Coenzyme Q$_9$ Provides Cardioprotection after Converting into Coenzyme Q$_{10}$

Istvan Lekli,[†,‡] Somak Das,[†] Samarjit Das,[†] Subhendu Mukherjee,[†]
Istvan Bak,[‡] Bela Juhasz,[‡] Debasis Bagchi,[§] Golakoti Trimurtulu,[||]
A. V. Krishnaraju,[||] Krishanu Sengupta,[||] Arpad Tosaki,[†,‡] and
Dipak K. Das*[,†,§]

Cardiovascular Research Center, University of Connecticut School of Medicine, Farmington,
Connecticut, Department of Pharmacy, University of Debrecen, Debrecen, Hungary, InterHealth
Nutraceuticals, Benicia, California, and Laila Impex R & D Center,
Vijayawada, Andhra Pradesh, India

Coenzyme Q$_{10}$ (CoQ$_{10}$) has been extensively studied as adjunctive therapy for ischemic heart disease, and its cardioprotective ability is well-established. The mitochondrial respiratory chain contains several coenzymes, including CoQ$_1$, CoQ$_2$, CoQ$_4$, CoQ$_6$, CoQ$_7$, CoQ$_8$, CoQ$_9$, and CoQ$_{10}$. It is not known whether other CoQs, especially CoQ$_9$, is equally cardioprotective as CoQ$_{10}$. The present study was designed to determine if CoQ$_9$ could protect guinea pig hearts from ischemia reperfusion injury. Guinea pigs were randomly divided into three groups: groups I and II were fed CoQ$_9$ and CoQ$_{10}$, respectively, for 30 days while group III served as control. After 30 days, the guinea pigs were sacrificed and isolated hearts were perfused via working mode were subjected to 30 min ischemia followed by 2 h of reperfusion. Cardioprotection was assessed by evaluating left ventricular function, ventricular arrhythmias, myocardial infarct size, and cardiomyocyte apoptosis. Samples of hearts were examined for the presence of CoQ$_9$ and CoQ$_{10}$. The results demonstrated that both CoQ$_9$ and CoQ$_{10}$ were equally cardioprotective, as evidenced by their abilities to improve left ventricular performance and to reduce myocardial infarct size and cardiomyocyte apoptosis. High performance liquid chromatographic (HPLC) analysis revealed that a substantial portion of CoQ$_9$ had been converted into CoQ$_{10}$. The results indicate that CoQ$_9$ by itself, or after being converted into CoQ$_{10}$, reduced myocardial ischemia/reperfusion-induced injury.

KEYWORDS: Coenzyme Q$_9$; coenzyme Q$_{10}$; heart; ischemic reperfusion injury

INTRODUCTION

Coenzyme Q$_{10}$ (CoQ$_{10}$), a member of the ubiquinone family, is an essential component of the mitochondrial electron transfer chain, which is required for ATP synthesis and functions as an antioxidant in cell membranes and lipoproteins (*1*). CoQ$_{10}$ is also a powerful antioxidant not only within the mitochondria but also in other organelle membranes containing CoQ (*2*). CoQ$_{10}$ is ubiquitously present in the mammalian tissues, especially in the heart. The fact that the levels of endogenous CoQ$_{10}$ in the heart decreases during ischemic heart disease including heart failure prompted clinical trials with CoQ$_{10}$ in patients that suffered from heart failure (*3*). Randomized, double-blind, placebo-controlled trials of oral administration of CoQ$_{10}$

have confirmed the effectiveness of CoQ$_{10}$ in improving angina episodes, arrhythmias, and left ventricular function in patients with acute myocardial infarction (*4*).

CoQ$_9$ is found in rodents like mice and rats, while CoQ$_6$, CoQ$_7$, and CoQ$_8$ are found in yeast and bacteria (*5, 6*). The majority of CoQ$_9$ in rat liver is present in its reduced form (ubiquinol), which exerts its antioxidative function (*7*). Similar to CoQ$_{10}$, CoQ$_9$ is not merely a compound responsible for energy transduction in mitochondrial membrane in rat heart; it also serves as a functional element in the cells and possesses ability for redox cycling. CoQ$_9$ differs from CoQ$_{10}$ with respect to the number of isoprenoid units in the tail: CoQ$_9$ has nine units in contrast to the presence of 10 units in CoQ$_{10}$.

Most of the CoQ$_{10}$ is found in mammalian hearts including human myocardium (*7*). CoQ$_{10}$ is not an essential nutrient, because it can be synthesized in the body. High amounts of CoQ$_{10}$ can also be found in several food products, including meat, fish, peanuts, and broccoli (*8*). Dietary intake of CoQ$_{10}$ is about 2−5 mg per day, which is inadequate for the body under physiological conditions (*2*).

* Corresponding author. Tel: (860) 679-3687. Fax: (860) 679-4606.
E-mail: DDAS@NEURON.UCHC.EDU.
[†] University of Connecticut School of Medicine.
[‡] University of Debrecen.
[§] InterHealth Nutraceuticals.
[||] Laila Impex R & D Center.

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Table 1. Cardiac Function in Isolated Ischemic/Reperfused Hearts Obtained from Guinea Pigs Treated with 5 mg/kg/day of CoQ_{10} and CoQ_9, Respectively, for 4 Weeks[a]

group	Before ISA				After 60 min of RE				After 120 min of RE			
	HR	CF	AF	LVDP	HR	CF	AF	LVDP	HR	CF	AF	LVDP
control	254 ± 5	24 ± 1	32 ± 2	104 ± 5	195 ± 4	15 ± 1	7 ± 1	49 ± 3	182 ± 4	15 ± 1	8 ± 1	45 ± 3
5 mg/kg CoQ_{10}	261 ± 6	25 ± 2	34 ± 2	100 ± 5	228 ± 3^b	20 ± 1^b	20 ± 2^b	71 ± 3^b	217 ± 3^b	19 ± 1^b	18 ± 2^b	64 ± 3^b
5 mg/kg CoQ_9	248 ± 5	23 ± 2	35 ± 2	106 ± 4	240 ± 4^b	22 ± 2^b	27 ± 1^b	80 ± 2^b	233 ± 4^b	25 ± 2^b	26 ± 1^b	75 ± 2^b

[a] $n = 6$ in each group. Abbreviations: heart rate (HR) in beats/min, coronary flow (CF) in mL/min, aortic flow (AF) in mL/min, left ventricular developed pressure (LVDP) in mmHg, ischemia (ISA), reperfusion (RE). [b] $p < 0.05$ compared to the values of the control group.
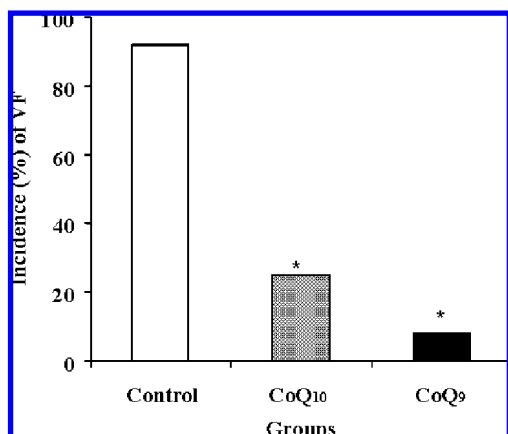
Figure 1. Incidence of reperfusion-induced ventricular fibrillation (VF). Guinea pigs were orally treated with a daily dose of 5 mg/kg of CoQ_{10} or CoQ_9 for 4 weeks, and then hearts were isolated and subjected to 30 min of global ischemia followed by 120 min of reperfusion. $N = 12$ in each group, $*p < 0.05$ compared to the drug-free control group.

Although CoQ_9 may also be present in the body, CoQ_{10} remains the only CoQ supplement that is commercially available. As mentioned earlier, CoQ_{10} is the essential component for ATP synthesis and acts as the redox link between flavoproteins and cytochromes that are needed for ATP synthesis. Hearts require additional CoQ_{10} for maintaining ATP levels under pathophysiological conditions such as ischemic heart diseases, including heart failure. Whether CoQ_9 can also perform the same task for the heart, especially if CoQ_9 supplementation can reduce myocardial ischemia/reperfusion, is not known. The present study compares the effects of CoQ_9 vs CoQ_{10} in the ischemic myocardium and determines that CoQ_9 could protect the ischemic heart to the same extent as CoQ_{10}. CoQ_9, however, was found to be bioconverted into CoQ_{10} and it is likely that CoQ_9 could fill up the gap for CoQ_{10} after being converted into CoQ_{10} as the bioavailability of CoQ_{10} is relatively poor.

MATERIALS AND METHODS

Materials. CoQ_9 and CoQ_{10} were generous gift from Liala Impex R & D Center, India. Guinea pigs were obtained from Charles River. The standards for CoQs were purchased from Sigma Chemical Co., St. Louis, MO. All other chemicals were of analytical grade and obtained from Sigma Chemical Co., St. Louis, MO.

Animals. Male Hartley guinea pigs of about 350−400 g body weight were randomly divided into three groups, control, CoQ_9, and CoQ_{10}. The guinea pigs were given vehicle only, CoQ_9, or CoQ_{10} by gavaging once a day at 5 mg/kg of body weight (dissolved 0.5 mL of water) for 30 days. The animals had free access to food and water. All animals received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institute of Health (NIH Publication No. 86−23, revised 1985).

Working Heart Preparation. After 30 days animals were anesthetized with sodium pentobarbital (70 mg/kg) and heparinized with 500 units/kg of intraperitoneal disodium heparin. After 5 min of heparin administration, the animals were sacrificed, and hearts were excised and cannulated via the aorta and perfused in the Langendorff mode at a constant perfusion pressure. The perfusion buffer consisted of a modified Krebs−Henseleit bicarbonate buffer (millimolar concentrations: NaCl 118, KCl 5.8, $CaCl_2$ 1.8, $NaHCO_3$ 25, KH_2PO_4 0.36, $MgSO_4$ 1.2, and glucose 5.0). Following the 5 min washout period of the Langendorff heart perfusion, the pulmonary vein was cannulated, and the heart was switched to the "working" mode via perfusion of the left atria (at a filling pressure of 17 cm of the buffer, 1.7 kPa) as described in detail elsewhere (*9, 10*). Global ischemia was imposed by clamping the atrial and aortic cannulas. After 30 min of ischemia, the reperfusion was initiated by unclamping the atrial inflow and aortic outflow lines and continued for another 120 min

Measurement of Infarct Size. Hearts for determination of infarct size were perfused, at the end of each experiment, with 25 mL of 1% triphenyltetrazolium chloride solution (TTC) in phosphate buffer (pH 7.4) via the side arm of the aortic cannula and then stored at −70 °C for later analysis. Frozen hearts were sliced transversely (*11*) in a plane perpendicular to the apico-basal axis into 3−4 mm thick sections, weighted, blotted dry, placed in between microscope slides, and scanned on a Hewlett-Packard Scanjet 5p single pass flat bed scanner (Hewlett-Packard, Palo Alto, CA). Using the NIH Image 1.61 image processing software, each digitalized image was subjected to equivalent degrees of background subtraction and brightness and contrast enhancement for improved clarity. Infarct zones of each slice were traced and the respective areas were calculated in terms of pixels. The areas were measured by computerized planimetry software, these areas were multiplied by the weight of each slice, and then the results were summed up to obtain the weight of the risk zone (total weight of left ventricle) and the infarct zone. Infarct size was calculated as the ratio, in percent, of the infarct zone to the risk zone.

Evaluation of Apoptosis. Immunohistochemical detection of apoptotic cells was carried out with TUNEL (*12*) using the APOPTAG kit (Oncor, Gaithersburg, MD). The heart tissues were immediately put in 4% formalin and fixed in an automatic tissue-fixing machine. The tissues were carefully embedded in the molten paraffin in metallic blocks, covered with flexible plastic molds, and kept under freezing plates to allow the paraffin to solidify. The metallic containers were removed and tissues became embedded in paraffin on the plastic molds. Prior to analyzing tissues for apoptosis, tissue sections were deparaffinized with xylene and washed in succession with different concentrations of ethanol (absolute, 95%, 70%). Then tissues were incubated again with mouse monoclonal antibody recognizing cardiac myosin heavy chain to specifically recognize apoptotic cardiomyocytes. The fluorescence staining was viewed with a confocal laser microscope. The number of apoptotic cells was counted and expressed as a percent of total myocytes population.

High Performance Liquid Chromatography (HPLC) and Mass Spectroscopy (MS) for the Determination of CoQ_9 and CoQ_{10}. *Preparation of CoQ_9 and CoQ_{10} Standard Solutions.* Standard solutions were prepared by weighing approximately 10 mg of CoQ_9 and CoQ_{10} standards respectively into a 100 mL volumetric flask and then dissolving it by using the mobile phase as a diluent. The stock solution was further diluted 1:10 to attain a final working concentration of 0.01 mg/mL. The CoQ_{10} stock solution had to be sonicated for 5 min for complete dissolution of the powder into solution.
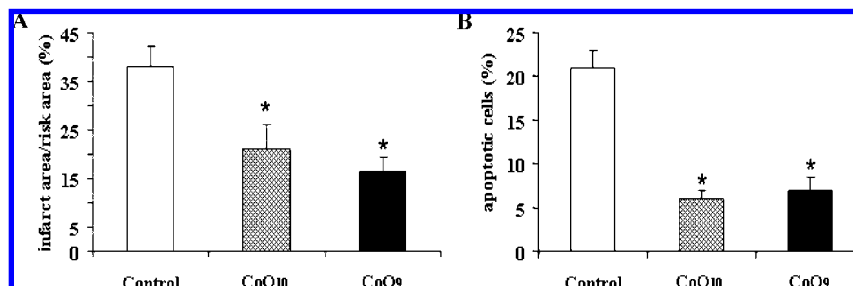
Figure 2. (**A**) Effects of CoQ$_{10}$ and CoQ$_9$ on infarct size in isolated guinea pig hearts subjected to 30 min of ischemia followed by 120 min of reperfusion. *$p < 0.05$ compared to the untreated age-matched ischemic/reperfused drug-free control value. (**B**) Effects of CoQ$_{10}$ and CoQ$_9$ on cardiomyocyte apoptosis in isolated guinea pig hearts subjected to 30 min of ischemia followed by 120 min of reperfusion. *$p < 0.05$ compared to the untreated age-matched ischemic/reperfused drug-free control value.
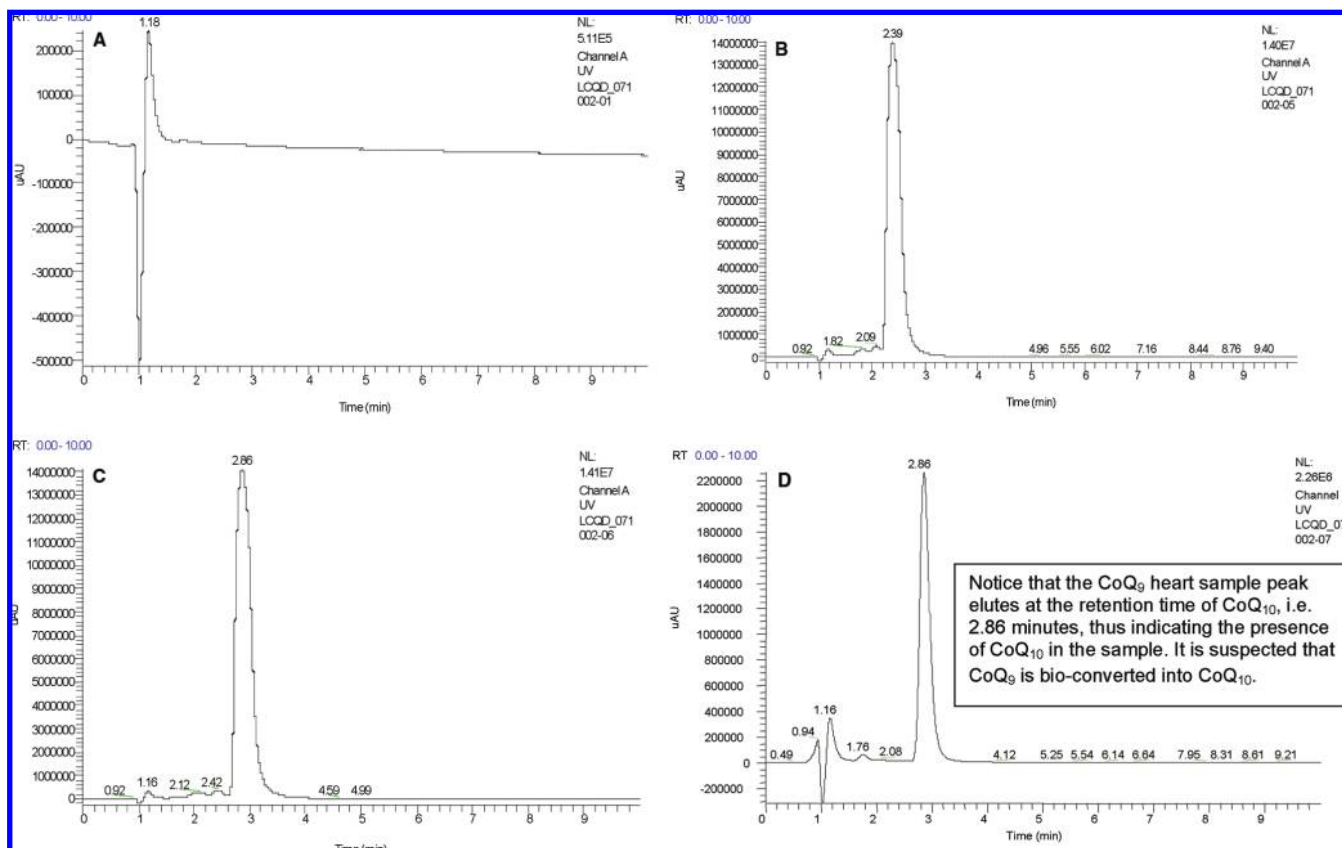

Figure 3. HPLC chromatograms of (**A**) blank (mobile phase), (**B**) CoQ$_9$ standard solution, (**C**) CoQ$_{10}$ standard solution, and (**D**) CoQ$_9$ heart sample. The standards and sample solutions were analyzed using an Agilent 1100 HPLC. The mobile phase was methanol−2-propanol−formic acid (45:55:0.05, v/v/v) containing methylamine at the concentration of 5 mmol/L. At a flow rate of 0.2 mL/min, 5 μL injections of the samples were done using the autosampler. The CoQ$_9$ peak eluted at 2.39 min and the CoQ$_{10}$ eluted at 2.86 min. However, the CoQ$_9$ heart sample peak eluted at 2.89 min, which is the same as CoQ$_{10}$, thus indicating the presence of CoQ$_{10}$ rather than CoQ$_9$ in the sample. Bioconversion of CoQ$_9$ into CoQ$_{10}$ was suspected and this was further verified using mass spectrometric analysis.

Preparation of CoQ$_9$ and CoQ$_{10}$ Heart Samples. The heart samples provided for analysis were centrifuged at 3000 rpm for 10 min. The supernatant was then transferred to another centrifuge tube and was evaporated to dryness using nitrogen, in order to obtain a more concentrated solution. The residue after dryness was then dissolved using 2 mL of mobile phase and was then transferred to an autosampler injection vial. The samples were analyzed immediately after preparation, and the remainder of the standard solutions was stored at 4 °C for future analysis.

HPLC Analysis of CoQ$_9$ and CoQ$_{10}$. The modular HPLC system consisted of an Agilent 1100 quaternary pump, Agilent 1100 autosampler, Agilent 1100 column heater, and Agilent 1100 UV detector. The analysis of CoQ$_9$ and CoQ$_{10}$ was performed by using a YMC Pro C18, 3 μm, 120 Å, 2.0 × 50 mm column, and the mobile phase consisted of methanol−2-propanol−formic acid (45:55:0.05, v/v/v), containing

methylamine at the concentration of 5 mmol/L. The flow rate was 0.2 mL/min and the column compartment was maintained at 40 °C. The injection volume was 5 μL (*13*).

Mass Spectroscopy for the Identification of the Peaks. Finnigan LCQ ion trap benchtop mass spectrometer (Thermo Fischer Scientific) interfaced with an Agilent 1100 HPLC system was used for analysis. Data processing was done in the Finnigan Xcalibur data system operating on a Windows NT PC-based system. The turbo ion spray interface and mass spectrometer were operated under the following conditions: positive ionization polarity, 4.8 kV spray voltage, 425 °C probe temperature, 2.8 × 10^{-5} Torr collision gas pressure (*13*). All parameters were adjusted for each analyte, using the tune method CoQ$_{10}$ EP071002 created by the analyst at the time of analysis with Xcalibur software. Divert valve and contact closure were not used during the run.
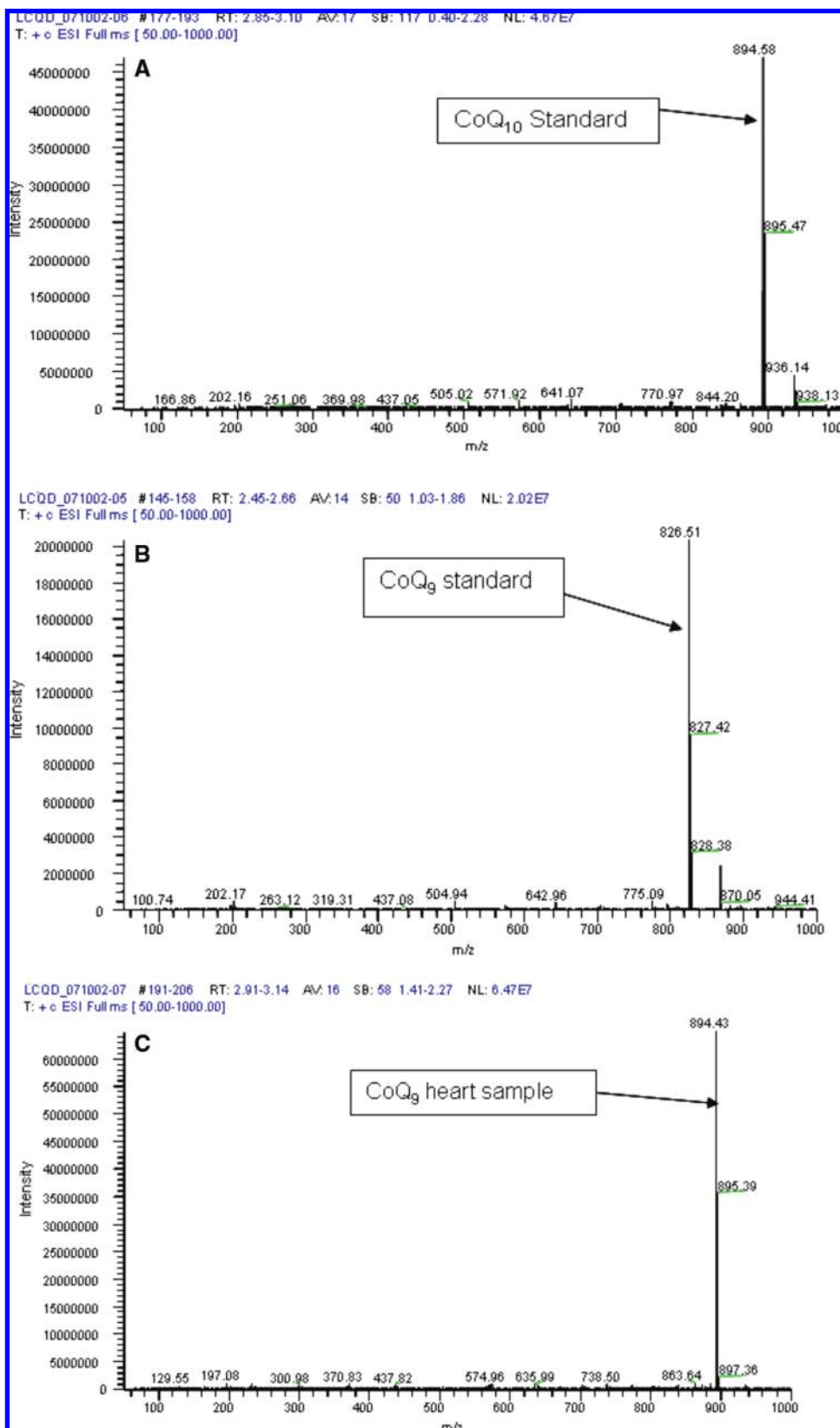
Figure 4. Mass Spectrometry (MS). (**A**) CoQ$_{10}$ standard, (**B**) CoQ$_{9}$ standard, (**C**) CoQ$_{9}$ heart sample. Methylamine was used in the mobile phase to obtain the methyl ammonium adduct molecules of CoQ$_{9}$ and CoQ$_{10}$. The sensitivity of the adduct ions $[M + CH_3NH_3]^+$ was much higher than that of the protonated ions $[M + H]^+$ (7). The MS spectra of both $[M + CH_3NH_3]^+$ at m/z 826.5 for CoQ$_{9}$ and m/z 894.6 for CoQ$_{10}$ were observed. However, the CoQ$_{9}$ heart sample indicated a mass peak at m/z 894.6, which matches the peak for CoQ$_{10}$ and not CoQ$_{9}$. Therefore, there was evidence of CoQ$_{10}$ in the CoQ$_{9}$ heart sample from the MS data. This confirmed the hypothesis of bioconversion of CoQ$_{9}$ to CoQ$_{10}$.

Statistics. The values of HR, CF, AF, LVDP, infarct size, and apoptotic cells were expressed as mean value ± SEM. A two-way analysis of variance was first carried out to test for any differences in mean values between groups. If differences were established, the values of the drug-treated groups were compared with those of the drug-free group by Dunnett's test. A different procedure, because of the nonparametric

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distribution, was used for the distribution of discrete variables, such as the incidence of VF. Thus, the χ^2 test was used to compare the incidence of VF between untreated-control and treated groups.

RESULTS

Effects of CoQ9/CoQ10 on the Recovery of Left Ventricular Function. Table 1 shows the recovery of postischemic cardiac function in isolated hearts subjected to 30 min of ischemia followed by 120 min of reperfusion obtained from guinea pigs treated with 5 mg/kg/day of CoQ10 and CoQ9, respectively, for 4 weeks. The results (**Table 1**) clearly show that postischemic recovery in HR, CF, AF, and LVDP were significantly improved in the CoQ10- and CoQ9-treated groups in comparison with the drug-free control values. Thus, for instance, after 30 min of ischemia followed by 120 min of reperfusion, aortic flow (**Table 1**) was significantly increased from its drug-free control value of 8.0 ± 1.0 to 18.0 ± 2.0 mL/min ($p < 0.05$) and 26.0 ± 1.0 mL/min ($p < 0.05$) in hearts obtained from guinea pigs treated with 5 mg/kg/day of CoQ10 and CoQ9, respectively. Similar postischemic recovery in HR, CF, and LVDP was observed (**Table 1**) in isolated hearts after 4 weeks of CoQ10 or CoQ9 treatment. The improvement in postischemic cardiac function (HR, CF, AF, and LVDP) was more pronounced in the CoQ9-treated group than it was registered in the CoQ10-treated group. However, before ischemia, cardiac function (HR, CF, AF, and LVDP) was not significantly changed in the CoQ10- or CoQ9-treated groups in comparison with the drug-free control values (**Table 1**).

Effects of CoQ9 or CoQ10 on the Development of Arrhythmias. The incidence of reperfusion-induced VF was significantly reduced by CoQ10 and CoQ9. As shown in **Figure 1**, and compared to untreated ischemic/reperfused drug-free group, reperfusion-induced VF was reduced from 92% to 25% ($p < 0.05$) and 8% ($p < 0.05$) with 5 mg/kg/day of CoQ10 and CoQ9, respectively.

Effects of CoQ9 or CoQ10 on Myocardial Infarct Size. **Figure 2A** shows the percentage of infarct size in isolated guinea pig hearts subjected to 30 min of global ischemia followed by 120 min of reperfusion. Drug-free ischemic/reperfused control hearts were associated with a $38 \pm 4.1\%$ infarct size (**Figure 2A**) which was consistently reduced by the dose of 5 mg/kg/day of CoQ10 and CoQ9 to $21.1 \pm 5\%$ ($p < 0.05$) and $16.3 \pm 3.2\%$ ($p < 0.05$), respectively.

Effects of CoQ9 or CoQ10 on Myocardial Apoptosis. As shown in **Figure 2B**, in the case of ischemic control group guinea pig (I/R), the cardiomyocyte apoptosis determined by the TUNEL method was about $21 \pm 2\%$ at the end of reperfusion. Both CoQ10 and CoQ9 treatment significantly reduced the number of apoptotic cardiomyocytes to $6 \pm 1\%$ and $7 \pm 1.5\%$.

HPLC Analysis of CoQ9 or CoQ10. CoQ9 and CoQ10 were observed at the retention times of 2.39 and 2.86 min, respectively. **Figure 3** shows chromatograms of CoQ9 and CoQ10 standard solutions. However, the retention time of CoQ9 heart sample indicated a retention time of 2.86 and not 2.39 min. The retention time of the CoQ9 heart sample matched that of CoQ10 rather than CoQ9. The qualitative analysis was done by identifying the compounds by their retention times. It was suspected that at this point CoQ9 was probably bioconverted to CoQ10. Further investigation was conducted by using mass spectrometry to verify the conversion of CoQ9 into CoQ10 in the heart sample.

Mass Spectrometry of CoQ9 and CoQ10. In Teshima's paper (*13*) it was reported that the analytical sensitivity for CoQ10

was low due to the poor ionization property of CoQ10. Optimization of the HPLC-MS method was done by introducing 5 mmol/L of methylamine in mobile phase [methanol−2-propanol−formic acid (45:55:0.05, v/v/v)], to enhance the sensitivity for the determination of CoQ9 and CoQ10. The standard and sample solutions were injected using an Agilent 1100 HPLC. The flow rate of 0.2 mL/min was maintained. A YMC Pro C18, 3 μm, 120 Å, 2.0 × 50 mm column was used. The HPLC was interfaced with the mass spectrometer. Electron spray ionization mass spectrometry (ESI-MS) was conducted for the identification of the compounds. A full MS scan from 50 to 1000 m/z was run for the compounds of interest, namely CoQ9 and CoQ10. No MS/MS or fragmentation was done at this point. In the presence of methylamine in the mobile phase, the product ion spectra of both $[M + CH_3NH_3]^+$ at m/z 826.5 for CoQ9 and m/z 894.6 for CoQ10 were observed (see **Figure 4**). However, the CoQ9 heart sample indicated a mass peak at m/z 894.6 (see **Figure 4**), which matches the m/z peak for CoQ10 and not CoQ9. Therefore, there was evidence of CoQ10 in the CoQ9 heart sample from the MS data. This confirms the bioconversion of CoQ9 into CoQ10.

DISCUSSION

Several salient features are apparent from the present study. The first is that CoQ9 and CoQ10 provided identical amounts of cardioprotection, as evidenced from the comparable degree of the postischemic ventricular recovery and reduction of myocardial infarct size and cardiomyocyte apoptosis. Both CoQ9 and CoQ10 reduced the incidence of ventricular arrhythmias. LC−MS results revealed complete bioconversion of CoQ9 into CoQ10, and no CoQ9 could be detected in the heart, as most of the CoQ9 was detected as CoQ10. The results, thus, raises the interesting possibility that nutritionally supplemented CoQ9 could replace CoQ10 and CoQ9 could provide cardioprotection after being converted into CoQ10.

CoQ10, an endogenously synthesized provitamin present in the mitochondrial electron transport chain, has been found to be cardioprotective and used as adjunct therapy for ischemic heart disease (*15–18*). The mitochondrial respiratory chain contains several coenzymes, including coenzymes Q1, Q2, Q4, Q6, Q7, Q8, Q9, and Q10. Coenzymes Q6, Q7, and Q8 exist in yeast and bacteria, whereas CoQ10 is prevalent in humans. CoQ10 is present ubiquitously in most mammals including humans, except for rodents, where CoQ9 is the predominant form of CoQ. For this reason, we choose guinea pigs as experimental animals to study the effect of CoQ9, as the hearts of this animal does not contain any CoQ9. Feeding the guinea pigs CoQ9 for 4 weeks provided a similar degree of cardioprotection as CoQ10. Since most of the CoQ9 was found as CoQ10, it could not be ascertained whether CoQ9 by itself or after being converted into CoQ10 provided cardioprotection. However, this study provides valuable information that nutritional supplementation of CoQ9 should be adequate for the animals needing CoQ10 supplementation.

It is not known whether exogenous CoQ9 could be equally cardioprotective as CoQ10 in the animals where CoQ9 is totally absent. A recent study has indicated that reduced CoQ9 could act as a potential antioxidant, regardless of its cellular concentration (*19*). Reduced CoQ9, together with α-tocopherol, were found to act as potential antioxidant in guinea pig hepatocytes when incubated with AAPH, while reduced CoQ10 mainly exhibited its antioxidant activity in cells containing CoQ10 as the predominant CoQ homologue (*20*). Another related study has demonstrated a significant decrease of CoQ9 in heart mitochondria of diabetic rats, suggesting that reduced CoQ9

could be responsible for the increased susceptibility of diabetic heart to oxidative damage (*21*). Yet another study indicated that myocardial reperfusion decreased the mitochondrial content of ubiquinone and stimulated CoQ_9 biosynthesis in young rats but not in aged rats (*22*). The synthesis of CoQ_9 was found to be increased in the liver in hyperthyroidism (*23*). A recent study indicated that coenzyme Q_9 could regulate the aging process in *Caenorhabditis elegans* mitochondria (*24*). Similar to CoQ_{10}, CoQ_9 participates in the electron transport inside the mitochondria of the cell, and in case of rodents, where CoQ_9 is the predominant coenzyme Q, it serves as an essential component for the ATP synthesis.

It has been known for a long time that liver possesses the ability to convert coenzyme Qs into CoQ_{10} by breaking down the side chains from basic coenzyme Q molecule (*25*). The liver can then reassemble them to form CoQ_{10}. However, the creation of CoQ_{10} is a complex process requiring many cofactors (e.g., vitamin B_6, B_{12}, folic acid) and several chain reactions. In the present study, prior to subjecting the hearts to ischemia/reperfusion protocol, the majority of CoQ_9 was found to be present as CoQ_{10}.

CoQ_{10} is an essential cofactor for the mitochondrial respiratory chain as well as for the proper functioning of uncoupling proteins (*26*). It is also an important redox component for both mitochondria and lipid membrane, as it can function directly to ATP synthesis and in its reduced form (ubiquinol) as an antioxidant to protect biological membranes and serum LDL from lipid peroxidation (*27, 28*). Under normal condition, the body may not require any exogenous CoQ_{10}, since it can biosynthesize CoQ_{10}. However, in certain pathophysiological conditions, such as hypertension, cardiomyopathy, angina, heart failure, muscular dystrophy, and aging (*29, 30*), de novo production of CoQ_{10} may be reduced and, hence, tissues require an exogenous supply of CoQ_{10}. In contrast, the level of CoQ_{10} is increased under certain conditions, such as physiological exercise (*31*). A recent study showed that supplementation of CoQ_{10} could reduce myocardial ischemia/reperfusion injury in pigs on cardiopulmonary bypass (*15*).

In most countries CoQ_{10} is widely used as a nutritional supplement. Despite extensive studies having been performed on the role of exogenous CoQ_{10} in the body, its precise function still remains obscure. It is generally accepted that most of the exogenously administered CoQ_{10}, either as nutritional supplement or derived from CoQ_{10}-rich foods, is taken up by the liver and blood components, and only a small amount goes to other organs, such as heart. In the present study, we were able to detect appreciable amounts of CoQ_{10} and some amount of CoQ_9 after 4 weeks of CoQ_{10} or CoQ_9 supplementation. As mentioned earlier, in addition to its major role in mitochondria, its antioxidant role also appears to be important for the lipids. Additionally, recent studies indicate a novel role of exogenous CoQ_{10} in the induction and transcription of genes involved in cell signaling, metabolism, and transport (*26*). Another recent study has indicated CoQ_{10} as a modulator of transition pore, suggesting its role in apoptosis (*32*).

In summary, the results of the present study demonstrate for the first time that nutritional supplementation of CoQ_9 can reduce myocardial ischemia/reperfusion injury to the same extent as CoQ_{10}. However, whether the cardioprotection was achieved from CoQ_9 or after its bioconversion into CoQ_{10} was not established from this study. Nevertheless, the finding that CoQ_9 and CoQ_{10} can provide the same degree of cardioprotection appears to be important, due the fact that only very little exogenous CoQ_{10} is taken up by the heart, while a significant amount of CoQ_9/CoQ_{10} was detected in the heart after 4 weeks of CoQ_9 feeding. It is tempting to speculate that heart may be able to better utilize CoQ_9 than CoQ_{10}.

ABREVIATIONS USED

HR, heart rate; AF, aortic flow; CF, coronary flow; LVDP, left ventricular developed pressure; VF, ventricular fibrillation; IR, ischemia/reperfusion; ISA, ischemia; RE, reperfusion; HPLC, high performance liquid chromatography; MS, mass spectroscopy; CoQ_9, coenzyme Q_9; CoQ_{10}, coenzyme Q_{10}.

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