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10-(6′-Plastoquinonyl)decyltriphenylphosphonium (SkQ1) Does Not Increase the Level of Cytochromes *P450* in Rat Liver and Human Hepatocyte Cell Culture

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Abstract—Mitochondria-targeted antioxidant SkQ1 did not increase the content of cytochromes *P450* in livers of rats that were given SkQ1 in drinking water for 5 days in a dose (2.5 μmol per kg body weight) that exceeded 10 times the SkQ1 therapeutic dose. SkQ1 did not affect the levels of cytochrome *P450* forms CYP1A2, CYP2B6, and CYP3A4 in monolayer cultures of freshly isolated human hepatocytes, while specific inducers of these forms (omeprazole, phenobarbital, and rifampicin, respectively) significantly increased expression of the cytochromes *P450* under the same conditions. We conclude that therapeutic doses of SkQ1 do not induce cytochromes *P450* in liver, and the absence of the inducing effect cannot be explained by poor availability of hepatocytes to SkQ1 *in vivo*.

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When xenobiotics (chemical substances foreign to the human organism) enter a human body as medications or pollutants (narcotics, toxins, etc.), they undergo oxidation in human liver to facilitate their excretion from the organism. Biotransformation of xenobiotics is catalyzed by a group of autooxidizable heme-containing liver proteins named cytochromes *P450* (CYPs) due to the absorption maximum at 450 nm in the spectrum when CYP in its reduced state is complexed with carbon monoxide. Aside from the liver, CYPs (encoded by over 50 genes) have been found in other human organs and tissues, where they predominantly oxidize endogenous substrates and are involved in the biosynthesis and metabolism of a number of physiologically active compounds [1-6].

Liver CYPs, that are located mostly in the endoplasmic reticulum membranes of hepatocytes, display unusual catalytic properties – a phenomenon that has not yet received a proper explanation. These CYPs exhibit extremely broad substrate specificity and do not follow Michaelis–Menten kinetics. Beside substrate hydroxylation of the monooxygenase type, liver CYPs can catalyze N-, S-, and O-dealkylation, heteroatom oxygenation, cleavage of ester and amide bonds, lipid peroxidation, desaturation, isomerization, etc. Due to these properties, CYP3A4, which is the most studied CYP form, can metabolize up to 50% of drugs that exist on the modern pharmacological market [1, 2, 6]. Xenobiotics are known to increase the levels of cytochromes *P450* in liver by stimulating biosynthesis of one or several CYP forms in hepatocytes [5].

For a long time, cytochromes *P450* were considered as components of the major detoxifying system in mammals that hydroxylates (hence, makes more hydrophilic) chemical compounds to facilitate their excretion from the organism. In some cases, however, the oxidation products are more toxic that the original compounds. Thus, hydroxylation activates carcinogenic properties of polycyclic aromatic hydrocarbons (for example, benzo(a)pyrene), heterocyclic amines (additives in smoked meat), and aromatic amines (pesticides, tobacco smoke components, many drugs) [1, 2, 6]. Therefore, it is important to study cytochrome *P450* induction

Abbreviations: CYP, cytochrome *P450*; SkQ1, 10-(6′-plastoquinonyl)decyltriphenylphosphonium.

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Table 1. Cytochrome *P450* content (nmol per mg protein) in liver microsomes from SkQ1-treated and control rats (daily dose, 2500 nmol per kg body weight; 5 days)

Control	SkQ1
0.68	0.62
0.79	0.67
0.59	0.76
0.70	0.58
0.62	0.69
0.60	0.61
mean 0.66 ± 0.08	mean 0.65 ± 0.07

by newly developed medications before recommending them for treatment of the corresponding pathologies.

Here and in our earlier studies, we investigated induction of cytochromes *P450* in liver by the mitochondria-targeted antioxidant SkQ1. SkQ1 was synthesized in our laboratory in 2008 [7]. It is a synthetic derivative of plastoquinone conjugated with the membrane-penetrating cation decyl(triphenyl)phosphonium. SkQ1 had passed preclinical and clinical trials [7-15] and was recommended for the therapy of various pathological conditions; it has been on the market since 2012.

Earlier, we demonstrated that therapeutic doses of SkQ1 did not affect the levels of cytochromes *P450* in rat liver. The animals received SkQ1 for 5 days, i.e. for a period commonly used to test cytochrome *P450* induction by a studied substance. As a positive control, rats were treated with the classical CYP inducer phenobarbital that considerably increased CYP content in the liver [16]. We also demonstrated that the total amount of cytochromes *P450* in liver microsomes isolated from rats that had received therapeutic doses of SkQ1 in drinking water for several (up to 24) months did not differ from the cytochrome *P450* content in livers of the control animals [17].

In this work, we repeated our previous experiments described in [16], except that the dose of SkQ1 was increased 10 times. Outbred albino male rats (body weight, 200-220 g) were given SkQ1 in drinking water (2500 nmol per kg body weight) for 5 days. The animals were sacrificed; their livers were perfused with cold physiological solution and then homogenized. Liver microsomes were isolated from the homogenate by differential centrifugation as described in [16, 17]. The total amount of cytochromes *P450* in the microsomes was determined by the classical method of Omura and Sato [18] from the absorption of the reduced cytochrome *P450* complex with carbon monoxide at 450 nm using the molar extinction coefficient of 91 $mM^{-1} \cdot cm^{-1}$. Protein was determined by Lowry's method [19]. Table 1 shows the amounts of cytochromes *P450* (nmol/mg protein) in microsomes from the livers of control and experimental rats. The results clearly indicate that SkQ1 did not induce cytochrome *P450* biosynthesis even when used is amounts that exceeded many times its therapeutic doses.

SkQ1 did not induce cytochrome *P450* expression when added directly to the monolayer cultures of freshly isolated human hepatocytes [20, 21] from donors. Hepatocytes were incubated with different SkQ1 concentrations for 72 h with daily change of the medium. The SkQ1-containing medium was then removed and replaced with solutions containing standard substrates of the CYP1A2, CYP2B6, and CYP3A4 forms (phenacetin, bupropion, and midazolam, respectively). After 15- or 30-min incubation, the concentrations of the reaction products, i.e. hydroxylated derivatives of the above-mentioned substrates, were measured by the LC-PDA-MS method. The results are presented in Table 2 as the ratios between the amounts of hydroxylated products produced

Table 2. Activity of cytochrome *P450* forms (CYP1A2, CYP2B6, and CYP3A4) in hepatocyte monolayer cell cultures (measured using corresponding cytochrome *P450* standard substrates and presented as a ratio between the concentrations of hydroxylation products in treated and untreated cells): 1) after 72-h incubation with SKQ1; 2) after incubation with inducers of cytochrome *P450* forms

Tested compound		Concentration	Activity of cytochrome *P450* forms in hepatocyte cell cultures from three donors		
			1A2	2B6	3A4
1	SkQ1	0.1 μM	0.8-1.0	0.9-1.1	1.0-1.1
	SkQ1	1 μM	1.1-1.3	1.1	0.9-1.2
	SkQ1	5 μM	0.5-0.7	0.6-1.0	0.3-0.7
2	Omeprazole	50 μM	14-16	–	–
	Phenobarbital	1 mM	–	9.8-12	–
	Rifampicin	10 μM	–	–	3.3-4.3

by cytochromes *P450* in the treated cells to the amounts of reaction products in the corresponding untreated controls. At all the concentrations tested, SkQ1 did not increase the concentration of hydroxylation products in the hepatocyte cultures from all three donors – the ratios between the amounts of the reaction products in the experimental and control cells were close to 1. Treatment of hepatocytes with CYP inducers considerably increased the ratio between the amounts of the reaction products in the treated and control cells, which proved the ability of studied hepatocyte cultures to respond to CYP inducers with a significant increase in the rates of substrate hydroxylation.

Based on the results of this work and data from our previous publications, we conclude that the SkQ1 cation does not induce cytochromes *P450* in liver when used in therapeutic doses and, therefore, SkQ1 does not exhibit negative (toxic or carcinogenic) side effects. The results obtained in hepatocyte cell culture exclude the possibility that the absence of the SkQ1 effect on liver cytochromes *P450* is due to poor delivery of this compound to the cells.

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