Neurobiology of Disease 82 (2015) 504–515

Contents lists available at ScienceDirect

Neurobiology of Disease

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

A nutrient combination designed to enhance synapse formation and function improves outcome in experimental spinal cord injury

Patrick N. Pallier [a,1], Laura Poddighe [b,1], Virginia Zbarsky [a], Milosz Kostusiak [a], Rasall Choudhury [a], Thomas Hart [a], Miguel A. Burguillos [a], Omar Musbahi [a], Martine Groenendijk [c], John W. Sijben [c], Martijn C. deWilde [c], Marina Quartu [b], John V. Priestley [a], Adina T. Michael-Titus [a,*]

[a] Centre for Neuroscience and Trauma, The Blizard Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, 4 Newark Street, Whitechapel, London E1 2AT, UK
[b] University of Cagliari, Sardinia, Italy
[c] Nutricia Research — Nutricia Advanced Medical Nutrition, Utrecht, The Netherlands

ARTICLE INFO

Article history:
Received 19 June 2015
Revised 14 September 2015
Accepted 16 September 2015
Available online 24 September 2015

Keywords:
Fortasyn connect
Spinal cord injury
Dietary supplementation
Neuroprotection
Neuroplasticity

ABSTRACT

Spinal cord injury leads to major neurological impairment for which there is currently no effective treatment. Recent clinical trials have demonstrated the efficacy of Fortasyn® Connect in Alzheimer's disease. Fortasyn® Connect is a specific multi-nutrient combination containing DHA, EPA, choline, uridine monophosphate, phospholipids, and various vitamins. We examined the effect of Fortasyn® Connect in a rat compression model of spinal cord injury. For 4 or 9 weeks following the injury, rats were fed either a control diet or a diet enriched with low, medium, or high doses of Fortasyn® Connect. The medium-dose Fortasyn® Connect-enriched diet showed significant efficacy in locomotor recovery after 9 weeks of supplementation, along with protection of spinal cord tissue (increased neuronal and oligodendrocyte survival, decreased microglial activation, and preserved axonal integrity). Rats fed the high-dose Fortasyn® Connect-enriched diet for 4 weeks showed a much greater enhancement of locomotor recovery, with a faster onset, than rats fed the medium dose. Bladder function recovered quicker in these rats than in rats fed the control diet. Their spinal cord tissues showed a smaller lesion, reduced neuronal and oligodendrocyte loss, decreased neuroinflammatory response, reduced astrocytosis and levels of inhibitory chondroitin sulphate proteoglycans, and better preservation of serotonergic axons than those of rats fed the control diet. These results suggest that this multi-nutrient preparation has a marked therapeutic potential in spinal cord injury, and raise the possibility that this original approach could be used to support spinal cord injured patients.

1. Introduction

Spinal cord injury (SCI) has an annual incidence of fifteen to forty cases per million (Sekhon and Fehlings, 2001). Despite the increased survival due to advances in emergency medicine, there are no neuroprotective or neuroregenerative treatments available, and many SCI patients suffer from lifelong motor and sensory impairment, with an array of other clinical manifestations (loss of body temperature control, respiratory, bowel and bladder, and sexual dysfunction, as well as neuropathic pain and spasticity). A high proportion of SCI patients have some degree of bladder voiding disturbance (Manack et al., 2011), and improving bladder and bowel function is the first to second highest priority in SCI patients, remaining a top research priority (van Middendorp et al., 2014).

In SCI, the initial primary damage due to physical trauma is followed by a secondary wave of injury leading to widespread tissue damage and degeneration, including the destruction of axonal tracts that control motor, sensory, and autonomic functions (Anderson and Hall, 1993; Profyris et al., 2004; Priestley et al., 2012). In human and in experimental SCI, cystic cavities develop, surrounded by a glial scar, and both form a powerful barrier against axonal regrowth and reconnection (Yiu and He, 2006). Because axons in the central nervous system regenerate poorly, neurological recovery is limited, but can be substantially improved when sparing even a few axons (<12%) (Fehlings and Tator, 1995; Basso et al., 1996).

Abbreviations: APC, adenomatous polyposis coli tumour suppressor protein; BBB, Basso, Beattie, and Bresnahan locomotor scale; CSPG, chondroitin sulphate proteoglycan; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FC, Fortasyn® Connect; GFAP, glial fibrillary acidic protein; 5-HT, serotonin; Iba1, ionized calcium binding adapter molecule 1; MBP, Myelin basic protein; NeuN, Neuronal Nuclei; PSD-95, postsynaptic density protein 95; PUFA, polyunsaturated fatty acid; SCI, spinal cord injury; UMP, uridine 5′-monophosphate.

* Corresponding author at: Centre for Neuroscience and Trauma, The Blizard Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London E1 2AT, UK.
E-mail address: a.t.michael-titus@qmul.ac.uk (A.T. Michael-Titus).
[1] Joint first authors.
Available online on ScienceDirect (www.sciencedirect.com).

http://dx.doi.org/10.1016/j.nbd.2015.09.007
0969-9961/

Numerous pharmacological therapies have been evaluated in SCI, although none have met with substantial success. An ideal agent for treatment should protect the spinal cord against the secondary damage, and stimulate or support neurite growth. We have shown previously that long-chain omega-3 PUFAs, such as DHA and EPA, are neuroprotective when administered acutely following SCI in rodents (King et al., 2006; Lim et al., 2010). Acute administration combined with dietary supplementation of DHA for 6 weeks post-SCI led to even better functional outcome and tissue protection (Huang et al., 2007a). Work developed in the Wurtman laboratory over the last decade, focusing on the concept of supporting synaptogenesis in neurodegenerative disorders (Wurtman et al., 2006, 2009a), has resulted in a specific formulation of nutrients registered as Fortasyn® Connect (FC). FC includes the omega-3 PUFAs DHA and EPA, but also choline, UMP, phospholipids, folic acid, vitamins B6, B12, C, E, and selenium (van Wijk et al., 2014). These form the necessary precursors and supporting nutrients for enhancing membrane formation and function through the biosynthetic "Kennedy pathway" (Kennedy and Weiss, 1956). Dietary supplementation with this preparation results in an increase in synaptic proteins, neurites, and dendritic spines (Wang et al., 2005; Sakamoto et al., 2007; Cansev et al., 2009). FC is the neuroactive component of Souvenaid®, a multi-nutrient drink that has shown efficacy in Alzheimer's disease (Scheltens et al., 2010, 2012). We hypothesized that administering this specific mixture of nutrients after SCI would improve functional outcome through neuroprotection and the support of regenerative processes. We investigated its therapeutic potential in a rat model of compression SCI (Nystrom et al., 1988), validated previously (Huang et al., 2007a, b). Chronic supplementation with FC led to markedly enhanced neurological outcome and tissue protection. Our data suggest that increased sparing of white matter, progressive tissue repair, and changes in the glial scar induced by the FC treatment synergised to promote functional recovery.

2. Materials and methods

2.1. Experimental design

Two experiments (Study 1 and Study 2) were conducted in rats to assess the therapeutic potential of FC in SCI, using two durations of treatment, and three concentrations (Fig. 1). Adult Sprague Dawley (Study 1) or Wistar (Study 2) rats were used, depending on availability (Charles River Laboratories, Harlow, UK). Animal procedures were approved by the Animal Care Committee of Queen Mary, University of London, and the UK Home Office, and were in accordance with the UK Animals (Scientific Procedures) Act of 1986, and with the EU Directive 2010/63/EU for the protection of animals used for scientific purposes. Efforts were made to minimize the number of animals used. The ARRIVE guidelines for reporting research (http://www.nc3rs.org.uk/ARRIVE) were followed throughout the design and execution of all experiments.

Group size was decided on the basis of our experiments with neuroprotective compounds in rat SCI (King et al., 2006; Huang et al., 2007a, b). The assignment of rats to treatment groups was pseudo-randomized before surgery to achieve even distribution of rats of similar body weights across groups. All rats were fully paralyzed after waking up from surgery. Functional recovery was assessed in an open arena using the BBB locomotor scale (Basso et al., 1995). Any rat that did not reach a BBB score of 3 (extensive movements of two joints for each hindlimb) after 14 days of injury was removed from the experiment, according to the UK Home Office requirements for this model. No statistical outliers were excluded in any of the experiments. Experimenters were blind to treatment groups. BBB scoring was judged independently by two experimenters who agreed on a final score. A third experimenter scored weekly, for scoring consistency.

In Study 1 (Fig. 1A), injured rats were fed for 9 weeks after compression SCI with either a medium-dose FC-enriched diet or a control diet. At the end of the experiment, transverse tissue sections at the injury site were processed for immunofluorescence.

In Study 2 (Fig. 1B), injured rats were fed for 4 weeks after compression SCI with either a control diet or a diet enriched with one of three doses of FC (low, medium, or high dose). Four weeks was chosen as the endpoint because the functional recovery of Wistar rats treated with a control diet showed a plateau at this time point. At the end of the experiment, horizontal tissue sections at the injury site were processed for immunofluorescence.

2.2. Surgical procedures and post-operative care

A compression injury was delivered at vertebral thoracic level 12 (T12) (Nystrom et al., 1988; Huang et al., 2007a, b). The rats (200–250 g) were anaesthetized using 4% isoflurane (Abbott Laboratories, Berkshire, UK), and anaesthesia was maintained using 1.5–2% isoflurane

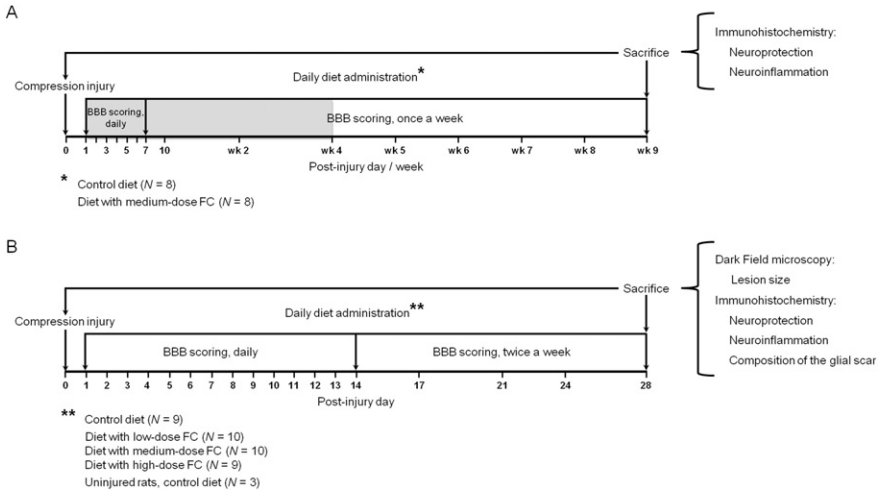

Fig. 1. Following compression SCI, rats were fed a FC-enriched diet or a control diet, for either 4 or 9 weeks. In study 1 (A), 16 female rats were fed for 9 weeks after compression SCI with either a control diet or a diet enriched with FC at the medium dose. Locomotor recovery was scored daily during the first week, and then once a week. At the end of the experiment, rats were sacrificed, and transverse sections of the compression area were processed for immunohistochemistry. The area highlighted in grey shows the corresponding length of study 2, for comparison. In study 2 (B), 41 female rats were fed for 4 weeks with either a control diet or a diet enriched with FC at one of three possible doses. Locomotor recovery was scored daily during the first 2 weeks, and then twice a week. At the end of the experiment, rats were sacrificed, and horizontal sections of the compression area were processed for dark field microscopy and immunohistochemistry. Tissues from uninjured rats were used as controls for the effects of injury and treatment.