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An investigation of the effects of MitoQ on human peripheral mononuclear cells

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Abstract

MitoQ is a ubiquinone derivative targeted to mitochondria which is known to have both antioxidant and anti-apoptotic properties within mammalian cells. Previous research has suggested that the age-related increase in oxidative DNA damage in T lymphocytes might contribute to their functional decline with age. This paper describes the impact of mitoQ on unchallenged or oxidatively challenged *ex vivo* human peripheral blood mononuclear cells from healthy 25–30 or 55–60 year old volunteers. When cells were challenged with hydrogen peroxide (H_2O_2), following mitoQ treatment (0.1–1.0 μM), the ratio of reduced to oxidized forms of glutathione increased, the levels of oxidative DNA damage decreased and there was an increase in the mitochondrial membrane potential. Low levels of mitoQ (0.1 or 0.25 μM) had no impact on endogenous DNA damage, whilst higher levels (0.5 and 1.0 μM) of mitoQ significantly reduced endogenous levels of DNA damage. The results of this investigation suggest that mitoQ may have anti-immunosenescent potential.

Keywords: *MitoQ, oxidative stress, DNA damage, T cell immonosenescence*

Introduction

The important role of mitochondria in the life and death of a cell has been well established [1]. A decline in mitochondrial bioenergetic function with age in various human tissues has been implicated as a factor contributing to the onset of a range of diseases [2]. Studies have revealed that damage to mitochondria could contribute to an age-related immunodeficiency [3]. Damage can lead to the release of reactive oxygen species (ROS) [4], with the potential to contribute to the age-related increase in nuclear DNA damage and mutations in human T lymphocytes [5,6]. This increase in DNA damage may result in cell cycle arrest or apoptosis [7,8], leading to a decline in immune system competency.

A major difficulty in treating diseases caused by mitochondrial dysfunction is the challenge of targeting bioactive molecules to mitochondria *in vivo*. This problem has been addressed by the development of mitochondria-targeted antioxidants such as MitoQ [2,9,10]. Previous studies have demonstrated the ability of mitoQ to decrease H_2O_2-induced caspase activation and apoptotic cell death in Jurkat cells on acute exposure [10]. Other studies have revealed anti-oxidant potential on long-term exposure to mitoQ, evidenced by decreased telomere shortening in human fibroblasts [11]. In this paper, we describe the impact of a range of doses of mitoQ administered acutely or chronically, on endogenous oxidative stress or on H_2O_2-induced oxidative stress in human peripheral blood mononuclear cells *ex vivo*. As glutathione (GSH) is an important antioxidant *in vivo* and it becomes oxidized to GSSG under oxidative stress, the intracellular GSH:GSSG ratio [12] was used as an

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indicator of oxidative stress levels in the cells. Levels of oxidative damage to nuclear DNA were measured as a marker of oxidative stress-induced biomolecule damage.

Materials and methods

Subject selection

Ethical approval was granted by Nottingham Trent University for this study, conditional on informed consent by each of the study subjects. The human blood samples for this study were drawn from 10 healthy, non-smoking males fasted overnight, five each in the age groups of 25–30 years and 55–60 years. The subjects were not on any form of medication, they had not suffered any recent illness nor were they taking any form of prescribed/unprescribed medication (e.g. dietary supplements). The number of study subjects were sufficient to provide data which was scientifically meaningful and which are based on a range of other studies which have been published by this research group [13].

Sample collection

Twenty millilitre human blood samples were drawn in the morning between 8.00–9.00 am in a tube containing heparin to prevent coagulation. The samples were kept on ice and taken to the laboratory within 5 min for separation of peripheral blood mononuclear cells.

Separation and cryostorage of peripheral blood mononuclear cells

Peripheral blood mononuclear cells were isolated from fresh heparinized venous blood by density gradient centrifugation on Ficoll-Isopaque (PAA Laboratories, Somerset, UK) in LeucoSep lymphocyte separation) tubes. Following separation, cell samples were cryopreserved in a medium made up of 10% DMSO (Sigma-Aldrich, Dorset, UK.), 20% FBS (Invitrogen, Paisley, UK) and 70% X-Vivo 10 and stored in liquid nitrogen until thawed for further analysis.

Acute/chronic exposure of human peripheral blood mononuclear cells ex vivo *to mitoQ*

The relevant scientific literature was consulted in order to confirm the dose range of the antioxidants and the oxidant. In the investigations undertaken by Hampton and Orenius [14], Dumont et al. [15] and Kelso et al. [10], Jurkat cells (or T-cells in the case of Kelso et al.) were treated with 0–500 μM H_2O_2 to produce an effective oxidative challenge. Saretzki et al. [11] and Horton et al. [16] used a concentration of 37 μM vitamin E in their studies. We also used 74 μM in this investigation. Kelso et al. [10] demonstrated that 1 μM mitoQ completely blocked caspase activation in Jurkat cells induced by 150 μM H_2O_2 under acute exposure conditions to MitoQ. In the studies of Saretzki et al. [11], where the effects of chronic exposure to MitoQ were investigated in fibroblasts, 10–20 nM MitoQ was used. In the investigations described in this paper, 10–30 nM MitoQ was used in the chronic exposure experiments.

In relation to the investigations described in this paper, a range of MitoQ concentrations were tested out for cytotoxicity, anti-/pro-oxidative potential. None of the concentrations used in the experiments elicited pro-oxidant properties.

Thawed cells were maintained in culture separately under the conditions that have been detailed previously [8]. For acute exposure to antioxidants/control agents, cultured cells were incubated separately with mitoQ or decyl triphenyl phosphonium cation (DTPP) (0, 0.1, 0.25, 0.5 or 1.0 μM)—a non-antioxidant mitochondria-targeted control compound which allowed for the non-specific effects of the TPP component of MitoQ to be examined or Vitamin E (Sigma-Aldrich, Dorset, UK.) (37 or 74 μM)—a lipophilic non-mitochondrial-targeted antioxidant, as controls, for 3 or 6 h. Following this treatment, 0–250 μM H_2O_2 (Sigma-Aldrich, Dorset, UK) was exogenously applied for a 15 min period. The H_2O_2 treatment was performed at 4°C to minimize DNA repair.

For chronic exposure to mitoQ, freshly isolated human peripheral blood mononuclear cells from six healthy males, three each from each of the two age groups detailed above, were maintained in culture separately for 3 weeks as detailed previously [8]. The cells were supplemented with 0–30 nM mitoQ or DTPP and with 400 U/ml recombinant IL-2 on days 1 and 4 of the cycle. On day 7 a new culture cycle was set up with fresh medium and RJK853 feeder cells. The samples required for further analysis were cryo-preserved at the end of a 7 day growth cycle [8]. Only DTPP was used as a control for mitoQ for this part of the investigation.

Determination of levels of oxidative damage to DNA in human peripheral blood mononuclear cells ex vivo pre-treated with mitoQ/DTPP/Vitamin E

The levels of oxidative damage to DNA (DNA single strand breaks, alkali labile sites and oxidatively modified purines and pyrimidines) in treated/control cultures of human peripheral blood mononuclear cells *ex vivo* were determined using the modified alkaline comet assay as previously described [8,17,18]. Ethidium bromide stained slides were digitially analysed using UV microscopy and Komet 5.5 software (Andor Technology, Belfast, Northern Ireland), counting 50 cells per slide. The Comet assays for the samples were

performed in duplicate and DNA damage levels were expressed as the % DNA in comet tail.

Intracellular GSH:GSSG ratio and total glutathione levels in human peripheral blood mononuclear cells ex vivo *treated with mitoQ/DTPP/Vitamin E*

The ratio of the reduced and oxidized form of glutathione and total glutathione levels were assessed using a GSH: GSSG ratio assay kit (Calbiochem, UK). This technique incorporates a thiol-scavenging reagent, 1-methyl-2-vinylpyridinium trifluoromethane-sulphonate1 (M2VP) at a level that rapidly scavenges GSH, but does not interfere with the glutathione reductase in recycling GSSG into GSH. Whole cell extracts were prepared from harvested cells (2×10^6 cells/ml for GSH and 5×10^6 cells/ml for GSSG), according to the manufacturer's instructions. The total glutathione levels and the GSH: GSSG ratio were calculated from the GSH and GSSG concentrations of the cells, photometrically measured at 412 nm in μM concentrations. The samples were analysed in duplicate.

Assessment of mitochondrial membrane potential in ex vivo *human peripheral blood mononuclear cells exposed to mitoQ*

All TPP compounds can lead to mitochondrial depolarization at high concentrations and this is a non-specific effect [19]. The experiments reported in this paper were designed to avoid this potential artefact by ensuring that the concentrations of MitoQ used are too low to cause this problem. In addition, parallel experiments were carried out with the same concentration of decylTPP which may also non-specifically disrupt mitochondria to the same extent as MitoQ. By inclusion of these controls, it is unlikely that any of the mitochondrial effects are due to this non-specific uncoupling.

The JC-1 staining kit assay (Invitrogen, Paisley, UK) was used to assess the mitochondrial membrane potential in 1.5×10^6 human peripheral blood mononuclear cells *ex vivo*. Cells subjected to mitoQ treatment were suspended in warm (25°C) PBS (Oxoid Ltd, Cambridge, UK) and stained with JC-1 dye according to manufacturer's instructions. The mean fluorescent intensity of green signal (depolarized mitochondria/dead cells) was compared to the mean fluorescent intensity of red signal (percentage of live cells). Intensity values were determined using a flow cytometer at absorption maxima of 488 nm. Membrane potential was assessed using JC1 staining in control cells, the addition of MitoQ or decylTPP had no impact on the red/green fluorescence values.

The results were expressed as mean ratio values or mitochondrial depolarization values. The mitochondrial depolarization value was calculated by dividing the mean value of green signal by the mean value of red signal. A higher depolarization value represents lower mitochondrial membrane potential due to higher levels of oxidative stress and increased number of dead/dying cells. A lower depolarization value or a value closer to basal levels represents higher mitochondrial membrane potential due to low levels of oxidative stress and hence increased levels of live cells.

Statistical analysis

The results were tested for significance using paired two-sample Student's *t*-tests assuming equal variances; *p*-values are presented as appropriate.

Results

Impact of mitoQ on levels of nuclear DNA damage in human peripheral blood mononuclear cells ex vivo

Figure 1 shows the effect of 0.5 μM mitoQ on levels of endogenous DNA damage and on DNA damage levels following treatment with H_2O_2 in cells derived from five healthy donors aged 25–30 years. There was a significant (Student's *t*-test, 95% confidence level) decrease in endogenous levels of oxidative DNA damage following a 3-h treatment with 0.5 μM mitoQ.

H_2O_2 treatment (150 and 250 μM) significantly increased oxidative DNA damage levels, compared to untreated cells (endogenous DNA damage levels). In addition, 0.5 μM mitoQ treatment afforded significant protection against H_2O_2 (150 and 250 μM) induced DNA damage. Levels of DNA damage, detected using the alkaline comet assay, and levels of oxidized pyrimidines were significantly lower in 0.5 μM mitoQ treated cells following challenge with 50 μM H_2O_2. Further studies investigating the effect of a higher dosage of mitoQ (1 μM) revealed similar antioxidant potential to that of 0.5 μM mitoQ (Table I).

To confirm the antioxidant potential of the ubiquinone in MitoQ, the results obtained showed that neither 0.5 nor 1 μM DTPP had significant impact on endogenous oxidative DNA damage levels. However, rather surprisingly, DTPP treatment (for 3 h) resulted in significant protection against H_2O_2 induced DNA damage (Table I). There was no significant difference between the extent of the protective effective against H_2O_2 following 0.5 or 1 μM DTPP treatment. The effects of 6 h of supplementation with 0.5 or 1 μM mitoQ/DTPP on the levels of endogenously produced or exogenously applied DNA damage were not significantly different from the antioxidant potential after 3 h of supplementation (Table I).

Furthermore, a much lower concentration of mitoQ (0.1 or 0.25 μM) after 6 h of treatment had no significant impact on endogenous levels of oxidative DNA. However, at these lower doses, mitoQ

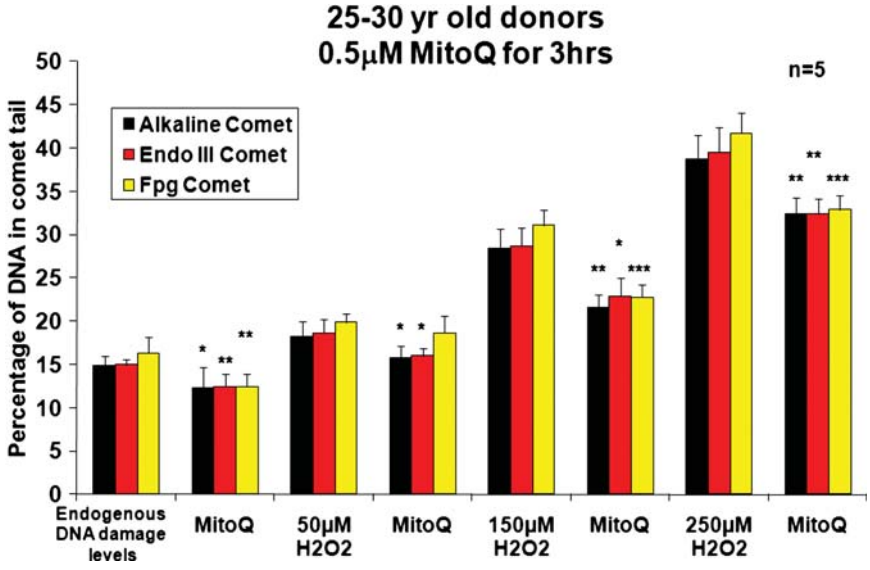

Figure 1. The effect of 0.5 μM mitoQ on levels of oxidative DNA damage in human peripheral blood mononuclear cells isolated from fresh human blood derived from each of five different healthy donors 25–30 years. The bars indicate the mean ± SD. Values statistically different from their controls (Student's *t*-test, 95% confidence level) are indicated with an asterisk. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

treatment resulted in significant protection against H_2O_2 induced DNA damage (Table I). Low concentrations of DTPP (0.1 or 0.25 μM) had no impact on DNA damage levels, endogenous or induced. None of the concentrations of vitamin E used in this investigation had an effect on endogenous or H_2O_2 induced DNA damage levels (Table I).

There was no significant difference in the levels of oxidative DNA damage, effect of H_2O_2 treatment or the effect of all the concentrations of mitoQ/DTPP on the levels of oxidative DNA damage between the cells derived from donors from the different age groups (Table I). In all cases, there was a consistent increase in levels of oxidative DNA damage with increase in concentration of H_2O_2 (0–250 μM) treatment (e.g. Figure 1). The levels of oxidative DNA damage observed between samples of the human peripheral blood mononuclear cells derived from each of the five different donors in each of the above cases were not significantly different.

The effect of mitoQ on intracellular GSH:GSSG ratio and total glutathione levels in human peripheral blood mononuclear cells

The effect of 0.5 μM mitoQ on the GSH:GSSG ratio (± H_2O_2 treatment) in peripheral blood mononuclear cells derived from five healthy, non-smoking males in the 25–30 years age group is illustrated in Figure 2.

H_2O_2 treatment alone (150 and 250 μM) resulted in marked lowering of the GSH:GSSG ratio, in a

Table I. Summary of the results of the effect of different concentrations of mitoQ or DTPP or Vitamin E supplementation, on levels of oxidative DNA damage in human peripheral blood mononuclear cells isolated from fresh human blood derived from each of 10 different healthy donors, five each in the age groups of 25–30 and 55–60 years.

Donor age(years)	Concentration of mitoQ/DTPP/vitamin E supplementation	MitoQ/DTPP/vitamin E supplementation time	Mean % change in endogenous DNA damage levels in mitoQ/DTPP/vitamin E treated cells compared to untreated cells (controls)	DNA damage levels in mitoQ/DTPP/vitamin E treated cells subjected to exogenously applied ROS (250 μM H_2O_2 treatment) compared to cells treated only with exogenously applied ROS (controls)
25–30 or 55–60	0.5 or 1 μM mitoQ	3 or 6 h	14.5*	21.44*
25–30 or 55–60	0.1 or 0.25 μM mitoQ	6 h	1.22 nsd	26.7*
25–30 or 55–60	0.5 or 1 μM DTPP	3 or 6 h	0.6 nsd	24.5*
25–30 or 55–60	0.25 or 0.1 μM DTPP	6 h	1.1 nsd	0.72 nsd
25–30 or 55–60	37 or 74 μM vitamin E	3 or 6 h	1.1 nsd	0.26 nsd

*significantly reduced, $p < 0.01$.
nsd, no significant difference vs control.

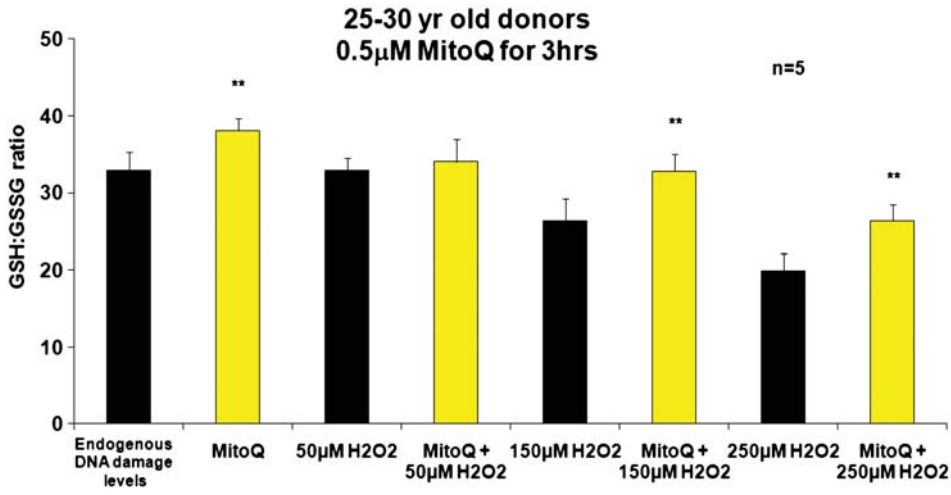

Figure 2. The effect of 0.5 μM mitoQ on intracellular GSH:GSSG ratio in human peripheral blood mononuclear cells isolated from fresh human blood derived from each of five different healthy donors, aged 25–30 years. The bars indicate the mean ± SD. Values statistically different from their controls (Student's *t*-test, 95% confidence level) are indicated with an asterisk. $^{*}p < 0.05$, $^{**}p < 0.01$.

dose-dependent trend (Figure 2). Figure 2 also shows that 0.5 μM mitoQ treatment (3 h) resulted in a significantly higher GSH:GSSG ratio in peripheral blood mononuclear cells following these two doses of H_2O_2 treatment; 50 μM H_2O_2 treatment did not have any impact on GSH:GSSG ratio. The effect of 1 μM mitoQ on the GSH:GSSG ratio in peripheral blood mononuclear cells was similar to the effect of 0.5 μM mitoQ (Table II). Further experiments revealed no significant changes in GSH:GSSG ratio on treatment with 1 μM DTPP. However, 1 μM DTPP treatment resulted in a significantly higher GSH:GSSG ratio in cells treated with a range of doses of H_2O_2. The effect of 0.5 μM DTPP on the GSH:GSSG ratio in human peripheral blood mononuclear cells was not significantly different from the effect of 1 μM DTPP (Table II). The effect of 0.5 or 1 μM mitoQ/DTPP after 6 h of supplementation was not significantly different from the results obtained after 3 h of supplementation (Table II).

Furthermore, neither 0.1 nor 0.25 μM mitoQ resulted in significant changes in GSH:GSSG ratio. However, these doses resulted in a significantly higher GSH:GSSG ratio in cells treated with a range of doses of H_2O_2 (Table II). Neither 0.1 nor 0.25 μM DTPP had significant impact on the GSH:GSSG ratio in cells ± H_2O_2. None of the concentrations of vitamin E used in this investigation had any effect on GSH:GSSG ratio ± H_2O_2 (Table II).

There were no significant differences in the GSH:GSSG ratio, the effect of H_2O_2 treatment or effect of all the concentrations of mitoQ/DTPP on the GSH:GSSG ratio in cells derived from donors of the two age groups (Table II). In all cases, there was a consistent decrease in the GSH:GSSG ratio with an increase in concentration of H_2O_2 (0–250 μM) treatment. Furthermore, the GSH:GSSG ratios observed between cells derived from each of the five different donors were not significantly different.

Table II. Summary of the results of the effect of different concentrations of mitoQ or DTPP or vitamin E supplementation, on the GSH:GSSG ratio in human peripheral blood mononuclear cells isolated from fresh human blood derived from each of 10 different healthy donors, five each in the age groups of 25–30 and 55–60 years.

Donor age(years)	Concentration of mitoQ/DTPP/vitamin E supplementation	MitoQ/DTPP/vitamin E supplementation time	Mean % change in basal GSH:GSSG ratio in mitoQ/DTPP/vitamin E treated cells compared to untreated cells (controls)	Mean % change in GSH:GSSG ratio in mitoQ/DTPP/vitamin E treated cells subjected to exogenously applied ROS (250 μM H_2O_2 treatment) compared to cells treated only with exogenously applied ROS (controls)
25–30 or 55–60	0.5 or 1 μM mitoQ	3 or 6 h	15.7*	31.3*
25–30 or 55–60	0.1 or 0.25 μM mitoQ	6 h	1.9 nsd	26.7*
25–30 or 55–60	0.5 or 1 μM DTPP	3 or 6 h	4.7 nsd	26.5*
25–30 or 55–60	0.1 or 0.25 μM DTPP	6 h	1.7 nsd	0.6 nsd
25–30 or 55–60	37 or 74 μM vitamin E	3 or 6 h	1.17 nsd	3.4 nsd

*significantly higher, $p < 0.01$.
nsd, no significant difference vs control.

In terms of total glutathione levels, there were no consistent changes on supplementation with any of the concentrations of mitoQ (0.1, 0.25, 0.5, 1 μM) or DTPP (0.1, 0.25, 0.5, 1 μM) or vitamin E (37 or 74 μM) in cells derived from donors from either age group, treated with and without a range of doses of H_2O_2 (results not shown).

The effect of mitoQ on mitochondrial membrane potential in human peripheral blood mononuclear cells

The effect of 0.1 μM mitoQ on the mitochondrial membrane potential in cells derived from three males aged 25–30 is illustrated in Figure 3. The mitochondrial membrane potential was measured ± H_2O_2 treatment after 6 h of supplementation with mitoQ.

The results in Figure 3 show that there were no significant changes in mitochondrial membrane potential on treatment with 0.1 μM mitoQ alone. H_2O_2 treatment (all doses) resulted in an increase in mitochondrial depolarization values (reflecting a decrease in the average number of living cells in conditions of oxidative stress). However, mitoQ treatment resulted in a significant decrease in mitochondrial membrane depolarization in cells treated with a range of doses of H_2O_2, when compared to those treated with H_2O_2 alone. There was no significant difference in the extent of the impact of 0.5 μM mitoQ compared to the effect of 0.1 μM mitoQ (± H_2O_2 treatment), in mitochondrial membrane depolarization values. The mitochondrial membrane depolarization value in cells derived from each of the three donors in each of the above cases was not significantly different.

Discussion

A decline in mitochondrial bioenergetic function with age in various human tissues has been implicated as a major factor contributing to the onset of a wide range of diseases [2]. The mitochondria targeted antioxidant, mitoQ, was developed to treat such diseases [8,9]. In light of the suggested role of oxidative biomolecule damage in the ageing of the T-cells of the immune system, this investigation was undertaken to investigate the potential anti-immunosenescent effects of mitoQ.

The results of this investigation revealed that there were no significant differences in endogenous or H_2O_2 induced DNA damage levels between the samples from the 25–30 year age group, compared to those within samples from the 55–60 year age group. These findings are in line with previously published data from our group [6] and imply the absence of a significant increase in oxidative DNA damage levels or in the ability to protect against H_2O_2 induced DNA damage within the age range of the donor subjects.

MitoQ supplementation (0.5 or 1 μM) decreased oxidative stress, evidenced by the significantly higher GSH:GSSG ratios, and significantly decreased levels of endogenous oxidative DNA damage in cell samples derived from both age groups, compared to controls. Lower concentrations of mitoQ (0.1 or 0.25 μM) did not have any impact on the GSH:GSSG ratio or on the levels of endogenous oxidative DNA damage, in cell samples from the donors of both age groups.

All concentrations of mitoQ used in this investigation (0.1, 0.25, 0.5 or 1 μM) resulted in a significantly higher GSH:GSSG ratio and a significant decrease in oxidative DNA damage levels following treatment

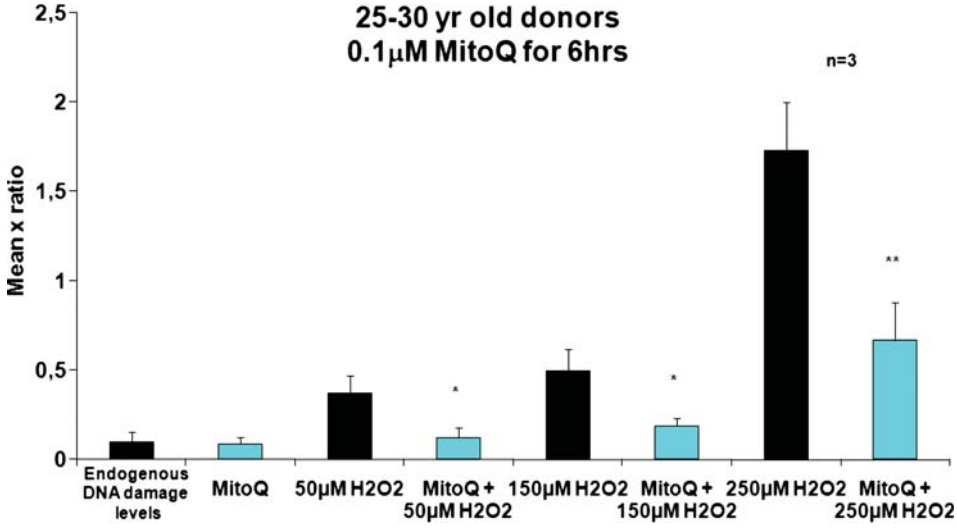

Figure 3. The effect of 0.1 μM mitoQ on mitochondrial membrane potential in human peripheral blood mononuclear cells isolated from fresh human blood derived from each of three healthy donors aged 25–30 years. The bars indicate the mean ± SD. Values statistically different from their controls (Student's *t*-test, 95% confidence level) are indicated with an asterisk. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

with H_2O_2. The results were interesting because although mitoQ is a mitochondria targeted antioxidant, it demonstrated significant protection against oxidative damage in the cytosol and nucleus, suggesting that reactive oxygen species emanating from the mitochondria contribute to nuclear and cytosolic oxidative damage. Mitochondrial ROS are generally thought to be a significant source of ROS in these cells, as for most eukaryotic cells [20], although we cannot exclude contributions from other sources, such as plasma membrane NADPH-oxidases.

MitoQ is likely to act primarily to prevent oxidation damage within the mitochondrial inner membrane by decreasing lipid peroxidation. This is turn will protect mitochondrial function from damage. In our view the most likely way in which MitoQ prevents the decrease in the GSH:GSSG ratio and protects against nuclear DNA damage is that oxidative damage to mitochondria disrupts mitochondrial function and in the damaged mitochondria there is increased leakage of superoxide from the respiratory chain. This forms hydrogen peroxide which in turn then leaks out of the mitochondria and oxidizes the cytosolic GSH pool and damages nuclear DNA. Therefore we feel that the mode of action of MitoQ is to the rest of the cell, preventing damage to mitochondria that in turn would lead to an increase in ROS leakage. The antioxidant potential of 1 μM concentration of mitoQ in the cytosol, evidenced by its protective effect against oxidative DNA damage induced by exogenously applied ROS, in this investigation was in line with the results of previous findings revealing blockage of 150 μM H_2O_2 induced caspase activation in Jurkat cells by 1 μM mitoQ [10].

The ability of mitoQ at specific concentrations to scavenge radicals, resulting in a significantly higher GSH:GSSG ratio, compared to controls, is an important finding, considering the role of intracellular redox status (GSH:GSSG ratio) as a mediator of apoptosis in several cell systems [12,21,22].

DTPP is a lipophilic mitochondria targeted compound having a similar structure to mitoQ, except for the ubiquinone derivative in mitoQ. The ubiquinone derivative is responsible for the antioxidant capability of mitoQ. Rather surprisingly, DTPP (0.5 or 1 μM) significantly decreased the DNA damage levels and prevented a decrease in the GSH:GSSG ratio following H_2O_2 treatment in cell samples from both age groups. It may be that this protection is due to non-specific interactions of DTPP with the cells and thus part of the protection seen by high concentrations of MitoQ may also arise from non-specific interactions with the cells.

The lipophilic nature of vitamin E enables it to act as a potent antioxidant in membranes and lipoproteins [23]. In this investigation, the impact of concentrations of vitamin E (a non-mitochondria targeted antioxidant) on the levels of free radicals and oxidative DNA damage, in cell samples, was investigated. Neither 37 nor 74 μM resulted in significant changes to the levels of oxidative DNA damage or GSH:GSSG ratio in cells treated ± H_2O_2. These results suggest that radicals arising intracellularly—from the cytosol and/or mitochondria—or indeed as a result of intracellular breakdown of H_2O_2 were not scavenged by the vitamin E embedded in the lipophilic cell membrane layers. It may have been that vitamin E entered the cell relatively poorly over the time scale of these experiments. Its uptake into mitochondrial is also known to be poor.

Mitochondrial membrane depolarization value is a marker of T-cell integrity. The results of our investigation indicated increased mitochondrial membrane potential on treatment with 0.1 or 0.5 μM mitoQ after exposure to a range of doses of H_2O_2 in cells derived from donors of both age groups. On the basis of these results, future research could examine the anti-immunosenescent effect of mitoQ *in vivo*. MitoQ is rapidly taken up by tissues, so a blood concentration of 0.5 μM mitoQ would not be easy to maintain. Furthermore, it may be that any potential anti-senescent properties operate at the level of haematopoietic stem cells and not on cells in the circulation.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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