Abstract No. 385

Oxidative stress in multiple sclerosis: Effect of dietary supplementation with coenzyme Q10

Marcello Moccia[1], Antonio Capacchione[2], Roberta Lanzillo[1], Fortunata Carbone[3], Francesco Saccà[1], Anna De Rosa[1], Antonio Carotenuto[1], Roberto Albero[1], Giuseppe Matarese[3-4], Raffaele Palladino[5-6], and Vincenzo Brescia Morra[1]

1. Multiple Sclerosis Clinical Care and Research Center, Department of Neuroscience, Reproductive Science and Odontostomatology, Federico II University, Naples, Italy. 2. Medical Affairs Department, Merck, Italy. 3. Institute of Experimental Endocrinology and Oncology, National Research Council (IEOS-CNR), Naples, Italy. 4. Department of Molecular Medicine and Medical Biotechnologies, Federico II University, Naples, Italy. 5. Department of Primary Care and Public Health, Imperial College, London, UK. 6. Department of Public Health, Federico II University, Naples, Italy.

INTRODUCTION

- Experimental evidence suggests that oxidative stress has an important role in the pathogenesis of multiple sclerosis (MS) [1].
  - In relapsing remitting MS (MS), oxidative stress seems strictly associated with inflammatory activity.
  - In progressive MS, neurodegenerative aspects of MS can further amplify oxidative damage [1].
- The measurement of oxidative biomarkers in accessible body fluids (i.e., serum) seems particularly promising in tracking the MS disease course [1-5].
- Investigations that focused on natural compounds with antioxidant activity, such as uric acid (UA) and bilirubin, provide indirect evidence of increased oxidative stress in MS [2-3].
  - UA is a powerful free radical scavenger and is easily detectable in human serum; UA levels are reduced in MS patients compared with controls, and are associated with clinical relapses, progression of disability and a deterioration in cognitive outcomes [2-3].
- Advanced oxidative protein products are markers of oxidative damage, are increased in MS patients compared with controls, and are directly associated with MS-related disability [4].
- Neuro-inflammation can be affected by oxidative stress and levels of cytokines such as Tumor Necrosis Factor (TNF) or Interleukin (IL)-17 can be modified [5].
- The use of antioxidants is expected to change the course of neuroinflammation, which should impact MS-related outcomes [6].
  - **Coenzyme Q10 (CoQ10)**, an essential compound present in nearly every cell of the human body, seems particularly promising as it has been shown to delay the onset and alleviate the progression of MS in an experimental mouse model, with statistically significant differences seen in the expression of IL-4, IL-10, IL-12 and TNF [5-6].
  - There is preliminary evidence of reduced CoQ10 levels in MS patients compared with controls. In addition, CoQ10 levels showed an inverse relationship with MS severity. In two clinical studies, dietary supplementation with CoQ10 has been shown to decrease markers of oxidative stress and neuro-inflammation in patients with relapsing-remitting MS (RRMS) [7-8].
  - Previous studies of CoQ10 in MS are limited as they included a restricted number of markers of oxidative damage and neuro-inflammation, and they did not evaluate the possible activity of CoQ10 in preventing or reducing oxidative damage on serum proteins or nucleic acids [7-10].
  - There is preliminary evidence for the potential efficacy of CoQ10 in treating depressive symptoms and fatigue in MS [8-10].

OBJECTIVES

- To evaluate possible associations between laboratory markers of oxidative stress and CoQ10 dietary supplementation, in a population of MS patients treated with subcutaneous high-dose interferon beta-1a (44 mcg, three times per week).
- To evaluate possible associations between different markers of oxidative stress and MS clinical characteristics in MS patients, with or without CoQ10 dietary supplementation at different timepoints.

METHODS

Study design

- MS patients were recruited at the MS Clinical Care and Research Centre of the "Federico II" University (Naples, Italy). Details of the study design are presented in Figure 1A.

Population

- We extracted from our clinical and biological database patients who had dietary supplementation with CoQ10. Dietary supplementation with CoQ10 was performed at a daily dose of 200 mg for a 3-month period, in accordance with current indications and preliminary evidence for CoQ10 dietary supplementation in MS. The formulation of CoQ10 supplement is currently available on the Italian market (Skatto® 100 mg/mL, Chiesi Farmaceutici SpA).
- Inclusion criteria:
  - Clinical and radiological diagnosis of RRMS;
  - Age >18 years old;
  - Treatment with subcutaneous high-dose interferon beta-1a (44 mcg, three times per week).
- Exclusion criteria:
  - Recent corticosteroid treatment (<6 months);
  - Exposure at any time to azathioprine, cladribine tablets, cyclophosphamide, cyclosporine A, methotrexate, or any other immunosuppressive agent;
  - History of malignancy, major systemic disease or other illness that would in our opinion interfere with the interpretation of study results.

Variables of interest

- Laboratory outcomes:
  - *Markers of free radical scavenging activity*: UA and bilirubin;
  - *Markers of oxidative damage*: 8-hydroxydeoxyguanosine (a product of the oxidative DNA damage), protein carbonyls (a common protein oxidative modification), and oxidation occurring within lymphocytes;
  - *Markers of neuroinflammation*: IL-2, IL-4, IL-6, IL-10, TNF, interferon-gamma and IL-17A.
- Clinical outcomes:
  - Demographic features (age, gender);
  - Concomitant diseases and treatments;
  - MS clinical features (disease duration, occurrence of relapses, Expanded Disability Status Scale [EDSS]).
- Patient-reported outcomes:
  - MS Neuropsychological Questionnaire (MSNQ);
  - Visual Analogic Scale (VAS) for the presence of pain, fatigue and headache;
  - Modified Fatigue Impact Scale (MFIS);
  - Beck's Depression Inventory (BDI).

Statistics and sample size calculation

- Using linear regression to analyse the main outcome, a sample of 60 subjects for a total of 180 records was considered suitable to obtain an acceptable estimate (α=0.05; power=0.8; OR=2.0).
- Preliminary comparisons were performed with χ^2 test, Fisher's exact test or t-test, as appropriate.
- Mixed-effect linear regression models were used to assess the difference between groups in laboratory measures and clinical findings over time, considering the presence or absence of treatment. Results are presented as coefficient (Coeff) and 95% confidence intervals (95% CI). All the variables included in the model were tested for multicollinearity (variance inflation factor [VIF] smaller than 2.5).
- Covariates included in the statistical models were age, gender, disease duration, duration of interferon treatment, baseline EDSS and, for analysis of UA levels, creatinine.
- Stata 12.0 and Microsoft Excel were used for data processing and analysis. Results have been considered statistically significant if p<0.05.

Table 1. Demographic, clinical and laboratory characteristics of the two study groups at baseline. Data are presented as mean ± standard deviation, median (min−max), number (%), as appropriate. P-values are presented from t-test, chi-square test and Fisher's exact test.

	Group receiving CoQ10 first (n=31)	Group receiving CoQ10 later (n=30)	P-value
Age, *years*	42.1±1.8	40.9±1.6	0.640
Gender, *female*	20 (64.5%)	19 (63.3%)	0.7
Disease duration, *years*	10.9±1.2	11.1±1.5	0.953
EDSS	2.5 (1.0-5.5)	2.5 (1.0-6.0)	0.497
Duration of interferon β-1a treatment, *years*	5.2±0.7	4.5±0.9	0.556
Naïve to interferon β-1a treatment	15 (48.3%)	15 (50%)	
Relapses in previous 2 years	0.24±0.1	0.24±0.1	0.237
Uric acid, *mg/dL*	4.6±0.2	4.6±0.2	0.898
MSNQ	38.6±2.6	38.0±2.1	0.858
VAS pain	6.0±0.4	5.9±0.4	0.882
VAS fatigue	5.2±0.5	5.3±0.4	0.929
VAS headache	4.7±0.6	5.1±0.5	0.559
MFIS	26.4±1.8	28.4±1.8	0.252
BDI	11.5±1.2	13.6±1.9	0.349

BDI, Beck's Depression Inventory; **CoQ10**, coenzyme Q10; **EDSS**, Expanded Disability Status Scale; **MFIS**, Modified Fatigue Impact Scale; **MSNQ**, Multiple Sclerosis Neuropsychological Questionnaire; **VAS**, Visual Analogic Scale.

RESULTS

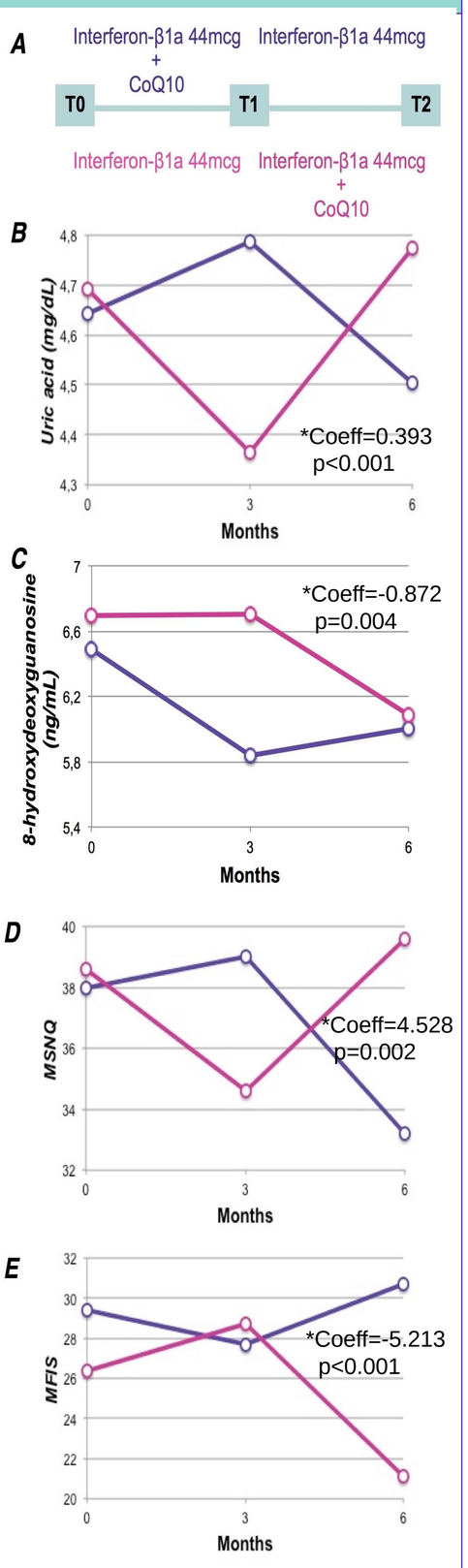
Baseline characteristics

- Demographic, clinical and laboratory characteristics in the groups receiving CoQ10 in the first part of the study (from T0 to T1) or later (from T1 to T2) are reported in Table 1.
- No statistically significant differences were observed.

Laboratory and clinical outcomes

- Patients receiving CoQ10 supplement presented with increased UA (Coeff=0.393; 95% CI=0.220−0.566; p<0.001), compared with non-CoQ10 supplementation status.

Figure 2. Study design with cross-over between groups (A), and profile plots showing variations of uric acid levels (B), 8-hydroxydeoxyguanosine (C), MSNQ (D), and MFIS (E) at different time points (T0 = 0 months, T1 = 3 months, T2 = 6 months).



CoQ10, coenzyme Q10; **MFIS**, Modified Fatigue Impact Scale; **MSNQ**, Multiple Sclerosis Neuropsychological Questionnaire.

- Patients receiving CoQ10 supplement presented with reduced 8-hydroxydeoxyguanosine (Coeff=-0.872; 95% CI=-1.479−-0.275; p=0.004), and with a not-significant reduction protein carbonyls Coeff=-0.169; 95% CI=-0.953−0.614; p=0.672), and oxidated lymphocytes (Coeff=-1.496; 95% CI=-10.500−-7.507; p=0.745) , compared with non-CoQ10 supplementation status.
- After receiving CoQ10 supplement, patients presented with improved scores on the MSNQ (Coeff=4.528; 95% CI=1.644−7.411; p=0.002), and on MFIS (Coeff=-5.213; 95% CI=-7.775−-2.650; p<0.001), compared with non-CoQ10 supplementation status.
- EDSS variations were associated with oxidated lymphocytes (Coeff=0.007; 95% CI=0.001-0.013; p=0.024). MSNQ variations were associated with UA (Coeff=2.496; 95% CI=0.018 -4.975; p=0.038)
- No statistically significant differences were detected for relapses and EDSS variations.

CONCLUSIONS

- **Restoring an appropriate oxidative balance with CoQ10 dietary supplementation in combination with disease-modifying treatment may be responsible for an improvement in patient-related outcomes such as cognition and fatigue.**
- **In the long-term, it is possible to hypothesise that a reduction in oxidative stress might exert positive effects on the disease course of MS and, in particular, on disability.**
- **Future studies on larger populations and with longer follow-up are required to confirm the present findings.**

REFERENCES

1. Ljubisavljevic S. *Mol Neurobiol*. 2016;53:744-758.
2. Moccia M, et al. *J Neurol*. 2015;262:961-967.
3. Moccia M, et al. *Clinical Chem Lab Med*. 2015;53:753-759.
4. Oliveira SR, et al. *J Neurol Sci*. 2012;321:49-53.
5. Soleimani M, et al. *Iran Biomed J*. 2014;18:203-211.
6. Mao P, et al. *Biochim Biophys Acta*. 2013;1832:997-1003.
7. Gironi M, et al. *J Immunol Res*. 2014;2014:961863.
8. Sanoobar M, et al. *Int J Neurosci*. 2013;123:776-782.
9. de Bustos F, et al. *Acta Neurol Scand*. 2000;101:209-211.
10. Lazzarino G, et al. *Mol Neurobiol*. 2016.

ACKNOWLEDGEMENTS

The authors acknowledge Merck-Serono for the support during the study.

DISCLOSURES

This research was partially supported by Merck Serono S.p.A. (Italy), an affiliate of Merck KGaA, Darmstadt, Germany.

Presented at Italian Society of Neurology, October 14th-17th, 2017