In Vitro Effects of the Reduced Form of Coenzyme Q$_{10}$ on Secretion Levels of TNF-α and Chemokines in Response to LPS in the Human Monocytic Cell Line THP-1

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Summary Ubiquinol-10 (QH$_2$), the reduced form of Coenzyme Q$_{10}$ (CoQ$_{10}$) serves as a potent antioxidant of lipid membranes. Because many antioxidants reveal potent anti-inflammatory effects, the influence of QH$_2$ on lipopolysaccharide (LPS)-induced pro-inflammatory cytokines and chemokines were determined in the human monocytic cell line THP-1. Stimulation of cells with LPS resulted in a distinct release of Tumour necrosis factor-alpha (TNF-α), Macrophage inflammatory protein-1 alpha (MIP-1α), Regulated upon activation, normal T cell expressed and secreted (RANTES) and Monocyte chemotactrant protein-1 (MCP-1). The LPS-induced responses were significantly decreased by pre-incubation of cells with QH$_2$ to 60.27 ± 9.3% ($p = 0.0009$), 48.13 ± 6.93% ($p = 0.0007$) and 74.36 ± 7.25% ($p = 0.008$) for TNF-α, MIP-1α and RANTES, respectively. In conclusion, our results indicate anti-inflammatory effects of the reduced form of CoQ$_{10}$ on various proinflammatory cytokines and chemokines in vitro.

Key Words: coenzyme Q$_{10}$, ubiquinol-10, inflammation, monocytes

Introduction Exposure of cells to the pro-inflammatory lipopolysaccharide (LPS) triggers TLR4-dependent phosphorylation cascades which lead to activation of NFkB. This central transcription factor induces the expression and subsequent secretion of various pro-inflammatory cytokines and chemokines [1–3]. Reactive oxygen species (ROS) are important for the activity of the TLR4-signalling pathway [4]. Accordingly, antioxidants are described as anti-inflammatory agents [5, 6]. Because Coenzyme Q$_{10}$ (CoQ$_{10}$) is a potent antioxidant, we postulated that this molecule possesses anti-inflammatory properties. More recently was shown that CoQ$_{10}$ supplementation minimizes oxidative stress during statin drug therapy [7]. Indeed, we found a reduction of LPS-induced cytokine release by CoQ$_{10}$ in murine and human monocytic cell lines [8]. In the latter study, we treated cells with the oxidized form of CoQ$_{10}$ (ubiquinone-10) which is converted intracellular to ubiquinol-10 (QH$_2$). As only the reduced form of CoQ$_{10}$ can act as an antioxidant, here we studied effects on secretion of the cytokine TNF-α and different chemokines in LPS-stimulated THP-1 cells that were directly incubated with QH$_2$.

Material and Methods Reagents Lipopolysaccharide (LPS, E.coli O55:B5) was obtained from Sigma-Aldrich (Taufkirchen, Germany). The aqueous solutions of ubiquinol-10 (PEG-60 hydrogenated castor oil, ubiquinol-10, glycerol, water) and the corresponding vehicle (no ubiquinol-10 supplement) were received from KANEKA Corporation (Osaka, Japan).
Cell culture

Cultivation of THP-1 cells occurred routinely in RPMI medium 1640 supplemented with 10% FCS and 1% antibiotics (penicillin/streptomycin) in a humidified incubator containing 5% CO₂ at 37°C. For determination of TNF-α and chemokines, cells were plated at a density of 0.5 × 10⁶ cells in a 12-well plate for 24 h before pre-incubation. Subsequently, cells were preincubated with either 10 μM ubiquinol-10 or the reference substances pyrrolidine-dithiocarbamate (PDTC) or N-acetyl-cysteine (NAC), or the respective vehicle control. After 24 h, cell culture medium was removed and fresh LPS-containing medium (1 μg/ml) was added for 4 h. Finally, for cytokine determination via ELISA, supernatants were kept and stored at −80°C. For protein determination via the BRADFORD method, cells were collected into NET-buffer.

Cytotoxicity

For determination of cell viability, the Cell-Titer Glo® Luminescent Assay was used. Thus, total ATP levels were measured as an index of the viable cell number. The luminescence was detected on a GloMax® (Promega, Mannheim, Germany).

Determination of TNF-α and chemokines

Using TNF-α as an internal control, this cytokine was determined by DuoSet ELISA (R&D Systems, Wiesbaden, Germany) as well as multiplex suspension array technology (BioRad, Munich, Germany) according to the manufacturer’s instructions. The chemokines MCP-1, MIP-1α and RANTES were determined by the multiplex suspension array system.

Protein concentration

Cells were collected into NET-buffer (50 mM TRIS [pH 7.5], 150 mM NaCl, 1 mM EDTA [pH 8.0], 0.5% NP-40) and the cell suspension was treated with ultrasonics and then centrifuged by 14000 rpm at 4°C for 20 min. Determination of protein concentration occurred in the resulting supernatant by the Bradford method according to the manufacturer’s instructions.

Statistics

All data are results of two (PDTC, NAC) or three (QH₂) independent biological experiments performed in duplicate and expressed as means ± standard error of the mean (SEM). Results were analyzed by an unpaired two-sided Student’s t-test using SPSS 11.5 for Windows and GraphPad Prism 4.0 software. p-values less than or equal to 0.05 were considered statistically significant.

Results and Discussion

NFκB is a multisubunit transcription factor that is ubiquitously expressed in different cell types and can be activated by several agents such as LPS, TNF-α or the oxidant hydrogen peroxide (H₂O₂) [9]. This activation process includes phosphorylation of the IκB subunit and its dissociation from the inactive cytoplasmic complex. Thus, the active dimer of p50 and p65 translocates into the nucleus where specific target genes of pro-inflammatory mediators and cytokines become immediately up-regulated [9, 10]. However, this NFκB-activating cascade was shown to be inhibited by antioxidants such as PDTC and NAC. This has led to the hypothesis that oxygen radicals are key players in the activation of NFκB through an redox-dependent mechanism [9, 11, 12]. Because many antioxidants reveal potent anti-inflammatory effects, the influence of QH₂ on LPS-induced pro-inflammatory cytokines and chemokines was determined in the human monocyte cell line THP-1. All experiments were performed with the well known radical scavengers PDTC and NAC to validate the putative anti-inflammatory effects of QH₂. To implement culture conditions that do not lead to unspecific side effects, cell vitality was measured at different medium concentrations of QH₂. As shown in Table 1, no cytotoxic effects were found for PDTC (100 μM), NAC (100 μM) and QH₂ (1, 10, 100 μM). For further experiments we used 10 μM QH₂, because this concentration leads to a significantly higher intracellular QH₂ content in THP-1 cells and is also achievable in human serum through QH₂ supplementation (unpublished results). As shown in Figure 1A-D, unstimulated THP-1 monocyes secrete low amounts of the pro-inflammatory cytokine TNF-α and chemokines MIP-1α, RANTES and MCP-1 into the medium. However, stimulation with LPS induces approximately 58-(TNF-α), 7-(MIP-1α), 2-(RANTES) and 4-(MCP-1) fold higher levels of these pro-inflammatory

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agents in the cell culture medium within 4 h, respectively. Next, we tested the effect of pre-incubation of cells with 10 μM QH\(_2\) for 24 h. Thus, as shown in Figure 1A–C, the LPS-induced responses were significantly decreased to 60.27 ± 9.3%, 48.13 ± 6.93% and 74.36 ± 7.25% for TNF-α, MIP-1α and RANTES, respectively (Fig. 1A–C). No significant effect was found for MCP-1 (Fig. 1D). Pre-incubation of cells with 10 μM PDTC or 10 μM NAC decreased TNF-α levels significantly to 47.69 ± 19.07% and 54.43 ± 17.64%, respectively (Fig. 1A). No significant effects of PDTC and NAC were found on LPS-induced secretion levels of other pro-inflammatory mediators.

Inflammation has been related to the pathogenesis of various diseases, such as atherosclerosis [13]. Monocytes play an important role in the response to inflammatory agents, particularly to those derived from gut bacteria and are able to enter the circulation, such as bacterial endotoxins. Thus, endotoxins circulate at low concentrations in the blood of all healthy individuals, but are also increased after a high-fat meal [14]. However, elevated levels are associated with an increased risk of atherosclerosis or sepsis [14–16]. For our experiments we used LPS, a compound of gram-negative bacteria that is also relevant in vivo to trigger a serious medical inflammatory process in vitro. Finally, stimulation of monocytes with LPS induces production of ROS, which in turn activate the transcription factor NFκB [4, 9] that triggers a large amount of genes encoding for inflammatory mediators and cytokines [17]. Numerous studies in monocytes revealed natural occurring antioxidants as compounds with anti-inflammatory effects [18, 19]. Here we used CoQ\(_{10}\) in order to study its putative anti-inflammatory effect in the human monocytic cell line THP-1. For this purpose, we used the reduced form of CoQ\(_{10}\), QH\(_2\), which functions as an antioxidant. To our knowledge, effects of QH\(_2\) on inflammatory markers have not been investigated so far, because this form of CoQ\(_{10}\) is not commonly available. Thus, only a few studies were published using QH\(_2\) in vivo. These studies indicate effects

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Fig. 1. Effects of pre-treatment of ubiquinol on LPS-induced release of TNF-α (A), MIP-1α (B), RANTES (C) and MCP-1 (D) in THP-1 cells.

Cells were either pre-treated with 10 μM QH\(_2\) or the respective reference substances PDTC or NAC, or medium and vehicle for 24 h. Afterwards, media were removed and cells were treated with LPS (1 μg/ml medium) for 4 h. The resulting concentrations (pg/μg cellular protein) of TNF-α, MIP-1α, RANTES and MCP-1 of the vehicle controls (+LPS) were set to 100% for QH\(_2\)-pretreated cells and the other values were referenced to it. Values from PDTC- and NAC-pretreated cells were related to medium controls (+LPS) taken as 100%. Statistically significant data (*, \(p \leq 0.05\); **, \(p \leq 0.01\); ***, \(p \leq 0.001\)) are means ± SEM of four (ubiquinol, vehicle) or two (PDTC, NAC, medium) independent experiments performed in duplicate.
of QH: on safety and bioavailability [20], sperm kinetic features [21], oxidative imbalance in children with Trisomy 21 [22] and neuroprotection in an animal Parkinson model [23]. In THP-1 cells, we found that QH2 reduces significantly the secretion of the pro-inflammatory agents TNF-α, MIP-1α, and RANTES in response to LPS. This putative anti-inflammatory effect of QH2 could be due to its anti-oxidant property in cell membranes, because LPS-induced ROS production occurs very closely to the membrane [4]. One important membrane-associated complex that is relevant for generation of ROS in monocytic cells [24] is the NADPH oxidase. This complex is described to consist of four proteins, whereas Rac is the most critical component for a functional NADPH oxidase. Rac is regulated by small GTP-binding proteins [25]. In this context it was shown that LPS induced Rac activity and moreover, the NADPH oxidase-dependent ROS formation [26]. Thus it seems that LPS directly initiates the NADPH oxidase activity by downstream signalling pathways. Results from a further study indicate an up-regulation of the NADPH oxidase complex through a NFκB-activation via reduction of H2O2 [9], an important reactive oxygen intermediate (ROI) of the NADPH oxidase pathway. These strong radical scavenging effects are also described for PDTC [27], which we used as an additional internal control to describe the putative anti-inflammatory effects of the reduced form of CoQ10 (QH2), a compound with strong anti-oxidant properties. In general it seems that QH2 mediates stronger anti-inflammatory effects on the tested pro-inflammatory compounds than PDTC and NAC, two well known radical scavengers mediating its anti-inflammatory properties through a diminished NFXB activation. Thus it seems that the reduced form of CoQ10 (QH2) mediates its anti-inflammatory effects at least in part through its strong antioxidant properties. However, these effects may be additionally mediated by gene expression. It has been shown in skeletal muscle of humans [28], heart of mice [29], CaCo-2 [30], and HeLa cells [31] that CoQ10 influences the expression of different genes. These hypotheses should be tested in future studies. In conclusion, our results indicate anti-inflammatory effects of the reduced form of CoQ10 on various proinflammatory cytokines and chemokines in vitro.

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References

[1] Bubici, C., Papa, S., Pham, C.G., Zazzeroni, F., and Franzoso, G: The NF-kappaB-mediated control of ROS and JNK signaling, *Histol. Histopathol.*, 21, 69–80, 2006.

[2] Takashiba, S., Van Dyke, T.E., Amar, S., Murayama, Y., Sokolne, A.W., and Shapiro, L.: Differentiation of monocytes to macrophages primes cells for lipopolysaccharide stimulation via accumulation of cytoplasmic nuclear factor kappaB. *Infect. Immun.*, 67, 5573–5578, 1999.

[3] Handa, O., Naito, Y., Takagi, T., Shimozawa, M., Kokura, S., Yoshida, N., Matsui, H., Cepinskas, G., Kvietys, P.R., and Yoshikawa, T.: Tumor necrosis factor-alpha-induced cytokine-induced neutrophil chemoattractant-1 (CINC-1) production by rat gastric epithelial cells: role of reactive oxygen species and nuclear factor-kappaB. *J. Pharmacol. Exp. Ther.*, 309, 670–676, 2004.

[4] Sanlioglu, S., Williams, C.M., Samavati, L., Butler, N.S., Wang, G., McCray, P.B. Jr, Ritchie, T.C., Hunninghake, G.W., Zandi, E., and Engelhardt, J.F.: Lipopolysaccharide induces Rac1-dependent reactive oxygen species formation and coordinates tumor necrosis factor-alpha secretion through IkK regulation of NF-kappaB B. *J. Biol. Chem.*, 276, 30188–30198, 2001.

[5] Parmentier, M., Hirani, N., Rahman, I., Donaldson, K., MacNee, W., and Antonicelli, F.: Regulation of lipopolysaccharide-mediated interleukin-1beta release by N-acetylcysteine in THP-1 cells. *Eur. Respir. J.*, 16, 933–939, 2000.

[6] Geronikaki, A.A. and Gavalas, A.M.: Antioxidants and inflammatory disease: synthetic and natural antioxidants with anti-inflammatory activity. *Comb. Chem. High Throughput Screen*, 9, 425–442, 2006.

[7] Kettawan, A., Takahashi, T., Kongkachuvichai, R., Charoenkiatkul, S., Kishi, T., and Okamoto, T.: Protective effects of coenzyme q(10) on decreased oxidative stress resistance induced by simvastatin. *J. Clin. Biochem. Nutr.*, 40, 194–202, 2007.

[8] Mitsui, Y., Schmelzer, J.D., Zollman, P.J., Mitsui, M., Tritschler, H.J., and Low, P.A.: Alpha-lipoic acid provides neuroprotection from ischemia-reperfusion injury of peripheral nerve. *J. Neurol. Sci.*, 163, 11–16, 1999.

[9] Schreck, R., Rieber, P., and Baeuerle, P.A.: Reactive oxygen intermediates as apparently widely used messengers in the activation of the NF-kappa B transcription factor and HIV-1. *Embo. J.*, 10, 2247–2258, 1991.

[10] Ghosh, S. and Baltimore, D.: Activation in vitro of NF-kappa B by phosphorylation of its inhibitor I kappa B. *Nature*, 344, 678–682, 1990.

[11] Schreck, R., Bevec, D., Dukor, P., Baeuerle, P.A., Chedid, L., and Bahr, G.M.: Selection of a muramyl peptide based on its lack of activation of nuclear factor-kappa B as a potential adjuvant for AIDS vaccines. *Clin. Exp. Immunol.*, 90, 188–193, 1992.

[12] Schreck, R. and Baeuerle, P.A.: A role for oxygen radicals as second messengers. *Trends Cell Biol.*, 1, 39–42, 1991.

[13] Ross, R.: The pathogenesis of atherosclerosis: a perspective
for the 1990s. *Nature*, 362, 801–809, 1993.

[14] Erridge, C., Attina, T., Spickett, C.M., and Webb, D.J.: A high-fat meal induces low-grade endotoxemia: evidence of a novel mechanism of postprandial inflammation. *Am. J. Clin. Nutr.*, 86, 1286–1292, 2007.

[15] Bruunsgaard, H., Pedersen, A.N., Schroll, M., Skinhoj, P., and Pedersen, B.K.: Impaired production of proinflammatory cytokines in response to lipopolysaccharide (LPS) stimulation in elderly humans. *Clin. Exp. Immunol.*, 118, 235–241, 1999.

[16] Peng, T., Shen, E., Fan, J., Zhang, Y., Arnold, J.M., and Feng, Q.: Disruption of phospholipase C Gammal signalling attenuates cardiac tumor necrosis factor-alpha expression and improves myocardial function during endotoxemia. *Cardiovasc. Res.*, 78, 90–97, 2008.

[17] Shakhov, A.N., Collart, M.A., Vassalli, P., Nedospasov, S.A., and Jongeneel, C.V.: Kappa B-type enhancers are involved in lipopolysaccharide-mediated transcriptional activation of the tumor necrosis factor alpha gene in primary macrophages. *J. Exp. Med.*, 171, 35–47, 1990.

[18] Antonicelli, F., Brown, D., Parmentier, M., Drost, E.M., Hiran, N., Rahman, I., Donaldson, K., and MacNee, W.: Regulation of LPS-mediated inflammation in vivo and in vitro by the thiol antioxidant Nacystelyn. *Am. J. Physiol. Lung Cell Mol. Physiol.*, 286, L1319–1327, 2004.

[19] Sacanella, E., Vazquez-Agell, M., Mena, M.P., Antunez, E., Fernandez-Sola, J., Nicolas, J.M., Lamuela-Raventos, R.M., Ros, E., and Estruch, R.: Down-regulation of adhesion molecules and other inflammatory biomarkers after moderate wine consumption in healthy women: a randomized trial. *Am. J. Clin. Nutr.*, 86, 1463–1469, 2007.

[20] Hosoe, K., Kitano, M., Kishida, H., Kubo, H., Fujii, K., and Kitahara, M.: Study on safety and bioavailability of ubiquinol (Kaneka QH) after single and 4-week multiple oral administration to healthy volunteers. *Regul. Toxicol. Pharmacol.*, 47, 19–28, 2007.

[21] Balercia, G., Mosca, F., Mantero, F., Boscaro, M., Mancini, A., Riccardi-Lamonica, G., and Littarru, G.: Coenzyme Q10 supplementation in infertile men with idiopathic asthenozoospermia: an open, uncontrolled pilot study. *Fertil. Steril.*, 81, 93–98, 2004.

[22] Miles, M.V., Patterson, B.J., Chalfonte-Evans, M.L., Horn, P.S., Hickey, F.J., Schapiro, M.B., Steele, P.E., Tang, P.H., and Hotze, S.L.: Coenzyme Q10 (ubiquinol-10) supplementation improves oxidative imbalance in children with trisomy 21. *Pediatr. Neurol.*, 37, 398–403, 2007.

[23] Cleren, C., Yang, L., Lorenzo, B., Calingasan, N.Y., Schomer, A., Sirci, A., Wille, E.J., and Beal, M.F.: Therapeutic effects of coenzyme Q10 (CoQ10) and reduced CoQ10 in the MPTP model of Parkinsonism. *J. Neurochem.*, 104, 1613–1621, 2008.

[24] Gauss, K.A., Nelson-Ovort, L.K., Siemsen, D.W., Gao, Y., DeLeo, F.R., and Quinn, M.T.: Role of NF-kappaB in transcriptional regulation of the phagocyte NADPH oxidase by tumor necrosis factor-alpha. *J. Leukoc. Biol.*, 82, 729–741, 2007.

[25] Bokoch, GM.: Regulation of the phagocyte respiratory burst by small GTP-binding proteins. *Trends Cell Biol.*, 5, 109–113, 1995.

[26] Hsu, H.Y. and Wen, M.H.: Lipopolysaccharide-mediated reactive oxygen species and signal transduction in the regulation of interleukin-1 gene expression. *J. Biol. Chem.*, 277, 22131–22139, 2002.

[27] Chandel, N.S., Trzyna, W.C., McClintock, D.S., and Schumacker, P.T.: Role of oxidants in NF-kappa B activation and TNF-alpha gene transcription induced by hypoxia and endotoxin. *J. Immunol.*, 165, 1013–1021, 2000.

[28] Linnane, A.W., Kopsidas, G, Zhang, C., Yarovaya, N., Kovalenko, S., Papakostopoulos, P., Eastwood, H., Graves, S., and Richardson, M.: Cellular redox activity of coenzyme Q10: effect of CoQ10 supplementation on human skeletal muscle. *Free Radic. Res.*, 36, 445–453, 2002.

[29] Lee, C.K., Pugh, T.D., Klopp, R.G., Edwards, J., Allison, D.B., Weindruch, R., and Prolla, T.A.: The impact of alpha-lipoic acid, coenzyme Q10 and caloric restriction on life span and gene expression patterns in mice. *Free Radic. Biol. Med.*, 36, 1043–1057, 2004.

[30] Gronenbor, D.A., Kindermann, B., Alhammer, M., Klapper, M., Vormann, J., Littarru, G.P., and Doring, F.: Coenzyme Q10 affects expression of genes involved in cell signalling, metabolism and transport in human CaCo-2 cells. *Int. J. Biochem. Cell Biol.*, 37, 1208–1218, 2005.

[31] Gorelick, C., Lopez-Jones, M., Goldberg, G.L., Romney, S.L., and Khabele, D.: Coenzyme Q10 and lipid-related gene induction in HeLa cells. *Am. J. Obstet. Gynecol.*, 190, 1432–1434, 2004.