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Meteorin reverses hypersensitivity in rat models of neuropathic pain

Jesper Roland Jørgensen [a,*], Xiao-Jun Xu [b], H. Moore Arnold [c], Gordon Munro [d], Jing-Xia Hao [b], Blake Pepinsky [c], Carol Huang [c], Bang Jian Gong [c], Zsuzsanna Wiesenfeld-Hallin [b], Lars U. Wahlberg [a], Teit E. Johansen [a]

[a] NsGene A/S, Baltorpvej 154, 2750 Ballerup, Denmark
[b] Department of Physiology and Pharmacology, Section of Integrative Pain Research, Karolinska Institutet, Stockholm, Sweden
[c] Biogen Idec, Inc., 14 Cambridge Center, Cambridge, MA 02142, USA
[d] Department of Pharmacology, NeuroSearch A/S, 93 Pederstrupvej, DK-2750, Ballerup, Denmark

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ABSTRACT

Neuropathic pain is caused by a lesion or disease to the somatosensory nervous system and current treatment merely reduces symptoms. Here, we investigate the potential therapeutic effect of the neurotrophic factor Meteorin on multiple signs of neuropathic pain in two distinct rat models. In a first study, two weeks of intermittent systemic administration of recombinant Meteorin led to a dose-dependent reversal of established mechanical and cold hypersensitivity in rats after photochemically-induced sciatic nerve injury. Moreover, analgesic efficacy lasted for at least one week after treatment cessation. In rats with a chronic constriction injury (CCI) of the sciatic nerve, five systemic injections of Meteorin over 9 days dose-dependently reversed established mechanical and thermal hypersensitivity as well as weight bearing deficits taken as a surrogate marker of spontaneous pain. The beneficial effects of systemic Meteorin were sustained for at least three weeks after treatment ended and no adverse side effects were observed. Pharmacokinetic analysis indicated that plasma Meteorin exposure correlated well with dosing and was no longer detectable after 24 hours. This pharmacokinetic profile combined with a delayed time of onset and prolonged duration of analgesic efficacy on multiple parameters suggests a disease-modifying mechanism rather than symptomatic pain relief. In sciatic nerve lesioned rats, delivery of recombinant Meteorin by intrathecal injection was also efficacious in reversing mechanical and cold hypersensitivity. Together, these data demonstrate that Meteorin represents a novel treatment strategy for the effective and long lasting relief from the debilitating consequences of neuropathic pain.

Introduction

Neuropathic pain can arise as a result of lesion to or disease within the somatosensory system and is associated with a diverse range of disease states including diabetes, cancer, autoimmunity, viral infections and stroke (Haanpaa et al., 2011; Treede et al., 2008). Patients typically present with any number of symptoms as typified by spontaneous pain, pain evoked by normally innocuous sensory stimuli (allodynia), or exacerbated pain in response to noxious stimuli (hyperalgesia). Multiple underlying mechanisms (e.g. nociceptor sensitization, local inflammation, ectopic discharges, loss of descending inhibitory controls, spinal disinhibition) contribute to the behavioral manifestation of neuropathic pain (Costigan et al., 2009). In the clinic, antiepileptic and antidepressant drugs form the first line of treatment, albeit they have proven to be at best, symptomatic and only partially effective.

Neurotrophic factors are critical during development and for maintenance of the adult nervous system. Accordingly, molecules in this group are of clinical interest in relation to neurological disorders including neuropathic pain (Ossipov, 2011; Sah et al., 2005). During development, nerve growth factor (NGF) supports the survival of TrkA-receptor expressing peripheral sensory neurons. Thereafter, a proportion of these neurons lose responsiveness to NGF, instead becoming responsive to glial cell line-derived neurotrophic factor (GDNF). Both neurotrophic factors then continue to play an important role in the normal functioning of the adult nervous system (Pezet and McMahon, 2006). Although NGF has some neuroprotective properties after nerve injury, its clinical use is limited due to well characterized pronociceptive effects of exogenously administered NGF (Apfel et al., 2000), thereby reflecting the complex role of NGF as an endogenous mediator of pain. Accordingly, inhibiting the underlying pronociceptive mechanisms using antibodies against NGF alleviates pain in preclinical and clinical studies, but unfortunately not without side effects in patients (Cattaneo, 2010; Lane et al., 2010). Prolonged intrathecal infusion of GDNF in rats with spinal nerve ligation (SNL) reverses mechanical as well as thermal hypersensitivity (Boucher et al., 2000). Unfortunately, significant side effects are also associated with GDNF treatment likely reflecting the relatively broad

Abbreviations: CCI, chronic constriction injury; DRG, dorsal root ganglion; GDNF, glial cell line-derived neurotrophic factor; NGF, Nerve growth factor; SC, subcutaneous; SNL, spinal nerve ligation.

* Corresponding author at: NsGene, Baltorpvej 154, 2750 Ballerup, Denmark.
E-mail address: JRJ@NSGENE.DK (J.R. Jørgensen).

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distribution of its receptor GFRα1. Artemin (Baloh et al., 1998) belongs to the GDNF family and its receptor, GFRα3, is found almost exclusively on nociceptive afferents (Orozco et al., 2001). As the ligand specificity of GDNF and Artemin is mediated by GFRα1 and GFRα3 respectively (Carmillo et al., 2005) but the RET receptor tyrosine kinase is a common signaling component (Airaksinen and Saarma, 2002), neurotrophic support by Artemin may be expected to have fewer off-target effects than GDNF. Consistent with this interpretation, repeated intermittent systemic injection of Artemin in SNL rats, dose-dependently reverses mechanical and thermal hypersensitivity concomitant with normalization of neurochemical as well as morphological features of primary sensory neurones (Gardell et al., 2003). Importantly no adverse effects were observed in the studies by Gardell et al. even at high doses and with continued treatment of SNL rats. Even though the beneficial effects of GDNF and Artemin persist only for the duration of the administration regimen, these studies demonstrate the potential of neurotrophic factors in relation to peripheral neuropathy.

Meteorin (Nishino et al., 2004) and the recently described molecule Cometin (Jorgensen et al., 2012) constitute a unique family of neurotrophic factors. This family is unrelated to the GDNF family and other known neurotrophic factors. During mouse development, Meteorin is widely expressed within the nervous system, including the dorsal root ganglia (DRG). In primary DRG explant cultures Meteorin promotes extensive neurite outgrowth of small and intermediate nociceptive sensory neurons (Jorgensen et al., 2009; Nishino et al., 2004). Interestingly, this seems to be a glia mediated effect rather than a direct neuronal effect. However, the receptor and exact mechanism of action is currently unknown but Meteorin has been shown to use the gp130 co-receptor as an upstream transducer of Jak-STAT3 signalling (Lee et al., 2010). In the central nervous system, Meteorin protects against quinolinic acid-mediated excitoxicity (Jorgensen et al., 2010) and has furthermore been reported to participate in cerebral angiogenesis (Park et al., 2008) as well as neurogenesis (Wang et al., 2012). On this basis, we investigated the therapeutic potential of Meteorin in relation to peripheral neuropathy and associated pain.

In the current set of experiments, we demonstrate that intermittent systemic administration of recombinant Meteorin dose-dependently reverses hypersensitivity in two distinct rat models of neuropathic pain. Following a slow onset, the beneficial effects last for weeks after cessation of treatment, which together with the pharmacological profile indicate a modification of the underlying neuropathy rather than symptomatic pain relief. Hence, Meteorin is a new candidate for treatment of neuropathic pain through a novel mechanism.

Materials and methods

Recombinant Meteorin

Recombinant Meteorin was manufactured in collaboration with R&D Systems Inc. (Minneapolis, MN). Briefly, the sequence encoding mouse Meteorin (Q8C1Q4) was cloned and expressed in an NS0 mouse myeloma cell line. The recombinant mouse Meteorin was purified from the conditioned medium by ion exchange, hydrophobic interaction and size exclusion chromatography. The buffer was exchanged into PBS and the protein solution stored at −80 °C. The purity of the preparation, as judged by densitometry scan, was 95%.

Photochemically induced sciatic nerve injury

All procedures involving animals were reviewed and approved by Institutional Animal Care and Use Committees of the respective institutions (Karolinska Institutet and Biogen Idec), and were in accordance with the US National Institutes of Health guidelines.

Photochemically induced sciatic nerve injured rats are known to develop allodynia to both mechanical and cold stimulation (Kupers et al., 1998). For this model, 280–330 g male Sprague–Dawley rats (Taconic, Denmark) were used. Briefly, under general anesthesia (chloral hydrate 300 mg/kg), the left sciatic nerve was exposed at mid-thigh level and irradiated for 1.5 min with an argon laser operating at 514 nm at an average power of 0.17 W. Erythrosin B (32.5 mg/kg dissolved in 0,9% saline) was injected intravenously through the tail vein just prior to irradiation. The resulting local ischemic damage to the sciatic nerve leads to a highly reproducible allodynia within 7 days. Hypersensitive animals were next randomly divided into four groups ($n=8$) and subcutaneously (s.c.) injected with either saline as the negative control or Meteorin at 0.05, 0.2 or 0.8 mg/kg. Each rat received six injections over a two week period on days 7, 9, 11, 14, 17 and 21 counting from the time of nerve injury. Behavioral assessments were conducted before each injection during the treatment period and on days 28 and 35.

In a second study, male Sprague–Dawley rats (Harlan, The Netherlands) weighing 400–450 g were fitted with a chronic intrathecal catheter with the tip at the lumbar enlargement (Storkson et al., 1996). Proper location of the catheter was verified by intrathecal injection of 10 μl lidocaine (Xylocain 50 mg/ml, Astra, Sweden) and a corresponding transient block of sensory and motor function. Three to five days after catheter implantation, ischemic sciatic nerve injury was produced as described above. Baseline responses were evaluated after catheter implantation and again before sciatic nerve irradiation. Rats that developed hypersensitivity to mechanical and cold stimulation 7 days after nerve injury were randomly divided into four groups ($n=8$) which were given saline as negative control or recombinant Meteorin (0.5, 2 or 6 μg) in a volume of 10 μl intrathecally. Each rat received six injections over a two week period (on days 7, 9, 11, 14, 16 and 18 counting from the time of nerve injury). Behavioral testing was conducted prior to intrathecal injection on respective treatment days and furthermore on days 21, 25, 28 and 35 following treatment cessation.

For evaluation of mechanical hypersensitivity, a set of calibrated nylon monofilaments (von Frey hairs, Stoelting, IL) was applied to the glabrous skin of the paws in ascending order from the lowest to the highest monofilament used. Each monofilament was applied 5 times at successively increasing force and the withdrawal threshold was determined as the force at which the animal withdrew the paw from at least 3 out of 5 consecutive stimuli of the same force. The response to cold was tested with ethyl chloride, which was briefly (<1 s) sprayed on the plantar surface of the hindpaw. The response was scored as the following: 0 = no response, 1 = startle-like response, no hindpaw withdrawal (normal), 2 = brief withdrawal of the stimulated hindpaw (mild response), 3 = sustained or repeated withdrawal of the stimulated hindpaw, brief licking or shaking (strong response) (Wu et al., 2006). Animals were observed and body weight followed throughout the study. All tests were performed by an experimenter who was blind with respect to the experimental conditions. No animals were removed from the study.

Chronic constriction injury (CCI)

Thirty male Sprague–Dawley rats weighing 250–280 g underwent surgery to produce a chronic constriction of the left sciatic nerve (Bennett and Xie, 1988). Rats were anesthetized via inhalation of isofluorane gas and received a skin incision just caudal to the biceps femoris at mid-thigh level on the left hindlimb. A small incision was then made into the underlying muscle layer and separated gently using hemostats with care taken not to disturb the sciatic nerve. The sciatic nerve was freed of adhering tissue and slightly elevated using 45° angle forceps. Four pieces of 4-0 chromic gut suture material (previously washed in sterile saline) were brought under the nerve and then each loosely tied around the nerve into a square knot allowing for a chronic constriction of the nerve without cutting off the blood supply. The knots were spaced 1 mm apart. Muscle