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Animal and human studies with the mitochondria-targeted antioxidant MitoQ

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As mitochondrial oxidative damage contributes to a wide range of human diseases, antioxidants designed to be accumulated by mitochondria *in vivo* have been developed. The most extensively studied of these mitochondria-targeted antioxidants is MitoQ, which contains the antioxidant quinone moiety covalently attached to a lipophilic triphenylphosphonium cation. MitoQ has now been used in a range of *in vivo* studies in rats and mice and in two phase II human trials. Here, we review what has been learned from these animal and human studies with MitoQ.

Keywords: mitochondria; antioxidants; MitoQ; oxidative damage

Introduction

Mitochondria are a major source of reactive oxygen species (ROS) and are also particularly susceptible to oxidative damage.[1,2] Consequently, mitochondria accumulate oxidative damage that contributes to mitochondrial dysfunction and cell death and this is related to a range of diseases.[1,2] To decrease mitochondrial oxidative damage a number of mitochondria-targeted antioxidants have been developed[3–7] and form the basis for new pharmaceuticals. Ideally, mitochondria-targeted antioxidant should be pharmaceutically tractable and stable small molecules with acceptable oral bioavailability that are selective taken up by mitochondria within organs where they can control oxidative damage and, in the ideal situation, can be recycled back to the active antioxidant form.[5] The best characterized mitochondria-targeted antioxidant to date is MitoQ, which consists of a quinone moiety linked to a triphenylphosphonium (TPP) moiety by a 10-carbon alkyl chain.[5,8,9] Here, we outline the *in vitro* properties of MitoQ before discussing the knowledge obtained from *in vivo* studies using this compound.

In vitro *properties of MitoQ*

Lipophilic TPP cations can pass easily through phospholipid bilayers because their charge is effectively dissipated and surrounded by a protective organic chemical array enabling their accumulation into the mitochondrial matrix in response to the mitochondrial membrane potential.[10,11] The Nernst equation indicates that, under normal biological conditions, the uptake of lipophilic cations into mitochondria increases 10-fold for every 61.5 mV of membrane potential, leading to 100–1,000 fold accumulation.[10] Uptake into cells is also driven by the plasma membrane potential.[10] The TPP moiety on MitoQ thus enables its accumulation within mitochondria—driven by the membrane potential. Once within mitochondria, nearly all the accumulated MitoQ is adsorbed to the matrix surface of the inner membrane where it is continually recycled to the active quinol antioxidant form by complex II in the respiratory chain.[8,12–14] MitoQ cannot restore respiration in mitochondria lacking coenzyme Q because the reduced quinol form of MitoQ is not oxidized by complex III[13] and therefore cannot act as an electron carrier, consequently, most of the

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effects of MitoQ that occur *in vitro* are likely to be due to the accumulation of the antioxidant quinol form, although the quinone form may also react directly with superoxide.[15] When the quinol form of MitoQ acts as an antioxidant it is oxidized to the quinone form, which is then rapidly re-reduced by complex II, restoring its antioxidant efficacy.[12] As MitoQ is largely found adsorbed to the mitochondrial inner membrane, and its linker chain enables its active quinol antioxidant component to penetrate deeply into the membrane core, it was anticipated to be an effective antioxidant against lipid peroxidation, which has been confirmed for isolated mitochondria.[8,14] MitoQ has also been shown to protect against peroxynitrite damage although, as with other quinols, its reactivity with hydrogen peroxide is negligible.[12]

The uptake of MitoQ by cells in culture has been extensively studied and has been shown to be adequately described by the Nernst equation.[8,16] These studies indicate that there is rapid equilibration of MitoQ across the plasma membrane, driven by the plasma membrane potential, followed by the accumulation of MitoQ into mitochondria within the cells.[16] Consistent with this, preventing the uptake of MitoQ by dissipating the mitochondrial membrane potential with the uncoupler carbonylcyanide-*p*-trifluoromethoxyphenylhydrazone (FCCP) abolishes the protection afforded by MitoQ in a cell model of Friedreich's ataxia.[17] As is expected from its uptake and activation within cells in culture, MitoQ has been used in a large number of cell models of mitochondrial oxidative (reviewed in Ref. 5), where it has shown protection against damage. These findings that MitoQ is protective in a range of isolated mitochondria and cell studies led to it being extended to studies *in vivo*, as described in the next sections.

Targeting MitoQ to mitochondria *in vivo* and its effects on whole animal metabolism

To function as a potentially therapeutic mitochondria-targeted antioxidant *in vivo* it must be shown that MitoQ can be administered safely long-term to animals without toxicity and that *in vivo* it accumulates within mitochondria at sufficient concentrations to be protective. The first studies established the intravenous (iv) toxicity of MitoQ in mice.[9] There was no toxicity at 750 nmol MitoQ/mouse (~20 mg MitoQ/kg) but toxicity was evident at 1,000 nmol MitoQ/mouse (~27 mg MitoQ/kg).[9] To measure oral toxicity mice were administered MitoQ in their drinking water and was shown to be well tolerated up to 500 μM, with toxicity evident at higher concentrations.[9] Since then MitoQ has been administered to mice in their drinking water at 500 μM for up to 28 weeks with no evident toxicity.[18] In this study the amount of MitoQ consumed corresponds to an oral dose of ~3.2 μmol MitoQ/day/mouse, or to 95–138 μmol MitoQ/day/kg.[18] Therefore substantial amounts of MitoQ can be administered to mice by iv or oral routes without adverse toxic effects.

The uptake of MitoQ into various tissues was initially investigated using [^{3}H]MitoQ and iv injection.[9] These experiments showed that MitoQ was rapidly cleared from the plasma and accumulated in the heart, brain, skeletal muscle, liver, and kidney.[9] Orally administered [^{3}H]MitoQ was taken up into the plasma and from there into the heart, brain, liver, kidney, and muscle.[9] Similar studies indicated that MitoQ was excreted in the urine and bile as unchanged MitoQ and also with sulfation and glucuronidation of the quinol ring, with no indication of other metabolites.[16,19]

Initial determination of the concentration of MitoQ in various tissues using [^{3}H]MitoQ indicated that, following oral administration, about 100–700 pmol/g wet weight was present in tissues.[9] This technique has since been superseded by the development of methods to measure MitoQ by a liquid chromatography tandem mass spectrometry (LC/MS/MS) assay, relative to a deuterated internal standard (IS), d_3-MitoQ, by multiple reaction monitoring (MRM) using the transitions 583.3 > 441.3 for MitoQ and 586.3 > 444.3 for d_3-MitoQ.[18] This assay could detect 0.1 pmol MitoQ/100 mg tissue. For mice fed 500 μM MitoQ drinking water for 4–6 months the steady-state accumulation of MitoQ was ~113 pmol MitoQ/g in the heart, ~20 pmol MitoQ/g in the liver, and ~2 pmol/g in the brain. In other studies rats fed MitoQ for 2 weeks accumulated ~20 pmol MitoQ/g in the heart[20], and rats fed MitoQ for 12 weeks accumulated ~40 pmol MitoQ/g in the heart, and ~200 pmol MitoQ/g in the liver.[21] Therefore, long-term administration of MitoQ in the drinking water led to the substantial steady-state accumulation of MitoQ within mouse heart and liver, with significantly less in the brain.

The effects of long-term *ad libitum* oral administration of MitoQ levels on the behavior, metabolism, gene expression, and accumulation of oxidative damage markers of young C57BL/6 mice, has been investigated.[18] There were no changes in the physical activity, O_2 consumption, food consumption, and respiratory quotient (RQ) of mice that had been administered 500 μM MitoQ for 24–28 weeks when assessed by a Comprehensive Lab Animal Monitoring System (CLAMS). There was a slight decrease in the RQ in the MitoQ-fed mice. The effect of MitoQ on motor coordination and balance was investigated by the Rotarod test and in this MitoQ led to a small, overall improvement in the performance. Feeding MitoQ led to no differences in the lean mass of treated mice, but there was a decrease in the percentage of body fat due to decreases in some fat depots. DEXA analysis showed that there was no effect of MitoQ on bone mineral density or mineral content. MitoQ administration led to a decrease in liver triglyceride content and also decreased white adipocyte size. Consumption of MitoQ did not affect plasma cholesterol or free fatty acid levels, but significantly decreased plasma triacylglyceride content. MitoQ administration did not affect glucose or insulin levels in the fed and fasted states, and glucose and insulin tolerance tests also showed no differences.

To assess how oral MitoQ administration affected gene expression, RNA levels in heart and liver tissue were compared between MitoQ-treated and control mice fed MitoQ for ~20 weeks, using the Affymetrix GeneChip MouseGene array of 28,853 genes.[18] The overall level of gene expression in both tissues was not markedly affected by MitoQ. The small number of changes in that occurred were analyzed using the DAVID Functional Annotation Clustering tool and showed that no biological process terms were significantly over-represented. Therefore the changes in gene expression in the heart and liver from long-term exposure to MitoQ were relatively minor and appeared to be unrelated to any particular cellular process. Importantly, there were negligible alterations to mitochondrial or antioxidant gene expression. The lack of change in gene expression on MitoQ administration also enables us to eliminate the possibility that the long-term administration of an antioxidant leads to a compensatory decrease in the expression of endogenous antioxidant defences. Similarly, we can exclude the possibility that the protective effects of MitoQ seen *in vivo* might have been due to hormesis, by which an increase in ROS production up-regulates the expression of antioxidant defence genes. These findings are consistent with MitoQ having relatively little impact on the levels of antioxidant defences *in vivo* in young, wild-type mice when administered at levels that are protective against a range of pathologies.

To determine whether administration of MitoQ affected oxidative stress levels, the accumulation of a number of mitochondrial oxidative damage markers were measured in liver and heart mitochondria from mice fed MitoQ for ~20 weeks.[18] These included measurement of oxidative damage to the phospholipid cardiolipin (CL),[22] the accumulation of protein carbonyls,[23,24] the activity of mitochondrial respiratory complexes, mtDNA copy number and damage to mtDNA assessed by a quantitative PCR assay.[25] Together these data indicated that long-term exposure to MitoQ had no effect on a range of markers of oxidative damage in wild-type mice. Long-term exposure to MitoQ also had no effect on the expression of the mitochondrial matrix enzyme manganese superoxide dismutase, MnSOD, encoded by the *Sod2* gene. As expression of this gene is sensitively up-regulated in response to increased mitochondrial ROS production,[26,27] this indicates that MitoQ does not increase oxidative stress or the flux of ROS within mitochondria *in vivo*.

The demonstration that MitoQ was not prooxidant *in vivo* is important, as all quinols can potentially redox cycle to produce superoxide in an aqueous environment.[28] The factors that lead to superoxide production by quinones are reduction to the quinol followed by deprotonation to the quinolate (pK_a ~11), making it thermodynamically possible to reduce oxygen to superoxide.[28,29] Quinones can also produce superoxide by undergoing one-electron reduction at the flavin site of complex I,[30] and possibly other flavoenzymes,[31] with the ubisemiquinone radical then reacting with oxygen to give superoxide.[30] It is possible to establish conditions *in vitro* where all quinols produce superoxide,[29,31–33] however, our data indicate that this does not occur *in vivo* within mice fed MitoQ. Thus while prooxidant reactions of MitoQ and other targeted quinones are measureable *in vitro*, they do not occur *in vivo*.

These findings indicate that MitoQ can be given safely long-term to young, wild-type mice at

levels that are protective in pathological models. This suggests that the effects of MitoQ *in vivo* are due to their antioxidant properties and not to other factors and provides a firm basis for the ongoing use of MitoQ in the investigation of mitochondrial ROS metabolism *in vivo*.

Protective effects of MitoQ in animal models of human diseases

The studies discussed earlier indicate that long-term administration of MitoQ to mice is safe. The next step is to determine whether the accumulation of MitoQ within the mitochondria of these animals *in vivo* can act as a protective treatment in animal models of diseases that involve mitochondrial oxidative damage. A number of *in vivo* studies with MitoQ have now been carried out in several different laboratories,[20,21, 34–38] and these are outlined here.

The first study of the protective effects of MitoQ was against cardiac ischemia/reperfusion (I/R) injury.[20] In this study, 500 μM MitoQ was administered to rats in their drinking water for 2 weeks and the hearts were then isolated and exposed to I/R injury in a Langendorff perfusion system. This study showed that MitoQ gave protection against heart dysfunction, tissue damage, and mitochondrial function compared with methylTPP or short chain quinol as independent controls of the two different functional groups in MitoQ.[20] Since then a similar study also showed that MitoQ was protective in I/R injury in the heart.[37]

MitoQ was protective against the damage to endothelial cells *in vivo* associated with chronic exposure to nitroglycerin, due to protecting against oxidative damage to nitroglycerin-metabolising enzymes within mitochondria.[35] MitoQ was protective against an increase in blood pressure in a spontaneously hypertensive rat model in which the increase in blood pressure is thought to arise from elevated mitochondrial oxidative damage in endothelial cells.[21]

Sepsis is another pathology in which there is considerable evidence that mitochondrial oxidative damage contributes to the tissue damage associated with the disorder.[39,40] Preadministering MitoQ to rats or mice prior to induction of sepsis by endotoxin led to extensive protection against cardiac damage.[38] This was associated with less induction of apoptosis, decreased markers of protein oxidative damage as well significant protection against damage to mitochondrial function.[38] In a study by a different group using the lipopolysaccharide model of sepsis, infusion of MitoQ at the same time as induction of sepsis led to significant protection against liver damage.[36]

MitoQ administered by intraperitoneal injection was protective against heart damage associated with the anti-cancer compound adriamycin.[34] In a rodent model of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) toxicity, MitoQ protected against *substantia nigra* damage, preserved locomotor activity and dopamine content as well as decreased mitochondrial markers of oxidative damage.[41] A number of other studies that are currently underway or have been completed and submitted for publication suggest that MitoQ may also show protection in further animal models of diseases involving mitochondrial oxidative damage, including fatty liver disease, kidney damage in type I diabetes, kidney ischemia-reperfusion injury, and neurodegeneration. Together these findings suggest that MitoQ is protective against pathological changes in a number of animal models of mitochondrial oxidative damage that are relevant to human diseases.

Human studies with MitoQ

The positive animal studies indicated that MitoQ was an attractive candidate for intervention in human diseases. Consequently, Antipodean Pharmaceuticals Inc. (http://www.antipodeanpharma.com/) developed MitoQ as a pharmaceutical. For a stable formulation it was found beneficial to make MitoQ with the methanesulfonate counteranion and to complex this with β-cyclodextrin. This preparation was readily made into tablets that passed through conventional animal toxicity. The oral bioavailability was determined at about 10% and major metabolites in urine were glucuronides and sulfates of the reduced quinol form along with demethylated compounds. In human Phase 1 trials MitoQ showed good pharmacokinetic behavior with oral dosing at 80 mg (1 mg/kg) resulting in a plasma maximal concentration of 33.15 ng/mL and after ∼1 h.

As there are multiple lines of evidence pointing to mitochondrial oxidative stress as a potential pathogenic cause for Parkinson's disease (PD), MitoQ was trialled to see if it could slow disease progression in this disease.[42] This was the

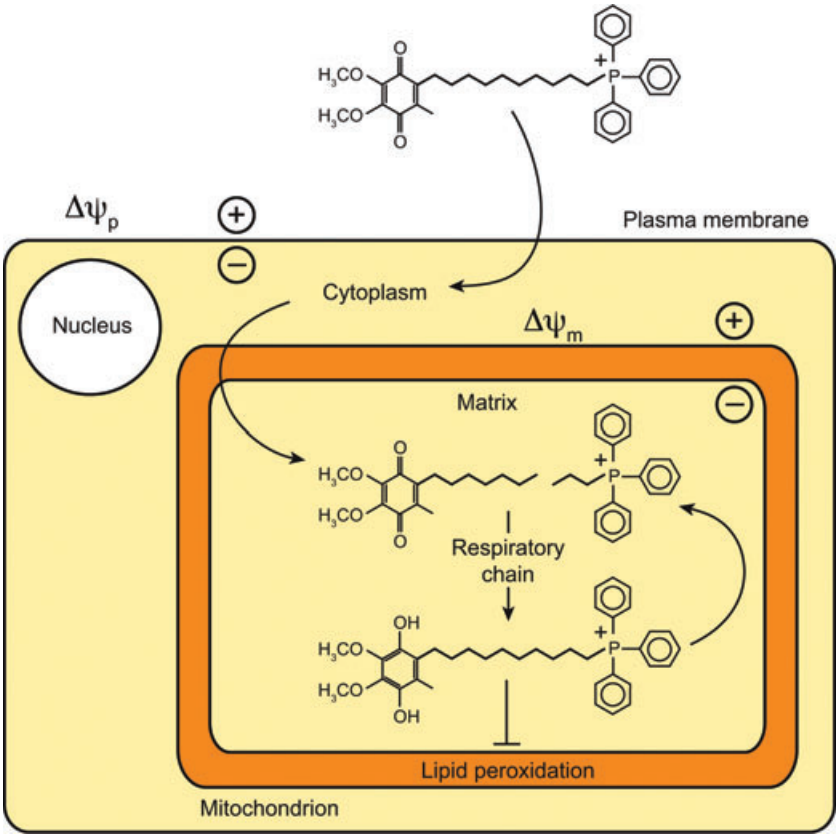

Figure 1. Uptake of MitoQ by mitochondria within cells. This schematic shows the uptake of MitoQ into the cytoplasm from the extracellular environment driven by the plasma membrane potential ($\Delta\psi_p$). From the cytoplasm the compound is further accumulated into mitochondria, driven by the mitochondrial membrane potential ($\Delta\psi_m$).

PROTECT study, which was registered on www.clinicaltrials.gov as NCT00329056. In this 13-centre study in New Zealand and Australia 128 newly diagnosed untreated patients with PD were enrolled in a double-blind study of two doses of MitoQ (40 and 80 mg/day) compared with placebo to see whether, over 12 months, MitoQ would slow the progression of PD as measured by the Unified Parkinson Disease Rating Scale. This study showed no difference between MitoQ and placebo on any measure of PD progression.[42] There are several possible explanations for this finding, although methodological problems such as inadequate sample size or inappropriate outcome measures seem unlikely.[42] The most probable explanation for the lack of effect is that by the time parkinsonism is clinically evident, approximately 50% of dopaminergic neurons are lost. It is possible that at diagnosis the fate of the remaining neurons is already determined and neuroprotection at this stage cannot prevent their death. The lack of efficacy of MitoQ might also be due to insufficient brain penetration. However, when MitoQ is administered orally to rodents it does accumulate to some extent in the brain.[9,18] Even so, it may be that there was too little to protect brain mitochondria against oxidative damage, and we cannot exclude this possibility entirely. Although in a rodent model of MPTP toxicity, MitoQ protected against *substantia nigra* damage, preserved locomotor activity and dopamine content as well as decreasing mitochondrial markers of oxidative damage.[41] While there was no therapeutic efficacy, this study did provide important saftey data for the long-term administration of MitoQ in humans and demonstarted that MitoQ can be safely administered as a daily oral tablet to patients for a year.

The second human trial carried out to date with MitoQ was the CLEAR trial on chronic hepatitis C

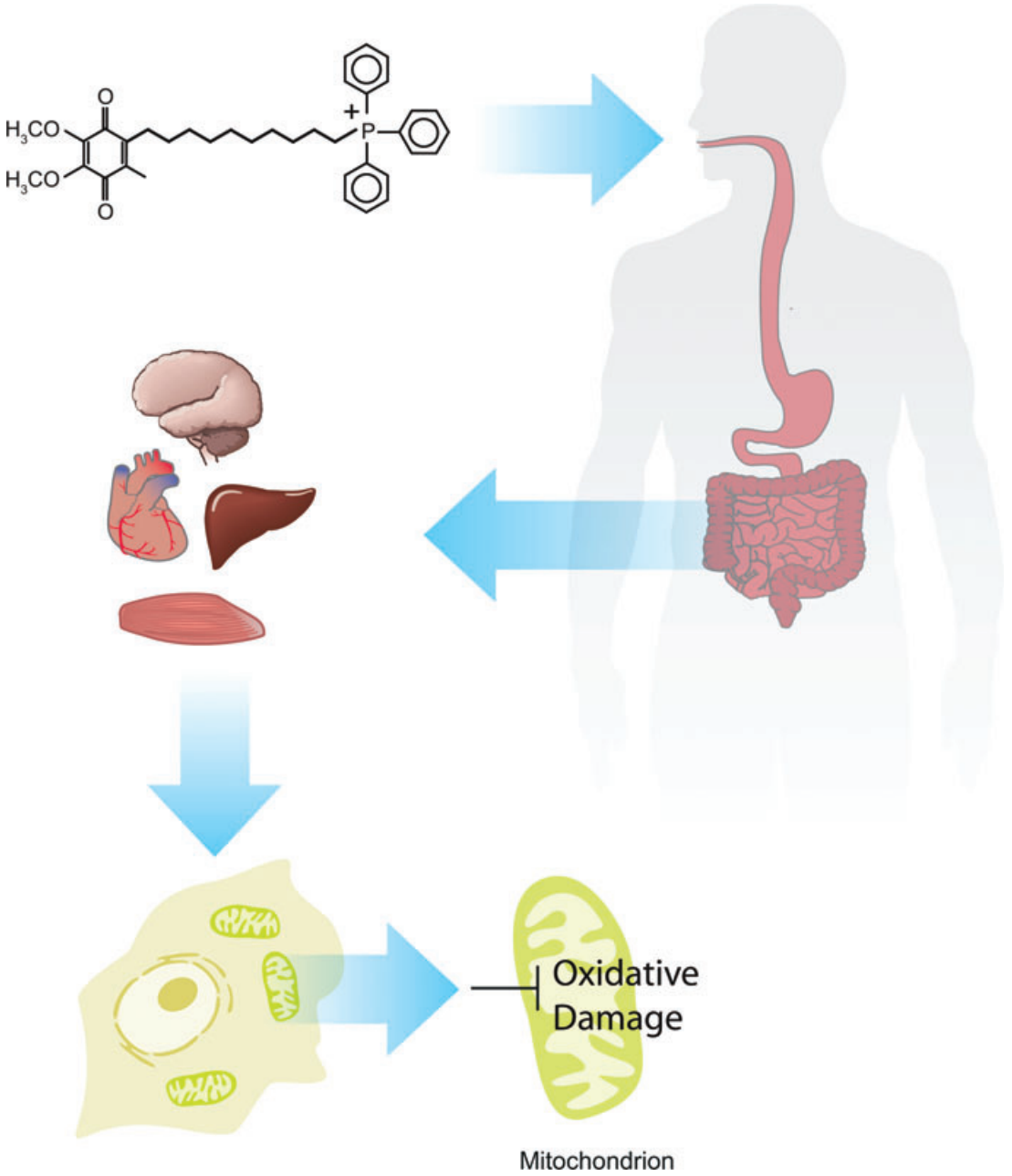

Figure 2. **Oral uptake and distribution of MitoQ. To be an ideal mitochondria-targeted antioxidant, MitoQ should be orally bioavailable, being rapidly taken up into the blood stream from the gut. From there it would pass into cells within those tissues affected by mitochondrial damage, such as the heart, brain, liver, and muscle. MitoQ would then accumulate within mitochondria, protecting them from oxidative damage. Ideally, MitoQ should then be recycled back to its active antioxidant quinol form after having detoxified a ROS.**

virus (HCV) patients.[43] This study was registered on www.clinicaltrials.gov as NCT00433108. HCV patients who were unresponsive to the conventional HCV virus treatments were chosen because in this group of patients there is evidence for increased oxidative stress and subsequent mitochondrial damage playing an important role in liver damage. Therefore, the effect of oral MitoQ on serum

aminotransferases and HCV RNA levels in HCV infected patients was assessed in a double-blind, randomized, parallel design trial of two different doses of MitoQ and of placebo in patients with a documented history of chronic HCV infection. Participants were randomized 1:1:1 to receive either 40 mg, 80 mg, or matching placebo for 28 days. Both treatment groups showed significant decreases in serum alanine transaminase (ALT) from baseline to treatment day 28. There was no effect of MitoQ on viral load, indicating that the mitochondria-targeted antioxidant was only affecting the liver damage associated with HCV infection and was not inhibiting the ability of the virus to replicate within the liver. These data suggest that MitoQ can reduce liver damage in HCV infection. More generally, this study is the first report of a potential clinical benefit from the use of mitochondria-targeted antioxidants in humans. Coupled with the 1 year's safety data for MitoQ from the Parkinson's Disease study, this suggests that the efficacy of MitoQ for other chronic liver disease that are thought to involve mitochondrial oxidative damage, such as non-alcoholic fatty liver disease are worthy undertakings.

Importantly from the pharmaceutical development viewpoint no severe adverse event was reported in either study. The most common treatment-related adverse was mild nausea that was dose-dependent. As there was no dose dependence for efficacy in the liver study, in future studies it should be possible to limit nausea while retaining efficacy by lowering the MitoQ dose. It is possible that the administration protocol involving overnight fast before taking the drug and the subsequent 1 h without food after its administration may have exacerbated the nausea, therefore in future studies it may be preferable to take the drug with some foods while carefully assessing whether this affects its bioavailability.

Conclusions

Animal experiments have indicated that MitoQ has antioxidant efficacy in a number of tissues *in vivo*. It has also been shown that MitoQ can be formulated into an effective pharmaceutical that can be successfully delivered orally to humans. Human studies to date indicate that MitoQ can be safely delivered to patients for up to a year and that these doses are effective in decreasing liver damage. These findings open up the use of MitoQ in longer duration and larger phase II B studies in liver disorders such as fatty liver disease. More generally, these findings suggest that orally administered MitoQ and related mitochondria-targeted antioxidants may also be applicable to the wide range of human pathologies that involve mitochondrial oxidative damage. Hopefully work over the next few years will indicate whether MitoQ and related compounds they can decrease mitochondrial oxidative damage in a range of diseases, and whether this improves the outcome for the patient.

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Conflicts of interest

M.P.M and R.A.J.S. hold intellectual property in the area of mitochondria-targeted antioxidants and act as consultants for Antipodean Pharmaceuticals Inc.

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