Influence of mitochondria-targeted antioxidant SkQ1 on expression of transcription factor Nrf2 gene and ARE-controlled genes of antioxidant enzymes in leucocytes of blood rats under hyperoxia-induced oxidative stress.

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Oxygen supplementation, or hyperoxia, is clinically practiced in treating premature babies and patients with cardiovascular and pulmonary diseases. However, prolonged exposure to hyperoxia in humans and animals results in increase in reactive oxygen species (ROS) generation and oxidant-induced tissue injury. Hyperoxia-induced oxidative stress activates the expression of wide range of genes that mediate the pathogenic effect of ROS or are required for the detection and detoxification of the oxidants. Hyperoxia induces the expression of several antioxidant enzymes and phase 2 detoxifying enzymes in tissues which depends on nuclear factor-erythroid 2-related factor 2 (Nrf2) [Cho et al., 2007, 2010]. Nrf2 transcriptionally induces antioxidant and defense enzyme genes by binding to the antioxidant response element (ARE). However, the regulative mechanisms of gene Nrf2 and ARE-controlled genes expression have been investigated insufficiently. Consequently the purpose of this study was to assess the effect of SkQ1 on expression of transcription factor Nrf2 gene and ARE-controlled genes of antioxidant enzymes in leucocytes of rat blood under hyperoxia-induced oxidative stress. The mitochondria-targeted antioxidants SkQ1 (plastoquinonyl-decyl-triphenylphosphonium) is a conjugate of a lipophilic decyltriphenylphosphonium cation with an antioxidant moiety of a plastoquinone [Skulachev, 2007].

The level of Nrf2 genes and genes of some antioxidant enzymes (SOD1, SOD2, SOD3, catalase and GPO4) expression in rat blood leukocytes were investigated. Rats were randomly divided into four equal groups: group 1- control; group 2 - SkQ1-treated rats received 50 nmol SkQ1/kg within 5 days; group 3 – rats after exposure under hyperbaric oxygenation (HBO; high oxygen pressure equal to 0.5 MPa during 90 min); group 4 - SkQ1-treated rats after exposure under high oxygen pressure (0.5 MPa for 90 min). The investigations in 3 and 4 groups were conducted 12 hours after HBO influence.

Transcript levels were quantified by real-time PCR using gene-specific primers. Differences in measurements of gene expression data between groups were analyzed using the Student t-test, and significance was established at $p<0.05$.

The reduction of nuclear transcription factor Nrf2 gene expression level in 49% in rats blood has been established. Nrf2 plays a critical role in protection against an oxidative stress. On the background of Nrf2 gene suppression under hyperbaric hyperoxia-induced oxidative stress decreasing in mRNA expression of ARE-controlled antioxidant enzymes genes occurs: SOD1,

better known as copper–zinc superoxide dismutase (decreased by 44%), catalase (decreased by 62%), GPO4 (decreased by 40%). However, there were no significant changes in genes SOD2 and SOD3 transcript levels compared to control group. So, it has been established that hyperoxia-induced oxidative stress caused reduction in Nrf2 and some ARE-controlled genes of antioxidant enzymes (SOD1, CAT, GPO4) expression in rats blood.

It was found that pretreatment of rats with mitochondria-targeted antioxidant SkQ1 significantly increased the level of Nrf2 gene and some ARE-controlled genes of antioxidant enzymes expression: gene Nrf2 by 216%, gene SOD1 by 70%, gene SOD2 by 77%, gene catalase by 95%, gene GPO4 by 156%. The research has shown that mitochondria-targeted antioxidant SkQ1 is a positive regulator of Nrf2 gene and the related genes of antioxidant enzymes transcript activity. SkQ1 can promote increasing antioxidant potential of leukocytes both at normoxia and hyperoxia. Revealed that the transcript activity of a gene Nrf2, as well as expression level of some ARE-controlled genes of antioxidant enzymes in rats blood comes back to norm during 12 hours after HBO-induced oxidative stress. However, an increase by 99% and 80% in genes SOD2 and GPO4 level expression after exposure under oxygen pressure in rats blood was registered.

Thus, it has been established that pretreatment of rats with mitochondria-addressed antioxidant SkQ1 promotes activation of Nrf2 gene expression and expression of Nrf2-dependent genes (SOD1, SOD2, catalase, GPO4) under the physiological norm condition, whereas SkQ1 prevents suppression of Nrf2 gene transcript activity and supports the stationary level of ARE-controlled genes expression or its activation under hyperoxia-induced oxidative stress.