[Chem. Pharm. Bull. 27(5)1101—1105(1979)]

UDC 577.158.04 : 547.567.04.09

Effects of Exogenous Ubiquinone-10 on Endogenous Ubiquinone-10 in Canine Plasma and on Electron Transport Enzymes in Leucocytes

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(Received October 12, 1978)

The distribution of exogenous ubiquinone-10 in canine plasma was examined using deuterium-labeled ubiquinone-10 in conjunction with a computerized gas chromatograph–mass spectrometry system. Electron transport enzymes in canine leucocytes were also studied. An increase in the plasma level of exogenous ubiquinone-10 did not affect the level of endogenous ubiquinone-10, and it was reflected in increased activities of the electron transport enzymes (succinate dehydrogenase Co Q reductase, succinate cytochrome c reductase and succinate oxidase) in leucocytes.

Keywords——deuterium labeled ubiquinone-10; exogenous ubiquinone-10; endogenous ubiquinone-10; direct inlet mass fragmentography; succinate dehydrogenase Co Q reductase; succinate cytochrome c reductase; succinate oxidase; canine plasma; canine leucocytes

Ubiquinone is known to be an essential co-factor for the mitochondrial electron transfer processes of respiration and coupled phosphorylation in organisms.[2] The biosynthetic route of ubiquinone has also been studied extensively.[3] The distribution in mitochondria of endogenous ubiquinone is thought to be functionally homogenous.[4] With respect to the metabolic fate and biological role of exogenous ubiquinone, it is not yet clear whether or not exogenous ubiquinone equilibrates with endogenous ubiquinone.

Ramasarma *et al.*[5,6] reported various results based on a biological approach. The therapeutic utilization of exogenous ubiquinone-10 (UQ-10) has been examined by measuring succinate dehydrogenase Co Q reductase activity in leucocytes of hypertensive patients.[7,8] The effect of exogenous UQ-10 on the ubiquinone pool of liver and spleen has also been described.[9]

In this paper the effect of orally administered UQ-10 on canine plasma levels was studied using deuterium-labeled ubiquinone-10 (UQ-10-d_5). The quantitative determination of UQ-10 and UQ-10-d_5 was carried out by direct-inlet mass fragmentography, using a computerized gas chromatograph–mass spectrometry system. In addition, the relationship between the canine plasma UQ-10 level and leucocyte enzyme activities was investigated. The activities of electron transport enzymes, such as succinate dehydrogenase Co Q reductase, succinate cytochrome c reductase and succinate oxidase, in leucocytes were determined after oral administration of UQ-10-d_5. The inhibitory effects of specific inhibitors[10] of the enzyme activities were also determined.

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Materials and Methods

All-*trans* UQ-10[11] and all-*trans* 2,3,5-trimethyl-6-decaprenyl-*p*-benzoquinone[12] were prepared at the laboratories of Eisai Co., Ltd. All-*trans* UQ-10-d_5 (2-C^2H_3-1'-C^2H_2-UQ-10) was synthesized by condensing 2,3-dimethoxy-5-C^2H_3-*p*-benzohydroquinone and 1-C^2H_2-decaprenol. The procedures of condensation and purification were as described previously.[11] The isotope chemical purity of UQ-10-d_5 was over 94% as determined by nuclear magnetic resonance spectroscopy. The following chemicals were used as received: 2,6-dichloroindophenol sodium salt (DCIP) and 2-thenoyltrifluoroacetone (TTFA) (Tokyo Chemical Industry Co., Ltd.), antimycin A (ICN Pharmaceuticals Inc., U.S.A.), sodium azide (E. Merck, Germany), cytochrome c (Type III, Sigma Chemical Company, U.S.A.), rotenone and potassium succinate (Nakarai Chemicals, Ltd., Japan). Other chemicals of the highest available quality were purchased commercially. Assays for individual determinations of UQ-10 and UQ-10-d_5 were performed on a JEOL JMS-D 300 gas chromatograph-mass spectrometer unit equipped with a computer system (JMA-2000). The ionizing voltage and ionizing current were 70 eV and 300 μA, respectively. The chamber temperature was kept at 300°. The sample temperature was raised linearly from 100° to 430° within 3 min. During that time, mass fragments from *m/e* 824 to *m/e* 900 were scanned and integrated. The absorption spectra were measured with a Hitachi 156 double-wavelength spectrophotometer. Male beagle dogs, weighing approximately 10 kg were used. They were caged individually in an air-conditioned, artificially lighted room and were fed a chow diet (CLEA Japan CD-1, 250 g/day).

Three or four beagles were used as a group. UQ-10-d_5 (1.5 mg/kg body weight) was given in capsules with 50 ml of milk. The control group was given only the capsule without UQ-10-d_5. The plasma sample for analysis was pretreated with a mixture of pancreatin (Type II, Sigma Chemical Company, U.S.A.) and sodium taurocholate (BDH Chemicals, U.K.) at 37° for 2 hours. These mild hydrolysis conditions were essential to obtain a clear mass fragmentogram.[13]

The preparation of leucocyte suspensions was performed as described in the previous paper.[14] For the assays of succinate dehydrogenase Co Q reductase[14] and succinate cytochrome c reductase[15,16] the leucocytes were sonified with a Branson B-12 sonifier, 1/8 horn, 80 W (Branson Sonic Power Company, U.S.A.) for 10 sec intermittently with chilling. Succinate oxidase[17] was measured in terms of oxygen consumption with a rotating platinum electrode (Yanagimoto PO-100 A, Japan) using unsonified leucocytes prepared immediately after exsanguination.

Results and Discussion

The mass spectrum of UQ-10-d_5 is shown in Fig. 1. The molecular ion peak appeared at *m/e* 869 (M+2), while that of UQ-10 appeared at *m/e* 864 (M+2). For individual analysis, a multiple ion detector adjusted to *m/e* 869 for exogenous UQ-10, *m/e* 864 for endogenous UQ-10 and to *m/e* 832 (M+2) for the internal standard (2,3,5-trimethyl-6-decaprenyl-*p*-benzoquinone) was used. The peak ratio was plotted against the internal standard. The results of integrating mass fragmentography of a mixture of standard compounds are shown in Fig. 2. The calibration curves of UQ-10 and UQ-10-d_5 are shown in Fig. 3. The minimum quantity of UQ-10 necessary for detection was established as 20 ng/ml plasma.

The levels of canine plasma UQ-10 were examined after oral administration of 1.5 mg/kg of UQ-10-d_5 in capsules. The time course (Fig. 4) shows an increase of the exogenous UQ-10 level, which reached a peak 6 hours after administration and then started to decrease. During observation for 24 hours the endogenous UQ-10 level did not change significantly. No daytime variation of endogenous UQ-10 in the plasma of the control group was observed.

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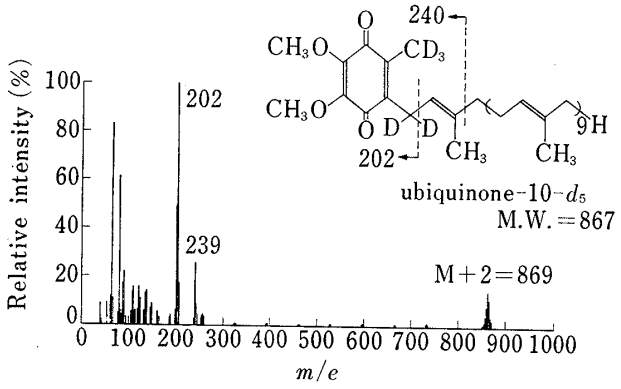

Fig. 1. Mass Spectrum of UQ-10-d_5

The conditions of measurement are described in "Materials and Methods."

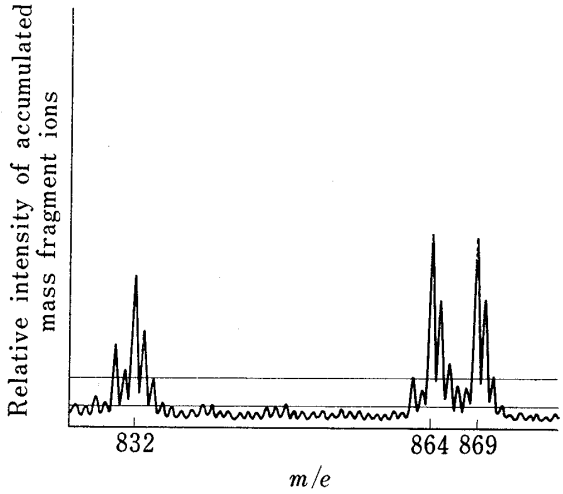

Fig. 2. Integrating Mass Fragmentography of a Mixture of 2,3,5-Trimethyl-6-decaprenyl-*p*-benzoquinone (*m/e* 832), UQ-10 (*m/e* 864) and UQ-10-d_5 (*m/e* 869)

The authentic compounds (50 ng each) were mixed and injected. The fragmentogram was obtained after 1276 scannings.

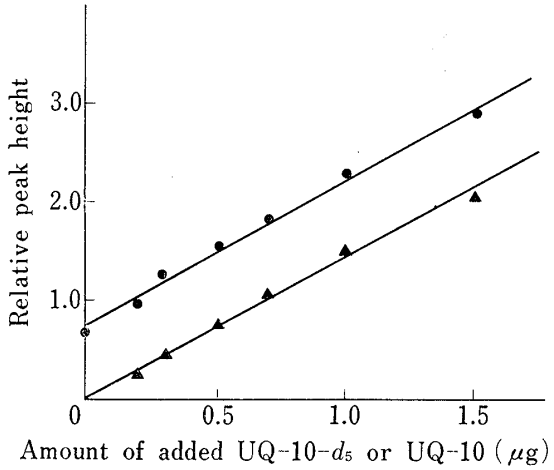

Fig. 3. Calibration Curve of UQ-10 and UQ-10-d_5 in Canine Plasma

The peak ratio was plotted against the internal standard (I.S., 2,3,5-trimethyl-6-decaprenyl-*p*-benzoquinone). UQ-10-d_5/I.S. (——●——), UQ-10/I.S. (——▲——). Endogenous UQ-10 was deducted in the case of added UQ-10 analysis.

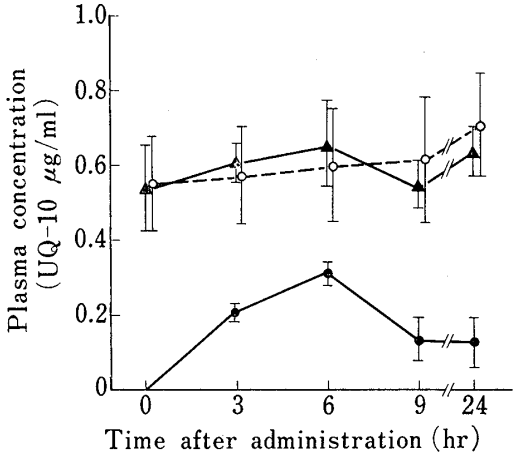

Fig. 4. Variation of the Canine Plasma UQ-10 Level of the Control Group (----○----) and UQ-10-d_5 (1.5 mg/kg) Orally Administered Group

The plasma levels are the sum of exogenous UQ-10 (——●——) and endogenous UQ-10 (——▲——). The points represent the means and S.E.M. of 3 individuals.

The activities of the electron transport enzymes in leucocytes were measured using leucocytes contained in corresponding blood samples. The variations of enzyme activities, such as succinate dehydrogenase Co Q reductase (Fig. 5A), succinate cytochrome c reductase (Fig. 5B) and succinate oxidase (Fig. 5C), are shown in the in the figures cited. The loss of exogenous UQ-10 and decrease of enzyme activities in leucocytes occurred 9 hours after administration.

The inhibitory effects of specific inhibitors on particular sites of electron transport were determined when the enzyme activities reached the highest level, 6 hours after UQ-10-d_5 administration. As shown in Table I, the activities of the enzymes were inhibited. TTFA

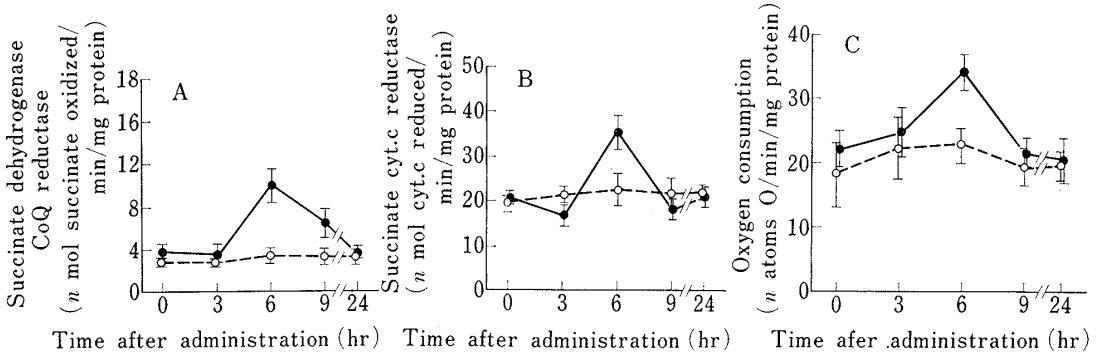

Fig. 5. Effect of Oral Administration of UQ-10-d_5 on Canine Leucocyte Electron Transport Enzymes

Control group (----○----) and UQ-10-d_5 (1.5 mg/kg) orally administered group (——●——). The points represent the means and S.E.M. of 4 individuals.

(A): Succinate dehydrogenase Co Q reductase. Approximately 0.4 mg of protein of sonified leucocyte suspension (0.1 ml) was used. To the leucocyte suspension, 1.0 ml of 0.15 M potassium phosphate buffer (pH 7.0), 0.2 ml of 0.01% 2,6-dichloroindophenol and 0.08 ml of water added. The reaction mixture was incubated at 37° for 10 min. After the addition of 0.1 ml of 0.3 M potassium succinate, the absorbance at 600 nm was recorded.

(B): Succinate cytochrome c reductase. The same treatment and the same amount of leucocyte suspension as in A were used for the assay. To the leucocyte suspension, 0.3 ml of 0.05 M potassium phosphate buffer (pH 7.4), 0.1 ml of 0.015 M sodium azide, 0.6 ml of water and 0.3 ml of 0.2% cytochrome c in 0.05 M potassium phosphate buffer (pH 7.4) were added. The reaction mixture was incubated at 37° for 5 min. Just after the addition of 0.1 ml of 0.3 M potassium succinate, the absorption change at 550—534 nm was measured at 10 sec intervals for 1 min.

(C): Succinate oxidase. A mixture of 1.0 ml of 0.3 M mannitol, 0.5 ml of 0.035 M Tris–HCl buffer (pH 7.4), 50 μl of 0.2 M $MgCl_2$, 0.2 ml of 0.5 M potassium phosphate buffer (pH 7.4) and 0.2 ml of water was kept at room temperature for 2 min. Approximately 1.0 mg protein containing unsonified leucotyte suspension (0.1 ml) was then added to the mixture, which was kept at room temperature for 2 min. The oxygen consumption was measured after the addition of 0.1 ml of 0.3 M potassium succinate and 50 μl of 0.05 M ADP to the mixture.

Table I. Effect of Inhibition of Electron Transport Enzymes in Leucocytes 6 Hours after Oral Administration of UQ-10-d_5

Enzyme system[a)]	Inhibitor (μM)	Percent inhibition
Succinate dehydrogenase Co Q reductase	Rotenone (10)	0
	TTFA (1000)	85—90
Succinate cytochrome c reductase	Antimycin A (0.025)	100
Succinate oxidase	Potassium cyanide (1000)	100

a) Assay conditions are described in the legend to Fig. 5.

was used as an inhibitor for succinate dehydrogenase Co Q reductase, antimycin A for succinate cytochrome c reductase and potassium cyanide for succinate oxidase. Rotenone was used to determine whether the electron flow observed here plays a role in the NADH→ complex I→ubiquinone route or not.

It has been shown that the intake of UQ-9 did not affect the incorporation of *p*-hydroxybenzoic acid in rat liver.[6] Further, in this paper it was shown that the increase of exogenous UQ-10 did not affect the endogenous UQ-10 level in the plasma. The effect of the increased UQ-10 level in plasma over certain critical levels was observed as enhanced activity of enzymes such as succinate dehydrogenase Co Q reductase, succinate cytochrome c reductase and succinate oxidase in leucocytes. These results appear to represent typical physiological effects of exogenous UQ-10 on both the plasma and leucocytes. It would be interesting to examine tissues other than leucocytes in future experiments. The phenomena observed here

appear to support the concept that exogenous UQ-10 would be therapeutically effective when local or systemic shortages of UQ-10 occur in tissues.[18] There may be some question as to whether or not the flow of electrons increased by exogenous UQ-10 passes through the expected route. However, the present experimental results (Table I) clearly show that the increase of electron flow does pass through the expected pathway (succinate→complex II→ubiquinone→complex III→cytochrome c→complex IV→oxygen). The ineffectiveness of inhibition by rotenone means that the electron pathway, NADH→complex I→ubiquinone, did not contribute to the increased electron flow. TTFA did not inhibit the electron flow completely, but did inhibit it by up to 90%. The inhibitory site of TTFA in the succinate dehydrogenase Co Q reductase assay is considered to be between ubiquinone and DCIP.[19,20]

The purpose of this paper has been to examine the effect of plasma UQ-10 level on electron transport enzyme activity in leucocytes as a model to clarify the biological action of exogenous UQ-10 in tissues and organs. To define the true physiological significance of the increased enzyme activity in leucocytes after administration of UQ-10, further investigation will be necessary.

Acknowledgement The authors are indebted to Dr. S. Ohtake, Director, Experimental Therapeutic Research Laboratories, Eisai Co., Ltd., for his valuable advice and also Messrs. K. Sugiyama, F. Sagami, K. Sakaguchi and Miss M. Kuroki of Eisai Research Laboratories for their skillful technical assistance.

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