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Long-term efficacy of Docosahexaenoic acid (DHA) for Spinocerebellar Ataxia 38 (SCA38) treatment: an open label extension study

Manes M[1], Alberici A[1], Di Gregorio E[2,3], Boccone L[4], Premi E[1], Mitro N[5], Pasolini MP[6], Pani C[4], Paghera B[7], Orsi L[8], Costanzi C[9], Ferrero M[3], Tempia F[10], Caruso D[5], Padovani A[1], Brusco A[2,3], Borroni B[1]

[1] Neurology Unit, Department of Clinical and Experimental Sciences, University of Brescia, Brescia, Italy

[2] Medical Genetics Unit, Città della Salute e della Scienza University Hospital, Turin, Italy

[3] Department of Medical Sciences University of Turin, Turin, Italy

[4] Ospedale Regionale Microcitemie, AOBrotzu, Cagliari, Italy

[5] Department of Pharmacological and Biomolecular Sciences, University of Milan, Milan, Italy,

[6] Neurophysiology Unit, "Spedali Civili", Brescia, Italy

[7] Department of Nuclear Medicine, University of Brescia, Brescia, Italy

[8] Neurologic Division 1 Department of Neuroscience and Mental Health AOU Città della Salute e della Scienza di Torino, Turin, Italy

[9]Neurology Unit, Cremona Hospital, Cremona, Italy

[10]Neuroscience Institute Cavalieri Ottolenghi (NICO) and Department of Neuroscience, University of Turin, Turin, Italy

Corresponding author:
Barbara Borroni, MD
Centre for Ageing Brain and Neurodegenerative Disorders,
Neurology Unit, Department of Clinical and Experimental Sciences
University of Brescia
Piazzale Spedali Civili 1, Brescia, Italy
Email: bborroni@inwind.it

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Abstract

Background: Spinocerebellar Ataxia 38 (SCA38) is caused by *ELOVL5* gene mutation, with significant reduction of serum docosahexaenoic acid (DHA) levels. DHA supplementation has been proven effective at short-term follow-up.

Objective: To evaluate long-term safety and efficacy of 600 mg/day oral DHA in SCA38 by a 2-year open label extension study.

Methods: Nine SCA38 patients underwent standardised clinical assessment at 62 (T1), 82 (T2) and 104 (T3) weeks, and compared to pre-treatment scores (T0). Brain 18-Fluorodeoxyglucose Positron Emission Tomography and electroneurography were performed at T0 and T3.

Results: We found a significant maintenance of clinical symptom improvement at each follow-up time-point (p<0.001) as compared to T0, a sustained increase of cerebellar metabolism at T3 as compared to T0 (p=0.013), and no worsening of neurophysiological parameters. No side effect was recorded.

Conclusions: Long-term DHA supplementation is an eligible treatment for SCA38.

Introduction

We have recently suggested supplementation with docosahexaenoic acid (DHA) as an effective treatment in Spinocerebellar ataxias 38 (SCA38), an autosomal dominant disorders phenotypically characterized by gait and limb ataxia, followed by dysarthria, dysphagia, and ophtalmoparesis, and in which *pes cavus* and hyposmia may be considered distinctive associated features (Borroni et al.; Manes Ann Neurol).

SCA38 is caused by mutations in the *ELOVL5* gene (Di Gregorio), which encodes an elongase enzyme involved in the synthesis of very long-chain fatty acids with a high and specific expression in Purkinje cells (Tempia). The mutations lead to a reduction of serum DHA of the omega-3 polyunsaturated fatty acid class (Di Gregorio).

Given the reduction of DHA levels in SCA38 patients, we have previously evaluated the safety and efficacy of DHA supplementation, and we carried out a double-blind randomised placebo-controlled study for 16 weeks, followed by an open-label study with overall 40-week DHA treatment. We demonstrated that oral DHA supplementation is a safe and effective treatment, exerting clinical efficacy and ameliorating cerebellar metabolism (Manes et al).

Here, we report the results of the subsequent long-term open-label extension study, aimed at evaluating the long-term safety and efficacy of oral DHA supplementation (600 mg/day) on clinical disease course, cerebellar metabolism and neurophysiological parameters up to two years follow-up.

Methods

This is a 104-week open-label extension study of a previous 16-week double blind, randomised, placebo-controlled trial followed by a 40-week open label phase. The patients included in the previous trial have been periodically evaluated at the Centre for Ageing Brain and Neurodegenerative Disorders, Department of Clinical and Experimental Sciences, University of Brescia, Italy up to two-years. We considered 9 patients out of 10 with the *ELOVL5* c.689G>T (p.Gly230Val) variant, as one died during the study (death not related to the disease). Mean age was 48.7±10.8, and the mean age at onset was 38.4±6.8; six patients were females.

The study was approved by the ethics committee of the Brescia Hospital, Italy (NP1821) and was conformed to the Declaration of Helsinki principles. Written informed consent was obtained from all patients. Trial was registered at ClinicalTrials.gov (NCT03109626).

During this 104-week extension, each patient received 600 mg/day b.i.d. of a fish oil derived DHA (Sofedus srl, Milan, Italy). Inclusion and exclusion criteria were previously published (Manes).

Each patient underwent standardised clinical assessment at 62 week (T1), 82 week (T2) and 104 week (T3) DHA supplementation follow-up, as compared to baseline (T0); moreover, we assessed cerebellar metabolism and neurophysiological parameters at T3, as compared to T0 (see **Figure 1**, study design).

Standardised clinical assessment included Scale for the Assessment and Rating of Ataxia (SARA, range 0-40)[8] and the International Cooperative Ataxia Rating Scale, (ICARS, range 0-100)[9]. As this was an open label study, to ensure blindness in the clinical assessment scoring, at each time-point (T0, T1, T2 and T3) neurological examination was video-recorded and analysed in blindness by AA, who was unaware of time-point, as the videos were presented randomly.

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Cerebellar metabolism was assessed by brain 18-fluorodeoxyglucose (FDG) Positron Emission Tomography (PET) scan, as previously reported (Manes). Briefly, processing and statistical analyses were carried out running on MATLAB (http://it.mathworks.com/products/matlab/) (Mathworks Inc., Sherborn, Mass., USA) and Statistical Parametric Mapping (SPM, http://www.fil.ion.ucl.ac.uk/spm/software/SPM12/), a fully automated, unbiased and operator independent software. Cerebellar metabolism changes were evaluated by non-parametric permutation

test (10,000 permutation, Statistical nonParametric Mapping 13, http://warwick.ac.uk/snpm; T0 vs. T3), and the threshold set at $p<0.05$, Family-Wise Error (FWE) cluster-level corrected[11].

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Electromyography/electroneurography (EMG/ENG) examination was performed according to standard procedures.

To assess the effect of DHA treatment over time, we applied one-way mixed ANOVA with TIME as within-subjects factors. Mauchly's test was used to test for assumption of sphericity, while Greenhouse–Geisser epsilon determination was used to correct in case of sphericity violation. Results were expressed as mean values±standard error (SE), unless otherwise specified. Statistical analyses were performed using SPSS version 21 (SPSS, Inc., Chicago, IL, USA).

Evaluation of *ELOVL5* expression was assessed by quantitative real-time PCR on retrotranscribed mRNA extracted from nine patients' PAX-gene blood at different time points of DHA supplementation (see REFERENCE): 62 week (T1), 82 week (T2) and 104-week (T3). The mean and S.E. was calculated and normalised to baseline (T0). Data analysis was performed by GraphPad using a second degree polynomial regression curve.

Results

Nine patients completed the 104-week follow-up. No discontinuations occurred, and no side effects were reported during DHA supplementation at established dosage.

We found a significant maintenance of improvement of clinical symptoms over time. Repeated measures ANOVA performed on total SARA score revealed a main effect of time ($F(3,24)$=9.82, p<0.001, partial η^2 =0.55). Post-hoc analysis showed a significant reduction of SARA score values at T1 (6.0±1.4, p=0.001), T2 (6.4±1.8, p=0.01) and T3 (6.4±1.8, p=0.013) compared to baseline (8.9±1.4) (**Figure 2, panel A**). No significant differences among T1, T2 and T3 time-points were reported.

The same main effect of time was shown for ICARS score ($F(1.35,10.86)$=8.07, p=0.012, partial η^2 =050). Post-hoc analysis showed a significant reduction of ICARS score values at T1 (13.1±3.9, p=0.008), T2 (13.0±4.0, p=0.012) and T3 (13.8±4.3, p<0.001) compared to baseline (18.7±4.3) (**Figure 2, panel A**). No significant differences among T1, T2 and T3 time-points were reported.

A significant difference in cerebellar metabolism between T0 and T3 was observed, with a sustained significant increase of cerebellar metabolism at T3 as compared to T0 in the left exterior cerebellar lobe (x,y,z= -44, -60, -26; T=10,26; p=0.013, cluster size=227) (see **Figure 2, panel B**). Importantly, no significant differences in the opposite contrast (T0>T3) were found at the pre-established threshold, ruling out worsening of regional cerebellar metabolism after 104-week DHA treatment.

At EMG/ENG, motor and sensory conduction velocities did not significantly worse during 104-week DHA treatment.

We showed a slight but significant reduction of *ELOVL5* expression in blood comparing each time point with baseline, although a high degree of variability was present. An increased expression was also present in all cases at T3 (r^2: 0.8989)(Figure 3).**Discussion**

In a double-blind, randomised, placebo-controlled study followed by a short-term open label phase, we have previously demonstrated that oral DHA is a safe and effective treatment for SCA38 patients (Manes). However, we did not know whether the beneficial effect of this treatment might be extended over time, with no long-term side effects and with no tolerance mechanisms occurring.

In the present study, by a long-term follow-up, evaluating patients up to 2 years, we have demonstrated that oral DHA at a dosage of 600 mg/day is the eligible treatment for SCA38. By a comprehensive assessment, we reported that DHA was able to stabilize clinical symptoms, as measured by SARA and ICARS scales, to maintain the significant amelioration of cerebellar metabolism, and to avoid the worsening of neurophysiological measures. Moreover, we have further proven that DHA supplementation was well tolerated, affordable and easy to administer dietary intervention, with no side effect and with a relatively low cost.Clinical findings were in line with the slightly decreased expression of *ELOVL5* in patients' blood. We also noted a final increment of *ELOVL*5 expression, which might suggest a compensatory mechanism after long-term administration and possibly an adjustment of the therapy if prolonged for many years..

The use of DHA supplementation in SCA38 stemmed from the initial observation that *ELOVL5* mutations lead to an increased amount of the encoded protein with a mislocalization of the aberrant form in the perinuclear area instead of endoplasmic reticulum and by a decrease of its final products, in particular DHA, in patients' serum (Di Gregorio et al., 2014). Thus, DHA supplementation can act by compensating the decrease of very long chain fatty acids and by lowering ELOVL5 aberrant protein via a transcriptional feedback loop.

Given the open-label study design, we tried to minimize possible biases in the clinical evaluation scoring, by video-recording and randomising the clinical assessments and making them evaluating by a neurologist who was unaware of the follow-up time-points. Furthermore, we used an unbiased,

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automated, and operator independent software for analysing imaging data, which allowed us to exclude regional worsening of cerebellar metabolism at 2 year follow-up.

Even though carried out in a small sample of patients, this study suggests that DHA supplementation is an effective treatment approach in SCA38. Its intake for the whole lifewould be beneficial but now we have no sufficient data to exclude the development of tolerance associated to a prolonged administration. Based on our observation, we might speculate that DHA treatment is even beneficial in the presymptomatic stage of the disease to delay disease onset and slow the progression of symptoms.

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Author Contributions

B.A., and B.B. contributed to the concept and study design. M.M., A.A., D.G.E., B.L., P.E., M.N., P.M.P., P.C., P.B., O.L., C.C., F.M., T.F., C.D., P.A., B.A., B.B. contributed to the data acquisition and analysis. M.M., A.A., B.A., and B.B. drafted the manuscript and figures, and all authors approved the final version.

Potential Conflicts of Interest

The authors do not have any potential conflict of interest.

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Legend to figures.

Figure 1. Study design of the open-label extension study with DHA supplementation in SCA38.
Black blocks indicate the time points of clinical assessment; black arrows indicated the time points of brain 18-fluorodeoxyglucose Positron Emission Tomography (FDG-PET) and electroneurography (ENG). T0: baseline; T1: 62 weeks follow-up; T2: 82 weeks follow-up; T3: 104 weeks follow-up. T*: previously published open label follow-up study (Manes et al., 2017) at 40 weeks follow-up.
wk: weeks.

Figure 2. Clinical assessment and cerebellar metabolism in the open-label extension study.
Panel A. SARA and ICARS scores at baseline (T0), after 62 weeks (T1), 82 weeks (T2) and 104 weeks (T3) DHA treatment in SCA38 patients.
*p-value<0.05.
Panel B. Pattern of cerebellar metabolism in SCA38 patients at T0 vs. T3 (T0<T3) ($p<0.05$, FWE corrected). See results for details. The results are superimposed on a 3D standardized template template.
P: posterior, L: left; R: right.

Figure 3. *ELOVL5* expression analysis in DHA-treated patients' blood. Expression was evaluated by Quantitative real-time PCR on retrotranscribed mRNA extracted from PAX-gene blood at different time points (62 week (T1), 82 week (T2) and 104 week (T3)). *ELOVL5* was normalized against *TBP* gene. The average value and S.E. is shown. Statistical analysis showed the data had a best fit with a second degree polynomial regression curve ($r^2= 0.8989$), with an initial reduction of expression and a final increase to levels close to baseline.