Pharmaceutical Chemistry Journal, Vol. 49, No. 11, February, 2016 (Russian Original Vol. 49, No. 11, November, 2015)

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

MULTI-DAY MONITORING OF UBIDECARENONE LEVEL IN RAT PLASMA AND TISSUES AFTER A SINGLE INTRAVENOUS INJECTION

E. I. Kalenikova,[1,*] E. A. Gorodetskaya,[1] O. G. Tokareva,[1] M. A. Belousova,[1] O. Yu. Kulyak,[1] M. M. Artem'eva,[1] and O. S. Medvedev[1]

Translated from Khimiko-Farmatsevticheskii Zhurnal, Vol. 49, No. 11, pp. 3 – 7, November, 2015.

Original article submitted May 28, 2015.

The pharmacokinetics of ubidecarenone (CoQ10) in rat plasma and the kinetics of its organ levels were studied for the first time for 8-16 d after a single i.v. injection at a dose of 30 mg/kg. The pharmacokinetics followed a classical two-compartment curve. The main pharmacokinetic parameters for plasma (C_0, $AUC_{0\to\infty}$, K_α, K_{el}, $t_{1/2\alpha}$, $t_{1/2el}$, Cl_T, V_d) and several organs (C_{max}, T_{max}, $AUC_{0\to t}$, f_{tiss}) were calculated. The results proved that CoQ_{10} injected i.v. rapidly attained and maintained for a long time high levels in plasma, myocardium, brain, liver, and kidneys. This was potentially important for treating acute ischemic states.

Keywords: ubidecarenone, pharmacokinetics, acute ischemic states.

Coenzyme Q_{10} (CoQ_{10}), which is also known as ubidecarenone, is a lipophilic molecule found in the membranes of almost all tissues and organs. CoQ_{10} plays a fundamental role in the metabolism of mitochondria and cellular bioenergetics [1]. Many cardiovascular diseases are associated with reduced CoQ_{10} levels [2]. Experimental and clinical studies showed that correction of a CoQ_{10} deficit provides cardiac and neural protection [3 – 6].

Most dosage forms of CoQ_{10} are preparations for internal use. The bioavailability with this administration route is known to be extremely low because of limited absorption due to low solubility and the high molecular weight [7 – 9]. Parenteral administration modes can increase rapidly CoQ_{10} levels in plasma and tissues. This can be especially important for urgent care of ischemia and myocardial infarct. It was shown earlier that myocardial infarct induced after experimental coronary occlusion can be reduced considerably in size by i.v. administration of CoQ_{10} at a dose of 30 mg/kg [10]. Injection (i.v.) of CoQ_{10} at this same dose in an ischemia model in rats decreased brain damage and improved the neurological state [3]. Thus, the development and preclinical trials of a parenteral CoQ_{10} dosage form for use in treating acute cardiovascular states are exceedingly crucial. Pharmacokinetic studies are the most important stage of preclinical trials. CoQ_{10} is known to be slowly eliminated [9, 11]. However, the reported characteristics of processes for reducing its *in vivo* concentration after injection are contradictory because of different and insufficiently long study protocols. CoQ_{10} levels in plasma and very important organs after its i.v. injection have not been monitored over long periods.

The goal of the present work was to study CoQ_{10} pharmacokinetics for several days after its i.v. injection.

[1] Department of Fundamental Medicine, M. V. Lomonosov Moscow State University, 31/5 Lomonosovskii Prosp., Moscow, 119192 Russia.
[*] e-mail: eikaleni@fbm.msu.ru.

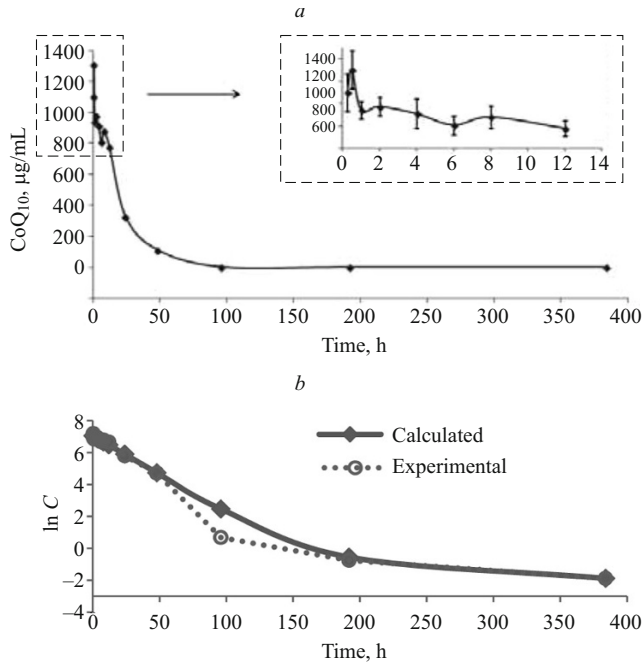

Fig. 1. Kinetic curves of CoQ_{10} in rat blood plasma for 16 d after i.v. injection of solubilized CoQ_{10} at a dose of 30 mg/kg: kinetic concentration—time curve, kinetic curve in the first 12 h in the inset (a); kinetic curve in semi-logarithmic coordinates (dashed line denotes plasma concentrations; solid line, calculated values).

TABLE 1. Mean Increase of CoQ_{10} Plasma Level in Rat Groups After a Single i.v. Injection of Solubilized CoQ_{10} Solution at a Dose of 30 mg/kg

Time after injection, h	CoQ_{10} increase, μg/mL	
	experimental value	calculated value
0.25	1102 ± 170*#	1144
0.5	1306 ± 175*	1130
1	941 ± 78*#	1103
2	976 ± 81*	1051
4	910 ± 135*	954
6	807 ± 87*	865
8	882 ± 103*	785
12	778 ± 67*#	647
24	328 ± 38*#	361
48	110 ± 61*#	113
96	2.0 ± 1.0*#	12
192	0.48 ± 0.26*#	0.58
384	0.16 ± 0.05*#	0.15

* Statistically significant differences vs. the control group not receiving CoQ_{10} (0.13 ± 0.06) μg/mL, $p < 0.05$;
\# statistically significant vs. the preceding time point, $p < 0.05$.

EXPERIMENTAL PART

The study used 72 male Wistar rats (300 – 350 g) according to applicable good laboratory practice requirements and prior approval of the local bioethics committee. Anesthetized (sodium pentobarbital, 45 mg/kg i.p.) animals with catheters implanted into thigh veins were injected (i.v.) once, depending on the group, with normal saline (1 mL/kg) (seven control animals) or solubilized CoQ_{10} solution (Kudesan® drops for internal use, 3%, OOO Vneshtorg Farma) at a dose of 30 mg/kg (five animals for each time point). Blood and tissues from the left ventricle (LV), brain, liver, and kidneys were collected 48 h after injection of normal saline and 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24, 48, 96, and 192 h after CoQ_{10} injection. Only blood was collected from an additional animal group 384 h (16 d) after the injection. Plasma and tissue samples were frozen and stored at –20°C before CoQ_{10} quantitative analysis by HPLC with electrochemical detection [12].

The main pharmacokinetic parameters were calculated using the experimental results. A regression equation for the elimination phase was derived based on the final (mono-exponential) portion of the pharmacokinetic curve using least-squares methods:

$$\ln C_t = \ln C_0 - K_{el} t, \quad (1)$$

where K_{el} is the elimination rate constant calculated using the formula:

$$K_{el} = \frac{n\sum_{i=1}^{n}(xy) - \sum_{i=1}^{n}(x)\sum_{i=1}^{n}(y)}{\sum_{i=1}^{n}(x^2) - (\sum_{i=1}^{n}(x))^2}, \quad (2)$$

where n is the number of experimental time points; x, a time point; y, the logarithm of the concentration (μg/mL).

The area under the pharmacokinetic curve of plasma concentration vs. time ($AUC_{0\to\infty}$) was calculated by a trapezoid method considering the finite portion of the area after the last blood-collection point. Total clearance (Cl_T) was calculated using the formula:

$$Cl_T = \frac{D}{AUC_{0\to\infty}}, \quad (3)$$

where D (μg/kg) is the single injected dose of the test compound; $AUC_{0\to\infty}$ (μg · h/mL), the area under the pharmacokinetic curve.

The elimination half-life ($t_{1/2el}$) was calculated as $t_{1/2el} = 0.693/K_{el}$. The apparent initial concentration (C_0) was determined from the derived bi-exponential concentration—time function at $t = 0$. The total distribution volume (V_d) was calculated using the formula:

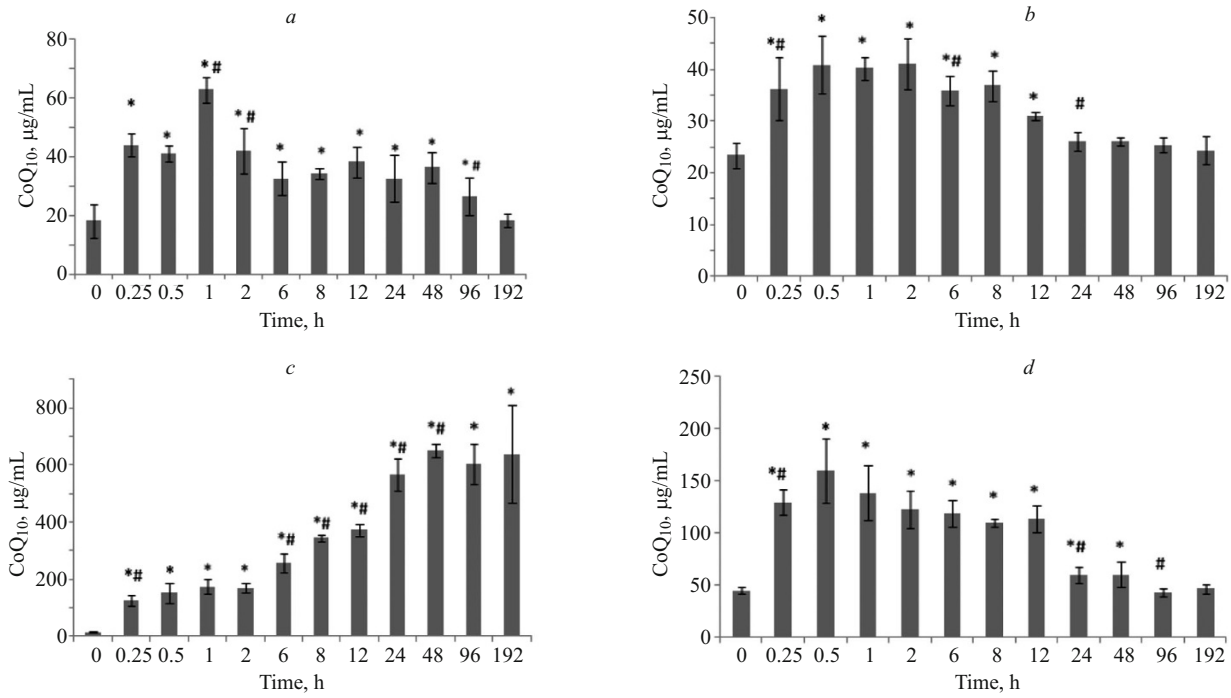

Fig. 2. CoQ_{10} content in myocardium (a), brain (b), liver (c), and kidneys (d) at each time point for 8 d after i.v. injection of a solution of solubilized CoQ_{10} at a dose of 30 mg/kg. * $p < 0.05$ vs. the control group (point "0"); # $p < 0.05$ vs. the preceding time point.

$$V_d = D/C_{0el},$$

where D (mg/kg) is the single injected dose of the test compound; C_{0el}, the apparent concentration for the elimination phase at time zero that was calculated using Eq. (1). Tissue availability (f_{tiss}) was calculated as the ratio of $AUC_{0\to t}$ in the tissue to the corresponding $AUC_{0\to t}$ value in plasma.

Results were processed statistically using Statistica 6.0 software and were presented as means with standard deviations. The non-parametric Mann—Whitney U-criterion was used to compare groups. Differences were considered statistically significant for $p < 0.05$.

RESULTS AND DISCUSSION

Table 1 presents the mean increases of CoQ_{10} blood-plasma concentrations after i.v. injection.

Elevated CoQ_{10} plasma levels were observed during the whole test period after i.v. injection. These decreased gradually but were still 2.2 times above background by the 16th day.

Pharmacokinetic curves based on the results were constructed in absolute and semi-logarithmic coordinates (Fig. 1*a* and 1b). The obtained kinetic curve for CoQ_{10} blood-plasma concentration as a function of time after injection obeyed satisfactorily the bi-exponential equation

$$C_t = 1157.25e^{-0.0486t} + 1.468e^{-0.0059t},$$

which corresponded to a two-compartment distribution model. The CoQ_{10} blood-plasma concentrations that were calculated using this equation agreed well with the experimental results (Table 1).

The first phase of the CoQ_{10} kinetic curve corresponded to distribution of the drug in internal organs and tissues and continued for ~96 h. Then, the rate of decrease of the blood concentration slowed considerably, indicating the start of the next phase, i.e., elimination.

The main pharmacokinetic parameters were calculated from the obtained results (Table 2).

The results showed that the drug distributed slowly ($t_{1/2\alpha} = 14.14$ h) and was eliminated very slowly ($t_{1/2el} = 117.5$ h). The low total clearance (1.18 mL/h/kg) was also indicative of prolonged elimination of CoQ_{10}. The distribution volume (20.4 L/kg) reflected the ability of the drug to penetrate extensively into organs and tissues. Figure 2 shows tissue accumulations of CoQ_{10} in potential target or-

TABLE 3. Pharmacokinetic Parameters of CoQ_{10} Distribution in Tissues for 8 d After i.v. Injection to Rats at a Dose of 30 mg/kg

Organ	T_{max}, h	C_{max}, μg/g	$AUC_{0\to t}$, μg·h/g	f_{tiss}
Heart (left ventricle)	1	62.7	1896	0.075
Brain	2	41.1	542	0.02
Liver	48	649.1	110425	4.36
Kidneys	0.5	160.2	2087	0.082

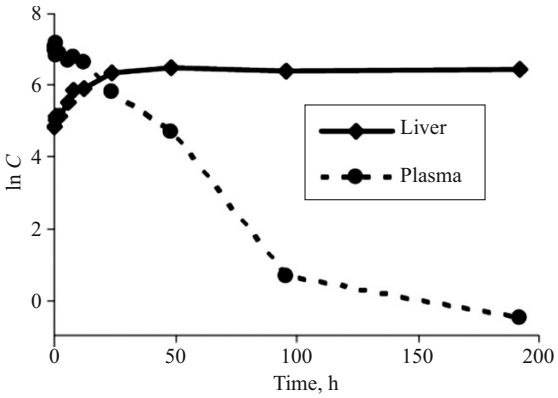

Fig. 3. Kinetic concentration—time curves for rat plasma and liver for 8 d after i.v. injection of solubilized CoQ_{10} at a dose of 30 mg/kg.

TABLE 2. Pharmacokinetic Characteristics of CoQ_{10} After i.v. Injection to Rats at a Dose of 30 mg/kg

Parameter	Value
Area under the curve $AUC_{0(\infty)}$, μg · h/mL	25403.07
Apparent initial concentration C_0, μg/mL	1158.72
Distribution constant K_α, h^{-1}	0.0486
Distribution half-life $t_{1/2\alpha}$, h	14.14
Elimination constant K_{el}, h^{-1}	0.0059
Elimination half-life $t_{1/2el}$, h	117.5
Total clearance Cl, mL/h/kg	1.18
Distribution volume V_d, L/kg	20.4

gans such as heart and brain and in organs of elimination such as the liver and kidneys.

The CoQ_{10} concentration in heart after a single i.v. injection increased by 2.5 times already in the first minutes and reached the maximum values after 1 h (242% increase). The increase remained at 80 – 111% in the next interval from 2 to 48 h; at 50%, after 4 d; and reached background values only after 8 d. The tissue availability (f_{tiss}) of CoQ_{10} for the LV was 0.075.

Brain levels of CoQ_{10} were increased statistically significantly after a single i.v. injection by 55, 75, 76, 53, and 32% after 15 and 30 min and 2, 6, and 12 h, respectively. The CoQ_{10} brain levels at later time points were at the background values. The f_{tiss} value of CoQ_{10} in brain was 0.02.

The CoQ_{10} content in liver increased in the first 48 h and reached a maximum of 649.05 μg/g, which was six times greater than the CoQ_{10} content in the control group. The attained CoQ_{10} level in liver was maintained for the next 6 d. Thus, the distribution in liver persisted for at least 48 h after i.v. injection. This agreed with the kinetic data for blood plasma. The f_{tiss} value for CoQ_{10} in liver was 4.36, which indicated that the drug accumulated in this organ.

The kidney content of CoQ_{10} increased (189%) already at the first time point (15 min) after injection. The maximum increase of CoQ_{10} concentration (256%) was reached at 0.5 h. The CoQ_{10} kidney contents remained elevated for 48 h and returned to background values 96 h after injection. The tissue bioavailability of CoQ_{10} in kidneys was 0.082.

Table 3 presents the pharmacokinetic parameters for CoQ_{10} accumulation in organs after i.v. injection in rats.

Reported pharmacokinetic data for CoQ_{10} were obtained primarily after internal administration [7 – 9]. Anecdotal studies after intravascular injection spanned less than 2 d [9, 13]. The pharmacokinetics of the drug in plasma and the changes in its levels in organs over 8 – 16 d were studied for the first time. The results showed a classical two-compartment pharmacokinetic curve. The main pharmacokinetic parameters for plasma and several organs were calculated. The results indicated that CoQ_{10} administered i.v. penetrated into brain and myocardium and provided prolonged elevated tissue levels. This was potentially important for treating acute ischemic states.

Plots of tissue bioavailability and a comparison of CoQ_{10} kinetic profiles in plasma and liver (Fig. 3) showed that the liver was a storage organ for CoQ_{10}. One of the physiological functions of the liver is to synthesize CoQ_{10} and secrete it into the circulation. Therefore, CoQ_{10} accumulation in the liver can keep its plasma levels elevated for a long time. This hypothesis agrees with previous results for the maintenance of elevated CoQ_{10} levels in plasma, myocardium, and liver even three weeks after a single i.v. injection [10]. CoQ_{10} liver accumulation with subsequent periodic release into the blood can produce a characteristic wave-like pharmacokinetic curve in the first 12 h (Fig. 1*a*).

Thus, a single i.v. injection produced a rapid increase and prolonged maintenance of high CoQ_{10} levels in plasma, myocardium, brain, liver, and kidneys. The experimental results and those obtained earlier for the cardio- and neuroprotective efficacy of CoQ_{10} [3 – 6] confirmed that the development of parenteral CoQ_{10} dosage forms is promising for treating acute cardiovascular states.

ACKNOWLEDGMENTS

The studies were sponsored by a grant of the Russian Scientific Foundation (Project No. 14-15-00126).

REFERENCES

1. G. P. Littarru and L. Tiano, *Mol. Biotechnol.*, **37**(1), 31 – 37 (2007).
2. S. L. Molyneux, C. M. Florkowski, P. M. George, et al., *J. Am. Coll. Cardiol.*, **52**(18), 1435 – 1441 (2008).
3. M. Belousova, O. Povarova, E. Gorodetskaya, et al., *Eur. Neuropsychopharmacol.*, **24**, Suppl. 2, S202 (2014).
4. M. Mancuso, D. Orsucci, L. Volpi, et al., *Curr. Drug Targets*, **11**(1), 111 – 121 (2010).

5. M. Zamani, M. Katebi, M. Mehdizadeh, et al., *Basic Clin. Neurosci.*, **3**(5), 5 – 10 (2012).
6. J. Garrido-Maraver, M. D. Cordero, M. Oropesa-Avila, et al., *Front. Biosci. (Landmark Ed.),* **19**, 619 – 633 (2014).
7. H. N. Bhagavan and R. K. Chopra, *Free Radical Res.*, **40**(5), 445 – 453 (2006).
8. M. V. Miles, *Mitochondrion*, 7 Suppl., S72-S77 (2007).
9. A. Nishimura, H. Yanagawa, N. Fujikawa, et al., *J. Health Sci.*, **55**(4), 540 – 548 (2009).
10. A. Ivanov, O. Tokareva, E. Gorodetskaya, et al., *J. Clin. Exp. Cardiol.*, **5**(4), 1 – 6 (2014).
11. N. K. Swarnakar, A. K. Jain, R. P. Singh, et al., *Biomaterials*, **32**(28), 6860 – 6874 (2011).
12. G. K. Budnikov and S. Yu. Garmonov (eds.), *Pharmaceutical Analysis* [in Russian], Vol. 16, Argamak-Media, Moscow (2013), pp. 616 – 653.
13. E. I. Kalenikova, E. V. Kharitonova, E. A. Gorodetskaya, et al., *Biomed. Khim.*, **61**(1), 125 – 131 (2015).