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Interleukin-10 resolves pain hypersensitivity induced by cisplatin by reversing sensory neuron hyperexcitability

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Abstract

Understanding the mechanisms that drive transition from acute to chronic pain is essential to identify new therapeutic targets. The importance of endogenous resolution pathways acting as a "brake" to prevent development of chronic pain has been largely ignored. We examined the role of IL-10 in resolution of neuropathic pain induced by cisplatin. In search of an underlying mechanism, we studied the effect of cisplatin and IL-10 on spontaneous activity (SA) in DRG neurons. Cisplatin (2 mg/kg daily for 3 days) induced mechanical hypersensitivity that resolved within 3 weeks. In both sexes, resolution of mechanical hypersensitivity was delayed in *Il10*$^{-/-}$ mice, in WT mice treated intrathecally with neutralizing anti-IL-10 antibody, and in mice with cell-targeted deletion of IL-10R1 on advillin-positive sensory neurons. Electrophysiologically, small to medium-sized DRG neurons from cisplatin-treated mice displayed an increase in the incidence of spontaneous activity. Cisplatin treatment also depolarized the resting membrane potential, and decreased action potential voltage threshold and rheobase, while increasing ongoing activity at −45 mV and the amplitude of depolarizing spontaneous fluctuations (DSFs). *In vitro* addition of IL-10 (10 ng/ml) reversed the effect of cisplatin on SA and on the DSFs amplitudes, but unexpectedly had little effect on the other electrophysiological parameters affected by cisplatin. Collectively, our findings challenge the prevailing concept that IL-10 resolves pain solely by dampening neuroinflammation and demonstrate in a model of chemotherapy-induced neuropathic pain that endogenous IL-10 prevents transition to chronic pain by binding to IL-10 receptors on sensory neurons to regulate their activity.

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Conflict of Interest statement.

The authors declare no conflict of interest

Resolution of pain depends on endogenous IL-10 signaling, IL-10 binds to its receptor on sensory neurons to reverse cisplatin induced-neuronal hyperactivity and -pain hypersensitivity.

Introduction.

Chronic pain affects between 30% and 50% of the population worldwide [50]. The immune system has been shown to play a pivotal role in the onset and maintenance of chronic pain [14,20,46]. Immune cells release pro-inflammatory mediators that sensitize peripheral sensory neurons and spinal cord neurons via binding to receptors for cytokines, chemokines and alarmins on these neurons [7,13,42,43,67]. Mice lacking receptors for these mediators are protected from neuropathic pain [1,53,63].

The immune system is, however, not always detrimental in chronic pain. An emerging body of literature indicates that the resolution of pain is an active process that depends on the participation of immune cells [3,9,23,25,26,28,62]. For example, we and others have shown that the lack of IL-10-producing macrophages and/or CD8+ T cells prevents the resolution of mechanical hypersensitivity induced by inflammation or chemotherapeutic drugs [3,9,23,26,28,62].

The anti-nociceptive effect of exogenous administration of IL-10 after nerve injury, inflammation, and paclitaxel treatment is well-established [16,23,30,37,58,65,74]. In addition, endogenous IL-10 is essential for exercise-induced analgesia [2,31,49], prevents neuropathic pain in young rodents [35] and promotes axon regeneration after injury [36].

IL-10 signals via binding to the IL-10 receptor (IL-10R), a hetero-tetramer of two IL-10R1 and two IL-10R2 subunits. IL-10R1 is the ligand binding subunit and is necessary for cells to be responsive to IL-10 [38]. IL-10R2 is shared with receptors for other cytokines. IL-10R is expressed by many different cell types [38,48]. The mechanisms via which IL-10 alleviates pain, including the identity of the target cells, remain elusive. Most of the previous studies have attributed beneficial effects of IL-10 on pain to its capacity to suppress the production of pro-inflammatory cytokines. Indeed, IL-10 administration suppresses neuroinflammation and microglia activation in models of chronic pain [16,37,58]. There is also evidence that IL-10 acts directly on sensory neurons [23,48]. However, it is not known whether direct effects of IL-10 on sensory neurons are required for its beneficial effects on chronic pain and whether IL-10 signaling to sensory neurons affects their activity.

Chronic pain is associated with neuronal hyperactivity, including spontaneous activity (SA) recorded *in vivo* from somatosensory neurons of humans [22] and rodents [8,15,45,60,64]. The SA associated with pain is often generated in the somata of DRG neurons *in vivo* [5,59]. This SA persists *in vitro,* both in rodent [4,5,33,51,71] and in human DRG neurons [39,57] and is strongly implicated in driving persistent pain [39,69]. Nociceptor SA generated at resting membrane potential (RMP) in the absence of extrinsic stimulation or ongoing activity (OA) resulting from extrinsic stimulation both depend upon enhancement of depolarizing spontaneous fluctuations (DSFs). DSFs were recently recognized as important contributors to nociceptor hyperactivity in painful conditions [40]. It is unknown whether cisplatin-induced neuropathic pain is associated with SA and enhancements of DSFs in DRG neurons.

Here we hypothesized that cisplatin-induced neuropathic pain is associated with hyperactivity of DRG neurons, and that a direct action of IL-10 on sensory neurons is

required for resolution of cisplatin-induced neuronal hyperactivity and mechanical hypersensitivity.

Methods

All experimental protocols were approved by the Animal Care and Use Committee at The University of Texas MD Anderson Cancer Center and conformed to the US National Institutes of Health guidelines on the ethical care and use of animals and ARRIVE guidelines.

Animal model.

Male and female 9-week old WT (JAX#000664) and *Il10*$^{-/-}$ (JAX#002251) mice were purchased from Jackson laboratory (Maine). To generate the *Avil*$^{Cre+/-}$;*Il10ra*^{floxflox} (*Il10ra*$^{DRG-KO}$) mice, *Avil*$^{Cre+/-}$ mice [27,75] (generously provided by Dr. Hui-Lin Pan, MD Anderson Cancer Center) were bred with *Il10ra*^{floxflox} [41] mice. Both strains were on C57BL/6 background. Experimental animals were obtained from *Avil*$^{Cre+/-}$;*Il10ra*^{floxflox} sir crossed with *Il10ra*^{floxflox} dam mice. The breeding generated 50% of *Avil*$^{Cre+/-}$;*Il10ra*^{floxflox} (*Il10ra*$^{DRG-KO}$) pups and 50% of control *Il10ra*^{floxflox} pups. No sex difference was observed in the proportion of each genotype. CIPN was induced by intraperitoneal administration of 2 mg/kg cisplatin (Teva pharmaceuticals) daily for 3 days (identified as days 0, 1 and 2 in figures) as previously described [26].

Experimental design.

Mice were randomly assigned to experimental groups. The sample size was estimated based on previous publications [4,23,26,40]. *Il10*$^{-/-}$ mice were compared to WT mice obtained from Jackson labs at the same time. *Il10ra*$^{DRG-KO}$ were compared to their Cre-negative control littermates (*Il10ra*^{floxflox}).

Mechanical pain sensitivity was assessed using von Frey filaments as previously described [11,19,26]. Briefly, mice were placed in opaque boxes (10 × 10 × 10 cm). After a 30 min habituation period, von Frey filaments were applied, and the paw withdrawal threshold was calculated using the “up & down” method. Female and male mice were tested separately, and the data were pooled as no differences were detected between sexes. Behavioral testing was performed by experimenters blinded to treatment and genotype.

Intrathecal injection.

Neutralizing anti-IL-10 (10 μg Sigma, #15145) antibody or control IgG (Sigma) were injected intrathecally under anesthesia (isoflurane 2.5%) as previously described [23,24,29] on days 8, 9 and 10 after the first dose of cisplatin.

Double immunofluorescence staining.

On day 25 after the first cisplatin injection, animals were euthanized with CO_2 and perfused with ice-cold PBS and4% paraformaldehyde in PBS. Lumbar DRG were fixed in paraformaldehyde for 48h and cryoprotected with sucrose. DRG were sliced with a Leica CM3050 S Cryostat to 8 μm sections. Slices were stained with mouse monoclonal anti-IL-10

(Santa Cruz, sc-28371, 1:100) and polyclonal rabbit anti-NeuN (Abcam #ab177487, 1:500) overnight at 4°C followed by Alexa Fluor 488 Goat-anti-mouse and Fluor 594 Goat-anti-rabbit (Alexa, Invitrogen, A-11029, 1:500 and A-11037, 1:500) [16]. Two controls were used: omission of the primary antibody and DRG slices from *Il10ra*$^{DRG\text{-}KO}$ mice. Images were captured with a CTR4000 Leica confocal microscope.

Sensory neuron isolation.

On day 10 after the first injection, PBS- and cisplatin-treated WT mice were euthanized with $C0_2$ and perfused with ice-cold PBS. DRGs were quickly and carefully removed and trimmed in ice-cold DMEM, digested with Trypsin (0.3 mg/ml, Worthington, #LS003702) and collagenase D (1.5 mg/ml, Sigma, #11088858001) for 40 min at 34°C. Debris was removed by two successive centrifugations (6 minutes at 600 rpm) and cells were plated onto coverslips coated with 0.01% poly-L-ornithine (Sigma, P4957) and incubated overnight at 37°C with 5% CO2 in serum free DMEM as previously described [4].

Electrophysiology.

Patch-clamp recordings were performed as previously reported [4,40]. Small to medium size neurons (diameter < 30 μm) were recorded with whole-cell patch-clamp configuration at room temperature (~21 °C) with an EPC10 amplifier (HEKA Elektronik GmbH). Patch pipettes were pulled from borosilicate glass capillaries with a P-97 puller (Sutter Instruments) and fire polished to achieve a final electrode resistance of 3–8 MΩ when filled with a solution containing the following (in mM): 134 KCl, 1.6 $MgCl_2$, 13.2 NaCl, 3 EGTA, 9 HEPES, 4 Mg-ATP, and 0.3 Na-GTP (pH 7.2 adjusted with KOH, 300 mOsM, adjusted with sucrose). The bath solution contained (in mM): 140 NaCl, 3 KCl, 1.8 $CaCl_2$, 2 $MgCl_2$, 10 HEPES, and 10 glucose (pH 7.4 adjusted with NaOH, 320 mOsM). The whole-cell patch-clamp configuration was achieved under voltage-clamp by rupture of the membrane after a tight seal (>1 GΩ) was obtained. Membrane capacitance was then measured, and capacitance and series resistance were electronically compensated afterward. The calculated liquid junction potential was 4.3 mV and was not corrected. Spontaneous activity (SA) was measured in current-clamp mode and was defined as at least 1 spike occurring within 1 min with no current injection (I = 0). Ongoing activity (OA) during modest depolarization was then tested by injecting sufficient current to hold neurons at −45 mV (± 2 mV) for 30 seconds. Discharge of one or more action potentials was considered OA. Neurons were identified as rapidly accommodating (RA) or nonaccomodating (NA) by injecting a series of 2-second depolarizing pulses of depolarizing current (5 pA increments) from a holding potential of −60 mV up to twice rheobase as described previously (Odem et al., 2018). We excluded from our analysis RA neurons because they never exhibit SA [40]. RA neurons represented 4% of the 26 neurons patched from PBS-treated wild-type mice). The remaining NA neurons had electrophysiological properties identical to those shown previously in rats to be highly enriched in capsaicin-sensitive cells that are likely to be primary nociceptors [40].

For IL-10 treatments, cells isolated from cisplatin-injected mice were incubated for 15–30 min with 10 ng/ml of recombinant IL-10 (BioVision #4156–10) before recording. Recombinant IL-10 was reconstituted in 3 mM Tris PH8 and diluted into PBS following

manufacturer instructions. We acknowledge that the lack of a carrier protein may have reduced the working concentration of IL-10 *in vitro.* This dose was chosen based on its ability to inhibit microglia and astrocyte activation *in vitro* (without the use of protein carrier) [16].

Quantitative-real-time polymerase chain reaction (qPCR).

Sciatic nerve (SN), lumbar DRG and spinal cord tissues were quickly dissected and snap-frozen in liquid nitrogen. RNA was isolated using the Trizol-Chloroform (#15596–026, Invitrogen) method. One μg of RNA was reverse transcribed into cDNA using High Capacity cDNA Reverse Transcription kit (#4368813, Applied Bioscience). PCR was performed using PrimeTime Gene Expression Master Mix (#1055770, Integrated DNA Technologies, Coralville, IA) and validated PrimeTime qPCR primer assays for *Gapdh, Il10* and *Il10ra* (Integrated DNA Technologies).

Statistical analysis.

All data are presented as mean ± S.E.M. For behavioral experiments differences between groups were assessed using repeated measures two-way ANOVA followed by Bonferroni correction. For biochemical experiments unpaired t tests were used. For electrophysiological experiments, differences in incidence of SA or OA between populations were assessed by Fisher's exact test with Bonferroni correction. Differences between groups for the other electrophysiological modalities were tested with one-way ANOVA with Sidak's correction for multiple comparisons for normally distributed data, otherwise a Kruskal-Wallis test with Dunn's correction for multiple comparisons was used. Differences were considered statistically significant at $p<0.05$. In graphs, statistical differences are indicated as *=$p<0.05$, **=$p<0.01$ and ***=$p<0.001$. Prism (GraphPad software, La Jolla, CA) was used for plotting graphs and statistical analysis.

Results

Endogenous IL-10 is necessary for the resolution of cisplatin-induced mechanical pain hypersensitivity

Administration of cisplatin (2 mg/kg for 3 days) induced mechanical hypersensitivity that resolved after 3 weeks (Figure 1A). Resolution of pain hypersensitivity in cisplatin-treated mice was associated with an increase in *Il10* mRNA in the spinal cord but not in the sciatic nerve (Figure 1B, unpaired t test, $F(3, 4) = 6.98$, $p<0.05$). *Il10* mRNA was not detectable in the DRG. To determine the contribution of endogenous IL-10 to the resolution of mechanical hypersensitivity, we used female and male WT and $Il10^{-/-}$ mice. The maximal intensity of mechanical hypersensitivity was similar in WT and $Il10^{-/-}$ mice but the resolution of mechanical pain hypersensitivity was delayed in $Il10^{-/-}$ mice (Figure 1A, repeated measure two-way ANOVA, interaction genotype x time $F(11, 110) = 5.55$, $p<0.001$). No differences were found between female and male mice (Figure 1C). To test whether IL-10 was acting at the level of the spinal cord/DRG to promote resolution of hypersensitivity, we intrathecally injected a neutralizing anti-IL-10 antibody on day 8, 9 and 10 after the first injection of cisplatin. Inhibition of IL-10 signaling in the spinal cord/DRG markedly delayed the resolution of mechanical pain hypersensitivity in female and male

mice (Figure 1D, repeated measures two-way ANOVA, interaction genotype x antibody $F(39, 208) = 7.14$, $p<0.001$).

Isolated DRG neurons from cisplatin-treated mice exhibit spontaneous and evoked hyperactivity

To test whether IL-10 may regulate neuronal hyperactivity associated with neuropathic pain, we first tested the hypothesis that cisplatin treatment induces hyperactivity of DRG neurons. We isolated neurons from lumbar DRG of PBS- and cisplatin-treated mice on day 10 and cultured the cells overnight. DRG neurons from cisplatin-treated mice showed a dramatic increase in the incidence of SA (Figure 2A–B, Fisher's exact test, $p<0.001$). Cisplatin treatment also increased the incidence of OA as observed when neurons were artificially depolarized from their normal resting membrane potential (RMP) to −45 mV for 30 sec (Figure 2C Fisher's exact test, $p<0.001$).

In principle, SA or OA in the absence of transient depolarizing inputs can be promoted by one or more of only three general electrophysiological alterations [40]: (i) depolarization of RMP, (ii) hyperpolarization of the voltage threshold for generating action potentials (APs), and (iii) enhanced amplitude of depolarizing spontaneous fluctuations (DSFs) of membrane potential that bridges the gap between RMP and AP threshold. The results in Figures 3 show that cisplatin induced all three alterations. We observed sustained depolarization of the RMP (Figure 3A, PBS vs. cisplatin: one-way ANOVA followed by Sidak's multiple correction test $p<0.05$), hyperpolarization of the AP voltage threshold (Figure 3B, one-way ANOVA followed by Sidak's multiple correction test $p<0.01$, PBS vs. cisplatin: $p<0.05$), which together help explain the decrease in AP current threshold (rheobase) (Figure 3C, Kruskal-Wallis test with Dunn's correction for multiple comparisons $p=0.001$; PBS vs. cisplatin: $p=0.001$). In addition, neurons isolated from cisplatin-treated mice exhibited greater mean DSF amplitudes as measured either at RMP or when artificially depolarized to −45 mV compared to DRG neurons from PBS-treated mice (Figure 4A, Kruskal-Wallis test with Dunn's correction for multiple comparisons, $p<0.05$ at rest and $p=0.01$ at −45 mV). The increase in DSF amplitudes in DRG neurons of cisplatin-treated mice was voltage dependent, being most prominent at RMP. (Figure 4B, between −55 and −40 mV: Kruskal-Wallis test with Dunn's correction for multiple comparisons $p<0.001$, respectively for each bin compared with the next depolarized bin, $p<0.10$, $p<0.001$, $p<0.001$, $p>0.10$). This is in line with our previous observations in DRG neurons from rats [40] and mice [6] after spinal cord injury.

IL-10 reverses cisplatin-induced neuronal hyperactivity primarily by reducing the amplitude of the DSFs

Given that IL-10 is required for resolution of pain, we tested whether bath application of IL-10 could reverse the hyperactivity observed in DRG neurons from cisplatin-treated mice. Bath application of recombinant IL-10 (10 ng/ml) to DRG neurons from cisplatin-treated mice for 15–30 min significantly reduced the incidence of SA at RMP from 52% to 13% of neurons (Figure 2, SA Fisher's exact test, $p<0.01$). Bath application of IL-10 had no significant effect on OA at −45 mV (Figure 2). IL-10 also did not reverse the effects of cisplatin treatment on RMP, AP voltage threshold, and rheobase (Figure 3).

IL-10 bath application reduced DSF amplitudes in neurons from cisplatin-treated mice having RMPs between −55 and −41 mV, compatible with the average RMP recorded (Figure 4B, Kruskal-Wallis test with Dunn's correction for multiple comparisons for −55 to −51 mV and −50 to −46 mV bins with respectively p=0.05 and p<0.001, and one-way ANOVA followed by Sidak's multiple correction test for −45 to −41 mV bin with p<0.001). IL-10 did not significantly affect the overall DSF amplitudes recorded at RMP or when neurons were depolarized artificially to −45 mV (Figure 4A).

Cisplatin increased IL-10R1 expression in sensory neurons

Because IL-10 has a direct effect on neuronal activity, we next investigated whether resolution of cisplatin-induced mechanical hypersensitivity was associated with changes in the expression of IL-10R1 in DRG and spinal cord. *Il10ra* mRNA levels increased in the DRG and sciatic nerve but not in spinal cord in response to cisplatin administration (Figure 5A–C, for DRG unpaired t test, $F(6,7) = 2.91$ $p<0.05$; for SN unpaired t test, $F(7,5) = 10.8$ $p<0.05$). Next, we performed double immunofluorescence analysis to assess IL-10R1 expression on sensory neurons. IL-10R1 is expressed by sensory neurons and satellite glial cells in naïve mice (Figure 5D). The percentage of IL-10R1-positive neurons increased after cisplatin treatment (Figure 5E, Unpaired t test, $F(3,3) = 1.56$ $p<0.05$). To validate the specificity of the staining, we used mice that do not express IL-10R1 on Advillin-positive sensory neurons ($Il10Ra^{\text{DRG-KO}}$ mice) (Figure 5D). IL-10R1 staining was not detectable on sensory neurons in the DRG of these mice, while staining on cells around the neurons, most likely satellite glia, was not affected. The expression of IL-10R1 is upregulated at mRNA and protein level from day 8 and persisted for a least 2 weeks.

Sensory neuron IL-10R1 is critical for resolution of cisplatin-induced mechanical pain hypersensitivity

Because IL-10R1 is expressed on sensory neurons (Figure 5D) and *in vitro* addition of IL-10 reduced spontaneous activity in sensory neurons isolated from cisplatin-treated mice, we determined the contribution of IL-10R1 on sensory neurons to the resolution of pain hypersensitivity. We monitored the resolution of mechanical hypersensitivity in control ($Il10ra^{\text{flox}}$) and $Il10^{\text{DRG-KO}}$ mice treated with cisplatin. The lack of IL-10R1 in sensory neurons delayed the resolution in female and male mice (Figure 6A, repeated measure two-way ANOVA interaction time x genotype $F(8,72) = 5.09$, $p<0.001$). No differences were observed between sexes (Figure 6B). Baseline pain thresholds and severity of mechanical pain were similar in both genotypes (Figure 6A).

Discussion

It is well established that sensory neurons respond to proinflammatory cytokines such as IL-1β or TNFα produced by glia in the spinal cord or by infiltrating immune cells in spinal cord, DRG or nerve with increased excitability and pain hypersensitivity [7,17,21]. There is also ample evidence that exogenous administration of IL-10 reduces pain-related signaling and this is thought to be mediated through suppression of the production of proinflammatory cytokines by glia. Here we demonstrate for the first time that signaling by the anti-inflammatory cytokine IL-10 to specific IL-10 receptors expressed on sensory neurons is

required for resolution of mechanical hypersensitivity in cisplatin-treated male and female mice. We also show for the first time that cisplatin induces hyperactivity, including SA, in DRG neurons, a phenomenon closely linked to the maintenance of neuropathic pain [15,39,57,69]. Notably, IL-10 reverses the increase in SA induced by treatment of mice with cisplatin. Our present findings reinforce the concept that resolution of pain is an active mechanism that involves neuroimmune interactions [3,9,18,23,24,26]. These findings also challenge the concept that glia and/or immune cells are the main target cells mediating resolution of pain in response to IL-10.

We did not observe sex differences in the pro-resolution effect of endogenous IL-10 signaling. The lack of sex effects is consistent with previous findings on the role of T cells in the resolution of chemotherapy-induced peripheral neuropathy (CIPN), nerve injury-induced pain sensitivity, and the analgesic effect of IL-10 observed in both sexes following spared nerve injury [18,26,65]. The data in the figure 1D suggest that IL-10 actively inhibits pain hypersensitivity, because the hypersensitivity resolved after we stopped dosing the neutralizing antibody. The kinetics of the resolution after the last dose of anti-IL-10 are consistent with the described half-life of ~10 days for IgG [34].

Resolution of cisplatin-induced pain hypersensitivity is associated with upregulation of *Il10* in the spinal cord and *Il10ra* in the DRG and sciatic nerve indicating that resolution is an active mechanism and not the result of a simple disappearance of the triggers of pain. Our data clearly show that peripheral sensory neurons express IL-10R1 and that expression of these receptors in Advillin-positive neurons is required for the resolution of mechanical pain hypersensitivity. While Advillin is expressed by both sensory and sympathetic neurons, single cell RNA-seq data show that *Il10ra* is expressed in sensory but not sympathetic neurons [70] ruling out a potential effect of sympathetic neurons in our model. It should be noted that there is some delayed reduction in allodynia in the IL-10R1$^{DRG\text{-}KO}$ mice. This may be due to some residual expression of IL-10R1 on sensory neurons. However, we cannot exclude the possibility that IL-10R1 on other cells contributes to recovery as well.

Our findings expand previous findings by us and others that indicated that IL-10 reduces pain via a direct action on sensory neurons [23,48,68]. This neuronal effect of IL-10 adds to the proposed ability of this cytokine to suppress pain by suppressing production of proinflammatory cytokines by microglia thereby reducing neuroinflammation [55,58,74]. Moreover, our findings open up the possibility that reduction of microglial activity in response to IL-10 is mediated in part by suppression of SA in sensory neurons [61,66]. Consistent with our present findings, endogenous IL-10 is also necessary for the resolution of inflammatory pain [3,62]. In contrast to our findings in CIPN and inflammatory pain models, resolution of pain hypersensitivity after chronic constriction injury (CCI) is not altered in *Il10*$^{-/-}$ mice [56]. These data indicate that the role of endogenous IL-10 in resolution of pain may be disease/pain model specific. This is perhaps not surprising; the mechanisms underlying pain development differ among pain models, and therefore it is likely that mechanisms underlying resolution also differ.

We and others showed previously that chemotherapy-induced peripheral neuropathy is not associated with microglial activation, making it unlikely that suppression of microglial

activity contributes to the beneficial effects of IL-10 reported here [10,12,44,72,73]. Our current findings reveal a novel and direct mechanism of action of IL-10 on sensory neuron activity to resolve pain. We performed the first comprehensive electrophysiological characterization of isolated sensory neurons from cisplatin-treated mice. Our study reveals that cisplatin treatment induced strong hyperactivity in sensory neurons as shown by the enhanced incidence of SA, a phenomenon previously observed in models of peripheral and central neuropathic pain induced by surgical damage to the nerve [8,15,45,60,64]. Cisplatin also induced sustained OA measured at −45 mV, a novel measure of neuronal hyperactivity [40]. These findings indicate that cisplatin-induced neuronal hyperactivity is associated with the same fundamental alterations of neuronal excitability as previously described in nociceptors isolated from rats and mice after spinal cord injury, *i.e.* depolarization of RMP, lowered AP voltage threshold, and increased DSF amplitudes [6,40]. The mechanisms underlying large, irregular, low-frequency DSFs are complex and currently under investigation. DSF mechanisms in small, capsaicin-sensitive and/or isolectin B4-binding DRG neurons (probable nociceptors) (Odem, 2018) clearly differ from the mechanisms of high-frequency, sinusoidal, subthreshold membrane potential oscillations (SMPOs) described by Devor and colleagues in large DRG neurons (primarily non-nociceptive) [34].

Bath application of IL-10 for 15–30 min was sufficient to reverse SA in sensory neurons from cisplatin-treated mice. We showed previously that perfusion of DRG neurons from paclitaxel-treated rats with IL-10 blocked SA already after a few seconds, but the dose of IL-10 used in that study was an order of magnitude higher than in the present study [23]. IL-10 can alter the mRNA expression of ion channels implicated in the regulation of neuronal excitability, such as $Na_v1.8$ and $Ca_v2.2$ channels [48,68]. However, the rapid effect of IL-10 on SA observed in our current and previous study suggests a non-genomic effect of IL-10. To our knowledge, direct effects of IL-10 on the function of $Na_v1.8$ and $Ca_v2.2$, or other channels regulating neuronal excitability have not been reported. It is noteworthy that IL-10 has been reported to regulate neuronal activity in the hippocampus as well. Similarly to our observations, IL-10 reduced hippocampal neuronal activity within 10 min [32,52]. In these studies, IL-10 stimulates the activation of potassium channel BKα1 and the GABA release.

Intriguingly, IL-10 reduced SA by reversing the cisplatin-induced increase in DSF amplitudes without substantially altering RMP, rheobase, and AP voltage threshold. This result reinforces growing evidence that enhancement of DSFs is particularly important for nociceptor hyperactivity [6,40]. Our findings support the hypothesis that IL-10 may regulate the activity and/or trafficking of ion channels involved in generating or modulating DSFs.

In 20%−30% of patients treated with chemotherapy, neuropathy persists after cessation of treatment and this reduces the quality of life of cancer survivors [47]. It is important to note that a study comparing patients with painful neuropathy, painless neuropathy and controls showed that the circulating levels of IL-10 were higher in patients with painless neuropathy than in the other groups (patients with neuropathies induced by chemotherapy and other etiologies were included in this study) [54].

In conclusion, we report for the first time that IL-10 acts directly on sensory neurons to resolve neuronal hyperactivity and pain hypersensitivity induced by cisplatin. On the basis of these findings, we propose that stimulation of IL-10 signaling, or administration of IL-10 may be developed into a safe therapeutic strategy to prevent or treat chronic chemotherapy-induced neuropathic pain.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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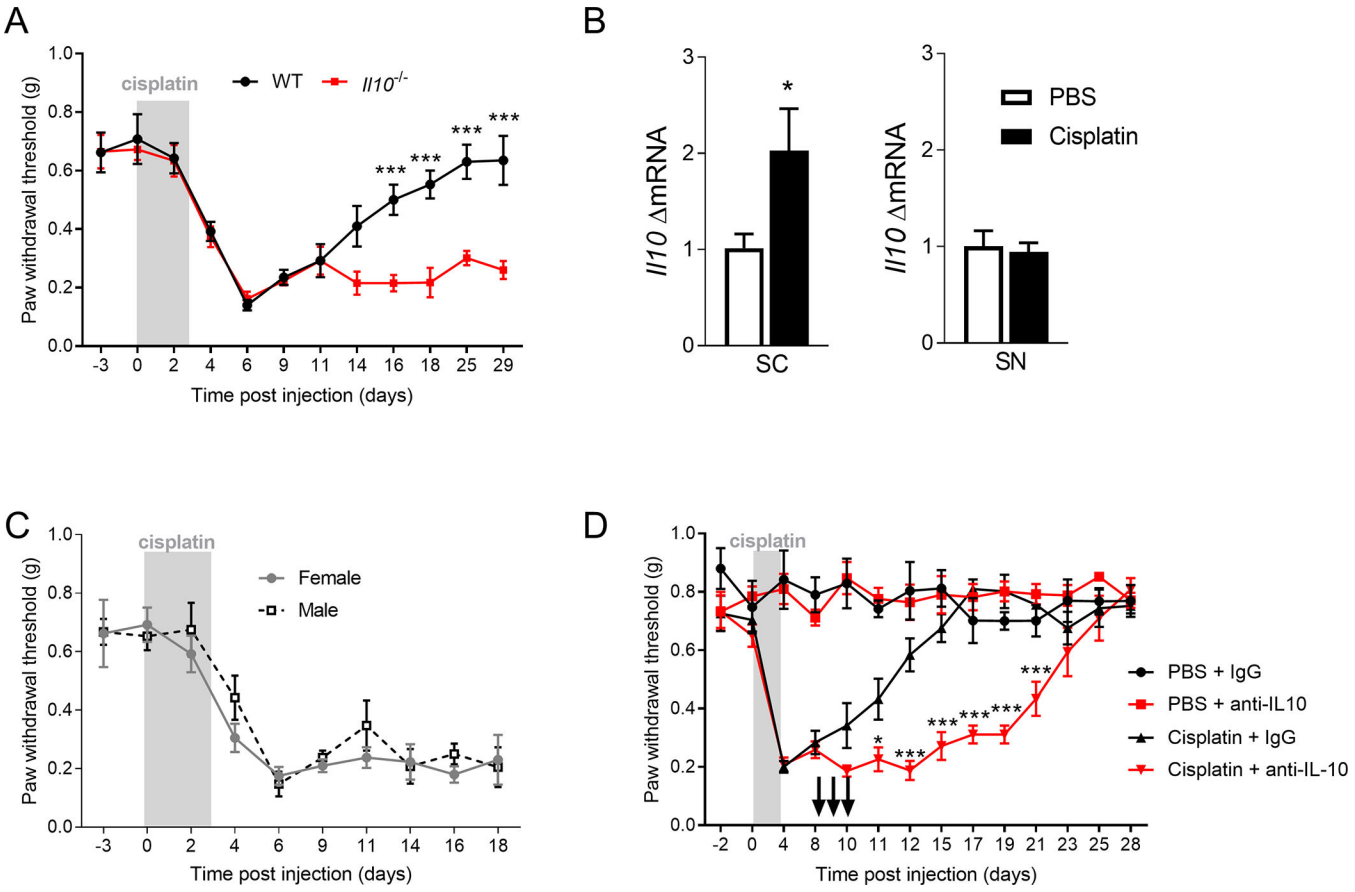

Figure 1.

IL-10 is critical for resolution of cisplatin-induced mechanical pain hypersensitivity. **A**. Mechanical sensitivity measured by von Frey hairs in WT and *Il10*$^{-/-}$ mice in response to cisplatin (2 mg/kg daily for 3 days) (n=4M+4F/group), repeated measure two-way ANOVA, interaction genotype x time F(11, 110) = 5.55, p<0.0001. **B**. mRNA levels of *Il10* in spinal cord (SC) and sciatic nerve (SN) on day 8 after start of cisplatin treatment (n=5/groups), for SC unpaired t test, F (3, 4) = 6.98, p=0.044. **C**. Mechanical sensitivity measured by von Frey hairs in female and male *Il10*$^{-/-}$ mice in response to cisplatin (n=4/group). **D**. Effect of intrathecal administration of neutralizing anti-IL-10 antibody or control IgG (10 μg/mouse/day, on day 8, 9 and 10 after start of cisplatin treatment) on mechanical sensitivity after cisplatin or PBS administration in female and male mice (n= 4F+4M/group), repeated measure two-way ANOVA, interaction genotype x antibody F (39, 208) = 7.14, p<0.0001. The arrows represent the intrathecal injection. Data are shown as mean ± SEM.

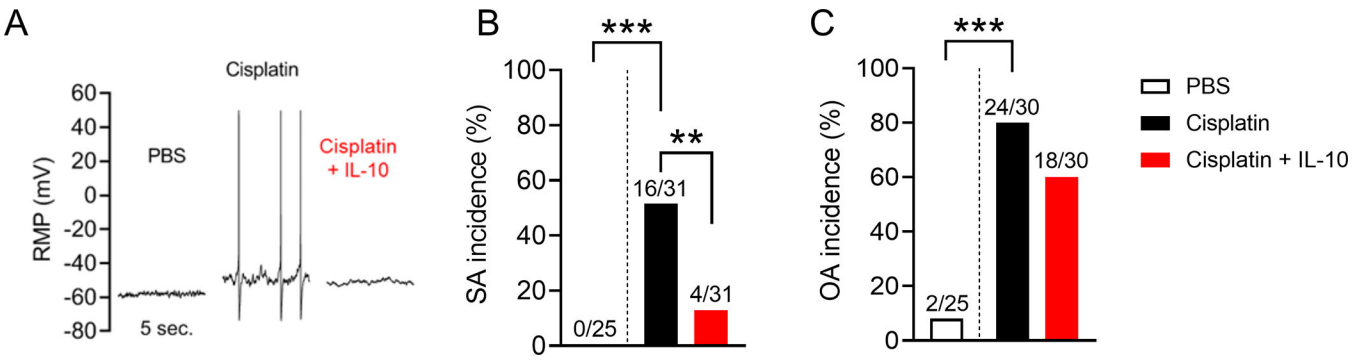

Figure 2.

IL-10 reverses cisplatin treatment-induced nociceptor hyperactivity by reducing the incidence of **s**pontaneous activity (SA) in dissociated sensory neurons. **A**. Examples of SA in isolated DRG neurons 10 days after the first injection of PBS or cisplatin. Recombinant IL-10 (10 ng/ml) was added 15–30 min before recording. **B, C**. Percentage of neurons exhibiting SA at RMP (**B**) and ongoing activity (OA) when held at −45 mV (**C**) are increased in cisplatin-treated mice. IL-10 bath application significantly attenuated the effects of cisplatin on the incidence of SA but not OA (**B**). The ratio above each bar indicates the number of neurons with SA or OA/total sampled (PBS-treated mice n=3 and cisplatin n=6). For SA incidence comparisons, Fisher's exact test, $p<0.001$ PBS vs cisplatin and $p<0.01$ cisplatin vs cisplatin + IL-10. For OA, Fisher's exact test, $p<0.0001$ PBS vs cisplatin. Data are shown as mean ± SEM.

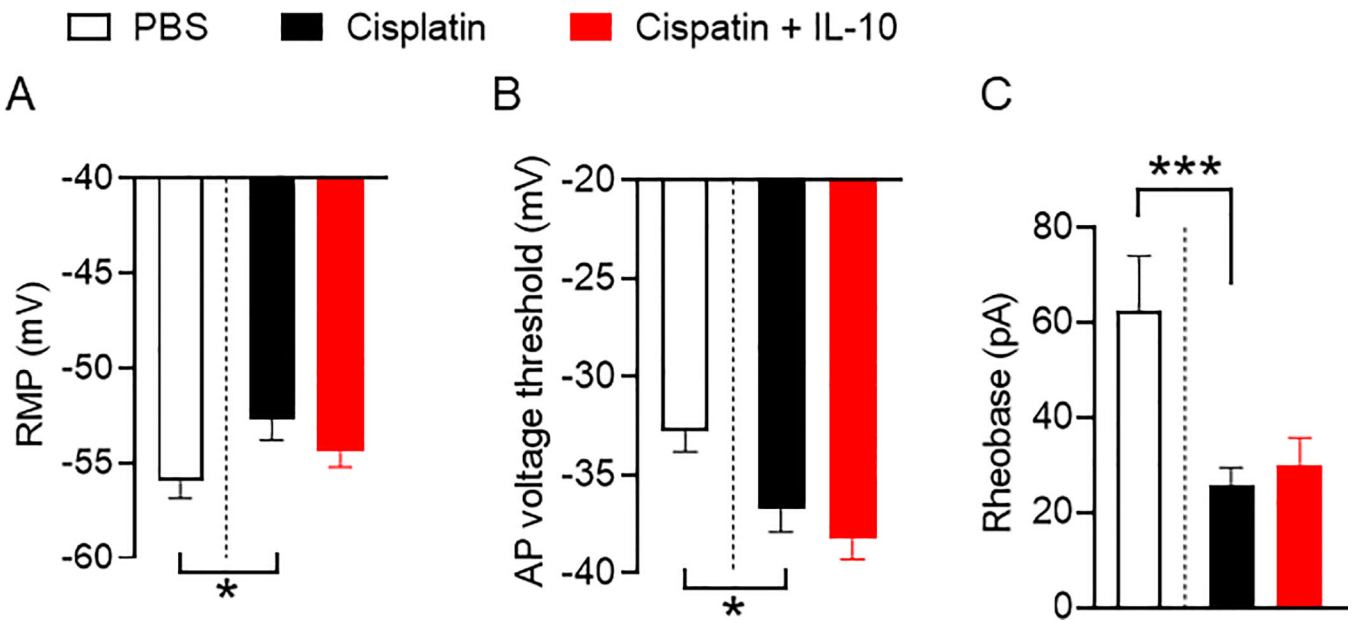

Figure 3.

Cisplatin treatment promotes neuronal hyperactivity by multiple electrophysiological alterations. Data were collected from DRG neurons isolated 10 days after the first injection of PBS or cisplatin. Recombinant IL-10 (10 ng/ml or 0.5 nM) was added 15–30 min before recording. **A**. Resting membrane potential (RMP, mV). PBS vs. cisplatin: one-way ANOVA followed by Sidak's multiple correction test p=0.038. **B**. Action potential (AP) voltage threshold (mV): one-way ANOVA followed by Sidak's multiple correction test p=0.0022, PBS vs. cisplatin: p=0.025. **C**. Rheobase (pA) Kruskal-Wallis test with Dunn's correction for multiple comparisons p=0.0003; PBS vs. cisplatin: p=0.0005. Data are shown as mean ± SEM.

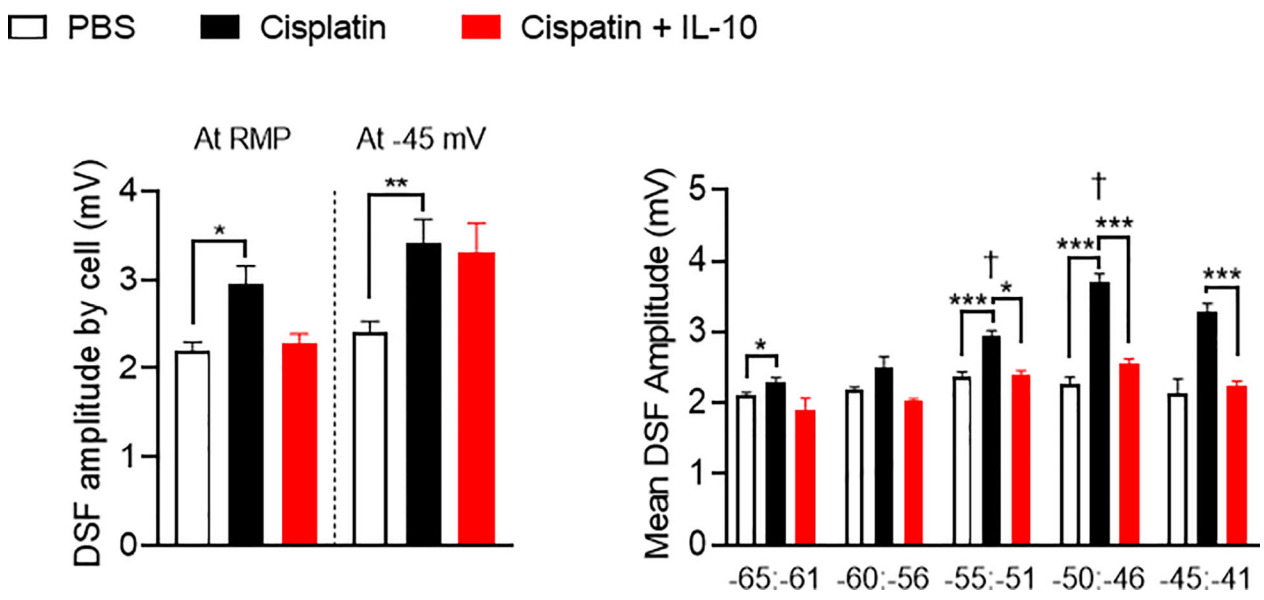

Figure 4.

Amplitudes of depolarizing spontaneous fluctuations (DSFs) are enhanced by *in vivo* cisplatin treatment and normalized by *in vitro* IL-10 incubation at depolarized membrane potentials. **A**. Average DSF amplitudes per neuron increased in cisplatin-treated mice at RMP and when neurons were artificially depolarized to −45 mV, (mice: PBS n=3 and cisplatin n=6) without being reversed by IL-10 incubation. Kruskal-Wallis test with Dunn's correction for multiple comparisons p=0.0195, PBS vs. cisplatin: p=0.0155. **B**. Mean DSF amplitudes binned by RMP ranging from −65 and −41 mV from DRG neurons isolated from PBS and cisplatin treated mice exposed to vehicle or IL-10 (10 ng/ml) *in vitro.* f denotes statistical significance for cisplatin-treated neurons from the marked bin compared with the previous one. Data are shown as mean ± SEM.

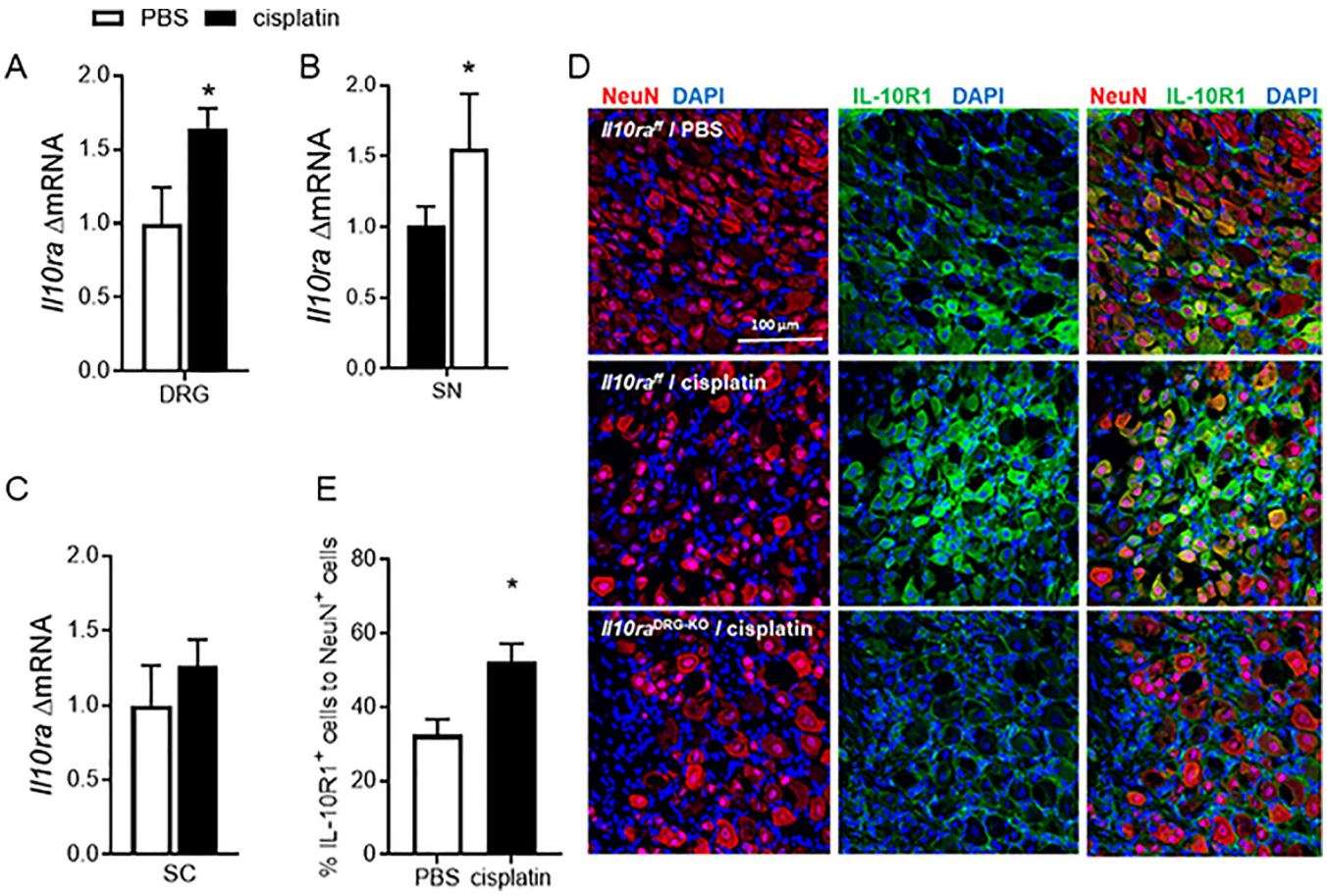

Figure 5.

Cisplatin increases IL-10R1 expression on sensory neurons **A**. mRNA levels of *Il10ra* in dorsal root ganglia (DRG) (n=8/group) unpaired t test, F(6,7) = 2.91 p=0.03, **B** sciatic nerve (SN) (n=8/group) unpaired t test, F(7,5) = 10.8 and**C**, spinal cord (SC) (n=9/group) on day 8 after start of cisplatin treatment.**D**, Immunofluorescence analysis of IL-10R1 on DRG neurons. Representative images of DRG sections labeled with anti-IL-10R1 (green) and anti-NeuN antibodies (red) to identify neuronal cells in control (*Il10ra*^{floxflox}) and *Il10*$^{DRG-KO}$ mice on day 25 after cisplatin or PBS administration. **E**. Quantification of the percentage of IL-10R1- and NeuN-positive cells (n= 3F+3M/group) Unpaired t test, F (3,3) = 1.56 p=0.021. Data are shown as mean ± SEM.

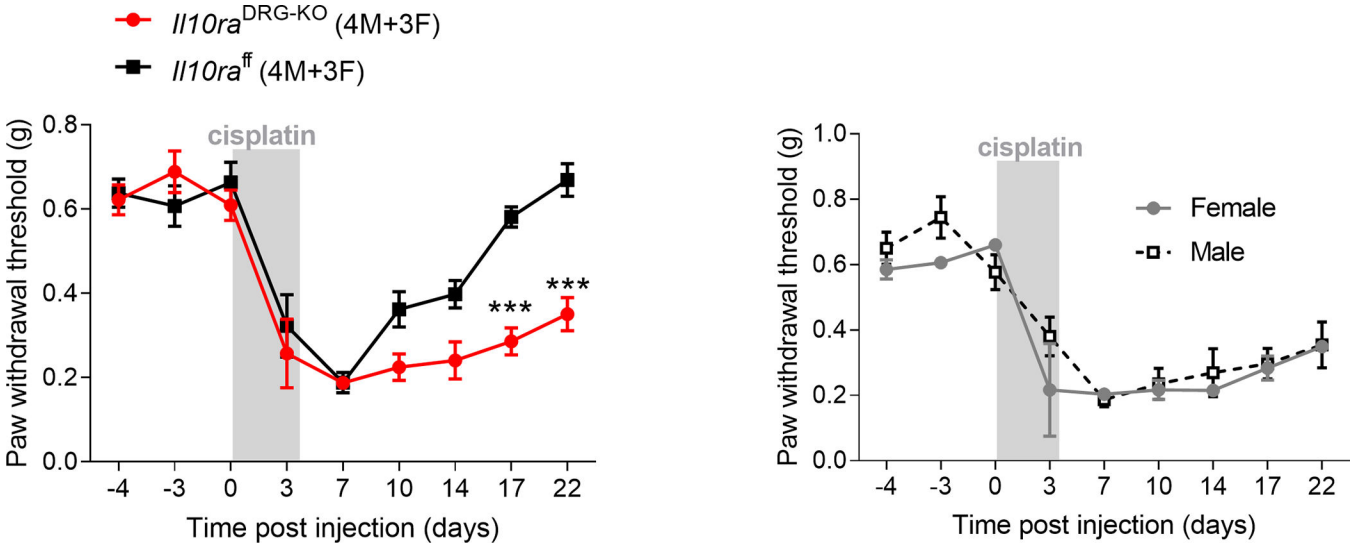

Figure 6.

Genetic deletion of *Il10ra* from Advillin-positive sensory neurons delays the resolution of cisplatin-induced mechanical hypersensitivity. **A**. Mechanical pain threshold in control (*Il10ra*^{floxflox}) and *Il10ra*$^{DRG-KO}$ mice after cisplatin treatment (2 mg/kg daily for 3 days) (n= 3F+4M/group), repeated measure two-way ANOVA interaction time x genotype $F(8,72) = 5.09$, $p<0.0001$. Resolution of mechanical pain threshold is delayed in mice that lack IL-10R1 on sensory neurons while baseline pain thresholds and severity of mechanical pain were similar in both genotypes. **B**. Mechanical sensitivity measured by von Frey hairs in female and male *Il10ra*$^{DRG-KO}$ mice in response to cisplatin. Data are shown as mean ± SEM.