Experimental Neurology 239 (2013) 13–27

Contents lists available at SciVerse ScienceDirect

Experimental Neurology

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

Improved outcome after spinal cord compression injury in mice treated with docosahexaenoic acid

Siew-Na Lim [a,b], Wenlong Huang [a,c], Jodie C.E. Hall [a,d], Adina T. Michael-Titus [a], John V. Priestley [a,*]

[a] Centre for Neuroscience and Trauma, Blizard Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London, UK
[b] Department of Neurology, Chang Gung Memorial Hospital and Chang Gung University College of Medicine, Taipei, Taiwan
[c] Pain Research Group, Department of Surgery and Cancer, Imperial College London, SW10 9NH, UK
[d] Center for Brain and Spinal Cord Repair, The Ohio State University, Columbus, OH 43210, USA

ARTICLE INFO

Article history:
Received 17 May 2012
Revised 20 September 2012
Accepted 24 September 2012
Available online 28 September 2012

Keywords:
Trauma
Spinal cord injury
Polyunsaturated fatty acid
Docosahexaenoic acid
Neuroprotection

ABSTRACT

In this study we have characterised the locomotor recovery, and temporal profile of cell loss, in a novel thoracic compression spinal cord injury (SCI) in the mouse. We have also shown that treatment with docosahexaenoic acid (DHA) is neuroprotective in this model of SCI, strengthening the growing literature demonstrating that omega-3 polyunsaturated fatty acids are neuroprotective after SCI.

Compression SCI in C57BL/6 mice was produced by placing a 10 g weight for 5 min onto a 2 mm × 1.5 mm platform applied to the dura at vertebral level T12. Mice partly recovered from complete hindlimb paralysis and by 28 days post-surgery had plateaued at an average BMS locomotor score of 4.2, equivalent to weight support with plantar stepping. During the same period, neuronal loss at the epicentre increased from 26% of ventral horn neurons by day 1, to 68% by day 28. Delayed loss of oligodendrocytes was also seen (e.g. 84% by day 28 in the dorsal columns) and microglia/macrophage activation was maximal at 7 days. In contrast, axonal damage, judged by a decrease in the non-phosphorylated form of 200 kD neurofilament, was an early event, as the loss was seen by day 1 and did not change markedly over time.

Mice that received an intravenous (i.v.) injection of 500 nmol/kg DHA 30 min after SCI, showed improved locomotor recovery and, at 28 day survival, reduced neuronal, oligodendrocyte and neurofilament loss, and reduced microglia/macrophage activation. For some of these indices of SCI, enrichment of the diet with 400 mg/kg/day DHA led to further improvement. However, dietary DHA supplementation, without the initial i.v. injection, was ineffective.

Introduction

Traumatic spinal cord injury (SCI) has devastating consequences for patients, and current treatments, such as acute decompression (Fehlings and Perrin, 2006) or administration of methylprednisolone (Hurlbert and Hamilton, 2008), are controversial or of limited efficacy. In preclinical studies, major progress has been made in both the fields of neuroprotection and neuroregeneration (Kwon et al., 2010), but translation into the clinic has been disappointing. Neuroprotective strategies are particularly appealing because they aim to stop the spread of injury, and the complex cascade of events that follow the primary insult means there are many possible targets for neuroprotective agents. However, although there are promising new agents under development (Kwon et al., 2011a), many clinical trials have failed, either because of limited efficacy or unexpected toxicity (Tator and Fehlings, 1999). There is, therefore, still a need for the development of new neuroprotective agents, which are safe and effective when delivered after spinal cord injury.

Recently, a number of preclinical studies have demonstrated that omega-3 (n−3) polyunsaturated fatty acids (PUFAs) are neuroprotective when administered after SCI. n−3 PUFAs are essential fatty acids that have crucial roles in the development and mature functioning of the nervous system. Particularly important is docosahexaenoic acid (DHA), a long chain (22 carbon) n−3 PUFA that accounts for approximately 50% of the PUFAs in central nervous system (CNS) membranes. We have shown in rat hemisection and compression models of SCI that DHA administered as an intravenous bolus 30 min after injury leads to increased neuronal, oligodendrocyte and axonal survival at the lesion epicentre, and improved locomotor function (Huang et al., 2007b; King et al., 2006; Ward et al., 2010). When the acute bolus is combined with dietary DHA supplementation for several weeks following injury, additional cell and axonal survival is seen, and further improvement of functional outcome (Huang et al., 2007b; Ward et al., 2010). Most recently we have shown that a multi-nutrient dietary formulation containing n−3 PUFAs is also neuroprotective (Zbarski-Barquero et al., 2012). Neuroprotection has also been reported in studies involving treatment with DHA prior to SCI (Figueroa et al., 2012) or treatment after SCI with

* Corresponding author at: Centre for Neuroscience and Trauma, Blizard Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, 4 Newark Street, London, UK. Fax: +44 20 78822218.
E-mail address: j.v.priestley@qmul.ac.uk (J.V. Priestley).

0014-4886/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.expneurol.2012.09.015

fenretinide, a synthetic retinoid derivative which increases levels of endogenous DHA (Lopez-Vales et al., 2010). DHA has also been shown to be neuroprotective if administered after traumatic brain injury in rodents (Bailes and Mills, 2010; Wu et al., 2011). The exact mechanism underlying these neuroprotective effects is not known, but it is likely to involve multiple pathways. Actions of DHA include effects on membrane properties such as fluidity and permeability (Stillwell and Wassall, 2003), effects on membrane proteins including receptors (Lafourcade et al., 2011) and ion channels (Lauritzen et al., 2000), effects on gene transcription by direct binding of non-esterified DHA to peroxisome proliferator activated receptors (PPARs) (Jump, 2002), reduction in the levels of pro-inflammatory n−6 PUFAs by competition for metabolic and catabolic pathways, and effects of DHA metabolites, including the anti-inflammatory and pro-homeostatic neuroprotectin D1 (NPD1) (Bazan et al., 2011). These multiple actions, and the established safety profile of existing n−3 PUFA formulations (Heller et al., 2006), make DHA a very attractive candidate for the clinical treatment of acute SCI.

Before DHA is tested in clinical trials, it should be validated in a wide range of preclinical SCI models, including different types of injury (e.g. contusion and compression) and different species (e.g. rat and mouse). Efficacy in multiple models has been identified as an important criterion before translation to the clinic (Kwon et al., 2011b). Most of our studies of n−3 PUFAs have been carried out in a rat thoracic compression model of SCI (Hall et al., 2012; Huang et al., 2007b; Lim et al., 2010; Ward et al., 2010), a model in which cell death extends over a longer period than in contusion injury models (Huang et al., 2007a). In the present study we have therefore developed a mouse compression model, which is similar in principle to our rat model (Huang et al., 2007a) and used it to investigate the neuroprotective properties of acute and dietary DHA treatment. It is well-established that mice exhibit a very different response to SCI, both in terms of functional recovery, and also in the tissue pathological changes and inflammatory reaction, compared to that seen in other mammals. In particular, mice do not exhibit the progressive necrosis and larger central cavitation that is so dramatic in rats and other mammals, in which a rim of anatomically preserved white matter surrounds a fluid-filled cystic cavity at the injury epicentre (Grossman et al., 2001; Huang et al., 2007a). In contrast, the injured mouse spinal cord is completely filled-in with dense fibrous connective tissue, and if present, there are only very small cavities (microcysts) in the lesion site (Farooque, 2000; Joshi and Fehlings, 2002a,b; Ma et al., 2001). The use of a mouse compression model therefore allows us to test DHA in a very different pathology, and provides a baseline for future studies in transgenic mice (e.g. Lim et al., 2012).

Material and methods

Compression SCI

C57BL/6 female mice (19–21 g) were deeply anaesthetized with 4% isoflurane (Merial, Essex, UK), as evidenced by lack of response to a nociceptive stimulus. Subsequent anaesthesia throughout the procedure was maintained using 1.5–2% isoflurane, with oxygen and nitrous oxide at a 1:1 ratio. A laminectomy was performed at vertebral level T12, leaving the dura undisturbed. The T11 and T13 transverse processes were clamped in a spinal compression frame, and the compression was applied by suspending the base of a compression platform onto the exposed cord, under microscopic control. Then a weight was applied to the platform for a specific period of time. The platform was then removed, the muscle layers were sutured and the skin layers closed with wound clips. In preliminary experiments, platforms of 1 mm×1 mm and 2 mm×1.5 mm were tested with a 10 g weight applied for 10 min. The 2 mm×1.5 mm platform was further tested using a 10 g weight applied for 5 min, or a 5 g weight applied for 10 min. The 10 g weight applied to a 2 mm×1.5 mm platform for 5 min, was judged to give the most reproducible results and BMS recovery profile indicative of a moderate SCI, and so was used in all the experiments described in this study. After surgery, animals were given buprenorphine intramuscularly (0.12 mg/kg; Reckitt Benckiser, UK) and then were placed in warmed cages to recover from anaesthesia. Manual bladder expression was performed twice a day until the establishment of reflex voiding. The mice were given free access to food and tap water, and were maintained on a 12 hour light/dark cycle at 21 °C±1 °C.

Treatment with DHA

SCI surgery was performed in several groups of animals, which received various types of DHA treatment. To define the optimum intravenous (i.v.) DHA treatment dose for mice, first we carried out a dose–response study. DHA for acute i.v. injection was diluted from 1 M stock aliquots (5 μl) of free fatty acid (Sigma, Dorset, UK) made up in absolute ethanol and stored at −80 °C until use. On the day of surgery, the stock aliquot solutions of fatty acids were diluted to the required concentration in sterilized saline (NaCl, 0.9%) and adjusted to pH 7.4. Mice received the following treatments via a tail vein 30 min after SCI: (i) saline vehicle (n=5); or (ii) DHA 100 nmol/kg (n=6); (iii) or DHA 250 nmol/kg (n=6); or (iv) DHA 500 nmol/kg (n=6), in a volume of 5 ml/kg, injected over 10 s. In subsequent studies, we explored a combined acute and chronic DHA treatment regime. Mice received the following treatment: (i) an i.v. injection of saline vehicle 30 min after SCI and a control diet (n=9); or (ii) an i.v. injection of DHA (500 nmol/kg) 30 min after SCI and a control diet (n=9); or (iii) an i.v. injection of saline vehicle 30 min after SCI and a DHA-enriched diet (400 mg/kg/day) (n=8); or (iv) an i.v. injection of DHA (500 nmol/kg) 30 min after injury and a DHA-enriched diet (400 mg/kg/day) (n=8). All animals were maintained for 4 weeks until sacrifice. Groups were allocated randomly and the experimenter was blinded to the treatment groups throughout the surgery and the behavioural testing period.

Two weeks prior to the start of the DHA dietary enrichment studies, the animals' diet was changed from the standard pelleted form to a ground form, in order to allow habituation to the different food texture. The control diet consisted of 5KB3 Certified EURodent maintenance diet 14% (LabDiet, IPS International Product Supplies Limited, London). The DHA-enriched diet consisted of the 5KB3 diet to which oil enriched in DHA (Incromega DHA 700E SR; Croda Healthcare, England) was added, resulting in the mice receiving a daily dose of 400 mg DHA per kilogramme of animal body mass. The oil contained 70–75% DHA, 10% EPA and the remainder contained arachidonic acid (AA) and other fatty acids. Oil-enriched diet was prepared weekly under nitrogen by thoroughly mixing the oil with the 5KB3 powder diet for a minimum of 10 min, ensuring complete and even dispersal of the oil throughout the diet. While still under nitrogen the food was placed in air-tight bags and then stored at −20 °C until used. Animals were found to consume less food for the first week after SCI (20–50% less), but later they resumed normal consumption of the amount of the diet provided. The full dose received was recalculated based on these findings.

Functional assessment

We used the Basso mouse scale (BMS) open-field test (Basso et al., 2006) to assess functional improvement after SCI. The scale consists of 9 categories which reflect the different stages of recovery. A BMS score of zero indicates that the animal has complete hindlimb paralysis, whereas a BMS score of 9 indicates the animal has no locomotor deficits (uninjured animal). The 11-point BMS subscores, which evaluate finer aspects of locomotor control, were also assessed. The behaviour was assessed and scored in blind, daily for the first week and either daily, or every 3 days thereafter until sacrifice.