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Effect of Plastoquinone Derivative 10-(6′-Plastoquinonyl)decyltriphenylphosphonium (SkQ1) on Contents of Steroid Hormones and NO Level in Rats

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Abstract—Introduction of the plastoquinone derivative 10-(6′-plastoquinonyl)decyltriphenylphosphonium (SkQ1) into male Wistar rats once a day for two weeks in doses of 25 and 250 nmol/kg led to elevation of 17β-estradiol level in blood serum by 33 and 41%, respectively. At the same time, nitrate and nitrite contents in the rat blood serum increased by 49 and 34%, respectively. ESR spectroscopy with diethyldithiocarbamate–iron complex as a spin trap showed more than twofold increase in NO production in lungs, but not in blood, liver, and intestines, following the SkQ1 daily introduction at a dose of 25 nmol/kg.

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Key words: SkQ1, penetrating cations, steroid hormones, estradiol, nitrate, nitrite, nitric oxide

Studies of geroprotector characteristics of the plastoquinone cationic derivative 10-(6′-plastoquinonyl)decyltriphenylphosphonium (SkQ1) revealed its influence on the mammalian reproductive system, particularly increasing fertility and duration of reproductive period of white female mice [1, 2]. So, studies followed on the effect of SkQ1 on the hormonal status of animals. The primary attention was focused on steroid hormones that are most tightly associated with reproduction. SkQ1 induces elevation of estradiol level in male rats.

One of the functions of estradiol is regulation of antioxidant defense [3-5]. Estradiol binds to a mitochondrial receptor and via MAP-kinase and transcription factor NF-κB enhances expression of manganese superoxide dismutase and selenium glutathione peroxidase.

Estradiol inhibits the UV-induced release of cytochrome *c* from mitochondria, decrease in their transmembrane potential, reactive oxygen species (ROS) production, and a series of other features of apoptosis [6]. Its capability of enhancing activity of natural mitochondrial antioxidant defense is considered as one of major factors determining higher longevity of females in many mammals including humans [3].

Another important function of estradiol is regulation of nitric oxide level via increase in activity of NO synthase (NOS) [7]. An effect of estrogens on blood vessel wall is known to occur through a series of transcription-independent factors (so-called non-genomic pathway). The data of several studies are indicative of significant increase in NO concentration at the peak of ovulation-associated secretion of estrogens into the blood [7, 8]. Estradiol (but not progesterone) increased activity of calcium-dependent NOSs in both male and female guinea pig tissues [9]. Taken at physiological concentrations, 17β-estradiol led to a dose-dependent increase in activity of constitutive NOSs in endothelial cell and arterial segment cultures in both men and women because of elevation of the intracellular calcium level [10]. Thus, there is a strong presumption for the triggering role of estradiol and other estrogens in regulatory cascades activating transport of calcium required for NOS activation [11]. In connection

Abbreviations: DETC, sodium diethyldithiocarbamate; MNIC, mononitrosyl iron complex; NOS, NO-synthase; ROS, reactive oxygen species; SkQ1, 10-(6′-plastoquinonyl)decyltriphenylphosphonium; StAR, steroidogenic acute regulatory protein.

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with this, we studied effects of SkQ1 on contents of NO and its metabolites in rat blood serum and other tissues.

MATERIALS AND METHODS

Male Wistar rats (outbred stock) were 45-day-old when delivered from the Nursery for Laboratory Animals of the Pushchino Branch of the M. M. Shemyakin and Yu. A. Ovchinnikov Institute of Bioorganic Chemistry of the Russian Academy of Sciences.

The rats were administered 25 and 250 nmol/kg of SkQ1 once a day for 14 days. The calculated dose was dissolved in 100 μl of 0.2% aqueous solution of ethanol and injected into the animal's cheek pouch. Animals administered with 100 μl of pure 0.2% aqueous solution of ethanol once a day for 14 days were used as a control.

17β-Estradiol in blood serum was determined using the Estradiol ELISA test-system (Cat. No. EIA-2693, DRG, Germany; http://www.drg-diagnostics.de), progesterone, testosterone, and cortisol using the steroidEIA-progesterone-01, steroidEIA-testosterone-01, and steroidEIA-cortisol-01 kits, respectively (AlkorBio, Russia; http://www.alkorbio.ru). The measurements were performed on an Alisei automated analyzer (Italy).

Peroxynitrite is one of most toxic NO metabolites. Its amount is estimated from the level of nitrotyrosine, a stable product of its reduction [12]. Nitrotyrosine in blood serum was determined using a nitrotyrosine ELISA kit (Hycult Biotechnology; http://www.hbt.nl/Site/), and nitrite/nitrate (NO_x) level in blood serum was determined by the method of Golikov et al. [13] by reduction of nitrate to nitrite using granulated cadmium followed by nitrite determination using the Griess reagent.

NO levels in rat organs and tissues were determined in the N. N. Semenov Institute of Chemical Physics (Moscow) by ESR spectroscopy with a spin trap. For this method, animals are administered exogenous traps for nitric oxide—dithiocarbamates and Fe^{2+} salts. In this work we used a lipid-soluble trap DETC and ferrous sulfate [14]. When interacting *in situ* with nitric oxide, these substances form mononitrosyl iron complexes (MNICs) directly in animal tissues. These complexes are paramagnetic, which allows their ESR detection both at 77°K and at room temperature. The tissue levels of NO were estimated by comparing the peak areas determined by double integration of ESR spectra of experimental and standard samples. Preliminary deoxygenated sodium DETC and Fe^{2+} sulfate solutions of known concentration treated with gaseous nitric oxide in Thunberg flasks were used as standards.

On completion of administration with SkQ1, 500 mg/kg of DETC was injected intraperitoneally, and Fe^{2+}/citrate complex was injected subcutaneously into a hip ($FeSO_4$, 37.5 mg/kg; Na^+ citrate, 187.5 mg/kg body weight). The animals were decapitated 30 min after injection of the spin trap components, blood was collected, and required organs were quickly, within 2-3 min, extracted and frozen in liquid nitrogen.

ESR signals were recorded using an ECS-106 spectrometer (Bruker, Germany) equipped with a TE-102 resonator. Spectral data were processed using an ESR spectrometer program module (Motorola computer with special software). The data were quantified by the double integration method with comparison of the data with a standard. The mononitrosyl complex of iron dithiocarbamate synthesized anaerobically and containing known number of spins was used as the standard.

Statistical processing of the data was performed according to the usual equations, and the standard error of the mean (SEM) was used as a criterion of dispersion in all experiments [15]. Distribution normality was tested using the Kolmogorov–Smirnov test (K-S test). Mean values were compared using Student's *t*-test. The difference was regarded as statistically significant at $p < 0.05$. Statistical data processing was performed using Statistica 5.5 and Excel 2007 program packages. Ten animals were used in each experiment.

RESULTS

The levels of progesterone, testosterone, and cortisol in rat blood serum following administration with two different doses of SkQ1 differed insignificantly from control. In contrast, the level of estradiol in male rat blood serum increased on average by 33 and 41% in response to the SkQ1 in doses of 25 and 250 nmol/kg per day, respectively (Fig. 1).

These data suggest that the SkQ1-induced elevation of estradiol level is sufficient for activating the system of NO production. In particular, introduction of SkQ1 in doses of 25 and 250 nmol/kg per day led to elevation of nitrate/nitrite level in rat blood serum by 49 and 34%, respectively (Fig. 2). The growth of nitrate/nitrite level

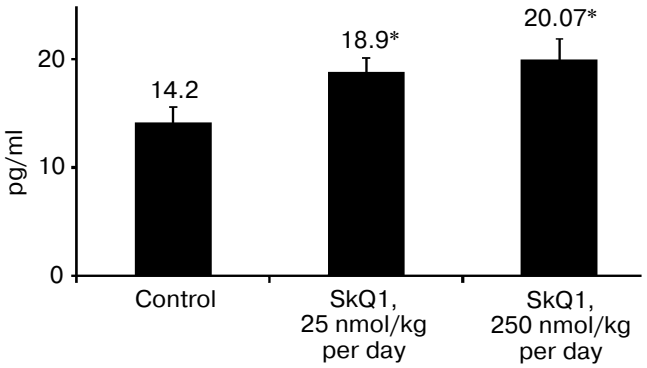

Fig. 1. Effect of SkQ1 on level of estradiol in male rat blood serum. * Here and in Figs. 2 and 4 difference from control is statistically significant (*t*-test; $p < 0.05$, $n = 10$).

may reflect enhanced NO production and other alterations in nitrogen metabolism as well. So, we carried out additional experiments on direct determination of the NO level in rat tissues by ESR.

Figure 3 displays typical spectra recorded in the experiment. The spectra *1* and *2* are ESR signals of MNIC-DETC and some other paramagnetic centers of experimental and control animals, respectively. Initial spectra in the part of interest are a superposition of copper–DETC complex (Fig. 3, curve *4*), manganese, and MNIC-DETC. Following subtraction, we observe a clear triplet signal of MNIC-DETC characterized by the g-factor value 2.035. Spectrum *4* is a "pure" ESR signal of MNIC-DETC; the ESR signal from the standard is shown for comparison (Fig. 3, curve *3*). Note that the ESR signal from the control animal is a guide to the basal level of nitric oxide: one can see a small triplet signal of MNIC-DETC.

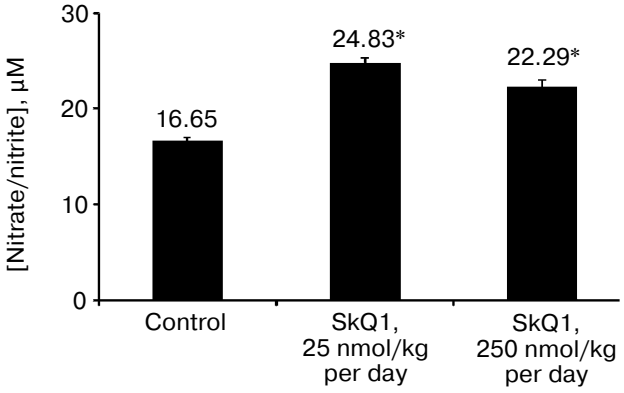

Fig. 2. Effect of SkQ1 on nitrate/nitrite level in rat blood serum.

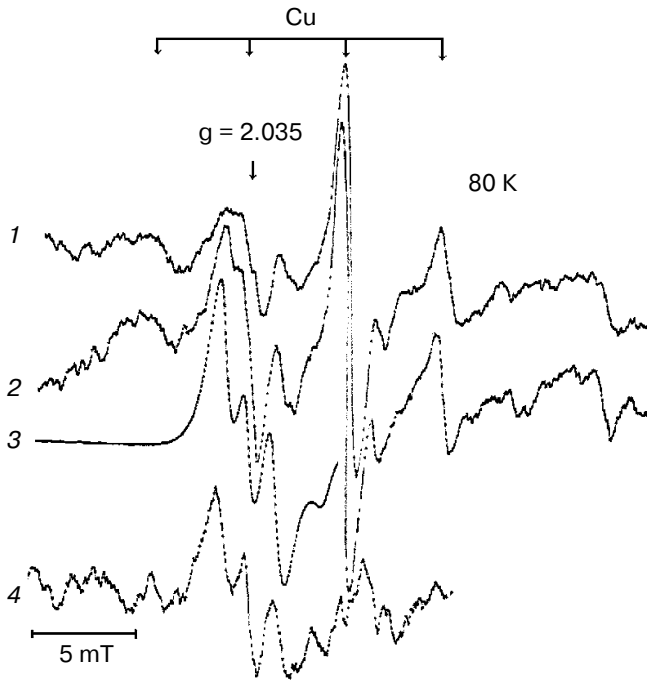

Fig. 3. ESR spectra of rat lung: *1*) animal after administration of 25 nmol/kg SkQ1; *2*) control animal (solvent without SkQ1 was introduced); *3*) ESR spectrum of MNIC-DETC standard; *4*) difference ESR spectrum of MNIC-DETC that it the result of subtracting spectrum (*2*) from spectrum (*1*). ESR conditions are as follows: static magnetic field, 336 mT; sweep width, 20 mT; microwave power 10-20 mW; amplification, 10^3-10^5; modulation amplitude, 0.5 mT; sweep time, 0.163 sec; temperature, 77°K.

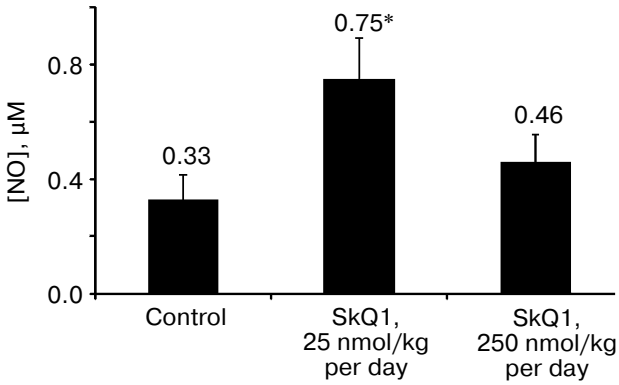

Fig. 4. Effect of SkQ1 on the NO level in rat lung.

More than twofold increase in the level of NO was observed in rat lung after introduction of SkQ1 at a dose of 25 nmol/kg per day (Fig. 4). No statistically significant difference from control was found in other tissues. Nitric oxide is an unstable substance whose lifetime in aqueous phase is several milliseconds. It is likely that elevation of nitrate and nitrite contents in blood is due to elevation of NO level in lungs. Interestingly, the aggregate nitrate/nitrite level that was determined in the first experiment also underwent greater elevation in response to the lower dose of SkQ1.

The test for peroxynitrite estimated from the level of nitrotyrosine in rat blood serum did not reveal any hazardous level of its production in response to SkQ1 (data not shown; nitrotyrosine content was below the threshold of sensitivity).

DISCUSSION

The level of any substance implicated in metabolism is determined by the ratio between the rates of its synthesis and decomposition or excretion from the body. To view possible mechanisms of elevation of estradiol level in the presence of SkQ1 in this aspect, one should keep in mind that one of the key stages of steroid synthesis in male mammals, conversion of cholesterol into pregnenolone, occurs in mitochondria, where the side chain of incoming cholesterol is cleaved by P-450scc (cholesterol 20,22-hydroxylase, cholesterol 20,22-desmolase). The rate of this process depends on the rate of cholesterol transport

between the outer and inner mitochondrial membranes by labile phosphoprotein StAR (steroidogenic acute regulatory protein) rather than on P-450scc activity [16]. It is also known that SkQ1, as well as its structural analog dodecyl triphenylphosphonium, stimulate transmembrane transport of fatty acid cations [17]. So, we may suppose the same effect of SkQ1 on the transport of newly synthesized StAR across the outer mitochondrial membrane. We also cannot exclude that SkQ1 molecules carrying both hydrophobic and hydrophilic domains can facilitate cholesterol insertion into a "hydrophobic tunnel" within the StAR protein globule [18].

Modification of estradiol preceding its conversion into hydrophilic metabolites to be eliminated from the body passes through stages associated with redox cycles and ROS production [19]. SkQ1 as antioxidant can inhibit these processes, thus increasing the level of estradiol.

As for nitric oxide and its metabolites, one should take in account that not only estradiol can influence such important physiological index as their levels. The level of NO in lung and the level of nitrate/nitrite in blood serum can increase for many reasons. In particular, NO can directly react with $O_2^{\cdot-}$ to form peroxynitrite. Hence, a decrease in ROS level in cells would benefit conservation of some portion of produced NO, thus elevating its accessibility. A complexity of mechanisms coordinating the levels of estradiol and NO is particularly evidenced in the fact that the higher dose of SkQ1 causing increase of the former index has no statistically significant effect on the latter one. The reason for this phenomenon deserves especial study.

The main phenomenological result of our study is demonstration of the fact that a fortnight daily introduction of SkQ1 at a dose of 25 nmol/kg provides a mild elevation of both estradiol and NO levels. Antioxidant effect of close doses of this substance was shown in many experiments, both *in vitro* and *in vivo* [1, 2, 20]. This combination of effects is rather rare and may be promising in therapy of a number of diseases that are accompanied by oxidative stress.

We suppose that earlier described protective effect of SkQ1 in ischemia-reperfusion injury of kidney, heart, and brain [20] also can be associated (at least in part) with a protective effect of NO induction.

The data of this work were obtained from experiments with males, which was necessary for unifying the experimental scheme in studies of effect of SkQ1 on different parameters. It is known that both metabolism and functions of estradiol differ significantly between the two sexes. A check for the ability of SkQ1 to increase the level of this hormone in females is of certain interest and we hope will be the subject of further studies.

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