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Short Communication

Evaluation of anti-angiogenic, anti-inflammatory and antinociceptive activity of coenzyme Q_{10} in experimental animals

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Abstract

Objectives This work aimed to assess some pharmacological activities of coenzyme Q_{10} (CoQ_{10}) in animal experimental models.

Methods The chick chorioallantoic membrane assay was used to evaluate anti-angiogenic activity of CoQ_{10}. Anti-inflammatory activity of CoQ_{10} was confirmed using two animal models of inflammation. These were the vascular permeability and air pouch models, models of acute and sub-acute inflammation, respectively. Antinociceptive activity was assessed by the acetic acid-induced abdominal constriction response.

Key findings CoQ_{10} dose-dependently displayed inhibition of chick chorioallantoic membrane angiogenesis. In the acetic acid-induced vascular permeability model in mice, CoQ_{10} at 50, 100 and 200 mg/kg reduced vascular permeability from 0.74 ± 0.01 (A_{590}) to 0.67 ± 0.01 ($P < 0.01$), 0.46 ± 0.02 ($P < 0.01$) and 0.30 ± 0.01 ($P < 0.01$), respectively. In the carrageenan-induced inflammation in the air pouch, CoQ_{10} was able to diminish exudate volume, the number of polymorphonulcear leucocytes and nitrite content in the air pouches. CoQ_{10} at 25, 50 and 100 mg/kg significantly reduced acetic acid-induced abdominal constriction in mice from 27.0 ± 2.00 (number of abdominal constrictions) to 17.7 ± 0.33 ($P < 0.01$), 9.3 ± 0.67 ($P < 0.01$) and 1.3 ± 0.33 ($P < 0.01$), respectively, suggesting a strong antinociceptive activity.

Conclusions CoQ_{10} possessed considerable anti-angiogenic, anti-inflammatory and antinociceptive activity, possibly via down-regulating the level of nitric oxide, which partly supported its use as a dietary supplement and in combination therapy.

Keywords anti-angiogenic; anti-inflammatory; antinociceptive; coenzyme Q_{10}; mitochondria

Introduction

Coenzyme Q_{10} (CoQ_{10}), also called ubiquinone or ubidecarenone, is an essential fat-soluble substance for electron transport in oxidative phosphorylation of mitochondria. It acts as an electron carrier between the NADH and succinate dehydrogenases and the cytochrome systems.[1] In addition to its role as an electron carrier, CoQ_{10} serves as an antioxidant in lipid membranes and lipoproteins. The reduced form of CoQ_{10}, also called ubiquinol-10 (QH_2), holds electrons rather loosely, so this CoQ_{10} readily gives up one or two electrons and thus acts as an antioxidant. Since many antioxidant compounds display anti-inflammatory activity in a variety of experimental models, CoQ_{10} has been considered to show anti-inflammatory activity.[2] QH_2 significantly decreased lipopolysaccharide (LPS)-induced release of a pro-inflammatory cytokine, tumour necrosis factor-α (TNF-α), and chemokines, such as macrophage inflammatory protein-1α (MIP-1α) and regulated upon activation, normal T cell expressed and secreted (RANTES), in a human monocytic cell line.[3] CoQ_{10} reduced the lipopolysaccharide (LPS)-induced TNF-α response via nuclear factor (NF)-κB1-dependent gene expression in the murine macrophage cell line RAW264.7 transfected with pro-inflammatory apolipoprotein E4.[4] CoQ_{10} was able to play an effective role for preventing ischaemia reperfusion injury, a complex inflammatory process based on the reintroduction of fully oxygenated blood into the targeted areas subjected to ischaemia, by increasing glutathione peroxidase and superoxide dismutase levels but decreasing malondialdehyde level.[5] Treatment with carni Q-gel (CoQ_{10} and

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L-carnitine) in patients with heart failure in association with increased pro-inflammatory cytokines was able to cause a significant reduction in the pro-inflammatory cytokines.[6] However, until quite recently, anti-inflammatory activity of CoQ_{10} was not evaluated independently of other medications using experimental animals. We have demonstrated anti-inflammatory and related anti-angiogenic and antinociceptive activity of CoQ_{10} using experimental animal models.

Materials and Methods

Chemicals and fertilized eggs

CoQ_{10}, retinoic acid, Evans blue, indometacin, dexamethasone and Griess reagent were obtained from Sigma Chemical Co. (St Louis, MO, USA). Fertilized brown Leghorn eggs were purchased from Pulmuone Food Co., Seoul, Korea. All other chemicals used were of highest grade commercially available.

Experimental animals

Male ICR mice (5-weeks-old, 25 ± 3 g) were obtained from Samtaco Animal Farm, Osan, Korea. The animal room was maintained at 23 ± 2°C with a 12-h light/dark cycle. Food and tap water were freely available. At least seven mice were used in each experimental group. The ethical guidelines, described in the National Institutes of Health Guide for Care and Use of Laboratory Animals, were followed throughout the experiments. Animal experiments performed in this work were approved under the reference number KNU1209 by the Ethical Committee, Kangwon National University, Chuncheon, Korea.

Chick chorioallantoic membrane assay

Anti-angiogenic activity of CoQ_{10} was determined using the chick chorioallantoic membrane (CAM) assay as described previously.[7] The fertilized chicken eggs were kept in a humidified egg incubator at 37°C. After incubation for three and a half days, approximately 2 ml albumen was aspirated from the eggs through a small hole drilled at the narrow end of the eggs, allowing the small chorioallantoic membrane and yolk sac to drop away from the shell membrane. The shell covering the air sac was punched out and removed by forceps. In the 4.5-day-old chick embryo, a sample-loaded Thermanox coverslip was applied onto the CAMs. Two days after returning the chick embryo to the incubator, an appropriate volume of 10% fat emulsion was injected into a 6.5-day-old embryo chorioallantois. The eggs were then observed under a microscope. The branching pattern of each egg was graded as 0, 1+ or 2+. Convergence of a few vessels toward the CAM surface was denoted as 1+, and 2+ reflected an increased density and length of vessels toward the CAM face.

Acetic acid-induced vascular permeability

According to a slight modification of the method of Whittle[8], an acetic acid-induced vascular permeability test was performed. Fifty minutes after oral administration of the vehicle (corn oil), CoQ_{10} (50, 100 or 200 mg/kg) or indometacin (positive control; 10 mg/kg), 0.1 ml/10 g 2% Evans blue solution was injected intravenously in each mouse. Ten minutes later, 0.1 ml/10 g 0.7% acetic acid in saline was injected intraperitoneally. Twenty minutes after the injection of acetic acid, the mice were killed by cervical dislocation. After 10 ml saline was injected into the peritoneal cavity, the washing solutions were collected. The concentration of Evans blue leaked into the peritoneal cavity was determined by reading the absorbance at 590 nm.

Carrageenan-induced inflammation in the air pouch

Based on the procedure of Ghosh *et al.*,[9] λ-carrageenan-induced inflammation model in the air pouch was performed. Six days before drug treatment, the air pouch was formed in the intrascapular region of the mice by initial subcutaneous injection of 4 ml sterile air and successive injections of 2 ml sterile air every three days to sustain its patency. On day 0, vehicle (corn oil), CoQ_{10} (0.03, 0.1 or 0.3 mg/pouch) or dexamethasone (0.01 mg/pouch) was administered into the air pouch directly after injection of λ-carrageenan (1 ml 2.0% solution). After 16 h, the pouch cavity was opened and the exudates were collected. The exudate volumes were measured using a graduated tube. Samples were diluted with Turk solution, and the polymorphonuclear leucocytes were counted in a standard haemocytometer chamber.

Acetic acid-induced abdominal constriction response

Antinociceptive activity of CoQ_{10} was determined as described previously.[10] Nociception was induced by intraperitoneal injection of 0.7% acetic acid solution at a dose of 0.1 ml/10 g body weight. Each experimental group of mice was treated orally with vehicle (corn oil), CoQ_{10} (25, 50 or 100 mg/kg) or indometacin (10 mg/kg; positive control). One hour after the oral administration, 0.7% acetic acid solution was injected. Ten minutes after this, the number of abdominal constrictions during a 10-min period was counted.

Nitrite analysis

Accumulated nitrite (NO_2^-) in the exudates obtained from the air pouches was determined based on the Griess reaction.[11] The samples (100 μl) were reacted with 100 μl Griess reagent (6 mg/ml) at room temperature for 10 min, and then NO_2^- concentration was determined by measuring the absorbance at 540 nm.

Statistical analyses

The results were expressed as mean ± SE. Comparison between experimental groups was performed by analysis of variance followed by the Tukey's multiple range tests. P values less than 0.05 were considered to be significant. The concentrations required to achieve 50% inhibition, the IC50 values, were calculated from the dose/response linear regression plots.

Results

The chick chorioallantoic membrane (CAM) assay was used to evaluate anti-angiogenic activity of CoQ_{10}. When CoQ_{10} at 0.01, 0.03, 0.1 or 0.3 μg/egg was applied onto the CAMs, their inhibition percentages in CAM angiogenesis were 33.3,

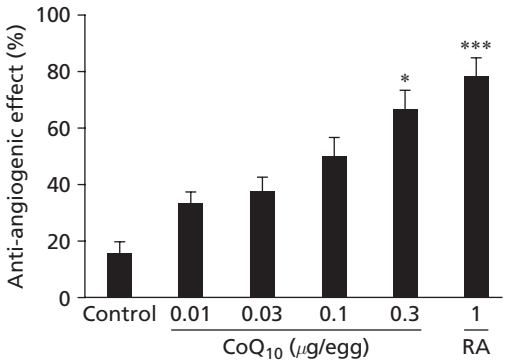

Figure 1 Dose-dependent anti-angiogenic activity of coenzyme Q_{10} in the chick embryo chorioallantoic membrane assay. Retinoic acid (RA; 1 μg/egg) was used as a positive control. Each group contained at least 25 eggs. CoQ_{10}, Coenzyme Q_{10}. Each column represents mean ± SE of the three independent experiments. $^{*}P < 0.05$, $^{***}P < 0.001$ compared with the control group.

37.5, 50.0, and 66.7%, respectively (Figure 1). Its IC50 value was 0.28 μg/egg.

CoQ_{10} at oral doses of 50, 100 or 200 mg/kg showed an inhibition of 8.9, 38.2 and 60.1%, respectively in the acetic acid-induced vascular permeability model (Table 1). The IC50 value for acetic acid-induced vascular permeability was determined to be 147.2 mg/kg. The finding suggested that an acute anti-inflammatory activity of CoQ_{10} partly emerged from its preventive action against the release of inflammatory mediators at the early phase.

In the air pouch model, CoQ_{10} at 0.03, 0.1 or 0.3 mg/pouch gave rise to inhibition of 12.0, 16.0 and 22.7%, respectively, with respect to the control exudate volume (Table 2). Total numbers of polymorphonuclear leucocytes in the air pouches were also diminished by CoQ_{10} at 0.03, 0.1 and 0.3 mg/pouch, the inhibitory percentages of which were 17.0, 32.7 and 42.8, respectively (Table 2).

The effect of CoQ_{10} at oral doses of 25, 50, and 100 mg/kg on the abdominal constriction response in mice is shown in Figure 2. In a dose-dependent manner, CoQ_{10} markedly inhibited the abdominal constriction response induced by acetic acid. CoQ_{10} at 100 mg/kg exhibited an inhibition of 95.1% in the number of abdominal constrictions compared with the control group (Figure 2).

Table 1 Inhibitory effects of coenzyme Q_{10} in the acetic acid-induced vascular permeability model in mice

Group	Dose (mg/kg)	A_{590}
Control	–	0.74 ± 0.01
CoQ_{10} 50	50	0.67 ± 0.01 (8.9)
CoQ_{10} 100	100	0.46 ± 0.02** (38.2)
CoQ_{10} 200	200	0.30 ± 0.01** (60.1)
Indometacin	10	0.20 ± 0.03** (73.1)

Indometacin (10 mg/kg) was used as a positive control. Coenzyme Q_{10} (CoQ_{10}; 50, 100 and 200 mg/kg) was orally administered. Vascular permeability was represented by the absorbance at 590 nm (A_{590}). The results are expressed as mean ± SE. Each group contained seven mice. This experiment was performed in triplicate. Figures in parentheses indicate inhibitory percentage with respect to the control group treated with corn oil only. $^{**}P < 0.01$ compared with the control group.

Discussion

Angiogenesis, defined as the growth of new blood vessels from pre-existing capillaries and post-capillary venules, is closely linked with a variety of diseases, and especially plays a crucial role in the growth and metastasis of various tumours and chronic inflammatory diseases, such as rheumatoid arthritis and proliferative diabetic retinopathy.[12] Anti-angiogenic molecularly targeted therapies, such as monoclonal antibodies or tyrosine kinase inhibitors, are being applied for the treatment of malignant gliomas.[13] FR-118487, an angiogenesis inhibitor of the fumagillin family, has a chemopreventive effect in addition to causing dormancy of hepatocellular carcinoma associated with chronic liver diseases.[14] In conjunction with conventional chemotherapy, anti-angiogenic therapy has great potential for curing tumours.[15] Supplementation with CoQ_{10}, riboflavin and niacin to breast cancer patients undergoing tamoxifen therapy gave rise to a further significant reduction in pro-angiogenic marker levels and an increase in the levels of anti-angiogenic factors, which suggested anti-angiogenic potential of CoQ_{10}.[16] Oxidative stress, such as hydrogen peroxide, was shown to stimulate angiogenesis, and many natural antioxidants, such as pedicularioside G and rosmarinic acid were able to inhibit angiogenesis.[12,17–19] Antioxidant activity of CoQ_{10} may have been responsible also for its anti-angiogenic activity. Taken together, CoQ_{10} contained significant anti-angiogenic activity.

Anti-inflammatory activity of CoQ_{10} was examined using two in-vivo experimental models, the mouse vascular

Table 2 Effects of coenzyme Q_{10} on carrageenan-induced inflammation in the mouse air pouch model

Group	Dose (mg/pouch)	Volume of exudate (ml)	Number of total leucocytes ($\times 10^7$ cells)	Content of nitrite (μM)
Control	–	2.50 ± 0.02	4.80 ± 0.56	22.59 ± 0.28
CoQ_{10} 0.03	0.03	2.20 ± 0.12 (12.0)	3.99 ± 0.27 (17.0)	21.92 ± 0.60 (3.0)
CoQ_{10} 0.1	0.1	2.10 ± 0.03** (16.0)	3.23 ± 0.23* (32.7)	18.63 ± 3.91 (17.6)
CoQ_{10} 0.3	0.3	1.93 ± 0.13** (22.7)	2.75 ± 0.06** (42.8)	13.56 ± 1.45* (40.0)
Dexamethasone	0.01	0.67 ± 0.03** (73.3)	1.54 ± 0.02** (67.9)	6.38 ± 0.58** (71.8)

The results are expressed as mean ± SE of $n = 8$. Figures in parentheses indicate inhibitory percentages with respect to the corresponding control. Dexamethasone was used as a positive control. The control group was treated with corn oil only. Coenzyme Q_{10}, CoQ_{10}. This experiment was repeated in triplicate. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the control group.

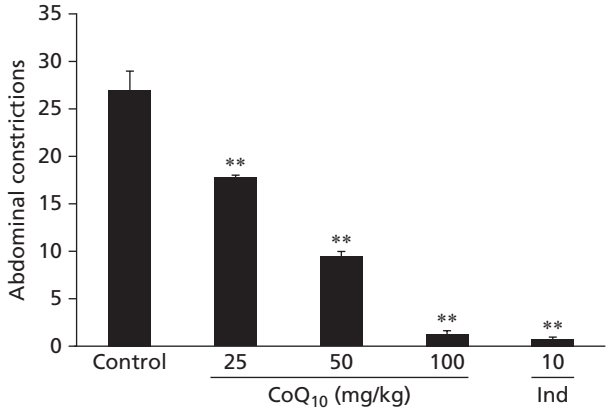

Figure 2 Inhibitory effect of coenzyme Q_{10} on the acetic acid-induced abdominal constriction response in mice. Indometacin (Ind, 10 mg/kg) was used as a positive control. Coenzyme Q_{10} (CoQ_{10}) was orally administered. The results are expressed as mean ± SE. Each group contained seven mice. This experiment was performed in triplicate. $^{**}P < 0.01$ compared with the control group.

permeability and air pouch models. The acute inflammatory response is associated with an increase in vascular permeability and cellular infiltration.[20] The acetic acid-induced vascular permeability model is used to evaluate the inhibitory activity of samples against increased vascular permeability, which is induced by acetic acid through releasing inflammatory mediators from mast cells.[21] Since nitric oxide (NO) is an important intracellular pro-inflammatory mediator, changes in the NO level in the air pouches were determined after treatment with CoQ_{10}.[22] NO reacts with superoxide radical to generate a peroxynitrite ion, which induces excess inflammatory activity and leads to a variety of pathological conditions, such as arthritis, sepsis, ulcerative colitis and systemic lupus erythematosus.[23] Suppression of NO production is thought to be closely linked with an anti-inflammatory action. The accumulated nitrite, as an index for NO level, in the air pouches was decreased by the treatment with CoQ_{10} at 0.03, 0.1 and 0.3 mg/pouch, the inhibitory percentages of which were 3.0, 17.6 and 40.0, respectively (Table 2). Taken together, CoQ_{10} possessed significant acute and sub-acute anti-inflammatory activity, possibly through suppressing NO generation.

Antinociceptive activity of CoQ_{10} was evaluated using the acetic acid-induced abdominal constriction response, which was used to detect a general antinociceptive activity of the sample under study. In a dose-dependent manner, CoQ_{10} markedly inhibited the abdominal constriction response induced by acetic acid. CoQ_{10} at 100 mg/kg exhibited an inhibition of 95.1% in the number of constrictions compared with the control group (Figure 2), which was comparable with that of indometacin (10 mg/kg), used as a positive control. An antinociceptive activity of CoQ_{10} was observed at relatively low doses of CoQ_{10} compared with the doses used in the acetic acid-induced vascular permeability model. The finding proposed that CoQ_{10} possessed an antinociceptive activity in addition to an anti-inflammatory activity.

Since, under inflammatory conditions, high reactive oxygen species (ROS) level converts the cellular redox balance toward oxidative stress, anti-inflammatory drugs are used to suppress the generation and accumulation of intracellular ROS.[24] CoQ_{10}, known as a potent endogenous antioxidant, has been considered to contain an anti-inflammatory activity. Some CoQ_{10} mimics were shown to contain antioxidant and subsequently anti-inflammatory activity. MitoQ, an orally active antioxidant that has the ability to target mitochondrial dysfunction, mimics the role of the endogenous mitochondrial CoQ_{10} and augments substantially the antioxidant capacity of CoQ_{10} to supraphysiological levels.[25] In an LPS–peptidoglycan model of sepsis, MitoQ decreased oxidative stress and protected mitochondria from damage as indicated by a lower rate of ROS formation and by maintenance of the mitochondrial membrane potential, and also suppressed pro-inflammatory cytokine release from the cells while increasing the production of the anti-inflammatory cytokine interleukin-10.[26] Idebenone, a synthetic analogue of CoQ_{10} and similar to CoQ_{10} in its antioxidant properties, was shown to inhibit arachidonic acid metabolism in astrocyte homogenates.[27] The results from this work further support anti-inflammatory and related activity of CoQ_{10} and its mimics and analogues.

Conclusions

CoQ_{10} possessed strong anti-angiogenic activity in the chick chorioallantoic membrane assay. CoQ_{10} was shown to have in-vivo anti-inflammatory activity using the acetic acid-induced vascular permeability and air pouch models, and was shown to have antinociceptive activity using the acetic acid-induced abdominal response.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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