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SkQ1 Controls *CASP3* Gene Expression and Caspase-3-Like Activity in the Brain of Rats under Oxidative Stress

S. B. Panina[1], O. I. Gutsenko[1], N. P. Milyutina[1,a]*, I. V. Kornienko[1], A. A. Ananyan[1], D. Yu. Gvaldin[1], A. A. Plotnikov[1], and V. V. Vnukov[1]

[1]*Southern Federal University, Academy of Biology and Biotechnology, Department of Biochemistry and Microbiology, 344090 Rostov-on-Don, Russia*
[a]*e-mail: natmilut@rambler.ru*

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Abstract—Here, we studied the effect of the mitochondria-targeted antioxidant SkQ1 (plastoquinone cationic derivative) on the *CASP3* gene expression and caspase-3 activity in rat cerebral cortex and brain mitochondria under normal conditions and in oxidative stress induced by hyperbaric oxygenation (HBO). Under physiological conditions, SkQ1 administration (50 nmol/kg, 5 days) did not affect the *CASP3* gene expression and caspase-3-like activity in the cortical cells, as well as caspase-3-like activity in brain mitochondria, but caused a moderate decrease in the content of primary products of lipid peroxidation (LPO) and an increase in the reduced glutathione (GSH) level. HBO-induced oxidative stress (0.5 MPa, 90 min) was accompanied by significant upregulation of *CASP3* mRNA and caspase-3-like activity in the cerebral cortex, activation of the mitochondrial enzyme with simultaneous decrease in the GSH content, increase in the glutathione reductase activity, and stimulation of LPO. Administration of SkQ1 before the HBO session maintained the basal levels of the *CASP3* gene expression and enzyme activity in the cerebral cortex cells and led to the normalization of caspase-3-like activity and redox parameters in brain mitochondria. We hypothesize that SkQ1 protects brain cells from the HBO-induced oxidative stress due to its antioxidant activity and stimulation of antiapoptotic mechanisms.

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Keywords: mitochondria-targeted antioxidant, brain, mitochondria, *CASP3* gene expression, caspase-3, hyperoxia

In the last few years, numerous studies have demonstrated that oxidative stress leads to different forms of cell death, including apoptosis, which is the most important cause of neuronal loss in neurodegenerative disorders, ischemia, and brain trauma [1-3]. Hyperbaric oxygenation (HBO) is a commonly used model of oxidative stress that allows studying stress-induced impairments in the free radical homeostasis and programmed cell death in the nervous and other tissues [4]. It has been established that oxidative stress developing during hyperoxia initiates mitochondrial and receptor-mediated apoptotic pathways. Apoptosis is now believed to be the most important mechanism of the damaging action of HBO. HBO-induced apoptosis has been demonstrated in various cell lines [5-7] and animal models [8-10].

Different programmed cell death pathways in the nervous tissue result in the activation of the caspase cascade. The key role in the classical mechanism of neuronal apoptosis belongs to the effector caspase-3 [11]. It is known that caspase activity can be regulated at different levels [12]. Apparently, redox signaling and oxidative stress play an important role in the activation of initiator and effector caspases [3, 13].

The neuroprotective effect of mitochondria-targeted antioxidants in ischemia/reperfusion, brain trauma, and neurodegenerative diseases have been shown by many research groups [14-16]. Thus, antioxidants of the SkQ family display the antiapoptotic effect in the development of different pathological conditions associated with oxidative stress [10, 17-19]. However, little is known on the effect of SkQ antioxidants on the functioning of caspase-3, which many authors believe to be a target critical for neuroprotection in various neurological disorders [20, 21]. Hence, studying the antiapoptotic effect and neuroprotective activity of SkQ compounds in the oxidative stress with different etiology is undoubtedly interesting.

Abbreviations: DC, diene conjugate; EPO, erythropoietin; GR, glutathione reductase; GSH, glutathione; HBO, hyperbaric oxygenation; LPO, lipid peroxidation; MDA, malonic dialdehyde; SB, Schiff bases.

* To whom correspondence should be addressed.

The purpose of this work was to elucidate the SkQ1 effect on the *CASP3* gene expression and caspase-3 activity in mitochondria and cerebral cortex in hyperoxia-induced oxidative stress.

MATERIALS AND METHODS

Experiments were performed in *Rattus norvegicus* male rats (body weight, 180-200 g). All experimental animals were divided into six groups: group 1 (control) included intact animals kept under standard conditions; group 2 (SkQ1) was injected for 5 days with SkQ1 in a dose of 50 nmol/kg body weight; group 3 (HBO) was subjected to HBO (0.5 MPa, 90 min) and decapitated immediately after the HBO session; group 4 (HBO12) was subjected to HBO and decapitated 12 h after the HBO session; group 5 (SkQ1+HBO) was injected with SkQ1 (50 nmol/kg body weight for 5 days), subjected to HBO 1 h after the last injection, and decapitated immediately after the HBO session; group 6 (SkQ1+HBO12) was injected with SkQ1 (50 nmol/kg body weight for 5 days), subjected to HBO 1 h after the last injection, and decapitated 12 h after the HBO session. For HBO, experimental animals were placed into a 60-liter pressure chamber equipped with an alkaline carbon dioxide absorber. After ventilation with pure oxygen for 3 min, the pressure in the chamber was increased to 0.5 MPa. The compression and decompression were performed at the rate of 0.2 MPa/min, the isopression was for 90 min. This regimen of HBO (0.5 MPa for 90 min) is known to cause an acute oxidative stress [22]. SkQ1 was dissolved in 100 μl of 0.2% ethanol and injected into the cheek pouches of the animals. The dose and mode of SkQ1 administration were chosen according to [23] and our previous studies.

mRNA expression was analyzed by reverse transcription followed by quantitative polymerase chain reaction (RT-qPCR) with specific primers. Total RNA was isolated from 30 mg of cerebral cortex by the guanidine thiocyanate/phenol/chloroform method using commercial RIBO-zol-V kit (InterLabServis, Russia). The quality of isolated RNA was assessed by electrophoresis in 1.2% agarose gel; RNA concentration was determined from the absorbance at 260 nm. cDNA was synthesized using a Reverse Transcription kit (Syntol, Russia) containing MMLV-RT (Moloney Mouse Leukemia Virus Revertase), Random-6 primers, deoxyribonucleotide triphosphate mixture (dNTPs), and RNase inhibitor.

The expression levels of genes for caspase-3 (*CASP3*), transcription factor Nrf2 (*Nrf2*), Cu,Zn superoxide dismutase SOD1 (*SOD1*), Mn superoxide dismutase SOD2 (*SOD2*), extracellular superoxide dismutase E-SOD (*SOD3*), catalase (*CAT*), and glutathione peroxidase 4 (*Gpx4*) were determined by the real-time PCR (qPCR) in the presence of the intercalating EVA Green dye (Molecular Probes, USA) with a qPCR kit (Syntol) using an iQ5 real-time PCR detection system (Bio-Rad Laboratories, USA). β-Actin (*BACT*) was used as a reference gene. The primers for qPCR were chosen with the Primer BLAST and Primer 3 programs (Table 1).

Table 1. Primers for PCR-RT and efficiency of the primer pairs used

Gene	Nucleotide sequences of forward (*f*) and reverse (*r*) primers	Primer pair	Efficiency, E
BACT	f: 5'-agccatgtacgtagccatcc-3' r: 5'-tcggaaccgctcattgccg-3'	*BACT-f, BACT-r*	0.953
CASP3	f: 5'-caacaacgaaacctccgtgg- 3' r: 5'-ctgctccttttgctgtgatc-3'	*CASP3-f, CASP3-r*	0.842
Nrf2	f: 5'-atgtcaccagctcaagggcacagtgc-3' r: 5'-ccatcctccccgaacctagtt-3'	*Nrf2-f, Nrf2-r*	0.970
SOD1	f: 5'-aaccagttgtggtgtcagga-3' r: 5'-ctcctgagagtgagatcaca-3'	*SOD1-f, SOD1-r*	0.930
SOD2	f: 5'-taacgcgcagatcatgcag-3' r: 5'-gtcacgcttgatagcctcca-3'	*SOD2-f, SOD2-r*	0.873
SOD3	f: 5'-aggctctttctcaggcctc-3' r: 5'-agatctccaggtctttggag-3'	*SOD3-f, SOD3-r*	0.933
CAT	f: 5'-ttctacactgaagatggtaactg-3' r:5'-gaaagtaacctgatggagagac-3'	*CAT-f, CAT-r*	0.903
Gpx4	f: 5'-ggctacaatgtcaggtt-3' r: 5'-ttatcaatgagaaacttggtaa-3'	*Gpx4-f, Gpx4-r*	0.780

The efficiency (*E*) of each primer pair was evaluated by PCR at different cDNA dilutions (1 : 1, 1 : 2, 1 : 4, and 1 : 8) with subsequent calculation of average ΔCt (Table 1).

qPCR was performed as follows: denaturation at 95°C for 5 min and 40 cycles of annealing/synthesis at 58-60°C for 50 s and denaturation at 95°C for 15 s. The specificity of the amplification products was confirmed by recording their melting curves.

Accumulation of the PCR products was analyzed using the Bio-Rad IQ5 Optical System Software version 2.0; qPCR data were processed with the iCycler IQ5 software (Bio-Rad). The expression levels of the analyzed genes were calculated by the ΔCt method (using the reference gene).

For the biochemical studies, the rats were sacrificed by decapitation. The brains were isolated and washed in 0.9% ice-cold NaCl solution; the cortices were isolated on ice (cortices from two animals was combined into one sample). The tissue was homogenized at a 1 : 10 ratio (w/v) in buffer (100 mM Tris-HCl, 150 mM NaCl, 0.5 mM EDTA, pH 7.4) with a Potter S homogenizer (LKB, Sweden) for 2 min at 1500 rpm. The resulting homogenates were treated with Triton X-100 (final concentration, 0.1%), incubated for 10 min at 37°C, and then centrifuged for 15 min at 1000*g*. The resulting supernatant was used for determination of the caspase-3 activity.

Mitochondria were isolated by differential centrifugation [24]. Isolated cerebral hemispheres were washed in cold PBS at 4°C, dried with a filter paper, and weighed. Ten milliliters of the isolation medium (0.25 M sucrose containing 0.2 M Tris-HCl and 1 mM EDTA, pH 7.4) was added to 1 g of the brain tissue. The brain tissue was then homogenized and centrifuged at 4°C for 10 min at 1000*g* to remove nondisintegrated cells and nuclei. The resulting supernatant was centrifuged at 14,000*g* for 10 min at 4°C. The pelleted mitochondria were washed with 5 ml of the isolation medium without EDTA and then lysed by adding 2 ml of buffer solution supplemented with a non-ionic detergent (0.1 M potassium phosphate buffer, pH 7.8, containing 0.1% Triton X-100) at 4°C. The mitochondrial lysate was assayed for the activity of caspase-3, reduced glutathione (GSH) content, glutathione reductase (GR) activity, and concentration of molecular products of lipid peroxidation (LPO).

Caspase-3 activity was determined using a Caspase 3 Assay Kit, Colorimetric (Sigma, USA). The colorimetric assay of caspase-3 activity is based on hydrolysis of the peptide substrate acetyl-Asp-Glu-Val-Asp-*p*-nitroanilide (Ac-DEVD-pNA) with production of the chromophore *p*-nitroaniline (pNA) with the maximum absorbance at 405 nm. Because the substrate DEVD is also cleaved with caspase-7, this type of activity is called caspase-3-like or DEVDase activity [25].

The intensity of lipid peroxidation (LPO) was determined in the brain mitochondrial fraction from the accumulation of LPO products. The content of diene conjugates (DC) was determined spectrophotometrically at 233 nm in the chloroform extract of mitochondria [26]. The content of malonic dialdehyde (MDA) was determined by the reaction with thiobarbituric acid [27]. The content of Schiff bases (SB) was determined spectrofluorimetrically (excitation maximum, 360 nm; emission maximum, 440 nm) [28]. The chloroform extract was prepared as described by Bligh and Dyer [29]. The GR activity was assessed from the rate of NADPH oxidation [30]. The GSH content was determined by the reaction with dithio-bis(2-nitrobenzoic acid) [31].

The data were analyzed using the Statistica 10 StatSoft program package. The normality of distribution was verified with the Shapiro–Wilk test. The groups were compared using the two-factor analysis of variance ANOVA with subsequent pair comparisons of the group means (the Newman–Keuls test). Multiple linear regression, including all predictors, was used to evaluate potential relationship between the dependent variable (*CASP3* gene expression) and expression of the *Nrf2* and Nrf2-controlled genes of the antioxidant enzymes (*SOD1*, *SOD2*, *GPx4*, *CAT*). The differences were considered significant at $p < 0.05$; at $0.05 < p < 0.1$, there was considered a trend to significant differences.

RESULTS

The effect of SkQ1 on *CASP3* expression and caspase-3-like activity in the cerebral cortex was studied under physiological conditions and in the HBO-induced oxidative stress. We found that under physiological conditions, SkQ1 administration of (50 nmol/kg for 5 days) did not change the level of *CASP3* expression or the effector caspase activity (Figs. 1 and 2).

However, both *CASP3* expression and caspase-3-like activity were significantly influenced by the SkQ1 administration in the hyperoxia-subjected rats. HBO-induced oxidative stress increased the level of *CASP3* mRNA expression in the cortical cells of HBO12 rats by 57% ($F = 9.96$; $p = 0.0002$) as compared to the control. Caspase-3 cleaves many vitally important protein substrates during the HBO-induced oxidative stress. Injection of SkQ1 for 5 days before the HBO session normalized transcriptional activity of the *CASP3* gene; however, in comparison with the HBO12 group, the level of *CASP3* mRNA in the SkQ1+HBO group was decreased by 47% ($F = 9.33$; $p = 0.003$) (Fig. 1).

Similar results were observed for the caspase-3-like activity in the rat cortex. Thus, the caspase-3-like activity in the HBO12 rats increased by 89% ($F = 62.15$; $p < 0.0001$) as compared to the control. SkQ1 administration for 5 days before the HBO session normalized caspase-3-like activity and decreased it by 39% as compared to the HBO12 group ($F = 36.08$; $p < 0.0001$) (Fig. 2).

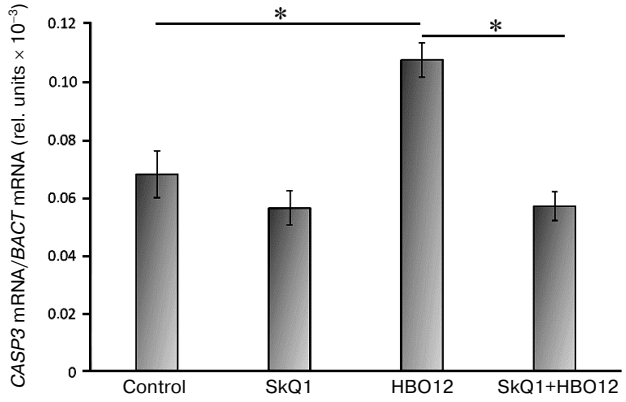

Fig. 1. The effect of SkQ1 on the level of *CASP3* mRNA in the rat cortex under normal conditions and after HBO-induced oxidative stress (M ± m). Each group included 20 animals; * $p < 0.05$ (ANOVA with *post hoc* Newman–Keuls).

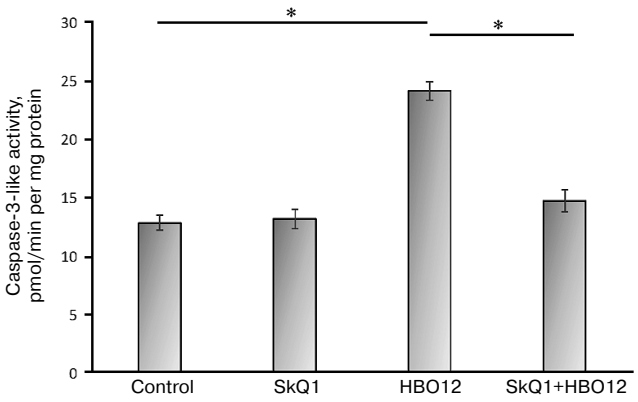

Fig. 2. The effect of SkQ1 on the caspase-3-like activity in the rat cortex under normal conditions and after HBO-induced oxidative stress (M ± m). Each group included 10 animals; * $p < 0.05$ (ANOVA with *post hoc* Newman–Keuls).

There is a direct correlation between the *CASP3* expression level and caspase-3-like activity in the cerebral cortex ($R = 0.51$; $p = 0.02$).

To elucidate the specific features of the SkQ1 effect on the caspase-3-like activity in hyperoxia, the enzyme activity and the levels of oxidative stress biomarkers were studied in the brain mitochondria immediately after the HBO session.

We found that SkQ1 administration under physiological conditions did not affect the caspase-3-like activity in the mitochondria and the cytosol of the cortical cells. However, in rats subjected to HBO, SkQ1 injection increased the caspase-3-like activity by 130% ($F = 143.9$; $p < 0.0001$) and 114% ($F = 66.02$; $p < 0.0001$) in the mitochondrial and cytoplasmic fraction, as compared to the control (Fig. 3, a and b). SkQ1 administration for 5 days before the HBO session normalized the enzyme activity in both mitochondrial and cytosolic fractions and decreases in the caspase-3-like activity by 54% in the mitochondria ($F = 115.48$; $p < 0.0001$) and by 48% in the cytosol ($F = 47.08$; $p < 0.0001$), as compared to the HBO group.

Oxidation by free radicals is one of the most important mechanisms of programmed cell death; therefore, we studied the contents of LPO molecular products in the cortical mitochondria from the control and HBO-subjected rats and found that both SkQ1 and hyperoxia significantly affected the contents of LPO products in brain mitochondria.

SkQ1 injection under physiological conditions decreased the level of DCs and MDA in the brain mitochondria by 12-15% with respect to the control ($F = 33.93$; $p < 0.0001$), whereas the content of SB did not significantly differ from the value observed in the control group (Fig. 4, a-c).

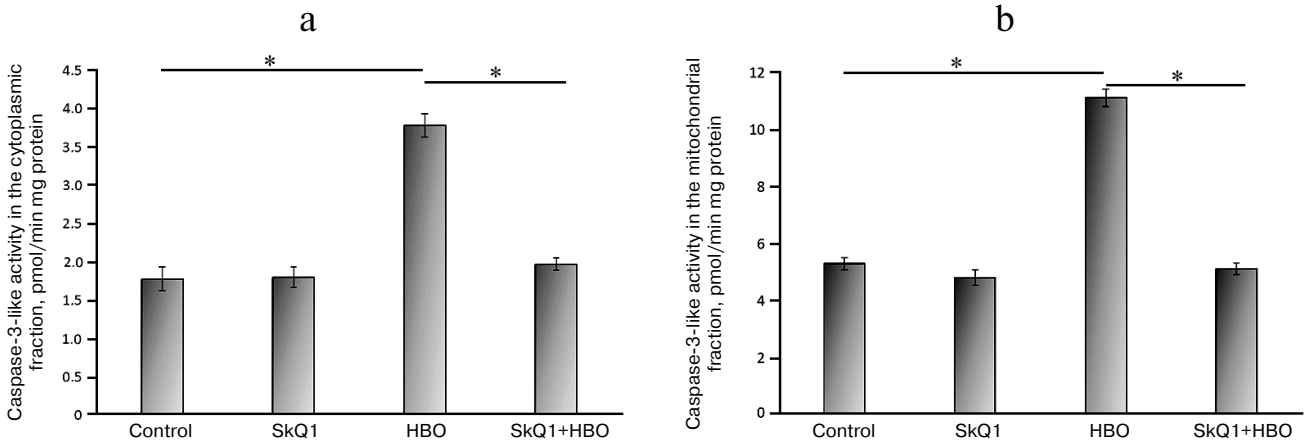

Fig. 3. The effect of SkQ1 on the caspase-3-like activity in the mitochondrial (a) and cytoplasmic (b) fractions of the rat cortex under normal conditions and after HBO-induced oxidative stress (M ± m). Each group included 14 animals; * $p < 0.05$ (ANOVA with *post hoc* Newman–Keuls).

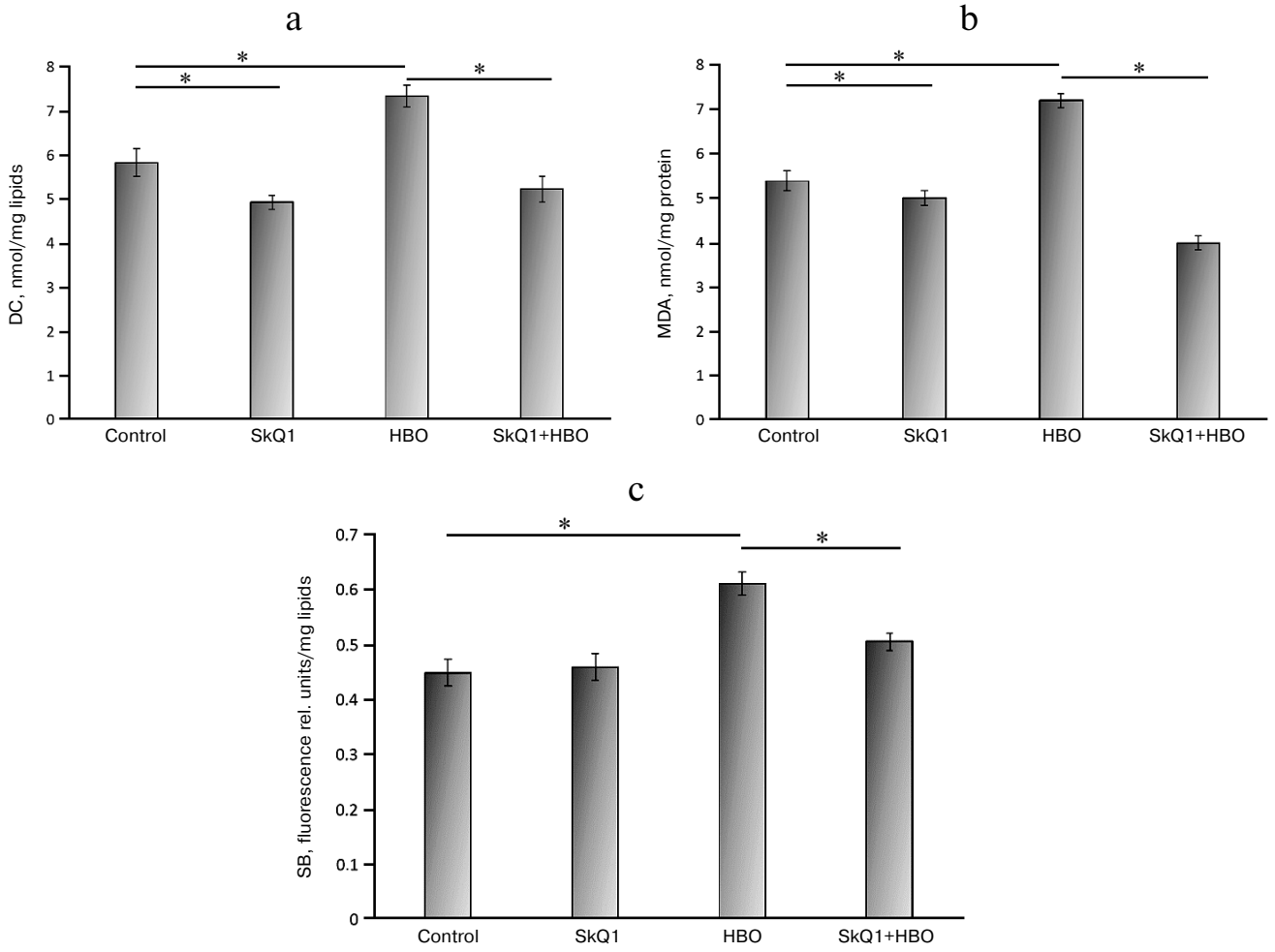

Fig. 4. The effect of SkQ1 on the contents of LPO products (a) DC, (b) MDA, and (c) SB in rat brain mitochondria under normal conditions and after HBO-induced oxidative stress (M ± m). Each group included 14 animals; * $p < 0.05$ (ANOVA with post hoc Newman–Keuls).

As compared to the control, HBO-induced oxidative stress increased the content of primary LPO products in the mitochondrial fraction by 26% ($F = 12.33$; $p = 0.001$). The content of secondary LPO products increased by 34% ($F = 4.78$; $p = 0.033$), and the content of terminal LPO products increased by 36% ($F = 23.02$; $p < 0.0001$). SkQ1 administration before the HBO led to the normalization of the LPO product levels. The content of DC decreased by 29% ($p = 0.0001$), the content of MDA decreased by 45% ($p = 0.0002$), and the content of SB decreased by 17% ($p = 0.001$), as compared to the HBO group. Note that the MDA content in the SkQ1+HBO group was by 26% lower ($F = 4.78$; $p = 0.033$) than in the control.

GSH is one of the most important redox compounds regulating the extent of apoptosis [1]. Therefore, we estimated the levels of GSH and the activity of glutathione reductase (GR) responsible for its regeneration in the brain mitochondria of the experimental animals.

It was found that HBO-induced oxidative stress decreased the levels of both GSH and GR activity in the mitochondria by 20% (1.67 ± 0.18 μmol/g protein vs. 2.04 ± 0.21 μmol/g protein in the control; $p < 0.05$) and by 24% (10.72 ± 1.34 IU/g protein vs. 14.16 ± 0.68 IU/g protein in the control; $p < 0.01$), respectively. Note that in the SkQ1+HBO group, SkQ1 was able to maintain the high level (3.15 ± 0.29 μmol/g protein) of GSH that was by 54% higher ($p < 0.05$) than in the control, whereas the GR activity was 12.77 ± 0.64 IU/g protein, i.e., close to normal ($p > 0.1$).

Our next goal was to analyze a potential relationship between the *CASP3* gene expression and expression levels of the *Nrf2* and Nrf2-controled genes of the antioxidant enzymes (*SOD1*, *SOD2*, *Gpx4*, *CAT*) as possible predictors using the linear multiple regression. We found that the most significant regression model ($R = 0.750$, $R^2 = 0.562$) was the one, in which a probable influence of the predictors on the *CASP3* gene expression level was assessed under the hyperoxia conditions (Table 2).

Table 2 shows that under the hyperoxia conditions, *CASP3* expression could be significantly influenced by the

Table 2. Regression analysis results: the model with the *CASP3* gene expression level as the dependent variable under hyperoxia conditions of expression levels of the *Nrf2*, *SOD1*, *SOD2*, *Gpx4* and *CAT* genes as independent predictors

Predictor	B coefficient	SE(B)*	β coefficient	t	*p*	95% CI for B	
						lower limit	upper limit
(Constant)	0.149	0.036		4.164	0.001	0.071	0.227
Nrf2	−3.371	3.176	−0.215	−1.061	0.309	−10.290	3.548
SOD1	0.059	0.091	0.126	0.642	0.533	−0.140	0.257
SOD2	−0.320	0.241	−0.445	−1.329	0.208	−0.844	0.204
CAT	−3.431	1.517	−0.542	−2.261	**0.043**	−6.736	−0.125
Gpx4	0.156	0.054	0.819	2.894	**0.013**	0.038	0.273

Note: Significant predictors are shown in bold ($p < 0.05$).
* B coefficient standard error.

transcriptional levels of two (*CAT* and *Gpx4*) out of five predictor genes. The activation of *CASP3* expression is associated with the decrease in the *CAT* gene expression (B = −3.43; 95% CI (−6.74 and −0.13), $p = 0.043$) and, on the contrary, with the increase in the gene *Gpx4* expression (B = 0.16; 95% CI (0.04 and 0.27), $p = 0.013$). The other analyzed regression models did not include significant predictors and were characterized by low determination coefficients.

DISCUSSION

Here, we found that administration of the mitochondria-targeted antioxidant SkQ1 under physiological conditions does not lead to the changes in the *CASP3* expression and caspase-3-like activity in the brain cortical cells and in the mitochondrial and cytoplasmic fractions of brain cells of the experimental animals.

Caspase-3 is an intracellular effector cysteine protease with highly conserved active site that hydrolyzes peptide bonds after aspartic acid residues. The enzyme is located in the cytoplasm and mitochondrial intermembrane space and acts as a central effector in many apoptotic pathways, as well as at the apoptosis terminal stage in neurons and glial cells [12, 32].

The intracellular localization of active and inactive forms of caspases has been intensively studied under physiological conditions and in various pathologies [33]. It has been shown that caspases, including caspase-3, are located in the cytosol and different subcellular components, including mitochondria, nucleus, synaptosomes, myelin, and endoplasmic reticulum, thereby suggesting a ubiquitous character of apoptosis in various cell types [34-36]. It was supposed that localization of caspases within a particular compartment under apoptotic and non-apoptotic conditions should facilitate their role as main targets in different types of stress and in the regulation of different apoptotic pathways. It was established [37, 38] that caspase-3 is not a cytosol-specific enzyme; its constitutive activity was detected in the mitochondrial and nuclear fractions obtained from the neocortex, cerebellum, and hippocampus of rats.

The *CASP3* gene is located in human chromosome 4 (4q35.1), its promoter does not have the TATA-box and contains sites for binding of different transcription factors [39]. The activity of caspase-3 is regulated at multiple levels, including transcription, proteolytic processing, modulation of the enzymatic function, and degradation [12].

We established that under conditions of hyperoxia-induced oxidative stress, the expression of *CASP3* in the brain cortex is significantly upregulated, which to some extent represents activation of the programmed cell death.

Obviously, such increase in the transcriptional activity of the effector caspase-3 gene in oxidative stress can be caused by various factors, among which transcription factors play an important role. Analysis of the *CASP3* gene promoter revealed the presence of binding sites for various transcription factors: Sp1 clusters, Ets1-like elements [39], sites for binding AP1, NF-κB, p53, etc. [11]. These and other transcription factors are activated in hyperoxia, which could upregulate the *CASP3* expression and promote apoptosis [40, 41]. Note that oxidative stress leads to a significant increase in the expression of Sp1 and Ets factors, thereby resulting in the activation of caspase-3 function and facilitation of apoptosis in various types of cells [39, 42, 43].

Oxidative stress induced by hyperoxia causes a decrease in the transcriptional activity of the *Nrf2* gene in the rat brain cortex [44] that can also facilitate the programmed cell death. It is known that Nrf2 binds to the antioxidant response elements (ARE) in the promoters of genes for the antiapoptotic proteins of the Bcl-2 family (Bcl-2, Bcl-xL) and regulates their expression [45, 46]. The deficiency of the antiapoptotic proteins of the Bcl-2

family can lead to the increase in the mitochondrial outer membrane permeability (MOMP), release of cytochrome *c*, apoptosome formation, and initiation of the caspase cascade with subsequent cleavage and activation of caspase-3. In this study, we showed that caspase-3 activation in the brain cortex and in the mitochondria and cytosol of brain cells can be promoted by the HBO-induced oxidative stress.

Using regression analysis, we demonstrated the dependence between the expression levels of *CASP3* and genes encoding antioxidant enzymes. The reverse relationship between the *CASP3* and *CAT* gene expression in hyperoxia might be related to the important role of hydrogen peroxide (H_2O_2) in the apoptosis induction [13]. On the other hand, it cannot be excluded that the direct relationship between the expression levels of the *CASP3* and *Gpx4* is a compensatory response of the genome to the oxidative stress. Gpx4 is synthesized as short and long isoforms (20 and 23 kDa, respectively); the long isoform is located in mitochondria and plays a leading role in the cell protection against oxidative stress and apoptosis [47]. The deletion of the *Gpx4* gene not only causes embryonic and neonatal lethality in mice but also results in the death of adult animals. Moreover, Gpx4-deficient mice display mitochondrial dysfunctions, increased levels of apoptosis, and neurodegeneration [48].

An important result of this work is a discovery of the direct dependence between the *CASP3* expression and caspase-3-like activity in the cortex of rats subjected to the HBO-induced oxidative stress. The observed increase in the caspase-3-like activity in the mitochondria and cortical cells can be associated with the increase in the *CASP3* gene expression, as well as with the LPO intensification, decrease in the GSH level, and suppression of the activity of GR that regenerates GSH.

It should be emphasized that LPO activation and oxidation of polyunsaturated acyl chains of cardiolipin (mitochondrial phospholipid marker) catalyzed by the cardiolipin complexes with cytochrome *c* can be considered proapoptotic signals that trigger mitochondrial dysfunction, metabolic reprogramming, and mitophagy [49]. Reactive oxygen and nitrogen species (ROS/RNS) can oxidize cellular GSH or induce its export leading to the impairments in the intracellular redox homeostasis and activation of the apoptotic signaling cascade [1].

Preliminary administration of SkQ1 (cationic derivative of plastoquinone) for 5 days before the hyperoxia session provided the maintenance of the normal levels of the *CASP3* gene expression and caspase-3-like activity. This result deserves a special attention because the retention of the basal levels of caspase activities in oxidative stress might be a promising strategy in neuroprotection [7].

It is likely that transcriptional activation of the *Nrf2* and Nrf2-controled genes encoding antioxidant enzymes (shown earlier in our studies [44]) is one of the factors that mediate the effect of SkQ1 on the *CASP3* gene expression and enzyme activity in the rat brain in the HBO-induced oxidative stress. On one hand, this leads to a decrease in the extent of HBO-induced oxidative stress and normalization of the *CASP3* gene transcriptional activity that is known to be activated by redox-sensitive transcription factors. On the other hand, an increase in the Nrf2-regulated expression of the antiapoptotic proteins Bcl-2 and Bcl-xL prevents MOMP and formation of apoptosome, accompanied by caspase-9 activation by the induced approach mechanisms with subsequent activation of caspase-3.

We should also mention the study by Chernyak et al. [19], in which subnanomolar concentrations of the mitochondria-targeted antioxidants of the SkQ family were shown to prevent the TNF-induced apoptosis in the endothelial EAhy926 cells through inhibition of the Bid-dependent release of cytochrome *c* into the cytoplasm and following cleavage of caspase-3 and its substrate, the PARP protein. Moreover, SkQs caused an increase in the content of the antiapoptotic protein Bcl-2 and decreased the levels of the proapoptotic proteins Bax and p53.

The stimulating effect of SkQR1 on erythropoietin (EPO) production by kidneys and its neuroprotective action in cerebral ischemia demonstrated in the works by Zorov et al. [50, 51] have open new possibilities for elucidating the mechanisms of mitochondria-targeted antioxidant action on the caspase-mediated apoptosis in oxidative stress. On one hand, it is known that recombinant EPO (rEPO) has a protective effect in hyperoxia that is associated with suppression of the oxidative stress in the rat brain [52]. In *in vitro* experiments, rEPO efficiently inhibited formation of $OH^{\bullet}$ through chelating catalytically active iron (Fe^{2+}) and suppressed $LO_2^{\bullet}$ generation [53]. EPO inhibits apoptosis and displays neuroprotective effects in neurodegeneration in various types of cells, including neurons. On the other hand, EPO was shown to induce expression of the transcription factor Nrf2 which is the most powerful antiapoptotic regulator of redox balance in the cells [54, 55].

Hence, it can be assumed that the basal levels of the *CASP3* gene expression and enzyme activity in the cortex and brain mitochondria in animals treated with SkQ1 were maintained due mostly to the antioxidant activity of SkQ1. This suggestion is confirmed, in particular, by a decrease in the LPO intensity and normalization of its level in the brain mitochondria of rats subjected to HBO after preliminary SkQ1 administration, demonstrated in this work.

In this connection, it should be noted that SkQ1 and other SkQ compounds completely abolish cardiolipin peroxidation in mitochondria by interrupting the chain reaction of cardiolipin unsaturated fatty acid peroxidation [17, 18]. Moreover, SkQ1 strongly inhibits the peroxidase activity of the cytochrome *c* complex with cardio-

lipin and the permeability of phosphatidylcholine/cardiolipin liposomes. It was suggested that the antioxidant and antiapoptotic effects of plastoquinone cationic derivatives are associated with the prevention of cytochrome *c* interaction with cardiolipin [56].

In conclusion, the antiapoptotic effect of SkQ1 in the brain tissue in hyperoxia is associated with the prevention of the increase in the *CASP3* gene expression in the cortex and in the caspase-3-like activity in the brain cortex and mitochondria. The protective effect of SkQ1 in the HBO-induced stress is mediated through a diverse spectrum of antioxidant effects and stimulation of antiapoptotic factors.

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Conflicts

The authors do not have conflict of interests in the financial and other spheres.

Approval of the Ethics Committee

All procedures on animals were performed according to the principles of the European Convention about Protection of Vertebrates Used in Experiments or Science (Strasburg, March 18, 1986) and the Directive 2010/63/EU of the European Parliament and European Union Council from September 22, 2010, on the protection of animals used for scientific purpose (paragraph 27).

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