CURRENT THERAPEUTIC RESEARCH

VOLUME 70, NUMBER 6, DECEMBER 2009

Effect of Pretreatment With Coenzyme Q_{10} on Isoproterenol-Induced Cardiotoxicity and Cardiac Hypertrophy in Rats

Arvindkumar E. Ghule, MPharm[1]; Chetan P. Kulkarni, MPharm[1]; Subhash L. Bodhankar, PhD[1]; and Vijaya A. Pandit, MD[2]

[1]Department of Pharmacology, Poona College of Pharmacy, Bharati Vidyapeeth University, Pune, India; and [2]Department of Pharmacology, Bharati Medical College, Bharati Vidyapeeth University, Pune, India

ABSTRACT

BACKGROUND: Coenzyme Q_{10} (CoQ_{10}) is a lipid-soluble, vitamin-like substance found in the hydrophobic interior of the phospholipid bilayer of most cellular membranes. It appears to be involved in the coordinated regulation between oxidative stress and antioxidant capacity of heart tissue when the heart is subjected to oxidative stress in various pathogenic conditions.

OBJECTIVE: The objective of the present study was to investigate the effect of pretreatment with CoQ_{10} (100 mg/kg) on isoproterenol (ISO)-induced cardiotoxicity and cardiac hypertrophy in rats.

METHODS: Albino male Wistar rats (250–300 g) were evenly divided by lottery method into 1 of the following 3 groups: the ISO group (olive oil 2 mL/kg orally for 18 days and ISO 1 mg/kg IP from days 9–18); the CoQ_{10} + ISO group (CoQ_{10} 100 mg/kg orally for 18 days and ISO 1 mg/kg IP from days 9–18); and the control group (olive oil 2 mL/kg orally for 18 days and water IP from days 9–18). Twenty-four hours after the last dose of water or ISO, the rats were anesthetized and an ECG was recorded. Blood was withdrawn by retro-orbital puncture for estimation of serum creatine kinase-MB (CK-MB) isoenzyme levels, lactate dehydrogenase (LDH) levels, and aspartate aminotransferase activities. The animals were euthanized using an overdose of ether. The hearts of 6 animals from each group were used for estimation of superoxide dismutase (SOD) activity, reduced glutathione (GSH) concentration, lipid peroxidation (LPO), malondialdehyde (MDA), and total protein concentration. Histopathology of the 2 remaining hearts in each group was carried out by a blinded technician.

RESULTS: A total of 24 rats (8 in each group) were used in this study; all rats survived to study end. Compared with the control group, the ISO-treated rats had a significant change in heart to body weight ratio ($P < 0.001$); significant changes in the endogenous antioxidants (ie, significantly higher myocardial MDA concentration [$P < 0.001$]; significantly lower myocardial GSH concentration [$P < 0.001$] and SOD activity [$P < 0.01$]); and significantly higher serum activities of marker enzymes (eg,

Accepted for publication August 18, 2009.
doi:10.1016/j.curtheres.2009.12.004
© 2009 Excerpta Medica Inc. All rights reserved.
0011-393X/$ - see front matter

CK-MB [$P < 0.001$] and LDH [$P < 0.001$]). Compared with the ISO group, the CoQ_{10} + ISO group had a significant change in heart to body weight ratio ($P < 0.001$); significant changes in the endogenous antioxidants (ie, significantly lower MDA concentration [$P < 0.05$]; significantly higher myocardial GSH concentration [$P < 0.001$] and SOD activity [$P < 0.05$]); and significantly lower serum activities of marker enzymes (eg, CK-MB [$P < 0.05$] and LDH [$P < 0.01$]).

Conclusion: Pretreatment with CoQ_{10} (100 mg/kg) for 18 days was associated with moderate protection against ISO-induced cardiotoxicity and cardiac hypertrophy, and with lower myocardial injury by preserving endogenous antioxidants and reducing LPO in rat heart. (*Curr Ther Res Clin Exp.* 2009;70:460–471) © 2009 Excerpta Medica Inc.

Key words: antioxidants, cardiac hypertrophy, coenzyme Q_{10}, isoproterenol.

INTRODUCTION

Cardiac hypertrophy is an adaptive response of the heart muscle to a variety of intrinsic and extrinsic stimuli.[1] Although hypertrophy of the heart muscle is initially beneficial during early growth, prolonged hypertrophy is potentially harmful, causing dilated cardiomyopathy and heart failure, which are associated with significant morbidity and mortality. Sorting out the molecular mechanisms involved in cardiac hypertrophy has become a primary focus of research to develop rational pharmacologic approaches for preventing pathologic changes associated with this disease.

Isoproterenol (ISO), a β_1-adrenergic agonist, has been reported to be associated with oxidative stress in the myocardium, resulting in infarct-like necrosis of the heart muscle.[2] It is also associated with increases in the concentrations of serum and myocardial lipids, which in turn leads to coronary heart disease.[3] ISO is known to generate free radicals and to stimulate lipid peroxidation (LPO), which is a causative factor for irreversible damage to the myocardial membrane,[4] and thus favors the deposition of myocardial lipids. Enhanced free radical formation and lipid peroxide accumulation have been proposed as a possible biochemical mechanism for myocardial damage and cardiac hypertrophy during ISO-induced myocardial infarction.[2]

Coenzyme Q_{10} (CoQ_{10}), or ubiquinone, is a lipid-soluble, vitamin-like substance found in the hydrophobic interior of the phospholipid bilayer of most cellular membranes.[1] It consists of a quinone head attached to a chain of 9 or 10 isoprene units, depending on the mammalian species.[5] CoQ_{10} is known to enhance mitochondrial activity related to the synthesis of adenosine triphosphate.[6] It also plays a role in the inhibition of LPO by scavenging reactive oxygen species (ROS) directly or in conjunction with α-tocopherol.[7] A principal function of CoQ_{10} is to act as an electron carrier between nicotinamide dinucleotide and succinate dehydrogenases and the cytochrome system.[8] During mitochondrial electron transport, ubiquinone also occurs as semiquinone and ubiquinol, the fully reduced form of ubiquinone. Semiquinone has a role in the generation of superoxide anions during mitochondrial respiration,[9]

whereas ubiquinol functions as an intracellular antioxidant, presumably by preventing both the initiation and propagation of LPO. CoQ_{10} appears to be involved in the coordinated regulation between oxidative stress and antioxidant capacity of heart tissue when the heart is subjected to oxidative stress in various pathogenic conditions.[10]

The objective of the present study was to investigate the effect of pretreatment with CoQ_{10} (100 mg/kg) on ISO-induced cardiotoxicity and cardiac hypertrophy in rats.

MATERIALS AND METHODS

Animals

Albino male Wistar rats (250–300 g) were obtained from the National Toxicological Centre (Pune, India). The rats were housed under standard conditions: temperature, 25°C; relative humidity, 60%; and a light–dark cycle of 12 hours. A pellet diet (Chakan Oil Mills, Pune, India) and water were provided ad libitum.

All study protocols were approved by the Institutional Animal Ethics Committee of the Poona College of Pharmacy (Bharati Vidyapeeth University, Pune, India).

Chemicals

CoQ_{10} was supplied by Medicines Pvt. Ltd. (Mumbai, India) and the ISO solution was supplied by Samarth Life Sciences Pvt. Ltd. (Mumbai, India). Epinephrine hydrochloride, superoxide dismutase (SOD), and malondialdehyde (MDA) were purchased from Sigma Chemical Co. (St. Louis, Missouri). Reduced glutathione (GSH), 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB), and thiobarbituric acid (TBA) were obtained from Himedia Laboratories (Mumbai, India). All chemicals were analytical grade.

Experimental Procedure

Albino male Wistar rats were evenly divided by lottery method into 1 of the following 3 groups: ISO group (olive oil 2 mL/kg orally for 18 days and ISO 1 mg/kg IP from days 9–18); CoQ_{10} + ISO group (CoQ_{10} 100 mg/kg orally for 18 days and ISO 1 mg/kg IP from days 9–18); and the control group (olive oil 2 mL/kg orally for 18 days and water IP from days 9–18). Olive oil was chosen as the vehicle because CoQ_{10} is soluble in olive oil. The weighed quantity of CoQ_{10} was dissolved in olive oil to make a final solution of 100 mg/mL. The dose of CoQ_{10} (100 mg/kg) was selected on the basis of previous unpublished pilot dose–response studies. Changes in body weight were recorded daily.

Twenty-four hours after the last injection of ISO and vehicle or water, the rats were anesthetized using anesthetic ether and an 8-channel ECG was recorded (Power Lab System, AD Instruments Pty. Ltd., Bella Vista, Australia). Blood was drawn via retro-orbital puncture and centrifuged at 7500 rpm for 15 minutes at 0°C. Then serum was transferred using a micropipette into microcentrifuge tubes (Eppendorf, Tarsons Products Pvt. Ltd., Kolkata, India) and stored at 4°C until analyzed.

The animals were euthanized using an overdose of anesthetic ether, and the hearts were isolated and weighed. Hearts from 2 animals in each group were randomly selected by lottery method for histopathologic examination. The hearts from the remaining 6 animals were cut into small pieces, placed in a chilled sucrose solution (0.25 M), and blotted on filter paper. The tissues were then homogenized in 10% chilled tris hydrochloride buffer (10 mM, pH 7.4) using a tissue homogenizer (Remi Motors, Mumbai, India) and centrifuged at 7500 rpm for 15 minutes at 0ºC using a high-speed cooling centrifuge (Eppendorf 5810-R, Eppendorf AG, Hamburg, Germany). The clear supernatant was used for the estimation of SOD activity and GSH, LPO, MDA, and total protein concentration.

The ECG recording, determination of organ weight, and measurement of myocardial MDA and GSH concentrations and SOD activity were done by a single investigator (A.E.G.) who was not blinded to treatment group. Serum creatine kinase-MB (CK-MB) isoenzyme levels, lactate dehydrogenase (LDH), and aspartate aminotransferase (AST) activities were measured by a blinded technician who was not involved in the study.

Serum Parameters

Serum activities of CK-MB, LDH, and AST were measured using an automated chemistry analyzer (Micro Lab 300, Merck & Co., Inc., Whitehouse Station, New Jersey) and reagent kits (CK-MB [Randox Laboratories Ltd., Antrim, United Kingdom] and LDH and AST [Ecoline, Merck Ltd., Mumbai, India]).

Tissue Parameters

Lipid Peroxidation Assay (MDA Concentration)

The LPO assay was used to measure TBA-reactive substances, as described by Slater and Sawyer.[11] Two milliliters of freshly prepared 10% w/v trichloroacetic acid (TCA) was added to 2 mL of the tissue homogenate (supernatant). The mixture was allowed to stand in an ice bath for 15 minutes and was then centrifuged at 2500 rpm for 15 minutes at 0°C. Two milliliters of clear supernatant solution was mixed with 2 mL of freshly prepared 0.67% w/v TBA. The resulting solution was heated in a boiling water bath for 10 minutes and was then immediately cooled in an ice bath for 5 minutes. Color absorbance was measured using an ultraviolet-visible spectrophotometer (JASCO-V-530, JASCO Corp., Tokyo, Japan) at 532 nm using 1,1,3,3-tetraethoxypropane as a standard.

Estimation of Glutathione Concentration

Myocardial GSH concentration was determined by the method described by Moron et al.[12] One milliliter of tissue homogenate (supernatant) and 1 mL of 20% w/v TCA were mixed and centrifuged at 2500 rpm for 15 minutes at 0°C. Then, 2 mL of DTNB (0.6 M) reagent was added to 0.25 mL of supernatant. The final volume was increased to 3 mL with phosphate buffer (pH 8.0). The color developed was read at 412 nm against a blank reagent. Different concentrations (10–50 µg) of standard GSH were processed as mentioned previously to construct a standard curve. The amount of reduced GSH was expressed as µg/g protein.

Estimation of Superoxide Dismutase Activity

Myocardial SOD activity was determined using the method of Misra and Fridovich,[13] in which 0.5 mL of heart homogenate, 0.5 mL of cold distilled water, 0.25 mL of ice-cold ethanol, and 0.15 mL of ice-cold chloroform were mixed well using a cyclomixer for 5 minutes and centrifuged at 2500 rpm for 15 minutes at 0°C. Then 1.5 mL of carbonate buffer (pH 10.2) and 0.5 mL of 0.4 M ethylenediaminetetraacetic acid solutions were added to 0.5 mL of supernatant. The reaction was initiated by the addition of 0.4 mL of epinephrine bitartrate (3 mM) and the change in optic density per minute was measured at 480 nm against a blank reagent. SOD activity was expressed as U/mg protein. The change in optical density per minute at 50% inhibition of epinephrine to adrenochrome transition by the enzyme was used as the enzyme unit. The calibration curve was prepared using 10 to 125 units of SOD.

Determination of Total Protein Concentration

Myocardial protein concentration was determined using the method of Lowry et al.[14] Diluted membrane fraction aliquots (0.1 mL) were placed in test tubes. To this, 0.8 mL of 0.1 M sodium hydroxide and 5 mL of Lowry C reagent (a freshly prepared mixture of 1 mL of copper sulfate [0.5% w/v] in 1% sodium potassium tartrate into 50 mL of sodium carbonate [2% w/v] in 0.1 M sodium hydroxide) was added and the solution was allowed to stand for 15 minutes. Then, 0.5 mL of 1 N Folin phenol reagent was added and the contents were mixed well by vortex mixer. The color that developed was measured at 640 nm against a blank reagent containing distilled water instead of sample. Different concentrations (40–200 µg) of standard protein bovine serum albumin were processed as mentioned previously for preparation of a standard curve. The values were expressed as mg protein/g wet tissue (mg/g).

Histopathologic Studies

Hearts were quickly excised, preserved in 10% formalin, processed, and embedded in paraffin. Then 4-µm–thick paraffin sections were cut on glass slides and stained with hematoxylin and eosin reagents and observed under a light microscope by an independent investigator who was blinded to treatment group to assess myocardial injury. Injury was assessed on a 4-point scoring method: 0 = no injury; 1 = mild injury; 2 = moderate injury; and 3 = severe injury.

Estimation of Organ Weight Ratio

In each group, heart to body weight ratio and left ventricle to body weight ratio were determined. Body weight was the weight on the day the rats were euthanized. Heart weight was measured after keeping the heart in cold saline and squeezing out the blood.

Statistical Analysis

The statistical analysis was done using GraphPad Prism software version 4.03 (GraphPad Software Inc., La Jolla, California). Analysis was made using 1-way ANOVA followed by post hoc Bonferroni adjustment to control for multiple comparisons. $P < 0.05$ was considered statistically significant. It was determined that 6 animals in each

group would be needed to make a statistically meaningful comparison of serum parameters. Additional animals were added for the histopathologic analysis.

RESULTS

Electrocardiographic Changes

At posttreatment, the mean (SEM) QT interval was significantly higher in the ISO group compared with the control group (77.40 [1.00] vs 62.70 [1.00] msec, respectively; $P < 0.001$); the ST interval was also significantly higher in the ISO group (63.00 [1.00] vs 44.08 [0.80] msec; $P < 0.001$). The QT and ST intervals were significantly lower in the CoQ_{10} + ISO group compared with the ISO group (69.00 [0.50], $P < 0.01$ and 54.10 [1.00], $P < 0.001$, respectively) (**Table I**).

Serum Enzyme Activities

Serum CK-MB activity was significantly higher in the ISO group compared with the control group (989.2 [70.28] vs 569.8 [73.13] IU/L, respectively; $P < 0.001$); serum LDH activity was also significantly higher in the ISO group (1787.0 [82.55] vs 953.5 [48.62] IU/L; $P < 0.001$). Serum CK-MB and LDH activities were significantly lower in the CoQ_{10} + ISO group compared with the ISO group (805.3 [101.9] IU/L, $P < 0.05$ and 1420.3 [98.40] IU/L; $P < 0.01$). Serum AST activity was significantly higher in the ISO group compared with the control group (1058.2 [96.80] vs 674.6 [97.34] IU/L; $P < 0.05$). Serum AST activity in the CoQ_{10} + ISO group was not significantly different compared with the ISO group (**Table II**).

Myocardial Endogenous Antioxidants

Myocardial MDA concentration was significantly higher in the ISO group compared with the control group (4.42 [0.18] vs 2.68 [0.18] nM MDA/mg protein, respectively; $P < 0.001$). The MDA concentration was significantly lower in the CoQ_{10} + ISO group compared with the ISO group (3.27 [0.24] nM MDA/mg protein; $P < 0.05$). Myocardial GSH concentration was significantly lower in the ISO group compared with the control group (17.79 [1.00] vs 27.50 [0.71] µg GSH/mg protein; $P < 0.001$). Myocardial GSH concentration was significantly higher in the CoQ_{10} + ISO group than in the ISO group (26.9 [0.71] µg GSH/mg protein; $P < 0.001$). Myocardial SOD activity was significantly lower in the ISO group compared with the control group (3.59 [0.96] vs 9.49 [1.14] U/mg protein; $P < 0.01$), whereas myocardial SOD activity in the CoQ_{10} + ISO group was significantly higher than in the ISO group (8.85 [0.77] U/mg protein; $P < 0.05$) (**Table III**).

Organ Weight Determination

Heart to body weight ratio was significantly higher in the ISO group compared with the control group (4.00 [0.09] vs 3.18 [0.08] mg/g, respectively; $P < 0.001$); left ventricle to body weight ratio was also significantly higher in the ISO group (2.04 [0.03] vs 1.50 [0.09] mg/g; $P < 0.05$). Heart to body weight ratio was significantly lower in the CoQ_{10} + ISO group compared with the ISO group (3.19 [0.04] mg/g; $P < 0.001$), as was left ventricle to body weight ratio (1.72 [0.06] mg/g; $P < 0.05$) (**Table IV**).

Table I. Posttreatment ECG findings by treatment group (N = 24). Data are mean (SEM) msec.

Interval	ISO	CoQ_{10} + ISO	Control
QT	77.40 (1.00)*	69.00 (0.50)†	62.70 (1.00)
ST	63.00 (1.00)*	54.10 (1.00)‡	44.08 (0.80)

ISO = isoproterenol; CoQ_{10} = coenzyme Q_{10}.
*$P < 0.001$ versus the control group.
†$P < 0.01$ versus the ISO group.
‡$P < 0.001$ versus the ISO group.

Table II. Posttreatment serum enzyme activities by treatment group (n = 18). Data are mean (SEM) IU/L.

Enzyme Activity	ISO	CoQ_{10} + ISO	Control
CK-MB	989.2 (70.3)*	805.3 (101.9)†	569.8 (73.1)
LDH	1787.0 (82.6)*	1420.3 (98.4)‡	953.5 (48.6)
AST	1058.2 (96.8)§	861.3 (56.6)	674.6 (97.3)

ISO = isoproterenol; CoQ_{10} = coenzyme Q_{10}; CK-MB = creatine kinase-MB; LDH = lactate dehydrogenase; AST = aspartate aminotransferase.
*$P < 0.001$ versus the control group.
†$P < 0.05$ versus the ISO group.
‡$P < 0.01$ versus the ISO group.
§$P < 0.05$ versus the control group.

Table III. Myocardial malondialdehyde (MDA) and glutathione (GSH) concentrations and superoxide dismutase (SOD) activity by treatment group (n = 18). Data are mean (SEM).

Activity	ISO	CoQ_{10} + ISO	Control
MDA, nM/mg protein	4.42 (0.18)*	3.27 (0.24)†	2.68 (0.18)
GSH, µg/mg protein	17.79 (1.00)*	26.90 (0.71)‡	27.50 (0.71)
SOD, U/mg protein	3.59 (0.96)§	8.85 (0.77)†	9.49 (1.14)

ISO = isoproterenol; CoQ_{10} = coenzyme Q_{10}.
*$P < 0.001$ versus the control group.
†$P < 0.05$ versus the ISO group.
‡$P < 0.001$ versus the ISO group.
§$P < 0.01$ versus the control group.

Table IV. Heart to body weight and left ventricle to body weight ratios by treatment group (n = 18). Data are mean (SEM) mg/g.

Ratio	ISO	CoQ_{10} + ISO	Control
Heart to body weight	4.00 (0.09)*	3.19 (0.04)†	3.18 (0.08)
Left ventricle to body weight	2.04 (0.03)‡	1.72 (0.06)§	1.50 (0.09)

ISO = isoproterenol; CoQ_{10} = coenzyme Q_{10}.
* $P < 0.001$ versus the control group.
† $P < 0.001$ versus the ISO group.
‡ $P < 0.05$ versus the control group.
§ $P < 0.05$ versus the ISO group.

Histopathologic Changes

The ISO-treated rats had marked tissue injury (inflammation, nuclear pyknosis, cytoplasmic vacuoles, and cytoplasmic eosinophilia). In the CoQ_{10} + ISO group, these changes were less, though no statistical comparisons were made.

DISCUSSION

Teerlink et al[15] suggested that changes in the left ventricular (LV) shape caused due to hypertrophy leads to reduction in heart functionality. ISO-induced hypertrophy produces increases in the LV cavity that is disproportionate to the weight of the heart. Studies in both experimental models and humans have indicated that not only are these increases in LV volume important, but changes in the LV geometry may independently play an important role in the observed decrease in cardiac performance.[16–18] Other studies have suggested that these alterations in LV geometry may predict impairment of LV function.[19,20]

Evidence has been found of the involvement of oxidative stress in cardiac diseases, including cardiac hypertrophy. ISO-induced cardiac hypertrophy and cardiotoxicity serve as a standardized model to study the effects of many drugs on cardiac function.[1] Oxidative stress originating from ISO injection is mediated primarily via β_1-adrenergic receptors. Stimulation of β_1-adrenergic receptors rapidly generates ROS as well as depresses total cellular antioxidant capacity.[21] Adrenoceptor activation produced by ISO may occur through an oxidation mechanism.[22] Significant reductions in cardiac tissue levels of protective antioxidant enzymes, such as SOD and GSH, have been observed in ISO-induced cardiotoxicity.[1,21,23] Dhalla et al[24] reported that excess catecholamines affect the calcium transport mechanism primarily via oxidative reactions involving free radical–mediated damage and that antioxidants may be indicated for the treatment of stress-induced heart disease.

In a clinical setting, coronary artery disease developes when the vascular supply to the heart is impeded. This may impair the supply of oxygenated blood to cardiac tissue to such a degree as to induce myocardial ischemia that, if severe and prolonged, may induce the death of the myocardial cell. Myocardial infarcts and, less commonly, ischemia can also happen if oxygen demand abnormally increases as may occur in severe

ventricular hypertrophy. In experimental studies, a similar situation of cardiac necrosis can be produced by injection of natural or synthetic sympathomimetic in high dosages.

The results of our study clearly support that there was increased myocardial injury, as indicated by the increase in QT and ST intervals in the ISO-treated group. Administration of CoQ_{10} prior to ISO (100 mg/kg) maintained or attenuated the prolongation of QT and ST intervals close to the values in the control group.

Shimomura et al[25] reported that, in rats after running downhill, the serum CK and LDH activity of the control group were significantly ($P < 0.01$ and $P < 0.05$, respectively) elevated immediately after exercise. However, in the CoQ_{10}-treated group, CK activity was at the same level as the sedentary group, indicating that CoQ_{10} treatment was associated with blocking elevated enzyme activity during exercise. Okamoto et al[26] cultured skeletal femoral muscle cells of neonatal rats. Continuous electric field stimulation produced rhythmic contraction and relaxation of cells for 4 hours. Lactate and lactate dehydrogenase release was elevated after the onset of stimulation, whereas intracellular Ca^{2+} contents increased gradually at relaxation. Addition of 5 µM CoQ_{10} appeared to protect the cells against these biochemical changes after stimulation. Prince and Rajadurai[3] found significant ($P < 0.05$) increases in the activities of CK and LDH in the serum of ISO-treated rats compared with controls. ISO damages the myocardial cells, which contain LDH, CK-MB, and AST, resulting in leakage of enzymes in the blood. These results are consistent with previous studies.[1,21,27,28] In the present study, the serum activities of CK-MB, LDH, and AST in the CoQ_{10}-treated group were significantly lower than in the ISO-treated group.

It has been reported that ROS are generated due to β_1-adrenergic receptor stimulation, while total cellular oxidant capacity is reduced. This event downregulates copper-zinc–SOD enzyme activity and protein and mRNA concentration while also reducing GSH concentration.[29] As a result, integrity of the membrane is lost, reducing myocyte toxicity and resulting in myocardial necrosis.[29,30]

The most abundant ROS generated in living cells are superoxide anion and its derivatives, particularly the highly reactive and damaging hydroxyl radical, which induces peroxidation of cell membrane lipids.[31] In this respect, any increase in organ SOD activity appears to be beneficial in the event of increased free radical generation. In the present study, administration of ISO (1 mg/kg) was associated with a significant increase in MDA concentration (an index of LPO) in cardiac tissues and decrease in myocardial GSH concentration and SOD activity compared with the control group, suggesting an increase in oxidative stress. These results are consistent with previous studies that reported the involvement of oxidative stress and LPO in ISO-induced cardiac hypertrophy and cardiotoxicity.[29,30,32] Administration of CoQ_{10} improved the biochemical markers, indicating decreased oxidative stress, manifested as increased GSH concentration and SOD activity with decreased LPO (MDA concentration) compared with ISO treatment alone.

In the present study, histopathologic analysis found inflammation, nuclear pyknosis, cytoplasmic vacuoles, and cytoplasmic eosinophilia in the ISO-treated rat hearts. Similar findings were reported in earlier studies of ISO-induced cardiotoxicity and

cardiac hypertrophy.[5,28–30] In the present study, histopathologic analysis revealed less damage in the CoQ_{10} + ISO group than in the ISO group.

Limitations

Some experiments and the drug administration were not blinded. However, serum determinations and histopathologic assessments were blinded.

CONCLUSION

Pretreatment with CoQ_{10} (100 mg/kg) for 18 days was associated with moderate protection against ISO-induced cardiotoxicity and cardiac hypertrophy, and with lower myocardial injury by preserving endogenous antioxidants and reducing LPO in rat heart.

ACKNOWLEDGMENTS

The authors acknowledge Dr. S.S. Kadam, Vice Chancellor, and Dr. K.R. Mahadik, Principal, Poona College of Pharmacy (Pune, India) for their interest in this work. We thank V. Mohan and Sunil Bhaskaran of Indus Biotech (Pune, India) for procuring and donating the CoQ_{10}, and Drs. S. Joshi, M. Hassanpour Fard, and S.K. Kushawaha for their help in this study.

The authors have indicated they have no other conflicts of interest regarding the content of this article.

REFERENCES

1. Choudhary R, Mishra KP, Subramanyam C. Prevention of isoproterenol-induced cardiac hypertrophy by eugenol, an antioxidant. *Ind J Clin Biochem*. 2006;21:107–113.
2. Ennis IL, Escudero EM, Console GM, et al. Regression of isoproterenol-induced cardiac hypertrophy by Na+/H+ exchanger inhibition. *Hypertension.* 2003;41:1324–1329.
3. Prince PS, Rajadurai M. Preventive effect of Aegle marmelos leaf extract on isoprenaline-induced myocardial infarction in rats: Biochemical evidence. *J Pharm Pharmacol*. 2005;57: 1353–1357.
4. Sathish V, Ebenezar KK, Devaki T. Synergistic effect of nicorandil and amlodipine on tissue defense system during experimental myocardial infarction in rats. *Mol Cell Biochem*. 2003;243: 133–138.
5. Battino M, Ferri E, Gorini A, et al. Natural distribution and occurrence of coenzyme Q homologues. *Membr Biochem*. 1990;9:179–190.
6. Turunen M, Olsson J, Dallner G. Metabolism and function of coenzyme Q. *Biochim Biophys Acta*. 2004;1660:171–199.
7. Ernster L, Dallner G. Biochemical physiological and medical aspects of ubiquinone function. *Biochim Biophys Acta*. 1995;1271:195–204.
8. Sunamori M, Tanaka H, Maruyama T, et al. Clinical experience of coenzyme Q10 to enhance intraoperative myocardial protection in coronary artery revascularization. *Cardiovasc Drugs Ther*. 1991;5(Suppl 2):297–300.
9. Kagan V, Serbinova E, Packer L. Antioxidant effects of ubiquinones in microsomes and mitochondria are mediated by tocopherol recycling. *Biochem Biophys Res Commun*. 1990;169:851–857.
10. Das DK, Maulik N. Protection against free radical injury in the heart and cardiac performance. In: Sen CK, Packer L, Hänninen O, eds. *Exercise and Oxygen Toxicity.* New York, NY: Elsevier; 1994:359–388.

11. Slater TF, Sawyer BC. The stimulatory effects of carbon tetrachloride and other halogenoalkanes on peroxidative reactions in rat liver fractions in vitro. General features of the systems used. *Biochem J*. 1971;123:805–814.
12. Moron MS, Depierre JW, Mannervik B. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. *Biochim Biophys Acta*. 1979;582:67–78.
13. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *J Biol Chem*. 1972;247:3170–3175.
14. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem*. 1951;193:265–275.
15. Teerlink JR, Pfeffer J, Pfeffer MA. Progressive ventricular remodeling in response to diffuse isoproterenol-induced myocardial necrosis in rats. *Circ Res*. 1994;75:105–113.
16. Hamilton GW, Murray JA, Kennedy JW. Quantitative angiocardiography in ischemic heart disease. The spectrum of abnormal left ventricular function and the role of abnormally contracting segments. *Circulation*. 1972;45:1065–1080.
17. Kitamura S, Kay JH, Krohn BG, et al. Geometric and functional abnormalities of the left ventricle with a chronic localized noncontractile area. *Am J Cardiol*. 1973;31:701–707.
18. Hori M, Inoue M, Mishima M, et al. Infarct size and left ventricular ejection fraction in acute myocardial infarction. *Japan Circ J*. 1977;41:1299–1306.
19. Gibson DG, Brown DJ. Continuous assessment of left ventricular shape in man. *Br Heart J*. 1975;37:904–910.
20. Tomlinson CW. Left ventricular geometry and function in experimental heart failure. *Can J Cardiol*. 1987;3:305–310.
21. Wheatley AM, Thandroyen FT, Opie LH. Catecholamine-induced myocardial cell damage: Catecholamines or adrenochrome. *J Mol Cell Cardiol*. 1985;17:349–359.
22. Brodde OE. Beta 1- and beta 2-adrenoceptors in the human heart: Properties, function, and alterations in chronic heart failure [published correction appears in *Pharmacol Rev.* 1991;43:350]. *Pharmacol Rev.* 1991;43:203–242.
23. Remião F, Carmo H, Carvalho F, Bastos ML. Copper enhances isoproterenol toxicity in isolated rat cardiomyocytes: Effects on oxidative stress. *Cardiovasc Toxicol*. 2001;1:195–204.
24. Dhalla KS, Rupp H, Beamish RE, Dhalla NS. Mechanisms of alterations in cardiac membrane Ca2+ transport due to excess catecholamines. *Cardiovasc Drugs Ther*. 1996;10(Suppl 1):231–238.
25. Shimomura Y, Suzuki M, Sugiyama S, et al. Protective effect of coenzyme Q10 on exercise-induced muscular injury. *Biochem Biophys Res Commun.* 1991;176:349–355.
26. Okamoto T, Kubota N, Takahata K, et al. Protective effect of coenzyme Q10 on cultured skeletal muscle cell injury induced by continuous electric field stimulation. *Biochem Biophys Res Commun*. 1995;216:1006–1012.
27. Saravanan G, Prakash J. Effect of garlic (Allium sativum) on lipid peroxidation in experimental myocardial infarction in rats. *J Ethnopharmacol.* 2004;94:155–158.
28. Zhou R, Xu Q, Zheng P, et al. Cardioprotective effect of fluvastatin on isoproterenol-induced myocardial infarction in rat. *Eur J Pharmacol*. 2008;586:244–250.
29. Rathore N, John S, Kale M, Bhatnagar D. Lipid peroxidation and antioxidant enzymes in isoproterenol induced oxidative stress in rat tissues. *Pharmacol Res*. 1998;38:297–303.
30. Srivastava S, Chandrasekar B, Gu Y, et al. Downregulation of CuZn-superoxide dismutase contributes to beta-adrenergic receptor-mediated oxidative stress in the heart. *Cardiovasc Res*. 2007;74:445–455.
31. Hemnani T, Parihar MS. Reactive oxygen species and oxidative DNA damage. *Indian J Physiol Pharmacol*. 1998;42:440–452.

32. Banerjee SK, Sood S, Dinda AK, et al. Chronic oral administration of raw garlic protects against isoproterenol-induced myocardial necrosis in rat. *Comp Biochem Physiol C Toxicol Pharmacol*. 2003;136:377–386.

Address correspondence to: Subhash L. Bodhankar, PhD, Department of Pharmacology, Poona College of Pharmacy, Bharati Vidyapeeth University, Erandawane, Pune 411038, India. E-mail: sbodh@yahoo.com