M., O'Collins, V., Sena, E.S., Dirnagl, U., Bath, P.M., Buchan,A., van der Worp, H.B., Traystman, R., Minematsu, K., Donnan, G.A., Howells, D.W. Good laboratory practice: preventing introduction of bias at the bench. Stroke 2009, 40, e50ee52.

McCleane, G. Lacosamide for pain. Expert Opin Investig Drugs. 2010 Sep.; 19(9): 1129-1134.

Mogil, J.S. Animal models of pain: progress and challenges. Nat Rev Neurosci. 2009 Apr.; 10(4): 283-294.

Nicholson, B., Verma, S. Comorbidities in chronic neuropathic pain. Pain Med. 2004; 5 (Suppl 1): S9–S27.

Nozaki-Taguchi, N., Chaplan, S.R., Higuera, E.S., Ajakwe, R.C., Yaksh, T.L. Vincristine-induced allodynia in the rat. Pain 2001, 93, 69e76.

Ohayon, M.M. Relationship between chronic painful physical condition and insomnia. J Psychiatr Res. 2005; 39(2): 151–159.

Peltier, A.C., Russell, J.W. Recent advances in drug-induced neuropathies. Curr Opin Neurol. 2002 Oct.; 15(5): 633-638.

Postma, T.J., Benard, B.A., Huijgens, P.C., Ossenkoppele, G.J., Heimans, J.J. Longterm effects of vincristine on the peripheral nervous system. J. Neurooncol. 1993, 15, 23e27.

Quach, T.T., Duchemin, A.M., Rogemond, V., Aguera, M., Honnorat, J., Belin, M.F., Kolattukudy, P.E. Involvement of collapsin response mediator proteins in the neurite extension induced by neurotrophins in dorsal root ganglion neurons. Mol Cell Neurosci. 2004 Mar.; 25(3): 433-443.

Rao, R.D., Michalak, J.C., Sloan, J.A., Loprinzi, C.L., Soori, G.S., Nikcevich, D.A., Warner, D.O., Novotny, P., Kutteh, L.A., Wong, G.Y. Efficacy of gabapentin in the management of chemotherapy-induced peripheral neuropathy: a phase 3 randomized, double-blind, placebo-controlled, crossover trial (N00C3). Cancer 2007, 110, 2110e2118.

Rao, R.D., Flynn, P.J., Sloan, J.A., Wong, G.Y., Novotny, P., Johnson, D.B., Gross, H.M., Renno, S.I., Nashawaty, M., Loprinzi, C.L. Efficacy of lamotrigine in the management of chemotherapy-induced peripheral neuropathy: a phase 3 randomized, double-blind, placebo-controlled trial, N01C3. Cancer 2008, 112, 2802e2808.

Rauck, R.L., Shaibani, A., Biton, V., Simpson, J., Koch, B. Lacosamide in painful diabetic peripheral neuropathy: a phase 2 double-blind placebo-controlled study. Clin. J. Pain 2007, 23, 150e158.

Sareen J., Cox B.J., Clara I., Asmundson G.J. The relationship between anxiety disorders and physical disorders in the US National Comorbidity Survey. Depress Anxiety. 2005; 21(4): 193–202.

Savarese, D.M., Savy, G., Vahdat, L., Wischmeyer, P.E., Corey, B. Prevention of chemotherapy and radiation toxicity with glutamine. Cancer Treat. Rev. 2003, 29, 501e513.

Seltzer, Z., Dubner, R., Shir, Y. A novel behavioral model of neuropathic pain disorders produced in rats by partial sciatic nerve injury. Pain. 1990 Nov.; 43(2): 205-218.

Shaibani, A., Fares, S., Selam, J.L., Arslanian, A., Simpson, J., Sen, D., Bongardt, S. Lacosamide in painful diabetic neuropathy: an 18-week double-blind placebo-controlled trial. J. Pain 2009, 10, 818e828.

Sheets, P.L., Heers, C., Stoehr, T., Cummins, T.R. Differential block of sensory neuronal voltage-gated sodium channels by lacosamide [(2R)-2-(acetylamino)-N-benzyl-3-methoxypropanamide], lidocaine, and carbamazepine. J. Pharmacol. Exp. Ther. 2008, 326, 89e99.

Sullivan, K.A., Lentz, S.I., Roberts, J.L. Jr., Feldman, E.L. Criteria for creating and assessing mouse models of diabetic neuropathy. Curr Drug Targets. 2008 Jan.; 9(1): 3-13.

Tanner, K.D., Levine, J.D., Topp, K.S. Microtubule disorientation and axonal swelling in unmyelinated sensory axons during vincristine-induced painful neuropathy in rat. J. Comp. Neurol. 1998, 395, 481e492.

Verstappen, C.C., Koeppen, S., Heimans, J.J., Huijgens, P.C., Scheulen, M.E., Strumberg, D., Kiburg, B., Postma, T.J. Dose-related vincristine-induced peripheral neuropathy with unexpected off-therapy worsening. Neurology 2005, 64, 1076e1077.

Wall, P.D., Devor, M., Inbal, R., Scadding, J.W., Schonfeld, D., Seltzer, Z., Tomkiewicz, M.M. Autotomy following peripheral nerve lesions: experimental anaesthesia dolorosa. Pain. 1979 Oct.; 7(2): 103-111.

Wallace, V.C., Blackbeard, J., Pheby, T., Segerdahl, A.R., Davies, M., Hasnie, F., Hall, S., McMahon, S.B., Rice, A.S. Pharmacological, behavioural and mechanistic analysis of HIV-1 gp120 induced painful neuropathy. Pain. 2007 Dec. 15; 133(1-3): 47-63.

Wallace, V.C., Cottrell, D.F., Brophy, P.J., Fleetwood-Walker, S.M. Focal lysolecithin-induced demyelination of peripheral afferents results in neuropathic pain behavior that is attenuated by cannabinoids. J Neurosci. 2003 Apr. 15; 23(8): 3221-3233.

Wang, Y., Brittain, J.M., Jarecki, B.W., Park, K.D., Wilson, S.M., Wang, B., Hale, R., Meroueh, S.O., Cummins, T.R., Khanna, R. In silico docking and electro- physiological characterization of lacosamide binding sites on collapsin response mediator protein 2 (CRMP-2) identifies a pocket important in modulating sodium channel slow inactivation. J Biol Chem. 2010 August 13; 285(33): 25296–25307.

Wang, Y., Khanna, R. Voltage-gated calcium channels are not affected by the novel anti-epileptic drug lacosamide. Transl Neurosci. 2011 Mar.; 2(1): 13-22.

Weng, H.R., Cordella, J.V., Dougherty, P.M. Changes in sensory processing in the spinal dorsal horn accompany vincristine-induced hyperalgesia and allodynia. Pain 2003, 103, 131e138.

Wolf, S., Barton, D., Kottschade, L., Grothey, A., Loprinzi, C. Chemotherapy-induced peripheral neuropathy: prevention and treatment strategies. Eur. J. Cancer 2008, 44, 1507e1515.

Wolff, J.L., Starfield, B., Anderson, G. Prevalence, expenditures, and complications of multiple chronic conditions in the elderly. Arch Intern Med. 2002; 162(20): 2269–2276.

Woolf, C.J. Central sensitization: uncovering the relation between pain and plasticity. Anesthesiology. 2007; 106(4): 864–867.

Woolf, C.J. and Mannion, R.J. Neuropathic pain: aetiology, symptoms, mechanisms, and management. Lancet. 1999 Jun. 5; 353 (9168): 1959-1964. Review.

Wymer, J.P., Simpson, J., Sen, D., Bongardt, S. Efficacy and safety of lacosamide in diabetic neuropathic pain: an 18-week double-blind placebo-controlled trial of fixed-dose regimens. Clin. J. Pain 2009, 25, 376e385.

Xiao, W.H., Bennett, G.J. Chemotherapy-evoked neuropathic pain: abnormal spontaneous discharge in A-fiber and C-fiber primary afferent neurons and its suppression by acetyl-L-carnitine. Pain 2008, 135, 262e270.

Yoshimura, T., Kawano, Y., Arimura, N., Kawabata, S., Kikuchi, A., Kaibuchi, K. GSK-3beta regulates phosphorylation of CRMP-2 and neuronal polarity. Cell. 2005 Jan. 14; 120(1): 137-149.

Zagari, M.J., Mazonson, P.D., Longton, W.C. Pharmacoeconomics of chronic nonmalignant pain. Pharmacoeconomics 1996; 10(4): 356–377.

Ziegler, D., Hidvégi, T., Gurieva, I., Bongardt, S., Freynhagen, R., Sen, D., Sommerville, K. Efficacy and safety of lacosamide in painful diabetic neuropathy. Diabetes Care. 2010 Apr; 33 (4): 839-841.

6. Appendix

Lebenslauf

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Publikationen

Lacosamide has protective disease modifying properties in experimental vincristine neuropathy.
Geis C, Beyreuther BK, Stöhr T, Sommer C.
Neuropharmacology. 2011 Sep.;61(4):600-7.

Lacosamide: a review of preclinical properties.
Beyreuther BK, Freitag J, Heers C, Krebsfänger N, Scharfenecker U, Stöhr T.
CNS Drug Rev. 2007 Spring;13(1):21-42. Review.

Antinociceptive efficacy of lacosamide in rat models for tumor- and chemotherapy-induced cancer pain.
Beyreuther BK, Callizot N, Brot MD, Feldman R, Bain SC, Stöhr T.
Eur J Pharmacol. 2007 Jun 22;565(1-3):98-104. Epub 2007 Mar 3

Antihyperalgesic efficacy of lacosamide in a rat model for muscle pain induced by TNF.
Beyreuther BK, Geis C, Stöhr T, Sommer C.
Neuropharmacology. 2007 Apr;52(5):1312-7. Epub 2007 Feb 4.

Antinociceptive efficacy of lacosamide in the monosodium iodoacetate rat model for osteoarthritis pain.
Beyreuther B, Callizot N, Stöhr T.
Arthritis Res Ther. 2007;9(1):R14.

Antinociceptive efficacy of lacosamide in a rat model for painful diabetic neuropathy.
Beyreuther B, Callizot N, Stöhr T.
Eur J Pharmacol. 2006 Jun 6;539(1-2):64-70. Epub 2006 Apr 7.

Discovery and optimization of 2-aryl oxazolo-pyrimidines as adenosine kinase inhibitors using liquid phase parallel synthesis.
Bauser M, Delapierre G, Hauswald M, Flessner T, D'Urso D, Hermann A, Beyreuther B, De Vry J, Spreyer P, Reissmüller E, Meier H.
Bioorg Med Chem Lett. 2004 Apr 19;14(8):1997-2000.

Importance of the intracellular domain of NR2 subunits for NMDA receptor function in vivo.
Sprengel R, Suchanek B, Amico C, Brusa R, Burnashev N, Rozov A, Hvalby O, Jensen V, Paulsen O, Andersen P, Kim JJ, Thompson RF, Sun W, Webster LC, Grant SG, Eilers J, Konnerth A, Li J, McNamara JO, Seeburg PH.
Cell. 1998 Jan 23;92(2):279-89.

Tissue specific control regions of the N-methyl-D-aspartate receptor subunit NR2C promoter.
Suchanek B, Seeburg PH, Sprengel R.
Biol Chem. 1997 Aug;378(8):929-34.

Gene structure of the murine N-methyl D-aspartate receptor subunit NR2C.
Suchanek B, Seeburg PH, Sprengel R.
J Biol Chem. 1995 Jan 6;270(1):41-4.

Patente

Bettina Beyreuther, Thomas Stohr: Method for treating non-inflammatory musculoskeletal pain. SRZ Properties August 2007: US 20070197657

Martin Hendrix, Lars Barfacker, Bettina Beyreuther, Ulrich Ebert, Christina Erb, Frank-Thorsten Hafner, Heike Heckroth, Yan-Hong Liu, Dagmar Karthaus, Adrian Tersteegen, Franz-Josef van der Staay, Marja van Kampen: 6-Arylamino-5-cyano-4-pyrimidinones as pde9a inhibitors. Bayer HealthCare May 2007: US 20070105881

Bettina Beyreuther, Thomas Stohr: Method for treating non-inflammatory osteoarthritic pain. SRZ Properties March 2007: US 20070048372

Bettina Beyreuther, Thomas Stohr: Therapeutic combination for painful medical conditions. February 2007: US 20070043120

Martin HENDRIX, Lars BARFACKER, Bettina BEYREUTHER, Ulrich EBERT, Christina ERB, Frank-Thorsten HAFNER, Heike HECKROTH, Yan-Hong LIU, Dagmar KARTHAUS, Adrian TERSTEEGEN, Franz-Josef VAN DER STAAY, Marja VAN KAMPEN: 6-arylamino-5-cyano-4-pyrimidinones as pde9a inhibitors. Boehringer Ingelheim International April 2009: US 20090111838

Martin Hendrix, Lars Bärfacker, Bettina Beyreuther, Ulrich Ebert, Christina Erb, Frank-Thorsten Hafner, Heike Heckroth, Yan-Hong Liu, Dagmar Karthaus, Adrian Tersteegen, Franz-Josef van der Staay, Marja van Kampen: 6-arylamino-5-cyano-4-pyrimidinones as pde9a inhibitors. Boehringer Ingelheim International February 2009: US 7488733

Bettina Beyreuther, Thomas Stohr: Novel use of peptide compounds for treating bone cancer pain, chemotherapy-and nucleoside-induced pain. Schwarz Pharma March 2006: US 20060046957

Cara Heers, Thomas Stoehr, Bettina Beyreuther: Therapy for hyperexcitability disorders. Schwarz Pharma December 2010: US 20100324144

Bettina Beyreuther, Thomas Stohr: Methods for treating nucleoside-induced pain. Schwarz Pharma February 2010: US 20100029543

Thomas Stöhr, Bettina Beyreuther: Combination therapy for pain in painful diabetic neuropathy. Ucb Pharma October 2010: US 20100256179

Bettina Beyreuther, Dieter Scheller, Joachim Freitag, Josephe Bianchine: Method for treating pain using a substituted 2-aminotetralin compound. January 2008: US 20080008748

Bettina Beyreuther, Thomas Stohr: Novel Use of Peptide Compounds For Treating Muscle Pain. November 2008: US 20080280835

Bettina Beyreuther, Thomas Stohr, Joachim Freitag: Method for identifying crmp modulators. September 2009: US 20090241205

Bettina Beyreuther, Michael Hahn, Christopher Kallus, Joachim Kruger, Heinrich Meier, Elke Reibmuller, Leila Telan, Reilinde Nopper, Mathias Kroll: Tetrahydroquinoxalines acting as bradykinin antagonists. November 2004: US 20040235849

Bettina Beyreuther, Dieter Scheller, Joachim Freitag, Joseph Bianchine: Method for treating pain using a substituted 2-aminotetralin compound. Ucb Pharma May 2011: US 20110104281

Bettina Beyreuther, Thomas Stohr: Method for treating bone cancer pain or chemotherapy induced pain. Schwarz Pharma March 2010: US 7687553

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Tierversuchgenehmigung

The animal experiments were performed by PD. Dr. Christian Geis and Lydia Biko in Würzburg. All experiments were conducted according to Bavarian state regulations for animal experimentation, were approved by Bavarian State authorities and followed the guidelines in the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978). All efforts were made to minimize animal suffering and to reduce the number of animals used. The study has been performed in accordance with the ARRIVE guidelines for reporting animal research and the criteria for good laboratory practice (Kilkenny et al., 2010; Macleod et al., 2009).

Eidesstattliche Versicherung

Ich versichere an Eides statt, dass die Dissertation selbständig und ohne unzulässige fremde Hilfe erstellt worden ist und die hier vorgelegte Dissertation nicht von einer anderen Medizinischen Fakultät abgelehnt worden ist.

Düsseldorf, den