Targeting Antioxidants to Mitochondria by Conjugation to Lipophilic Cations

Michael P. Murphy[1] and Robin A.J. Smith[2]

[1]MRC Dunn Human Nutrition Unit, Wellcome Trust/MRC Building, Cambridge CB2 2XY, United Kingdom; email: mpm@mrc-dunn.cam.ac.uk

[2]Department of Chemistry, University of Otago, Dunedin, New Zealand; email: rajsmith@chemistry.otago.ac.nz

Annu. Rev. Pharmacol. Toxicol. 2007. 47:629–56

First published online as a Review in Advance on October 2, 2006

The *Annual Review of Pharmacology and Toxicology* is online at http://pharmtox.annualreviews.org

This article's doi: 10.1146/annurev.pharmtox.47.120505.105110

0362-1642/07/0210-0629$20.00

Annu. Rev. Pharmacol. Toxicol. 2007.47:629-656.

Key Words

MitoQ, triphenylphosphonium, oxidative damage, reactive oxygen species, ubiquinol

Abstract

Mitochondrial oxidative damage contributes to a range of degenerative diseases. Consequently, the selective inhibition of mitochondrial oxidative damage is a promising therapeutic strategy. One way to do this is to invent antioxidants that are selectively accumulated into mitochondria within patients. Such mitochondria-targeted antioxidants have been developed by conjugating the lipophilic triphenylphosphonium cation to an antioxidant moiety, such as ubiquinol or α-tocopherol. These compounds pass easily through all biological membranes, including the blood-brain barrier, and into muscle cells and thus reach those tissues most affected by mitochondrial oxidative damage. Furthermore, because of their positive charge they are accumulated several-hundredfold within mitochondria driven by the membrane potential, enhancing the protection of mitochondria from oxidative damage. These compounds protect mitochondria from damage following oral delivery and may therefore form the basis for mitochondria-protective therapies. Here we review the background and work to date on this class of mitochondria-targeted antioxidants.

INTRODUCTION

Oxidative damage: non-specific damage to biological molecules caused by reactive oxygen species

Mitochondria have many roles that are central to eukaryotic cell function and survival (1). Mitochondria are the cell's principal site of energy metabolism and the main source of ATP, as well as being involved in heme and iron sulfur center biosynthesis, amino acid and nitrogen metabolism, and calcium homeostasis modulation (1–3). Furthermore, mitochondria are critically involved in apoptotic cell death, being crucial in committing the cell to die by releasing proapoptotic factors from the intermembrane space (4). Finally, mitochondrial damage that disrupts ATP synthesis and calcium homeostasis is a key component of necrotic cell death (5). For all these reasons, mitochondrial malfunction disrupts the function of cells, tissues, and organs and contributes to a remarkably wide range of diseases (2, 6–9). Mitochondria can malfunction owing to defects in genes encoded by mitochondrial or nuclear DNA, and because mutations often affect the ability of the mitochondria to make ATP, these diseases generally present as defects in tissues with high ATP demand, such as neurons or muscles (7–11). As well as diseases in which an underlying genetic cause directly affects mitochondrial function, mitochondrial damage also arises in and contributes to the pathology of many other disorders, including Parkinson's disease (12, 13), Huntington's disease (14, 15), Alzheimer's disease (12, 15), amyotrophic lateral sclerosis (15), diabetes (16, 17), steatohepatitis (18), sepsis (19), retinopathies (20), and ischemia-reperfusion injury (12).

In nearly all cases where mitochondrial dysfunction contributes to disease, a major cause of damage is reactive oxygen species (ROS) produced by mitochondria, either directly or as a secondary consequence of other malfunctions (21–23) (**Figure 1**). The proximal ROS is superoxide, produced by the respiratory chain, probably at complexes I and III (21–23), although other superoxide sources within mitochondria include α-glycerophosphate dehydrogenase (24), the electron transfer flavoprotein/electron transfer flavoprotein-ubiquinone oxidoreductase system in β-oxidation (25), α-ketoglutarate dehydrogenase (26), and dihydroorotate dehydrogenase (27). The intermembrane space protein $p66^{Shc}$ and monoamine oxidase on the mitochondrial outer membrane can also produce hydrogen peroxide (28, 29). Superoxide itself is not particularly reactive (30), although it can react with aconitase to release ferrous iron (31) and also with nitric oxide to form the reactive and damaging oxidant peroxynitrite (32). Superoxide dismutates to hydrogen peroxide, which can react with ferrous iron to form the very reactive hydroxyl radical. Mitochondrial ROS cause damage to mitochondrial protein, lipid, and DNA, thereby disrupting mitochondrial function and also causing ROS to flow to the cytosol (30, 31, 33). There are a series of mitochondrial antioxidant defenses to intercept ROS and to minimize oxidative damage (2), but excessive production of ROS or disruption to the antioxidant defenses leads to extensive oxidative damage to mitochondria (22). As mitochondrial oxidative damage is either a primary cause or a significant secondary factor leading to cell damage and death in degenerative diseases, a general therapy to decrease mitochondrial oxidative damage would be of use in a range of clinical situations (2, 22, 34).

Mitochondrial oxidative damage can be decreased with clinically significant benefits by increasing the expression of mitochondrial antioxidant enzymes, or by

Annu. Rev. Pharmacol. Toxicol. 2007.47:629-656.

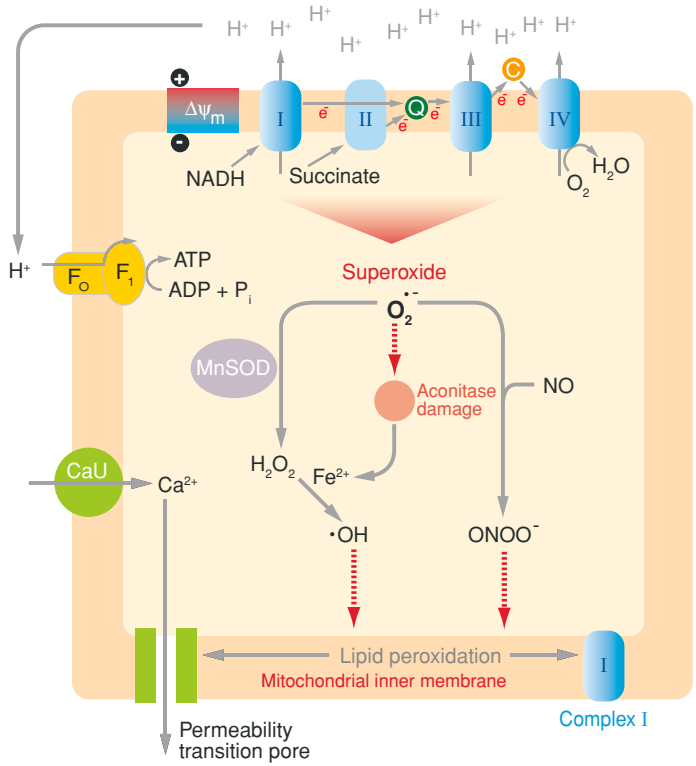

Figure 1

Mitochondrial oxidative damage. The respiratory chain (*blue*) carries electrons from NADH to oxygen and in so doing pumps protons across the mitochondrial inner membrane to build up a proton electrochemical potential gradient, mainly composed of the membrane potential ($\Delta\psi_m$, negative inside). This drives ATP synthesis through the ATP synthase (*orange*). The respiratory chain also releases superoxide, which can dismutate to hydrogen peroxide (H_2O_2) catalyzed by MnSOD (*purple*), react with aconitase (*red*) to release ferrous iron, or react with nitric oxide to form peroxynitrite ($ONOO^-$). Hydrogen peroxide can react with ferrous iron to form the hydroxyl radical ($^{\bullet}OH$), which, along with peroxynitrite, can cause oxidative damage to mitochondrial DNA, protein, or lipid. Lipid peroxidation, which damages complex I and also leads to induction of the mitochondrial permeability transition, is illustrated.

ectopically expressing antioxidant enzymes within mitochondria (35, 36). Therefore, increasing the antioxidant capacity of the mitochondrial compartment is a potential therapy, but to be pharmaceutically viable, a small-molecule antioxidant is required (2). However, most small-molecule antioxidants will distribute around the body, with only a small fraction being taken up by the mitochondria. This difficulty is well illustrated by the antioxidant vitamin E, which showed no benefits in Parkinson's disease (37, 38). An explanation for this disappointing result may be a combination of poor uptake into the body and limited delivery to the mitochondria (2, 39). This issue is further highlighted by the demonstration that very large doses of coenzyme Q_{10} are required to show any benefit in Parkinson's disease (40, 41).

Annu. Rev. Pharmacol. Toxicol. 2007.47:629-656.

Lipophilic cation: a cation with a large, hydrophobic surface area that enables it to pass through phospholipid bilayers and accumulate within mitochondria

In addition to its accumulation into mitochondria, the following questions must also be considered during the development of a therapeutic antioxidant (42): What biological molecule is the antioxidant supposed to protect and do sufficient quantities of the antioxidant reach its target to protect in vivo? How does the antioxidant protect—is it by scavenging ROS, preventing ROS formation, upregulating defenses, or aiding repair? If the antioxidant acts by scavenging ROS, can the resultant antioxidant-derived radicals cause damage themselves or can they be recycled? Can the antioxidants cause damage in other biological systems? Consequently, pharmaceutically tractable and stable small-molecule antioxidants are required that have the following properties: acceptable oral bioavailability, selective uptake by mitochondria within those organs most affected by mitochondrial oxidative damage, efficient blocking of oxidative damage within mitochondria, the ability to be recycled to the active antioxidant form within mitochondria, and action as a clinically effective antioxidant at concentrations well below those that cause toxic side effects. It would also be helpful for long-term administration if the compound had a natural process limiting its uptake and enabling it to come to a steady-state distribution.

One approach to addressing these challenges is to target antioxidants to mitochondria by conjugation to a lipophilic cation, such as the triphenylphosphonium (TPP) cation (**Figure 2**) (2, 39, 43–45). This procedure leads to orally bioavailable molecules, which accumulate in the cell, driven by the plasma membrane potential, and accumulate further into the mitochondria where the antioxidant moiety can protect from oxidative damage and also be recycled back to its active form (**Figure 2**). The theoretical background to these molecules, work on them to date, and future prospects for their development are discussed in this review.

ACCUMULATION OF LIPOPHILIC CATIONS BY CELLS AND MITOCHONDRIA

Two features of lipophilic cations make them effective at delivering antioxidants to mitochondria: They can pass directly through phospholipid bilayers without requiring a specific uptake mechanism and they accumulate substantially within mitochondria owing to the large membrane potential (46–48). Lipophilic cations can easily move through phospholipid bilayers because the activation energy for movement of lipophilic cations through the hydrophobic barrier of a biological membrane is far lower than for other cations (47, 49). The activation energy for transport is given by the energy required to move the cation from the aqueous phase to the hydrophobic core of the membrane, which is principally composed of the Born energy, which raises the activation energy, and the hydrophobic energy, which lowers it (50). The Born energy (W_B) in kJ/mol for a cation with Z charges is given by (46, 50)

$$W_B = \frac{339Z^2}{r}. \qquad 1.$$

Thus the enthalpy required to move the cation into the membrane is inversely proportional to the radius (r) of the cation. The hydrophobic energy is the energy required to move an uncharged molecule identical in size and hydrophobicity from the aqueous

Annu. Rev. Pharmacol. Toxicol. 2007.47:629-656.

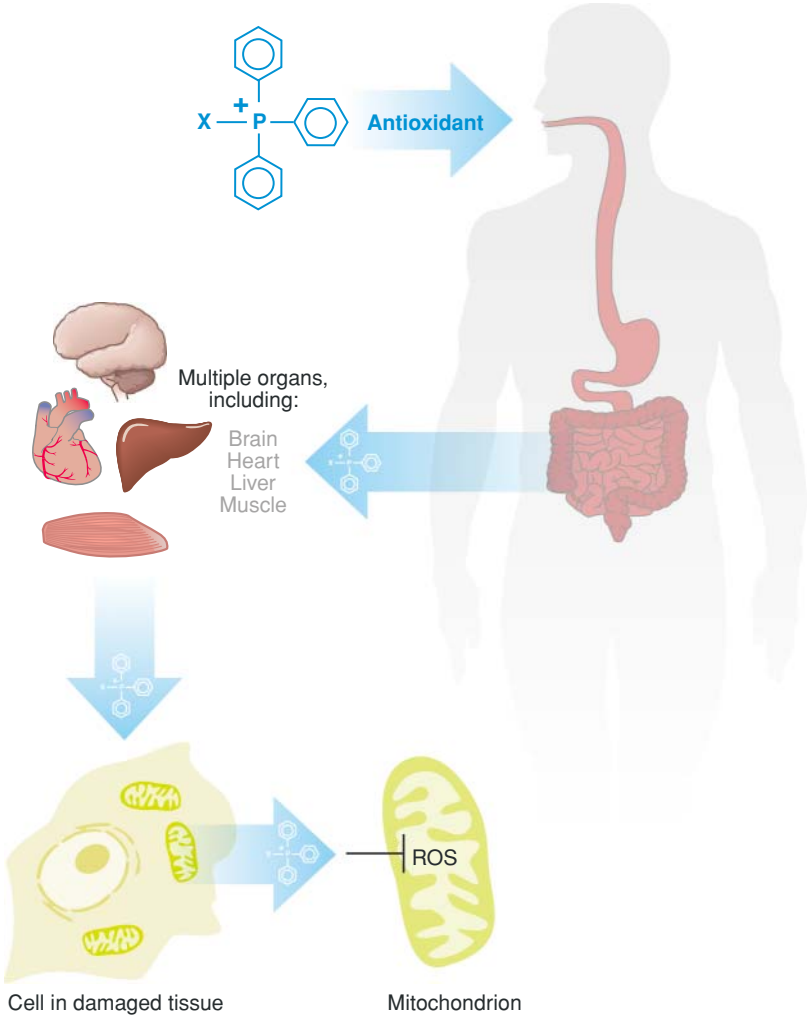

Figure 2

Oral uptake and distribution of a mitochondria-targeted antioxidant. An ideal mitochondria-targeted antioxidant would 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. The antioxidant would then accumulate within mitochondria, protecting them from oxidative damage. Ideally, the compound would be recycled back to its active antioxidant form after having detoxified a ROS.

Annu. Rev. Pharmacol. Toxicol. 2007.47:629-656.

environment into the lipid core of the membrane (46, 47, 50). This is an attractive force and the greater the hydrophobic surface area of the cation, the larger the hydrophobic effect. Thus the large, hydrophobic radius of the TPP cation (about 4.2 Å) enables it to pass easily through the phospholipid bilayer relative to other cations. In addition, further increasing the hydrophobicity of the compound should increase its rate of uptake through biological membranes.

We have a detailed understanding of how lipophilic cations, such as triphenylmethylphosphonium (TPMP), move through phospholipid bilayers (46, 47, 50). The distinctive energy profile for their movement through a phospholipid bilayer is shown in **Figure 3**. In addition to the energy barrier at the center of the membrane, the other notable feature is the potential energy wells close to each membrane surface

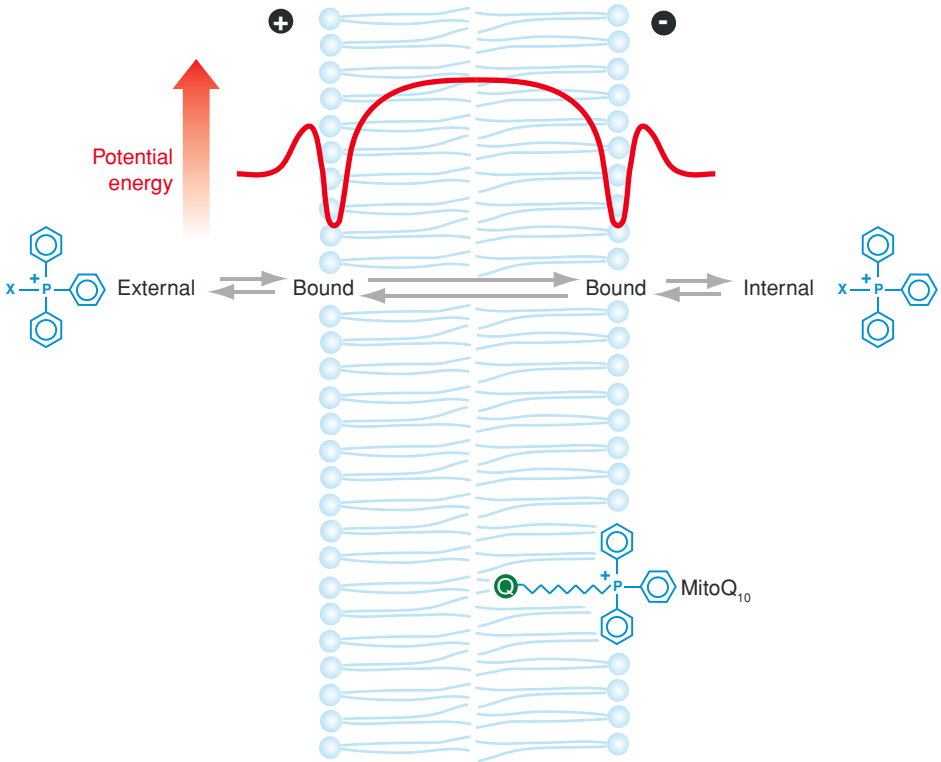

Figure 3

Uptake of lipophilic cations through phospholipid bilayers. The TPP cations will initially bind to the potential energy well on the outside of the membrane, then pass through to the potential energy well on the inner surface of the membrane. MitoQ$_{10}$ (*illustrated*) will be largely present adsorbed to the inner surface of the inner membrane, with the TPP moiety in the potential energy well and the alkyl Q moiety inserted into the membrane.

(47). These wells arise because the attractive hydrophobic force becomes large very close to the membrane surface, whereas the repulsive electrostatic forces increase more gradually on moving through the membrane (47). The binding of alkylTPP cations within this potential energy well is on the hydrophobic side of the lipid/water interface of the membrane at about the level of the carbonyls of the phospholipid fatty acyl groups (47, 49–52). The enthalpy for binding is positive (i.e., repulsive) owing to electrostatic forces, and binding is driven by the increase in entropy owing to the hydrophobic effect (49). During membrane transport the cations initially adsorb to the membrane in the potential energy well on the outer surface of the membrane. They then pass rapidly through the hydrophobic core of the membrane to the potential energy well on the other membrane surface, followed by desorbing from the membrane (**Figure 3**).

Lipophilic cations are taken up from a positively charged compartment into a negatively charged compartment until a sufficiently large concentration gradient is

Annu. Rev. Pharmacol. Toxicol. 2007.47:629-656.

built up to equalize the electrochemical potential of the molecules in the two compartments. At this point, compound uptake has equilibrated with the membrane potential ($\Delta\psi$) and the ratio of the concentrations of the free, unbound cations in the two compartments is described by the Nernst equation:

$$\Delta\psi = \frac{2.303RT}{F}\log_{10}\left(\frac{[cation_{in}]}{[cation_{out}]}\right). \quad 2.$$

As 2.303RT/F is 59.5–61.5 mV at 25°C–37°C, there will be an approximately tenfold accumulation of the cation within mitochondria for every ~60 mV increase in $\Delta\psi$. In addition, as the plasma membrane potential is approximately 30–60 mV (negative inside), lipophilic cations accumulate 5–10-fold into the cytoplasm. As the mitochondrial membrane potential in cells is typically 140–180 mV (53, 54), the cations within the cytosol further accumulate several-hundredfold within mitochondria, selectively localizing within the mitochondria. The overall conclusion is that it should be possible for correctly designed antioxidants, linked to lipophilic cations, to be taken up into cells within the body and then be further accumulated into mitochondria, thus selectively protecting mitochondria against oxidative damage (**Figure 4**).

MitoQ: a mitochondria-targeted derivative of ubiquinone that is accumulated within mitochondria and reduces oxidative damage

INTERACTION OF TRIPHENYLPHOSPHONIUM CATIONS WITH MITOCHONDRIAL MEMBRANES

Many different lipophilic cations have a sufficiently large hydrophobic surface area to permeate membranes and accumulate within mitochondria. For example, fluorescent lipophilic cations, such as rhodamine, JC-1, and the MitoTracker compounds, are widely used to visualize mitochondria selectively within cells (55–57). In principle, any lipophilic cation could be used to direct an attached antioxidant to mitochondria; however, to date, only modified TPP cations have been successfully prepared and evaluated as mitochondria-targeted antioxidants and hence form the main focus of this review. The uptake of TPP cations by energized mitochondria was introduced more than three decades ago by Skulachev and coworkers to investigate the mitochondrial $\Delta\psi$ (58, 59), and since then TPP compounds have been routinely used to measure the mitochondrial $\Delta\psi$ (53, 54). The interaction of TPP cations with phospholipid bilayers has also been studied extensively to probe the structures and electrostatics of biological membranes (47, 49–52). There are a number of synthetic chemical routes to making a range of variously substituted alkyltriphenylphosphonium compounds (**Figure 5**) (44) so the TPP cation was selected as the primary carrier for the initial work in examining the possibility of targeting antioxidants to mitochondria.

The model for the uptake of TPP cations through membranes, which has been described above and illustrated in **Figures 3** and **4**, has a number of important implications for the interactions of TPP cations with mitochondria. First, the steady-state concentration of lipophilic cations in the hydrophobic core of the membrane is negligible even though they move relatively easily through the membranes. This is supported by the very low solubility of even highly hydrophobic TPP cations, such as $MitoQ_{10}$ in cyclohexane, a mimic of the low dielectric environment at the membrane core (60). Second, these compounds have a strong tendency to adsorb as

Annu. Rev. Pharmacol. Toxicol. 2007.47:629-656.

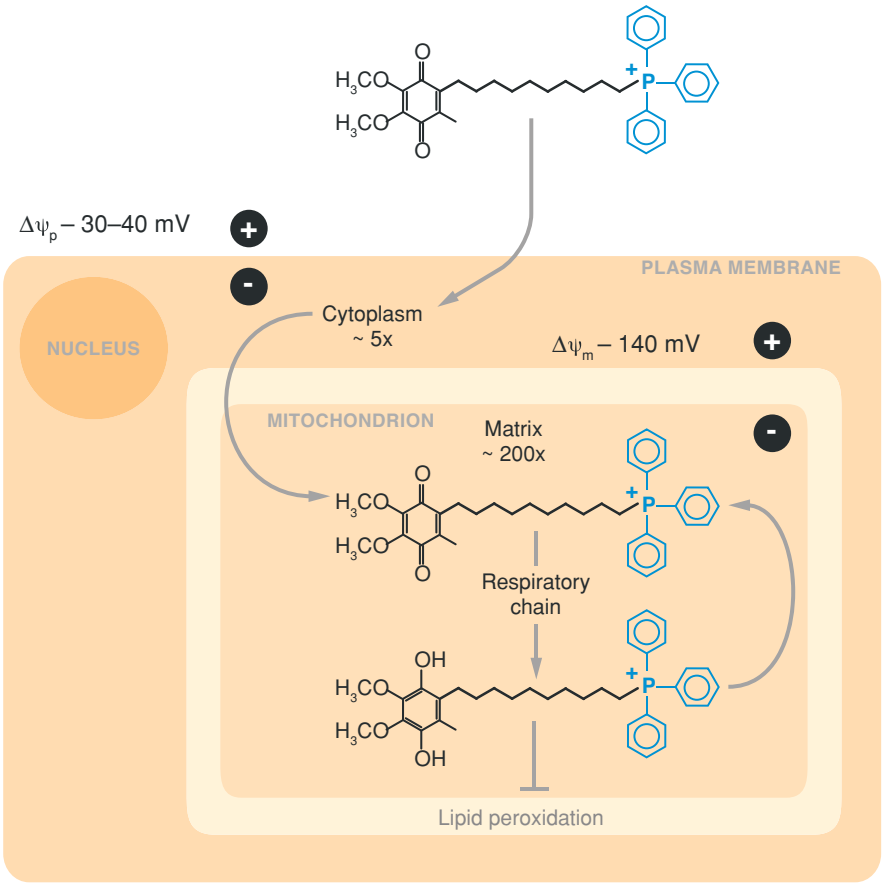

Figure 4

Accumulation of MitoQ$_{10}$ into cells and mitochondria. MitoQ$_{10}$ will first pass through the plasma membrane and accumulate in the cytosol driven by the plasma membrane potential ($\Delta\psi_p$). From there it will be further accumulated several-hundredfold into the mitochondria, driven by the mitochondrial membrane potential ($\Delta\psi_m$). There it is reduced to the active antioxidant ubiquinol by complex II. In preventing oxidative damage, it is oxidized to the ubiquinone, which is then rereduced by complex II.

a monolayer onto the surface of phospholipid bilayers owing to the local potential energy well. At low concentrations, this adsorption is approximately described by a Langmuir adsorption isotherm, which assumes that the cations adsorb independently to the surface binding sites (46, 53, 61, 62). Third, the more hydrophobic alkyltriphenylphosphonium cations have a stronger membrane adsorption tendency. Furthermore, the TPP component is always found at the same position in the potential energy well on the membrane surface, whereas the hydrophobic alkyl chain is inserted into the hydrophobic core of the membrane (44, 52, 61). Consequently, the extent to which the attached molecule can penetrate into the membrane is determined

Annu. Rev. Pharmacol. Toxicol. 2007.47:629-656.

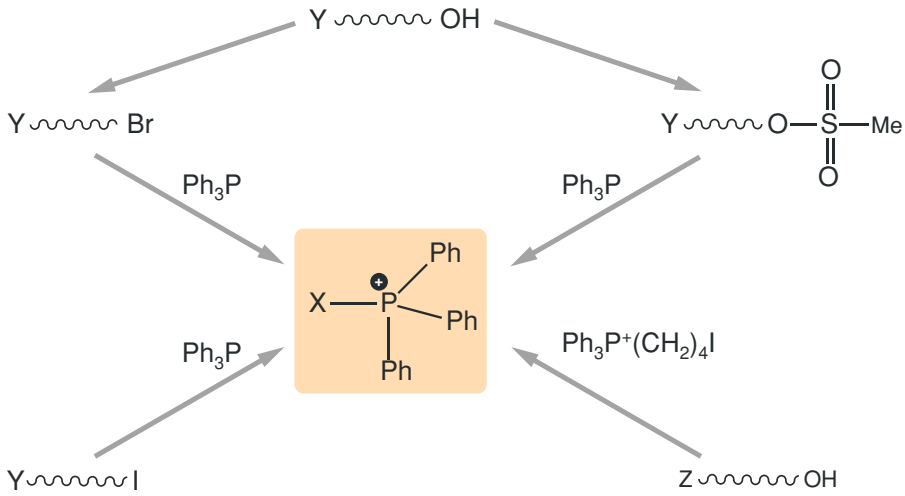

Figure 5

Schematic outline of the chemical syntheses of alkyltriphenylphosphonium cations, with a range of substituents indicated here by X. Leaving groups (-OSO_2Me, -Br, -I) connected to an antioxidant moiety (Y) are displaced by triphenylphosphine (Ph_3P) to form the triphenylphosphonium cation conjugated to Y, or an existing haloalkyltriphenylphosphonium salt such as $Ph_3P^+(CH_2)_4I$ reacts with an antioxidant moiety (Z) connected to a hydroxyl to form the TPP cation conjugated to Z via a 4-carbon alkyl chain and an ether bond. Ph = C_6H_5.

by the length of the alkyl chain connected to the TPP moiety (63, 64). This is illustrated for the mitochondria-targeted antioxidant $MitoQ_{10}$ in **Figure 3**. Finally, the high volume of matrix-facing mitochondrial membrane relative to that of the matrix means that a very large proportion of the alkyltriphenylphosphonium cation within mitochondria is membrane-bound, far greater than the proportion bound to the outside of the mitochondria (46). This is true even for the most hydrophilic cations, such as TPMP, where 60% of the TPMP in the matrix is membrane-bound (54). Increasing the hydrophobicity only slightly, e.g., to $MitoE_2$, results in 84% being membrane-bound (65). For the more hydrophobic compounds, such as $MitoQ_{10}$, an even greater proportion of the compound taken up into the mitochondria is thought to be membrane-bound (66).

In summary, TPP cations should be taken up by mitochondria, and increasing the length of the carbon bridge connecting the TPP moiety to the antioxidant component should increase the rate of uptake, the extent of association to the inner membrane surface, and the depth of penetration of the antioxidant moiety into the membrane.

MITOCHONDRIA-TARGETED ANTIOXIDANTS

In principle, a wide range of antioxidants could be targeted to mitochondria by conjugation to the TPP moiety, and antioxidants targeted to all components of the intramitochondrial ROS cascade have been developed (**Figure 6**). As lipid

Annu. Rev. Pharmacol. Toxicol. 2007.47:629-656.

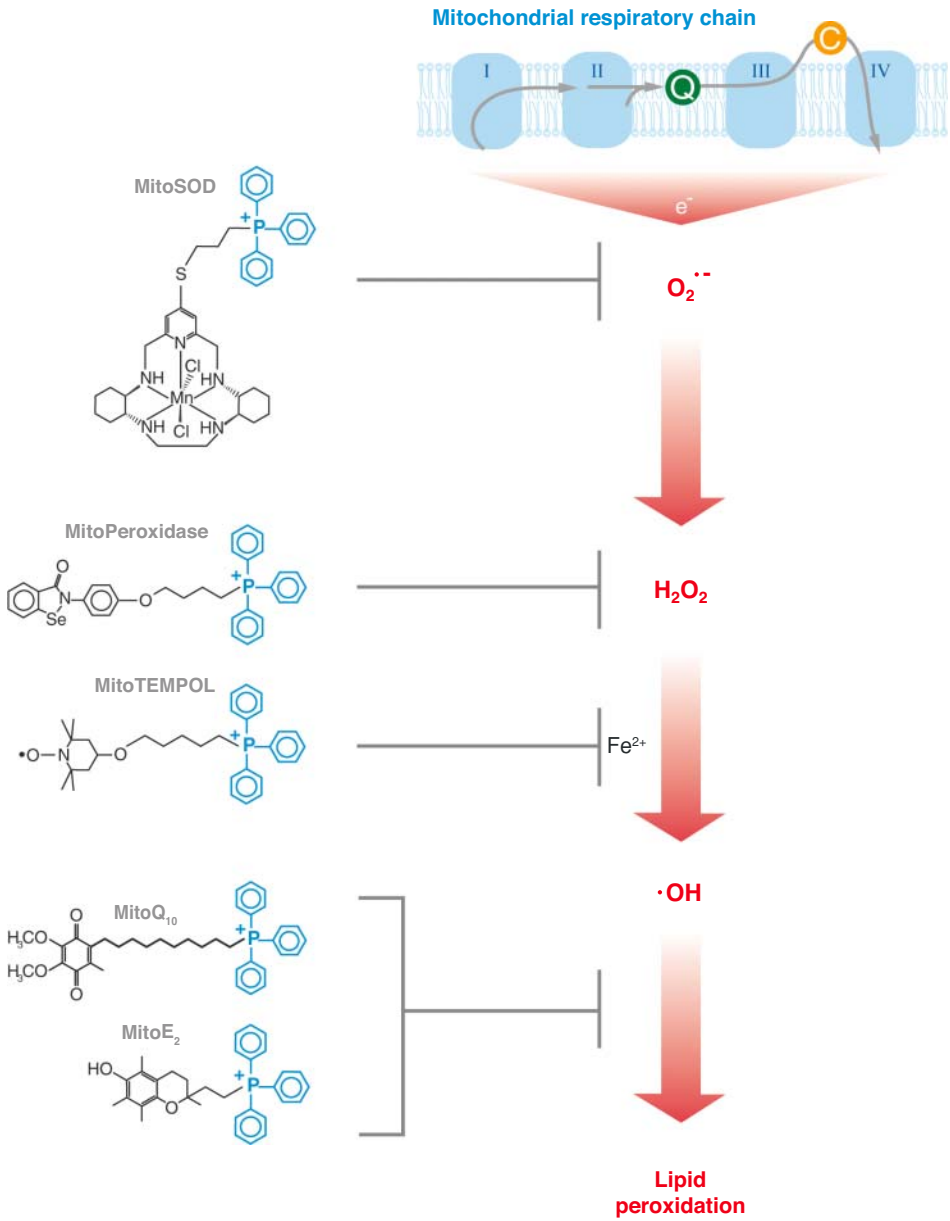

Figure 6

Mitochondria-targeted antioxidants. The mitochondrial respiratory chain produces superoxide, which can release ferrous iron, dismutate to hydrogen peroxide, and then react with ferrous iron to form the hydroxyl radical and initiate lipid peroxidation. A series of mitochondria-targeted antioxidants designed to decrease superoxide (MitoSOD), hydrogen peroxide (MitoPeroxidase), ferrous iron (MitoTEMPOL), and lipid peroxidation ($MitoQ_{10}$, $MitoE_2$) are shown.

Annu. Rev. Pharmacol. Toxicol. 2007.47:629-656.

peroxidation is important in many forms of mitochondrial oxidative damage, and because the alkylTPP conjugates strongly associate with the mitochondrial inner membrane, the initial focus has been on antioxidants that are effective against lipid peroxidation. In particular, targeted versions of ubiquinol (MitoQ) and to a lesser extent α-tocopherol, the main active component of natural vitamin E (MitoE), are discussed in this review. The subscript in the compound (e.g., $MitoQ_{10}$) refers to the number of carbons in the alkyl chain connecting the TPP and antioxidant moieties.

$MitoQ_{10}$ and $MitoE_2$ in Isolated Mitochondria

The first mitochondria-targeted antioxidant investigated was $MitoE_2$, which comprises the α-tocopherol moiety of vitamin E conjugated to a TPP cation by a two-carbon chain (**Figure 6**) (65). The α-tocopherol moiety is an effective chain-breaking antioxidant that prevents lipid peroxidation with the resulting α-tocopheroxyl radical then being recycled by the endogenous mitochondrial coenzyme Q pool (67, 68). $MitoE_2$ was taken up rapidly by isolated mitochondria, where it was more effective at preventing lipid peroxidation than an untargeted α-tocopherol (65). This approach was extended to targeting the ubiquinol moiety of coenzyme Q to mitochondria, as ubiquinol is known to be an effective chain-breaking antioxidant that can be recycled by the respiratory chain (68). In this case, the initial work was carried out on a molecule in which the ubiquinol moiety was linked to the TPP cation by a ten-carbon bridge to form $MitoQ_{10}$ (66). This compound has since been extensively characterized and its interaction with mitochondria is now reasonably well understood (**Figures 3** and **4**).

$MitoQ_{10}$ is taken up rapidly by isolated mitochondria driven by the $\Delta\psi$, and within mitochondria nearly all the accumulated $MitoQ_{10}$ is adsorbed to the matrix surface of the inner membrane (66). $MitoQ_{10}$ is reduced to the active ubiquinol antioxidant by complex II in the respiratory chain, but it is not a good substrate for complex I (64) or electron transfer flavoprotein-ubiquinone oxidoreductase (F.E. Frerman, unpublished observations). $MitoQ_{10}$ can also be reduced by α-glycerophosphate dehydrogenase, but as the active site for this enzyme is on the outer surface of the mitochondrial inner membrane, it is unlikely to be an important $MitoQ_{10}$ reduction site in vivo when $\Delta\psi$ is substantial (64). $MitoQ_{10}$ cannot restore respiration in mitochondria lacking coenzyme Q because the reduced form of $MitoQ_{10}$ is poorly oxidized by complex III; consequently, all the effects of $MitoQ_{10}$ are likely to be due to the accumulation of the antioxidant ubiquinol form (64). Furthermore, when the ubiquinol form of $MitoQ_{10}$ acts as an antioxidant, it is oxidized to the ubiquinone form, which is then rapidly reduced by complex II, restoring antioxidant efficacy (64). This is important because the recycling of an antioxidant back to its active form after it has neutralized a ROS is a critical factor in the efficacy of many antioxidants (68–71). As $MitoQ_{10}$ is largely found adsorbed to the mitochondrial inner membrane, and its side chain enables it to penetrate deeply into the membrane core, it was anticipated to be an effective antioxidant against lipid peroxidation. This has been confirmed for isolated mitochondria (64, 66, 72). $MitoQ_{10}$ has also been shown to detoxify peroxynitrite and it can react with superoxide, although, as with other ubiquinols, its reactivity with hydrogen peroxide is negligible (64, 66).

Annu. Rev. Pharmacol. Toxicol. 2007.47:629-656.

The dependence of efficacy on chain length was tested by creating and examining a series of MitoQ molecules with different numbers of carbons (3, 5, 10, 15) in their linker chains. It was found that the shorter-chain MitoQ compounds were less effective antioxidants than $MitoQ_{10}$ (64, 72) and this was due, in part, to the slower reduction to the ubiquinol form. This may be a consequence of poor access of the short-chain analogs to the active sites of ubiquinone reductases in the respiratory chain (64). In addition, as illustrated in **Figure 3** for $MitoQ_{10}$, the antioxidant moiety can access most of the hydrophobic core of the membrane, whereas the shorter-chain compounds would have far more limited access. As yet, only short-chain MitoE analogs have been tested and they have been found to be less effective than $MitoQ_{10}$ (73), but the above model predicts that the longer-chain MitoE analogs currently under development should be far more effective than $MitoE_2$ at preventing lipid peroxidation (65). In summary, $MitoQ_{10}$ seems to fulfill most of the requirements, in isolated mitochondria, for a mitochondria-targeted antioxidant.

Interaction of $MitoQ_{10}$ and $MitoE_2$ with Cells

Toxicity is the first issue to examine in determining whether $MitoQ_{10}$ or $MitoE_2$ can act as antioxidants in cells. The extensive accumulation of lipophilic cations within isolated mitochondria at concentrations approaching millimolar levels can disrupt membrane integrity, respiration, and ATP synthesis (45, 53, 54, 59). These effects are thought to be largely a result of adsorption of the lipophilic cations to the matrix surface of the inner membrane, disrupting membrane permeability and affecting enzyme and transporter activity. Supporting this idea, the more hydrophobic TPP cations can disrupt mitochondrial function at lower concentrations, and the degree of disruption correlates with the amount of compound adsorbed to the inner membrane. Thus, $MitoQ_{10}$ starts to increase the respiration rate of isolated mitochondria when 2.5–5 μM of the compound is added to the incubation, whereas 5–10-fold higher concentrations of TPMP are required to show the same effects (66). The nonspecific effects of $MitoQ_{10}$ on mitochondria are assessed using the control compound decylTPP, which is similar in hydrophobicity to $MitoQ_{10}$ [octanol-PBS partition coefficients of 5000 (64) and 2760 (72), respectively] but lacks the antioxidant ubiquinol moiety (64). We find that the nonspecific mitochondrial disruption of $MitoQ_{10}$ and decylTPP occurs at similar concentrations (74). These nonspecific effects on mitochondrial function will always limit the amounts of TPP-derived targeted antioxidants that can be used, and therefore it is essential that the compounds are effective antioxidants at concentrations well below those that disrupt function.

In addition to the nonspecific disruption of mitochondria, there are reports of more specific inhibition by lipophilic cations. Low micromolar concentrations of the tetraphenylphosphonium cation can inhibit the mitochondrial Na^+/Ca^{2+} exchanger (75) and 2-oxoglutarate dehydrogenase (76). However, replacing a phenyl group of tetraphenylphosphonium with a methyl group greatly decreases inhibition (75) and there is no evidence that decylTPP or $MitoQ_{10}$ affects these processes. Although the lipophilic cation rhodamine can inhibit the F_oF_1-ATP synthase (77), there are no reports of TPP cations doing so.

Annu. Rev. Pharmacol. Toxicol. 2007.47:629-656.

In moving to cells from isolated mitochondria, the targeted antioxidants will be accumulated into the cytosol 5–10-fold relative to the extracellular environment by the plasma membrane potential (3, 45, 54). Consequently, a given concentration of targeted antioxidant added to the incubation will disrupt mitochondria in cells more than for isolated mitochondria. In yeast, toxicity owing to mitochondrial disruption can be assessed by observing the effects on cell growth on nonfermentable medium. Under these conditions, $MitoQ_{10}$ and decylTPP show similar toxicity, with no effects evident at 0.1 μM, but at 1 μM toxicity is apparent and becomes severe at 10 μM. In comparison, TPMP is far less toxic (74). For mammalian cells in culture, we find that concentrations in the range 0.1–1 μM generally avoid short-term toxicity (M.F. Ross & M.P. Murphy, unpublished observations). However, this varies considerably with cell density, type, and incubation conditions and should be checked for all new experimental arrangements. In establishing these parameters for the nonspecific toxic effects of $MitoQ_{10}$, we find decylTPP to be a useful, routine control (74), although another group uses a long-chain hydroxyalkyl, TPP (78). Finally, in many cancer cells, expression of the plasma membrane P-glycoprotein, the product of the multidrug resistance (MDR-1) gene, in the plasma membrane leads to resistance to anticancer drugs (79). As this protein can export tetraphenylphosphonium and TPMP from cells (80, 81), it might also decrease accumulation of alkyltriphenylphosphonium cations by cells. To date, there is no evidence for $MitoQ_{10}$ efflux from cells by this pathway; however, the possible role of glycoprotein-P in anomalous cation uptake should always be considered.

Uptake and Distribution of $MitoQ_{10}$ and $MitoE_2$ Within Cells

It is well known that tetraphenylphosphonium and TPMP can be taken up into cells through the plasma membrane (53, 54). The uptake by $MitoQ_{10}$ is faster than that of TPMP, presumably owing to its greater hydrophobicity lowering its activation energy for passage through the plasma membrane (66). $MitoQ_{10}$ uptake into cells is largely blocked by abolishing the mitochondrial membrane potential by the uncoupler carbonylcyanide-p-trifluoromethoxyphenylhydrazone (FCCP), consistent with uptake being primarily into the mitochondria and not to other cell compartments (66). These data are consistent with rapid equilibration of $MitoQ_{10}$ across the plasma membrane followed by accumulation into mitochondria. Rapid cell subfractionation in 143B cells indicated that at least 90% of $MitoE_2$ was in the mitochondria (65). However, it is technically difficult to confirm that a lipophilic cation taken up by cells is actually located within mitochondria, as the mitochondria depolarize and rapidly release the compounds during cell subfractionation. An alternative way of visualizing TPP cations within cells is by using the 4-iodobutyltriphenylphosphonium (IBTP) cation in which TPP is linked to an iodoalkyl system that reacts with protein thiols to form a stable thioether linkage (82). This chemical bond prevents loss of the functionalized TPP cation on cell fixation, and the location can be visualized by using TPP-specific antiserum. The results from these experiments indicate there is almost total mitochondrial uptake within cells with very little remaining outside the mitochondria (82). This suggests that in cultured cells, nearly all accumulated lipophilic

Annu. Rev. Pharmacol. Toxicol. 2007.47:629-656.

cations are present within mitochondria; however, this approach may underestimate nonmitochondrial uptake (82). One further indication of the membrane potential-dependent mitochondrial concentration of TPP-containing molecules within cells was the FCCP-sensitive protection afforded by $MitoQ_{10}$ in a cell model of Friedreich's ataxia, whereas FCCP did not affect the potency of decylQ or idebenone (73). This is fully consistent with $MitoQ_{10}$ protecting against the damage in this cell model owing to its $\Delta\psi$-dependent uptake into mitochondria. Therefore, there exists strong evidence that, on incubation with cells in culture, $MitoQ_{10}$ is predominantly accumulated within the mitochondria, but the amount of $MitoQ_{10}$ present throughout the cell is currently difficult to quantify.

Protection Against Oxidative Damage by $MitoQ_{10}$ and $MitoE_2$ in Cells

$MitoQ_{10}$ and $MitoE_2$ have been used in a large range of mitochondrial and cell models, summarized in **Supplemental Table 1**, where they show protection against oxidative damage. In several cases, $MitoQ_{10}$ and $MitoE_2$ decreased levels of cytoplasmic ROS, as assessed by measuring the fluorescence of cytosolic dichlorofluorescein (78, 87–92, 99, 102, 104). Increased dichlorofluorescein fluorescence is generally interpreted as increased hydrogen peroxide production, suggesting that $MitoQ_{10}$ decreases hydrogen peroxide efflux from mitochondria. However, the mechanism is unclear as $MitoQ_{10}$ does not react with hydrogen peroxide (64). $MitoQ_{10}$ may be acting upstream of mitochondrial hydrogen peroxide production; alternatively, it could be that the ROS produced by mitochondria under these situations is not hydrogen peroxide but some other compound, such as peroxynitrite or a lipid peroxidation breakdown product, that reacts with dichlorofluorescein. The interaction of $MitoQ_{10}$ with mitochondrial ROS within rotenone-treated fibroblasts has been studied in detail (90). $MitoQ_{10}$ did not decrease superoxide production as measured by dihydroethidium oxidation but it did prevent lipid peroxidation as measured by the fluorescent probe C11-BODIPY (90). This finding is consistent with the model for $MitoQ_{10}$ action developed from studies with isolated mitochondria, namely that the main antioxidant action of $MitoQ_{10}$ is to prevent lipid peroxidation. It remains to be seen if this is the major mechanism by which $MitoQ_{10}$ acts as a protective agent in all cell types and forms of oxidative stress.

There are a number of other mechanisms by which $MitoQ_{10}$ might affect mitochondrial ROS metabolism. The accumulation of lipophilic cations can decrease the mitochondrial $\Delta\psi$, thus lowering ROS production by reverse electron transport (105, 106). In vitro, both $MitoQ_{10}$ and decylTPP can decrease mitochondrial ROS production by this mechanism; however, there are no indications that this is the case in cells. $MitoQ_{10}$, in common with all ubiquinols, can deprotonate in water (pK_a = 11.3) to form the ubiquinolate anion, which can facilitate the formation of superoxide from oxygen (68). However, this superoxide production pathway is insignificant if ubiquinol deprotonation is prevented by maintaining it in the lipid phase (68). This seems to be the case with $MitoQ_{10}$, which produces less superoxide than idebenone in the presence of mitochondrial membranes (64). In yeast mitochondria, any

Annu. Rev. Pharmacol. Toxicol. 2007.47:629-656.

superoxide produced by $MitoQ_{10}$ does not cause damage (64), and in fibroblasts $MitoQ_{10}$ does not increase superoxide production (90). Even so, it may be that such a nondamaging flux of ROS from $MitoQ_{10}$ could increase expression of antioxidant defense enzymes and thus increase protection, a process called hormesis (107). Future work will examine how antioxidant defenses respond to the long-term presence of $MitoQ_{10}$. To summarize, current evidence indicates that $MitoQ_{10}$ acts predominantly as an antioxidant through preventing lipid peroxidation to the mitochondrial inner membrane. However, more experimentation is required to determine if this model applies to all cell types and forms of oxidative stress.

ADMINISTRATION AND DISTRIBUTION OF TRIPHENYLPHOSPHONIUM CATIONS IN VIVO

To function as therapies, mitochondria-targeted antioxidants must be delivered to mitochondria within cells in patients, preferably following oral administration (**Figure 2**). Because TPP cations pass easily through phospholipid bilayers, they should be able to pass from the gut to the bloodstream and from there to most tissues. It has been shown that the TPMP cation is taken up into energized mitochondria within the perfused heart (108, 109), liver (110), and skeletal muscle (111–113). When simple alkylTPP compounds, $MitoE_2$, or $MitoQ_{10}$ are administered to mice by intravenous injection, they are rapidly cleared from the plasma and accumulate in the heart, brain, skeletal muscle, liver, and kidney (114–116). These experiments clearly indicate that once in the bloodstream, alkylTPP compounds rapidly redistribute into organs; however, it is difficult to be sure where the compound is located within the cell owing to the rapid redistribution from the mitochondria upon homogenizing the tissue. One surrogate approach is to use IBTP (82), and when IBTP was given to mice by intravenous injection there was accumulation into mitochondria, but not cytosol, of the heart, consistent with predominant mitochondrial localization of TPP cations taken up into tissues from the bloodstream (114).

Importantly, TPP-derived compounds are orally bioavailable to mice, as was shown by feeding mice tritiated TPMP, $MitoE_2$, or $MitoQ_{10}$ in their drinking water, which led to uptake into the plasma and from there into the heart, brain, liver, kidney, and muscle (114). The TPMP was shown to be cleared from all organs at a similar rate by a first-order process with a half-life of approximately 1.5 days (114). Therefore, these studies are consistent with orally administered alkylTPP compounds distributing to all organs owing to their facile permeation through biological membranes.

The nonspecific toxicity of alkylTPP cations found in mitochondria and cells will also occur in vivo, and this will probably be the major factor limiting the amounts of the compound that can be administered safely. In crude toxicity assessments (114), TPMP and $MitoE_2$ showed no toxicity at 300 nmol intravenous (~4–6 mg/kg) but did show toxicity at 500 nmol (~6–10 mg/kg). $MitoQ_{10}$ was better tolerated, with no toxicity at 750 nmol (~20 mg/kg) but toxicity was evident at 1000 nmol (~27 mg/kg). Administering 500 μM TPMP, $MitoE_2$, or $MitoQ_{10}$ to mice in their drinking water could be maintained for several weeks: No toxic effects were noted for TPMP for

Annu. Rev. Pharmacol. Toxicol. 2007.47:629-656.

at least 43 days, $MitoE_2$ for at least 14 days, or $MitoQ_{10}$ for at least 14 days (114). The similarities in toxicities are consistent with the hypothesis that the toxic effects are largely due to nonspecific disruption to mitochondria caused by accumulation of large amounts of the lipophilic cation. Although the published investigations outlined above are relatively limited proof-of-principle studies, more formal toxicity determinations have also been undertaken in developing $MitoQ_{10}$ for clinical trials, and these are outlined below under the pharmaceutical development of $MitoQ_{10}$. To summarize, it is possible to administer alkyltriphenylphosphonium compounds to animals orally and they are taken up into the plasma with reasonable bioavailability and then rapidly cleared from the plasma accumulating in mitochondria within tissues.

EFFICACY OF MITOCHONDRIA-TARGETED ANTIOXIDANTS IN VIVO

Having shown that the long-term administration of mitochondria-targeted antioxidants is possible, the next step is to determine whether the amount of compound accumulated is sufficient to act as an antioxidant in vivo. To date, only a few trials of this nature have been published. When 500 μM $MitoQ_{10}$ was administered to rats in their drinking water for 2 weeks and the hearts then isolated and exposed to ischemia-reperfusion injury in a Langendorff perfusion system, there was protection against the loss of heart function, tissue damage, and mitochondrial function compared with TPMP or short-chain quinol controls (117). The most probable reason for the observed protection in these experiments is that lipid peroxidation in the mitochondrial inner membrane was being prevented by $MitoQ_{10}$ (118). However, this has yet to be confirmed by showing that $MitoQ_{10}$ can block an increase in markers of oxidative damage in mitochondria.

Infusion of $MitoE_2$ into the striatum of rats does not protect against loss of striatal medium-spiny neurons following acute perinatal ischemia-reperfusion injury (119), suggesting that mitochondrial oxidative damage is not important for this kind of cell death or that the acute ROS damage overwhelmed the capacity of $MitoE_2$ (119). Feeding *Drosophila melanogaster* $MitoQ_{10}$ had no effect on the normal aging process although there appeared to be some protection against various forms of oxidative stress. The protection was found to be sex specific and TPMP also offered protection in these situations, which suggests that the increased survival may be a fly-specific effect (120).

OTHER MITOCHONDRIA-TARGETED ANTIOXIDANTS

Most work on mitochondria-targeted antioxidants has been on $MitoE_2$ and $MitoQ_{10}$, which are designed to prevent lipid peroxidation. However, targeting to mitochondria by conjugation to the lipophilic TPP cation can clearly be applied to many other small, neutral antioxidants. A range of further mitochondria-targeted antioxidants has been developed against each aspect of the ROS cascade emanating from the respiratory chain, which starts with superoxide, going on to hydrogen peroxide, then to

Annu. Rev. Pharmacol. Toxicol. 2007.47:629-656.

the hydroxyl radical (in the presence of ferrous ions), and finally to lipid peroxidation (**Figure 6**). These are a mitochondria-targeted version of the SOD mimetic M40403 (121), MitoSOD, which can degrade superoxide; a mitochondria-targeted version of the peroxidase mimetic ebselen, MitoPeroxidase (122); and a mitochondria-targeted version of the nitroxide TEMPOL. The MitoTEMPOL molecule may act as a SOD mimetic and also promote the detoxification of ferrous iron, by oxidizing it to ferric iron, while simultaneously being reduced to a hydroxylamine, which can then be converted back to a nitroxide. We have also prepared a mitochondria-targeted version of the spin trap phenylbutylnitrone (MitoPBN), and this has been shown to interact with mitochondrial carbon-centered radicals (123). Clearly, many other antioxidants can be targeted to mitochondria by this approach, which has been exploited by a number of other groups (78, 88, 124, 125). Work is continuing toward developing an understanding of the interaction of these molecules with mitochondria and determining which ones have useful properties. The ultimate aim is to create a suite of mitochondria-targeted antioxidants that can be used to intervene at several stages of the mitochondrial ROS cascade and perhaps be used in a complementary fashion (**Figure 6**).

ALTERNATIVE TARGETING STRATEGIES

In addition to targeting antioxidants to mitochondria, conjugation to the TPP cation can also be used to introduce a range of other potentially useful molecules to mitochondria (2, 45). The use of TPP to facilitate the delivery of thiol probes is well established (82, 126, 127) and the incorporation of TPP is a key aspect of the commercially available mitochondria-specific superoxide probe, MitoSOX. In addition, it should be possible to conjugate TPP to a range of drugs so they can interact with specific sites within the mitochondria. It may also be possible to use this process to deliver and hold a pool of active compound within the cell. Finally, TPP should be able to deliver active functions to mitochondria using a cleavable connector motif that is slowly degraded in the mitochondria, leaving a cargo in the matrix. An analogous strategy using a disulfide as a cleavable link has been developed to deliver compounds to the cytosol (128).

To date, the TPP cation has been the targeting component of most of the compounds examined. The TPP cation is clearly a promising approach as its interactions with mitochondria are reasonably well understood, the molecules are chemically tractable, and one exemplar has been taken through to Phase II trials. However, the properties of easy passage through phospholipid bilayers and accumulation within mitochondria are shared by many other lipophilic cations, and, in principle, these could also be used. For example, rhodamine was used to direct cisplatin to mitochondria as an anticancer treatment (55). The rationale for using molecules such as rhodamine is less compelling as they are chemically complicated and their uptake is not as well understood as that for the TPP compounds. However, many other simple lipophilic cations could be used in place of TPP. Alkyl triphenylarsonium-based compounds should behave similarly to those of TPP, although triphenylarsine is less reactive than triphenylphosphine and consequently the chemical synthesis of the requisite

Annu. Rev. Pharmacol. Toxicol. 2007.47:629-656.

cations would be more challenging. Triphenylamine exists but is a poor nucleophile, and, consequently, alkyl triphenylammonium cations are difficult to prepare and are rather unstable. Benzylammonium cations are much more chemically accessible and these are taken up by mitochondria, albeit far less effectively than TPP derivatives (129, 130). In addition to varying the cation, it should also be possible to vary substituents on the phenyl groups of the TPP function to modulate hydrophobicity and other properties. These modifications may be useful to fine-tune the hydrophobicity of the molecule or to lessen any toxicity or to prevent the induction of the glycoprotein-P efflux pathway.

PHARMACEUTICAL DEVELOPMENT OF TRIPHENYLPHOSPHONIUM CATIONS

The development of $MitoQ_{10}$ as a pharmaceutical is somewhat different from that of most other pharmaceuticals. Typically, in medicinal chemistry a large number of compounds are investigated that are based on a lead compound that interacts with a specific target, such as a receptor binding site. In assessing these compounds, the "rule of five" is often used as a preliminary screen to ensure that drug candidates are soluble, bioavailable, and can pass through phospholipid bilayers (131). However, mitochondria-targeted antioxidants based on TPP lipophilic cations are less constrained by these traditional guidelines as they have the unusual property of being both relatively water soluble and membrane permeant. Even though the molar mass of $MitoQ_{10}$ is relatively large for a pharmaceutical and it has a high octanol/PBS partition coefficient (72), it is readily bioavailable and passes easily through biological membranes. A further unusual feature of the TPP compounds is that they are targeted to an organelle to interfere in a general rather than a specific process, i.e., oxidative damage. Therefore, if lipophilic cations such as $MitoQ_{10}$ prove to be effective pharmaceuticals, it represents a previously unexplored approach to medicinal chemistry and drug discovery.

$MitoQ_{10}$ is now being developed as a pharmaceutical (132). For a commercially satisfactory stable formulation it was found beneficial to prepare the compound with the methanesulfonate counter anion and, to facilitate handling, long-term storage, and manufacture, it is adsorbed onto β-cyclodextrin. This preparation was readily made into tablets and has passed through conventional animal toxicity screening with no observable adverse effects at a level of 10.6 mg/kg. The oral bioavailability was determined at approximately 10%, and major metabolites in urine are glucuronides and sulfates of the reduced, hydroquinone form, along with demethylated compounds. In Phase I trials, $MitoQ_{10}$ showed good pharmacokinetic behavior with oral dosing at 80 mg (1 mg/kg), resulting in a plasma $C_{max} = 33.15$ ng/ml and $T_{max} \sim 1$ h. This formulation has good pharmaceutical characteristics and is proceeding to Phase II trials in Parkinson's disease and Friedreich's ataxia (132). This development work with $MitoQ_{10}$ has established a satisfactory pharmaceutical profile for the TPP moiety and will undoubtedly be the basis for future pharmaceutical formulations of other mitochondria-targeted antioxidants and related compounds.

Annu. Rev. Pharmacol. Toxicol. 2007.47:629-656.

PERSPECTIVE

The use of the TPP cation to increase the antioxidant defenses of mitochondria has been demonstrated to be a viable strategy in vitro. It has also been shown that compounds such as $MitoQ_{10}$ can be formulated into pharmaceuticals that can be successfully delivered orally to humans. Animal experiments have indicated that $MitoQ_{10}$ has antioxidant efficacy in tissues and therefore the scene is set for testing this and related compounds in human diseases. It will be important to ascertain definitively whether these chemicals are acting as effective antioxidants in vitro, and whether by so doing, they improve the outcome of the disease pathology. An intriguing aspect of the use of mitochondria-targeted antioxidants is that they could, in principle, be applied to a range of diseases and organs because mitochondrial oxidative damage contributes to so many disorders. In addition, they could be applied to acute injuries such as ischemia-reperfusion injury during surgery; to semiacute situations such as liver damage from steatohepatitis; or to chronic degenerative disease such as Parkinson's disease, Friedreich's ataxia, or type II diabetes. It may even be possible to administer these compounds prophylactically. Hopefully, work over the next few years will indicate in which organs these compounds are effective, whether they can decrease mitochondrial oxidative damage in diseases, and whether this positively affects the outcome for the patient. Finally, there is considerable scope to fine-tune the chemical biology of these compounds to target specific ROS and other mitochondrial targets.

SUMMARY POINTS

1. Decreasing mitochondrial oxidative damage is a useful therapeutic approach for a wide range of human disorders.
2. Lipophilic cations can be used to target antioxidants to mitochondria in vivo, and this approach is now being tested in patients with degenerative diseases.

DISCLOSURE STATEMENT

Both authors hold patents in the areas covered by the review. Some of these patents are being developed by a company, Antipodean Pharmaceuticals Inc., with a view to being used as therapies for human diseases. Both authors are consultants to Antipodean Pharmaceuticals Inc. In addition, through the system operated by the University of Otago, New Zealand (where both authors were employed when the original patent was filed), the authors may be assigned a proportion of any income arising from the patents.

ACKNOWLEDGMENTS

Work on these topics in the authors' laboratories has been supported over several years by the Health Research Council of New Zealand, the Marsden Fund administered by the Royal Society of New Zealand, the Foundation of Research Science and

Annu. Rev. Pharmacol. Toxicol. 2007.47:629-656.

Technology of New Zealand, Antipodean Pharmaceuticals Inc., the Friedreich's Ataxia Research Alliance, and the Medical Research Council. This work was supported by the European Community's sixth Framework Program for Research, Priority 1 "Life sciences, genomics and biotechnology for health" Contract LSHM-CT-2004–503116. We thank Dr. Meredith Ross for making the figures, Dr. Kenneth Taylor of Antipodean Pharmaceuticals Inc. for his support and encouragement, and numerous coworkers for their insights and contributions to the work described here.

LITERATURE CITED

2. An overview of the rationale for targeting antioxidants to mitochondria.

1. Scheffler IE. 1999. *Mitochondria*. New York: Wiley-Liss
2. **Murphy MP, Smith RAJ. 2000. Drug delivery to mitochondria: the key to mitochondrial medicine. *Adv. Drug Deliv. Rev*. 41:235–50**
3. Nicholls DG, Ferguson SJ. 2002. *Bioenergetics 3*. London: Academic
4. Green DR, Kroemer G. 2004. The pathophysiology of mitochondrial cell death. *Science* 305:626–29
5. Zong WX, Thompson CB. 2006. Necrotic death as a cell fate. *Genes Dev*. 20:1–15
6. Wallace DC. 1999. Mitochondrial diseases in man and mouse. *Science* 283:1482–88
7. Smeitink J, van den Heuvel L, DiMauro S. 2001. The genetics and pathology of oxidative phosphorylation. *Nat. Rev. Genet*. 2:342–52
8. Leonard JV, Schapira AH. 2000. Mitochondrial respiratory chain disorders I: mitochondrial DNA defects. *Lancet* 355:299–304
9. Leonard JV, Schapira AH. 2000. Mitochondrial respiratory chain disorders II: neurodegenerative disorders and nuclear gene defects. *Lancet* 355:389–94
10. Taylor RW, Turnbull DM. 2005. Mitochondrial DNA mutations in human disease. *Nat. Rev. Genet*. 6:389–402
11. Taylor RW, Chinnery PF, Clark KM, Lightowlers RN, Turnbull DM. 1997. Treatment of mitochondrial disease. *J. Bioenerg. Biomembr*. 29:195–205
12. Mattson MP, Magnus T. 2006. Ageing and neuronal vulnerability. *Nat. Rev. Neurosci*. 7:278–94
13. Abou-Sleiman PM, Muqit MMK, Wood NW. 2006. Expanding insights of mitochondrial dysfunction in Parkinson's disease. *Nat. Rev. Neurosci*. 7:207–19
14. Squitieri F, Cannella M, Sgarbi G, Maglione V, Falleni A, et al. 2006. Severe ultrastructural mitochondrial changes in lymphoblasts homozygous for Huntington disease mutation. *Mech. Ageing Dev*. 127:217–20
15. Beal MF. 2005. Mitochondria take center stage in aging and neurodegeneration. *Ann. Neurol*. 58:495–505
16. Green K, Brand MD, Murphy MP. 2004. Prevention of mitochondrial oxidative damage as a therapeutic strategy in diabetes. *Diabetes* 53:S110–18
17. Brownlee M. 2001. Biochemistry and molecular cell biology of diabetic complications. *Nature* 414:813–20
18. Pessayre D, Berson A, Fromenty B, Mansouri A. 2001. Mitochondria in steatohepatitis. *Semin. Liver Dis*. 21:57–69

Annu. Rev. Pharmacol. Toxicol. 2007.47:629-656.

19. Crouser ED, Julian MW, Blaho DV, Pfeiffer DR. 2002. Endotoxin-induced mitochondrial damage correlates with impaired respiratory activity. *Crit. Care Med.* 30:276–84
20. Wright AF, Jacobson SG, Cideciyan AV, Roman AJ, Shu X, et al. 2004. Lifespan and mitochondrial control of neurodegeneration. *Nat. Genet.* 36:1153–58
21. Raha S, Robinson BH. 2000. Mitochondria, oxygen free radicals, disease and ageing. *Trends Biochem. Sci.* 25:502–8
22. Finkel T. 2005. Radical medicine: treating ageing to cure disease. *Nat. Rev. Mol. Cell Biol.* 6:971–76
23. Balaban RS, Nemoto S, Finkel T. 2005. Mitochondria, oxidants, and aging. *Cell* 120:483–95
24. Miwa S, St-Pierre J, Partridge L, Brand MD. 2003. Superoxide and hydrogen peroxide production by *Drosophila* mitochondria. *Free Radic. Biol. Med.* 35:938–48
25. St-Pierre J, Buckingham JA, Roebuck SJ, Brand MD. 2002. Topology of superoxide production from different sites in the mitochondrial electron transport chain. *J. Biol. Chem.* 277:44784–90
26. Starkov AA, Fiskum G, Chinopoulos C, Lorenzo BJ, Browne SE, et al. 2004. Mitochondrial alpha-ketoglutarate dehydrogenase complex generates reactive oxygen species. *J. Neurosci.* 24:7779–88
27. Forman HJ, Kennedy J. 1976. Dihydroorotate-dependent superoxide production in rat brain and liver. A function of the primary dehydrogenase. *Arch. Biochem. Biophys.* 173:219–24
28. Giorgio M, Migliaccio E, Orsini F, Paolucci D, Moroni M, et al. 2005. Electron transfer between cytochrome c and p66shc generates reactive oxygen species that trigger mitochondrial apoptosis. *Cell* 122:221–33
29. Sandri G, Panfili E, Ernster L. 1990. Hydrogen peroxide production by monoamine oxidase in isolated rat-brain mitochondria: its effects on glutathione levels and Ca^{2+} efflux. *Biochim. Biophys. Acta* 1035:300–5
30. Sawyer DT, Valentine JS. 1981. How super is superoxide? *Acc. Chem. Res.* 14:393–400
31. Vasquez-Vivar J, Kalyanaraman B, Kennedy MC. 2000. Mitochondrial aconitase is a source of hydroxyl radical. An electron spin resonance investigation. *J. Biol. Chem.* 275:14064–69
32. Beckman JS, Beckman TW, Chen J, Marshall PA, Freeman BA. 1990. Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. *Proc. Natl. Acad. Sci. USA* 87:1620–24
33. Fridovich I. 1997. Superoxide anion radical ($O_2^{-\cdot}$), superoxide dismutases, and related matters. *J. Biol. Chem.* 272:18515–17
34. Skulachev VP. 2005. How to clean the dirtiest place in the cell: cationic antioxidants as intramitochondrial ROS scavengers. *IUBMB Life* 57:305–10
35. Schriner SE, Linford NJ, Martin GM, Treuting P, Ogburn CE, et al. 2005. Extension of murine life span by overexpression of catalase targeted to mitochondria. *Science* 308:1909–11

Annu. Rev. Pharmacol. Toxicol. 2007.47:629-656.

36. Chen Z, Siu B, Ho YS, Vincent R, Chua CC, et al. 1998. Overexpression of mnSOD protects against myocardial ischemia/reperfusion injury in transgenic mice. *J. Mol. Cell Cardiol.* 30:2281–89
37. The Parkinson Study Group. 1993. Effects of tocopherol and deprenyl on the progression of disability in early Parkinson's disease. *N. Engl. J. Med.* 328:176–83
38. The Parkinson Study Group. 1998. Mortality in datatop: a multicenter trial in early Parkinson's disease. *Ann. Neurol.* 43:318–25
39. Murphy MP. 2001. Development of lipophilic cations as therapies for disorders due to mitochondrial dysfunction. *Exp. Opin. Biol. Therapy*. 1:753–64
40. Shults CW, Beal MF, Song D, Fontaine D. 2004. Pilot trial of high dosages of coenzyme Q_{10} in patients with Parkinson's disease. *Exp. Neurol.* 188:491–94
41. Shults CW, Oakes D, Kieburtz K, Beal MF, Haas R, et al. 2002. Effects of coenzyme Q_{10} in early Parkinson disease: evidence of slowing of the functional decline. *Arch. Neurol.* 59:1541–50
42. Andersson CM, Hallberg A, Hogbberg T. 1996. Advances in the development of pharmaceutical antioxidants. *Adv. Drug Res.* 28:65–180
43. Smith RAJ, Kelso GF, Blaikie FH, Porteous CM, Ledgerwood EC, et al. 2003. Using mitochondria-targeted molecules to study mitochondrial radical production and its consequences. *Biochem. Soc. Trans.* 31:1295–99
44. Smith RAJ, Kelso GF, James AM, Murphy MP. 2004. Targeting coenzyme Q derivatives to mitochondria. *Meth. Enzymol.* 382:45–67
45. Murphy MP. 1997. Targeting bioactive compounds to mitochondria. *Trends Biotechnol.* 15:326–30
46. Ross MF, Kelso GF, Blaikie FH, James AM, Cocheme HM, et al. 2005. Lipophilic triphenylphosphonium cations as tools in mitochondrial bioenergetics and free radical biology. *Biochemistry* 70:222–30
47. Ketterer B, Neumcke B, Laeuger P. 1971. Transport mechanism of hydrophobic ions across through lipid bilayers. *J. Membr. Biol.* 5:225–45
48. Liberman EA, Topali VP, Tsofina LM, Jasaitis AA, Skulachev VP. 1969. Mechanism of coupling of oxidative phosphorylation and the membrane potential of mitochondria. *Nature* 222:1076–78
49. Flewelling RF, Hubbell WL. 1986. Hydrophobic ion interactions with membranes. *Biophys. J.* 49:531–40
50. Honig BH, Hubbell WL, Flewelling RF. 1986. Electrostatic interactions in membranes and proteins. *Annu. Rev. Biophys. Biophys. Chem.* 15:163–93
51. Cafiso DS, Hubbell WL. 1981. EPR determination of membrane potentials. *Annu. Rev. Biophys. Bioeng.* 10:217–44
52. Ono A, Miyauchi S, Demura M, Asakura T, Kamo N. 1994. Activation energy for permeation of phosphonium cations through phospholipid bilayer membrane. *Biochemistry* 33:4312–18
53. Azzone GF, Pietrobon D, Zoratti M. 1984. Determination of the proton electrochemical gradient across biological membranes. *Curr. Topics Bioenerg.* 13:1–77
54. Brand MD. 1995. Measurement of mitochondrial protonmotive force. In *Bioenergetics—A Practical Approach*, ed. GC Brown, CE Cooper, pp. 39–62. Oxford: IRL

46. A detailed explanation of the physical chemistry underlying the interaction of lipophilic cations with mitochondria.

48. The first demonstration that the membrane potential leads to the uptake of lipophilic cations into mitochondria.

Annu. Rev. Pharmacol. Toxicol. 2007.47:629-656.

55. Chen LB. 1988. Mitochondrial membrane potential in living cells. *Annu. Rev. Cell Biol*. 4:155–81
56. Cossarizza A, Baccarani CM, Kalashnikova G, Franceschi C. 1993. A new method for the cytofluorimetric analysis of mitochondrial membrane potential using the J-aggregate forming lipophilic cation 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolcarbocyanine iodide (JC-1). *Biochem. Biophys. Res. Commun*. 197:40–45
57. Poot M, Gibson LL, Singer VL. 1997. Detection of apoptosis in live cells by mitotracker red CMXROS and SYTO dye flow cytometry. *Cytometry* 27:358–64
58. Liberman EA, Skulachev VP. 1970. Conversion of biomembrane-produced energy into electric form. IV. General discussion. *Biochim. Biophys. Acta* 216:30–42
59. Bakeeva LE, Grinius LL, Jasaitis AA, Kuliene VV, Levitsky DO, et al. 1970. Conversion of biomembrane-produced energy into electric form. II. Intact mitochondria. *Biochim. Biophys. Acta* 216:13–21
60. Koehorst RB, Spruijt RB, Vergeldt FJ, Hemminga MA. 2004. Lipid bilayer topology of the transmembrane alpha-helix of M13 major coat protein and bilayer polarity profile by site-directed fluorescence spectroscopy. *Biophys. J.* 87:1445–55
61. Demura M, Kamo N, Kobatake Y. 1985. Determination of membrane potential with lipophilic cations: correction of probe binding. *Biochim. Biophys. Acta* 820:207–15
62. Flewelling RF, Hubbell WL. 1986. The membrane dipole potential in a total membrane potential model. Applications to hydrophobic ion interactions with membranes. *Biophys. J*. 49:541–52
63. James AM, Sharpley MS, Frerman FE, Hirst J, Smith RAJ, Murphy MP. 2006. Interaction of the mitochondria-targeted antioxidant $MitoQ_{10}$ with phospholipid bialyers and ubiquinone reductases. *J. Biol. Chem.* Submitted
64. James AM, Cocheme HM, Smith RAJ, Murphy MP. 2005. Interactions of mitochondria-targeted and untargeted ubiquinones with the mitochondrial respiratory chain and reactive oxygen species. Implications for the use of exogenous ubiquinones as therapies and experimental tools. *J. Biol. Chem*. 280:21295–312
65. Smith RAJ, Porteous CM, Coulter CV, Murphy MP. 1999. Targeting an antioxidant to mitochondria. *Eur. J. Biochem*. 263:709–16
66. Kelso GF, Porteous CM, Coulter CV, Hughes G, Porteous WK, et al. 2001. Selective targeting of a redox-active ubiquinone to mitochondria within cells. *J. Biol. Chem*. 276:4588–96
67. Maguire JJ, Wilson DS, Packer L. 1989. Mitochondrial electron transport-linked tocoperoxyl radical reduction. *J. Biol. Chem*. 264:21462–65
68. James AM, Smith RAJ, Murphy MP. 2004. Antioxidant and prooxidant properties of mitochondrial coenzyme Q. *Arch. Biochem. Biophys*. 423:47–56
69. Frei B, Kim MC, Ames BN. 1990. Ubiquinol-10 is an effective lipid-soluble antioxidant at physiological concentrations. *Proc. Natl. Acad. Sci. USA* 87:4879–83

64. In this paper the mode of reduction of $MitoQ_{10}$ by mitochondria was determined.

65. The synthesis and characterization of the first mitochondria-targeted antioxidant, $MitoE_2$.

66. The synthesis and characterization of $MitoQ_{10}$.

68. A detailed review of how ubiquinols act as antioxidants.

Annu. Rev. Pharmacol. Toxicol. 2007.47:629-656.

70. Sies H. 1993. Strategies of antioxidant defense. *Eur. J. Biochem.* 215:213–19
71. Ames BN, Shigenaga MK, Hagen TM. 1993. Oxidants, antioxidants, and the degenerative diseases of aging. *Proc. Natl. Acad. Sci. USA* 90:7915–22
72. Asin-Cayuela J, Manas AR, James AM, Smith RAJ, Murphy MP. 2004. Fine-tuning the hydrophobicity of a mitochondria-targeted antioxidant. *FEBS Lett.* 571:9–16
73. **Jauslin ML, Meier T, Smith RAJ, Murphy MP. 2003. Mitochondria-targeted antioxidants protect Friedreich ataxia fibroblasts from endogenous oxidative stress more effectively than untargeted antioxidants. *FASEB J.* 17:1972–74**
74. James AM, Cocheme HM, Murphy MP. 2005. Mitochondria-targeted redox probes as tools in the study of oxidative damage and ageing. *Mech. Ag. Dev.* 126:982–86
75. Wingrove DE, Gunter TE. 1986. Kinetics of mitochondrial calcium transport. II. A kinetic description of the sodium-dependent calcium efflux mechanism of liver mitochondria and inhibition by ruthenium red and by tetraphenylphosphonium. *J. Biol. Chem.* 261:15166–71
76. Mildaziene V, Baniene R, Marcinkeviciute A, Nauciene Z, Kalvenas A, Zimkus A. 1997. Tetraphenylphosphonium inhibits oxidation of physiological substrates in heart mitochondria. *Mol. Cell Biochem.* 174:67–70
77. Bullough DA, Ceccarelli EA, Roise D, Allison WS. 1989. Inhibition of the bovine-heart mitochondrial F_1-ATPase by cationic dyes and amphipathic peptides. *Biochim. Biophys. Acta* 975:377–83
78. Kalivendi SV, Konorev EA, Cunningham S, Vanamala SK, Kaji EH, et al. 2005. Doxorubicin activates nuclear factor of activated T-lymphocytes and FAS ligand transcription: role of mitochondrial reactive oxygen species and calcium. *Biochem. J.* 389:527–39
79. Gottesman MM, Pastan I. 1993. Biochemistry of the multidrug resistance mediated by the multidrug transporter. *Annu. Rev. Biochem.* 62:385–427
80. Gros P, Talbot F, Tang-Wai D, Bibi E, Kaback HR. 1992. Lipophilic cations: a group of model substrates for the multidrug-resistance transporter. *Biochemistry* 31:1992–98
81. Lampidis TJ, Shi YF, Calderon CL, Kolonias D, Tapiero H, Savaraj N. 1997. Accumulation of simple organic cations correlates with differential cytotoxicity in multidrug-resistant and -sensitive human and rodent cells. *Leukemia* 11:1156–59
82. Lin TK, Hughes G, Muratovska A, Blaikie FH, Brookes PS, et al. 2002. Specific modification of mitochondrial protein thiols in response to oxidative stress: a proteomics approach. *J. Biol. Chem.* 277:17048–56
83. Echtay KS, Murphy MP, Smith RAJ, Talbot DA, Brand MD. 2002. Superoxide activates mitochondrial uncoupling protein 2 from the matrix side. Studies using targeted antioxidants. *J. Biol. Chem.* 277:47129–35
84. Alleva R, Tomasetti M, Andera L, Gellert N, Borghi B, et al. 2001. Coenzyme Q blocks biochemical but not receptor-mediated apoptosis by increasing mitochondrial antioxidant protection. *FEBS Lett.* 503:46–50

73. A demonstration that mitochondria-targeted antioxidants are protective in a cell model of Friedreich's ataxia.

Annu. Rev. Pharmacol. Toxicol. 2007.47:629-656.

85. Hughes G, Murphy MP, Ledgerwood EC. 2005. Mitochondrial reactive oxygen species regulate the temporal activation of nuclear factor κB to modulate tumor necrosis factor-induced apoptosis: evidence from mitochondria-targeted antioxidants. *Biochem. J.* 389:83–89
86. Chen K, Thomas SR, Albano A, Murphy MP, Keaney JFJ. 2004. Mitochondrial function is required for hydrogen peroxide-induced growth factor receptor transactivation and downstream signaling. *J. Biol. Chem.* 279:35079–86
87. Schafer M, Schafer C, Ewald N, Piper HM, Noll T. 2003. Role of redox signaling in the autonomous proliferative response of endothelial cells to hypoxia. *Circ. Res.* 92:1010–15
88. Dhanasekaran A, Kotamraju S, Kalivendi SV, Matsunaga T, Shang T, et al. 2004. Supplementation of endothelial cells with mitochondria-targeted antioxidants inhibit peroxide-induced mitochondrial iron uptake, oxidative damage, and apoptosis. *J. Biol. Chem.* 279:37575–87
89. Saretzki G, Murphy MP, von Zglinicki T. 2003. MitoQ counteracts telomere shortening and elongates lifespan of fibroblasts under mild oxidative stress. *Aging Cell* 2:141–43
90. Koopman WJ, Verkaart S, Visch HJ, van der Westhuizen FH, Murphy MP, et al. 2005. Inhibition of complex I of the electron transport chain causes $O_2^{-\cdot}$-mediated mitochondrial outgrowth. *Am. J. Physiol. Cell Physiol.* 288:C1440–50
91. King A, Gottlieb E, Brooks DG, Murphy MP, Dunaief JL. 2004. Mitochondria-derived reactive oxygen species mediate blue light-induced death of retinal pigment epithelial cells. *Photochem. Photobiol.* 79:470–75
92. Barhoumi R, Faske J, Liu X, Tjalkens RB. 2004. Manganese potentiates lipopolysaccharide-induced expression of NOS2 in C6 glioma cells through mitochondrial-dependent activation of nuclear factor κB. *Brain Res. Mol. Brain Res.* 122:167–79
93. Sanjuan-Pla A, Cervera AM, Apostolova N, Garcia-Bou R, Victor VM, et al. 2005. A targeted antioxidant reveals the importance of mitochondrial reactive oxygen species in the hypoxic signaling of HIF-1α. *FEBS Lett.* 579:2669–74
94. Galeotti T, Pani G, Capone C, Bedogni B, Borrello S, et al. 2005. Protective role of mnSOD and redox regulation of neuronal cell survival. *Biomed. Pharmacother.* 59:197–203
95. Kim A, Murphy MP, Oberley TD. 2005. Mitochondrial redox state regulates transcription of the nuclear-encoded mitochondrial protein manganese superoxide dismutase: a proposed adaptive response to mitochondrial redox imbalance. *Free Radic. Biol. Med.* 38:644–54
96. Bianchi P, Pimentel DR, Murphy MP, Colucci WS, Parini A. 2005. A new hypertrophic mechanism of serotonin in cardiac myocytes: receptor-independent ROS generation. *FASEB J.* 19:641–43
97. Wang XF, Witting PK, Salvatore BA, Neuzil J. 2005. Vitamin E analogs trigger apoptosis in HER2/ERBB2-overexpressing breast cancer cells by signaling via the mitochondrial pathway. *Biochem. Biophys. Res. Commun.* 326:282–89
98. Ng Y, Barhoumi R, Tjalkens RB, Fan YY, Kolar S, et al. 2005. The role of docosahexaenoic acid in mediating mitochondrial membrane lipid oxidation and apoptosis in colonocytes. *Carcinogenesis* 26:1914–21

Annu. Rev. Pharmacol. Toxicol. 2007.47:629-656.

99. Siler-Marsiglio KI, Pan Q, Paiva M, Madorsky I, Khurana NC, Heaton MB. 2005. Mitochondrially targeted vitamin E and vitamin E mitigate ethanol-mediated effects on cerebellar granule cell antioxidant defense systems. *Brain Res*. 1052:202–11
100. Camello-Almaraz MC, Pozo MJ, Murphy MP, Camello PJ. 2006. Mitochondrial production of oxidants is necessary for physiological calcium oscillations. *J. Cell Physiol*. 206:487–94
101. Fearon IM. 2006. OxLDL enhances L-type Ca^{2+} currents via lysophosphatidylcholine-induced mitochondrial reactive oxygen species (ROS) production. *Cardiovasc. Res*. 69:855–64
102. Pletjushkina OY, Fetisova EK, Lyamzaev KG, Ivanova OY, Domnina LV, et al. 2005. Long-distance apoptotic killing of cells is mediated by hydrogen peroxide in a mitochondrial ROS-dependent fashion. *Cell. Death Differ.* 12:1442–44
103. Pletjushkina OY, Fetisova EK, Lyamzaev KG, Ivanova OY, Domnina LV, et al. 2006. Hydrogen peroxide produced inside mitochondria takes part in cell-to-cell transmission of apoptotic signal. *Biochemistry* 71:60–67
104. Hwang PM, Bunz F, Yu J, Rago C, Chan TA, et al. 2001. Ferredoxin reductase affects the p53-dependent, 5-fluorouracil-induced apoptosis in colorectal cancer cells. *Nat. Med.* 7:1111–17
105. Lambert AJ, Brand MD. 2004. Inhibitors of the quinone-binding site allow rapid superoxide production from mitochondrial NADH:Ubiquinone oxidoreductase (complex I). *J. Biol. Chem*. 279:39414–20
106. Lambert AJ, Brand MD. 2004. Superoxide production by NADH:Ubiquinone oxidoreductase (complex I) depends on the pH gradient across the mitochondrial inner membrane. *Biochem. J*. 382:511–17
107. Calabrese EJ, Staudenmayer JW, Stanek EJ. 2006. Drug development and hormesis: changing conceptual understanding of the dose response creates new challenges and opportunities for more effective drugs. *Curr. Opin. Drug Discov. Dev*. 9:117–23
108. Kauppinen R. 1983. Proton electrochemical potential of the inner mitochondrial membrane in isolated perfused rat hearts, as measured by exogenous probes. *Biochim. Biophys. Acta*. 725:131–37
109. Wan B, Doumen C, Duszynski J, Salama G, LaNoue KF. 1993. A method of determining electrical potential gradient across mitochondrial membrane in perfused rat hearts. *Am. J. Physiol. Heart Circ. Physiol*. 265:H445–52
110. Steen H, Maring JG, Meijer DK. 1993. Differential effects of metabolic inhibitors on cellular and mitochondrial uptake of organic cations in rat liver. *Biochem. Pharmacol*. 45:809–18
111. Rolfe DFS, Brand MD. 1996. Contribution of mitochondrial proton leak to skeletal muscle respiration and to standard metabolic rate. *Am. J. Physiol. Cell Physiol*. 271:C1380–89
112. Rolfe DFS, Brand MD. 1996. Proton leak and control of oxidative phosphorylation in perfused, resting rat skeletal muscle. *Biochim. Biophys. Acta* 1276:45–50
113. Rolfe DFS, Newman JM, Buckingham JA, Clark MG, Brand MD. 1999. Contribution of mitochondrial proton leak to respiration rate in working skeletal muscle and liver and to SMR. *Am. J. Physiol. Cell Physiol*. 276:C692–99

Annu. Rev. Pharmacol. Toxicol. 2007.47:629-656.

114. Smith RAJ, Porteous CM, Gane AM, Murphy MP. 2003. Delivery of bioactive molecules to mitochondria in vivo. *Proc. Natl. Acad. Sci. USA* 100:5407–12

115. Fukuda H, Syrota A, Charbonneau P, Vallois J, Crouzel M, et al. 1986. Use of ^{11}C-triphenylmethylphosphonium for the evaluation of membrane potential in the heart by positron-emission tomography. *Eur. J. Nucl. Med.* 11:478–83

116. Srivastava PC, Hay HG, Knapp FF. 1985. Effects of alkyl and aryl substitution on the myocardial specificity of radioiodinated phosphonium, arsonium, and ammonium cations. *J. Med. Chem.* 28:901–4

117. Adlam VJ, Harrison JC, Porteous CM, James AM, Smith RAJ, et al. 2005. Targeting an antioxidant to mitochondria decreases cardiac ischemia-reperfusion injury. *FASEB J.* 19:1088–95

118. Paradies G, Petrosillo G, Pistolese M, Di Venosa N, Federici A, Ruggiero FM. 2004. Decrease in mitochondrial complex I activity in ischemic/reperfused rat heart: Involvement of reactive oxygen species and cardiolipin. *Circ. Res.* 94:53–59

119. Covey MV, Murphy MP, Hobbs CE, Smith RAJ, Oorschot DE. 2006. Effect of the mitochondrial antioxidant, mito vitamin E, on hypoxic-ischemic striatal injury in neonatal rats: a dose-response and stereological study. *Exp. Neurol.* 199:513–19

120. Magwere T, West M, Riyahi K, Murphy MP, Smith RAJ, Partridge L. 2006. The effects of exogenous antioxidants on lifespan and oxidative stress resistance in *Drosophila melanogaster*. *Mech. Ag. Dev.* 127:356–70

121. Salvemini D, Wang ZQ, Zweier JL, Samouilov A, Macarthur H, et al. 1999. A nonpeptidyl mimic of superoxide dismutase with therapeutic activity in rats. *Science* 286:304–6

122. Filipovska A, Kelso GF, Brown SE, Beer SM, Smith RAJ, Murphy MP. 2005. Synthesis and characterization of a triphenylphosphonium-conjugated peroxidase mimetic: insights into the interaction of ebselen with mitochondria. *J. Biol. Chem.* 280:24113–26

123. Murphy MP, Echtay KS, Blaikie FH, Asin-Cayuela J, Cocheme HM, et al. 2003. Superoxide activates uncoupling proteins by generating carbon-centered radicals and initiating lipid peroxidation: studies using a mitochondria-targeted spin trap derived from alpha-phenyl-*n*-tert-butylnitrone. *J. Biol. Chem.* 278:48534–45

124. Dessolin J, Schuler M, Quinart A, De Giorgi F, Ghosez L, Ichas F. 2002. Selective targeting of synthetic antioxidants to mitochondria: towards a mitochondrial medicine for neurodegenerative diseases? *Eur. J. Pharmacol.* 447:155–61

125. Dhanasekaran A, Kotamraju S, Karunakaran C, Kalivendi SV, Thomas S, et al. 2005. Mitochondria superoxide dismutase mimetic inhibits peroxide-induced oxidative damage and apoptosis: role of mitochondrial superoxide. *Free Radic. Biol. Med.* 39:567–83

126. Burns RJ, Murphy MP. 1997. Labeling of mitochondrial proteins in living cells by the thiol probe thiobutyltriphenylphosphonium bromide. *Arch. Biochem. Biophys.* 339:33–39

114. Here it was shown that mitochondria-targeted antioxidants can be administered orally to mice and are then accumulated by mitochondria in tissues.

117. This is the first demonstration that mitochondria-targeted antioxidants can protect tissues from damage.

Annu. Rev. Pharmacol. Toxicol. 2007.47:629-656.

127. Burns RJ, Smith RAJ, Murphy MP. 1995. Synthesis and characterization of thiobutyltriphenylphosphonium bromide, a novel thiol reagent targetted to the mitochondrial matrix. *Arch. Biochem. Biophys.* 322:60–68
128. Filipovska A, Eccles MR, Smith RAJ, Murphy MP. 2004. Delivery of antisense peptide nucleic acids (PNAs) to the cytosol by disulphide conjugation to a lipophilic cation. *FEBS Lett.* 556:180–86
129. Muratsugu M, Kamo N, Kurihara K, Kobatake Y. 1977. Selective electrode for dibenzyl dimethyl ammonium cation as indicator of the membrane potential in biological systems. *Biochim. Biophys. Acta* 464:613–19
130. Kamo N, Muratsugu M, Hongoh R, Kobatake Y. 1979. Membrane potential of mitochondria measured with an electrode sensitive to tetraphenyl phosphonium and relationship between proton electrochemical potential and phosphorylation potential in steady state. *J. Membr. Biol.* 49:105–21
131. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. 1997. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Deliv. Rev.* 23:3–25
132. Antipodean Pharmaceuticals Inc. **http://www.antipodeanpharma.com/**

Annu. Rev. Pharmacol. Toxicol. 2007.47:629-656.

Annual Review of Pharmacology and Toxicology

Volume 47, 2007

Contents

Annu. Rev. Pharmacol. Toxicol. 2007.47:629-656.

Indexes

Errata

An online log of corrections to *Annual Review of Pharmacology and Toxicology* chapters (if any, 1997 to the present) may be found at http://pharmtox.annualreviews.org/errata.shtml