Lack of anti-inflammatory effect of coenzyme Q10 supplementation in the liver of rodents after lipopolysaccharide challenge

Audrey M. Neyrincka, Emilie Catry, Florence M. Sohet, Patrice D. Cani, Barbara D. Pachikian, Laure B. Bindels, Nathalie M. Delzenne

Metabolism and Nutrition Research Group, Louvain Drug Research Institute, Université catholique de Louvain, Brussels, Belgium

Walloon Excellence in Life Sciences and Biotechnology (WELBIO), Louvain Drug Research Institute, Université catholique de Louvain, Brussels, Belgium

Article info
Article history:
Received 2 June 2015
Accepted 16 July 2015
Available online 4 August 2015

Keywords:
Inflammation
LPS
Coenzyme Q10
Liver
Precision-cut liver slices

Background & aims: Sepsis is characterized by a systemic dysregulated inflammatory response and oxidative stress. A large body of evidence supports a key role of mitochondrial dysfunction during the various phases of sepsis (early and late-phase of sepsis-associated multiorgan failure). Coenzyme Q10 (CoQ10) is a key cofactor in the mitochondrial and respiratory chain, and is depleted in septic shock patients. However, its effect on acute sepsis remains unexplored. The reduced form of CoQ10 (Qx) has been shown to lessen pro-inflammatory response in macrophages or fibroblasts in culture. The aim of the study is to investigate the effect of Qx on hepatic inflammation in models of acute LPS-induced inflammation.

Methods: We have conducted 2 experiments: one in vitro using rat precision-cut liver slices (PCLS) incubated with LPS and Qx, and one in vivo experiment using mice fed with Qx for 2 weeks and killed 1 h after LPS administration.

Results: LPS challenge induced hepatic inflammatory stress both in vitro and in vivo (increased TNFα, nitrite and PGE2 release and/or expression). Incubation of PCLS with Qx fails to modulate hepatic inflammatory stress. Furthermore, Qx supplementation is not able to counteract hepatic LPS-induced acute inflammation in vivo.
1. Introduction

Severe sepsis and septic shock are major causes of morbidity and mortality. Among the tissues involved in the inflammatory response to sepsis, the liver plays a central role in response to inflammatory stresses. Liver immune cells are able to recognize bacterial components such as lipopolysaccharides (LPS) – the major Gram negative bacteria membrane component. In particular, Kupffer cells, known as fundamental macrophages, are involved in LPS detoxification and their stimulation leads to the secretion of inflammatory mediators such as tumor necrosis factor α (TNFα), prostaglandin E2 (PGE2), and the reactive intermediates NO.

Coenzyme Q10 (CoQ10) is an effective endogenously synthesized-lipid soluble antioxidant, acting either by prevention of lipid peroxidation (chain-breaking), by regeneration of vitamin E or by interaction with superoxide or other reactive oxygen species. CoQ10 is a key component of the oxidative phosphorylation process in the mitochondria. The major form of CoQ10 found in the living organism is the reduced form, ubiquinol (Qx), responsible for its antioxidant properties. Apart from its anti-oxidative function, CoQ10 appears to modulate immune functions by largely unknown mechanisms. The potential anti-inflammatory effect of CoQ10 or its reduced form has been suggested through in vitro studies, showing a decrease in IL-1 induced inflammation in human fibroblast and a decrease in the LPS-induced release of TNFα proinflammatory cytokines in human and animal macrophages. Schmelzer et al. also showed anti-inflammatory properties of Qx in vitro on the human monocyte cell line THP-1 (at a dose of 10 μM) on TNFα, macrophage inflammatory protein-1α, MIP1α, and RANTES release in the incubation medium after an LPS challenge.

In order to investigate the potential role of CoQ10 as an anti-inflammatory compound during acute sepsis, we have tested the potential modulation of acute hepatic inflammation induced in vitro in rat precision-cut liver slices (PCLS). The PCLS model was chosen because it allows to keep functional the different cell types—including immune liver cells— and respects the cell–cell interactions occurring in the liver tissue in vivo. Moreover we have tested the role of Qx oral supplementation on hepatic LPS-induced inflammation in vivo in mice 1 h after the injection of 0.1 mg/kg body weight LPS.

2. Material and methods

2.1. Reagents

LPS and bovine serum albumin were purchased from Sigma Chemical Co (St. Louis, MO, USA). Waymouth medium, penicillin, streptomycin and glucose were purchased from Gibco-invitrogen (Belgium). Qx was provided by Kaneka (Japan). Aspartate aminotransferase (AST) activity kit was purchased from Diasys, Diagnostic system GmbH (Holzeim, Germany). PGE2 Immunoassay assay kit (DE0100®) and Quantikine rat TNFα immunoassay kit (RTA00®) were purchased from R&D System (UK). TriPure isolation reagent was purchased from Roche Diagnostics (Belgium, Vilvoorde). Reverse transcription System was purchased from Promega (Leiden, The Netherlands).

2.2. In vitro study on PCLS

Housing conditions were as specified by the Belgian Law of 29 May 2013, on the protection of laboratory animals (Agreement LA 1230314). Male wistar rats (n = 5) weighing 240 g–280 g were
housed in individual cages in a room with 12 h light: dark cycle and were given free access to water and control diet (AO4, Safe, France).

Rat procedures were carried out under pentobarbital anesthesia (60 mg/kg body weight). Perfusion in situ of the liver was performed with oxygenated ice-cold Krebs–Ringer buffer prior to liver removal. Tissue cores (diameter 10 mm) were prepared from the freshly excised liver and PCLS (about 220 μm thickness) were rapidly prepared in oxygenated ice-cold Krebs–Ringer buffer using a Krumdiek slicer according to a procedure previously described [16,17]. After 30 min of preincubation at 4 °C in Waymouth medium supplemented with 100 IU/ml penicillin, 100 μg/ml streptomycin, 2.4 mM glutamine, 100 nM insulin and 0.3% bovine serum albumin, PCLS were transferred into fresh medium and incubation lasted for 2 or 20 h. Incubation medium was similar to preincubation medium except for 0.1% bovine serum albumin. According to the data published by Groneberg et al., it appeared that the optimal and non-toxic concentration of CoQ10 to study expression of genes involved in cell signaling, metabolism and transport on isolated CaCo-2 cells was 50 μM [18]. Taking into account that we have used a tissue culture model, which imposes diffusion to target the related cells, we have decided to use a dose of 100 μM; at this dose, no cytotoxic effects were found in the human monocytic cell line THP-1 [15]. Therefore, PCLS were additionally incubated in the presence or in the absence of Qx (100 μM) and/or LPS (10 μg/ml). A vial containing 2 PCLS (2 ml medium per PCLS) was used for each condition. Vials were all saturated with 95% O2–5% CO2 and placed in a shaking water bath at 37 °C. After the incubation, PCLS were frozen in liquid N2, medium was collected and medium and PCLS were then all kept at −20 °C for further analysis.

2.3. In vivo study

Ten-week-old male C57BL/6J mice (Charles River) were housed in groups of four mice per cage (eight mice per group) at 22 °C with sequential light/dark cycle (lights off 6 pm) and were given free access to diet and water. They were fed either the control diet (CT and CT-LPS groups; CE-2, CLEA Japan Inc., Japan) or a CE-2 diet supplemented with 0.4% or 1% Qx (Qx 0.4%-LPS and Qx 1%-LPS groups) for 2 weeks. We have chosen to test two different doses (0.4% and 1%) since we have previously tested those doses in models of obesity and/or diabetes (Ob/Ob mice, high-fat diet fed mice, streptozotocin-treated mice). In fact, a supplementation of CoQ10 at 1% has been shown to decrease a panel of markers of oxidative and inflammatory stress in the liver of high fat diet fed mice [19]. The energy content of the CE-2 diet is 342 kcal/100 g. The CE-2 diet contained the following (g/100 g diet): moisture 8.7, protein 24.8, fiber 3.5, ash 7, lipid 4. Food intake, taking into account spillage, was recorded three times a week. The mean value for the weekly assessment of food and energy intake was calculated.

At the end of the feeding period, mice were ip injected with 0.1 mg/kg body weight of LPS (CT-LPS, Qx 0.4%-LPS and Qx 1%-LPS groups) or NaCl 0.9% (CT group) and anaesthetized 1 h later by ip injection of ketamine (100 mg/kg)/xylazine (10 mg/kg). Cava vein blood samples were collected in EDTA tubes, and plasma was stored at −80 °C. Liver was excised, immediately clamped in liquid N2 and kept at −80 °C for mRNA analysis. The agreement of the animal experiments performed in this study was given by the local Ethical Committee and housing conditions were as specified by the Belgian Law of 29 May 2013, on the protection of laboratory animals (Agreement LA 1230314).

2.4. TNFa, PGE2 and nitrite measurements

PGE2 and TNFα concentrations were measured in frozen aliquots of incubation medium by immunoassay kit. Nitrite concentration was measured by the Griess reaction as a reflection of NO-production [3].

2.5. Real-time quantitative PCR

Total RNA was isolated from PCLS and liver using TriPure isolation reagent. cDNA was prepared by reverse transcription of 1 μg total RNA using the Kit Reverse transcription System. Real-time polymerase chain reactions (PCRs) were performed with the StepOnePlus instrument and software (Applied Biosystems) as described [20]. Ribosomal protein L19, RPL19, RNA was chosen as
housekeeping gene. The targeted mouse and rats genes, RPL19, TNF-\(\alpha\), inducible-NO synthase (iNOS), cyclooxygenase-2 (COX-2) and monocyte chemotactic protein-1 (MCP1) are available on request (audrey.neyrink@uclouvain.be).

2.6. Statistical analysis

Results are presented as Mean ± standard error of the mean (SEM). Statistical significance of difference between groups was assessed by one-way followed by post hoc Tukey's multiple comparison tests (GraphPad Prism Software; www.graphpad.com). Data with different superscript letters are significantly different (\(P < 0.05\)) according to the post hoc ANOVA statistical analysis.

3. Results

3.1. Effect of Qx towards LPS challenge performed in vitro using PCLS

TNF\(\alpha\), nitrite and PGE\(_2\) released in the medium and their related mRNA level (mRNA level of TNF\(\alpha\), iNOS and COX2 respectively) have been analyzed as markers of inflammation. Incubation of PCLS in control condition during 20 h versus 2 h increased TNF\(\alpha\), nitrites and PGE-2 release in the medium as well as the expression of iNOS and COX2 mRNA, independently of the treatment. LPS induces a significant increase of TNF\(\alpha\) released in the medium after 2 h and 20 h of incubation (Fig. 1A and B) and a

![Fig. 1. Effect of LPS and Qx on TNF\(\alpha\) released by PCLS (after 2 h – A- and 20 h – B) and TNF\(\alpha\) mRNA levels of PCLS (after 2 h – C- and 20 h – D). PCLS were incubated in the presence or absence of 100 \(\mu\)M Qx as well as in the presence or absence of 10 \(\mu\)g/ml LPS for 2 h or 20 h. Medium were as following: control medium (CT), medium supplemented with Qx (Qx), medium supplemented with LPS (CT-LPS) or medium supplemented with LPS and Qx (Qx-LPS). Data are mean ± SEM (n \(\geq\) 4). Data with different superscript letters are significantly different \(P < 0.05\), according to the post hoc ANOVA statistical analysis (One-way ANOVA). Qx, reduced form of coenzyme Q10; LPS, lipopolysaccharide; PCLS, precision-cut liver slices; TNF\(\alpha\), tumor necrosis factor-\(\alpha\).]
significant increase in TNFα mRNA level after 2 h, but not after 20 h (Fig. 1C and D). Qx does not affect neither TNFα release nor mRNA expression (Fig. 1).

Nitrite released in the medium is not modified by the treatments after 2 h of incubation (Fig. 2A), but its level increased by LPS after 20 h, without any changes following Qx treatment (Fig. 2B). After 2 h and 20 h of incubation (Fig. 2C and D), iNOS mRNA levels in PCLS shows the same pattern as nitrite release after 20 h, while the LPS-promoted induction of iNOS is statistically significant only after 20 h of incubation. Nevertheless, Qx incubation does not modify iNOS expression whatever conditions (Fig. 2).

PGE₂ released in the medium is increased after 2 h and 20 h of incubation with LPS but this increased is only significant after 2 h of incubation. PCLS incubated with Qx alone or Qx-LPS show intermediary level -without significance-between CT and LPS conditions (Fig. 3A and B). COX-2 mRNA level is not modified by the treatments (Fig. 3C and D).

Nor LPS, neither Qx addition in the incubation medium induce AST release in the medium, signing no cell lytic effect of the compounds at the tested dose. However, after 20 h of incubation, AST released in the medium is increased as compared to 2 h values, independently of LPS and Qx treatment (data not shown).

3.2. Effect of dietary enrichment with Qx towards LPS challenge performed in vivo

Markers of inflammation (TNFα, iNOS and COX-2 mRNA level), recruitment of macrophages (MCP1 mRNA level; Fig. 4) and toxicity (serum AST) have been assessed 1 h after LPS challenge (0.1 mg/kg, ip). LPS injection induces a huge and significant increase in liver TNFα, COX-2 and MCP-1 expression (Fig. 4A, C, 4D); the induction of iNOS expression being not significant (Fig. 4B). After 2 weeks of Qx

![Fig. 2. Effect of LPS and Qx on nitrite released by PCLS (after 2 h – A- and 20 h – B) and iNOS mRNA levels of PCLS (after 2 h – C- and 20 h – D). PCLS were incubated in the presence or absence of 100 μM Qx as well as in the presence or absence of 10 μg/ml LPS for 2 h or 20 h. Medium were as following: control medium (CT), medium supplemented with Qx (Qx), medium supplemented with LPS (CT-LPS) or medium supplemented with LPS and Qx (Qx-LPS). Data are mean ± SEM (n ≥ 4). Data with different superscript letters are significantly different p < 0.05, according to the post hoc ANOVA statistical analysis (One-way ANOVA). Qx, reduced form of coenzyme Q10; LPS, lipopolysaccharide; PCLS, precision-cut liver slices; iNOS, inducible NO synthase.]
dietary treatment, no significant changes of the expression of LPS-induced inflammatory markers occur, even at a dose of 1% of Qx. Of note, 1% Qx pretreatment tends to decrease AST level whereas LPS injection does not modify serum AST level (AST level (means ± SEM in UI/L): CT 64.48 ± 10.04, CT-LPS 77.57 ± 20.50, Qx 0.4%-LPS 73.61 ± 17.65 and Qx 1%-LPS 40.02 ± 5.39).

4. Discussion

The potential anti-inflammatory effect of CoQ10 or its reduced form has been suggested through in vitro and in vivo studies [8]. From in-silico study (50 μM LPS on Caco-2 cells during 24 h), Schmelzer et al. [21] showed the upregulation of genes controlling inflammation i.e. interleukine-5 or C reactive protein. They observed in vitro the modulation of inflammatory cytokines such as TNFα in human (THP-1) and murine monocyte (RAW264.7) cell lines after pretreatment with CoQ10 or Qx during 24 h and LPS challenge (1 or 5 μg/ml during 4 h) [13,15,22]. Another group showed the suppression of interleukin-1 (100 pg/ml)-induced inflammatory response in human dermal fibroblasts incubated for 24 h together with interleukin-1 in the presence of CoQ10 (10 μM) [12]. Here, we have investigated for the first time the anti-inflammatory potential of Qx, the reduced form of CoQ10 on liver inflammation induced by acute LPS challenge in the original model of PCLS maintain cell interactions. We confirmed that LPS challenge was able to produce inflammatory stress inside the liver tissue independently of any cell lysis. Indeed, LPS induced the expression of TNFα upon short term incubation with LPS, leading to the secretion of TNFα within 2 h of incubation; this effect being more pronounced after 20 h. Those
results are in accordance with studies performed by other authors \[1,4,20,23\]. Furthermore, we observed an increase in iNOS mRNA 20 h after the incubation with LPS, and the relevance of this effect was supported by the higher amount of nitrite (NO$_2$ metabolite) released in the medium at that time point. Elferink et al. showed an induction of iNOS mRNA expression within a period of time ranging from 3 h to 24 h of incubation in the presence of LPS in rat and human liver slices [23]. In vivo, the kinetics of increased iNOS expression was observed after LPS challenge in the liver of mice, with a 100-fold increase remaining 6 and 18 h after the LPS challenge [24]. Interestingly, we could also show an increase in the secretion of PGE$_2$ by PCLS treated with LPS. This was coordinated with the changes in COX-2 mRNA, and required also long-term incubation, in accordance with the previous data published by Neyrinck et al. [3]. The higher production of PGE$_2$ could contribute to the decrease in TNF$\alpha$ expression observed after 20 h of incubation, as previously described [2]. Whatever the parameter taken into account, we were unable to show any significant effect of Qx on major inflammatory markers such as a cytokine –TNF$\alpha$ –, a prostaglandin –PGE2– or a nitrogen species – nitrite-released upon LPS activation in our in vitro model.

Before concluding for the lack of anti-inflammatory effect of CoQ10 in the liver, we need to investigate LPS challenge in vivo. Indeed, other mechanisms involving different organ interactions (for
example the contribution of the spleen [25]) or cell recruitment (monocytes and neutrophils) may explain why the increase in TNFα mRNA expression upon LPS in PCLS remains really modest as compared to the 100 fold increased in TNFα mRNA expression observed in vivo 1 h after LPS injection in this study. The same observation can be made concerning COX2: 1 h after the injection of LPS, liver COX2 mRNA expression was 20 fold increased in this study, whereas we did not find any changes in these parameters upon LPS challenge in vitro. Therefore, we decided to test the implication of dietary pretreatment with CoQ10 on mice. Indeed, promising data have been obtained in mice models of obesity. In a model of high-fat induced obesity associated with inflammation, we have shown that mice fed orally with CoQ10 (1% in the diet during 8 weeks) exhibit a decrease in a panel of markers of oxidative and inflammatory stress in the liver [19]. Furthermore, intraperitoneal injection of CoQ10 (10 mg/kg/day ip during 13 days) modulated the expression of pro-inflammatory cytokines in the adipose tissue of ob/ob mice (decrease in TNFα mRNA level) [26]. Even if the bioavailability of ingested CoQ10 is low, we and others have shown that liver is a storage pool of CoQ10 [27,28]; COQ10 level being 40-fold higher following CoQ10 supplementation during 8 weeks at the dose of 1% in the diet [19].

In the present study, an acute load of LPS did not affect iNOS expression, but produced a huge increase in the expression of TNFα and COX-2 in the liver. Those increases in COX-2 and TNFα expression were associated with a recruitment of immune cells that can be dependent on the huge increase of liver MCP1 mRNA expression after LPS treatment. Pretreatment with Qx does not modify any key parameter involved in inflammatory stress induced by an acute load of LPS. Those data contrast with our previous data obtained in a model of high-fat fed mice with chronic low level inflammation, and showing that Q10 was able to lessen a global hepatic inflammatory score, based on the mRNA level of TNFα, interleukine-6, C reactive protein, STAMP2 and NADPH oxidase [19]. We can interpret those differences through the fact that here we used a massive LPS dose mimicking a septic shock. This LPS condition is really different of the low grade chronic inflammation observed in obese models. Several studies performed in animals with acute inflammation induced by LPS suggest beneficial effects due to CoQ10 supplementation (see [8] for review). For example, Abd El-Gawad and Khalifa showed that animals with LPS-induced brain inflammation (10 mg/kg ip) are protected against oxidative stress (lipid peroxidation and nitric oxide production) after 7-days pretreatment with CoQ10 (200 mg/kg per os) [29]. Lelli et al. found a protective effect of CoQ supplementation on free-radical-mediated lipid peroxidation assessed by fluorescent products during septic shock in dogs [30]. Sugino et al. showed that CoQ10 supplementation could reduce the LPS-induced lipid peroxidation in mice, which might have been associated with the enhanced survival of CoQ10-supplemented mice [31]. However, none of these studies demonstrated a lower hepatic inflammation due to CoQ10 treatment.

In conclusion, Qx is not able to lessen inflammatory stress occurring upon acute LPS challenge in the liver of rodents. However, in our experiment in vivo, we focused on the modifications of the expression of genes coding for pro-inflammatory markers. As a perspective, it could be interesting to take into account the fact that Qx is able to modulate post-transcriptional events, by changing namely the miRNA-146a expression [32]. Anyway, CoQ10 remains an interesting anti-inflammatory nutrient in certain contexts, since, for example, it can modulate liver inflammation in chronic pathologies characterized by a low grade inflammation such as obesity.

Conflict of interest

None declared.

Acknowledgments

This work was supported by Kaneka Inc. LBB is a Postdoctoral Researcher from the F.R.S.-FNRS (Fond National de la Recherche Scientifique, Belgium). PDC is a Research Associate from the F.R.S.-FNRS and is a recipient of ERC Starting Grant 2013 (European Research Council, Starting grant 336452-ENIGMO. NMD and PDC are recipient of grants from FNRS.
References