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Redox Status and Pharmacokinetics of Coenzyme Q_{10} in Rat Plasma after Its Single Intravenous Administration

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Abstract—The pharmacokinetics of the total pool of coenzyme Q_{10} (CoQ_{10}), its oxidized (ubiquinone) and reduced (ubiquinol, $CoQ_{10}H_2$) forms have been investigated in rats plasma during 48 h after a single intravenous injection of a solution of solubilized CoQ_{10} (10 mg/kg) to rats. Plasma levels of CoQ_{10} were determined by HPLC with spectrophotometric and coulometric detection. In plasma samples taken during the first minutes after the CoQ_{10} intravenous injection, the total pool of coenzyme Q_{10} and proportion of $CoQ_{10}H_2$ remained unchanged during two weeks of storage at −20°C. The kinetic curve of the total pool of coenzyme Q_{10} corresponds to a one-compartment model ($R^2 = 0.9932$), while the corresponding curve of its oxidized form fits to the two-compartment model. During the first minutes after the injection a significant portion of plasma ubiquinone undergoes reduction, and after 7 h the concentration of ubiquinol predominates. The decrease in total plasma coenzyme Q_{10} content was accompanied by the gradual increase in plasma ubiquinol, which represented about 90% of total plasma CoQ_{10} by the end of the first day. The results of this study demonstrate the ability of the organism to transform high concentrations of the oxidized form of CoQ_{10} into the effective antioxidant (reduced) form and justify prospects of the development of parenteral dosage forms of CoQ_{10} for the use in the treatment of acute pathological conditions.

Keywords: coenzyme Q_{10}, CoQ_{10}, ubiquinone, ubiquinol, redox status, intravenous administration, pharmacokinetics, HPLC

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INTRODUCTION

Coenzyme Q_{10} (coenzyme Q_{10}, CoQ_{10}, ubiquinone) is the only endogenously synthesized fat-soluble antioxidant involved in numerous reactions related to the redox status of the body [1, 2]. Structurally, CoQ_{10} is a 2,3-dimethoxy-5-methyl-6-decaprenyl-1,4-benzoquinone.

In human cells, CoQ_{10} is an obligate component of the mitochondrial respiratory chain, where it transfers electrons from NADH-dehydrogenase and succinate dehydrogenase complexes (complex I and complex II, respectively) to cytochrome b_5 (complex III) [2, 3]. In the body CoQ_{10} exists in the reduced (ubiquinol, $CoQ_{10}H_2$) and oxidized (ubiquinone, CoQ_{10}) forms:

$$CoQ + e^- + H^+ \rightarrow {}^{\cdot}QH$$

$$^{\cdot}QH + e^- + H^+ \rightarrow CoQH_2,$$

where $^{\cdot}QH$ is a ubisemiquinone radical.

In the reduced form CoQ_{10} acts as an antioxidant, which prevents damage of DNA, lipids, proteins and other molecules [4–6]. It is required for normal functioning of living organisms and, in particular, for the functioning of tissues with high levels of energy metabolism. The highest concentrations of CoQ_{10} have been found in cardiac muscle, kidney and liver [7, 8]. Needs of the body in ubiquinone are replenished by endogenous synthesis and dietary intake. Aging and pathological processes lead to a decrease in the levels of ubiquinone in blood and tissues [9–11] and therefore CoQ_{10} is recommended for the prophylaxis and as addition to the standard treatment of cardiovascular, neurological and other diseases. CoQ_{10} containing drugs and biologically active supplements for oral administration are characterized by extremely low bioavailability of CoQ_{10} [12]. Pharmacokinetics and the redox status of plasma CoQ_{10} after its intravenous administration, providing maximum bioavailability, have not been well-studied yet.

The aim of this study was to investigate the pharmacokinetics of the total pool of plasma CoQ_{10}, its oxidized and reduced forms after a single intravenous injection of a solution of solubilized CoQ_{10} to rats.

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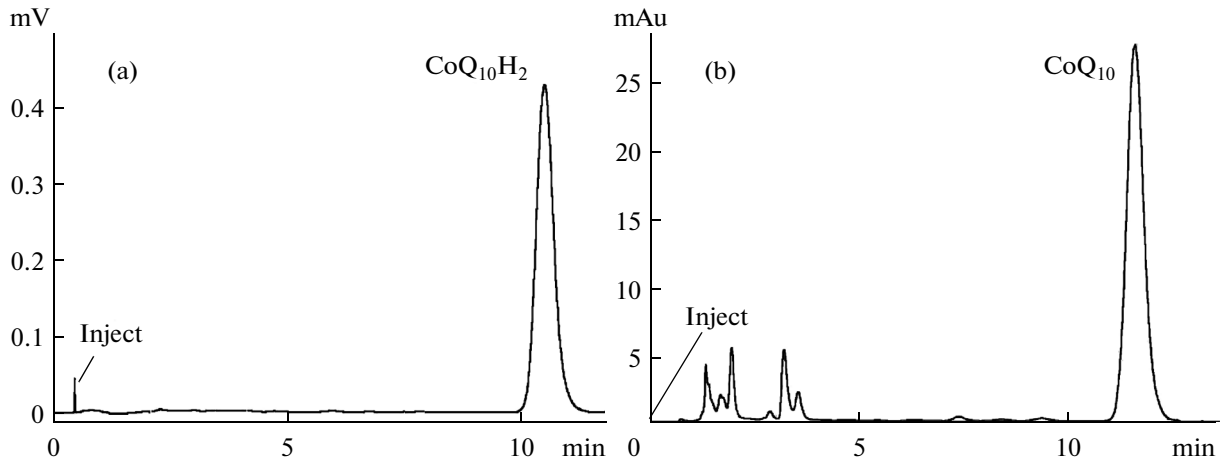

Fig. 1. Chromatogram of a standard dilution of ubiquinone (CoQ_{10}) in ethanol after its reduction to $CoQ_{10}H_2$; an electrochemical detector (a). Chromatogram of the same standard dilution of CoQ_{10} without the reduction; spectrophotometric detection at $\lambda_{max} = 275$ nm (b).

MATERIALS AND METHODS

The Pharmacokinetic Study Protocol

The study was performed in male Wistar rats ($n = 4$) weighing 250–300 g. Catheters were implanted into femoral vein and femoral artery of animals anesthetized with pentobarbital (50 mg/kg, i.p.) for injection of the drug agent and subsequent blood sampling, respectively. One day after surgery, overnight-fasted awake animals were injected intravenously with a solution of solubilized CoQ_{10} (10 mg/kg as Qudesan, produced by Akvion, Moscow, Russia). For two days after administration of CoQ_{10} animals were fasted with free access to water. Blood samples (0.3 mL) were taken before and after 0.1 h, 0.25 h, 1 h, 3 h, 5 h, 7 h, 9 h, 24 h, 32 h, and 48 h after the injection. Blood was immediately centrifuged, plasma collected, frozen, and stored up to two weeks at −20°C until analysis.

Analysis of CoQ_{10} in the Blood Plasma

CoQ_{10} was extracted from plasma as described by Lass and Sohal [13] with minor modifications [14]. Plasma was mixed with ethanol and *n*-hexane (1 : 2 : 5), stirred for 10 min, centrifuged (4000 *g*) for 3 min, and the upper *n*-hexane layer was collected. The extraction procedure was repeated by adding *n*-hexane. The extract portions were pooled, evaporated to dryness and dissolved in ethanol.

The chromatographic analysis was carried in an isocratic mode by reverse phase HPLC using a liquid chromatograph Stajer (Akvilon) using a Phenomenex column (Luna C18, 150 × 4.6 mm, 5 μm). The eluent flow rate was 1.4 mL/min.

The plasma total content of CoQ_{10} ($TCoQ_{10}$) was determined using an electrochemical (EC) detector, while the content of its oxidized form was determined by using the spectrophotometric (SF) detector. Ubiquinol concentrations were calculated for each sample by the difference between data of electrochemical and spectrophotometric determinations.

The total CoQ_{10} content was determined using a coulometric detector Coulochem II with the cell model 5011 (ESA, USA). The mobile phase consisted of 0.3% NaCl (w/v) in the mixture ethanol : methanol : 7% $HClO_4$ (975 : 15 : 10). Before injection into the column the oxidized form of the plasma extract was reduced to $CoQ_{10}H_2$ by addition of sodium tetrahydroborate solution in ethanol. Completeness of the reduction was controlled by repeated addition of the reducing solution. The retention time of $CoQ_{10}H_2$ was 10.0 min (Fig. 1a). EC-detection of $CoQ_{10}H_2$ was performed in the oxidative mode at voltages −50 mV/+350 mV on the first/second electrodes. Registration and processing of chromatographic data were performed using the computer software from Environmental Sciences Associate, Inc. (USA).

During SF-detection of ubiquinone (λ_{max} = 275 nm) 96% ethanol was used as an eluent; the retention time of CoQ_{10} was 11.85 min (Fig. 1b). Chromatographic parameters were calculated using the program Multichrom (version 3.X).

The SF-detector response was linear over the range of 0.2–500 mg/mL ($r = 0.9999$; Fig. 2a), the EF-detection response was linear in the range of 0.01–100 mg/mL ($r = 0.9995$; Fig. 2b). The detection limit of CoQ_{10} in the injected sample was 4 ng and 0.1 ng in the case of SF-detection and EC-detection, respectively.

Plasma CoQ_{10} content was determined using calibration curves prepared for model mixtures of ubiquinone in the blood plasma and determined by means of SF- and EC-detections (Fig. 3). Serial standard dilutions of CoQ_{10} in ethanol were prepared using ubidecarenone (Kaneka, Japan).

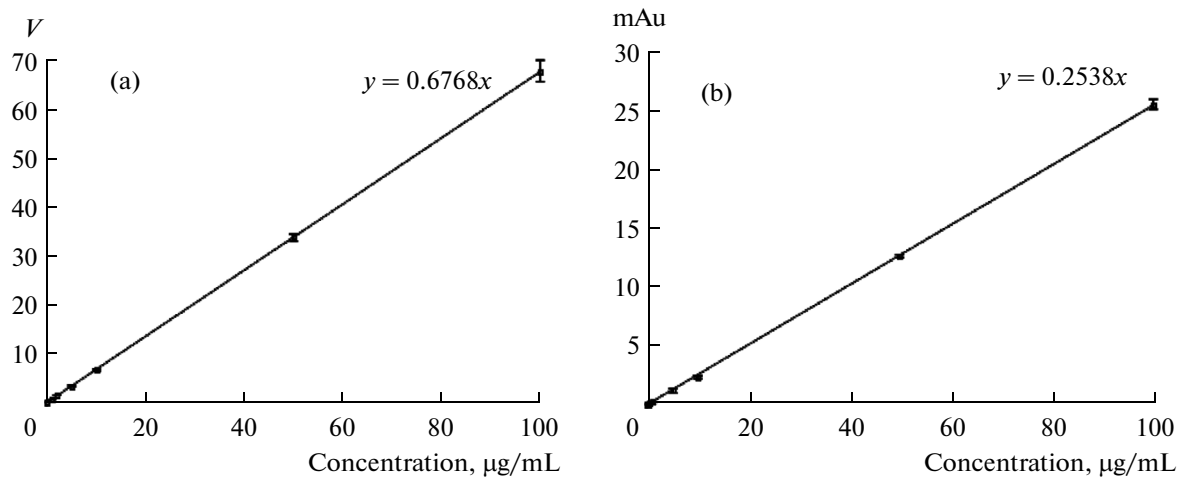

Fig. 2. Calibration curves of standard alcohol dilutions of CoQ_{10} obtained using an electrochemical detector (a) and a spectrophotometric detector at $\lambda_{max} = 275$ nm (b). Data represent mean values ± SEM ($n = 4$).

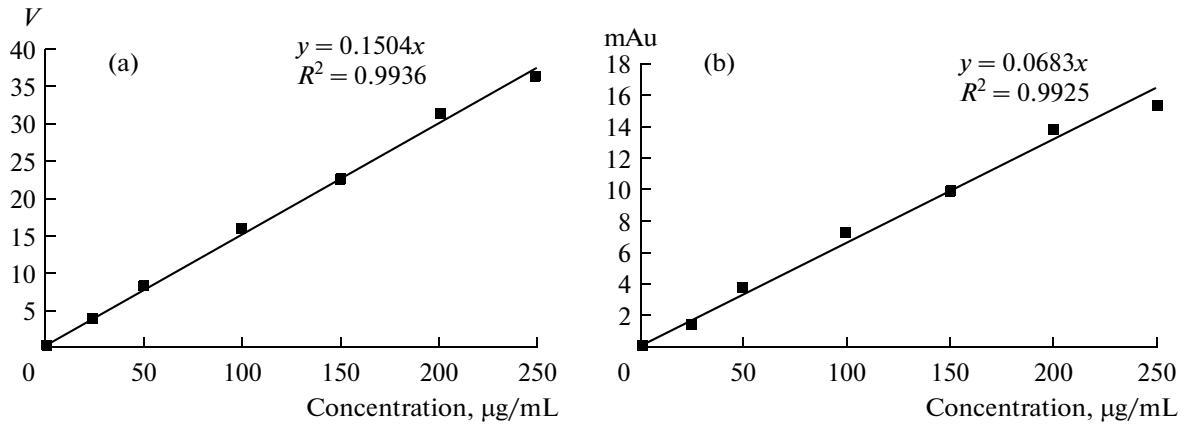

Fig. 3. Calibration curves obtained using HPLC data with electrochemical detection (a) and spectrophotometric detection $\lambda_{max} = 275$ nm (b) for analysis of model mixtures of ubiquinone in the blood plasma.

On the chromatogram reduced and oxidized forms of coenzyme Q_{10} can be determined spectrophotometrically by switching a wavelength detector from 290 nm (λ_{max} for $CoQ_{10}H_2$) to 275 nm (λ_{max} for CoQ_{10}) (Fig. 4). However, it is quite difficult to calibrate ubiquinol by means of $CoQ_{10}H_2$ additions to plasma, as it is impossible to completely prevent (and evaluate) its partial oxidation during sample preparation. Therefore, the evaluation of redox status of plasma CoQ_{10} in the blood plasma actually represents the evaluation of the redox status of CoQ_{10} in plasma extracts.

Stability Studies $CoQ_{10}H_2$ in Rat Plasma for Storage

Stability of plasma $CoQ_{10}H_2$ during storage at –20°C was studied in a separate series of experiments. A plasma sample taken within the first minutes after the intravenous administration of solubilized CoQ_{10} (10 mg/kg), was divided into aliquots of 100 μL. $CoQ_{10}H_2$ and $TCoQ_{10}$ were determined in plasma extracts immediately after blood sampling and after 2 days, 3 days, 4 days, 6 days, 9 days, and 14 days of storage.

In this series of experiments plasma extracts were analyzed using the electrochemical detector. Each extract was assayed twice: prior to addition of the reductant (determination of $CoQ_{10}H_2$) and after reduction of oxidized form by $NaBH_4$ (determination of total concentration of coenzyme Q_{10}). Data for each sample represent the mean of three determinations (Fig. 5). Results of the quantitative analysis represent mean ± SEM.

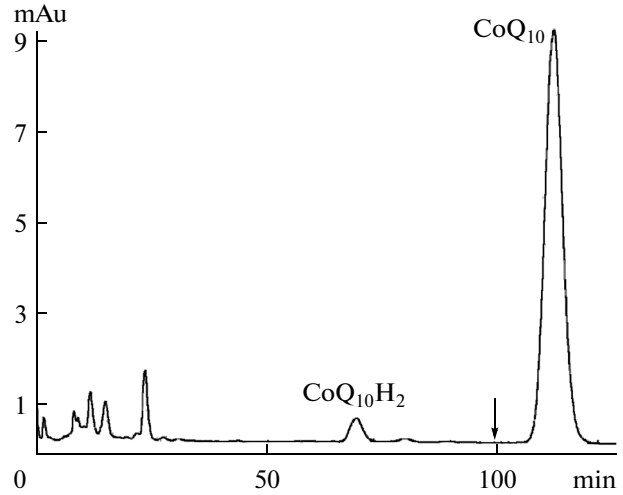

Fig. 4. Chromatogram of plasma extract containing oxidized and reduced forms of CoQ_{10}. Spectrophotometric detection with the wavelength switching from 290 nm to 275 nm (indicated by arrow).

RESULTS AND DISCUSSION

A graphical comparison of HPLC-SF and HPLC-EC results of determination of CoQ_{10} in serial plasma dilutions (in the range 1.7–200 μg/mL) showed high convergence of results ($y = 0.909x$; Fig. 6), allowing to combine these two techniques into assay of the same plasma sample.

Determination of the ratio of oxidized and reduced forms of CoQ_{10} in biological objects is a separate analytical task as ubiquinol is easily oxidized to ubiquinone under the influence of various factors. Lagendijk et al. [15] demonstrated that $CoQ_{10}H_2$ is unstable in blood plasma and in the isopropanol extract the ratio $CoQ_{10}H_2$: CoQ_{10} varies considerably within one hour after blood sampling. The authors do recommend to analyze CoQ_{10} immediately after sampling or to store plasma at –75°C (under these conditions the ratio $CoQ_{10}H_2$: CoQ_{10} remained unchanged for 13 months). However, there are studies in which the reduced form of CoQ_{10} was not oxidized within a certain period of time. For example, Tang et al. [16] studied $CoQ_{10}H_2$ stability in blood samples. The experiment was performed immediately after the sampling of venous blood in people and after 8 h of storage at 4°C. The results showed that during the storage of plasma samples for 8 h in a refrigerating chamber the $CoQ_{10}H_2$ content remained unchanged.

Wang et al. [17] demonstrated that the total concentration of CoQ_{10} and the ubiquinol : ubiquinone ratio remained the same in freshly prepared alcoholic extract from the plasma, and after 12 h of storage.

According to the authors of [18], at room temperature (22°C) the rate of ubiquinol oxidation was minimal (0.1%/h) during the day in organic solvents and in blood, while in plasma samples in was minimal for 3 h after centrifugation.

Figure 5 shows, that during two weeks of storage at –20°C of blood plasma collected shortly after the intravenous administration of solubilized CoQ_{10}, the total content of coenzyme Q_{10} and the proportion of $CoQ_{10}H_2$ remained unchanged. This result guaranties accurate determination of $TCoQ_{10}$ and $CoQ_{10}H_2$ in plasma samples taken in the pharmacokinetic experiment and stored until analysis in the freezer for 14 days.

Results of the analysis of plasma samples collected for 2 days after intravenous injection of ubiquinone were used for generation kinetic curves of total CoQ_{10}, its oxidized and reduced forms (Fig. 7a). The kinetic curve of total coenzyme Q_{10} after intravenous administration corresponds to an one-compartment model ($R^2 = 0.9932$), while its oxidized form fits to the two-compartment model (Fig. 7b). The first phase of the kinetic curve of ubiquinone continues within one day and is associated mainly with its intensive reduction

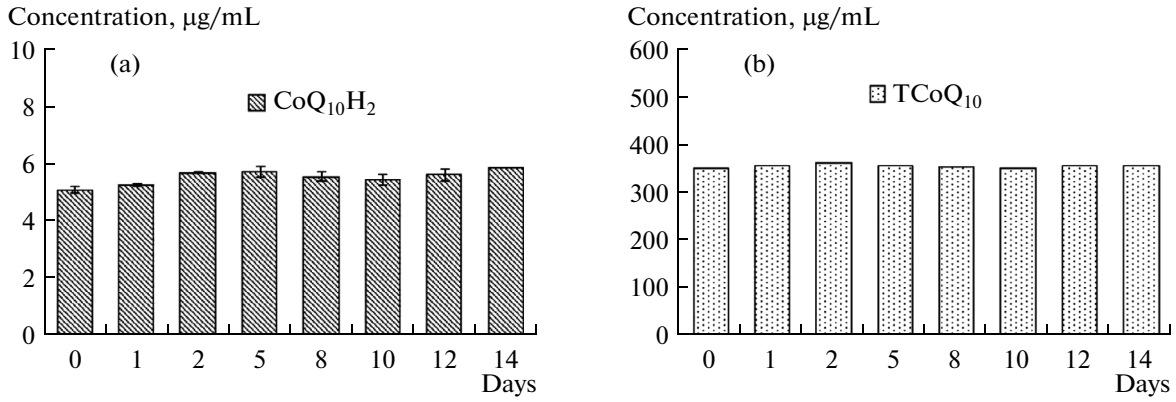

Fig. 5. Total content of CoQ_{10} ($TCoQ_{10}$) (total content of CoQ10 corresponds to Fig. 5b) and its reduced form ($CoQ_{10}H_2$) (corresponds to Fig. 5a) in blood plasma obtained after intravenous administration of solubilized CoQ_{10} (10 mg/kg) in rats. Data represent concentrations in plasma immediately after blood sampling and after subsequent storage the plasma for 14 days at –20°C.

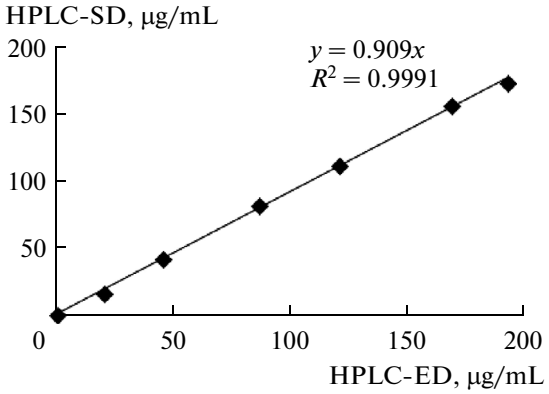

Fig. 6. Convergence of results of ubiquinone determination in model mixtures with blood plasma by HPLC with electrochemical (HPLC-ED) and spectrophotometric detection (HPLC-SD).

process (Fig. 8); the fraction of ubiquinol reaches maximum at 24 h post-injection and then the rate of the decrease of plasma ubiquinone concentration slows down.

It is known that in humans ubiquinol represents more than 90% of the plasma CoQ_{10} pool [19]. Plasma ubiquinol is mainly distributed among lipoproteins where it protects them against oxidation. It is believed that the most important function of lipoprotein $CoQ_{10}H_2$ consists in regeneration of α-tocopheroxyl radical, the oxidation product of α-tocopherol in the reaction with lipid radicals [20]. Recovery of oxidized forms of CoQ_{10} in plasma involves ascorbic acid.

$$\alpha\text{-TO}^{\cdot} + CoQH_2 \rightarrow {}^{\cdot}QH + \alpha\text{-TOH},$$

$$\alpha\text{-TO}^{\cdot} + {}^{\cdot}QH \rightarrow CoQ + \alpha\text{-TOH},$$

$$CoQ + HO\text{-Asc-}OH \rightarrow O = Asc = O + CoQH_2,$$

where α-TO$^{\cdot}$ is the tocopheroxyl radical.

In rats, intravenous administration of the oxidized form of ubiquinone, creating a plasma concentration, three orders of magnitude higher than the endogenous level of CoQ_{10}, may be considered as a test for capacity of reducing mechanisms.

The results of this study have shown that during the first minutes after the intravenous administration of ubiquinone a substantial portion of its plasma pool is reduced to ubiquinol, and after 7 h $CoQ_{10}H_2$ concentration predominantes. The decrease in the total CoQ_{10} content is accompanied by a gradual increase of $CoQ_{10}H_2$ from 19% (5 min after administration) to the maximal value (89%) one day after administration (Fig. 8). Two days after administration to rats the total concentration of plasma coenzyme Q_{10} was still much higher than the basal level.

Consequently, intravenous administration of solubilized CoQ_{10} allows to achieve almost immediately high concentration of ubiquinone that is reduced within a few hours and maintains a stably elevated level

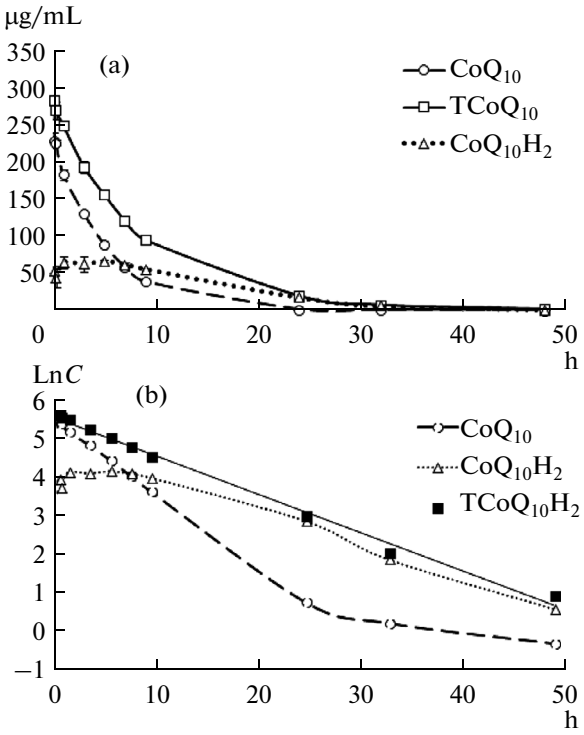

Fig. 7. Kinetic curves of plasma concentrations of ubiquinone (CoQ_{10}), ubiquinol ($CoQ_{10}H_2$) and the total CoQ_{10} pool ($TCoQ_{10}$) after intravenous administration of solution of solubilized CoQ_{10} (10 mg/kg) (a). Kinetic curves of total and oxidized form of CoQ_{10} represented in semi logarithmic plots (b).

of plasma ubiquinol, one of the most effective and safest endogenous antioxidants.

CONCLUSIONS

The pharmacokinetics of the total pool of coenzyme Q_{10} (CoQ_{10}), its oxidized (ubiquinone) and

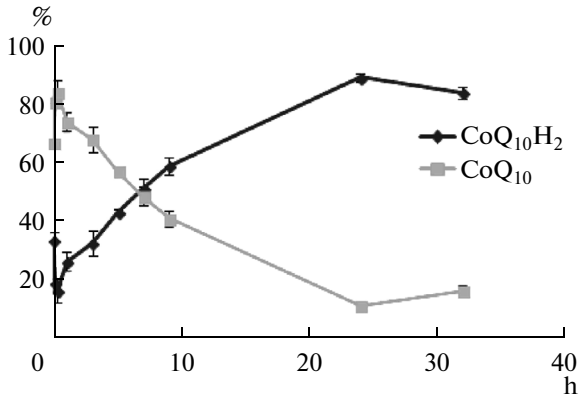

Fig. 8. Changes in the ratio of oxidized (CoQ_{10}) and reduced ($CoQ_{10}H_2$) forms of CoQ_{10} in rat plasma during 48 h after intravenous administration of Qudesan solution. Data represent mean values ± SEM.

reduced (ubiquinol, $CoQ_{10}H_2$) forms has been investigated in plasma during 48 h after a single intravenous injection of a solution of solubilized CoQ_{10} (10 mg/kg) to rats. The results obtained by HPLC with spectrophotometric and electrochemical detection, provide convincing evidence that the organism is able to transform the high concentration of the oxidized form of CoQ_{10} in the effective antioxidant (reduced) form. This justifies prospects for the development of parenteral dosage forms of CoQ_{10} for the use in the treatment of acute pathological conditions.

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