Behavioural Brain Research 320 (2017) 440–449

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Research report

Behavioral and electromyographic assessment of oxaliplatin-induced motor dysfunctions: Evidence for a therapeutic effect of allopregnanolone

O. Taleb*,[1], F. Bouzobra[1], H. Tekin-Pala, L. Meyer, A.G. Mensah-Nyagan[2], C. Patte-Mensah*,[2]

Biopathologie de la Myéline, Neuroprotection et Stratégies Thérapeutiques, INSERM U1119, Fédération de Médecine Translationnelle de Strasbourg (FMTS), Université de Strasbourg, Bâtiment 3 de la Faculté de Médecine, 11 rue Humann, 67 000 Strasbourg, France

HIGHLIGHTS

- We validated a rat model of oxaliplatin-evoked motor neuropathy.
- Wire suspension and balance beam tests showed motor deficit in OXAL-treated rats.
- Motor fiber conductance velocity was reduced in OXAL-treated rats.
- OXAL-treatment increased the compound muscle action potential duration.
- Allopregnanolone treatment successfully corrected OXAL-evoked motor dysfunctions.

ARTICLE INFO

Article history:
Received 21 March 2016
Received in revised form 20 October 2016
Accepted 23 October 2016
Available online 24 October 2016

Keywords:
Motor disorders
Nerve fiber dysfunction
Neuroprotection
Neurosteroid
Peripheral neuropathy

ABSTRACT

The antineoplastic oxaliplatin (OXAL) is pivotal for metastatic cancer treatments. However, OXAL evokes sensory and motor side-effects including pain, muscle weakness, motor nerve fiber dysfunctions/neuropathies that significantly impact patients' lives. Therefore, preclinical investigations are struggling to characterize effective analgesics against OXAL-induced painful/sensory symptoms but surprisingly, OXAL-evoked motor dysfunctions received little attention although these neurological symptoms are also disabling for patients. Here, we validated a rat model of OXAL-induced motor neuropathy by using (i) behavioral methods as the wire suspension and balance beam tests to assess muscle weakness and (ii) electrophysiological techniques to record the gastrocnemius electromyography (EMG). The conductance velocity of motor fibers was reduced and compound muscle action potential (CMAP) duration increased in OXAL-treated rats, leading to CMAP dispersion with no modification of the area under the curve, reflecting a heterogeneous demyelination of motor fibers. Functional motor unit analysis revealed a 50 % decrease of their estimated number which was compensated by a motor unit size increase. OXAL-induced motor weakness appeared as a combined consequence of motor fiber demyelination and motor axonopathy. Because we previously observed that allopregnanolone (AP) counteracted OXAL-evoked painful/sensory symptoms, we evaluated its action against OXAL-induced motor neurological dysfunctions. AP treatment successfully corrected motor behaviors, conductance velocity, CMAP duration, motor unit number (MUN) and motor unit size altered by OXAL-chemotherapy. These results, which are the first to show that AP efficiently rescues OXAL-induced motor neuropathy, consolidate the idea that AP-based therapy may be relevant for the treatment of both sensory and motor peripheral neuropathies.

1. Introduction

Antineoplastic-based chemotherapy remains the major strategy frequently used in oncology to treat various cancers. However, the effectiveness of this treatment is limited by serious adverse effects such as sensory and/or motor peripheral neuropathies which depend on the nature or on the cumulative dose of antineoplas-

* Corresponding authors.
E-mail address: cmensah@unistra.fr (C. Patte-Mensah).
[1] These authors contributed equally as the first authors.
[2] These authors contributed equally as the last authors.

http://dx.doi.org/10.1016/j.bbr.2016.10.040
0166-4328/

tic drugs, including taxanes, vinca-alcaloides and organoplatinium compounds. Chemotherapy-induced peripheral neuropathy (CIPN) significantly decreases patient quality of life, leading to a discontinuation of first-line treatments. For instance, oxaliplatin (OXAL), a third generation organoplatinium compound possessing a more favorable toxicological profile than the previous platinum drugs, is frequently used to treat colorectal cancer and other malignancies such as lung, breast and ovarian cancers [1–6]. However, OXAL evokes peripheral neuropathic side effects limiting the treatment effectiveness as the severity of neurological complications increases with the number of OXAL injections (i.e. cumulative dose). It has been demonstrated that OXAL accumulates in dorsal root ganglia neurons via the organic cation transporter 2 (OCT2) and causes sensory peripheral neuropathy by reducing the metabolism and axonal transport [7]. In clinic, muscle weakness and major motor deficit are also associated with OXAL-evoked symptoms, suggesting that OXAL can also accumulate in motor neurons and induce motor fiber dysfunction [8,9]. In support of this idea, motor neurons express OCT2 which participates at the neuromuscular junction to the recycling of acetylcholine, more precisely in choline uptake by cholinergic nerve terminals [10]. Thus, the localization and function of OCT2 may facilitate OXAL entry into motor nerve terminals and subsequently its accumulation in motor neurons by retrograde transport.

The normal or physiological activity of neuromuscular junctions requires the axonal transfer of action potential but also the vesicular transport to the endplate in order to provide the material necessary for the neuromuscular function. Therefore, toxic effects of OXAL on metabolism and axonal transport may induce alterations of endplate transmission and denervation of muscle fibers. It is well-known that acute motor axon loss results in reduced size of the compound muscle action potential (CMAP) following the nerve stimulation. In chronic lesion situations, there is compensatory collateral sprouting of surviving axons resulting in the re-innervation of denervated muscle fibers; this process may allow a relatively preserved maximal CMAP amplitude until the axon loss becomes severe with no sprouting of collaterals. Motor unit number (MUN) refers to the number of motor neurons or axons innervating and controlling a single muscle. MUN is a critical measure in every diseases involving injury, death of motor neurons or axon degeneration. Thus, an accurate estimation of MUN may provide valuable insights into the pathophysiological mechanisms of motor disorders [11–13]. Consequently, the determination of OXAL effects on MUN and CMAP parameters in rodents appears as an interesting approach to investigate motor abnormalities evoked by OXAL and this may allow the validation of a relevant preclinical model to investigate and identify effective therapies against OXAL-induced motor peripheral neuropathy. Indeed, although sensory and motor neurological symptoms are both clinically and frequently assessed in cancer patients during OXAL treatment [14–16], preclinical efforts to develop animal models which may serve to characterize effective drugs against OXAL-induced neuropathy have until now focused essentially on painful/sensory symptoms [17,18]. Just a little and unfruitful attention was devoted to OXAL-evoked motor dysfunctions at preclinical level [19] while a severe ascending motor neuropathy rendering a patient wheelchair-bound has been reported [20] in addition to several other motor neurological symptoms clinically identified in patients using EMG/electrophysiological measurements and/or neuropathy assessment scales [8,21,22]. Therefore, it appears that the validation of a reliable model of OXAL-induced motor dysfunctions may constitute a valuable preclinical tool to investigate therapeutic strategies against OXAL-induced motor neuropathy.

Allopregnanolone (AP) known also as 3α,5α-tetrahydroxyprogesterone is a naturally occurring neurosteroid synthesized in the central nervous system. [23–25]. AP has been reported to have potent neuroprotective effect in many diseases [26–28]. More importantly, we previously observed that AP efficiently counteracted painful/sensory symptoms including allodynia and hyperalgesia induced by OXAL treatment in rat [29]. Consequently, we hypothesized that AP therapy may probably be effective to treat OXAL-induced motor neuropathy in rat. The present study was therefore designed to check our hypothesis and, in a first step, we used behavioral, electrophysiological and immunohistochemical methods to validate a relevant preclinical model of OXAL-induced motor peripheral neuropathy. Motor weakness evoked by OXAL treatment was behaviorally evidenced in rat with the wire suspension test and the balance beam test. Afterwards, we applied the surface electrode electromyography (EMG) technique to evaluate neuromuscular junction abnormalities underlying motor deficits. Furthermore, the degeneration of motor axons induced by OXAL treatment was investigated by using immunohistochemical approach to assess the alterations of neurofilament 200 kDa (NF200) in peripheral nerve motor axons. Finally, we tested the potential of AP to improve OXAL-evoked motor behavior deficits and to reverse to normal values, EMG components, including CMAP and MUN, and NF200 level in sciatic nerve motor fibers affected by OXAL treatment.

2. Materials and methods

2.1. Animals

In these experiments we used adult male Sprague-Dawley rats weighting initially 250–300 g. Animal handling were performed according to the European Community Council Directives (86/609/EC) and a local agreement delivered by the Alsace Department of Veterinary Public Health (Agreement number 67-186). The animals were obtained from a commercial source (Janvier, Le Genest St Isle, France) and housed under standard laboratory conditions in a 12-h light/dark cycle with food and water ad libitum. Before starting the experiments, the animals were allowed a one-week acclimatization period after delivery in our animal house.

2.2. Animal treatments

OXAL and AP (Sequoia Research Products, UK) were used as previously described [29]. Briefly, OXAL was dissolved (4 mg/ml) in 5% glucose, used as vehicle (Veh_{gluc}), and intraperitoneally (i.p.) injected twice a week during 4 weeks, at a dose of 4 mg/kg/injection. Thus, the chemotherapy was performed at days D2, D5, D9, D12, D16, D19, D23 and D26. The cumulative OXAL dose was then 32 mg/kg for each treated animal. AP was diluted in water containing 0.3% hydroxypropylcellulose or HPC (Sigma Aldrich, St. Louis, MO) used as vehicle (Veh_{hpc}), and injected (i.p.) at a dose of 4 mg/kg each other day during 3 weeks.

Control animals were i.p. injected with the same liquid volume (1 ml/kg) used for treated animals that contained the same concentration of each vehicle (Veh_{gluc} or Veh_{hpc}). In summary, four treatments were used and each animal was injected with the same volume solution containing Veh_{glu}-Veh_{hpc}, OXAL-Veh_{hpc}, Veh_{gluc}-AP or OXAL-AP corresponding respectively to Veh, OXAL, AP and OXAL-AP groups of 6 animals each. The naive non injected animal controls were not used in these experiments because we have already shown that Veh_{gluc} and Veh_{hpc} did not affect sciatic nerve fiber morphology or functions [29].

2.3. Behavioral tests for motor weakness

2.3.1. Wire suspension test

Muscular strength and neuromuscular endurance of rats was assessed by evaluating the grip capability of the animals on a sus-