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Neuropathic pain and reactive gliosis are reversed by dialdehydic compound in neuropathic pain rat models

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HIGHLIGHTS

- We evaluate the effect of two different dialdehydic compounds on neuropathic pain.
- Sciatic spared nerve injury and paclitaxel evoked painful peripheral neuropathy.
- We investigate the allodynic and hyperalgesic neuropathic behaviour.
- We analyse the reactive macro and microgliosis by GFAP and Iba1 labeling.
- We hypothesize a general property of dialdehydes as analgesic and anti-gliotic molecules.

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ABSTRACT

The role of the purinergic system in the modulation of pain mechanisms suggests that it might be promising target for treating neuropathic pain. In this study we evaluated the effects of two different dialdehydic compounds: a modified stable adenosine (2-[1-(6-amminopurin-9-il)-2-osso-etossi]prop-2-enale, named MED1101), and oxidized ATP (Ox-ATP), in two different neuropathic pain rat models: the sciatic spared nerve injury (SNI) and paclitaxel evoked painful peripheral neuropathy (pPPN). Neuropathic animals were divided in groups as follows: (a) treated with intraperitoneal (i.p.) MED1101 or Ox-ATP for 21 days; (b) receiving vehicle (VEH) and (c) control (CTR) rats. The allodynic and hyperalgesic behavior was investigated by Von Frey filament test and thermal Plantar test, respectively. We evaluated by immunocytochemistry the astrocytic (GFAP) and microglial (Iba1) response on lumbar spinal cord sections. In either experimental models and using either substances, treated animals showed reduced allodynia and thermal hyperalgesia paralleled by a significant reduction of glial reaction in the spinal cord. These data prompt to hypothesize a potential role of dialdehydes as analgesic agent in chronic neuropathic pain and a possible role as anti-gliotic molecules.

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Neuropathic pain syndrome, characterized by allodynia and hyperalgesia, follows to peripheral nerve injury or the administration of anti-tumoral agents such as paclitaxel. Decades of morpho-functional studies have focused on mechanisms of neuropathic pain suggesting that peripheral sensitization of nerve terminals induces an imbalance of neuro-glial homeostasis in the spinal cord [26]. Moreover, as we recently demonstrated, neuropathic pain is paralleled by reactive changes both in microglia and in astrocytes in the central nervous system (CNS), a condition that is called *gliopathy* [8,19,25]. Several animal models have been proposed to study the mechanisms underlying neuropathic pain: evidence indicated the activation of the purinergic system as a mechanism modulating pain perception, suggesting that purinergic neuro-glial interactions are important modulators of sensory neurotransmission. In particular, ATP has been demonstrated to be a proinflammatory mediator [14,22] and released in large amounts after tissue injury. Both microglial cells [17,20] and astrocytes express different types of P2Y and P2X receptors under various conditions, suggesting that P2 receptors-mediated signaling critically contributes to the development and maintenance of neuropathic pain state. P2XR antagonists, therefore, may potentially reduce secondary damage both by directly inhibiting excitatory neuronal damage and by

Abbreviations: ATP, adenosine triphosphate; Ox-ATP, oxidized ATP; SNI, spared nerve injury; pPPN, paclitaxel evoked painful peripheral neuropathy; CNS, central nervous system.

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reducing both local and systemic inflammatory responses [30,35]. Among the ATP-sensitive purinergic receptors, the P2X7 receptor (P2X7R) plays a role in peripheral sensory transduction of pain perception [38]. It has been shown that oxidized ATP (Ox-ATP), a non selective antagonist of P2X7R, has analgesic effects in acute [12] and persistent pain [18] and attenuates glial reaction [28]. Ox-ATP is a dialdehydic compound, hence to evaluate if the aforementioned properties, were common to different dialdehydes, we compared the well defined effects of Ox-ATP with the new stable adenosine-like compound MED1101. Moreover to clearly focus on central mechanisms of maladaptive plasticity underlying neuropathic pain we study the effects of the compounds in two model showing very different mechanisms at the level of peripheral nerves and dorsal root ganglia (DRG) represented by: (i) persistent pain induced by SNI and (ii) paclitaxel induced neuropathic pain model (pPPN). We report that the either dialdehydic compound improves neuropathic behavior recovery and reduces glial activation, suggesting that it could be a general property of dialdehydes. It will open a new avenue in this field of research, looking for new tool to treat neuropathic pain and the more general mechanism underlying the maladaptive plasticity following gliopathy, crucial in several non-autonomous-cell diseases.

Adult male Sprague Dawley rats (250–300 g; Charles River, Calco, Italy) ($n = 40$) were maintained on a 12/12 h light/dark cycle and allowed free access to food and water. Each animal was housed under specific pathogen-free conditions in iron-sheet cages with solid floors covered with 4–6 cm of sawdust. All surgery and experimental procedures were performed during the light cycle and approved by the Animal Ethics Committee of The Second University of Naples. Animal care was in compliance with Italian (D.L. 116/92) and EC (O.J. of E.C. L358/1 18/12/86) regulations on the care of laboratory animals. All efforts were made to reduce animal numbers.

The adenosin-5′-triphosphate-2′,3′-dialdehyde sodium salt (Ox-ATP) was kindly provided by Medestea Research (Torino, Italy). The 2-[1-(6-amminopurin-9-il)-2-osso-etossi]prop-2-enale (MED1101), was synthesized according to the process, previously described by Grant and Lerner (1980) and kindly provided by Medestea Research (Torino, Italy).

For the SNI model, rats (group I, $n = 10$) were treated continuously with the tested compounds (6 mg/kg) starting from day 1 (the day after SNI) for 21 days and grouped into two experimental groups ($n = 5$), each receiving Ox-ATP (SNI-OxATP) and MED1101 (SNI-MED) at the same dose. For the pPPN model, rats (group II, $n = 10$) were treated continuously with one of the tested compounds when a peripheral neuropathy was clinically assessed by von Frey and plantar test. pPPN neuropathic rats were then grouped into two experimental groups ($n = 5$), each receiving Ox-ATP (PPN_PxATP) and MED1101 (PPN-MED) at the same dose (6 mg/kg). Vehicle group (VEH, $n = 10$) consisted of SNI ($n = 5$) or pPPN ($n = 5$) neuropathic rats treated with i.p. vehicle (DMSO) for 21 days. We assumed as control (CTR) group ($n = 10$) animals that were not treated with paclitaxel or nerve injured.

Spared sciatic nerve injury was made according to the methods of Decosterd and Woolf [11]. Briefly, rats ($n = 15$) were anaesthetized with chlorydrate tiletamine (40 mg/kg) during surgery. The skin on the lateral surface of the thigh was incised and a section made directly through the biceps femoris muscle exposing the sciatic nerve and its three terminal branches: the sural, common peroneal and tibial nerves. The common peroneal and the tibial nerves were then tight-ligated with 5.0 silk and sectioned distal to the ligation. Great care was taken to avoid any contact with or stretching of the intact sural nerve. Muscle and skin were closed in two layers.

Rats ($n = 15$) were treated by i.p. injections of paclitaxel 2 mg/kg on alternate days (day 0, 2, 4 and day 6) as described previously [31] The final cumulative paclitaxel dose administered was 8 mg/kg. Paclitaxel was prepared by diluting Taxol® (Bristol-Myers-Squibb) in a vehicle that is a mixture of saline solution and Cremophor EL 10% (a derivative of castor oil and ethylene oxide), used for paclitaxel injections at a concentration of 0.6 mg/ml. Animals were evaluated by von Frey and plantar test each day after the end of paclitaxel treatment. When neuropathic syndrome characterized by allodynia and thermal hyperalgesia became clinically evident (day 14), animals were divided into groups receiving Ox-ATP (Medestea Research, Torino, Italy) or MED1101 (Medestea Research, Torino, Italy).

In a pilot study, three different doses were tested: 2, 4, and 6 mg/kg. The dose of 6 mg/kg was the only effective compared to the other two doses tested (2 or 4 mg/kg), that did not show any behavioral or anti-gliotic effect (data not shown).

SNI rats received i.p. Ox-ATP or MED1101 (6 mg/kg) treatment starting from the day after peripheral nerve injury (day 1) to day 21. The Ox-ATP was dissolved in distilled water (dH_2O) and a total volume of 1 ml, containing 1.8 mg of Ox-ATP, was injected for each rat. The MED1101 was dissolved in DMSO and a total volume of 200 μl, containing 1.8 mg of MED1101, was injected for each rat. pPPN rats received either substances (6 mg/kg) i.p. starting from day 14 after the end of paclitaxel treatment to day 35. VEH animals were treated with a total volume of 200 μl of DMSO.

Behavioral tests to confirm the presence of mechanical allodynia and thermal hyperalgesia after SNI, were conducted on day 0 (the day of SNI), day 1 (the day of the first i.p. injection of compounds), day 7, 14, 21 and 28 after injury. On day 28 all animals were sacrificed.

For pPPN animals, behavioral tests for thermal and mechanical sensitivity were performed on day 1 (the day after the first injection of paclitaxel), day 7, day 14 (7 days after the last injection of paclitaxel), day 21, day 28,day 35 and day 42. Animals were sacrificed on day 42.

Mechanical allodynia was assessed by using the von Frey filaments (Ugo Basile) according to the method described by Chaplan et al. [3]. Animals were allowed to habituate for 30 min before testing. Filaments were applied in either ascending or descending strength as necessary to determine the filament closest to the threshold of response. The time of response to a progressive force applied to hind paw limb (30 g in 20 s) was evaluated six times using the injured hind limb, with an interval of 5 min between stimulations. The threshold was the lowest force that evoked a consistent, brisk, withdrawal response. Thermal nociceptive thresholds were measured using a device based on the design by Hargreaves et al. [15]. Animals were allowed to habituate for 30 min before testing. Paw-withdrawal latency in response to radiant heat (infra-red) was measured using the plantar test apparatus (Ugo Basile). The injured hind limb was tested twice at each time point, with an interval of 5 min between stimulations. All testing was performed blind.

At the end of treatments, rats were deeply anaesthetized by intraperitoneal injection of chloral hydrate (300 mg/kg body weight) and perfused transcardially with saline solution (Tris–HCl 0.1 M/EDTA 10 mM) followed by 4% paraformaldehyde in 0.01 M phosphate-buffer (PB), pH 7.4 at 4 °C. For light microscopy, spinal cords were removed and post-fixed 2 h in the same fixative, then soaked in 30% sucrose PBS and frozen in chilled isopentane on dry ice. Serial sections were cut at the slide microtome (25 μm thickness) and collected in cold PBS for immunohistochemistry.

The following antibodies have been used for immunodetection: mouse antibodies directed against Glial Fibrillary Acidic Protein (GFAP) (1:400; Sigma–Aldrich, Milano, Italy); rabbit antibodies to ionized calcium binding adaptor molecule 1 (Iba1) (1:500; Wako Chemicals, USA).

Lumbar (L4–L6) spinal cord sections were blocked in 10% normal serum (from animal species different from the species origin

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