Chapter 3

ARA 290, a peptide derived from the tertiary structure of erythropoietin, produces long-term relief of neuropathic pain. An experimental study in rats and β-common-receptor knockout mice

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Anesthesiology 2011; 115(5): 1084-92

Introduction

Neuropathic pain is a difficult-to-treat chronic pain disorder. It is characterized by allodynia (increased sensitivity to nonpainful stimuli) and hyperalgesia (increased sensitivity to painful stimuli) to mechanical (i.e., touch, pressure) and/or thermal (cold) stimuli.[1] The mechanisms of neuropathic pain are diverse and not fully understood. Key elements include central and peripheral sensitization, neuronal plasticity, and neurogenic inflammation.[2,3] These elements share intrinsic properties and pathways and ultimate behavioral effects on the perception of painful and nonpainful stimuli. Management of neuropathic pain is characterized by a trial-and-error approach, with interventions including pharmacologic treatment (opioids, antidepressants, antiepileptics, nonsteroidal anti-inflammatory drugs, and their combinations), spinal cord stimulation, and physiotherapy, often with limited success.

The effects of current pharmacologic approaches are limited with respect to efficacy, duration of effect, and the occurrence of often-unacceptable side effects.[1] Recent experimental studies examined the effect of exogenous erythropoietin in painful peripheral neuropathy models.[4-9] The results indicate that exogenous erythropoietin facilitates recovery of sensory and motor functions, including a reduction of allodynia. Erythropoietin possesses generalized tissue-protective and trophic properties that have been demonstrated in various tissues, including neural, cardiovascular, and renal tissues.[10-14] Erythropoietin produces its tissue-protective effects via activation of the erythropoietin receptor (EPOR)-β-common-receptor complex (EPOR-βcR complex), which is locally up-regulated after tissue injury.[11,15] Endogenous erythropoietin, produced in injured tissues, is considered a biologic antagonist of the pro-inflammatory cytokine tumor necrosis factor-α (TNF-α), which is produced by immune cells secondary to their activation after an initial tissue insult.[11] The tissue-protective effects of erythropoietin are distinct from its effects on hematopoiesis. The hematopoietic effect of erythropoietin is mediated through the EPOR homodimer ($EPOR_2$) present on erythrocyte precursor cells.[11] The affinity of erythropoietin for the $EPOR_2$ is 100 times greater than its affinity for the EPOR-βcR complex. Thus, using exogenous erythropoietin for tissue protection requires high circulating plasma concentrations. The use of exogenous erythropoietin has several disadvantages, including the activation of hematopoiesis and an increased risk of cardiovascular complications, including hypertension and thrombosis.[16]

The robust tissue-protective effects of erythropoietin prompted the development of erythropoietin analogs that retain their effect at the EPOR-βcR complex (and consequently their tissue-protective effects) but do not interact with the erythropoietin receptor homodimer (and thus do not cause erythropoiesis and cardiovascular complications). Various erythropoietin analogs have been produced that are

tissue-protective in vivo, including carbamylated erythropoietin and the small helix B surface peptide ARA 290.[11,17,18] ARA 290 is an 11-amino-acid peptide that mimics the tertiary structure of erythropoietin and has been shown to have tissue-protective properties without stimulating hematopoiesis.[11,17]

Because the ability of ARA 290 to treat neuropathic pain after peripheral nerve injury remains unknown, the current study was designed to explore the effect of ARA 290 on behavioral responses after unilateral nerve injury of the sciatic nerve in rats and mice and to determine whether the β-common receptor is involved by using mice lacking the β-common-receptor (βcR knockout or $\beta cR^{-/-}$ mice) and consequently lacking the EPOR-βcR complex.

Materials and Methods

Animals

The experimental protocol was approved by the Animal Ethics Committee (Dierethische Commissie) of the Leiden University Medical Center, Leiden, The Netherlands, and experiments were performed in accordance with the guidelines of the International Association for the Study of Pain.[19] The rats used in this study were 8-week-old female Sprague-Dawley rats (Charles River, Maastricht, The Netherlands) weighing 200–260 g. $\beta cR^{-/-}$ mice used for the experiments, as described previously, were obtained from Dr. Nimesh Patel, Ph.D. (Kidney Research United Kingdom Career Development Fellow, The William Harvey Research Institute, Centre for Translational Medicine & Therapeutics, London, United Kingdom).[20] Confirmation of $\beta cR^{-/-}$ was done as described by Robb et al.[20] using Southern blot analysis. Control strain-matched, wild-type mice (C57/BL6) were obtained from Charles River. The mice were 8–12 weeks of age when tested.

Animals were housed two per cage in individually ventilated cages for the duration of the entire experimental period under standard laboratory conditions with water and food ad libitum and a light–dark cycle (12:12 h; lights on 7:00 AM). At the end of the studies, the animals were killed by exsanguination during sevoflurane, 6%, anesthesia.

Surgery

Before surgery, animals were tested for baseline nociceptive thresholds as described below. Twenty-four rats, 16 $\beta cR^{-/-}$ mice, and 16 wild-type mice were surgically treated to receive an adapted spared nerve injury (SNI).[21] Animals were anesthetized with sevoflurane (6%) induction and maintenance (3%). A small incision was made in the lateral surface of the left hind limb of the animal, exposing the muscles. The

trifurcation of the sciatic nerve was revealed by blunt preparation between the two heads of the biceps femoris muscle. Next, the tibial and common peroneal nerves were tightly ligated with 5–0 silk in rats and 6–0 silk in mice and cut to remove 2–4 mm of the distal nerve. The sural nerve was left intact. To prevent spontaneous nerve reconnection, the transected nerves were displaced. During the surgical procedure, great care was taken not to stretch or touch the sciatic or sural nerves. The wound was closed in two layers with 4–0 silk in rats and 6–0 silk in mice, and a single dose of 0.01 and 0.05 mg/kg buprenorphine was administered in rats and mice, respectively, to relieve postoperative pain. Eight rats, eight $\beta cR^{-/-}$ mice, and eight wild-type mice received a sham operation. To that end, the animals were anesthetized and the sciatic nerve was exposed as described. After the exposure, no SNI was induced, and the wound was closed in two layers with 4–0 (rats) or 6–0 (mice) silk and a single dose of 0.01 (rats) or 0.05 (mice) mg/kg buprenorphine was administered to relieve postoperative pain. During the surgical procedure, great care was taken not to stretch or touch the exposed nerves.

Study Drugs

ARA 290 (Araim Pharmaceuticals, Ossining, NY) was dissolved in phosphate-buffered saline (PBS) at pH 7.4 to obtain a stock solution of 1 mg/ml. All animals treated with ARA 290 received injections with 30 µg/kg ARA 290 in 200 µl PBS. The peptide was stored at 4 °C between uses. Vehicle treatment consisted of 200 µl PBS at pH 7.4. Both ARA 290 and vehicle were injected intraperitoneally. The ARA 290 dosages used in this study are based on the work of a previous study on the effect of ARA 290 on motor function after sciatic nerve compression injury.[17]

Rat Study Design

The 24 rats that received the SNI were allocated randomly to one of the following treatment groups. Treatment was initiated 24 h after induction of the SNI. Group 1: n = 8; five 30 µg/kg ARA 290 intraperitoneal injections at 2-day intervals, followed by once-a-week maintenance therapy of 30 µg/kg ARA 290. Group 2: n = 8; five vehicle (PBS) intraperitoneal injections at 2-day intervals, followed by once-a-week maintenance therapy of vehicle. Group 3: n = 8; five 30 µg/kg ARA 290 intraperitoneal injections at 2-day intervals, with no maintenance therapy.

Mice Study Design

The 32 mice that received the SNI were randomly allocated to one of the following treatment groups. Treatment was initiated 24 h after induction of the SNI: Groups IA and IB: n = 8 βcR-/- and eight wild-type mice; five 30 µg/kg ARA 290 intraperitoneal injections at 2-day intervals, followed by once-a-week maintenance therapy of intra-

peritoneal injections of 30 µg/kg ARA 290. Group IIA and IIB: n = 8 βcR-/- and eight wild-type mice; five vehicle (PBS) intraperitoneal injections at 2-day intervals, followed by once-a-week maintenance therapy of intraperitoneal injections of vehicle. The follow-up was 4 weeks after surgery.

Measurement of Tactile and Cold Allodynia

Allodynia was assessed before surgery (baseline values) and during follow-up at 1-week intervals on the plantar surfaces of the affected (ipsilateral) and contralateral hind paws. To measure the two types of allodynia, the animals were placed in a see-through box on an increased wire mesh floor. Tactile allodynia was tested first, followed by testing for cold allodynia. Before testing, the animals were allowed to habituate for at least 10 min. When testing coincided with a treatment day, testing was performed before administration of ARA 290 or vehicle. Tactile allodynia was tested with the use of different von Frey hairs (Semmes-Weinstein Monofilaments, North Coast Medical Inc., San Jose, CA) with increasing stiffness (0.004–300 g), causing incremental forces to be exerted on the plantar surface of the affected and contralateral hind paws. The hairs were applied 10 times at intervals of 1–2 s to slightly different loci within the test area. The hind paw that was not surgically treated was tested first. When no response was observed, the ipsilateral hind paw was stimulated in a similar fashion. The force necessary to evoke a pain reflex by a brisk paw withdrawal was recorded, and no additional filaments were applied to the paw that showed a response. The experiment was continued until responses from both the ipsilateral and the contralateral paw were obtained. After a rest period, cold allodynia was tested. Twenty (rats) or 10 (mice) µl acetone was sprayed on the plantar surface of the hind paw, and the response was recorded using the following classification: 0 = no withdrawal, 1 = startle response lasting less than 1 s, 2 = withdrawal lasting between 1 and 5 s, 3 = withdrawal lasting between 5 and 30 s (with or without paw licking), and 4 = withdrawal lasting longer than 30 s (with or without licking and repeated shaking).

Statistical Analysis

A power analysis was based on data from a previous study on the effect of ketamine versus vehicle treatment on tactile allodynia in the rat SNI model.[22] We calculated a group size of at least eight animals was needed to detect a difference between treatments of at least 1 SD between the two groups, with a reliability of 5% and power more than 80%. To analyze the effect of treatment with ARA 290 over time on tactile allodynia, a two-way repeated measures analysis of variance (ANOVA) was used. The tests were followed by a Holm-Sidak test for post hoc comparisons when required. The effect of ARA 290 on cold allodynia was tested with nonparametric tests: Kruskal-

Wallis and post hoc Tukey tests. All statistical analyses were performed with SigmaPlot version 11 (Systat Software Inc., Chicago, IL). Hypothesis testing was two-tailed, with P values < 0.05 considered significant. Data are expressed as mean ± SEM.

Results

Effect of ARA 290 Maintenance in the Rat

After SNI, animals that received vehicle treatment showed the rapid development of tactile allodynia with the lowest applicable force of 0.004 g within 2 weeks after surgery. In contrast, intraperitoneal injections of ARA 290 produced long-term relief of tactile allodynia lasting at least 15 weeks (Figure 1A). The allodynic responses differed significantly between treatment groups (main effect: P < 0.001; post hoc: ARA 290 vs. vehicle P < 0.001, ARA 290 vs. sham P = 0.008). In addition to the development of tactile allodynia observed on the ipsilateral side, a decrease of the nociceptive threshold was observed in the contralateral paw (i.e., contralateral allodynia). Contralateral allodynia was greater in vehicle-treated than in ARA 290-treated animals (Figure 1B, main effect: P < 0.001; post hoc: ARA 290 vs. vehicle P < 0.001, ARA 290 vs.

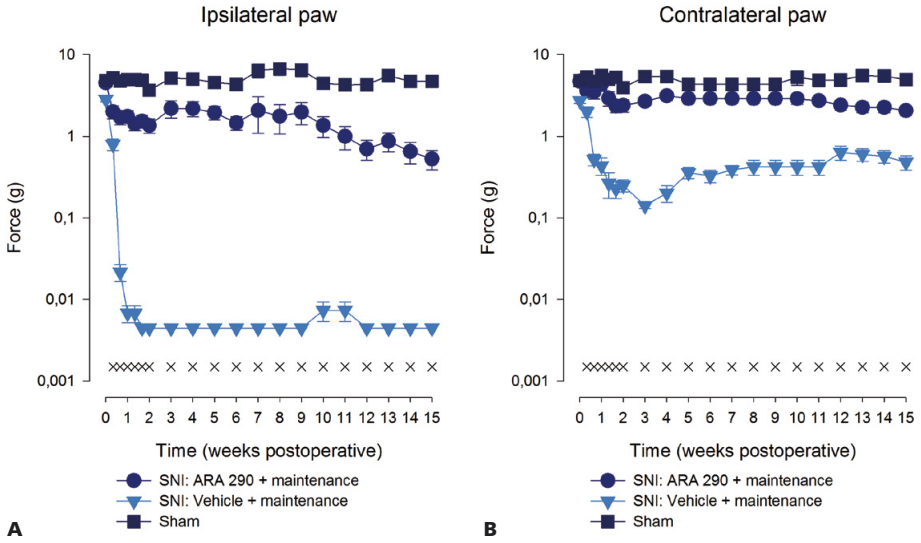

Figure 1: Effect of ARA 290 treatment during the 15 weeks after spared nerve injury (SNI) surgery on tactile allodynia. A: Ipsilateral paw. B: Contralateral paw. Thirty µg/kg ARA 290 was injected for 5 days at 2-day intervals (first injection within 24 h after surgery), followed by once-a-week maintenance therapy of 30 µg/kg ARA 290. ARA 290 produces significantly less tactile allodynia than does vehicle on ipsilateral (P < 0.001) and contralateral paws (P < 0.001). All treatments were given via the intraperitoneal route. X = treatment with either ARA 290 or vehicle.

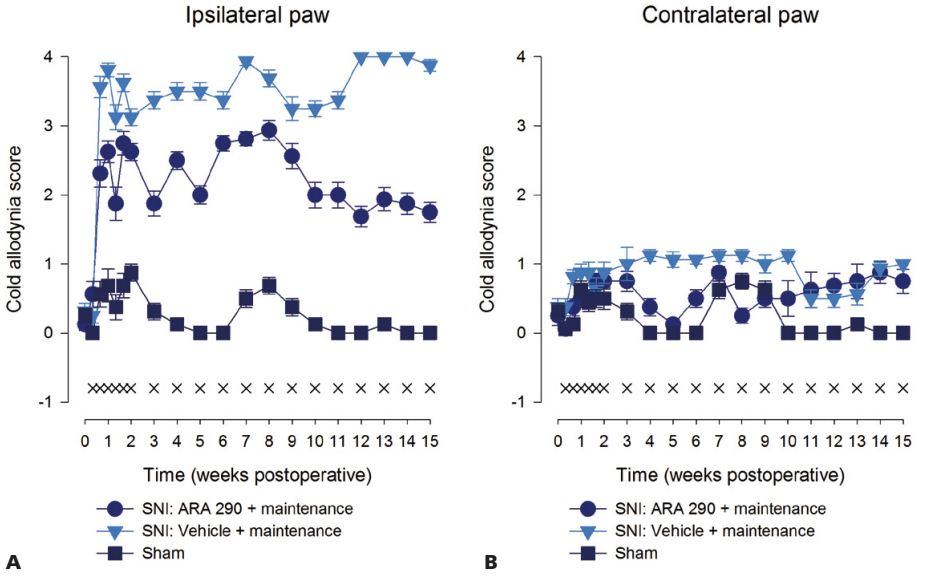

Figure 2: Effect of ARA 290 treatment during the 15 weeks after spared nerve injury (SNI) surgery on cold allodynia. A: Ipsilateral paw. B: Contralateral paw. Thirty µg/kg ARA 290 was injected for 5 days at 2-day intervals (first injection within 24 h after surgery), followed by once-a-week maintenance therapy of 30 µg/kg ARA 290. ARA 290 produces significantly less cold allodynia than does vehicle on ipsilateral ($P<0.001$) and contralateral paws ($P<0.001$). All treatments were given via the intraperitoneal route. X = treatment with either ARA 290 or vehicle.

sham $P<0.001$). Similarly, in animals treated with vehicle, cold allodynia developed rapidly after SNI surgery in the ipsilateral paw, with mean allodynia scores between 3 and 4 (4 is the maximum score) during the 15-week study period. Treatment with ARA 290 was associated with significantly less cold allodynia in the ipsilateral paw, with mean scores between 1.8 and 2.9 (Figure 2A, $P<0.001$; compared with vehicle-treated animals by post hoc test). Cold allodynia responses in the contralateral paw averaged to approximately 1 in vehicle-treated animals. A small but significant reduction in cold allodynia was observed during ARA 290 treatment in the contralateral paw (Figure 2B, $P<0.05$; compared with vehicle-treated animals by post hoc test).

Effect of 2-week versus Maintenance ARA 290 in the Rat

To assess the effect of early ARA 290 treatment, eight animals received five injections of 30 µg/kg ARA 290 during the initial 2 weeks after SNI surgery and no additional treatment. Animals treated according to this regimen showed a delay in the progression of tactile allodynia for the duration of follow-up but to a lesser extent than that of the group treated with weekly ARA 290 injections (maintenance therapy) ($P=0.018$, Figure 3A). Regardless of the therapy received, animals displayed comparable nociceptive thresholds in the contralateral paw (Figure 3B).

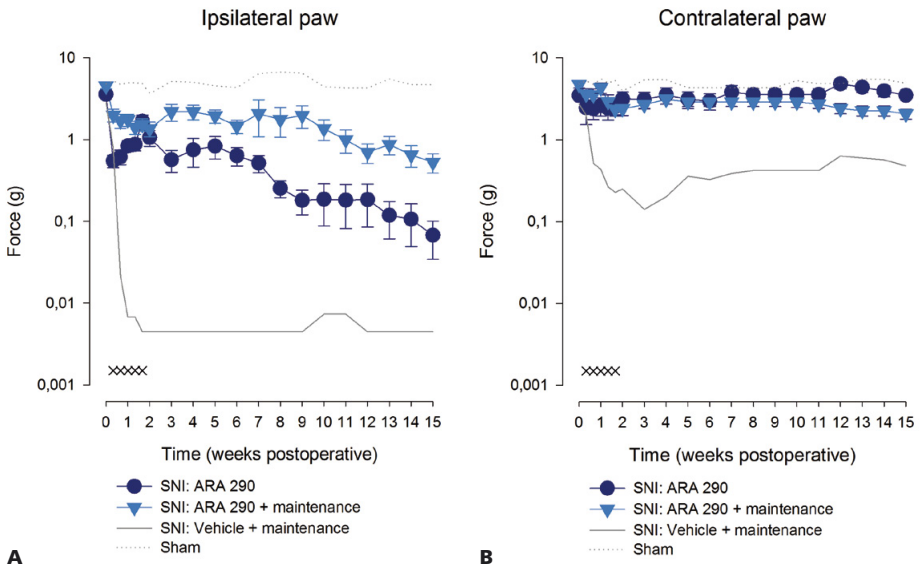

Figure 3: Effect of 2 week therapy versus weekly maintenance therapy on tactile allodynia. A: Ipsilateral paw. B: Contralateral paw. To guide the eye, data from vehicle-treated animals (continuous line) and sham-operated animals (dotted line) are added. Maintenance therapy produced less allodynia than 2-week treatment in the ipsilateral paw (P=0.02), but no difference was observed in the contralateral paw. All treatments were given via the intraperitoneal route. X=treatment with either ARA 290 or vehicle.

Omitting the maintenance therapy resulted in relief of cold allodynia but to a lesser extent than occurred after maintenance therapy (Figure 4A, P<0.001). No difference was observed in the contralateral paw (Figure 4B).

Effect of ARA 290 Maintenance in βcR$^{-/-}$ Mice

A treatment effect on tactile allodynia was observed in both genotypes (P<0.001). ARA 290 had no effect on tactile allodynia in βcR$^{-/-}$ mice (ARA 290 vs. vehicle: P=0.963, post hoc test). One week after SNI surgery, withdrawal of the affected paw occurred at the lowest possible force, 0.004 g, irrespective of treatment with ARA 290 or vehicle (Figure 5).

In contrast, wild-type animals did show an effect of ARA 290 treatment, with withdrawal responses occurring at 0.020 g versus 0.004 g in PBS-treated animals within 2 weeks after surgery (Figure 5, A and B, P=0.027 vs. vehicle-treated mice, post hoc test).

At the contralateral hind paw allodynia was observed that responded to ARA 290 treatment in wild-type animals (P=0.034 vs. vehicle, post hoc test) but not in βcR$^{-/-}$ mice (P=0.941 vs. vehicle, post hoc test) (Figure 5, C and D). In wild-type and βcR$^{-/-}$ animals, cold allodynia developed in the ipsilateral (main effect: P<0.001 in

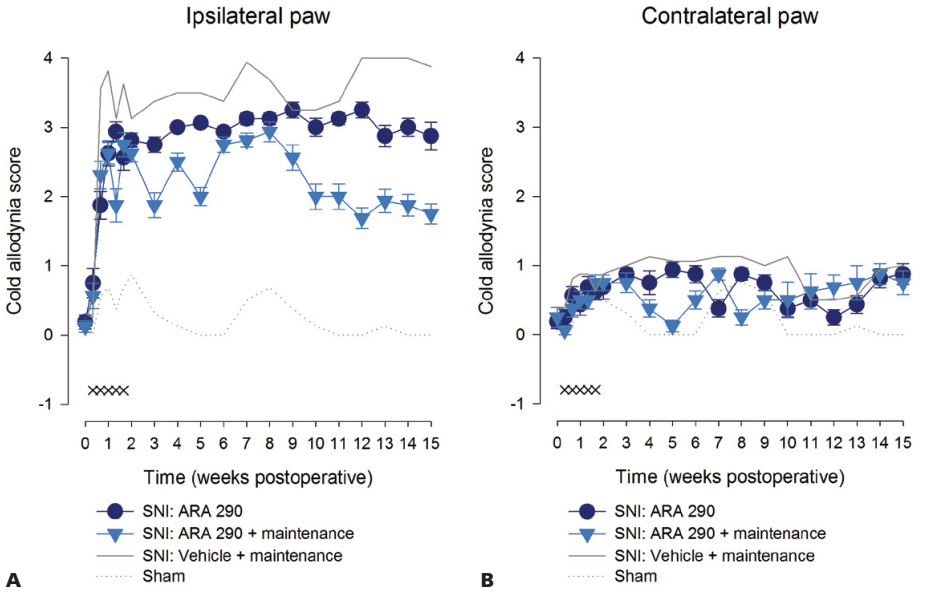

Figure 4: Effect of 2-week therapy versus weekly maintenance therapy on cold allodynia. A: Ipsilateral paw. B: Contralateral paw. To guide the eye, data from vehicle-treated animals (continuous line) and sham-operated animals (dotted line) are added. Maintenance therapy produced less allodynia than 2-week treatment in the ipsilateral paw ($P<0.001$), but no difference was observed in the contralateral paw. All treatments were given via the intraperitoneal route. X = treatment with either ARA 290 or vehicle.

both genotypes) but not contralateral hind paw (main effect: $P=0.068$ in $\beta cR^{-/-}$ and 0.087 in wild-type mice) (Figure 6). ARA 290 had a significant effect on cold allodynia responses in wild-type (Figure 6A, post hoc: ARA 290 vs. vehicle $P<0.05$ but not in $\beta cR^{-/-}$ mice (Figure 6B).

Discussion

The main findings of our studies are: (1) ARA 290 treatment in the 2 weeks after nerve injury produces effective, long-term relief of allodynia in rats; (2) in the same species, ARA 290 therapy was most effective when it was maintained at 1-week intervals; and (3) an effect of ARA 290 on nociceptive withdrawal responses was absent in mice with a homozygous deletion of the β-common-receptor ($\beta cR^{-/-}$), whereas reduced pain responses were observed in wild-type mice (mice with an intact heterodimer receptor). Our finding of a long-term antiallodynic effect of the ARA 290 peptide is novel and promising, but additional testing in humans is required to predict the effectiveness of ARA 290 in patients with neuropathic pain.

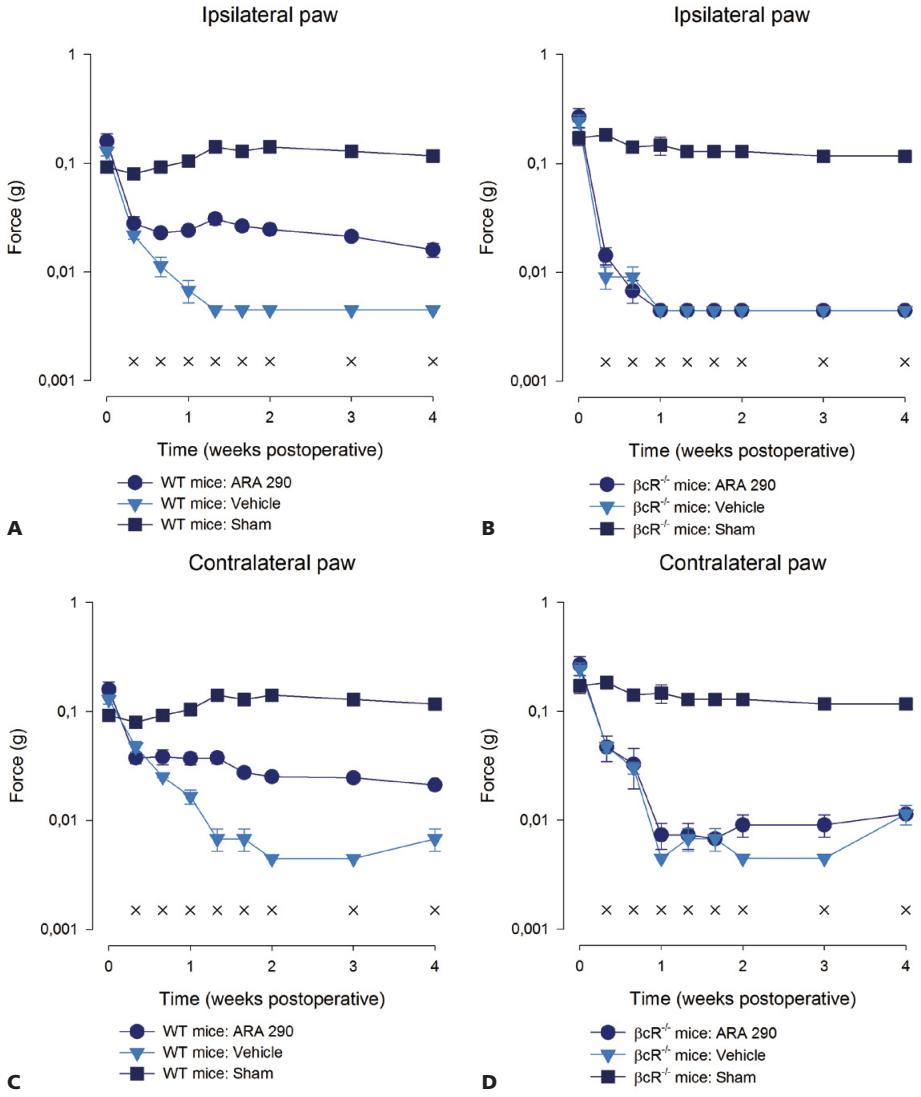

Figure 5: Effect of ARA 290 treatment on tactile allodynia measured in the ipsilateral hind paw (i.e., paw with nerve injury) and contralateral hind paw. A: Effect of ARA 290 therapy in mice with an intact β-common-receptor (wild-type mice), ipsilateral paw. B: Effect of ARA 290 therapy in mice lacking the β-common-receptor ($\beta cR^{-/-}$ mice), ipsilateral paw. C: Effect of ARA 290 therapy in wild-type mice, contralateral paw. D: Effect of ARA 290 therapy in $\beta cR^{-/-}$ mice, contralateral paw. ARA 290 caused a relief of allodynia compared with vehicle in wild-type but not $\beta cR^{-/-}$ animals (wild-type: ipsilateral P = 0.027, contralateral P = 0.034; $\beta cR^{-/-}$: ipsilateral P = not significant; contralateral P = not significant). All treatments were given via the intraperitoneal route. X = treatment with either ARA 290 or vehicle.

ARA 290 is a peptide derived from the erythropoietin molecule. In most tissues, including spinal cord and brain, the cytokine erythropoietin is produced in re-

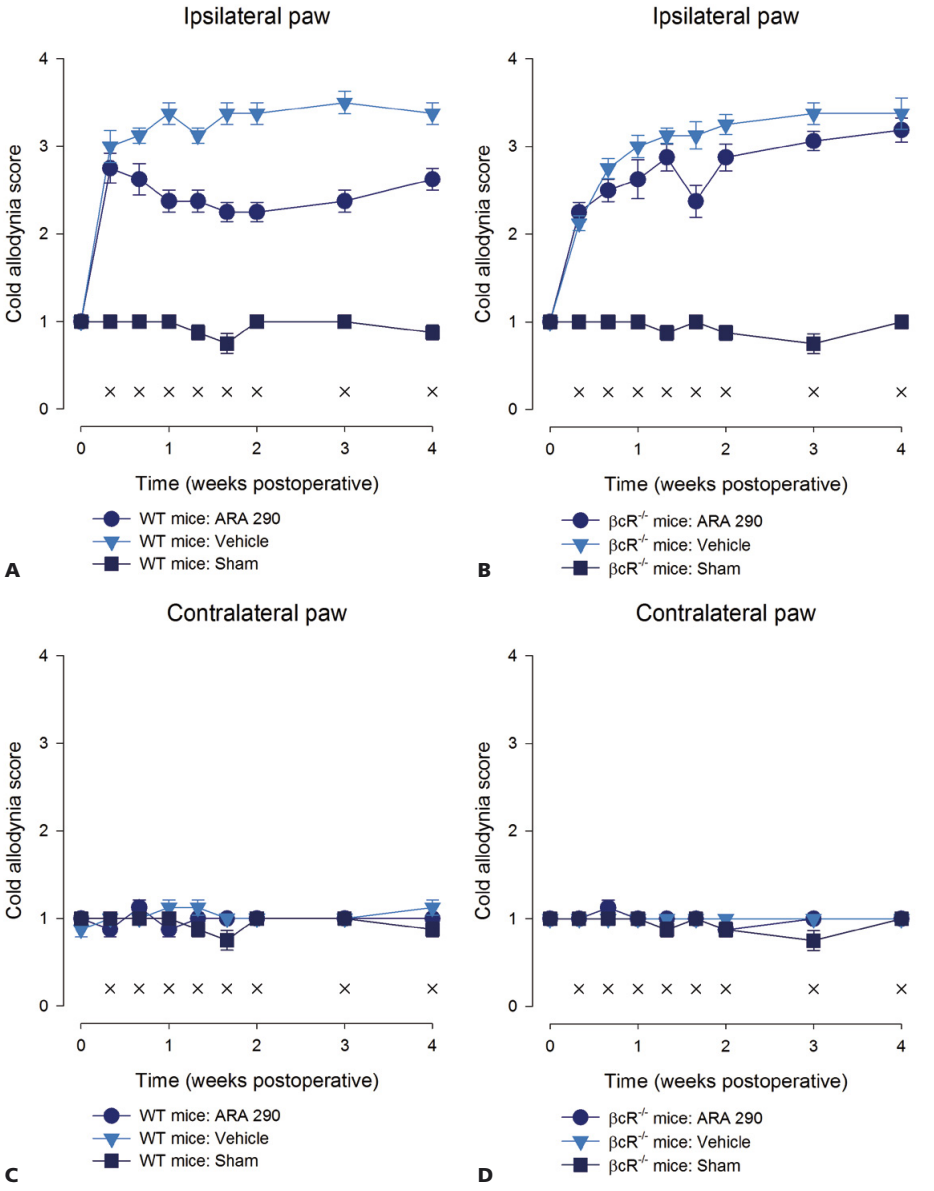

Figure 6: Effect of ARA 290 treatment on cold allodynia measured in the ipsilateral hind paw (i.e., paw with nerve injury) and contralateral hind paw. A: Effect of ARA 290 therapy in mice with an intact β-common-receptor (wild-type mice), ipsilateral paw. B: Effect of ARA 290 therapy in mice lacking the β-common receptor (βcR$^{-/-}$ mice), ipsilateral paw. C: Effect of ARA 290 therapy in wild-type mice, contralateral paw. D: Effect of ARA 290 therapy in βcR$^{-/-}$ mice, contralateral paw. A significant effect was observed in wild-type but not βcR-/- mice at the ipsilateral site only (wild-type: ipsilateral P=0.05, contralateral P=not significant; βcR$^{-/-}$: ipsilateral P=not significant, contralateral P=not significant). All treatments were given via the intraperitoneal route. X=treatment with either ARA 290 or vehicle.

sponse to local injury, counteracting the effects of proinflammatory cytokines.[11,23] Recent animal studies indicate that exogenously administered erythropoietin enhances the process of healing and effectively prevents overt tissue damage after injury.[10-14] For example, Brines et al.[12] showed that systemic administration of recombinant human erythropoietin (rhEPO, 5,000 units/kg) before or as long as 6 h after blunt trauma to the rat brain reduced concussive injury by 50–75%. Similarly, rhEPO reduced the infarct size after carotid artery occlusion in the rat.[12] These local tissue-protective effects are not mediated by the hematopoietic EPOR dimer but through the EPOR-βcR complex, which is locally up-regulated after tissue injury.[11,15,17] To activate this receptor, high local concentrations of erythropoietin are required because the EPOR-βcR complex exhibits a 100-fold lower affinity for erythropoietin than does the hematopoietic EPOR dimer.[11] High local concentrations of exogenously administrated erythropoietin are obtained only after high doses are injected systemically because tissue production of erythropoietin after injury is delayed significantly.[11] The use of high-dose exogenous erythropoietin has several disadvantages, including the activation of hematopoiesis and increased risk of cardiovascular complications (e.g., hypertension, thrombosis). For example, a clinical study on the effect of erythropoietin administration (40,000 units once/week for 4 weeks) to trauma patients admitted to the intensive care unit showed that although mortality was reduced by 50%, there was a 40% increased risk of thrombosis.[16]

Several nonhematopoietic erythropoietin analogues have been developed that selectively activate the EPOR-βcR complex and that have tissue-protective properties, such as carbamylated erythropoietin, asialoerythropoietin, and ARA 290.[11,17,18,24] Several preclinical studies have shown these compounds facilitate wound healing, limit the infarction volume in a stroke model, reduce collateral damage to surrounding tissue adjacent to the injury site in cardiomyopathy, and improve motor function after spinal cord compression.[11,17,24-27]

ARA 290 has been shown to up-regulate EPOR expression in injured tissue.[28] In the current study, we used ARA 290 to assess its effect on nociceptive responses after peripheral nerve injury. ARA 290 caused effective, long-term attenuation of ipsilateral and contralateral tactile and cold allodynia in a SNI model in the rat. The data obtained in $\beta cR^{-/-}$ mice point toward the β-common-receptor as the site of action of ARA 290 after nerve injury. Our findings are in agreement with previous observations on the effect of exogenous erythropoietin in various models of peripheral nerve injury (including chronic constriction injury, L5 spinal crush injury, and L5 spinal nerve transection).[4-9] In all models, erythropoietin effectively reduced pain behavior coupled with observations of reduced neuroimmune activation related to the anti-TNF activity of erythropoietin. In addition, the site of action of ARA 290 is similar to

that of erythropoietin (i.e., the EPOR-βcR complex) because the erythropoietin effect on motor function after spinal cord injury models is absent in $\beta cR^{-/-}$ mice.[15]
The neuroanatomical level of the effect of ARA 290 at the β-common-receptor in our experimental pain models remains unknown. We cannot exclude an effect at the (peripheral) site of nerve injury or centrally at spinal or supraspinal sites. However, a complete and prolonged block of the peripheral nerve by use of local anesthetics does not prevent the development of neuropathy, which suggests that central effects are predominant.[29] There is ample evidence that after peripheral nerve injury, as induced in our current study, an innate immune response is triggered in the spinal cord in which proinflammatory cytokines, including TNF-α, are released.[3,5,30-34] This neuroinflammatory response is highly self-amplifying, causing collateral damage to surrounding tissue and leading to sensitization of primary affected and secondary neurons, enhancing allodynia, hyperalgesia, and spontaneous pain. An important issue in this respect is the short half-life of ARA 290 (plasma half-life ≈ 2 min in rats and rabbits).[17] Although this suggests a peripheral rather than a central effect, there is ample evidence that ARA 290 passes the blood–brain barrier. For example, ARA 290 is able to cross the blood–brain barrier to exert its neuroprotective effects in ischemic stroke models and passes the blood–retinal barrier, reducing retinal edema in diabetic animals.[17] Asialoerythropoietin, a nonerythropoietic cytokine with a similarly short plasma half-life of 2 min, passes the blood– brain barrier and appears promptly in the cerebrospinal fluid after intravenous injection and binds to neurons in the hippocampus and cortex in a pattern corresponding to the distribution of the EPOR.[24] Regardless of the location of action of ARA 290, given its short half-life, it is reasonable to assume that ARA 290 initiated a cascade of events involving a series of transduction factors, of which activation of the EPOR-βcR complex is the first step (see also Brines and Cerami[11] Figure 4), that eventually result in the silencing or reduction of the inflammatory response. Evidence from such a sequence of events at central sites may be inferred from previous studies on rhEPO. Jia et al.[8] showed that rhEPO attenuates allodynia and reduces the spinal neuroimmune activation induced by L5 spinal nerve transection with reduced activation of glia cells and reduced production of proinflammatory cytokines (TNF-α, interleukin-1β) and NF-κB activation in the spinal cord. The same group showed that preemptive rhEPO attenuates mechanical and thermal hyperalgesia after L5 spinal nerve transection, as well as the cerebral expression of TNF-α, interleukin-1β, and NF-κB activation.[9] After dorsal root ganglion crush injury, rhEPO reduced local apoptosis and pain behaviors.[6] These data indicate a neuroprotective and anti-inflammatory role of rhEPO at central sites in a variety of neuropathic pain states, causing a significant amelioration of pain behavior. Given the observations in rhEPO-treated animals, the fact that AR290 is an erythropoietin analog acting at the EPOR- βcR complex, and that it is able to pass the blood–brain

barrier, our data may well be explained by an anti-inflammatory and neuroprotective effect of ARA 290 at spinal and possibly supraspinal sites. However, we again stress that a peripheral effect cannot be excluded. A peripheral effect of rhEPO has been observed in an animal model of diabetic neuropathy, where it prevents and reverses intraepidermal neuronal loss,[4] and in chronic constriction injury, rhEPO facilitates the recovery from neuropathic pain and reduces Schwann cell TNF-α expression at the nerve injury site.[5] Despite a large reduction of allodynia maintained during the intensive treatment period, a slow trend toward an increase in pain behavior was observed during the weekly ARA 290 dosing paradigm (Figure 3). This observation could suggest that because of the biologic half-life of ARA 290 of less than 1 week, more frequent dosing could prevent the trend for increased pain. An alternative explanation could be that noninflammatory processes slowly develop to foster proallodynic responses and gain in importance over time or that the inflammatory response becomes more resilient. If true, this suggests that treatment of neuropathic pain caused by nerve injury should be aimed at targeting multiple processes, of which suppression of the immune response is one that requires early (and continuous) treatment. It is not likely that decreasing the interval between nerve injury and the initiation of treatment or using ARA 290 as a preemptive measure results in a more effective relief of neuropathic pain because the EPOR-βcR complex is being up-regulated secondary to tissue damage.[11] Alternatively, more intense treatment during the initial phase (e.g., higher doses or injections at a 1-day interval) may be more effective in neutralizing the initial hit induced by the peripheral nerve injury.

We observed contralateral development of allodynia in mice and rats that was attenuated by ARA 290 treatment (Figures 1 and 5). These findings indicate the presence of neuroinflammation in the spinal cord and dorsal root ganglia at the site opposite from the severed peripheral nerves and suggest the presence of a more generalized inflammatory response in the central nervous system in our SNI animals. Indeed, in unilateral nerve damage, a bilateral increase in TNF-α and activated glia cells in bilateral homo-and heteronymous dorsal root ganglia is observed in a rat model of chronic constriction injury, suggesting a more generalized inflammatory response.[35,36]

In conclusion, our data indicate that the development of allodynia after peripheral nerve injury is effectively prevented for the long term by early treatment with ARA 290. Testing of ARA 290 in patients with chronic pain is required before any conclusions on the effectiveness of ARA 290 in humans may be drawn.

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