Rapid Communication

Modulation of spinal glial reactivity by intrathecal PPF is not sufficient to inhibit mechanical allodynia induced by nerve crush

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A B S T R A C T

Spinal glial reactivity has been strongly implicated in pain that follows peripheral nerve injury. Among the many therapeutic agents that have been tested for anti-allodynia through immune modulation is the atypical methylianthine propentofylline. While propentofylline shows a potent anti-allodynia effect after nerve transection injury, we here demonstrate that, when propentofylline is used intrathecally at the effective immune-modulatory dose, allodynia after rat nerve crush injury is completely preserved. Micogial/macrophage Iba-1 and astrocytic GFAP expression, increased in the dorsal horn of nerve crushed animals, was, however, effectively attenuated by propentofylline. Effective modulation of spinal glial reactivity is, thus, no assurance for anti-allodynia.

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As one of the most debilitating and difficult-to-treat pain conditions, neuropathic pain has an enormous socio-economic impact (Dworkin et al., 2010). Insights into cellular and molecular mechanisms have inspired a surge of experimental treatments, some of which have it to clinical trials (Berger et al., 2011b). Besides well-known neuronal pain mechanisms also the immune system has been largely implicated in the development and maintenance of neuropathic pain, with a key role assigned to immune competent cells within the central nervous system (CNS) (Scholz and Woolf, 2007). Propentofylline (PPF), a methylxanthine inhibiting phosphodiesterases has been endowed with potent immune modulatory effects, even though actions on neuronal cell populations have been reported as well (Flavin and Ho, 1999; Gwak et al., 2008). When used intrathecally at a dose of 10 μg PPF in nerve injured animals, spinal glial reactivity could be effectively reduced and neuropathic pain was well attenuated (Jaken et al., 2011; Raghavendra et al., 2003; Sweitzer et al., 2001). Clinically, however, PPF has not always been successfully used in treatment of neuropathic pain. Painful post-herpetic neuralgia patients were reported to not benefit from PPF, which was then attributed to human macrophages showing a different responsiveness to PPF compared to rodent macrophages (Landry et al., 2012). As the most potent anti-allodynia effects of PPF in animal studies have been reported after nerve transection (Raghavendra et al., 2003; Sweitzer et al., 2001), which typically leads to persistent allodynia, it remains unclear whether PPF has similar effects in models that are more moderate in pain. Nerve crush injury is frequently seen in the clinic (Sunderland, 1980) and its modeling in rodents shows that allodynia is relatively short-lived compared to nerve transection (Decosterd and Woolf, 2000). Therefore, the objective of this study was to investigate whether intrathecal PPF at a dose that effectively modulates spinal glial reactivity and allodynia after nerve transection shows similar results after nerve crush. As crush injuries are typically characterized by an immune-driven spontaneous regeneration and return of function we also determined whether the treatment interfered with the recovery process.

Animals were maintained in accordance with the Belgian legislation on animal protection and respecting the European Community Council directive of 24 November 1986 (86-609/ECC) and the decree of 20 October 1987 (87-848/EEC). Every attempt was made to minimize the number and suffering of animals. Adult female Sprague Dawley rats were housed socially (two-to-three animals per cage; n = 46) with ad libitum access to food and water. Animals were subjected to spared nerve crush injury (SNC) as previously described (Decosterd and Woolf, 2000), but using a non-serrated nerve clamp (Luis et al., 2007) (force of 54 N over...
a period of 30 s for both the tibial and common peroneal nerve branches). Animals received either daily intrathecal bolus injections with PPF \((n=15; 10 \mu l \text{ of PPF} (10 \mu g); \text{based on previously reported anti-allodynic effects for this dose (Jaken et al., 2011; Raghavendra et al., 2003)})\) followed by 10 \(\mu l\) of saline flush or saline \((n=15; \text{two times } 10 \mu l \text{ of saline}); \text{sham surgeries with vehicle injections } (n=10) \text{ and control animals with PPF injections served as control groups. A subset of animals in each group was sacrificed at 10d after surgery for histological purposes only. Treatment was given 1 h before surgery and then once daily (between 4:00 p.m. and 6:00 p.m.) for the next 10 days after surgery.}

Mechanical allodynia was assessed in the plantar sural nerve territory using the up-down method to determine the 50% paw withdrawal threshold (PWT) (Chaplan et al., 1994). Non-pain related physiological responses to nerve crush were assessed with the static sciatic index (SSI; a behavioral test that assays motor function) (Bozkurt et al., 2008), the pinprick test (a behavioral test to determine somatosensory function). For the latter test, a safety pin was used to stimulate (touch), but not penetrate the tibial nerve territory of the plantar glabrous hind paw surface. The latency of paw withdrawal was measured using an arbitrary time of 10 s as an upper cut-off value and a lower cut-off value of 0.5 s.

Immunohistochemistry was performed on 4% paraformaldehyde-perfused spinal cords of nerve injured \((n=8)\) or sham-operated \((n=4)\) animals. Briefly, L4-L5 lumbar spinal cords were cryosectioned transversely \((30 \mu m)\) after being mounted in Tissue-Tek Optimal Cutting Temperature solution (O.C.T., Sakura FineTek). Sections were collected on Superfrost® Plus object glass slides (Thermo Scientific, Gerhard Menzel GmbH,
Germany) with a 360 μm-intersection interval. Staining for the macrophage/microglial marker ionized calcium-binding adapter molecule 1 (Iba-1) and the astrocytic marker glial fibrillary acidic protein (GFAP) were performed separately as previously described (Berger et al., 2011a) using rabbit anti-Iba-1 (WAKO; 1:1000) and rabbit anti–GFAP (DAKO; 1:1000) as primary antibodies (overnight incubation) and goat anti-rabbit Alexa 594 (Invitrogen; 1:100) as the secondary antibody. Sections were examined under a digital inverted EVOS microscope (Advanced Microscopy Group, Mill Creek, Washington) that uses a light-emitting diode (LED) illumination system and was equipped with a Texas Red light cube. Ten photomicrographs were taken from the L4–L5 ipsilateral and contralateral dorsal horns of each animal. Using the NIH Image J analysis software (version 1.45k), the mean gray value of pixel intensities was measured after background subtraction and results were expressed as a ratio in gray value between the ipsilateral and contralateral dorsal horns. Data were processed and analyzed using GraphPad Prism–4.0. After verifying normal Gaussian distribution, statistical comparisons were performed with a one way analysis of variance (ANOVA) and Tukey’s post hoc correction. All data are expressed as mean ± standard-error-of-the-mean (SEM). A p-value of 0.05 was regarded as the level of statistical significance.

SNC induced a significant increase in lumbar Iba-1 expression at 10 days (Fig. 1B and E versus sham in Fig. 1A and D), which was most noticeable in the medial boundary of the dorsal horn that is occupied by primary afferent fiber terminals of the crushed tibial and common peroneal nerve branches (Swett and Woolf, 1985); PPF markedly reduced spinal Iba-1 up-regulation (Fig. 1C and F). These findings were confirmed on a quantitative level (Fig. 1G) and similar results were obtained for astrocytic GFAP expression (Fig. 1H).

SNC, but not sham-surgery, triggered mechanical hypersensitivity, evidenced by a significantly decreased ipsilateral PWT (Fig. 2A). This effect was maintained throughout the first 10 days of the follow-up period and appeared slightly more pronounced in vehicle-treated SNC animals than in PPF-treated SNC animals, even though no statistical difference was detected (Fig. 2A and B). PPF treatment in non-crushed control animals did not affect PWT either (Fig. 2C).

In line with previous work (Decosterd and Woolf, 2000), allodynia spontaneously resolved over the course of weeks after SNC (Fig. 3A and B). Indeed, after six weeks PWT had returned to baseline values, an effect that was not influenced by PPF treatment. Regarding motor function, standardized crush of the common peroneal and tibial nerve branches decreased SSI scores from around 0 at baseline to −110.5 ± 2.0 in the first week after injury (Fig. 3C). Over the course of the next two weeks, a gradual return to baseline SSI values could be observed. Nevertheless, nerve lesioned animals which had received PPF treatment showed a minor improvement in the recovery of motor function (Fig. 3C and D). PPF treatment did not alter SSI in non-crushed control animals (Fig. 3E). A transient impairment of sensory function and a recovery within a week after injury was similar in all animal groups (Fig. 3F).

PPF has been successfully used for the treatment of neuropathic pain in a range of animal models (Gwak et al., 2008; Jaken et al., 2011; Raghavendra et al., 2003; Sweitzer et al., 2001; Zhang et al., 2013). However, while previous reports showed potent anti-allodynic effects of daily 10 μl intrathecal PPF after nerve transaction injury (Jaken et al., 2011; Raghavendra et al., 2003; Sweitzer et al., 2001), we could not confirm this effect after nerve crush using the same treatment. At variance, a modulation of spinal glial activity through the daily 10 μl intrathecal PPF bolus injections was obtained in this crush lesion model, similar to what has been reported for nerve transaction injury (Jaken et al., 2011; Raghavendra et al., 2003; Sweitzer et al., 2001).

Differential pharmacological responsiveness across distinct neuropathic pain models is a well-known phenomenon (Kayser et al., 2010; Michot et al., 2012, 2015). Our data suggest that the efficacy of PPF is at least to some extent dependent on the type of peripheral nerve lesion. Moreover, we show a dissociation between microglial activation and allodynia. The immune responses that occur within the spinal dorsal horn after peripheral nerve injury have been strongly implicated in neuropathic pain (Scholz and Woolf, 2007). While microglia and astrocytes importantly contribute to these immune responses and glia-to-neuron communication is mechanistically involved in neuropathic pain, effective control of neuropathic pain would also require a restoration of physiological signal transmission by neurons. Our data on enduring pain despite PPF-induced modulation of glial reactivity suggests that pathological alterations in neurons persist during this particular pharmacological treatment. On the other hand, increasing evidence suggests that not all glial responses to nerve injury...
have adverse effects and/or contribute to pain, particularly not those related to spinal glia (Leinders et al., 2013; Milligan and Watkins, 2009). In fact, expression of astrocytic GFAP at late stages after nerve injury have previously been found to inversely correlate with allodynia (Deumens et al., 2009) and only a part of the many possible microglial ‘enhanced response states’ has been designated as being pain-related (McMahon and Malcangio, 2009). The pain-related state of spinal glia is likely to depend on the specific nature of the pathological context. Human neuropathic conditions are typically heterogeneous and a great variety of animal models has been developed over the years. Differential mechanisms of neuroplasticity and pain in these models are very likely to occur considering that the evolution of allodynia differs greatly among animal models. Nerve crush is for instance associated with a rapid-onset of allodynia that resolves spontaneously after several weeks, while allodynia after transection injury can be particularly persistent, lasting for many months (Decosterd and Woolf, 2000). Distinct mechanisms might then also ask for different requirements to effectively treat the ensuing pains. Although only limited comparative data exist to describe neuroplasticity in crush and transection injuries, the timing of macrophage responses within the dorsal root ganglia is known to show differences between these two types of injury (Fenzi et al., 2001). At this point, it remains elusive as to why PPF is without beneficial effect on nerve crush-induced allodynia at a dose that effectively attenuates nerve transection-induced allodynia. Certainly, we may not exclude that any direct influence on the neuronal nociceptive network could matter to this discrepancy. Indeed, PPF has not only been endowed with potent immune modulatory properties (Jung et al., 1997; Meiners et al., 2004; Yoshikawa et al., 1999), but also with neuronal modulatory action (Gwak et al., 2008). PPF effects on neurons and glia are likely mediated through the compound’s generic influence on phosphodiesterases (Meiners et al., 2009).
et al., 2004) and/or on the mammalian target of rapamycin pathway (Norstedt Gregory et al., 2013). Finally, the effective modulation of spinal glial activity by 10 μl PPP bolus injections did not cause any adverse effects on functional recovery, an observation with biological significance considering that especially immune-modulatory treatments bring a risk of such adverse effects (Nadeau et al., 2011).

Conflicts of interest

None declared.

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