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Antioxidant Effect of CoQ_{10} on N-nitrosodiethylamine-induced Oxidative Stress in Mice

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The antioxidant effect of CoQ_{10} on N-nitrosodiethylamine (NDEA)-induced oxidative stress was investigated in mice. Food intake and body weight were similar in both CoQ_{10} and control groups during the 3-week experimental period. NDEA significantly increased the activities of typical marker enzymes of liver function (AST, ALT and ALP) both in control and CoQ_{10} groups. However, the increase of plasma aminotransferase activity was significantly reduced in the CoQ_{10} group. Lipid peroxidation in various tissues, such as heart, lung, liver, kidney, spleen and plasma, was significantly increased by NDEA, but this increase was significantly reduced by 100 mg/kg of CoQ_{10}. Superoxide dismutase activity increased significantly upon NDEA-induced oxidative stress in both the control and CoQ_{10} groups with the effect being less in the CoQ_{10} group. Catalase activity decreased significantly in both the control and CoQ_{10} groups treated with NDEA, again with the effect being less in the CoQ_{10} group. The lesser effect on superoxide dismutase and catalase in the NDEA-treated CoQ_{10} group is indicative of the protective effect CoQ_{10}. Thus, CoQ_{10} can offer useful protection against NDEA-induced oxidative stress.

Key Words: Catalase, CoQ_{10}, Lipid peroxidation, Reactive oxygen species, Superoxide dismutase

INTRODUCTION

Coenzyme Q is a quinone structure that is widely biosynthesized in living organisms such including yeasts, plants, and animals (Nohl et al., 1998). In humans, this compound has 10 isoprene units (5 carbons each) in the side chain and is thus named coenzyme Q10 (CoQ_{10}, Ubiquinone-10, 2,3-dimethoxy, 5-methyl, 6-decaprenyl benzoquinone) (Crane, 2001). Two major physiological activities of CoQ_{10} have been reported: (a) mitochondrial electron-transport activity and (b) antioxidant activity. $CoQ_{10}H_2$, a reduced form of CoQ_{10}, is an effective fat-soluble antioxidant and an important cellular antioxidant (Crane, 2001). $CoQ_{10}H_2$ inhibits lipid peroxidation when cell membranes and low-density lipoproteins are exposed to oxidizing conditions outside the body. In isolated mitochondria, CoQ_{10} can protect membrane proteins and DNA from the oxidative damage that accompanies lipid peroxidation (Ernster and Dallner, 1995).

N-nitroso compounds, like N-nitrosodimethylamine, N-nitrosodiethylamine (NDEA) and N-nitrosopyrrolidine, constitute an important group of carcinogens and have been found in various foodstuffs, such as milk products, meat products, soft drinks and alcoholic beverages (Bansal et al., 2005). Nitroso compounds are readily formed in the human body by the reaction of nitrite with amines and amides (Ohkawa et al., 1979). NDEA has been suggested to cause oxidative stress and cellular injury due to involvement of free radicals (Ames et al., 1993; Beckman and Ames, 1998; Noguchi et al., 2000). There are reports that oxygen free radicals and related lipid peroxides play a key role in the pathogenesis of age-related chronic degenerative diseases (Marklund and Marklund, 1974). It is very important to investigate the protective role of CoQ_{10} on oxidative stress induced by NDEA, which may be formed in human body. Therefore, we measured the effect of CoQ_{10} on lipid peroxidation, superoxide dismutase and catalase activity in various tissues in NDEA-treated mice.

METHODS

Materials

CoQ_{10} powder was obtained from Daewoong Pharm. Co. Ltd. (Seoul, Korea). N-nitrosodiethylamine (NDEA), p-nitrophenylphosphate, thiobarbituric acid, hypoxanthine, nitroblue tetrazolium (NBT) and xanthine oxidase were purchased from Sigma Chemical Co. (St. Louis, MO, USA). 2',7'-Dichlorofluorescein diacetate (DCF-DA) was purchased from Molecular Probes (Leiden, Netherlands).

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ABBREVIATIONS: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CoQ_{10}, coenzyme Q10; NDEA, N-nitrosodiethylamine; ROS, reactive oxygen species; SOD, superoxide dismutase.

Animals

Six-week-old male BALB/c mice, weighing 24 to 31 g, were purchased from Han-Lim Animal (Hwasung-gun, Gyunggi-do, Korea) and were housed in an environmentally controlled animal room (temperature 24±2°C, humidity 50±5%, illumination 300~500 Lux).

Animal protocols were approved by the ethics committee for the care and use of laboratory animals at Chung-Ang University. The animals were randomized into two groups. CoQ_{10} in olive oil (100 mg/kg) was orally administered to the CoQ_{10} group (n=16) for 3 weeks, while an equal volume of olive oil was orally administered to the control group (n=16). After 3 weeks, oxidative stress was induced in half of the animals in each group by i.p. administration of NDEA (200 mg/kg body weight) in normal saline (0.5 ml) and the other half were administered an equal volume of normal saline.

Collection of samples

The mice were sacrificed by light ether anesthesia at 48 h after induction of oxidative stress, and blood was collected by cardiac puncture into test tubes rinsed with a saturated solution of EDTA. The organs including the liver, kidney, spleen, heart, and lung of each animal were removed, washed with normal saline, and weighed. The tissue was stored at −20°C until use.

Biochemical analysis

Plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were determined by the colorimetric method described by Reitman and Frenkel (1957). Alkaline phosphatase (ALP) activity was determined using p-nitrophenylphosphate as the substrate (King and Delory, 1959). The color that developed upon hydrolysis of p-nitrophenylphosphate to p-nitrophenol by ALP was read at 410 nm and was proportional to the enzyme activity.

Measurement of lipid peroxidation

The fluorescence of thiobarbituric acid reactive substances was used as a measure of lipid peroxidation (Boland et al., 2000). Tissues were homogenized in phosphate-buffered saline with a Polytron homogenizer. Trichloroacetic acid was added at a final concentration of 5%. Then, an equal volume of 0.325% 2-thiobarbituric acid in 50% glacial acetic acid was added. The solution was incubated at 95°C for 30 min. After cooling, the samples were vigorously mixed with 500 μl of isobutanol and centrifuged at 2,000 rpm for 10 min. The fluorescence of a 200 μl aliquot of the upper organic phase was quantified using excitation and emission wavelengths of 485 and 535 nm, respectively. Results were expressed as μg/mg protein. Protein concentration was measured by the bicinchoninic acid (BCA) method to correct for differences between preparations (Smith et al., 1987).

Superoxide dismutase (SOD) assay

The SOD level in each sample was measured spectrophotometrically via a hypoxanthine/xanthine oxidase generating system coupled with NBT reduction (Kirby and Schmidt, 1997). Reaction mixtures containing 50 mM potassium phosphate buffer (pH 7.4), 1 mM K-EDTA, 0.6 mM hypoxanthine, 0.2 mM NBT, 20 mU/ml xanthine oxidase and sample were incubated at 37°C for 20 min and the absorbance was measured at 590 nm (McCune and Johns, 2002).

Catalase assay

Catalase activity was directly determined by decomposition of H_2O_2 by recording the decrease in absorbance at 240 nm using UV spectrophotometer and was expressed as units per milligram of protein.

Measurement of reactive oxygen species (ROS) generation

RAW 264.7 cells obtained from the Korean Cell Line Bank (Seoul, Korea) were cultured in DMEM supplemented with 10% heat-inactivated FBS and antibiotic-antimycotic at 37°C with 5% CO_2. The level of intracellular ROS was quantified by fluorescence with DCF-DA. RAW 264.7 cells were suspended in 20 ml of Krebs buffer [137 mM NaCl, 2.7 mM KCl, 0.4 mM Na_2HPO_4, 0.5 mM $MgCl_2$, 10 mM HEPES (pH 7.4), 1.8 mM $CaCl_2$, 5 mM glucose] and incubated with 20 μM DCF-DA for 30 min at 37°C. The cells were washed twice with Krebs buffer and were suspended in Krebs buffer at a density of 10^6 cells/ml. The cells were incubated with CoQ_{10} for 10 min, and ROS generation was then induced with 1 mg/ml silica for 30 min at 37°C. The fluorescence intensity was measured using excitation and emission wavelengths of 485 and 535 nm, respectively (Woo et al., 2000).

Data analysis

The results were represented as means±SD and analyzed statistically by analysis of variance (ANOVA), and the differences between groups were determined with the Newman-Keuls test. The level of significance was set at less than 5%.

RESULTS

Effect of CoQ_{10} on NDEA-induced liver damage

Food intake and body weight were similar in both the control and CoQ_{10} groups during the 3-week experimental period. The body weights in the control and CoQ_{10} groups

Table 1. Protective effect of CoQ_{10} on NDEA (200 mg/kg)-induced liver damage in mice

Parameters	Control		CoQ_{10} (100 mg/kg)	
	Saline	NDEA	Saline	NDEA
AST (U/l)	15.0±3.2	58.3±6.8*	14.2±2.5	42.3±4.5*,#
ALT (U/l)	14.9±2.5	69.4±5.3*	12.8±3.3	57.3±6.3*,#
ALP (U/l)	48.5±6.0	83.3±7.2*	47.2±7.0	71.3±5.3*,#

Values are mean±SD; n=8. AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase. *significantly different from saline group ($p<0.05$), #significantly different from NDEA control group ($p<0.05$).

increased from 25.8±1.1 to 27.8±1.0 and from 26.3±1.3 g to 27.9±1.3 g, respectively. The activities of typical marker enzymes of liver function (AST, ALT and ALP) were also similar in both groups without NDEA (Table 1). NDEA significantly increased these activities both in the control and CoQ_{10} groups. However, the increase of plasma aminotransferase activity was significantly less in the CoQ_{10} group as compared with the control group.

Effect of CoQ_{10} on NDEA-induced lipid peroxidation

Lipid peroxidation induced by NDEA in various tissues, including heart, lung, liver, kidney, spleen and plasma, was significantly increased both in the control and CoQ_{10} groups. Lipid peroxidation was the most prominent in the heart. CoQ_{10} alone did not affect the lipid peroxidation level without NDEA treatment, whereas it significantly reduced the level of NDEA-induced lipid peroxidation in all tissues, including plasma, heart, lung, liver, kidney and spleen (Fig. 1).

Effect of CoQ_{10} on SOD activity in murine tissues treated with NDEA

In all tissues, SOD activity increased significantly upon induction of oxidative stress by NDEA both in the control and CoQ_{10} groups. In the control, the increase of SOD activity induced by NDEA in plasma, heart and lung was more prominent than that in liver, kidney and spleen. The increase of SOD levels by NDEA in all tissues was significantly reduced by treatment with 100 mg/kg CoQ_{10} (Fig. 2). In the normal group without NDEA, CoQ_{10} alone significantly increased the SOD level in plasma, whereas it did not affect SOD levels in other tissues.

Effect of CoQ_{10} on catalase activity in murine tissues treated with NDEA

Upon induction of oxidative stress by NDEA, catalase activity decreased significantly in the control and CoQ_{10}

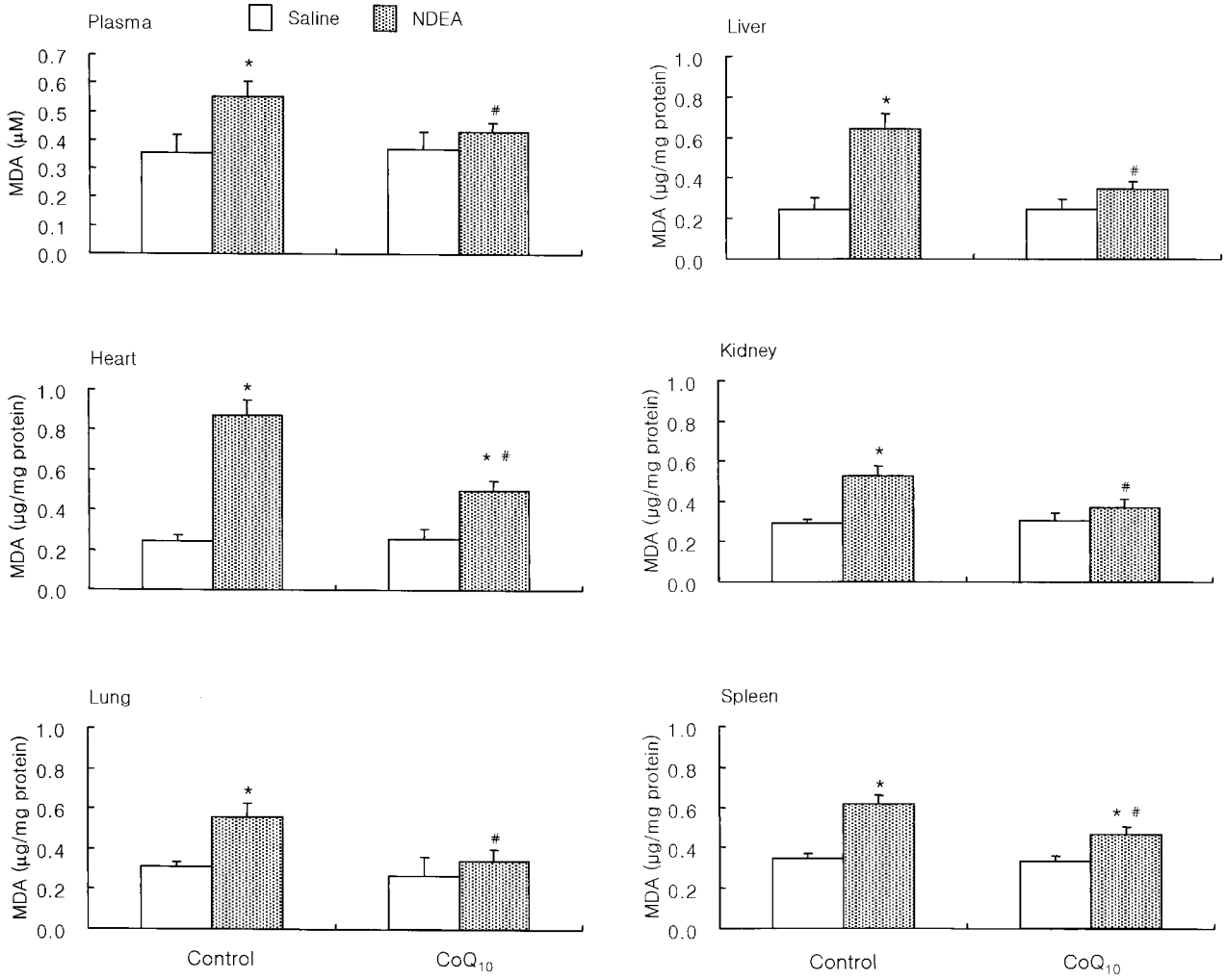

Fig. 1. The effect of CoQ_{10} on NDEA-induced lipid peroxidation in murine tissues. Malondialdehyde (MDA) is an index of lipid peroxidation in oxidative stress induced by 200 mg/kg NDEA. CoQ_{10} in olive oil (100 mg/kg) was orally administered to the CoQ_{10} group for 3 weeks, while an equal volume of olive oil was orally administered to the control group. Results are means±SD from 8 mice. *significantly different from saline group ($p<0.05$), #significantly different from NDEA control group ($p<0.05$).

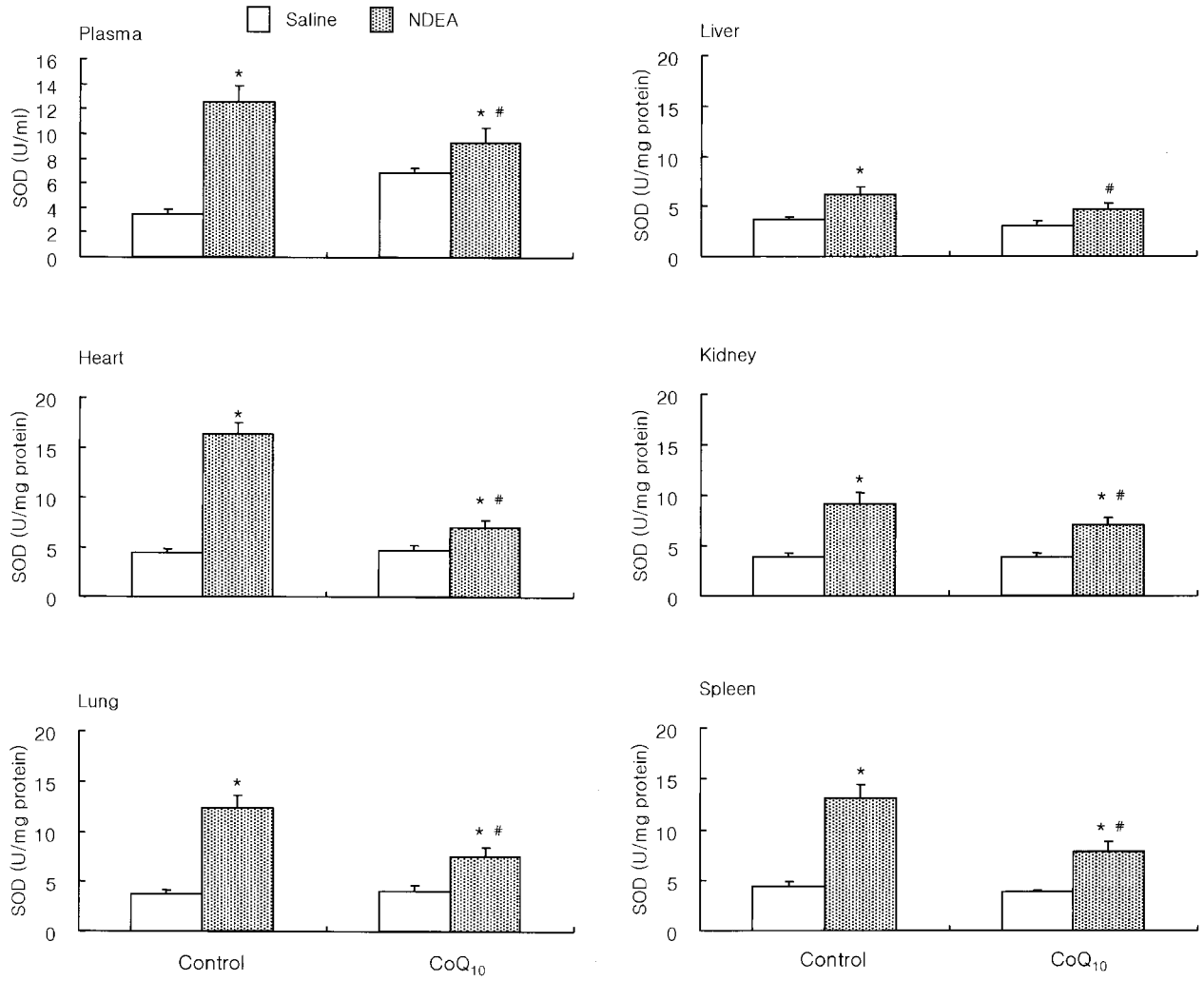

Fig. 2. The effect of CoQ_{10} on superoxide dismutase (SOD) activity in murine tissues treated with 200 mg/kg NDEA. CoQ_{10} in olive oil (100 mg/kg) was orally administered to the CoQ_{10} group for 3 weeks, while an equal volume of olive oil was orally administered to the control group. Results are means±SD from 8 mice. *significantly different from saline group ($p<0.05$), #significantly different from NDEA control group ($p<0.05$).

groups. CoQ_{10} significantly restored the level of catalase in all tissues treated with NDEA (Fig. 3). CoQ_{10} alone significantly increased the levels of catalase in heart and lung, but it did not affect the levels in liver, kidney and spleen. In a cell culture system, CoQ_{10} dose-dependently reduced ROS generation in RAW 264.7 cells stimulated with 1 mg/ml silica (Fig. 4).

DISCUSSION

The food intake and changes in body weight in the control and CoQ_{10} groups during the 3-week experimental period were similar, which indicate that CoQ_{10} does not produce any untoward effects. Liver is the typical detoxifying organ of the body and is profoundly affected by external chemicals. Aminotransferases (AST and ALT) and ALP have been used as markers of liver function. The increase in these activities by NDEA seems to be due to the hepatotoxicity of the chemical. It has been previously reported that hepatotoxic responses to NDEA result in increased levels of AST, ALT, and ketone bodies with nitroso compounds (Taniguchi et al., 1999). A relatively lower degree of hepatic damage in the CoQ_{10} group is indicative of the hepatoprotective nature of CoQ_{10}. While there is no direct evidence of the hepatoprotective effect of CoQ_{10}, it has been reported that maintenance of the normal level of CoQ_9H_2 in the S-allylmercaptocysteine pretreatment group may suppress acetaminophen-induced liver injury (Sumioka et al., 1998).

Our data showed that NDEA profoundly increased the level of lipid peroxidation. Free radicals, mostly ROS, cause cellular injury, the consequences of which are often exhibited and measured as lipid peroxidation (Spiteller, 1996). Lipid peroxidation is the oxidative deterioration of polyunsaturated lipids to form radical intermediates that cause oxidative damage. Malondialdehyde, the major endproduct of this reaction, is an index of lipid peroxidation and can be estimated as thiobarbituric acid reactive substances (Kohen and Nyska, 2002). Tremendous increases in malondialdehyde upon NDEA-induced oxidative stress

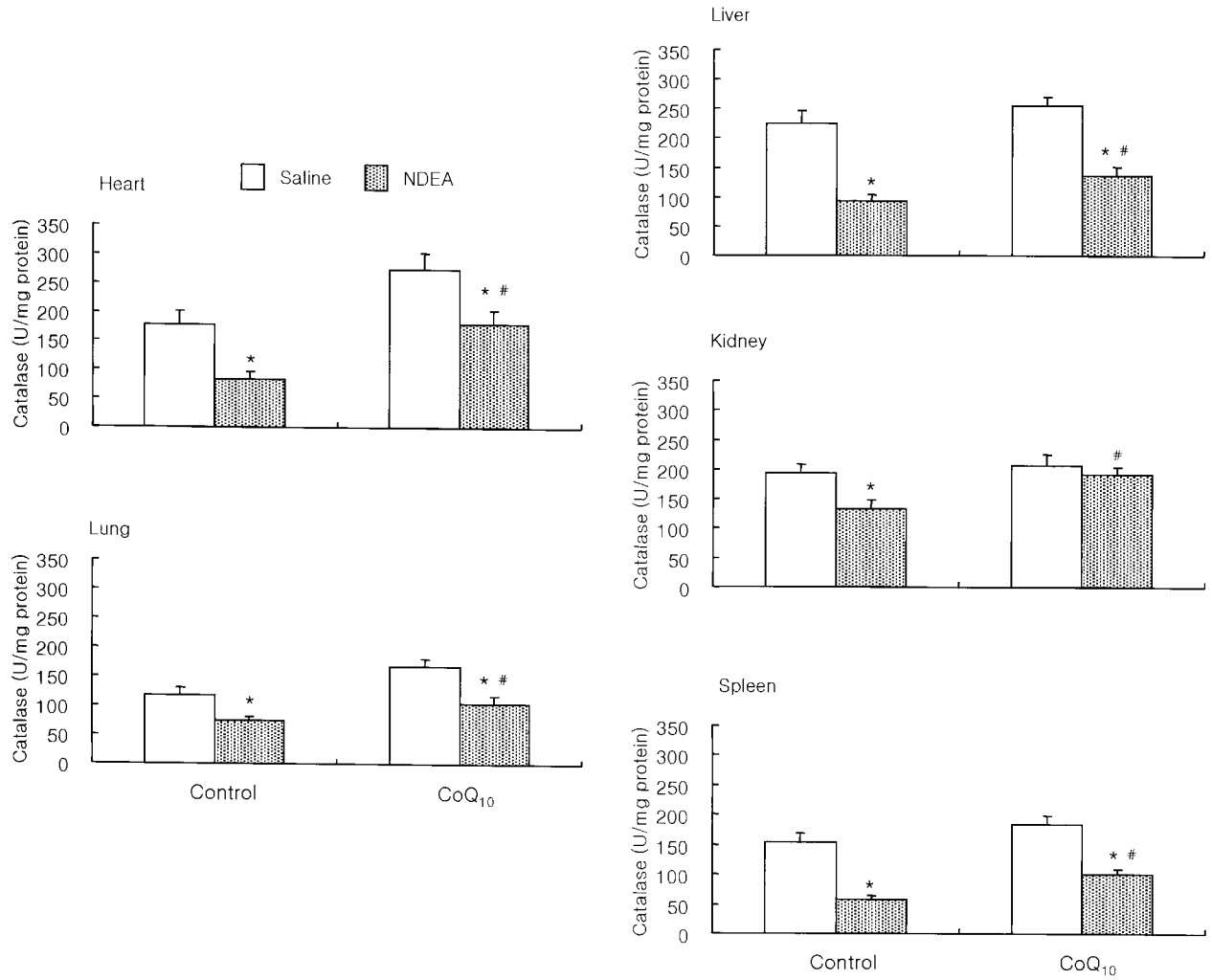

Fig. 3. The effect of CoQ_{10} on catalase activity in murine tissues treated with 200 mg/kg NDEA. CoQ_{10} in olive oil (100 mg/kg) was orally administered to the CoQ_{10} group for 3 weeks, while an equal volume of olive oil was orally administered to the control group. Results are means±SD from 8 mice. *significantly different from each saline group ($p<0.05$), #significantly different from NDEA group of control ($p<0.05$).

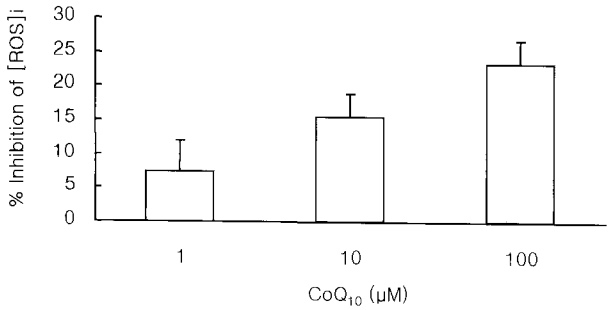

Fig. 4. The effect of CoQ_{10} on intracellular reactive oxygen species (ROS) production induced by 1 mg/ml silica in RAW 264.7 cells. Results are means±SD from 4 separate experiments.

could be caused by increased production of or decreased destruction of ROS. The lesser effect of CoQ_{10} on NDEA-induced lipid peroxidation in various tissues may be due to the presence of an antioxidant system associated with CoQ_{10} that increases anti-oxygenic potential. Supplementation of CoQ_{10} in diet is reported to help maintain a favorable antioxidant/pro-oxidant balance (Sohal and Forster, 2007). Recently, it has been also reported that CoQ_{10} significantly decreases the level of lipid peroxidation *in vivo* and *in vitro* (Sawicka and Dlugosz, 2008; Sena et al., 2008). This effect of CoQ_{10} may be related to its antioxidant activity since it significantly decreased ROS generation in silica-stimulated RAW 264.7 cells.

SOD scavenges the superoxide radical by converting it to H_2O_2, which in turn is converted to molecular oxygen by reactions catalyzed by peroxidase and catalase (Thomas, 2003). Catalase is ubiquitously present in all aerobic cells and is a major component of the primary antioxidant defense system. It is most abundant in liver and is responsible for catalytic decomposition of H_2O_2 to oxygen and water. In our studies, catalase activity decreased significantly upon NDEA-induced oxidative stress in control and CoQ_{10} groups, the effect being less in the CoQ_{10} group. Decreased

catalase activity indicates that NDEA-induced oxidative stress interferes with the anti-oxygenic potential in the tissues, thus resulting in increased generation of ROS. The H_2O_2 formed by SOD is decomposed by catalase. The decrease in catalase activity in animals administered NDEA may be caused by inactivation of catalase as superoxide anions have been shown to reduce catalase activity (Bartsch et al., 1989; Bansal et al., 1996). Similarly, exposure of erythrocytes to NDEA inhibited catalase activity through excess superoxide anion formation (Bansal et al., 2005). The lesser effect on catalase in the CoQ_{10} group during NDEA-induced oxidative stress is indicative of the protective effect of CoQ_{10}. Thus, CoQ_{10} can offer useful protection against NDEA-induced oxidative stress.

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