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Blockade of the spinal BDNF-activated JNK pathway prevents the development of antiretroviral-induced neuropathic pain

Maria Domenica Sanna, Carla Ghelardini, Nicoletta Galeotti*

Laboratory of Neuropsychopharmacology, Department of Neurosciences, Psychology, Drug Research and Child Health (NEUROFARBA), Section of Pharmacology, University of Florence, Florence, Italy

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ABSTRACT

Although antiretroviral agents have been used successfully in suppressing viral production, they have also been associated with a number of side effects. The antiretroviral toxic neuropathy induces debilitating and extremely difficult to treat pain syndromes that often lead to discontinuation of antiretroviral therapy. Due to the critical need for the identification of novel therapeutic targets to improve antiretroviral neuropathic pain management, we investigated the role of the JNK signalling pathway in the mechanism of antiretroviral painful neuropathy. Mice were exposed to zalcitabine (2′,3′-dideoxycytidine, ddC) and stavudine (2′,3′-didehydro-3′-deoxythymidine, d4T) that induced a persistent mechanical allodynia and a transient cold allodynia. Treatment with the JNK blocker SP600125 before antiretroviral administration abolished mechanical hypersensitivity with no effect on thermal response. A robust spinal JNK overphosphorylation was observed on post-injection day 1 and 3, along with a JNK-dependent increase in p-c-Jun and ATF3 protein levels. Co-immunoprecipitation experiments showed the presence of a heterodimeric complex between ATF3 and c-Jun indicating that these transcription factors can act together to regulate transcription through heterodimerization. A rise in BDNF and caspase-3 protein levels was detected on day 1 and BDNF sequestration prevented both caspase-3 and p-JNK increase. These data suggest that BDNF plays a role in the early stages of ddC-induced allodynia by promoting apoptotic events and the activation of a hypernociceptive JNK-mediated pathway. We illustrated the activation of a BDNF-mediated JNK pathway involved in the early events responsible for the promotion of neuropathic pain, leading to a better knowledge of the mechanisms involved in the antiretroviral neuropathy.

Summary: JNK blockade prevents antiretroviral-induced pain hypersensitivity. This may represent a potential prophylactic treatment of neuropathic pain to improve antiretroviral tolerability.

1. Introduction

Peripheral neuropathy is one of the most prevalent problems experienced by people exposed to potentially neurotoxic nucleoside reverse transcriptase inhibitors (NRTI) stavudine (d4T), zalcitabine (ddC) and didanosine (ddI) (Ances et al., 2009; Maritz et al., 2010; Ellis et al., 2010; Kamerman et al., 2012; Kokotis et al., 2013). Neuropathic pain associated with the use of NRTIs is clinically quite common with a greater prevalence than that reported for other common types of peripheral neuropathy (Morgello et al., 2004; Maritz et al., 2010), that have resulted in decreased NRTI use in developed countries (d'Arminio Monforte et al., 2000; Chen et al., 2003), although they are still widely used in underdeveloped countries (Kumarasamy et al., 2003, 2008; Hung et al., 2008; WHO et al., 2010). Unfortunately, analgesics used in other forms of neuropathic pain have proven ineffective (Phillips et al., 2010), and terminating NRTI therapy does not always reverse neuropathy and may actually exacerbate the pain (Berger et al., 1993). Despite decreased NRTI use today, many HIV patients have previously used NRTIs and continue to suffer from painful neuropathy, leading the antiretroviral neuropathic pain to become a major impact on

Abbreviations: ATF3, activating transcription factor 3; ddC, 2′,3′-dideoxycitidine; d4T, 2′,3′-didehydro-3′-deoxythymidin; DRG, dorsal root ganglia; i.p., intraperitoneal; i.t., intrathecal; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; NRTI, nucleoside reverse transcriptase inhibitors; PKC, protein kinase C; SDH, spinal dorsal horn.

* Corresponding author. Dept. NEUROFARBA, Viale G. Pieraccini 6, I-50139, Florence, Italy.
E-mail address: nicoletta.galeotti@unifi.it (N. Galeotti).

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quality of life in otherwise largely healthy individuals. Few efficacious treatments are available to relieve symptoms, so there is a critical need for the identification of novel therapeutic targets.

Therapeutic target identification has been slow because the causative mechanisms of NRTI-induced neuropathy remain unclear. Studies suggest that it is associated with structural damage, degeneration of small sensory axonal fibres (Dalakas, 2001; Polydefkis et al., 2002; Wallace et al., 2007), and indications of mitochondrial toxicity in damaged neurons (Dalakas, 2001; Bhangoo et al., 2007; Kamerman et al., 2012). The neuropathic pain-like behavioural and electrophysiological changes appear to have been related to increased calcium signalling in the nerve fibres (Joseph et al., 2004; Sanna et al., 2015) and activation of pro-apoptotic caspase pathways (Joseph and Levine, 2004); data which support a hypothesis of drug-induced mitochondrial damage leading to altered calcium homeostatis and activation of apoptotic pathways.

Damage to peripheral nerves leads to a rapid influx of Ca^{++} and Na^{+}, which elicits electrical responses that back-propagate to the cell body in the dorsal root ganglion (DRG) and contribute to the activation of several protein kinase pathways, including mitogen-activated protein kinases (MAPKs) (Ghosh and Greenberg, 1995; Matzner and Devor, 1994). MAPK family comprises three major members: extracellular signal-regulated kinase (ERK), p38, and c-Jun N-terminal kinase (JNK), all of which are implicated in pain processes. Compared to ERK and p38, less is known about JNK control of pain (Ji et al., 2009). However, increasing body of evidence suggests that the JNK cascade is a critical signalling pathway for the onset and the maintenance of neuropathic pain. JNK activation contributes to pain sensitization via both neuronal and glial mechanisms and JNK1 activation in spinal cord astrocytes contributes to the maintenance of chronic pain (Zhuang et al., 2006). JNK activation in astrocytes also leads to the production of reactive oxygen species that is known to have a role in neuropathic pain (Kim et al., 2004: Kawasaki et al., 2007). Furthermore, peripheral neuropathy is a common neurological symptom in AIDS patient. Treatment of neonatal DRG neurons with HIV envelope glycoprotein gp120 produces apoptosis, which can be blocked by inhibiting JNK pathway (Bodner et al., 2004).

At present the role of MAPK on antiretroviral-induced neuropathic pain is not elucidated. In order to improve pain management to limit discontinuation or abandon of antiretroviral therapy, the aim of the present study was to investigate the role of the MAPK signalling pathway in the mechanism of antiretroviral pain hypersensitivity. Specifically, we thought it worthwhile to investigate JNK-mediated mechanisms by focusing on events that occur in the spinal dorsal horn (SDH), and DRG.

2. Materials and methods

2.1. Animals

Male CD1 mice (20–22 g) from the Harlan Laboratories (Bresso, Italy) breeding farm were used. Mice were randomly assigned to standard cages, with four to five animals per cage. The cages were placed in the experimental room 24 h before behavioural testing for acclimatization. The animals were fed a standard laboratory diet and tap water ad libitum and kept at 23 ± 1 °C with a 12 h light/dark cycle, light on at 7 a.m. The experimental protocol was carried out after approval by the Animal Care and Research Ethics Committee of the University of Florence, Italy, under license from the Italian Department of Health (54/2014-B) and in compliance with the European Communities Council directive of 24 November 1986 (86/609/EEC). All studies involving animals are reported in accordance with the ARRIVE (McGrath et al., 2010).

2.2. Drug administration

Zalcitabine (2′,3′-dideoxycytidine, ddC) and stavudine (2′,3′-didehydro-3′-deoxythymidine, d4T) (Sigma, Milan, Italy) solution was freshly prepared in saline (0.9% NaCl) on the day of the experiment. Zalcitabine-, stavudine- and saline-treated groups were given a one-time intraperitoneal (i.p.) injection of zalcitabine, stavudine (25 mg/kg) or saline, respectively. The JNK inhibitor SP600125 (5 μg per mouse) (Calbiochem, Milan Italy) and the PKC blocker Calphostin C (0.2 μg per mouse) (Calbiochem, Milan Italy) were dissolved in 20% and 0.5% DMSO, respectively, and administered intrathecally (i.t.) 60 min before testing. Doses and administration schedule were chosen on the basis of time-course and dose–response curves performed in our laboratory. Drugs were administered i.t., as previously described (Sanna et al., 2015).

2.3. Behavioural testing

2.3.1. Mechanical allodynia

Mechanical allodynia was measured by using Dynamic Plantar Anesthesiometer (Ugo Basile) as previously described (Sanna et al., 2014). The mice were placed in individual Plexiglas cubicles (8.5 cm L 3.4 cm H 3.4 cm) on a wire mesh platform and allowed to acclimate for approximately 1 h, during which exploratory and grooming activity ended. After that, the mechanical stimulus was delivered to the plantar surface of the hind paw of the mouse from below the floor of the test chamber by an automated testing device. A steel rod (2 mm) was pushed with electronic ascending force (0–5 g in 35 s). When the animal withdrew its hind paw, the mechanical stimulus was automatically withdrawn and the force recorded to the nearest 0.1 g. Nociceptive response for mechanical sensitivity was expressed as mechanical paw withdrawal threshold. Each mouse served as its own control, the responses being measured both before and after antiretroviral drug administration. PWT was quantified by an observer blinded to the treatment.

2.3.2. Thermal hyperalgesia

Thermal hyperalgesia was measured by using the hot plate test (Sanna et al., 2015). Mice were placed inside a stainless steel container, which was set thermostatically at 52.5 ± 0.1 °C in a precision water-bath. The hot-plate apparatus (Ugo Basile Biological Research Apparatus, Varese, Italy) was 25 × 37 × 47(h) cm. Reaction times (s) were measured with a stopwatch before and 3, 7, 10, 14, 21 days after administration of the antiretroviral drug. The endpoint used was the licking of the fore or hind paws.

2.3.3. Cold allodynia

Cold allodynia was assessed as described (Galeotti and Ghelardini, 2013). Mice were placed on a cold plate that is maintained at a temperature of 4 ± 0.1 °C. Reaction times (s) were measured with a stopwatch before and 1, 2, 4, 5, 7 and 10 days after administration of the antiretroviral drug. The time between placements of a mouse on the plate and licking or lifting of a hind paw was measured with a digital timer. An arbitrary cut-off time of 60 s was adopted.

2.4. Western blot analysis

The lumbar spinal cord (L4-L6 segment) and DRG were removed 1, 3 and 7 days after zalcitabine or stavudine administration. Samples were homogenized on ice in lysis buffer. Protein concentration was quantified using the Bradford's method (protein assay kit, Bio-Rad Laboratories, Milan, Italy). Homogenates (10–20 μg) were separated on 10% SDS-PAGE and transferred onto nitrocellulose membranes (90 min at 120 V) using standard procedures.