Mitoquinone (mitoQ) exerts antioxidant effects independent of mitochondrial targeted effects in phorbol-12-myristate-13-acetate (PMA) or N-formyl-M-leucyl-L-phenylalanine (fMLP) stimulated polymorphonuclear leukocyte (PMN) superoxide (SO) release

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Introduction

PMNs have been identified as one of the main mechanisms underlying ischemia reperfusion (I/R) injury. Their tendency to injure the myocardium, coronary endothelium, and myocytes is caused by proinflammatory mediators such as tumor necrosis factor α and interleukin 8. PMNs contain an extensive cytoxic arsenal and their potential to destroy tissue is caused by degranulation and generation of reactive oxygen species (ROS). The activation of the PMN membrane-associated NADPH oxidase system by proinflammatory cytokines, C5a, platelet activating factor, and particulate stimuli initiates a respiratory burst characterized by a marked increase in cellular oxygen consumption and the generation of SO anions. Protein kinase C (PKC) activation of PMN NADPH oxidase is essential to generate PMN SO release (1). PML peptide activates PMN NADPH oxidase to stimulate NADPH oxidase via PKC. PKC is a lipid soluble broad spectrum PKC agonist that directly activates PKC (Fig. 1). Inhibition of PMN SO release attenuates inflammation mediated vascular injury (e.g. I/R).

Methods

Isolation of PMNs

Male Sprague-Dawley rats (350–400 g, Charles River, Springfield, MA), used as PMN donors, were anesthetized with isoflurane and given a 16 ml intraperitoneal injection of 0.5% glycerol (Sigma Chemical) dissolved in PBS. Rats were reanesthetized with isoflurane 6–18 h later, and the PMNs were harvested by peritoneal lavage in 30 ml of 0.9% NaCl as previously described (4). The peritoneal lavage fluid was centrifuged at 200 for 10 min at 4°C. The PMNs were then washed in 20 ml PBS and centrifuged at 200 g for 10 min at 4°C. Thereafter, the PMNs were resuspended in 5 ml PBS and concentrations were calculated. The PMN preparations were >90% pure and >95% viable according to microscopic analysis and 0.3% trypan blue, respectively.

Measurement of SO Release From Rat PMNs

The SO release from PMNs was measured spectrophotometrically (model 260 Gilford, Nova Biotech; El Cajon, CA) by the reducing of ferricytochrome c (4). The PMNs (1x10⁶) were resuspended in 450 µl PBS and incubated with ferricytochrome c (100 µM, Sigma Chemical) in a total volume of 900 µl PBS in the presence or absence of mitoQ (10-1 µM) or other CoQ analogs (10-80 µM) for 15 min at 37°C in spectrophotometric cells. The PMNs were stimulated with 100 nM PMA (Sigma Chemical) or 1 µM fMLP (Calbiochem) in a final reaction volume of 1.0 ml. Positive control samples were given SO dismutase (SOD; 10 µg/ml) just before the addition of PMA or fMLP. Absorbance was measured every 30 sec for up to 160 sec (peak response) for PMA and 180 sec for fMLP (peak response), and the change in absorbance (SO release) from PMNs was determined relative to time 0.

Cell Viability

Cell viability was determined by combining 0.5 ml of the samples from spectrophotometric release) from PMNs was determined relative to time 0.

Results

Isolation of PMNs

Figure 1. Schematic representation of PKC activation generating SO in a PMN. PMN chemotactic G-protein receptors are activated by (MLP), complement C5a, and IL-8. The G-protein subunits αq and β subunits dissociate after stimulation and activate phospholipase Cβ (PLC) and phospholipid insitol-3,4,5-trisphosphate (IP₃) to produce IP₃. IP₃ stimulates adenyl cyclase (DAG) and IP₃ binds Ca²⁺, respectively. Ins(1,4,5)P₃ stimulates Ca²⁺ release from the endoplasmic reticulum (ER). Ca²⁺ binds DAG and IP₃, directly activates PKC. PKC directly activates PKC. PKC phosphorylates NADPH oxidase to release SO anion (GS). Adapted from (1).

Hypothesis

We hypothesized that MitoQ and other CoQ analogs would dose-dependently attenuate both PMA and fMLP induced SO release in PMNs without affecting cell viability.

Figure 2. Illustration of the reduced and oxidized forms of mitoQ and CoQ analogs. The reduced form, ubiquinol, acts as an antioxidant free radical scavenger via hydrogen atom transfer.

Figure 3. The dose-dependent effects of MitoQ on PMN (100 nM) and fMLP (1µM)-induced SO release in PMNs. The peak response in PMN-induced SO release (black). The peak response in fMLP-induced SO release (blue). MitoQ (100 nM and mitoQ (200 nM) significantly attenuated PMN-induced SO release by 48% (*P<0.01) and 71% (**P<0.01) respectively compared to PMN. MitoQ (100 nM and mitoQ (200 nM) significantly attenuated fMLP-induced SO release by 90% (n=6, absorbance = 0.004 ± 0.000) and fMLP-induced SO release by 90% (n=4, absorbance = 0.016 ± 0.004).

Figure 4. The dose-dependent effects of CoQ analogs on PMN (100 nM) and fMLP (1µM)-induced SO release in PMNs. The peak response in PMN-induced SO release (black). The peak response in fMLP-induced SO release (blue). CoQ1, CoQ4, CoQ10, and DQ had no significant effect on cell viability. CoQ1 dose-dependently significantly attenuated PMA-induced SO release, DQ significantly inhibited PMA-induced SO release by 38% (**P<0.01) and 51% (**P<0.01) respectively compared to PMA.

Figure 5. The dose-dependent effects of CoQ analogs on PMN (100 nM) and fMLP (1µM)-induced SO release in PMNs. The peak response in PMA-induced SO release (black). The peak response in fMLP-induced SO release (blue). CoQ1, CoQ4, CoQ10, and DQ had no significant effect on cell viability. CoQ1 dose-dependently significantly attenuated PMA-induced SO release by 38% (**P<0.01) and 51% (**P<0.01) respectively compared to PMA.

Figure 6. The dose-dependent effects of CoQ analogs on PMN (100 nM) and fMLP (1µM)-induced SO release in PMNs. The peak response in PMA-induced SO release (black). The peak response in fMLP-induced SO release (blue). CoQ1, CoQ4, CoQ10, and DQ had no significant effect on cell viability. CoQ1 dose-dependently significantly attenuated PMA-induced SO release by 38% (**P<0.01) and 51% (**P<0.01) respectively compared to PMA.

Figure 7. The Effects of CoQ analogs on Cell Viability in PMA (100 nM) and fMLP (1 µM)-induced SO release in PMNs. Cell Viability in PMA-induced SO release (right) MitoQ (100 nM) and mitoQ (200 nM) significantly attenuated cell viability by 28% (**P<0.01) compared to PMA. MitoQ (100 nM) and mitoQ (200 nM) significantly attenuated cell viability by 38% (**P<0.01) compared to fMLP.

Summary of Results

MitoQ and CoQ10 dose-dependently attenuated both PMA and fMLP-induced SO release in PMNs. DCQ significantly inhibited fMLP-induced SO release by 48% (**P<0.01) and 67% (**P<0.01) respectively compared to PMA and fMLP. CoQ1, CoQ4, CoQ10, and DCQ significantly augmented the fMLP-induced SO release. CoQ1 significantly decreased the PMA-induced response. DQ and CoQ4 had no significant effect on PMN or fMLP-induced SO release (data not shown). MitoQ significantly decreased cell viability in both PMA (20 µM) and fMLP (10 µM) stimulated PMNs. CoQ1, CoQ4, and DQ had no significant effect on cell viability.

Conclusions

These results suggested that mitoQ exerted its antioxidant effects independent of the mitochondria and were related in part to cell viability. CoQ1 exerted its antioxidant effects independent of cell viability and suggests that the quinone group of the molecule was converted into its reduced form. DQ and CoQ4 exerted pro-oxidant effects in fMLP stimulated PMNs perhaps by augmenting receptor-mediated signal transduction causing NADPH oxidase activation. Whereas, CoQ10 dose-dependently inhibited fMLP-induced SO release, DQ had no significant effect on cell viability.

References