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EFFECT OF COENZYME Q_{10} SUPPLEMENTATION IN THE RAT MODEL OF ADJUVANT ARTHRITIS

Katarina Bauerova[a], Jarmila Kucharska[b], Danica Mihalova[a], Jana Navarova[a], Anna Gvozdjakova[b], Zuzana Sumbalova[b]

[a] *Institute of Experimental Pharmacology, Slovak Academy of Sciences, Bratislava, Slovak Republic,*
[b] *Pharmacobiochemical Laboratory, School of Medicine, Comenius University, Bratislava*
e-mail: Katarina.Bauerova@savba.sk

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Adjuvant arthritis (AA) is a model of chronic inflammation induced by *Mycobacterium butyricum* and characterized by similar pathophysiological and pathobiochemical changes as rheumatoid arthritis (RA) in humans. In this study the antirheumatic activity of coenzyme Q_{10} supplementation was tested not only as to its capability to suppress the inflammation edema of the hind paw and to improve the body weight of the arthritic animals, but also to improve so important biochemical parameters as markers of inflammation and oxidative stress, and of mitochondrial bioenergetics. Despite the unfavorable effects on the rheumatic processes observed by monitoring biometric parameters (hind paw volume, relative body weight, relative weight of spleen), a significant protective effect was observed on the level of mitochondrial energetic and antioxidant disbalance. This finding speaks in favor of CoQ_{10} supplementation in rheumatic patients, presumably as combinatory therapy with classical antirheumatics, e.g. NSAIDs.

INTRODUCTION

Adjuvant arthritis (AA) is a model of chronic inflammation induced by *Mycobacterium butyricum* and characterized by similar pathophysiological and pathobiochemical changes as rheumatoid arthritis (RA) in humans. Oxidative stress, involved in the etiopathogenesis of RA, may participate in disturbances of mitochondrial ultrastructure and function, leading to decreased efficiency and atrophy of the skeletal muscle. Previously[1] we found stimulation of skeletal muscle mitochondrial function and coenzyme Q_9 (CoQ_9) concentration in AA rats. Compounds with antirheumatic properties reversed these changes. Based on known antioxidant and bioenergetic properties, a beneficial effect of coenzyme Q_{10} was proposed in the pathogenesis of arthritis. In this study, the antirheumatic activity of coenzyme Q_{10} supplementation was tested not only as to its capability to suppress the inflammation edema of the hind paw and to improve the body weight of the arthritic animals, but also to improve so important biochemical parameters as markers of inflammation and oxidative stress, and of mitochondrial bioenergetics.

MATERIAL AND METHODS

Male Lewis rats were divided into four groups, each of 7–9 animals: C – controls, Q_{10} – control rats supplemented with CoQ_{10} during 28 days in daily dose 20 mg/kg body weight by intragastric tube, AA – adjuvant arthritis induced intradermally by *Mycobacterium butyricum* in incomplete Freund's adjuvant, AA + Q_{10} – AA rats supplemented with CoQ_{10}. All biometric and biochemical parameters monitored were measured on the 28th experimental day when the animals were sacrificed. For lipid peroxidation assessment, the plasmatic levels of thiobarbituric acid reactive substances (TBARS) were determined spectrophotometrically[2]. The plasmatic levels of carbonyls (CBN)[3,4], the plasmatic activity of *N*-acetyl-β-D-glucosaminidase (NAGA) (ref.[5]) and gamma-glutamyl transpeptidase (GGTP) (ref.[6]) were also assessed spectrophotometrically. Mitochondria of the skeletal muscle were isolated by differential centrifugation, respiratory chain function was measured using Clark oxygen electrode[7]. Concentrations of CoQ_9, CoQ_{10} and α-tocopherol (α-toc) in plasma and skeletal muscle mitochondria were determined by HPLC method[8,9]. Further the increase of the hind paw volume, decrease of the relative body weight and changes in the relative weight of the spleen were monitored.

The data were expressed as mean + standard error of mean (SEM). Statistical evaluation was performed using unpaired Student's t-test. For declaration of significance the two-tailed P value was taken into consideration.

RESULTS AND DISCUSSION

Adjuvant arthritis significantly induced lipoperoxidation in plasma (Table 1), significantly stimulated CoQ_9 and α-tocopherol concentration in skeletal muscle mitochondria and slightly stimulated mitochondrial function

in Complex I (Table 2). Supplementation with CoQ_{10} in AA rats restored α-tocopherol concentration in plasma but did not suppress lipoperoxidation (Table 1). Increased concentration of CoQ_9 in skeletal muscle mitochondria in AA rats was significantly reduced by CoQ_{10} supplementation. Activity of Complex I of the respiratory chain was decreased, function of Complex II was slightly stimulated (Table 2).

Due to the rheumatic processes, the plasmatic activity of NAGA and the level of plasmatic carbonyls were slightly increased. From the therapeutic aspect, the effect of supplementation with CoQ_{10} was positive in the case

Table 1. Parameters monitored in plasma: concentrations of antioxidants, thiobarbituric acid reactive substances (TBARS) and protein carbonyls (CBN), activity of NAGA

	C	Q_{10}	AA	AA + Q_{10}
CoQ_{10} (μmol/l)	n.d.	0.270 ± 0.032	n.d.	0.306 ± 0.045
CoQ_9 (μmol/l)	0.199 ± 0.033	0.191 ± 0.021	0.173 ± 0.087	0.230 ± 0.026
α-toc (μmol/l)	11.7 ± 1.2	17.2 ± 1.6*	13.1 ± 1.4	17.0 ± 0.9**
TBARS (μmol/l)	10.9 ± 0.6	11.2 ± 0.2	14.6 ± 0.5**	15.0 ± 0.3**
CBN (nmol/l)	0.635 ± 0.033	0.544 ± 0.057	0.665 ± 0.069	0.627 ± 0.053
NAGA activity	0.0656 ± 0.0065	0.0793 ± 0.0590	0.0758 ± 0.0067	0.0939 ± 0.0060*

Values are mean ± SEM; significance compared to control: **$p < 0.01$, *$p < 0.05$. NAGA activity was expressed as (μg 4-nitrophenol/min)/mg of proteins.

Table 2. Parameters monitored in skeletal muscle: mitochondrial function and antioxidants concentrations, activity of GGTP

	C	AA	AA + Q_{10}
S_3 – glutamate	46.6 ± 9.5	72.9 ± 11.3	29.3 ± 5.3
OPR- glutamate	130.0 ± 20.7	152.0 ± 26.8	57.6 ± 13.3*
S_3 – succinate	134.5 ± 22.7	127.4 ± 17.6	152.5 ± 14.4
OPR – succinate	173.6 ± 25.4	191.2 ± 27.4	204.1 ± 15.6
CoQ_{10} (μmol/l)	n.d.	0.108 ± 0.022	0.061 ± 0.023
CoQ_9 (μmol/l)	0.915 ± 0.128	2.400 ± 0.308**	1.440 ± 0.152*
α-toc (μmol/l)	0.165 ± 0.014	0.441 ± 0.113*	0.309 ± 0.045
GGTP activity	6.47 ± 1.09	5.41 ± 1.26	5.45 ± 1.33

S_3-stimulated mitochondrial respiration (nAtO/mg prot/min), OPR-oxidative phosphorylation rapidity [(nmol ATP/mg prot)/min], CoQ, α-toc (nmol/mg prot).
Values are mean ± SEM; significance compared to control **$p < 0.01$, *$p < 0.05$. GGTP activity was expressed as (nmol p-nitroaniline/min)/gww.
n.d. – under the detection limit, nAtO – nmol of atomic oxygen.

Table 3. Biometric parameters monitored: increase of hind paw volume (HPV), decrease of relative body weight (RBW), changes in relative weight of the spleen (RWS)

	C	Q_{10}	AA	AA + Q_{10}
HPV (%)	14.13 ± 2.11	18.58 ± 1.48	56.79 ± 12.92**	73.37 ± 7.21***
RBW (g)	91.43 ± 4.35	105.43 ± 2.52*	31.00 ± 13.93**	30.43 ± 4.50**
RWS (mg)	1.653 ± 0.054	1.643 ± 0.021	2.844 ± 0.288**	3.429 ± 0.368***

Values are mean ± SEM; significance compared to control *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$. HPV and RBW were calculated in relation to the first experimental day.

of plasma carbonyls and disputable for the activity of NAGA (Table 1). The effect of CoQ_{10} supplementation on GGTP activity in skeletal muscle was without significance (Table 2).

As seen from the Table 3, all biometric parameters were unfavorably affected due to the arthritic processes. The relative body weight was unaffected by the compound tested. The other parameters were higher, which could reflect indirectly a stimulation of inflammation processes. In conclusion, despite these unfavorable effects on the rheumatic processes, a significant protective effect was observed on the level of mitochondrial antioxidant disbalance. This finding may favor CoQ_{10} supplementation in rheumatic patients, presumably as a combinatory therapy with classical antirheumatics, e.g. NSAIDs.

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