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Glial role in oxaliplatin-induced neuropathic pain

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ABSTRACT

Oxaliplatin, a platinum-based chemotherapeutic agent, has become a standard treatment for advanced colorectal cancer. The dose-limiting toxicity of this compound is the development of peripheral neuropathy. A tangled panel of symptoms, sensory loss, paresthesia, dysesthesia and pain, may be disabling for patients and adversely affect their quality of life.

Recently, we described a characteristic glial activation profile in a rat model of oxaliplatin-induced neuropathy. Glial cells are considered a new pharmacological target for neuropathic pain relief but its relevance in chemotherapy-dependent neuropathies is debated. Aimed to evaluate the significance of glial activation in pain generated by oxaliplatin, the microglial inhibitor minocycline or the astrocyte inhibitor fluorocitrate were continuously infused by intrathecal route in oxaliplatin-treated rats. Both compounds significantly reduced oxaliplatin-evoked pain though the efficacy of fluorocitrate was higher revealing a prominent role of astrocytes. Immunohistochemical analysis of the dorsal horn confirmed the specific Iba1-positive cell inhibition caused by minocycline as well as the selectivity of fluorocitrate on GFAP-positive cells. The activation of astrocytes in minocycline-treated rats suggests a microglia-independent modulation of astrocytes by oxaliplatin neurotoxicity. Neither the selective activation of astrocyte after minocycline treatment nor the exclusive microglial response after fluorocitrate is able to evoke pain.

Morphometric and morphological determinations performed on dorsal root ganglia evidenced that the glial inhibitors did not prevent the oxaliplatin-dependent increase of eccentric nucleoli and multinucleolated neurons. The decrease of soma area was also unaltered.

In summary, these data highlight the role of central glial cells in oxaliplatin-dependent neuropathic pain. On the other hand, glial inhibition is not associated with neuroprotective effects suggesting the need for careful modulation of glial signaling to prevent the pathophysiology that leads to persistent neuropathic pain.

Introduction

Pain related to cancer treatments is of increasing importance. Chemotherapy has had a major impact, leading to increased survival time for many cancers; however, repeated treatments are associated with the development of painful peripheral polyneuropathies (Fallon, 2013). As regards platinum derivatives their relevance in therapy has been proven, in particular the addition of oxaliplatin appears to be associated with better survival among patients receiving adjuvant colon cancer treatment (Sanoff et al, 2012). Oxaliplatin-induced neurotoxicity is the major dose-limiting adverse event that negatively affects quality of life (Albers et al, 2011).

The difficulty to develop efficient drugs for this kind of neuropathy is strictly related to the peculiar alterations of the nervous tissue induced by oxaliplatin. In a rat model of oxaliplatin-induced neuropathy repeated administrations of this drug do not modify peripheral nerve morphology and only mild morphometric modifications are detectable in dorsal root ganglia (DRG). Characteristically, the peripheral nervous tissue lacks evident inflammatory responses (Di Cesare Mannelli et al, 2013a). Despite oxaliplatin's limited ability to cross the blood–brain barrier (Jacobs et al., 2005, 2010), in the rat spinal cord it was described to induce a dramatic oxidative damage (Di Cesare Mannelli et al, 2012) coincident with a significant increase in the activity of wide dynamic range neurons in the dorsal horn (Renn et al, 2011), glial cell activation and pain. A seven day oxaliplatin treatment induces a lowering of pain threshold combined with a significant increase in the number of Iba1 (microglia) and GFAP (astrocyte) immunoreactive cells in the spinal dorsal horn. Nevertheless, while microglia activates transiently and goes back to control values within 14 days, astrocytes show a reactive status up to 21 days of treatment. Glial activation also occurs in

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supraspinal areas involved in pain signaling such as anterior cingulate cortex, somatosensory area 1, neostriatum, ventrolateral periaqueductal gray, and nucleus raphe magnus, differently according to zone and treatment time-points (Di Cesare Mannelli et al, 2013a).

Nervous injuries induced by trauma or nerve entrapment evoke neuropathies characterized by strong activation of glial cells with changes in morphological phenotype (somatic hypertrophy, thickened and ramified branches, proliferation; Tsuda et al, 2013) and both microglia and astrocytes have recently been recognized as powerful modulators of pain and are emerging as a new target for drug development (Marchand et al, 2005; Milligan and Watkins, 2009; Scholz and Woolf, 2007). In comparison with trauma, chemotherapy-induced neuropathies show different characteristics of tissue injury and different responses to pharmacological treatments (Albers et al, 2011; Di Cesare Mannelli et al, 2013c; Kaley and DeAngelis, 2009). The increased density of glial cells in the presence of limited alteration of cell morphology does not allow the evaluation of the relevance of microglia and astrocytes in chemotherapy-induced neuropathic pain.

Aimed to verify the relationship between glial cell activation and oxaliplatin-dependent pain, we continuously infuse at the spinal level the microglial inhibitor minocycline (He et al, 2001; Yrjanheikki et al, 1998) or the astrocyte inhibitor fluorocitrate (Fonnum et al, 1997; Hassel et al, 1992; Szerb and Issekutz, 1987) by an intrathecal route in oxaliplatin-treated rats. Over three weeks of concomitant treatment, pain threshold is measured in comparison with the characterization of the spinal glial activation profile.

Materials and methods

Animals

Male Sprague–Dawley rats (Harlan, Varese, Italy), weighing approximately 200–250 g, at the beginning of the experimental procedure were used. Animals were housed in CeSAL (Centro Stabulazione Animali da Laboratorio, University of Florence) and used at least one week after their arrival. Four rats were housed per cage (size 26 × 41 cm); animals were fed with a standard laboratory diet and tap water ad libitum, and kept at 23 ± 1 °C with a 12 h light/dark cycle, light at 7 a.m. All animal manipulations were carried out according to the European Community guidelines for animal care (DL 116/92, application of the European Communities Council Directive of 24 November 1986 — 86/609/EEC). The ethical policy of the University of Florence complies with the Guide for the Care and Use of Laboratory Animals of the US National Institutes of Health (NIH Publication No. 85-23, revised 1996; University of Florence assurance number: A5278-01). Formal approval to conduct the experiments described was obtained from the Animal Subjects Review Board of the University of Florence. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Intrathecal catheterization

Rats were anesthetized with 2% isoflurane and intrathecal catheter was surgically implanted according to Yaksh and Rudy's (1976) method. Rats were shaved on the back of the neck and placed in the stereotaxic

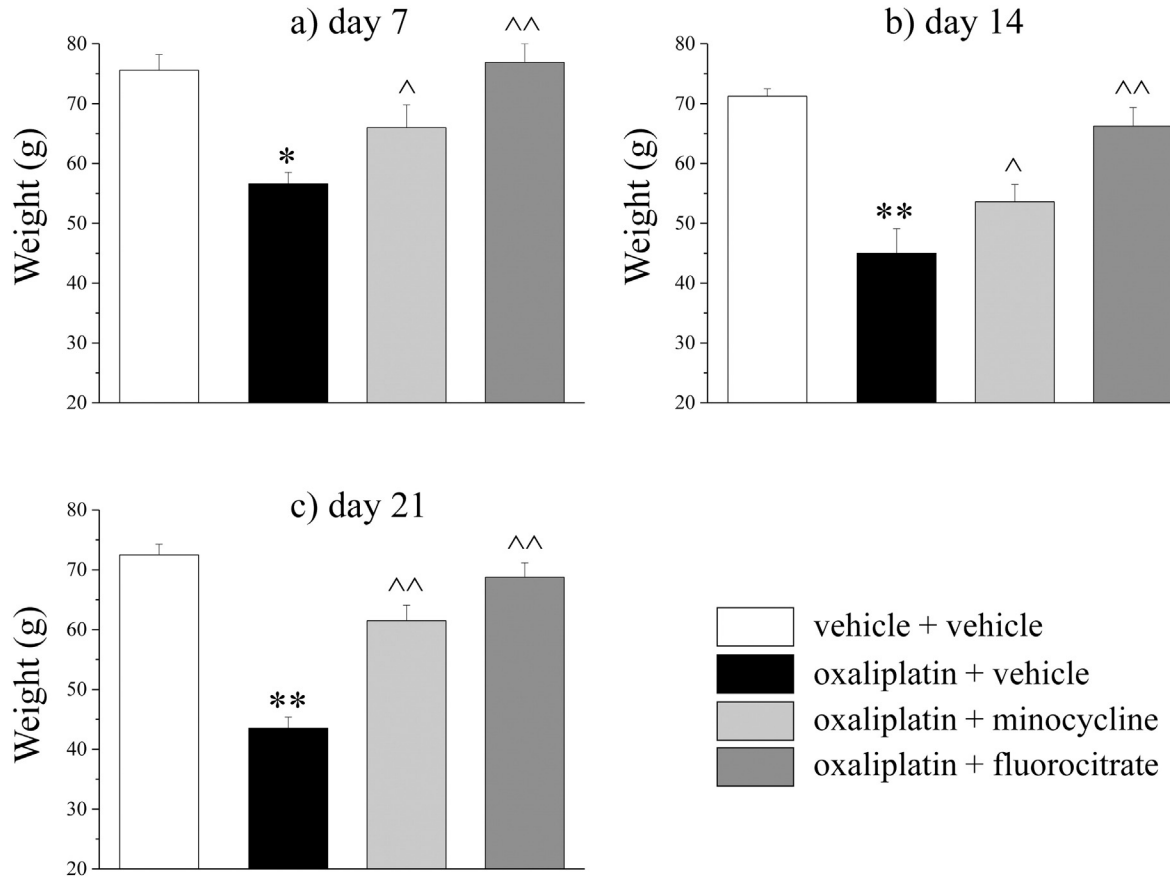

Fig. 1. Behavioral measures. *Pain: noxious stimuli*. The Paw-pressure test was used to measure the sensitivity to a mechanical stimulus. Animals were treated daily i.p. with 2.4 mg kg^{-1} of oxaliplatin. Minocycline (12.5 nmol/h) and fluorocitrate (1 nmol/h) were continuously infused i.t. starting from the first day of oxaliplatin administration and behavioral evaluations were performed after a) 7, b) 14 and c) 21 days of treatment. Control animals were treated with vehicle. Each value represents the mean ± S.E.M. of 12 rats per group, performed in 2 different experimental sets. *$P < 0.05$ and **$P < 0.01$ versus vehicle + vehicle; ^$P < 0.05$ and ^^$P < 0.01$ versus oxaliplatin + vehicle.