

Original Research Article

Coenzyme Q10 attenuates airway inflammation and oxidative stress in neonatal asthmatic rats

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Abstract

Purpose: To determine the therapeutic effect of coenzyme Q10 (CoQ10) on ovalbumin (OVA)-provoked asthma in neonatal rats.

Methods: Asthma was induced by exposing neonatal rats to OVA. The levels of SOD, CAT, GPx, GSH, MDA and MPO were estimated using standard biochemical kits, while ELISA was used to measure the concentrations of Ig E and Th2 cytokines. Gene expressions were assayed with qRT-PCR, and protein expressions were determined with western blotting.

Results: OVA treatment led to increases in levels of BALF inflammatory cells, lipid peroxidation, serum IgE and BALF Th2 cytokines, but it decreased antioxidant levels. Furthermore, the protein expression of NF- κ B and mRNA expression levels of proinflammatory cytokines and inducible nitric oxide synthase (iNOS) were upregulated in the asthmatic rats ($p < 0.05$). However, coenzyme Q10 supplementation significantly decreased lipid peroxidation, and reduced inflammatory cells and IgE levels, while the antioxidant levels were enhanced ($p < 0.05$). Moreover, coenzyme Q10 reduced the levels of Th2 cytokines and downregulated the expressions of NF- κ B, TNF- α , IL-6, and iNOS in the neonatal asthmatic rats ($p < 0.05$).

Conclusion: Coenzyme Q10 attenuates airway inflammation and oxidative stress in neonatal asthmatic rats. Thus, coenzyme Q10 has promising therapeutic potential in the management of asthma.

Keywords: Asthma, Neonatal, Coenzyme Q10, Th2, cytokines, Oxidative stress, Antiinflammation

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INTRODUCTION

Asthma is a long-term inflammatory and allergic airway pathology caused by infiltration of immune cells such as neutrophils, lymphocytes and macrophages into the lung tissues [1]. The mechanism underlying the pathology of asthma encompasses elevated mucus secretion, hyper-allergic response and constriction, and airway

inflammation [2]. The other clinical factors involved in asthma are high concentrations of IgE, infiltration of mast cells as a result of inflammation, and aggravated release of cytokines and chemokines. These factors result in accumulation of the inflammatory cells in the airway area, leading to generation of toxic free radicals. The free radicals reduce pulmonary

antioxidant defense system and modify the structure of vital biomolecules [3].

Bronchial hyperreactivity is mediated by Th2 cytokines, proinflammatory cytokines and chemokines. The Th2 cytokines implicated in asthma are IL-4, IL-5 and IL-13, with IL-4 as the important mediator involved in the maturation of Th2 cells, allergic reactions and IgE release [4]. Interleukin-5 (IL-5) orchestrates eosinophil activity, eosinophil infiltration into the lungs, and hyper mucus secretion [4]. Thus, Th2 cytokines play critical roles in airway hyperresponsiveness (AHR) during asthma. Nuclear factor kappa (NF- κ B) is an ROS-mediated transcription factor involved in the pathogenesis of various inflammatory diseases. Nuclear factor kappa B (NF- κ B) is a major inflammatory mediator which in turn activates other inflammatory molecules like TNF- α , IL-6, chemokines, adhesion molecules and MMPs [5]. The current management of asthma involves the use of corticosteroids, but this results in unwanted adverse effects [6]. Therefore, a viable therapeutic option is the treatment/prevention of asthma using alternative medicine.

Coenzyme Q10 (CoQ10), a naturally-occurring biomolecule with potent mitochondrial antioxidant and anti-inflammatory effects, is actively involved in the mitochondrial electron transport chain [7]. Several clinical studies have demonstrated the efficacy of CoQ10 in the prevention of asthma [8]. Thus, the current study was undertaken to determine the anti-asthmatic effect of CoQ10 in ovalbumin-induced neonatal asthma in rats.

EXPERIMENTAL

Drugs and chemicals

Coenzyme Q10 and OVA were purchased from Sigma Aldrich USA. The other reagents used in the study were of analytical grade.

Animals

Sprague Dawley rats aged 12 days old and weighing about 6 - 10 g, were used in the study. The animals were maintained on standard laboratory condition and fed neonatal diet. The experiment was conducted as per the international guidelines stated in the laboratory animal use and care [9]. The study was approved by the ethical committee of The Second People's Hospital of Huai'an, Huai'an 223002, Jiangsu Province, China University, China (approval no. SPH6782019).

Induction of asthma and drug treatment protocol

Asthma was induced in the neonatal rats via exposure to OVA (1 mg, i. p) adsorbed on 20 mg of gelatinous Al(OH)₃ at 7-day intervals i.e. on days 0, 7 and 14. Thereafter, the rats were sensitized with OVA (1.1 % in 100 μ L normal saline) using intratracheal instillation on day 21. Four groups of were used, with 6 rats/group (n = 6): group I (normal control), group II (asthmatic neonatal rats), Group III (asthmatic rats given CoQ10 alone at a dose of 10 mg/kg, p.o. daily for 15 weeks), and Group IV (asthmatic rats treated with CoQ10 at a dose of 10 mg/kg, p.o. daily for 15 weeks).

Sampling of blood and bronchoalveolar lavage fluid (BALF)

At the end of the final drug treatment, the animals were subjected to euthanasia using ketamine (100 mg/kg; i.p) and xylazine (10 mg/kg, i.p.). Blood was collected through retroorbital puncture, and the serum was subjected to IgE analysis. The BALF was collected by placing a cannula in the upper portion of the trachea and flushing thrice with 0.5 mL of cold PBS. The BALF was centrifuged, and the supernatant portion was used for assay of Th2 cytokines, while the cell pellets were used for the estimation of inflammatory cell count.

Determination of antioxidants in lung homogenate

The rats were euthanized and the lungs were isolated and homogenized using 50 mM Tris-HCl buffer. The homogenates were centrifuged at 10,000 rpm for 10 min at 4°C, and the resultant supernatants were used for the estimation of antioxidants.

The various biochemical markers of oxidative stress i.e. SOD, CAT, GPx, GSH, MDA and MPO were estimated using biochemical kits from Nanjing Jiancheng Bioengineering Institute, Nanjing, China.

Evaluation of levels of IgE and Th2

The serum concentrations of IgE and cytokines (IL-4, IL-5 and IL-13) in BALF fluid were estimated using their respective ELISA kits (Sigma Aldrich, USA).

Assay of expressions of TNF- α , IL-6 and iNOS

Total RNA was extracted from neonatal rat lung tissues and purified using Trizol reagent (Sigma,

USA) as per manufacturer's protocol. The purified RNA was reverse-transcribed to cDNA using oligoDT primers. A 30-cycle reaction procedure was followed, including denaturation for 30 sec at 95 °C, and annealing for 30 sec at 57 °C. At the end, a 1-min extension step was carried out at 72 °C. Then, each volume of sample was run in ethidium bromide-stained 1.5 % agarose gels to determine the degree of expression.

Assay of protein expression of NF- κ B

Lung tissues were excised and suspended in a lysis buffer at 4 °C, followed by temperature adjustment to 95 °C. Thereafter, the protein content of each lung cell extract was determined with the Bradford assay method. Equal amount of protein (40 μ g) was loaded from each sample and separated using SDS-PAGE before being transferred to polyvinylidene fluoride membranes. The membranes were then treated with TBS, and incubated overnight with primary antibodies (rabbit anti-NF- κ B and rabbit anti- β -actin) at 4 °C. Thereafter, the membranes were incubated with horseradish peroxidase-conjugated goat-anti rabbit secondary antibody at room temperature for 1 h. The protein expressions were visualized using enhanced chemiluminescence reagent.

Statistical analysis

Data are expressed as mean \pm SEM. Statistical analysis was done with SPSS software version 16. Comparison among different groups was carried out with ANOVA, followed by Tukey's post-hoc test procedure. Values of $p < 0.05$ were taken as indicative of statistically significant differences.

RESULTS

Effect of coenzyme Q10 on inflammatory cell count in neonatal rats

In OVA-exposed neonatal rats, there were marked increases in the populations of inflammatory cells, neutrophils, monocyte, eosinophils and basophils ($p < 0.05$). However, CoQ10 treatment significantly ($p < 0.05$) decreased inflammatory cell counts, and increased differential count to normal reference range (Table 1).

Coenzyme Q10 exerted protective effect against OVA-induced oxidative stress

The OVA-exposed neonatal rats had decreased level of the antioxidants SOD, CAT, GPx and

GSH ($p < 0.05$). However, administration of CoQ10 to the asthmatic rats significantly reduced the oxidative stress by increasing the levels of antioxidants. These results are shown in Table 2.

OVA induced increases in lipid peroxidation and MPO activity

As shown in Figure 1, there were significant increases in MDA and MPO activity in lung homogenate in OVA-exposed neonatal rats, as a result of lipid peroxidation ($p < 0.05$). However, CoQ10 administration produced marked reductions in lipid peroxidation and MPO activity to normal levels.

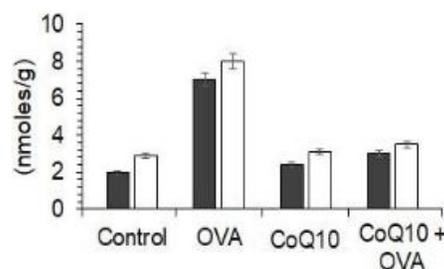


Figure 1: Effect of CoQ10 on the MDA (■) and MPO (□) levels in different groups. The results are presented as mean \pm SEM. Data were analyzed using One-way ANOVA, and comparison amongst groups was done with Tukey's comparison method. * $P < 0.05$ (control vs OVA; CoQ10 + OVA vs OVA)

Coenzyme Q10 reduced the serum level of OVA-specific Ig E

In neonatal rats, the OVA-induced allergic asthma significantly increased the serum level of IgE, as result of airway inflammation. However, treatment with CoQ10 significantly reduced the IgE level to normal and thus prevented airway inflammation. These results are presented in Figure 2.

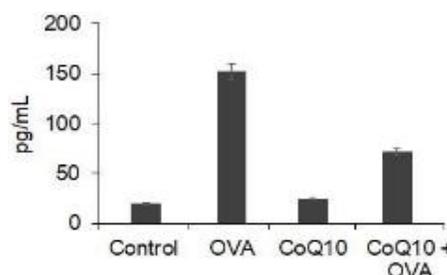


Figure 2: Effect of CoQ10 on IgE levels in different groups of rats. The results are presented as mean \pm SEM. Data were analyzed using One-way ANOVA, and comparison amongst groups was done with Tukey's comparison method. * $P < 0.05$ (control vs OVA; CoQ10 + OVA vs OVA)

Coenzyme Q10 decreased levels of Th2 cytokines in BALF

There were significant increases in the levels of Th2 cytokines (IL-4, IL-5 and IL-13) in the BALF fluid of OVA-exposed neonatal rats. However, CoQ10 administration significantly decreased the Th2 cytokines and restored their levels to normal. These results are shown in Figure 3.

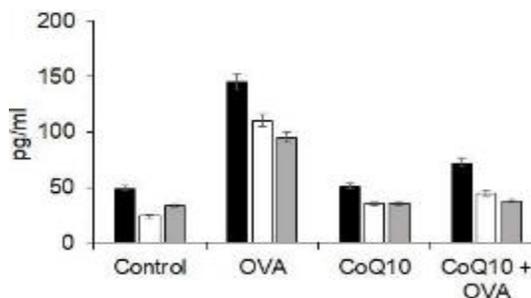


Figure 3: Effect of CoQ10 on levels of Th2 cytokines (IL-4, IL-5 and IL-13) in BALF of different rat groups. Data were analyzed using One-way ANOVA, and comparison amongst groups was done with Tukey's comparison method. **P* < 0.05 (control vs OVA; CoQ10 + OVA vs OVA)

Coenzyme Q10 downregulated the protein expression of NF-κB in neonatal asthmatic rats

Figure 4 shows that NF-κB protein expression was significantly increased in lung tissue of OVA-exposed neonatal asthmatic rats. However, CoQ10 treatment effectively suppressed NF-κB protein expression, when compared to OVA-exposed neonatal asthmatic rats. The relative protein expression of NF-κB was significantly higher in OVA-exposed neonatal rats than in control, but this was reversed by CoQ10 administration.

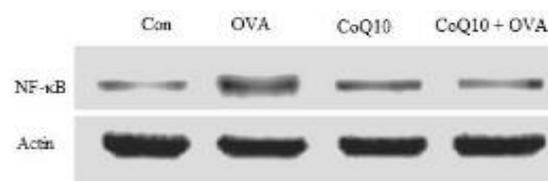


Figure 4: Western blot analysis showing the effect of CoQ10 on the protein expression of NFκB in different neonatal rat groups. The experiments were performed in triplicate

Coenzyme Q10 downregulated the mRNA expressions of TNF-α, IL-6 and iNOS

The expressions of TNF-α, IL-6 and iNOS mRNA were upregulated in lung tissue of OVA-exposed neonatal asthmatic rats. Treatment with CoQ10 effectively suppressed the mRNA expressions of TNF-α, IL-6 and iNOS, when compared to OVA-exposed neonatal asthmatic rats. The relative mRNA expression of NF-κB was significantly higher in OVA-treated neonatal rats than in the control, but CoQ10 treatment markedly decreased the mRNA expressions of the inflammatory cytokines and iNOS. These results are presented in Figure 5.

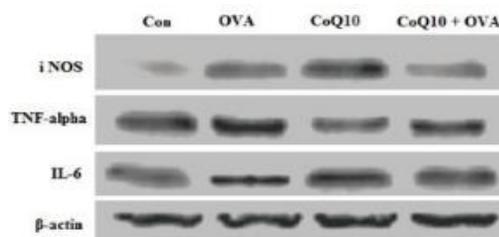


Figure 5: Semi-quantitative PCR analysis showing the effect of CoQ10 on the expressions of TNF-α, IL-6 and iNOS in different groups of neonatal rats. The experiments were performed in triplicate

Table 1: Effect of CoQ10 on inflammatory cell count in neonatal asthmatic rats

Group	Total leukocytes/ml BALF (x 10 ⁴)	Neutrophils/ml BALF (x 10 ⁴)	Eosinophils/ml BALF (x 10 ⁴)	Lymphocytes /ml BALF (x 10 ⁴)
Control	4 ± 1.12	1 ± 0.02	0.56 ± 0.01	20 ± 3.12
OVA	22 ± 3.45	135 ± 7.75	4 ± 5.7	80 ± 5.15
CoQ10	8 ± 1.87	4 ± 0.76	0.76 ± 0.03	24 ± 2.84
CoQ10+ OVA	12 ± 2.34	75 ± 5.32	1.34 ± 0.3	40 ± 4.65

The results are presented as mean ± SEM

Table 2: Effect of CoQ10 on antioxidant levels in neonatal asthmatic rats (U/mg protein)

Group	SOD	CAT	GPx	GSH
Control	10.56 ± 0.76	7.28 ± 0.56	15.25 ± 1.35	10.56 ± 1.12
OVA	4.28 ± 0.46	3.52 ± 0.28	5.76 ± .87	4.35 ± 0.65
CoQ10	9.45 ± 0.72	6.76 ± 0.42	14.25 ± 1.85	9.26 ± 1.42
CoQ10 + OVA	8.45 ± 0.54	6.12 ± 0.54	12.54 ± 1.76	8.65 ± 1.05

The results are presented as mean ± SEM

DISCUSSION

Exposure to a wide array of chemicals during pregnancy may lead to the development of asthma in neonates [10]. Earlier reports indicate that chemical exposure during maternity increases allergic responses and airway inflammation in a preclinical model of OVA-induced asthma [11]. Furthermore, exposure to chemicals released from smoke, organic reagents and air pollutants during maternity elevates the risk of childhood asthma [12]. Previous studies indicate that alterations in T helper 2 immune system, increased sensitivity to allergens, airway inflammation and bronchial hyperreactivity are implicated in the mechanisms involved in etiology of neonatal asthma [13]. Studies have also shown that oxidative insult is a prime factor in the development of asthma in children [14]. Furthermore, low levels of, or deficiency in natural antioxidants like glutathione and vitamins increase the risk of childhood asthma [15]. Coenzyme Q10 (CoQ10) is a mitochondrial antioxidant vitamin with anti-asthmatic properties [16]. The present study investigated the anti-asthmatic potential of CoQ10 in OVA-induced neonatal asthma in rats.

Airway inflammation is a critical pathological event in asthma [17]. During allergen exposure, Th2 cells are activated. This, in turn, triggers the activation of Th2-related cytokines such as IL-4, IL-5, and IL-13. Furthermore, the increased levels of these cytokines mediate the infiltration of airway epithelium by neutrophils and other inflammatory cells. Thus, the inflammatory cells recruited in the airway secrete various pro-inflammatory mediators and increase airway inflammation [18]. In the present study, OVA-exposed neonatal rats showed increased levels of inflammatory cells and Th2 cytokines in BALF fluid. However, treatment with CoQ10 significantly reduced the BALF inflammatory cells and levels of Th2 cytokines to normal, thereby minimizing airway inflammation.

Oxidative stress has been implicated in the pathogenesis of asthma. Exposure to OVA triggers infiltration of inflammatory cells, leading to the generation of free radicals. These free radicals damage vital cellular entities such as proteins and lipids. In this study, OVA-challenged neonatal rats had increased levels of MDA and MPO activities in the lung homogenates as a result of oxidative stress produced by superoxide anion and H₂O₂ in the lung alveoli [19]. However, treatment with CoQ10 significantly reduced the MDA and MPO levels by reducing the free radicals and lipid peroxidation [20].

There were decreased antioxidant levels in the OVA-challenged neonatal rats, relative to the control rats. The decreases in antioxidant levels are due to the attempt by the physiological antioxidant defense system to counteract the free radicals generated by OVA [21]. However, CoQ10 effectively increased the antioxidant levels, thereby ameliorating the oxidative stress generated by OVA in the neonatal rats.

Increased Ig-E level is the hallmark event during allergic diseases like asthma and allergic rhinitis. Thus, IgE assay is widely used for diagnosis of asthma [22]. Studies have revealed that Th2 cytokines IL-4 and IL-13 accelerate the release of Ig E from mast cells during asthma [23]. Similarly, in this study, neonatal rats exposed to OVA had increased IgE levels, when compared to control rats. However, CoQ10 administration markedly reduced the Ig E levels in the neonatal asthmatic rats.

It is known that NF- κ B is a cardinal transcription molecule which regulates the expressions of a wide range of cytokines involved in airway inflammatory conditions. In this study, a significant upregulation of NF- κ B protein expression was observed in OVA-exposed neonatal rats. However, CoQ10 administration downregulated the NF- κ B expression in neonatal rats, consistent with an earlier report [24].

Inducible nitric oxide synthase (iNOS) mediates vital processes in airway pathology in asthma and allergic rhinitis. Studies have indicated that iNOS expression is primarily due to the secretion of Th2 cytokines which mediate series of events like infiltration of inflammatory cells, proinflammatory cytokines release, and broncho-constriction [25]. The present study showed the OVA-induced significant upregulations in mRNA expressions of TNF- α , IL-6 and iNOS in lung tissue of neonatal rats were reversed by CoQ10 administration.

CONCLUSION

This study has demonstrated that OVA exposure increases inflammatory cell counts and lipid peroxidation, and decreased antioxidant levels in neonatal rat BALF. Furthermore, OVA exposure induced significant increases in levels of BALF Th2 cytokines and serum Ig E level, and upregulated the protein and mRNA expressions of NF- κ B and TNF- α , IL-6 and iNOS. However, CoQ10 exerted effective anti-asthmatic effect by reducing inflammatory cell count, lipid peroxidation, inflammation and iNOS, through its antioxidant properties. Thus, coenzyme Q10 may be of beneficial in the management of asthma.

DECLARATIONS

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Banghao Lu conceived and designed the study; Jingwei Peng, Jilong Ma, Lan Zhang collected and analyzed the data; Jingwei Peng wrote the manuscript. All authors read and approved the manuscript for publication.

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