ORIGINAL ARTICLE

Synergistic Effects of Coenzyme Q10 & L-Carnitine on Oligodendrocyte Necrosis and Myelination in a Rat Model of Multiple Sclerosis

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ABSTRACT

Objective: Determining the synergistic effect of Coenzyme Q10 & L-Carnitine on Oligodendrocyte necrosis and Myelination, in a rat model of Multiple sclerosis.

Study Design: Laboratory based experimental study.

Place and Duration of Study: The study was conducted at the Department of Anatomy, Islamic International Medical College Rawalpindi, Pakistan from March 2022 to May 2022 in collaboration with NIH Islamabad, for a duration of 12 weeks.

Methods: A total of fifty male Sprague Dawley rats were divided into five randomized groups, each with a distinct treatment plan. While Group 1 received a standard diet, the remaining four groups were induced with Multiple Sclerosis and administered 0.2% Cuprizone (CPZ) over a period of 12 weeks. After four weeks, Group 3 was given 150 mg/kg/day of Coenzyme Q10/Ubiquinone (CoQ10), Group 4 received 100 mg/kg/day of L-Carnitine (L-Car), and Group 5 was treated with a combination of both, all while still receiving CPZ. Upon completion of the 12-week protocol, the rats were sacrificed, and their brains were extracted. H & E staining was performed on coronal sections to assess any changes in oligodendrocyte necrosis, while Luxol Fast Blue (LFB) staining was utilized to visualize alterations in myelination.

Results: The combination of CoQ10 and L-Car was significantly better than the single agents in controlling the oligodendrocyte necrosis and controlling vacuolation of myelin, as evidenced by ANOVA and F-test.

Conclusion: This study has unequivocally demonstrated that taking CoQ10 and L-Car together has a greater effect on promoting myelination and preventing oligodendrocyte necrosis compared to using them individually. Therefore, it is highly recommended to prescribe both medications simultaneously for those with multiple sclerosis, as it can potentially provide greater advantages for patients.

Keywords: *L-Carnitine, Multiple Sclerosis, Oligodendrocyte, Ubiquinone*.

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Introduction

Multiple Sclerosis (MS) is a chronic neurodegenerative disease, targeting the CNS. It is believed to be autoimmune, mediated by autoreactive lymphocytes, that cross the blood-brain barrier, and enter the CNS, where they cause local inflammation, resulting in demyelination, oligodendrocyte loss, and axonal degeneration.[1] Myelin loss results in altered tissue excitability, leading to cognitive deficits, without obvious locomotor impairment.[2] Almost 50% of the total patients also experience depression-like symptoms in MS.[3] The rate of MS is 50-70 cases per 100,000 people worldwide, in Pakistan, although the rate is

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10 cases per 100,000 population, with the advances in diagnostic facilities, this number is increasing. It is considered as most common cause of neurological disability in young adults. There is no established treatment for MS, but some drugs are considered useful in enhancing myelination, thus helping minimize the symptoms and neurological deficits, especially the emerging role of antioxidants is considered to be revolutionary.[4]

Coenzyme Q10 (CoQ10), also known as ubiquinone, is an endogenous lipid. Mainly it is synthesized intracellularly, while a very small amount is obtained through some food items like fish, whole grains, and organ meat.[5] It is involved in the transport of electrons, playing a vital role in the mitochondrial respiratory chain. CoQ10 also can regenerate and recycle other antioxidants like vitamins C and E.[6] Because of its antioxidant effects on multiple systems of the body, CoQ10 is now considered the third most consumed supplement worldwide.[7] Behnam Khalilian and Soheila Madadi (2021) showed a positive effect of CoQ10 on remyelination and inflammation in the Corpus Callosum in the CPZ model of MS.[8]

L-carnitine, an amino acid, plays a crucial role in maintaining energy balance and facilitating metabolism in different tissues. Acting as a transporter, carnitine helps move long-chain fatty acids from the cytoplasm to the mitochondria, where they are broken down for energy in a process called beta-oxidation, saving a cell from oxidative damage.[9] Previous studies by Gharignia (2024) and Tayyaba Qureshi (2024) have shown the neuroprotective effects of L-carnitine in restoring normal histology in animal models of MS.[10,11] The aim of this study was to combine the two antioxidants and compare their synergistic effects on the oligodendrocyte necrosis, with the effect of individual agent.

Cuprizone, a well-known copper-chelating agent, is known to be harmful to the myelin sheaths. The effects of demyelination are observed three weeks after starting the cuprizone diet, and significant demyelination is seen worldwide by the sixth week. However, the replacement of the cuprizone diet with a normal one activates spontaneous remyelination. The success of this remyelination process depends on the maturity of oligodendrocyte precursor cells (OPCs). If the CPZ diet is sustained for 12 weeks, chronic demyelination ensues, without any activation of OPCs.[12]

Methods

The study was conducted at the Department of Anatomy, Islamic International Medical College Rawalpindi, Pakistan from March 2022 to May 2022 in collaboration with NIH Islamabad, for 12 weeks after approval from the Institutional Research Review Committee of Riphah International University Islamabad on date: 12th October 2021 vide letter no: Riphah/IRC/21/63.

For this study, 50 male Sprague Dawley rats, 4–6 weeks old, weighing 200–250 g, were kept in the standard laboratory conditions, with 12-h cycles of light and dark, room temperature of 23±1 C, and relative humidity of 45±1%, in the animal house of the National Institute of Health (NIH) Chak Shehzad Islamabad.

CPZ was obtained from Beijing Solarbio Science & Technology Co. Ltd., China. The control group, designated as Group 1, received a standard diet. All other groups functioned as multiple sclerosis (MS) models and were subjected to 0.2% CPZ w/w for a duration of 12 weeks. Beginning in the 5th week, Group 3 was given an additional dose of 100 mg/kg/day of L-Carnitine. Similarly, Group 4 started receiving a supplement of 150 mg/kg/day of CoQ10 from the 5th week onwards. Group 5, known as the synergistic group, was administered 100 mg/kg/day of L-Carnitine and 150 mg/kg/day of CoQ10 starting from the 5th week, while also being continuously exposed to CPZ poisoning throughout the entire 12-week period.

After the completion of the 12-week duration of the experiment, all the rats were dissected. Tissue sections were stained with H & E, and Luxol Fast Blue stains.[11] Slides were analyzed using a light microscope (Olympus), and necrotic oligodendrocytes were counted in the Corpus Callosum area using the multipoint tool of ImageJ. Myelination was assessed using LFB-stained slides. Four pictures from different zones of each slide were taken, and then the mean was calculated.[12] Data

were entered and analyzed in the statistical package for social sciences (SPSS version 25). The ANOVA test was performed to assess whether there were statistically significant differences in the severity of myelin lesions (grades) across the different groups. F-test was used to determine if the observed differences in means are statistically significant.

Results

As oligodendrocytes are destroyed by CPZ, their nuclei become pyknotic, exhibiting shrinkage of the cell cytoplasm, dense nuclear chromatin material, and fragmentation. (Figure-1). These changes lead to the formation of dark-staining nuclear material spheres.[13] Pyknosis was graded according to the following criteria. (Table-1).

The normal group did not have any pyknotic nuclei. CPZ caused grade 3 pyknosis in group 2. After administration of CoQ10, 60% of rats showed grade 2, and 40% showed grade 1 pyknosis. The L-Carnitine group showed 70% of rats in grade 2, and 30% in grade 1. The combination group showed 60% in grade 0, while 40% in grade 1. The ANOVA test gave highly significant values, $P \leq 0.001$, F-statistics gave a value of 53.72. (Table-2).

Myelination was assessed in LFB-stained slides. The severity of myelin lesions was graded according to the following scale (Table-3).[18]

The control group had only Grade 0 myelination, representing normal nerve fibers without any myelin damage. The CPZ-treated group showed predominantly grade 3 myelination, indicating severe vacuolation and disappearance of myelinated fibers. This confirms significant demyelination due to the CPZ treatment, as expected. CoQ10 and L-Car treatments showed predominantly Grade 2 myelination, suggesting moderate myelin damage, but less severe compared to the CPZ group. The combination of CoQ10 & L-Car treatments led to mixed results with some rats showing grade 1 and others grade 2, indicating a potential protective effect, though not complete prevention of myelin damage. ANOVA gave significant results with a *P*-value of <0.001, and F-test gave value of 75.66 (Table-4).

Table-1: Grades of Pyknotic nuclei

Parameter	Grade 0	Grade 1	Grade 2	Grade 3
Pyknotic nuclei	Absent 0 nucleus/hpf	Mild Occasional nuclei/hpf	Moderate 5-20 nuclei/hpf	Severe >20 nuclei/hpf

Table-2: Statistics of Pyknotic nuclei

Source of Variation	Sum of Squares (SS)	Degrees of Freedom (df)	Mean Square (MS)	F-statistic	*P*-value
Between Groups	1923.9	4	480.98	53.72	0.001
Within Groups	402.9	45	8.90		
Total	2326.82	49			

Table-3: Grading criteria for myelin lesions

Parameter	Grade 0	Grade 1	Grade 2	Grade 3
Severity of Myelin lesions	Normal Myelination	disarrangement of nerve fibers	Formation of marked vacuoles	Disappearance of myelinated fibers

Table-4: Statistics of Myelination

Source of Variation	Sum of Squares (SS)	Degrees of Freedom (df)	Mean Square (MS)	F-statistic	*P*-value
Between Groups	47.08	4	11.77	75.66	0.001
Within Groups	7.00	45	0.156		
Total	54.08	49			

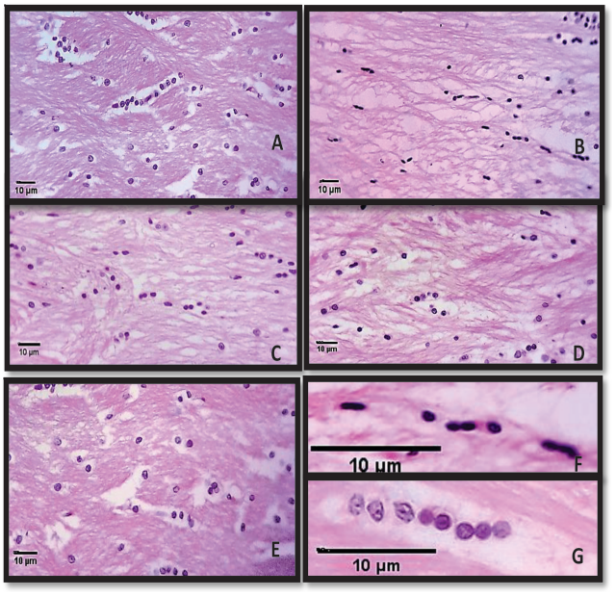

Fig.1: Photographs showing pyknotic nuclei (encircled) in H & E -stained sections (400X) A: All nuclei are normal in Group 1, no pyknosis is visible, B: Numerous nuclei are clumped together with condensed nuclear material, in Group 2. C: Pyknosis is visibly reduced in Group 3. D: Only a few pyknotic nuclei can be seen in Group 4, E: Occasionally damaged nuclei are visible in Group 5 F: Pyknotic nuclei, G: Normal Oligodendrocytes

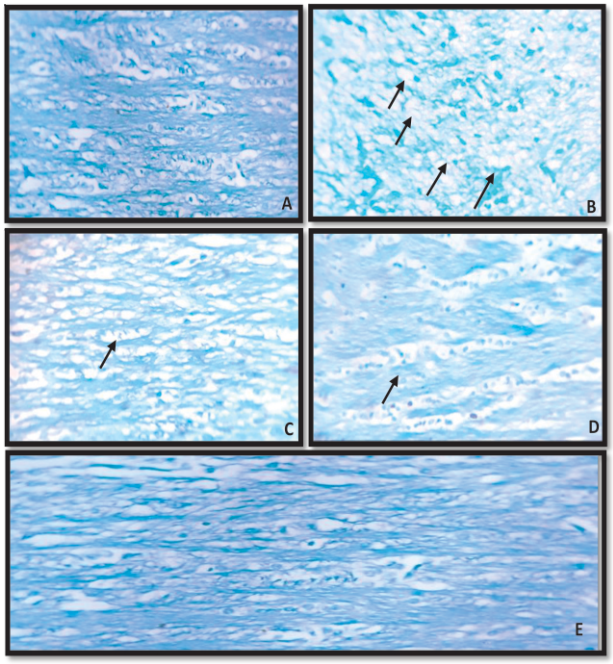

Fig. 2: Myelination in different groups A: Control Group showing normal myelination, B: CPZ group showing vacuolation (arrows), and demyelinated areas, C: Reduced vacuolation in CoQ10 group, D: L-Carnitine treated group showing enhanced myelination E: Combined group showing increased myelination

Discussion

Multiple sclerosis (MS) is an autoimmune inflammatory disease of the central nervous system (CNS) that causes oxidative damage at the mitochondrial level in oligodendrocytes, leading to a significant glial response.[1,12] Histologically, this is characterized by increased astrocytes and microglia, with activated cells secreting numerous pro-inflammatory cytokines, such as TNF-α and IL-6. This cascade of events results in neuroinflammation, myelin damage, and oligodendrocyte loss. The cuprizone (CPZ) model simulates MS by inducing similar inflammatory changes and glial responses. In this study, two antioxidants were administered to assess their ability to reverse the changes caused by CPZ poisoning. The effects of the individual agents on the myelination of nerve axons were compared to the combined effects of both agents to determine a more effective response.

The presence of pyknotic nuclei provides clear evidence of damage to oligodendroglia, indicated by a high F-statistics. CPZ has been found to cause oxidative harm to oligodendrocytes, resulting in nuclei becoming condensed. Group 2 exhibited a high number of pyknotic nuclei among the different treatment groups, but this significantly decreased in groups 3 and 4. Remarkably, Group 5 displayed the lowest number of pyknotic nuclei, indicating that the combination of CoQ10 and L-Carnitine has a greater impact in reversing the CPZ-induced damage. This reduction in pyknotic nuclei is due to the protective effect of CoQ10 and L-Carnitine at mitochondrial level that reduces oxidative stress, and neuroinflammation, caused by CPZ. In 2021, M Vaselbehagh indicated that CoQ10 reduced the number of pyknotic nuclei, in hippocampal methadone injury.[14] N Sharma in 2021 showed the neuroprotective effect of CoQ10 on oligodendrocyte apoptosis in a rat model of MS.[15] L-Carnitine was also beneficial in preserving the oligodendrocyte count, in a study by A Zidan in 2018.[16]

This study also assessed the effect of antioxidants on myelination, and its evidence was provided by the reduction of vacuolation in myelinated axons, by use of CoQ10 and L-Carnitine. The combination of these two agents demonstrated a better effect on myelination. A very low *P*-value (≤0.001) indicated

that the treatments had a clear and measurable impact on the severity of myelination lesions in the rats. Similar results were obtained previously, using CoQ10 in a rat model of MS, by B Khalilian in 2021, and N Pradhan in 2021.[5,8] In 2018, A Zidan provided histological evidence of improvement in myelination by L-Carnitine.[16] More recently, in 2024, S Gharighnia has shown the role of L-Carnitine on remyelination, in a mouse model of MS.[10]

Conclusion

The synergistic effect of CoQ10 and L-Carnitine is conclusively more effective in controlling oligodendrocyte necrosis, and myelination, as compared to individual agents. Therefore, it is recommended that prescribing these two drugs together in MS may be more beneficial for patients.

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Conflict of Interest: The authors declare no conflict of interest.

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Authors Contribution

TQ: Idea conception, manuscript writing and proofreading
SA: Study designing, manuscript writing and proofreading
AS: Data collection, data analysis, results and interpretation
HB: Data collection, data analysis, results and interpretation
TK: Data collection, manuscript writing and proofreading
TF: Data collection, manuscript writing and proofreading