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The particulars of certain drugs' effect on the endogenous coenzyme Q10 plasma level in patients with cardiovascular diseases

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Abstract

Objectives: Coenzyme Q10 (CoQ10) has many vital functions in human body and its endogenous level can be affected either by various diseases or by administrated drugs. This study reveals the effect of atorvastatin, amlodipine and ethoxidol on the endogenous CoQ10 plasma concentration.

Methods: It was determined the total plasma concentration of endogenous CoQ10 in the plasma of 54 healthy individuals and 62 patients with cardiovascular diseases during treatment with various drugs using high performance liquid chromatography with mass spectrometric detection (HPLC-MS/MS).

Results: It was found that CoQ10 plasma concentration in patients is statistically significantly lower (on average –49.0 Δ%) than in practically healthy individuals. The total CoQ10 plasma level in patients receiving atorvastatin in the complex therapy is statistically significantly lower (–15.2 Δ%), and in patients taking amlodipine or ethoxidol is statistically significantly higher (+18.2 and +20.2 Δ%, respectively) than in patients of control groups (a group of patients who receive the same drugs, except for the studied one).

Conclusions: The study showed that in patients with CVDs treated with various drugs the CoQ10 plasma level is statistically significantly lower than in practically healthy individuals. So, to avoid the adverse reactions connected with low CoQ10 plasma levels, it is recommended to adjust the therapy to maintain its constant level.

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Keywords: amlodipine; atorvastatin; clinical pharmacology; Coenzyme Q10; ethoxidol; HPLC-MS/MS.

Introduction

Coenzyme Q10 (CoQ10) was isolated in 1957 from a bull heart by an American, Frederick Crane at the Enzyme Institute of the University of Wisconsin [1]. Later he established that it is a part of the respiratory chain of electron transfer in mitochondria. In 1958 Volker (Merck, Sharp, Dohme) identified its chemical structure and functions. The main function of CoQ10 in the human body involves participation in the transfer of electrons in the respiratory chain of mitochondria [2]. Due to its lipophilicity, it interacts with dehydrogenases of the respiratory chain and transfers two electrons to cytochromes [3] and thus plays an important role in the synthesis of adenosine triphosphate (ATP) [4]. In addition, CoQ10 is a powerful antioxidant that protects the plasma membrane from lipid peroxidation [5–7]. In vitro studies have shown that CoQ10 inhibits the oxidation of low density lipoproteins to a far greater degree than other antioxidants: β-carotene or α-tocopherol [8]. CoQ-dependent plasma oxidoreductases may also be important for the regeneration of reduced forms of other antioxidants, which contributes to an increase in the overall antioxidant defense [9]. In such a way, CoQ10 is involved in the direct reduction of the tocopheryl radical [10].

Taking into account all the vital functions of CoQ10, the necessity of maintaining its constant endogenous level becomes obvious. The endogenous concentration of CoQ10 can be affected either by various diseases (cardiovascular, bronchopulmonary, endocrine) [11–16], or by administrated drugs. Diazoxide and propranolol were found to significantly inhibit CoQ10 succinate oxidase and CoQ10 NADH oxidase, respectively [17], and statins to inhibit the HMG-CoA reductase enzyme that simultaneously leads CoQ10 synthesis disruption [18].

Therefore, it seemed very relevant to study the effect of drugs of various chemical structures and pharmacological

effects on the endogenous plasma concentration of total coenzyme Q10 in patients with cardiovascular diseases.

Materials and methods

The study included 116 patients: 62 were patients with cardiovascular diseases, and 54 –practically healthy individuals. No significant differences in CoQ10 content were found by sex; thus, patients were not divided into groups by sex.

The group of patients with cardiovascular pathologies included patients with chronic heart failure, coronary heart disease and hypertension. The average age of patients was 64.2±6.98 years. In addition to cardiovascular diseases, the history of patients was not burdened with serious diseases of the gastrointestinal tract, urinary and other systems. According to the therapy, patients were divided into four groups - A, B, C, D (Table 1).

Group E included practically healthy individuals who didn't suffer any cardiovascular, endocrine and bronchopulmonary diseases and didn't administrate drugs. The average age in the group constituted 30±4.21 years. The study complied with the World Medical Association Declaration of Helsinki regarding ethical conduct of research involving human subjects and was confirmed by I.M. Sechenov First Moscow State Medical University Local Ethics Committee.

Blood for analysis was collected as follows: at 8:00 a.m. on an empty stomach, the patient was injected with a disposable catheter into the cubital vein and 5 mL of blood was taken into a tube containing EDTA. Plasma was separated by centrifugation and stored at –30 °C until analysis. The concentration of total CoQ10 in the plasma of patients was determined by high performance liquid chromatography with mass spectrometric detection (HPLC-MS/MS).

Chemicals and reagents

Methanol absolute and acetonitrile were purchased from Biosolve Chimie (France). Isopropanol, ethyl acetate, formic acid and ammonia solution where supplied by PanReac Applichem (Spain). Butylated hydroxyanisole (BHA) and 2,3-Dichloro-5,6-dicyano-p-benzoquinone (DDQ) were purchased from Sigma Aldrich (Germany). Ubiquinone was provided by Sigma Aldrich (Germany) and DL-α-tocopheryl acetate (internal standard) by MP Biomedicals (USA).

Instrumentation

It was used the following HPLC system used for the analyses: Nexera LC system with LCMS-8040 triple quadrupole mass spectrometric detector, Shimadzu (Japan).

Also it was used a vortex (Elmi, Latvia), an analytical balance AUW120D (Shimadzu, Japan) and a centrifuge (Eppendorf, Germany) for sample preparation.

Preparation of standard solution

A carefully weighed portion of ubiquinone was dissolved in isopropanol: ethyl acetate (1: 1 v/v) in such a way as to obtain a concentration of a standard solution of 50 μg/mL. By diluting the standard solution, working solutions were obtained with the following concentrations: 25 μg/mL, 10 μg/mL, 7.5 μg/mL, 5 μg/mL, 2.5 μg/mL, 1 μg/mL and 0.5 μg/mL. Standard solutions were stored at –30 °C.

Sample preparation

Ubiquinone was isolated from plasma by protein precipitation method using a mixture of isopropanol: ethyl acetate (1:1 v/v), followed by centrifugation. After the supernatant was transferred to the vial, ubiquinol was oxidized by adding 10 μL of formic acid and 10 μL of 1 mg/mL DDQ solution. An aliquot of the resulting solution was transferred to the chromatograph column.

HPLC method development

It was selected Luna C18 column (50×4.6 mm, 5 μm) for chromatographic separation in gradient mode. The temperature on the column was 40 °C. As the mobile phase, we used a mixture of formic acid solution (0.1% vol.) and concentrated ammonia solution (0.04% vol.)

Table 1: Study design.

№	Group of patients	Number of patients	Administrated dugs (pharmacological group)	Test group	Control group	Studied drugs (pharmacological group)
1	A	15	Atorvastatin (statins), Bisoprolol / Metoprolol (β-blockers)	B	+	–
2	B	17	Atorvastatin (statins), Bisoprolol / Metoprolol (β-blockers), Amlodipine (Ca channels blocker)	+	A	Amlodipine (Ca channels blocker)
				+	C	Atorvastatin (statins)
3	C	18	Bisoprolol / Metoprolol (β-blockers), Amlodipine (Ca channels blocker)	D	+	–
				B	+	–
4	D	12	Bisoprolol / Metoprolol (β-blockers), Amlodipine (Ca channels blocker) Ethoxidol (antioxidant)	+	C	Ethoxidol (antioxidant)
5	E	54	–	A B C D	+	–

in deionized water - eluent A and formic acid solution (0.1% vol.) and concentrated a solution of ammonia (0.04%, vol.) in a mixture of isopropanol: ethyl acetate (9:1) - eluent B. Separation was carried out in a gradient elution mode. The injection volume was 5 μL. The retention time of ubiquinone was 10.6±0.2 min, and of ubiquinol-8.0±0.2 min. During ionization, the ESI electrospray method in positive modewas used. Detection was carried out in the MRM mode (monitoring of multiple reactions). The precursor ions corresponded to (215)+880.7 m/z for ubiquinone and 882.7 m/z for ubiquinol, and the fragmention for both substances was the same - 197.1 m/z.

Quantitative determination was carried out by the method of internal standard using a solution of 4 μg/mL DL-α-tocopherol acetate as an internal standard. The detection limit of ubiquinone in plasma constituted 0.01 μg/mL, and the regression coefficient R^2 = 0.995 in the concentration range of 0.10–5.00 μg/mL of plasma.

Statistics

Statistical processing of the data was carried out using the program Statistical 6.0 and methods of descriptive statistics. The following statistical parameters were calculated: mean value, standard deviation of the average result, standard error, boundaries of the 95% confidence interval for the mean plasma concentration of CoQ10. The normality of the data distribution was evaluated using the non-parametric Kolmogorov-Smirnov test. The distribution was considered normal if p>0.05. Provided to the normal distribution of the data, the comparison of the samples was carried out using the Student's *t*-test for independent groups. In the absence of a normal distribution, the non-parametric Mann-Whitney test was used to assess statistically significant differences between the groups. The selected level of statistical significance was determined p≤0.05.

Results

The results of the quantitative determination of total CoQ10 in plasma of patients are represented in Table 2. The dynamics of the average plasma concentration of total CoQ10 in patients, expressed in Δ% compared with practically healthy individuals, is shown in Figure 1.

It was found that in patients with cardiovascular diseases the plasma concentration of endogenous coenzyme Q10 is statistically significantly lower (on average –49.0 Δ%) than in practically healthy individuals. The plasma level of total CoQ10 in patients receiving atorvastatin in the complex therapy is statistically significantly lower (–15.2 Δ%), and in patients administrating amlodipine or ethoxidol, it is statistically significantly higher (+18.2 and +20.2 Δ %, respectively) than in patients of control group.

Nevertheless the addition to the therapy with atorvastatin such drugs as amlodipine or/and ethoxidol significantly changes the concentration of CoQ10 (0.461 μg/mL in group A vs. 0.547 μg/mL in group B vs. 0.737 μg/mL in group D).

Discussion

The present study shows the influence of administrated drugs on CoQ10 level in plasma of patients with cardiovascular diseases. It was compared the concentration of

Table 2: Averaged values of the concentration of total CoQ10 in the plasma of patients (μg/mL).

Parameter	Studied drugs						Practically healthy individuals
	Amlodipine		Atorvastatin		Ethoxidol		
	Control group	Test group	Control group	Test group	Control group	Test group	
	A	B	C	B	C	D	E
n	15	17	18	17	18	12	54
Mean	0.461	0.527[a, b]	0.613	0.527[a, b]	0.613	0.737[a, b]	1.116
S.D.	0.064	0.030	0.053	0.030	0.053	0.063	0.423
S.E.	0.012	0.007	0.012	0.007	0.012	0.018	0.057
95% CI	0.405	0.504	0.586	0.504	0.586	0.697	1.001
	0.476	0.535	0.640	0.535	0.640	0.777	1.233
Kolmogorov-Smirnov test	0.156 p>0.10	0.136 p>0.10	0.164 p>0.10	0.136 p>0.10	0.164 p>0.10	0.165 p>0.10	0.302 p<0.10
Student's t-test	–	4.441[c] P = 0.0028	–	6.27[c] P = 0.0151	–	7.362[c] P<0.0001	–
Mann-Whitney U test	–	918[d] p<0.0001	–	1196[d] p<0.0001	–	660.5[d] p = 0.0025	–

[a] - statistically significant differences compared with the control group, [b] - statistically significant differences compared with practically healthy individuals, [c] - compared with the control group, [d] - compared with practically healthy individuals.

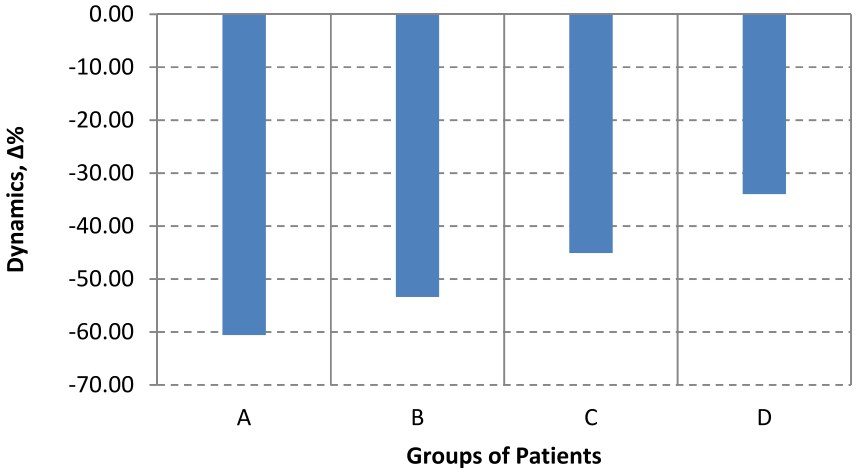

Figure 1: Dynamics of the plasma concentration of CoQ10 in different groups of patients with cardiovascular diseases in comparison with practically healthy individuals.

CoQ10 in the plasma of B (test group) and C (control group) groups of patients to determine the effect of atorvastatin. Both groups of data obeyed the normal Gaussian distribution, which was proved using the Kolmogorov-Smirnov test ($p>0.1$, the probability of error is not significant). An unpaired two-sample Student's *t*-test was used with Welch correction [19]. The obtained data (Figure 2, Table 2) indicate that in patients with cardiovascular diseases administration of atorvastatin as a part of complex therapy the plasma level of total CoQ10 is statistically significantly lower (–15.2 Δ%) than in patients of the control group receiving the same therapy, with the exception of statins. Our data are consistent with published data [20]. In such a way atorvastatin by inhibiting 3-hydroxy-3-methylglutaryl coenzyme A reductase significantly diminishes the plasma concentration of CoQ10. In its turn, it may lead to the impairment of heart function and myopathy. That's why monitoring the CoQ10 plasma level in patients who administrate statins is very important.

To clarify the effect of amlodipine (calcium channel blocker) on the CoQ10 endogenous level, a comparative analysis of its plasma concentration was performed in patients from groups B (test group) and A (control group). It was found that the concentration of total CoQ10 in the plasma of patients with cardiovascular diseases receiving amlodipine as a part of complex therapy is statistically significantly higher (+18.2 Δ%) than in patients of the control group receiving the same therapy, with the exception of amlodipine.

The obtained data allow us to conclude that amlodipine (calcium channel blocker) is able to neutralize the negative effect of statins on the redox balance of the body while they are prescribed with β-blockers to patients with cardiovascular diseases. It was previously established [21] that calcium channel blockers prevent, firstly, a decrease in

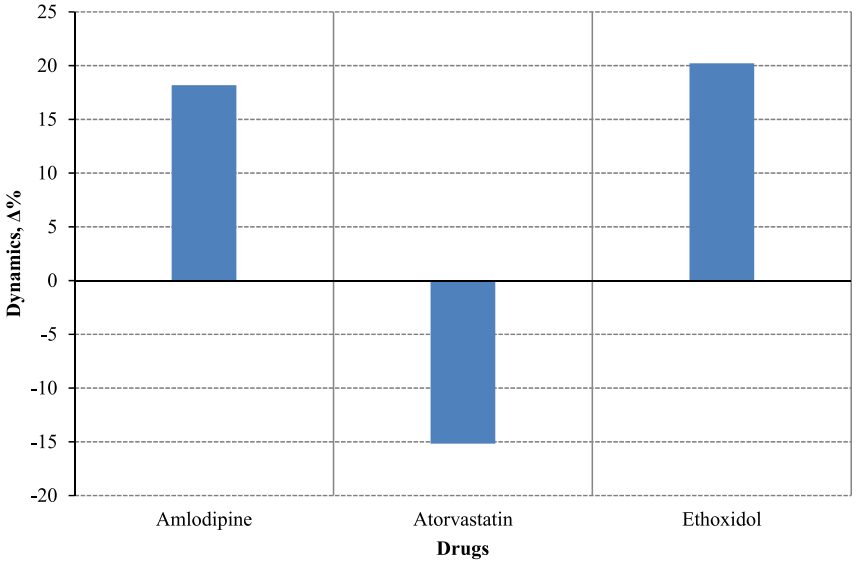

Figure 2: The dynamics of changes in the plasma concentration of CoQ10 in patients with cardiovascular diseases with the use of various drugs compared with the control group.

glutathione levels caused by oxidative stress, and, secondly, disturbances in the synthesis of prostacyclins characteristic of oxidative stress.

The properties of the ethoxidol (antioxidant) effect were evaluated by comparing the plasma concentration of total CoQ10 in patients from group B (test group) and group D (control group). According to the Kolmogorov-Smirnov test, the data obeys the normal distribution ($p>0.1$). It was revealed that the administration of ethoxidol during treatment with β-blockers and calcium channel blockers statistically significantly increases the concentration of CoQ10 in the plasma of patients with cardiovascular diseases (+20.2 Δ%). This fact may appeal due to the ability of antioxidants to absorb active oxygen species (ROS) and stop radical chain reactions. Also ethoxidol is engaged in preservation of the structural and functional organization of membranes by modulating the activity of membrane-bound enzymes and receptor complexes. Besides this mechanism of action of ethoxidol includes lipid peroxidation inhibition and superoxide dismutase' activity increase which implies a lower consumption of CoQ10.

Speaking of strong points of the research it must be marked that sample size of the patients is sufficiently large. There are some researches investigating statins effect on CoQ10 plasma concentration. But in our research we followed the difference between CoQ10 plasma level of patients administrating just statins and ß-blockers and statins, ß-blockers and calcium channel blockers. Also, for the first time it was determined the influence of antioxidant ethoxidol on CoQ10 plasma concentration. So, one of the future directions for the research may be the investigation of antioxidants effect on CoQ10 concentration in plasma.

There are a few limitations of the study: firstly the impossibility to choose patients who administrate drugs from just one pharmacological group. As cardiovascular pathologies are chronic ones, usually patients administrate a variety of drugs from different groups. That's why one of the future directions of the research may consist in narrowing the study groups to patients administrating drugs from only one group. Also, it would be appropriate to make the same research for a younger group of patients to understand the influence of age on CoQ10 level.

Conclusions

The study that in patients with cardiovascular diseases treated with various drugs, the plasma level of total CoQ10 is statistically significantly lower than in practically healthy individuals. It was found that taking atorvastatin (statins) statistically significantly reduces the endogenous concentration of total CoQ10 in plasma. Apparently, this occurs due to the fact that the drugs of this group inhibit HMG-CoA reductase, and thus reduce the synthesis of not only cholesterol, but also the synthesis of CoQ10. When amlodipine (a calcium channel blocker) is added to therapy, patients with cardiovascular diseases have a positive statistically significant effect on the overall concentration of CoQ10. This may happen due to the prevention of glutathione levels decrease, as well as impaired prostacyclin synthesis. When prescribing ethoxidol (antioxidant) to patients with cardiovascular diseases, the statistically significantly increased concentration of endogenous CoQ10 may occur due to increased overall antioxidant protection and lower consumption of CoQ10.

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Competing interests: Authors state no conflict of interest.

Ethical approval: The study complied with the World Medical Association Declaration of Helsinki regarding ethical conduct of research involving human subjects and was confirmed by I.M. Sechenov First Moscow State Medical University Local Ethics Committee.

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