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Involvement of microglial cells in the antinociceptive effects of metamizol in a mouse model of neuropathic pain

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ABSTRACT

Metamizol (also known as dipyrone or sulpyrine) is one of the non-opioid analgesics commonly used in clinical practice in the treatment of somatic and visceral pain. Here, our results give evidence that repeated twice daily intraperitoneal metamizol administration during 7 days diminished development of neuropathic pain symptoms in a mouse model of neuropathic pain. We observed that metamizol inhibited the activation of spinal microglia in neuropathic mice. Moreover, our findings provide evidence that pronociceptive (IL-1β, XCL1, and CCL2), but not antinociceptive (IL-1α, IL-1RA, and IL-18BP), factors play an important role in metamizol-induced antinociception. We observed that metamizol influences the spinal levels of the nociceptin receptor (NOP) but does not alter the expression of other members of the opioid receptor family (mu (MOP), delta (DOP) and kappa (KOP)), or other important nociception receptors (transient receptor potential vanilloid 1 (TRPV1) and transient receptor potential ankyrin 1 (TRPA1)). Metamizol administration did not affect the levels of the opioid prohormones (proopiomelanocortin (POMC), proenkephalin (PENK), prodynorphin (PDYN), and pronociceptin (PNOC)). However, we observed an enhanced antinociceptive effect of oxycodone, but not buprenorphine, after metamizol treatment. In conclusion, we found that metamizol-induced analgesia in neuropathy is associated with silencing microglia activation and, consequently, with a reduction in pronociceptive cytokines. These results provide evidence that metamizol may join the modest arsenal of effective remedies for neuropathic pain and may constitute part of a multimodal pain therapy.

1. Introduction

Metamizol (also known as dipyrone or sulpyrine) is one of the most commonly used non-opioid analgesics with antipyretic and antispasmodic properties. It was first introduced into clinical use in Germany in 1922, and for many years, it was available in many countries (Derry et al., 2010; Ramacciotti et al., 2007) However, despite its long-term therapeutic success, the mechanism of analgesic action of metamizol still remains partly unexplained. Several mechanisms have been proposed, including the involvement of the endogenous opioid system (Vanegas and Tortorici, 2002), stimulation of the PAG-RVM axis (Vazquez et al., 2007), inhibition of COX-1 and COX-2 (Hinz et al., 2007; Pierre et al., 2007) and interference with components of the endocannabinoid system (Rogosch et al., 2012). In 2015, Nassini et al. (2015) suggested that metamizol selectively inhibits calcium responses and currents in transient receptor potential ankyrin 1 (TRPA1)-expressing cells under neuropathic conditions.

Recently, a number of studies have suggested that spinal neuroimmune interactions underlie the development and maintenance of neuropathic pain. A disturbance of central nervous system (CNS) homeostasis causes rapid responses of microglia, which undergo a multistage activation process (Mika, 2008; Mika et al., 2013). Microglia were considered to function solely as phagocytotic cells within the CNS; however, it has recently been demonstrated that persistent activation of this cell type may contribute to neuropathic pain progression (Popiolek-Barczyk and Mika, 2016). The activated microglia change the profile of the production of pro- and/or antinociceptive factors (Popiolek-Barczyk and Mika, 2016). Zhang et al. (2011) found that metamizol reduces the level of Iba1 protein, which is a marker of microglia activation, and thus exerts a neuroprotective effect in an animal model of experimental brain ischaemia (Zhang et al., 2011). The influence of metamizol on spinal glial cells under neuropathic pain conditions remains unknown.

Current treatments for neuropathic pain are often ineffective and are frequently associated with undesirable side effects. Several lines of

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evidence show that disturbances in the spinal neuro-immune interactions underlie the reduced opioid analgesia in neuropathy. It needs to be mentioned that opioids are still the gold standard for the pharmacological treatment of severe pain, despite their unsatisfactory analgesic effects. A beneficial strategy to increase opioid effectiveness is to combine low quantities of opioids with glial modulators (Makuch et al., 2013; Mika et al., 2014, 2013, 2009, 2007; Wordliczek et al., 2000). It has been shown that repeated co-administration of morphine with metamizol results in delayed tolerance development, potentiated antinociceptive effects in naïve animals (Hernández-Delgadillo et al., 2003) and in chronic pain models (Domínguez-Ramírez et al., 2010; Taylor et al., 1998). Recently, Gaertner et al. (2017) have shown that metamizol can be recommended for patients with cancer pain in combination with morphine.

Based on these data, the objective of the present work was to study how pre-emptive and repeated (for 7 days) metamizol administration influences the development of tactile and thermal hypersensitivity in a mouse model of neuropathic pain (chronic constriction injury (CCI) to the sciatic nerve). Moreover, using RT-qPCR and/or Western blot analysis, we studied how metamizol administration influences the spinal mRNA and protein levels, respectively, of glial markers (C1q, Iba1 and GFAP). We also analysed pro- (XCL1, CCL2, and IL-1β) and antinociceptive (IL-1α, IL-1RA, and IL-18BP) factors, as well as opioid prohormones (POMC, PENK, PDYN, PNOC) and selected receptors, which are known to play a crucial role in nociception (TRPV1, TRPA1, MOP, DOP, KOP, and NOP). Additionally, we investigated how repeated intraperitoneal administration of metamizol influences opioid (oxycodone and buprenorphine) analgesia.

2. Materials and methods

2.1. Animals

Adult male Albino-Swiss CD-1 mice (weighing between 20 and 25 g) were purchased from Charles River Laboratories (Hamburg, Germany). The mice were housed in groups of ten in cages lined with sawdust under controlled conditions (temperature, 21 ± 2 °C; 12-h light/dark cycle – lights on at 6 AM) with *ad libitum* food and water. All experiments were carried out according to the recommendations of the International Association for the Study of Pain (Zimmermann, 1983) and the NIH Guide for the Care and Use of Laboratory Animals and were approved by the II Local Ethics Committee Branch of the National Ethics Committee for Experiments on Animals based at the Institute of Pharmacology, Polish Academy of Sciences (approval number 153/2016: Krakow, Poland). Care was taken to minimize animal suffering and minimize the number of animals used (3R policy).

2.2. Sciatic nerve surgery

Chronic constriction injury (CCI) to the sciatic nerve was performed under isoflurane anaesthesia (2% isoflurane in 100% oxygen with a flow of 1.5 L/min according to the procedure described by Bennett and Xie (1988) and modified for mice by Mika et al. (2007). An incision was made below the right hip bone in order to expose the sciatic nerve. Once the sciatic nerve was exposed, three ligatures (3/0 silk) were made around the nerve distal to the sciatic notch with 1-mm spacing until a brief twitch in the respective hind limb was observed. No procedure was conducted on the control animals (naïve). After CCI, the mice developed long-lasting allodynia and hyperalgesia.

2.3. Drug administration

The following drugs were used in the present study: metamizol (Met; 500 mg/kg; SANOFI, Germany), buprenorphine (B; 0.14 mg/kg; PolfaKutno, Poland), and oxycodone (O; 1 mg/kg; Norpharma A/S, Denmark). The dose of buprenorphine was chosen based on our own unpublished studies (Kocot-Kępska et al., *in preparation*), the oxycodone based on literature (Zhang et al., 2016). The drugs were dissolved in water (aqua pro-injection) and administered intraperitoneally (*i.p.*). The control group received vehicle (aqua pro-injection) according to the same protocol. No adverse side effects of metamizol treatment were observed during the experiments.

2.4. Behavioural tests

Behavioural experiments were performed between 8 a.m. and 12 p.m. Metamizol was injected 16 h and 1 h before CCI and then twice daily for 7 days. Behavioural evaluation was performed 60 min after the last morning metamizol administration. In the opioid experiments, metamizol was administered according to the same schedule, and after 1 h, each animal received a single *i.p.* oxycodone, buprenorphine or vehicle injection.

2.4.1. von Frey test

Mechanical sensitivity to non-noxious stimuli was measured using the von Frey test (Mika et al., 2007) by applying a set of calibrated nylon monofilaments (0.6–6 g; Stoelting) in serial increments to the mouse's ipsilateral hind paw midplantar surface until a behavioural response was observed. Mice were placed in plastic cages with a wire net floor 5 min before the experiment. The responses considered pain behaviours included rapid paw withdrawal, shaking and licking. In the von Frey test, the results are expressed as pressure [g] applied with the filament; the cut-off latency was 6 g.

2.4.2. Cold plate test

Sensitivity to noxious thermal stimuli was assessed using the cold plate test (Cold/Hot Plate Analgesia Meter, No. 05044, Columbus Instruments, USA) as previously described (Mika et al., 2007). The temperature of the plate was kept at 2 °C, and the cut-off latency was 30 s. The mice were placed on the cold plate, and the time until the injured paw was lifted was recorded. In CCI-exposed mice, the injured (right) paw always reacted first; therefore, the effect of the treatment is presented as the reaction of the ipsilateral hind paw.

2.5. RT-qPCR

The lumbar (L4–L6) spinal cords were dissected from neuropathic mice on day 7 after chronic metamizol or vehicle treatment and from naïve mice. The collected tissues were placed individually in tubes containing RNAlater (Qiagen Inc.) and then stored at −70 °C for RNA isolation. Total RNA extraction was performed using TRIzol reagent (Invitrogen) based on the Chomczynski and Sacchi protocol (Chomczynski and Sacchi, 1987). The RNA concentration was measured by DeNovix DS-11 (3411 Silverside Rd, Hanby Building Wilmington, DE 19810 USA). For reverse transcription reactions, an Omniscript Reverse Transcriptase Kit (Qiagen Inc., Hilden, Germany) was used. The reverse transcription reaction mix also contained RNase inhibitor (rRNasin, Promega, Mannheim, Germany) and an oligo-(dT16) primer (Qiagen Inc., Hilden, Germany). The reaction was performed at 37 °C for 60 min using 1 μg of total RNA. The obtained cDNA was diluted 1:10 with RNase/DNase-free H_2O, and from each sample, ~50 ng of cDNA was used for each quantitative real-time PCR (RT-qPCR) reaction. The RT-qPCR was conducted using Assay-On-Demand TaqMan probes based on the manufacturer's protocol (Applied Biosystems, Foster City, CA, USA) and run on an iCycler device (Bio-Rad, Hercules, Poland). The following TaqMan primers were used in the study: Mm01545399_m1 (*Hprt*), Mm00432142_m1 (*C1q*), Mm01253033_m1 (*Gfap*), Mm00443063_m1 (*Dop*), Mm01188089_m1 (*Mop*), Mm00440563_m1 (*Nop*), Mm01230885_m1 (*Kop*), Mm00457573_m1 (*Pdyn*), Mm00435874_m1 (*Pomc*); Mm01314909_m1 (*Pnoc*), Mm01212875_m1 (*Penk*), Mm00434228_m1 (*IL-1β*), Mm00434228_m1 (*XCL1*), Mm00441242_m1 (*CCL2*), Mm00439620_m1 (*IL-1α*),