

Preventive and Therapeutic Coenzyme Q10 Supplementation In Rat Subjected to Cerebrovascular Ischemia-Reperfusion Injury

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Abstract

It is known that oxidative stress and mitochondrial dysfunction plays an important role in animal models of brain ischemia. The present study was undertaken to test whether oral supplementation of coenzyme Q (CoQ) could protect against transient cerebral ischemia-induced mitochondrial damage in the rat brain. Rats were divided into four groups: 1- control; 2- ischemia-reperfusion; 3- CoQ before ischemia; 4- CoQ after ischemia. Transient cerebral ischemia was induced with three vessel occlusion (3-VO) for 50 min. CoQ (200 mg/kg/day, p.o.) was administered for 30 days before ischemia and/or for 30 days after ischemia. Brain mitochondria were used for the determination of oxidative phosphorylation (OXPHOS). Moreover, the concentrations of CoQ and tocopherols, and formation of TBARS were measured in brain mitochondria and in plasma. Ischemia-reperfusion injury revealed significant impairment of OXPHOS, decreased concentrations of brain and plasma endogenous antioxidants and increased formation of TBARS in plasma. When compared with ischemia-reperfusion group, preventive supplementation of CoQ was ineffective. However, positive effect of therapeutic CoQ supplementation was detected in RCI ($P<0.001$), S3 ($p<0.05$) and in OPR ($P<0.05$), as well as in the concentration of coenzyme Q₉ in brain mitochondria ($P<0.05$) and of α -tocopherol ($P<0.01$) in plasma. This suggests that the protection of CoQ₁₀ involve increased resistance to oxidative stress, when supplemented during reperfusion.

Introduction

Normal brain function is highly dependent on adequate blood flow and substrate delivery for production of ATP in mitochondria. Impaired cerebral perfusion originating in the microvasculature affects the optimal delivery of glucose and oxygen resulting in a breakdown of metabolic energy pathways in brain cells [1,2] Free radical formation has been demonstrated not only during cerebral ischemia [3,4], but much more at the onset of reperfusion after cerebral ischemia [5,6].

Many antioxidants are reported to reduce ROS-mediated reactions in animal models of cerebral ischemia, however, the preventive and therapeutic effect of CoQ₁₀ supplementation on cerebral ischemia and reperfusion has not been evaluated. Therefore, the present study was designed to investigate the effect of CoQ₁₀ supplementation on ischemia and reperfusion-induced mitochondrial injury in rats subjected to transient three-vessel occlusion (3-VO).

Materials and Methods

Animals

Adult (18 month old) male Wistar rats, weighing 460-550 g, obtained from VELAZ (Prag, Czech Republic), were maintained at 22 ± 2 °C, 45 relative humidity, 12 hour light/dark cycle in air conditioned room with free access to standard commercial rodent pellet diet ST 1 (TOP DOVO, Slovak Republic) and tap water ad libitum. The protocol of this study has been approved by Slovak Medical University Ethics Committee in compliance with the Guidelines of European Convention for the Protection of Vertebrate Animals Used for Experimental Purposes.

Experimental design

Rats were divided into four experimental groups

1. **C-** control group without treatment (n=6)
2. **IR-** 50-min 3-VO + 30-day reperfusion (n=6)
3. **Q₁₀+ IR-** preventive treatment with liposoluble CoQ₁₀ (Li-Q-SorbTM, Tishcon corp., USA) in daily dose 200 mg/kg bw for 30 days before IR injury (n=6)
4. **IR + Q₁₀-** therapeutic treatment with CoQ₁₀ by gavage in daily dose 200 mg/kg bw for 30 days after 50-min ischemia (n=6)

Transient brain ischemia was induced with occlusion of both, truncus brachiocephalicus and left common carotid artery (three vessel occlusion, 3-VO) for 50 minutes. At the end of 30-day reperfusion, animals were euthanized (Thiopental 160 mg/kg i.p.) and the brain was removed for biochemical studies.

Three cerebral vessel occlusion (3-VO)

Experiments were done in aged male Wistar rats under general anesthesia (ketamine 50 mg/kg b.w. and xylazine 4 mg/kg b.w.) placed in a supine position on the operating table, and left to respire spontaneously. Transient ischemia-reperfusion injury was accomplished by our original surgical procedure for three-vessel occlusion (3-VO) [7,8]. Briefly, minimally invasive transmanubrial approach was used for occlusion of both, the left common carotid and brachiocephalic trunk (including right common carotid and right vertebral artery) to eliminate cerebral blood flow through both common carotid and right vertebral arteries. At the end of the 50-min period, the microaneurysmal clips were released to restore cerebral blood flow. After completion of surgical procedure and recovery, the animals were shifted to their home cage.

In vitro mitochondrial respiration

After euthanasia (Thiopental, Spofa, Czech Republic, 150 mg/kg.b.w.), brain was removed and placed on ice-cold isolation solution containing (in mmol.l⁻¹) 225 manitol, 75 sucrose, 0.2 EDTA; pH 7.4. Tissue sample was minced and homogenized in the solution using a glass-teflon homogenizer. Brain mitochondria were isolated at 4°C by differential centrifugation [9]. Mitochondrial protein concentration was estimated by the method of Lowry et al [10] using bovine serum albumin as a standard. Respiratory chain function was measured in a respiratory buffer containing (in mmol.l⁻¹) 12.5 HEPES, 3 KH₂PO₄, 122 KCl, 0.5 EDTA and 2% dextran; pH 7.2 at 30°C, by means of Oxygraph Gilson 5/6H (USA) using Clark-type polarographic oxygen electrode. Sodium glutamate/malic acid (2.5 mmol/2.5 mmol) were used as a NAD substrate for complex I. To initiate state 3 respiratory activity, 500 nmol of ADP were added to the cuvette. When all the ADP was converted to ATP, state 4 respiration was measured. Parameters of oxidative phosphorylation, such as QO₂(S₃) [nAtO.mg prot⁻¹.min⁻¹], i.e., oxygen consumption rate in presence of ADP (state S₃), oxygen consumption rate without ADP (state₄); RCR [S₃.S₄], respiratory control ratio; ADP:O [nmol ADP . nAtO⁻¹], coefficient of oxidative phosphorylation; and OPR [nmol ATP.mg prot.min⁻¹], oxidative phosphorylation rate, were determined, respectively.

Determination of antioxidants

Concentrations of oxidized forms of CoQ₉, CoQ₁₀, α - and γ - tocopherols were determined by isocratic high-performance liquid chromatography (HPLC, LKB, Sweden) according to Lang et al. [11] with some modifications [12]. Plasma samples and isolated brain mitochondria were vortexed twice for 5 minutes with the mixture of hexane/ethanol (5/2, v/v, Merck, Germany). Collected organic layers were evaporated under nitrogen, the residues were taken up in ethanol and injected into Separon SGX C18 7 μ m 3x150 mm column (Tessek, Czech Republic). Elution was performed with methanol/ acetonitril/ethanol (6/2/2, v/v, Merck, Germany). The concentration of tocopherols were detected spectrophotometrically at 295 nm, concentration of coenzyme Q homologues at 275 nm using external standards (Sigma, Germany). Data were collected and processed using CSW 32 chromatographic station (Data Apex Ltd, Czech Republic). Concentration of compounds in plasma were calculated in μ mol.l⁻¹, in mitochondria in nmol.mg prot⁻¹.

Measurement of lipid peroxidation

Lipid peroxidation in plasma and brain homogenates was determined spectrophotometrically by measuring the formation of thiobarbituric acid reactive substances (TBARS) according to methods of Ohkawa et al. (13) and Janero and Burghardt (14). Plasma samples or brain homogenate were mixed with ice-cold 76% trichloacetic acid (TCA, Merck, Germany) and 1.07% thiobarbituric acid (TBA, Merck, Germany). Samples were incubated at 100°C and after cooling 90% TCA was added. After vortexing and centrifugation the absorbance of supernatant was measured at 532 nm using spectrophotometer Novaspec II Rapid (Pharmacia, LKB, Sweden). Concentration of lipid peroxides was expressed in plasma in μ mol.l⁻¹, in the brain tissue in nmol.g⁻¹.

Statistical analysis

The results were evaluated using Student's t-test for unpaired data, $p < 0.05$ was considered statistically significant. Data are expressed as means \pm standard deviation (S.D.). Statistical analysis was performed with one-way ANOVA followed by a Student's t-test. The value of P less than 0.05 was considered to be statistically significant.

Results

Effect of coenzyme Q on ischemia and reperfusion-induced mitochondrial dysfunction

The effects of 50 min cerebrovascular ischemia on rat brain mitochondria are shown in Tab. 1 and Fig. 1. Examination of oxidative phosphorylation revealed significant dysfunction of mitochondria from ischemia-reperfusion-treated group as demonstrated by the 21% decrease in oxygen consumption (QO_2S_3) and 24% decrease in the rate of ATP production (OPR), when compared with control group.

Preventive CoQ₁₀ supplementation for 30 days before 50-min cerebrovascular occlusion was ineffective as documented by 26% decrease in oxygen consumption and 26% decrease of ATP production when compared to control and to ischemia-reperfusion groups.

Therapeutic supplementation of CoQ₁₀ for 30 days after 50-min ischemia demonstrated normal values of oxygen consumption and ATP production as in control group.

Table 1. Mitochondria respiration. Effect of coenzyme Q10 supplementation on oxidative phosphorylation in brain mitochondria of rats subjected to ischemia-reperfusion injury. RCI [$S_3.S_4$], respiratory control index; QO_2S_3 , oxygen consumption rate in presence of ADP; OPR, oxidative phosphorylation rate. C, control group; IR, ischemia-reperfusion group; QIR, preventive supplementation of coenzyme Q10 for 30 days before cerebrovascular occlusion (3-VO); IRQ, therapeutic supplementation of coenzyme Q10 for 30 days of reperfusion. Values are expressed as mean \pm S.E.M. * = $P < 0.05$ versus control group; + = $P < 0.05$ versus ischemia-reperfusion group; x = $P < 0.05$ versus preventive supplementation of CoQ10.

Parameters of oxidative phosphorylation Complex I

	RCI ($S_3.S_4^{-1}$)	QO_2S_3 (nAtO.mg prot ⁻¹ .min ⁻¹)	OPR (nmol ATP.mg prot ⁻¹ .min ⁻¹)
Brain mitochondria			
C	3.38 \pm 0.26	62.80 \pm 2.53	201.47 \pm 8.42
IR	4.93 \pm 0.31**	49.93 \pm 5.76	153.55 \pm 17.58*
QIR	2.91 \pm 0.17 ⁺⁺⁺	46.76 \pm 1.16***	149.90 \pm 8.22**
IRQ	3.34 \pm 1.13 ⁺⁺⁺	67.21 \pm 5.37 ^{+x}	214.27 \pm 20.66 ^{+x}

*** $p < 0.001$, ** $p < 0.002$, * $p < 0.05$ vs C, ⁺⁺⁺ $p < 0.001$, ⁺ $p < 0.05$ vs IR, ^x $p < 0.05$ vs QIR

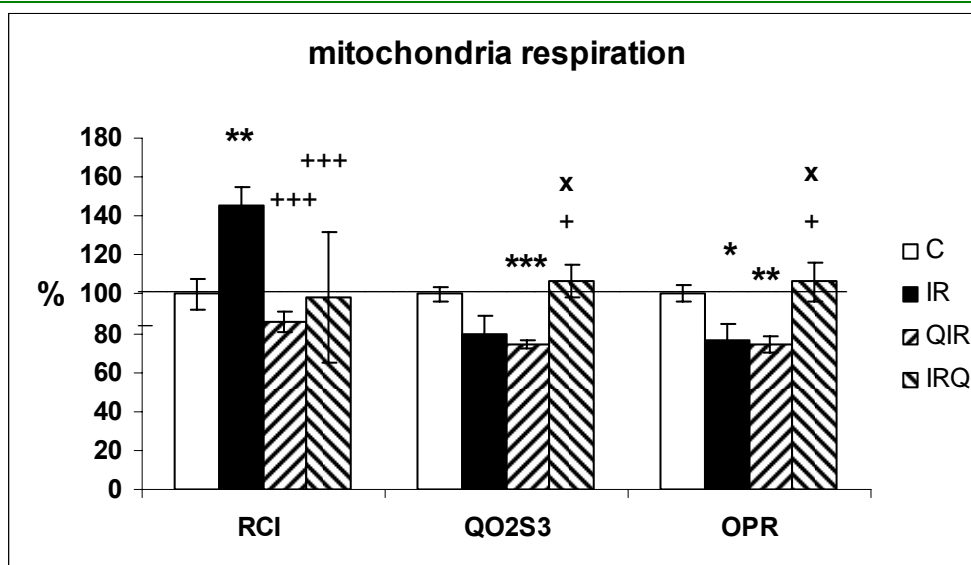


Figure 1. Effect of CoQ10 supplementation on parameters of brain mitochondria respiration in rats subjected to ischemia-reperfusion injury. RCI, respiratory control index ($S_3.S_4$); QO_2S_3 , oxygen consumption rate in presence of ADP (state 3); OPR, oxidative phosphorylation rate. C, control group; IR, ischemia (50 min 3-VO) – reperfusion(30 days) group; QIR, preventive supplementation of CoQ10 for 30 days before cerebral ischemia; IRQ, therapeutic supplementation of CoQ10 for 30 days after cerebral ischemia. Values are expressed as percentage of control group. * = $P < 0.05$ versus control group; + = $P < 0.05$ versus ischemia –reperfusion group; x = $P < 0.05$ versus preventive supplementation of CoQ10.

Table 2. Antioxidants. Effect of Coenzyme Q10 supplementation on the concentration of CoQ9, CoQ10, alpha and gamma tocopherol in brain mitochondria and in plasma of rats subjected to ischemia-reperfusion injury. C, control group; IR, ischemia-reperfusion group; QIR, preventive supplementation of CoQ10 for 30 days before cerebrovascular occlusion (3-VO); IRQ, therapeutic supplementation of Coenzyme Q10 for 30 days of reperfusion. Values are expressed as mean \pm S.E.M. * = $P < 0.05$ versus control group; + = $P < 0.05$ versus ischemia-reperfusion group; x = $P < 0.05$ versus preventive supplementation of CoQ10.

	CoQ9ox	CoQ10ox	α -tocopherol	γ -tocopherol
Brain mitochondria (nmol.mg prot⁻¹)				
C	0.70 \pm 0.03	0.34 \pm 0.03	0.34 \pm 0.06	0.05 \pm 0.01
IR	0.71 \pm 0.06	0.31 \pm 0.04	0.23 \pm 0.02	0.04 \pm 0.01
QIR	0.62 \pm 0.05	0.31 \pm 0.01	0.22 \pm 0.04	0.03 \pm 0.01
IRQ	0.95 \pm 0.07* + xx	0.34 \pm 0.02	0.32 \pm 0.02 +	
Plasma (μmol.l⁻¹)				
C	0.98 \pm 0.19	n.d.	13.49 \pm 1.90	0.08 \pm 0.01
IR	0.40 \pm 0.05*	n.d.	10.46 \pm 0.90	0.12 \pm 0.02
QIR	0.59 \pm 0.28	1.63 \pm 0.29	14.52 \pm 1.15 ⁺	0.06 \pm 0.01 ⁺
IRQ	0.50 \pm 0.11	1.00 \pm 0.28	13.21 \pm 1.32	0.04 \pm 0.01* ++

* $p < 0.05$ vs C, ⁺ $p < 0.05$ vs IR, ⁺⁺ $p < 0.005$ vs IR, ^{xx} $p < 0.005$ vs QIR, n.d. not detected

Table 3. TBARS. Effect of coenzyme Q10 supplementation on plasma and brain thiobarbituric acid reactive substances (TBARS) formation in rats subjected to transient ischemia-reperfusion injury using three cerebral vessels occlusion (3-VO). Values are expressed in mean ($n = 6$) \pm S.E.M. * = $P < 0.05$ vs. control group (C); + = $P < 0.05$ vs. ischemia reperfusion group (IR).

	Plasma (μ mol.l ⁻¹)	Brain homogenate (μ mol.kg ⁻¹)
C	9.18 \pm 0.51	94.24 \pm 8.14
IR	10.56 \pm 0.20*	93.16 \pm 4.02
QIR	10.08 \pm 0.27	104.96 \pm 6.71
IRQ	9.71 \pm 0.24 ⁺	

* $p < 0.05$ vs C, ⁺ $p < 0.05$ vs IR

Effect of coenzyme Q supplementation on ischemia-reperfusion induced impairment of endogenous antioxidants

The concentrations of CoQ and tocopherols in brain mitochondria are presented in Table 2 and in Figure 2.. Concentration of oxidized forms CoQ₉ (CoQ_{9 ox}) and CoQ₁₀ (CoQ_{10 ox}) in brain mitochondria after ischemia-reperfusion did not differ from control group, and decreased concentrations of α -tocopherol and γ -tocopherol are not significant.

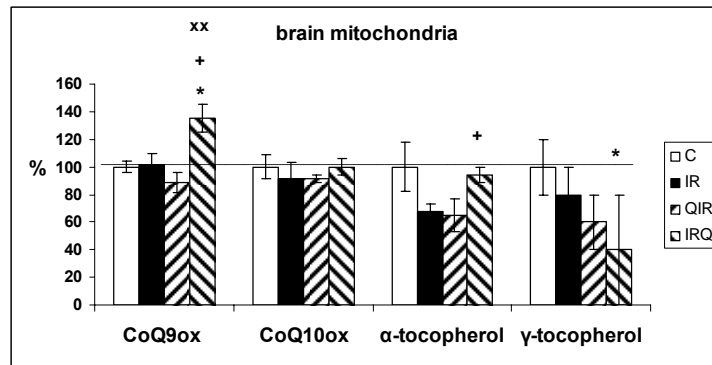


Figure 2. Effect of coenzyme Q10 (CoQ10) supplementation (200 mg/kg/day) on the concentrations of oxidized form coenzyme Q_{9-ox}, coenzyme Q_{10-ox}, α- and γ- tocopherols in brain mitochondria of rats subjected to transient cerebrovascular ischemia. C, control group; IR, brain ischemia (3-VO) for 50 min followed by reperfusion for 30 days; QIR, preventive CoQ10 supplementation for 30 days before ischemia reperfusion; IRQ, therapeutic CoQ10 supplementation for 30 days after 3-VO for 50 min. Values are expressed as percentage of control group. * = P < 0.05 vs. control group; + = P < 0.05 vs. ischemia-reperfusion group; x = P < 0.05

Similarly, concentration of CoQ_{9 ox} and CoQ_{10 ox} in brain mitochondria after preventive supplementation of CoQ₁₀ for 30 days before ischemia-reperfusion injury did not differ from control group and from ischemia-reperfusion group, and the concentrations of α-tocopherol and γ-tocopherol are not significant.

Therapeutic supplementation of CoQ₁₀ for 30 days after cerebrovascular occlusion shows significant increased concentration of CoQ_{9 ox} by 36% (P<0.05), normal values in the concentration of CoQ_{10 ox} and α-tocopherol, but significant decrease of γ-tocopherol (60%, P<0.05) when compared to control group.

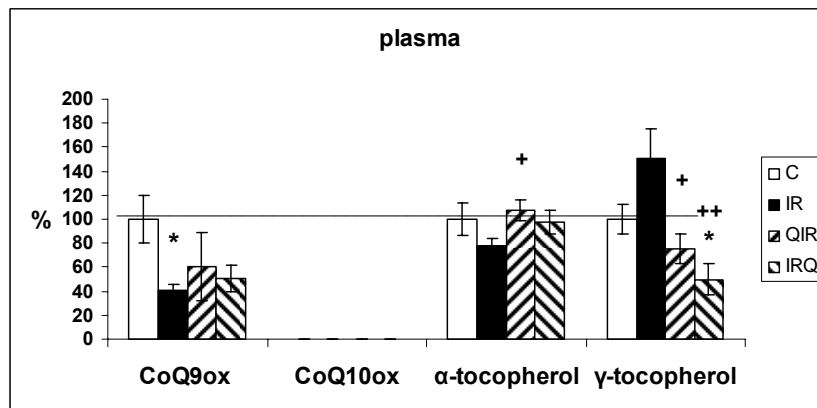


Figure 3. Effect of coenzyme Q10 (CoQ10) supplementation on plasma concentrations of oxidized forms of coenzyme Q₉ (CoQ_{9-ox}), coenzyme Q₁₀ (CoQ_{10-ox}), α- and γ- tocopherols in rats subjected to transient ischemia-reperfusion using three cerebral vessels occlusion (3-VO). C, control group; IR, ischemia for 50 minutes followed by reperfusion for 30 days; QIR, preventive CoQ10 supplementation for 30 days before ischemia-reperfusion; IRQ, therapeutic CoQ10 supplementation for 30 days after 50 min of cerebrovascular occlusion (3-VO). Values are expressed as percentage of control group (n = 6). * = P < 0.05 vs. control group; + = P < 0.05 vs. ischemia-reperfusion group.

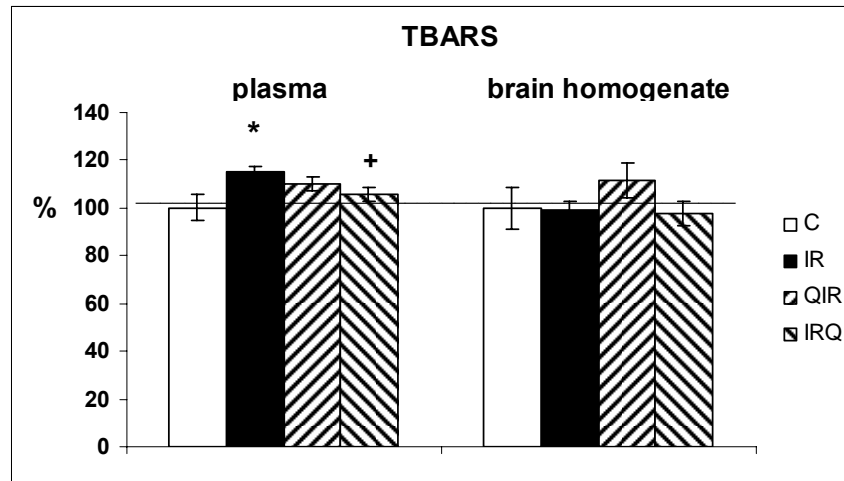


Figure 4. Effect of ischemia-reperfusion injury and CoQ10 supplementation on brain and plasma thiobarbituric acid reactive substances (TBARS) formation in rats. C, control group; IR, ischemia (50 min)-reperfusion (30 d) group; QIR, preventive CoQ10 supplementation for 30 d before ischemia-reperfusion; IRQ, therapeutic supplementation of CoQ10 for 30 d after ischemia. Values are expressed as percentage of control group. (n = 6). * = $P < 0.05$ vs. control; + = $P < 0.05$ vs. ischemia-reperfusion.

The effects of CoQ₁₀ supplementation on plasma concentrations of CoQ and tocopherols are presented on Tab. 2. and Fig. 3. Plasma concentration of CoQ_{9-ox} significantly decreased by 59% ($P < 0.05$) after ischemia-reperfusion; decrease of α -tocopherol and increase of γ -tocopherol are not significant when compared with control group.

Preventive supplementation with CoQ₁₀ led to normal concentration of α -tocopherol ($P < 0.05$), but to lower concentration of γ -tocopherol, compared with control and ischemia-reperfusion groups without CoQ₁₀ supplementation. Therapeutic supplementation with CoQ₁₀ keep normal level of α -tocopherol, but the concentration of CoQ_{9-ox} and γ -tocopherol had significantly lower levels ($P < 0.05$). CoQ_{10-ox} concentrations in plasma were not detectable.

Effect of coenzyme Q supplementation on TBARS formation

Plasma and brain values of thiobarbituric acid reactive substance (TBARS) formations as an index of oxidative stress are expressed in Tab. 3. and Fig. 4.

Ischemia-reperfusion produced significant increase in plasma formation of TBARS ($P < 0.05$), but not in brain homogenate. After preventive CoQ₁₀ supplementation the values of TBARS in plasma not differ from values of ischemia-reperfusion group, but in brain homogenate the values of TBARS tended to increase. After therapeutic CoQ₁₀ supplementation the values of TBARS significantly decreased ($P < 0.05$) when compared with ischemia-reperfusion group, and in brain homogenate the values of TBARS not differ from control groups.

Discussion

Normal function of the brain is dependent on adequate blood flow and substrate delivery for production of adenosine three phosphate (ATP) in mitochondria. Impaired delivery of glucose together with a deficient delivery of oxygen as energy substrates for aerobic glycolysis lead to mitochondrial dysfunction, oxidative stress and decreased adenosine three phosphate production [15]. Since brain tissue cannot store oxygen or glucose, energy failure can occur after acute or chronic, local or global ischemia resulting in the ischemic neuronal cell changes [16].

Several studies of ischemia/reperfusion-induced injury to mitochondrial energy-transducing processes have been reported. It is known that the brain damage produced by transient cerebral ischemia develops mainly during the reperfusion [17,18]. Sims & Pulsinelly [19] observed decreases in mitochondrial respiratory activity with 3 h of reperfusion following 30-min ischemia, and Sciamanna et al. [20] observed decrease in mitochondrial respiratory activity during 30-min cerebrovascular occlusion and much more decrease of activity after 5-hour of reperfusion. In the present study, we observed similar decrease in mitochondrial respiratory activity with 30-day of reperfusion and 50-min of ischemia induced by three cerebral vessels (3-VO) occlusion (Tab. 1, Fig. 1).

The mitochondrial disorders could be defined either as disorders due to defects of mitochondrial enzymes, or as disorders characterized by morphological abnormalities of mitochondria [1,21]. Brain is more vulnerable to ROS-

induced damage due to its high rate of oxygen consumption, high polyunsaturated lipid content, and relative lack of classic antioxidant enzymes [22,23]. In this study, mitochondrial and plasma levels of thiobarbituric acid reactive substances (TBARS) assay was employed as an index of oxidative stress. Cerebrovascular occlusion (3-VO) for 50 min followed by reperfusion for 30 days produce significant impairment in brain mitochondrial oxidative phosphorylation ($P<0.05$) and 15% increase in ($P<0.05$) in formation of thiobarbituric acid reactive substances in plasma as index of lipid peroxidation during ischemia-reperfusion injury of rat brain. These results are in agreement with other studies [24]. The overproduction of ROS can be detoxified by endogenous antioxidants [25,26].

Coenzyme Q (ubiquinone), an electron carrier in the inner mitochondrial membrane, may stabilize the respiratory chain components and act as an endogenous antioxidant.[27,28,29]. The present study was undertaken to test whether oral administration of exogenous coenzyme Q could protect against transient cerebral ischemia-induced mitochondrial damage in the rat brain. Zhang et al. [30,31] showed, that dietary coenzyme Q was taken up only into liver, spleen and plasma, and not into kidney, heart, muscle and brain. The beneficial effects exogenous coenzyme Q supplementation was detected only in subjects whose coenzyme Q levels have been depleted by defective synthesis [32].

Conclusion

The results of our study demonstrated that coenzyme Q supplementation for 30 days before the induction of cerebrovascular occlusion is not effective. However, oral supplementation of coenzyme Q during reperfusion significantly protected rats from reperfusion-induced brain injury. Significantly reduced concentration of coenzyme Q in brain and plasma could be explained by the consumption of coenzyme Q consumption due to scavenging of the rapidly generating ROS due to ischemia-reperfusion injury. These observations suggest that the supplementation of coenzyme Q may be potentially viable agent in the clinical therapy of stroke.

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