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MOLECULAR-BIOLOGICAL PROBLEMS OF DRUG DESIGN AND MECHANISM OF DRUG ACTION

PRECLINICAL STUDY OF THE PHARMACOKINETICS OF A NEW INTRAVENOUS DOSAGE FORM OF UBIQUINOL

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The pharmacokinetics of ubiquinol in a new dosage form for intravenous injection were studied for the first time. Biexponential kinetic curves were found using HPLC with electrochemical detection in 48-h experiments in rats. The main pharmacokinetic parameters, i.e., area under the kinetic curves, elimination half-life, and total clearance, were calculated. The pharmacokinetics were nonlinear in the studied dose range (5, 10, and 20 mg/kg) with a higher dose corresponding to lower clearance. Multiple administrations increased the clearance, which implied extensive uptake of the drug into organ tissues that was required to manifest the protective effects of ubiquinol. Gradual oxidation of ubiquinol in blood plasma indicated that it was involved in endogenous redox processes.

Keywords: ubiquinol, ubiquinone, pharmacokinetics, intravenous administration, redox status.

Overproduction of oxygen free radicals and exhaustion of antioxidant reserves are the most important factors determining the level of ischemic organ damage [1, 2]. The endogenous antioxidant coenzyme Q_{10} (CoQ_{10}) has attracted interest for a decade as a neuro- and cardioprotector [3 – 6]. Existing CoQ_{10} dosage forms are designed for internal use. CoQ_{10} is poorly absorbed in the intestines because it is insoluble in H_2O and has limited solubility in lipids and a relatively high molecular mass. The bioavailability for this administration mode is estimated as 0.1 – 3% for various pharmaceutical compositions of CoQ_{10} [7, 8]. Emergency therapy of acute ischemic myocardial and brain conditions uses intravenous (i.v.) administration of CoQ_{10}, which provides the fastest replenishment of tissue antioxidant reserves [9, 10]. However, injectable medicines based on CoQ_{10} are lacking. Endogenous CoQ_{10} occurs in living systems in two forms, i.e., oxidized (ubiquinone) and reduced (ubiquinol). Ubiquinol exhibits antioxidant properties and is oxidized to ubiquinone in free-radical reactions. Reverse reduction to ubiquinol is assisted by endogenous regeneration systems (ascorbate, tocopherol) [5, 11, 12]. The ratio of CoQ_{10} oxidized and reduced forms (redox status) is considered a biomarker of oxidative stress [13]. Obviously, the antioxidant properties acquired after *in vivo* conversion of ubiquinone to the reduced form ubiquinol are responsible for its leading role in producing protector effects. Isolated studies of the efficacy of ubiquinol for cardiovascular [14, 15] and neurodegenerative diseases [16 – 18] were conducted only with its internal administration. Parenteral administration of ubiquinol was used by only one research group on an experimental hemorrhagic shock model [19]. Experimental

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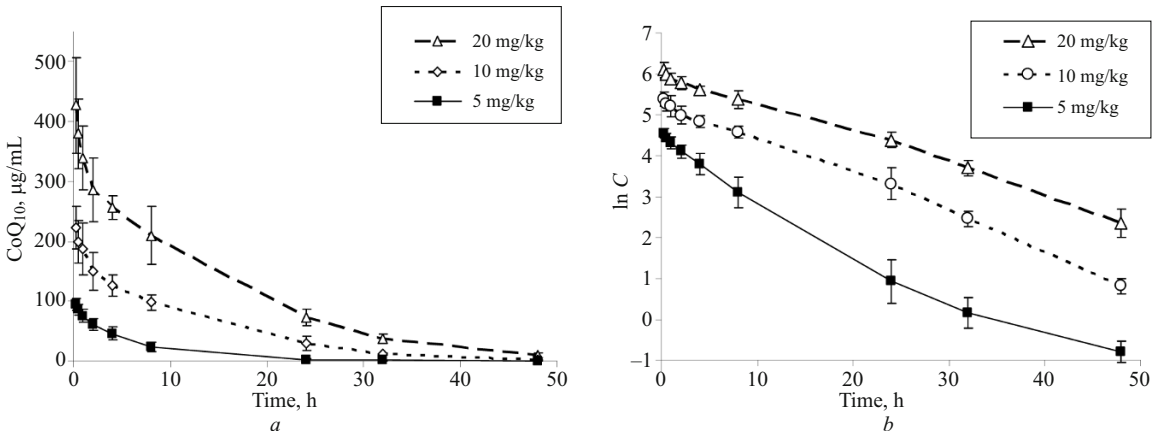

Fig. 1. Kinetic curves for total CoQ_{10} concentration in rat plasma for 48 h after a single i.v. administration at doses of 5, 10, and 20 mg/kg: concentration – time (*a*) and ln-concentration – time coordinates (*b*).

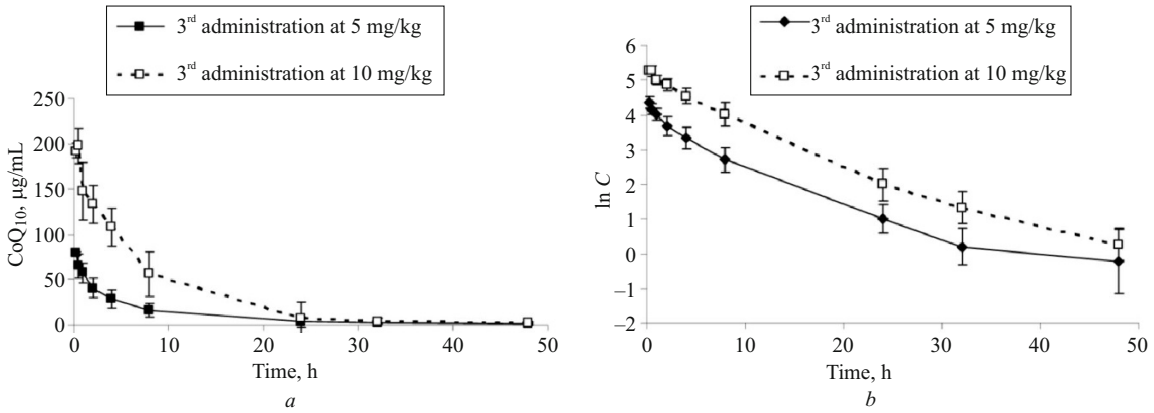

Fig. 2. Kinetic curves for total CoQ_{10} concentration in rat plasma for 48 h after a third i.v. administration at doses of 5 and 10 mg/kg: concentration – time (*a*) and ln-concentration – time coordinates (*b*).

results from our research group demonstrated convincingly the high therapeutic potential of ubiquinone with i.v. administration to treat developed ischemia of the myocardium and brain [20 – 23]. The efficacy if ubiquinol as an independent pharmacological agent could be much greater.

An innovative dosage form of ubiquinol, i.e., a solution of its solubilized substance for i.v. injection, has now been developed by researchers at Pharmacy House Corp. The goal of the research was to study the pharmacokinetics of the reduced form of CoQ_{10} (ubiquinol) in a dosage form for i.v. injection.

EXPERIMENTAL PART

The dosage form of ubiquinol for i.v. administration was an aqueous solution (1%) of solubilized substance. Tests were conducted on Wistar male rats (300 – 350 g) obtained from Stolbovaya nursery (SCBMT, Russia) according to Ministry of Health of Russia Order No. 199n dated Apr. 1, 2016, "On approval of Good Laboratory Practice Rules" and a resolution of the Bioethics Committee of M. V. Lomonosov Moscow State University. Anesthetized animals (chloral hydrate, 400 mg/kg) were fitted with catheters into the femoral vein for i.v. injection of the drug and into the femoral artery for collection of blood samples. Pharmacokinetics of the drug were studied with single and multiple injections. The drug was administered once at three doses (5, 10, and 20 mg/kg) for determining the linearity of the pharmacokinetics and multiple times at two doses (5 and 10 mg/kg) once per day for 3 d. Six animals were used for each dose. Blood samples were collected before and 0.25, 0.5, 1, 2, 4, 8, 24, 32, and 48 h after the last drug injection. Plasma samples were frozen until the quantitative determination. Kinetic curves of CoQ_{10} plasma concentration vs. time were constructed for each dose. Areas under the kinetic curves ($AUC_{0\to48}$ h), total clearance (Cl_T), and elimination half-life ($T_{1/2}$) were calculated. Pharmacokinetic parameters were calculated using the Kinetica 5.0 program (Thermo

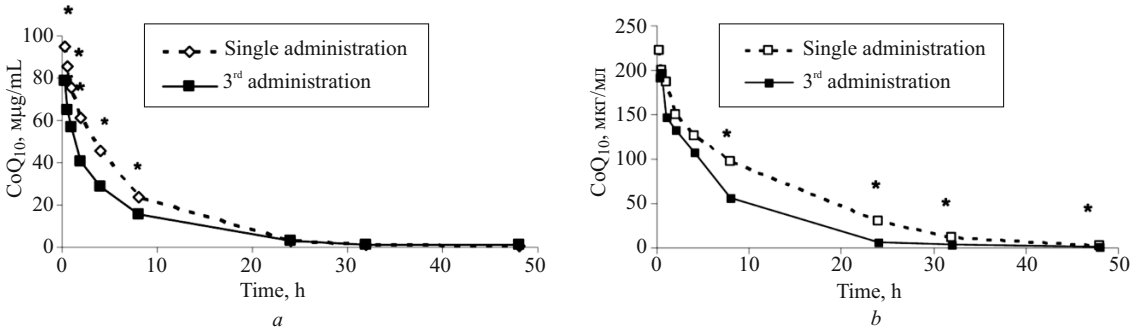

Fig. 3. Kinetic curves for CoQ_{10} in rat blood plasma after a single and third i.v. administration of ubiquinol at doses of 5 (*a*) and 10 mg/kg (*b*).

Fisher Scientific, USA). Approaches recommended by the *Handbook for Preclinical Drug Trials* were used to check the linearity [24].

Quantitative analysis of CoQ_{10} in plasma used a validated HPLC procedure with electrochemical detection on equipment of Environmental Science Associates, Inc. (USA) that included a model 580 pump, Coulochem II electrochemical detector, and isocratic mode over a column (150 × 4.6 mm) with C18 sorbent (5 μm) at eluent flow rate 1.4 mL/min. The mobile phase was NaCl (0.3%) in EtOH–MeOH–$HClO_4$ (7%) (970:20:10). Electrochemical detection was made in oxidative mode using a model 5011 analytical cell at a potential of –50 mV on the first pair of electrodes and +350 mV on the second pair. Chromatographic data were recorded and processed using a computer program of Environmental Science Associates, Inc. (USA). The lower limit of detection of CoQ_{10} was 0.25 μg/mL.

Analyte was extracted by adding EtOH (220 μL) and *n*-hexane (550 μL) to blood plasma (100 μL), mixing thoroughly for 10 min, and centrifuging at 3,000 rpm for 3 min. The upper layer of *n*-hexane was collected (500 μL). The remainder was treated with more *n*-hexane (550 μL). The extraction and collection procedure was repeated. The combined extract was evaporated to dryness and dissolved in EtOH. The efficiency of ubiquinol extraction from rat blood plasma was 91.7 – 113%. The extract was analyzed twice, i.e., before and after total reduction to ubiquinol (using addition of $NaBH_4$ solution in EtOH). Ubiquinol levels detected before reduction corresponded to the concentration of native unoxidized drug in rat blood plasma. Addition of reductant converted the oxidized form into the reduced form and enabled the concentration of total CoQ_{10} in blood plasma to be determined. The values for the concentration of total CoQ_{10} were used to construct kinetic curves and to calculate the pharmacokinetic parameters. According to the literature and our own results [25, 10], the content of endogenous CoQ_{10} in rat blood plasma was <0.1 μg/mL. This concentration was below the limit of quantitation. Therefore, the initial CoQ_{10} values were not determined quantitatively. Nevertheless, the value of the peak in the blank was subtracted from the values of peaks in all subsequent samples for each animal to account for background levels of CoQ_{10}. Results were processed statistically using the Statistica for Windows 6.0 program. The statistical significance of differences was determined using the Mann–Whitney and Wilcoxon non- parametric *U*-criterion. Differences were considered statistically significant for $p < 0.05$.

RESULTS AND DISCUSSION

Pharmacokinetic studies are an integral part of preclinical drug trials. Literature data on CoQ_{10} pharmacokinetics were obtained primarily after internal administration of its oxidized form [12, 26, 27, 28]. Data for intravascular injection of CoQ_{10} are scattered [29, 30].

Kinetic curves for the change of total CoQ_{10} concentration vs. time in absolute values and in logarithmic coordinates were plotted using results from a single administration of each drug dose (5, 10, and 20 mg/kg) (Fig. 1).

An analysis of ln-concentration–time kinetic curves revealed that they were bi-exponential for all three drug doses: for 5 mg/kg, the kinetics obeyed the equation $C = 50.09e^{-0.38t} + 79.02e^{-0.26t}$; 10 mg/kg, $C = 131.60e^{-0.09t} + 117.44e^{-0.14t}$; and 20 mg/kg, $C = 391.0e^{-0.07t} + 1285.6e^{-6.49t}$;

TABLE 1. Main Pharmacokinetic Parameters of CoQ_{10} After a Single i.v. Administration of Ubiquinol at Doses of 5, 10, and 20 mg/kg

Dose, mg/kg	Parameter		
	$AUC_{0\to48}$ h, mg·h/mL	Cl_T, mL/h	$T_{1/2}$, h
5	0.662 ± 0.285	2.98 ± 0.85	9.16 ± 3.65
10	2.263 ± 0.304 $^{\#}p < 0.01$	1.54 ± 0.22 $^{\#}p < 0.01$	7.81 ± 0.36
20	5.489 ± 0.574 $^{*}p < 0.01$, $^{\#p} < 0.01$	1.31 ± 0.13 $^{\#}p < 0.01$, $^{*}p < 0.05$	9.05 ± 1.96 $^{*}p < 0.05$

$^{\#}$ Difference from dose of 5 mg/kg; * difference from dose of 10 mg/kg.

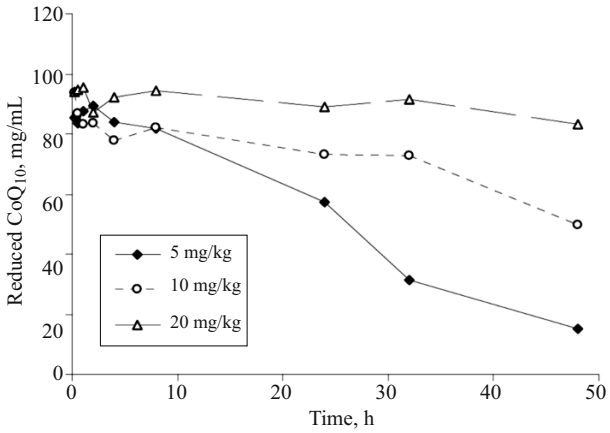

Fig. 4. Oxidation of ubiquinol in rat blood plasma after a single i.v. administration at three doses.

where t is the time (h); C, concentration (μg/mL). A similar bi-exponential kinetic curve was observed by us earlier after i.v. injection of ubiquinone [31]. It could be assumed by analogy with ubiquinone that the first short phase corresponded to rapid distribution of the drug into highly vascularized organs. The next phase was related mainly to continuation of accumulation and deposition of the drug in liver. Table 1 presents the main pharmacokinetic parameters of CoQ_{10} after a single injection at three doses.

The linearity check was the most important component of the pharmacological analysis because it allowed the change of concentration in response to a change of drug dose to be predicted. Ubiquinol clearance dropped with increasing dose, possibly because of a limited rate of excretion of CoQ_{10} mainly with secreted bile [26, 32]. Table 1 demonstrates that the elimination half-lives calculated for the different doses differed significantly. This was characteristic of nonlinear drug kinetics. However, the dependence between the areas under the concentration–time curves and the drug doses obeyed a linear regression ($y = 0.344x - 1.026$) with correlation coefficient 0.9997 ($p < 0.01$). This suggested that the pharmacokinetics were linear in the studied dose range. The kinetic curves were normalized to dose (linearity was assumed if the normalized and actual curves coincided) for further checking of the linearity [24]. The normalization used division of the concentration for each time point at a dose of 20 mg/kg by 2 and by 4 to obtain normalized curves for doses of 10 and 5 mg/kg, respectively. A comparison of the normalized and actual curves showed that they diverged significantly for both drug doses ($p < 0.05$). These results demonstrated that ubiquinol pharmacokinetics were nonlinear, analogously to those of ubiquinone [29].

The pharmacokinetics of the drug after multiple injections must be studied and compared with data for a single injection to reveal possible accumulation in blood plasma [24]. Kinetic curves of the change of total CoQ_{10} concentration vs. time were plotted from the results of three drug injections in absolute values and in logarithmic coordinates (Fig. 2).

Table 2 presents the main pharmacokinetic parameters for CoQ_{10} after three injections at two doses.

Table 2 reveals significant differences in the total clearance parameters calculated for the two doses. This also confirmed that the drug kinetics were nonlinear. The elimination half-lives were not statistically different between doses although the averages differed considerably because this parameter was highly variable at a dose of 5 mg/kg.

Figure 3 shows that the blood plasma concentrations after three injections were less than after a single injection. Statistically significant differences were obtained for a dose of 5 mg/kg in the first 8 h (Fig. 3*a*) and for a dose of 10 mg/kg, from 8 to 48 h (Fig. 3*b*) after injection. Correspondingly, the calculated clearances after three injections were considerably greater ($p < 0.05$) than after a single one, e.g., for a dose of 5 mg/kg, 4.09 vs. 2.91 mL/h; for a dose of 10 mg/kg, 2.45 vs. 1.54 mL/h. Considering that the primary excretion pathway of CoQ_{10}, i.e., secretion with bile, was saturated [26], the increasing clearance could be due to more extensive uptake in organ tissues. Multiple administrations in the studied dose range helped to increase the clearance which, in turn, suggested more efficient replenishment of tissues with the drug [3]. The higher drug dose corresponded to lower clearance for three injections, like for a single one.

The fraction of ubiquinol in the total CoQ_{10} plasma pool decreased gradually after injection of the drug and after 48 h was 15, 50, and 83% for doses of 5, 10, and 20 mg/kg, respectively (Fig. 4). Obviously, retention of the drug (in the reduced form) in plasma expressed in absolute value and percent increased with increasing dose. Oxidation of ubiquinol was a consequence of its involvement in endogenous redox processes, i.e., attested to its antioxidant activity. Redox activity of i.v. injected CoQ_{10} (in the oxidized form) was found by us earlier, e.g., ~10% of the ubiquinone with >90% present as ubiquinol remained in blood plasma after 48 h at a dose of 30 mg/kg [31].

Thus, the pharmacokinetics of an innovative dosage form of ubiquinol designed for i.v. injection were studied at doses of 5, 10, and 20 mg/kg for the first time. Bi-exponential kinetic curves were found. The main pharmacokinetic parameters, i.e., areas under the kinetic curves, elimination

TABLE 2. Pharmacokinetic Parameters of CoQ_{10} After Three Administrations at Doses of 5 and 10 mg/kg (24 h Interval Between Administrations)

Dose, mg/kg	Parameters		
	$AUC_{0\to48}$ h, mg·h/mL	Cl_T, mL/h	$T_{1/2}$, h
5	0.483 ± 0.08	4.09 ± 0.76	16.00 ± 8.40
10	1.618 ± 0.412[#]	2.45 ± 0.79[#]	10.33 ± 1.55

[*] Difference from dose of 5 mg/kg, $p < 0.01$.

half-lives, and total clearances were calculated. The pharmacokinetics of the drug in the studied dose range were nonlinear. Greater doses corresponded to lower clearances. Multiple injections increased the clearance. This suggested than the drug was taken up more extensively in organ tissues, which was required to manifest the protector effects of ubiquinol. Gradual oxidation of ubiquinol in blood plasma reflected involvement of the drug in endogenous redox processes. The results justified the potential of further clinical drug trials.

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