Neuroscience Letters 604 (2015) 69–74

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Research paper

Early treatment with UR13870, a novel inhibitor of p38α mitogenous activated protein kinase, prevents hyperreflexia and anxiety behaviors, in the spared nerve injury model of neuropathic pain

Iriana Galan-Arriero[a,b], Gerardo Avila-Martin[a], Agueda Ferrer-Donato[a], Julio Gomez-Soriano[a,c], Stefano Piazza[a,d], Julian Taylor[a,e,f,*]

[a] Sensorimotor Function Group, Hospital Nacional de Parapléjicos, SESCAM, Toledo, Spain
[b] Escuela Internacional de Doctorado (EID), Universidad Rey Juan Carlos I. Móstoles, Madrid, Spain
[c] Escuela de Enfermeria y Fisioterapia Universidad Castilla La Mancha, Toledo, Spain
[d] Neural Rehabilitation Group, Cajal Institute CSIC, Madrid, Spain
[e] Stoke Mandeville Spinal Research, National Spinal Injuries Centre, Buckinghamshire Healthcare Trust, Stoke Mandeville, Aylesbury, UK
[f] Harris Manchester College, University of Oxford, Oxford, UK

HIGHLIGHTS

- Microglial cell inhibition, through p38α MAPK, is a potential treatment for neuropathic pain.
- UR13870 is a highly specific p38α inhibitor.
- UR13870 prevents thermal and mechanical hyperreflexia and open field anxiety in the SNI model.
- UR13870 inhibits microglial reactivity within the lumbar dorsal horn after SNI.

ARTICLE INFO

Article history:
Received 19 June 2015
Received in revised form 28 July 2015
Accepted 30 July 2015
Available online 1 August 2015

Keywords:
Spared nerve injury
Neuropathic pain
p38α MAPK inhibitor
Antinociception
Microglia
OX-42
Place escape/avoidance paradigm
Open field induced anxiety test

ABSTRACT

Microglia cell activation plays a role in the development of neuropathic pain partly due to the activation of the p38α MAPK signaling pathway after nerve injury. In this study we assessed the effect of UR13870, a p38α MAPK inhibitor, in the "spared nerve injury" (SNI) model, to study its effects on modulation of spinal microglial activation and to test behavioral hyperreflexia responses and cerebral-mediated pain behavior. The effect of daily administration of UR13870 (10 mg/kg p.o.) and Pregabalin (50 mg/kg p.o.) on reflex hypersensitivity to mechanical and cold test stimuli and on affective related pain responses measured with the place escape avoidance paradigm and the open field-induced anxiety test, were evaluated after SNI in Sprague Dawley rats. Microglial reactivity in the ipsilateral lumbar laminae I/II dorsal horn was evaluated with OX-42 immunohistochemistry. UR13870 treatment significantly decreased hindlimb hyperreflexia to both mechanical and cold stimuli after SNI without loss of general motor function, in addition to a reduction in pain-related anxiety behavior at day 21 after SNI, accompanied by normalization of OX-42 immunoreactivity within the ipsilateral lumbar dorsal horn. Pregabalin treatment only reduced mechanical hyperreflexia and affected general motor function. Oral administration of the p38α MAPK inhibitor, UR13870, mediates antinociception to both mechanical and cold stimuli, and significantly restored inner-zone exploration in the open field test, accompanied by normalization in dorsal horn microglial activation in the SNI model.

* Corresponding author at: Finca "La Peraleda" s/n., 45071 Toledo, Spain. Fax: +34 925 247745.
E-mail addresses: igalan@jccm.es (I. Galan-Arriero), gavila@sescam.jccm.es (G. Avila-Martin), agata9364@hotmail.com (A. Ferrer-Donato), Julio.Soriano@uclm.es (J. Gomez-Soriano), stefano.piazza@csic.es (S. Piazza), jscott@sescam.org (J. Taylor).

http://dx.doi.org/10.1016/j.neulet.2015.07.044
0304-3940/

1. Introduction

Activation of microglia cells plays a central role in the development and maintenance of neuropathic pain, partly through the activation of the p38α mitogen activated protein kinase (MAPK) cell signaling pathway [1], and has been corroborated following central [2] and peripheral nerve injury [3]. The "spared nerve injury" (SNI) model is a peripheral neuropathic pain model which is widely used to study the effect of new analgesics in preclinical studies [3,4], particularly as it is characterised by clinically-relevant symptoms [5]. Reflex hypersensitivity to both mechanical and thermal stimuli develop soon after SNI [5,6] and are maintained at least up to several months after injury with the additional development of anxiety-like behavior [7]. Indeed affective pain-related behaviors have been shown to be important outcome measures of analgesia in chronic pain conditions [8]. SNI elicits microglia cell activation within the spinal dorsal horn which contributes to a change in sensory function with the observation of increased phosphorylation of p38α MAPK [3,9–12].

We have recently shown that the p38α MAPK inhibitor, UR13870, modulates affective responses after SCI through microglia cell inhibition [2], and these results linked to the previous studies, in which UR13870 demonstrated highly specific p38α inhibition, with a significant decrease in TNFα and cytokines releasing in microglial cells [13], suggest that this agent is a potential treatment for peripheral neuropathic pain conditions.

In the present study the objective is to assess the antinociceptive effect of an early treatment with oral UR13870 in reflex hypersensitivity responses to mechanical and cold stimuli, inhibition of microglial cell reactivity within the lumbar spinal cord dorsal horn following peripheral nerve injury in the SNI model, in addition to preventing the development of affective responses related to pain by specifically examining place escape avoidance behavior and open field-induced anxiety.

2. Material and methods

Following approval by the local Animal Welfare and Research Ethical Committee the "Spared Nerve Injury" (SNI) model was performed in anesthetized 10-week old male Sprague Dawley rats (200–250 g), by ligating and sectioning the peroneal and tibial branches of the left sciatic nerve [4]. Rats were randomly assigned into four groups: (i) Sham + Vehicle ($n = 8$); (ii) SNI + Vehicle ($n = 8$); (iii) SNI + UR13870 10 mg/kg, p.o. ($n = 8$); and (iv) SNI + Pregabalin 50 mg/kg, p.o. ($n = 8$). Drugs were administered daily by the oral gavage in a single dose. UR13870 (Palau Pharma, 10 mg/kg p.o.) and Pregabalin (Pfizer, 50 mg/kg p.o.) were dissolved in a vehicle solution of Gelatin B (5%, Sigma–Aldrich) and Mannitol (0.5%, Sigma–Aldrich) in 0.9% saline, prepared in a total volume of 0.5 ml. The UR13870 dose was chosen based on its functional and anti-inflammatory effect observed in the collagen-induced arthritis model [13]. Pregabalin was included in the study as a positive control for its pharmacological properties in peripheral nerve injury models.[14]

Motor and hyperreflexia tests were performed before the injury and at days 3, 5, 7, 10 and 14 after SNI. von Frey, Pin Prick and the Acetone tests were used to test reflex responses [15,16], applied directly to the spared sural nerve innervation area of the lateral plantar surface of the paw. Motor function was assessed with the Rota Rod device (4600, Ugo Basile).

Assessment of place escape avoidance paradigm (PEAP) behavior in animals with SNI, consisted of quantifying the time that the rat occupied a black-walled test chamber, during which an aversive stimulus was applied to the lateral plantar surface of the injured paw. A 60 × 30 × 30 cm chamber consisted of two different environments, one with black walls and the other with white walls, both equipped with a bank of infra-red light cells to automatically detect and process the time spent within each chamber and the number of crosses between them, and a grid floor to permit manual access to the plantar area of the paws (Cibertec S.A.). A 15 g von Frey filament (Stoelting Co.) was applied to the lateral surface of the paw ipsilateral to the SNI, but only when the rat was located within the black-walled chamber. In contrast when the animal moved to the white-walled chamber, the same filament was applied to non-injured contralateral paw. The stimulus was applied once every 15 s, for a total duration of 30 min [17,18]. The open field-induced anxiety test was also performed to evaluate the animalís behavior during the presentation of a novel situation at day 21 after SNI This test consisted of placing the animal in a 100 × 100 cm open field surrounded by a black curtain to exclude external cues, with a camera sited above the area connected to a commercial videotracking system (Ethovision XT software, Noldus Information Technology, Wageningen, Netherlands). The software delimited the total arena area into an inner (central area of 60 × 60 cm) and outer zone (remaining area). The rats were recorded and tracked for five minutes, and the software calculated the total time spent in each zone and the total distance moved. Anxiety behavior was confirmed when the animals demonstrated reduced exploration time within the inner zone also on day 21 after SNI [18,19].

Lumbar L4–L5 spinal cord tissue were extracted seven days after SNI, from anesthetized animals following intracardiac perfusion with 4% paraformaldehyde (Merck). Tissue was cut in 30 μm sections on a cryostat (HM560, Microm), and collected serially onto slides (Thermo Scientific). Spinal tissue was pre-incubated for 1 hour in a blocking solution and then incubated overnight with primary antibody against OX42 (Mouse anti-CD11b clone OX-42), followed by a secondary fluorescent antibody (1:1000 Goat anti-mouse Alexa 488 nm, Ref: A11001; Invitrogen). Microphotographs were performed with an epifluorescence microscope (Leica DM 5000B, Camera: DFC350 FX). OX-42 expression was quantified as previously described [2,20] using the Image J software (NIH Version 1.45s).

Statistical analysis was made with a two-way ANOVA test for reflex measures, and a one-way ANOVA test for the operant behavioral tests and immunohistochemistry, with the Bonferroni post-hoc test (GraphPad Prism, 5.1), in which the SNI-Vehicle treated group was the control ($p < 0.05^{*}$, $p < 0.01^{**}$ and $p < 0.001^{***}$). The power of the two-way ANOVA tests at $p < 0.05$ was identified as 0.99, with the exception of immunohistochemical analysis defined as 0.85 (GraphPad StatMate 2.0 Prism).

3. Results

3.1. Assessment of UR13870 and pregabalin modulation reflex after SNI

After SNI, a lower ipsilateral withdrawal reflex threshold was identified in response to von Frey filaments, reaching a minimum at day 14 after injury with a mean value of 2.0 ± 0.4 g in the SNI-Vehicle treated group, compared to 13.6 ± 1.1 g in the Sham-VEH group (Fig. 1a). UR13870 (10 mg/kg p.o.) prevented mechanical reflex hypersensitivity to von Frey filament testing after SNI, when compared to the SNI-VEH group ($p < 0.001$), with a mean value of 11.5 ± 1.5 g at day 14 (Fig. 1a). Daily treatment with Pregabalin (50 mg/kg p.o.) produced a slight decrease in withdrawal reflex threshold, which was significant from day 7 (9.3 ± 1.4, Fig. 1a). The pin prick noxious scores (Fig. 1b) in the SNI-UR13870 group oscillated between 1.0 ± 0.2 and 1.5 ± 0.2, and were significantly lower when compared to the SNI-Vehicle group (ranging from 2.4 ± 0.3 to 3.0 ± 0.1, $p < 0.001$). Pregabalin treatment also produced lower noxious scores at day 14 (1.8 ± 0.4 $p < 0.001$).