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CLINICAL STUDY

Benefit of mountain spa rehabilitation and ubiquinol treatment in patients with post-COVID-19 syndrome

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ABSTRACT

BACKGROUND: SARS-CoV-2 infection is associated with inflammation, decrease in antioxidants and oxidative damage. We aimed to investigate whether ubiquinol, reduced form of coenzyme Q_{10} (CoQ_{10}), with mountain spa rehabilitation (MR) will contribute to recovering of patients with post-COVID-19 syndrome.

METHODS: The study included 36 patients on MR lasting 16–18 days. Twenty-two patients were supplemented with ubiquinol 2x100 mg/day (MRQ), 14 underwent MR without supplementation. The control group consisted of 15 healthy volunteers. Concentrations of total CoQ_{10} (ubiquinone + ubiquinol) α- and γ-tocopherol were determined in platelets (PLT), in blood and plasma, also β-carotene was determined. Plasma concentration of thiobarbituric acid-reactive substances (TBARS) was used as the oxidative stress marker. Clinical symptoms were evaluated by questionnaire.

RESULTS: MRQ group showed a significant increase in CoQ_{10}, namely in PLT by 68 %, in blood by 194 %, and in plasma by 232 %. In MR group, CoQ_{10} stayed unchanged. In both groups, the initially increased concentrations of tocopherols in PLT returned nearly to the control values. β-carotene levels decreased in both groups while TBARS decreased slightly in the MRQ group. More clinical symptoms disappeared in the MRQ group.

CONCLUSION: Accelerated recovery of patients with post-COVID-19 syndrome was proven after mountain spa rehabilitation and ubiquinol supplementation. Increased systemic and cellular CoQ_{10} concentration alleviated clinical symptoms and improved antioxidant protection of the patients. We draw attention to the importance of monitoring and ensuring adequate levels of CoQ_{10} in post-COVID-19 syndrome *(Tab. 2, Fig. 1, Ref. 44)*. Text in PDF *www.elis.sk*

KEY WORDS: COVID-19, mountain spa rehabilitation, ubiquinol, coenzyme Q_{10}, vitamins, TBARS.

Introduction

COVID-19 infections are associated with an inflammatory process, redox imbalance, reduction in antioxidants and oxidative cell damage (1). In our previous study (2) we showed that SARS-CoV-2 virus modulated platelet mitochondrial respiration, oxidative phosphorylation, and reduced endogenous CoQ_{10} production in patients after acute COVID-19. Patients included in the study had a mild-to-moderate course of COVID-19 lasting 4–7 weeks after being diagnosed with SARS-CoV-2 virus infection, were non-hospitalized and non-vaccinated. The results partially confirmed our hypothesis that mitochondrial bioenergetics and endogenous coenzyme Q_{10} could be the targets of the new SARS-CoV-2 virus (3).

CoQ is a key component of mitochondrial respiratory chain essential for ATP (adenosine triphosphate) production. Antioxidant and antiinflammatory activities of CoQ_{10} include reduction in oxidative stress and cytokines production, which may modulate human immune function. Lowering effects of CoQ_{10} on circulatory levels of inflammatory markers, CRP, IL-6, and TNF-alpha were reported in a meta-analysis of 17 randomised controlled trials characterised by chronic inflammation. It was suggested that antiinflammatory effects of CoQ_{10} can lead to a decrease in nuclear factor kappa B gene expression (4, 5). To our knowledge, no studies with CoQ_{10} supplementation in patients with post-COVID-19 syndrome have been carried out. The idea that maintaining healthy mitochondrial system induced by a healthy lifestyle and treatments

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that could support mitochondrial function might be pivotal to the recovery from SARS-CoV-2 infection was reviewed (6), and the potential treatment or adjunct therapy with mitochondrial-targeted ubiquinone (MitoQ) was proposed (7).

It has been suggested there was a need to test the suppression of deleterious effects of reactive oxygen species (ROS) and reactive nitrogen species (RNS) by administering antioxidant vitamis to COVID-19 patients (9). The role of different fat-soluble vitamins in combating SARS-CoV-2 infection has been reviewed (8) and association between oxidative stress, inflammation and pathogenesis of SARS-CoV-2 infection has been confirmed in several studies (8, 10, 11).

Vitamin E is a fat-soluble antioxidant consisting of four tocopherols and four tocotrienols (α, β, γ, δ); all forms are potent antioxidants with lipoperoxyl radical-scavenging activities. The main forms in humans are alpha-tocopherol (αT) and gamma-tocopherol (γT), while γT differs from αT only in the number of methyl groups on the chromanol ring (one methyl group is missing) but has unique biological properties that αT does not have. It is able to trap deleterious nitrogen radicals, inhibit cyclooxygenase-2 (COX-2) activity and has antiinflammatory properties (12, 13). The γT form is metabolized by a cytochrome P450-dependent process in the liver, the activity of which is inhibited by interleukin and other proinflammatory cytokines. Thus, metabolism of γT may be altered under oxidative stress, which can lead to a decrease in degradation of γT and its increased concentrations. Humans cannot synthesize tocopherols. They can be obtained only from dietary sources (13). Soybean and corn oils, walnuts, pecans, pistachios and sesame seeds are rich sources of γT, while αT is predominantly found in sunflower seeds and oils. Some studies showed an inverse correlation between circulating γT and αT concentrations (14, 15).

Retinoids (found in animal products) and carotenoids (found in fruits and vegetables) are components of vitamin A. Beta-carotene (βCar), provitamin A is the most important carotenoid needed for transformation to vitamin A in human body. Vitamin A plays a vital role in regulating immune response and reducing susceptibility to infections. Several studies reported that vitamin A and related compounds have a potentially beneficial role in the management of COVID-19 (8, 16, 17). Inflammatory response in COVID-19 increases the risk of vitamin A depletion while consequently impairing the ability of the lungs to repair damaged epithelial surfaces, which leads to lung fibrosis and reduced pulmonary capacity. Vitamin A supplementation prior to infection and during recovery may be beneficial (18).

For patients with post-COVID-19 syndrome, it is recommended to undergo rehabilitation in thermal spa (19). Yet, another approach is to situate the spa rehabilitation in the mountains which is beneficial for chronic pulmonary diseases, and improves fatique, joint pain, psychological stress, sleep disorders, and quality of life of patients with various diseases. In the High Tatra Mountains in Slovakia, mountain spa rehabilitation with special effect on chronic pulmonary diseases of patients has been used since 1898. Sanatorium of Dr. Guhr, Tatranska Polianka is located at 1,005 m above the sea level, in a forest area with dry air, favorable solar radiation, reduced partial oxygen pressure, and relatively stable mild daily temperature. These climatic conditions of the High Tatra Mountains contribute to successful rehabilitation of chronic pulmonary diseases (20).

The aim of our study was to investigate whether special spa rehabilitation in the High Tatra Mountains including exercise and physical therapy would affect antioxidant and bioenergetic capacity, oxidative stress and clinical parameters of patients after COVID-19. In addition, we wanted to find out if the simultaneous supplementation of ubiquinol would enhance the benefits and contribute to the accelerated recovery of patients after COVID-19.

Material and methods

Subjects

In May and June 2021, 36 non-vaccinated patients who were 3-6 months after hospitalization for COVID-19, were included in the study. The main clinical symptoms of the patients, such as fatique, cough, loss of smell, impaired breathing during exercise, loss of hair, depression, loss of appetite and weight are described in details (Tab. 1).

The patients underwent a rehabilitation stay in the High Tatras, Tatranska Polianka, Sanatorium of Dr. Guhr in Slovakia. MR lasted 16- 18 days. They were divided into two groups: Group 1 was composed of patients (14 men, 8 women, mean age 57.8±2.5 years) who, during their stay, in addition to physical rehabilitation, exercise and outdoor activity were supplemented with ubiquinol

Tab. 1. Selected clinical symptoms affected by MR and MRQ of patients with post-COVID-19 syndrome.

	MR1 n=10	MR2 n=10	MR1 %	MR2 %	MRQ1 n=20	MRQ2 n=20	MRQ1 %	MRQ2 %
Dry cough	3	3	30	30	9	2	45	10
Shortness of breath	6	6	60	60	13	6	65	30
Difficulty breathing	6	3	60	30	13	8	65	40
Chills	2	1	20	10	3	0	15	0
Heart palpitations	3	1	30	10	10	4	50	20
Respiratory support with Q_2	0	0	0	0	3	0	15	0
Weakness	0	0	0	0	2	1	10	5
Overall fatigue	7	2	70	20	17	7	85	35
Malaise	2	2	20	20	1	0	5	0
GIT problems	0	0	0	0	1	1	5	5
Diarrhea	1	1	10	10	3	1	15	5
Chest pain	3	1	30	10	9	3	45	15
Muscle and joint pain	10	5	100	50	11	4	55	20
Headache	4	0	40	0	8	2	40	10
Weight loss	1	1	10	10	4	0	20	0
Hearing impairment	2	0	20	0	4	0	20	0

Before MR (MR1), after MR (MR2), n = number of patients with symptoms
Before MRQ (MRQ1), after MRQ (MRQ2), n = number of patients with symptoms
% = percentage evaluation of symptoms in patient groups

2x100 mg/day. Blood sampling was performed at the beginning of the stay (MRQ1) and at the end of the rehabilitation (MRQ2). Group 2 was composed of 14 patients (8 men, 6 women, mean age 58.7±2.64 years) who underwent MR under the same conditions as group 1 but without ubiquinol supplementation. Blood sampling was performed at the beginning of their stay (MR1) and at the end of the rehabilitation (MR2). The control group (C) consisted of fifteen healthy individuals (6 men, 9 women, mean age 51.3±2.3 years). The inclusion criteria for healthy subjects were absence of chronic medication and no history of COVID-19. Exclusion criteria were diseases, obesity, smoking, cancer, regular alcohol consumption.

The study was carried out according to the principles expressed in the Declaration of Helsinki, and the study protocol was approved by the Ethics Committee of Derer´s Hospital in Bratislava, Number: EK/012/2021/UNB. Written informed consent form was obtained from each subject before enrollment in this study. Study is registered at ClinicalTrials.gov ID: NCT05178225.

Platelets isolation

Platelets (PLT) were isolated from whole blood (21) as described previously (22) and counted on hematological analyzer Mindray BC-3600 (Mindray, China).

Coenzyme $Q_{10\text{-}TOTAL}$, α-tocopherol, γ-tocopherol, and β-carotene determination in blood and plasma

Concentrations of $CoQ_{10\text{-}TOTAL}$ (ubiquinone + ubiquinol) and lipophilic vitamins in whole blood and plasma were determined simultaneously by a modified HPLC method with spectrophotometric detection (23, 24). For the oxidation of ubiquinol to ubiquinone, 100 µL of 1,4-benzoquinone (2 mg/1 ml double-distilled water) was added to a tube with 500 µl of blood or plasma and vortexed for 10 seconds (25). After 10 minutes of incubation at room temperature, 2 ml of a mixture of hexane/ethanol (5/2 v/v) was added. The tubes were shaken for 5 minutes and centrifuged at 1,000 g for 5 minutes. The hexane layer was separated and extraction procedure was repeated with 1 ml of the extraction mixture. Collected organic layers were evaporated under nitrogen at 50 °C. The residues were taken up in 99.9 % ethanol and injected into a reverse phase HPLC column. Elution was performed with methanol/acetonitrile/ethanol (6/2/2 v/v/v) at a flow rate of 0.9 ml/min. The concentrations of $CoQ_{10\text{-}TOTAL}$, tocopherols and β-carotene were detected with an UV-detector at 275 nm, 295 nm, and 450 nm, respectively, using external standards. Data were collected and processed with a CSW32 chromatographic station (DataApex Ltd). Concentrations of analyzed substances were calculated in $\mu mol.l^{-1}$.

Coenzyme $Q_{10\text{-}TOTAL}$, α-tocopherol and γ-tocopherol determination in platelets

Isolated human platelets (150–250 millions) were disintegrated with 500 µl of cold methanol (26). Oxidation of ubiquinol to ubiquinone was performed with 1,4-benzoquinone as described for plasma extraction. The cell suspension was extracted with 2 ml hexane by shaking for 5 minutes. After centrifugation, organic layer was separated, evaporated and measured as described above. Concentrations of analyzed substances were calculated in $pmol.10^{-9\ PLT}$.

TBARS determination in plasma

A parameter of oxidative stress, thiobarbituric acid-reactive substances (TBARS), was determined by a spectrophotometric method (27).

Data analysis

The differences between patient groups and control group were evaluated by unpaired Student's t-tests. Paired Student´s t-tests were used for the evaluation of differences between MR1 and MR2 or MRQ1 and MRQ2. The results are expressed as mean±standard error of mean (sem). Values of $p < 0.05$ were considered statistically significant.

Results

Effect of MRQ and MR on selected clinical symptoms of patients with post-COVID-19 syndrome evaluated by questionnaire

After MRQ, many clinical symptoms have improved. In MRQ group the questionnaire was completed by 20 patients out of 22. At the beginning of rehabilitation, dry cough occured in 45 % of patients, at the end in 10 %, shortness of breath decreased from 65 % to 30 %, and breathing difficulty from 65 % to 40 %. Muscle and joint pain decreased from 50 % to 20 %, chest pain from 45 % to 15 %, weakness from 10 % to 5 %, overall fatigue from 85 % to 35 %, diarrhea from 15 % to 5 %, and headache from 40 % to 10 %. After MRQ2, no patient needed respiratory support with O_2, none had malaise, chills, weight loss or hearing impairment. GIT problems not affected by MRQ, occured in 5 % of patients before and after MRQ (Tab. 1).

In the MR group, the questionnaire was completed by 10 out of 14 patients. The effect of MR without ubiquinol supplementation was not noted in clinical symptoms such as dry cought, shortness of breath, malaise, diarrhea and weight loss. Breathing difficulty decreased from 60 % to 30 % , chills from 20 % to 10 %, overal fatigue from 70 % to 20 %, muscle and joint pain from 100 % to 50 %. Headache and hearing imparment disappeared after MR in all patients.

Effect of MRQ and MR on coenzyme Q_{10}, α-tocopherol and γ-tocopherol concentrations in platelets of patients with post-COVID-19 syndrome

Before starting the mountain spa rehabilitation, the concentration of $CoQ_{10\text{-}TOTAL}$ in plateletes in both groups of patients did not differ statistically from the control values in healthy volunteers (Tab. 2). Ubiquinol supplementation 2x100 mg/day for 16–18 days increased the concentration of $CoQ_{10\text{-}TOTAL}$ in PLT to 163.7±10.4 $pmol.10^{-9\ PLT}$ as compared to control values (+68 %, $p < 0.0001$) and MRQ1 ($p < 0.0001$). The concentration of CoQ_{10} in PLT was not affected by MR. Baseline concentration of α-tocopherol in PLT in MRQ1 group was higher than in the control group (6020.0±307.2 vs 2546.5 $pmol.10^{-9\ PLT}$, $p < 0.0001$). After MRQ it decreased to

Tab. 2. Effect of MR and MRQ on coenzyme Q_{10}, vitamins in platelets, whole blood plasma and TBARS in patients with post-COVID-19 syndrome.

	Control	MR1	MR2	MRQ1	MRQ2
Platelets					
Coenzyme Q_{10} pmol.$10^{-9\ PLT}$	84.1±5.56	93.9±5.92	91.5±7.11	97.5±5.73	163.7±10.4 p<0.0001 vs C p<0.0001 vs MRQ1
α-tocopherol pmol.$10^{-9\ PLT}$	2456.5±411.0	3594.5±310.4 p=0.051 vs C	2699.3±268.7 p=0.020 vs MR1	6020.0±307.2 p<0.0001 vs C	3578.6±151.5 p=0.012 vs C p<0.0001 vs MRQ1
γ-tocopherol pmol.$10^{-9\ PLT}$	288.2±21.1	632.7±68.8 p=0.0003 vs C	194.2±30.5 p=0.019 vs C p=0.00017 vs MR1	916.3±162.0 p=0.001 vs C	179.8±19.5 p=0.001 vs C p=0.0005 vs MRQ1
Blood					
Coenzyme Q_{10} μmol.l^{-1}	0.313±0.020	0.366±0.035	0.315±0.017	0.399±0.031	1.17±0.095 p<0.0001 vs C p<0.0001 vs MRQ1
α-tocopherol μmol.l^{-1}	20.8±1.67	20.3±1.12	21.1±1.03	19.4±0.83	19.0±0.98
γ-tocopherol μmol.l^{-1}	1.08±0.126	1.33±0.156	1.52±0.134 p=0.025 vs C	1.05±0.074	1.26±0.089
β-carotene μmol.l^{-1}	0.292±0.046	0.161±0.016 p=0.014 vs C	0.122±0.015 p=0.025 vs C p=0.012 vs MR1	0.185±0.023 p=0.026 vs C	0.123±0.016 p=0.003 vs C p<0.0001 vs MRQ1
Plasma					
coenzyme Q_{10} μmol.l^{-1}	0.516±0.032	0.586±0.050	0.576±0.052	0.596±0.047	1.98±0.139 p<0.0001 C p<0.0001 vs MRQ1
α-tocopherol μmol.l^{-1}	28.2±1.21	33.3±1.62 p=0.023 vs C	35.4±1.49 p=0.001 vs C	33.1±1.70 p=0.049 vs C	30.0±1.35 p=0.053 vs MRQ1
γ-tocopherol μmol.l^{-1}	1.63±0.178	2.23±0.228 p=0.048 vs C	1.88±0.146	1.91±0.195	1.89±0.164
β-carotene μmol.l^{-1}	0.414±0.071	0.208±0.029 p=0.016 vs C	0.140±0.171 p=0.002 vs C p=0.013 vs MR1	0.208±0.033 p=0.016 vs C	0.140±0.020 p=0.002 vs C p=0.013 vs MR1
TBARS μmol.l^{-1}	4.80±0.180	4.65±0.161	4.52±0.171	4.86±0.149	4.51±0.171 p=0.10 vs MRQ1

MR1, MRQ1 – measurements at the beginning of the study; MR2, MRQ2 – measurements at the end of the study; C – control group; TBARS – thiobarbituric acid-reactive substances. Statistical difference between groups is expressed by p-values

3578.6±151.5 pmol.$10^{-9\ PLT}$ ($p < 0.0001$) but it was still higher than control value ($p = 0.012$). The increased MR1 baseline concentration of α-tocopherol in MR group decreased after MR to control values. The initially increased MRQ1 and MR1 concentrations of γ-tocopherol in both MRQ1 and MR1 groups were suppressed during rehabilitation to values bellow the control group value 288.2±21.1 pmol.$10^{-9\ PLT}$ (Tab. 2).

Effect of MRQ and MR on coenzyme Q_{10}, α-tocopherol, γ-tocopherol and β-carotene concentrations in whole blood and plasma, TBARS in plasma of patients with post-COVID-19 syndrome

Baseline concentration of $CoQ_{10\text{-TOTAL}}$ in the MRQ group was similar to healthy controls in the whole blood (0.399±0.031 vs 0.313±0.021 μmol.l^{-1}), and also in plasma (0.596±0.047 vs 0.516±0.032 μmol.l^{-1} (Tab. 2). After MRQ, the $CoQ_{10\text{-TOTAL}}$ concentration increased in blood to 1.17±0.095 μmol.l^{-1} (+194 %, $p < 0.00001$), and in plasma to 1.98±0.139 μmol.l^{-1} (+232 %, $p < 0.00001$). In MR group, the $CoQ_{10\text{-TOTAL}}$ concentrations in blood and plasma initially did not differ from control, and stayed not affected by MR. The concentrations of αT and γT in blood and plasma at the beginning of the study (MRQ1, MR1) were not diferrend from those of the control. The concentration of γT in blood after MR was higher as compared to the control ($p = 0.025$) (Tab. 2). Baseline concentrations of β-carotene in blood and plasma were lower in comparison with control group, both in MRQ1 and MR1 group, and after MRQ and MR decreased even more (Tab. 2). The indicator of oxidative stress, lipid peroxidation (TBARS) slightly decreased after MRQ (to 92.8 %, $p = 0.10$), but was not affected after MR (Tab. 2).

Discussion

Several viruses induce oxidative stress, which facilitates their replication inside the cell (28). In patients with SARS-CoV-2 infection, the association between oxidative stress, cytokine storm,

coagulopathy and cell hypoxia were documented and therapeutic strategy to reduce oxidative stress using antioxidants was suggested (29). Mitochondrial dysfunction contributes to higher mitochondrial ROS production and inflammation in COVID-19 disease (30). Mitochondrial health induced by a healthy lifestyle and exercise is supposed to be protective against SARS-CoV-2 infection [6], while the benefit of regular exercise in post-COVID-19 syndrome has been reviewed (31). The authors consider physical exercise to have a favourable effect on the most frequent clinical manifestation of post-COVID-19 syndrome, i.e., those affecting psychological, neurological, cardiovascular, respiratory, musculoskeletal and immune systems.

Our hypothesis, that mitochondrial bioenergetics and endogenous coenzyme Q_{10} could be the targets of the new SARS-CoV-2 virus (3) has been partially proved in studies showing reduced mitochondrial bioenergetics in monocytes and peripheral blood mononuclear cells of patients with COVID-19 (32, 33). Platelets isolated from peripheral blood are an accessible source of mitochondria and their use for assessment of mitochondrial health is extensively studied. SARS-CoV-2 virus may manipulate mitochondrial function, and the manipulation may persist for an extended time after acute COVID-19 (3). In our pilot study, patients who had been 4–7 weeks after acute COVID-19 showed modulation of platelet mitochondrial respiration and oxidative phosphorylation and reduction in endogenous CoQ_{10} concentrations (2). Sufficient concentration of coenzyme Q_{10} is essential for mitochondrial bioenergetics, as well as antioxidant, antiinflammatory and immune functions.

The importance of CoQ_{10} (34) and fat-soluble vitamins A, D, E, K (8) for immune response and reducing the severity of COVID-19 infection has been reviewed. Since the deficiency in one or more of these vitamins modulates the immune response, it is believed that administration of antioxidants can be beneficial in the prevention and treatment of COVID-19 (9). Rehabilitation in thermal spa is also recommended (19).

Coenzyme Q_{10} (CoQ_{10}) is an inevitable component of mitochondrial respiratory chain, necessary for ATP production, it has also antioxidant and antiinflammatory properties. In contrast to other lipophilic antioxidants, the concentrations of CoQ_{10} depend on endogenous synthesis as well as on food intake. Up to date, we did not find any information on the effects of SARS-CoV-2 infection on concentration of coenzyme Q_{10} and other lipophilic vitamins in patients after COVID-19. In our study we analyzed concentrations of CoQ_{10}, and lipophilic vitamins, namely α-tocopherol, γ-tocopherol and β-carotene measured at the beginning and end of mountain spa rehabilitationin patients with post-COVID-19 syndrome, who were either supplemented with ubiquinol (MRQ) or not (MR). After MRQ, the concentration of CoQ_{10} in PLT increased significantly (by 68 %), thus confirming the high bioavailability of the administered ubiquinol. There was no change in CoQ_{10} concentration in the MR group. Higher plasma CoQ_{10} concentrations are necessary to facilitate its uptake by peripheral tissues and brain, and allow the uptake of CoQ_{10} by mitochondria. This has implications for therapeutic applications and is beneficial in human diseases (35). In this respect, the concentration of CoQ_{10} in platelets may reflect its intracellular concentration. For the uptake of supplemented CoQ_{10} by mitochondria, professor Frederick Loring Crane, a discoverer of CoQ_{10}, proposed a hypothesis about the alternative function and a new binding site of CoQ_{10} in the outer membrane of mitochondria on VDAC (voltage-dependent anion channel). It is proposed that coenzyme Q can regulate permeability transition pore opening and nutrition uptake through VDAC (36, 37) (Fig. 1).

Our preliminary results showed that platelet mitochondrial Complex I-linked oxidative phosphorylation (OXPHOS) and electron transfer (ET) capacity was markedly reduced in patients with post COVID-19 syndrome. After 16–18 days of MR, these parameters improved more in the group of patients supplemented with ubiquinol than in the non-supplemented group. The increase in OXPHOS and ET capacity correlated with the increase in CoQ_{10} in platelets and there was a trend to positive correlation with the improvement in pulmonary function (38).

Also selected clinical symptoms as cough, shortness of breath, breathing difficulty, and muscle, joint and chest pain, as well as weakness, fatigue, malaise, chills, and weight loss have been improved more markedly after the mountain rehabilitation with ubiquinol supplementation (MRQ) as compared to MR alone (Tab. 1).

The concentration of CoQ_{10} in whole blood and plasma after MRQ increased significantly (by 192 and 232 %, respectively), proving high bioavailability of administered ubiquinol. Increased concentrations of CoQ_{10} in circulation enhanced also its intracellular concentration, and improved mitochondrial bioenergetic functions, as proven by us in isolated platelets (41). There were no changes in concentrations of CoQ_{10} in patients after MR.

In both groups of patients with post-COVID-19 syndrome (MRQ, MR), we found higher baseline concentrations of tocopherols in PLT in comparison with the values in healthy volunteers. We suppose that it could be caused by vitamins supplementation during the disease. Further, γT is degraded by the cytochrome P450 enzyme in the liver whose activity is inhibited by inflammation which occurs in COVID-19, and thus, the γT concentration can be elevated. Subsequent mountain spa rehabilitation could improve cytochrome P450 function and reduce γT concentration. Ubiquinol treatment had no effect on tocopherols concentration in PLT; they were reduced in both groups near controls. Recently we found elevated concentrations of γT in plasma and skeletal muscle tissue of arthritic rats together with increased markers of inflammation (39), and treatment with CoQ_{10} corrected the increased γT concentration and improved the mitochondrial function (40).

Vitamin E and its components, α- and γ-tocopherols, have a ROS-scavenging potential; they protect cells from oxidative damage, increase the integrity of cell membranes, and improve the adaptive response of the immune system to viral infections (42). In our study, the concentrations of tocopherols in blood did not change significantly after MR. The concentration of αT in plasma slightly decreased after MRQ, probably as a consequence of increased CoQ_{10} concentration reducing oxidative damage and decreasing requirement for tocopherol mobilization.

Concentrations of β-carotene in blood and plasma were lower in patients with post- COVID-19 syndrome at the beginning of

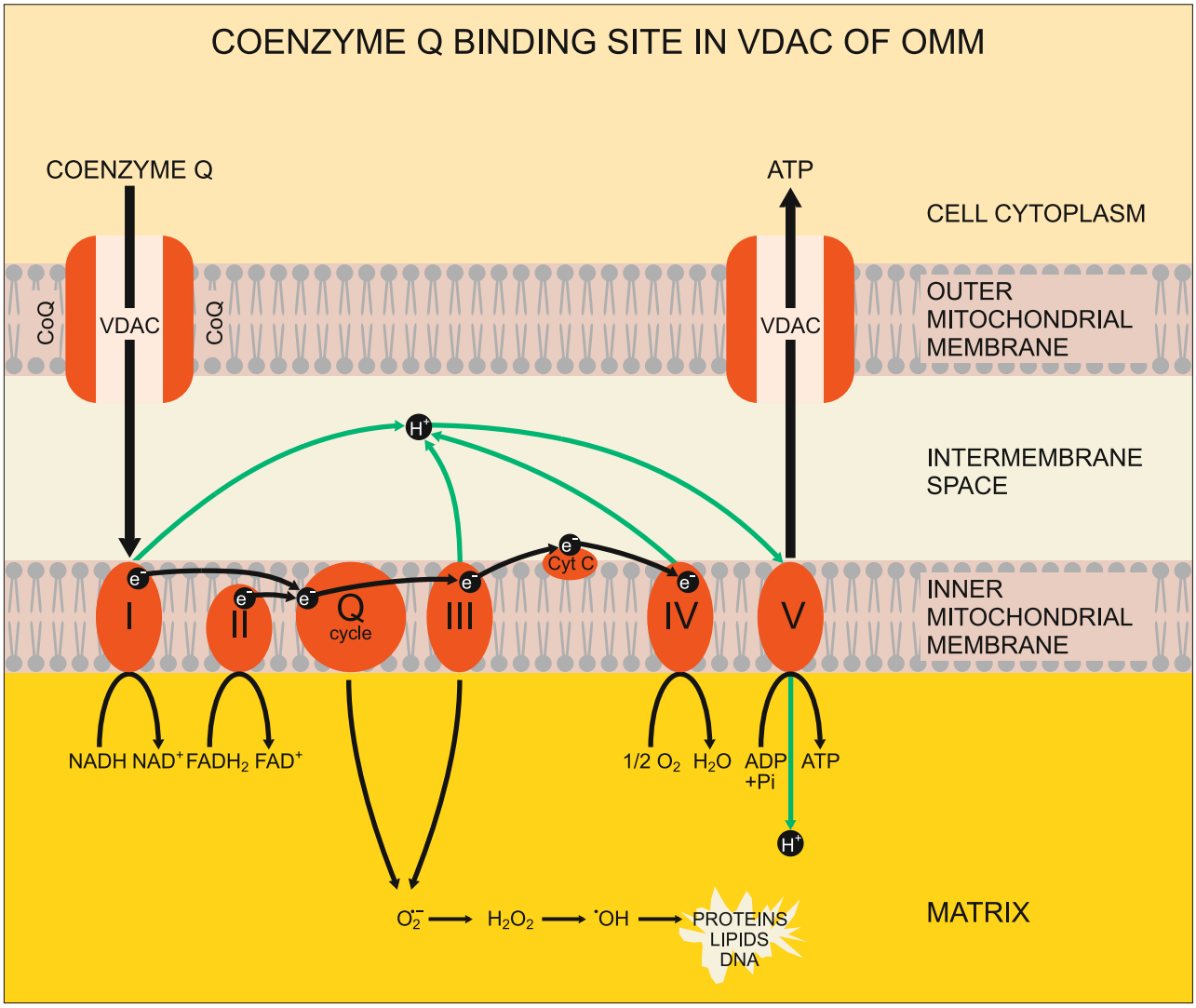

Fig. 1. Proposed coenzyme Q binding site in the VDAC of OMM (36, 37). CoQ – coenzyme Q; VDAC – voltage-dependent anionic channel; ATP – adenosine triphosphate; ADP – adenosine diphosphate; Pi – inorganic phosphorus; I, II, III, IV, V – respiratory chain complexes; H^+ – proton; e– – electron; Q-cycle – Coenzyme Q cycle; Cyt c – cytochrome c; NADH – reduced nicotinadenine dinucleotide; NAD^+ – nicotinadenine dinucleotide; $FADH_2$ – reduced flavinadenine dinucleotide; FAD^+ – flavinadenine dinucleotide; $O_2^{\cdot-}$ – superoxide radical; H_2O_2 – hydrogen peroxide; $OH^{\cdot}$ – hydroxyl radical; H_2O – water; O_2 – oxygen.

the study as compared to controls, and decreased even more during rehabilitation in both groups, which may indicate a persistent inflammatory process caused by virus infection. Supplementation with ubiquinol did not affect the β-carotene status. Beta-carotene has important immunomodulatory properties; it can scavenge ROS, enhance T-lymphocyte response, natural killer cell activity, and interleukin 2 production and thus suppress viral replication in COVID-19 patients. Gastrointestinal symptoms, liver abnormalities, and reduced food intake may contribute to the deficit in βCar and vitamin A. The roles of certain vitamins in the prevention and treatment of COVID-19 are still being researched (43, 44).

TBARS concentrations in both groups of patients before and after rehabilitation did not differ from control values. The slight decrease of TBARS concentration in MRQ2 vs MRQ1 group could be caused by higher antioxidant defense due to increased CoQ_{10} concentration.

Conclusion

Beneficial effect of mountain spa rehabilitation on clinical symptoms of the patients with post-COVID-19 syndrome was proven. Supplementation with ubiquinol increased CoQ_{10} concentration in blood, plasma and cells (reflected by platelets) and decreased oxidative stress (reflected by TBARS concentration). As a consequence of increased intracellular concentration of CoQ_{10} and antioxidant protection, the bioenergetics of organs was improved and the incidence of clinical symptoms was more reduced in the MRQ group. Increased systemic and cellular coenzyme

Q_{10} concentration and mountain spa rehabilitation supported the recovery of patients with post- COVID-19 syndrome. We draw attention to the importance of monitoring and ensuring adequate levels of CoQ_{10} in post-COVID-19 syndrome.

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