Review

Targeting lipophilic cations to mitochondria

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ARTICLE INFO

ABSTRACT

Mitochondrial function and dysfunction contributes to a range of important aspects of biomedical research. Consequently there is considerable interest in developing approaches to modify and report on mitochondria in cells and in vivo. One approach has been to target bioactive molecules to mitochondria by conjugating them to lipophilic cations. Due to the large mitochondrial membrane potential, the cations are accumulated within mitochondria inside cells. This approach had been used to develop mitochondria-targeted antioxidants that selectively block mitochondrial oxidative damage and prevent some types of cell death and also to develop probes of mitochondrial function. Here we outline some of the background to the development of these compounds.

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1. Introduction

Mitochondria contribute to many aspects of cell function and dysfunction. In particular, mitochondria are susceptible to oxidative damage and are also a major source of superoxide, consequently mitochondria accumulate oxidative damage contributing to mitochondrial dysfunction and cell death in a range of degenerative diseases [1–4]. Despite this, antioxidants have had limited success in preventing the progression of diseases involving mitochondrial oxidative damage. One possible reason for this may be that most small molecule antioxidants distribute around the body, with only a small fraction being taken up by the mitochondria. Thus pharmaceutically tractable and stable small molecule antioxidants are required that are selectively taken up by mitochondria within those organs most affected by mitochondrial oxidative damage where they block oxidative damage. One approach to preventing mitochondrial oxidative damage that may satisfy these criteria is to selectively target antioxidants to mitochondria by conjugation to lipophilic cations such as triphenyl phosphonium (TPP) [5–7] (Fig. 1).

The large membrane potential of 150–180 mV (negative inside) across the mitochondrial inner membrane can be used to deliver molecules to mitochondria [5]. Lipophilic cations pass easily through lipid bilayers because their charge is dispersed over a large surface area and the potential gradient drives their accumulation into the mitochondrial matrix [8–11]. The uptake of lipophilic cations into mitochondria increases 10 fold for every 61.5 mV of membrane potential at 37 °C leading to 100–500 fold accumulation, and uptake into cells is also driven by the plasma membrane potential (30–60 mV, negative inside) (Fig. 1).

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2. Mitochondria-targeted antioxidants

A wide range of antioxidants could be targeted to mitochondria by conjugation to the TPP moiety, and those that have been employed to date include TPP-conjugated derivatives of ubiquinone [12–15], tocopherol [16], lipoic acid [17], spin traps [18] and the peroxidase mimetic Ebselen [19]. As lipid 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 which are effective against lipid peroxidation. In particular, the targeted version of ubiquinol (MitoQ) has been used most extensively and is the best understood member of the family (reviewed in [7]). To summarize the work to date on MitoQ, it is taken up rapidly by isolated mitochondria driven by the $\Delta\psi$, and within mitochondria nearly all the accumulated MitoQ is adsorbed to the matrix surface of the inner membrane. MitoQ is reduced to the active ubiquinol antioxidant by complex II in the respiratory chain, but it is not a good substrate for complex I or electron transfer flavoprotein-ubiquinone oxidoreductase. MitoQ cannot restore respiration in mitochondria lacking coenzyme Q because the reduced form of MitoQ is poorly oxidised by complex III; consequently, all the effects of MitoQ are likely to be due to the accumulation of the antioxidant ubiquinol form. Furthermore, when the ubiquinol form of MitoQ acts as an antioxidant it is oxidised to the ubiquinone form which is then rapidly reduced by complex II, restoring antioxidant efficacy. As MitoQ 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. MitoQ has also been shown to detoxify peroxynitrite and it can react with superoxide although, as with other ubiquinols, its reactivity with hydrogen peroxide is
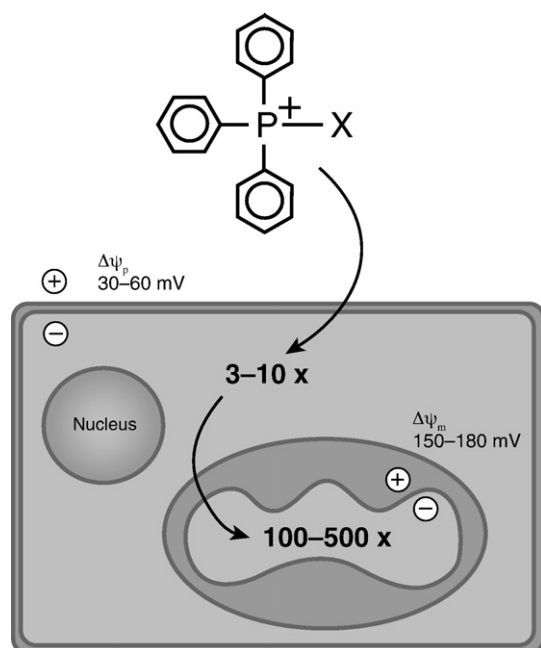
Fig. 1. Uptake of triphenylphosphonium cations by mitochondria within cells. This schematic shows the uptake of a triphenylphosphonium moiety attached to a moiety (X) into the cytoplasm from the extracellular environment driven by the plasma membrane potential. From the cytoplasm the compound is further accumulated into mitochondria, driven by the mitochondrial membrane potential. The mitochondrial and plasma membrane potentials ($\Delta\psi$) are indicated. The moiety, X, could be an antioxidant or a probe of mitochondrial function.

negligible. In summary MitoQ seems to fulfil most of the requirements, in isolated mitochondria, for a mitochondria-targeted antioxidant.

3. Targeting antioxidants to mitochondria in cells

In moving from isolated mitochondria to cells, toxicity is the first issue to be considered. The extensive accumulation of lipophilic cations within isolated mitochondria can disrupt membrane integrity, respiration and ATP synthesis as a result of adsorption of the lipophilic cations to the matrix surface of the inner membrane. 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. The non-specific effects of MitoQ on mitochondria are assessed using the control compound decylTPP which is similar in hydrophobicity to MitoQ (octanol-PBS partition coefficients of 5000 and 2760 respectively) but lacks the antioxidant ubiquinol moiety. We find that the non-specific mitochondrial disruption of MitoQ and decylTPP occurs at similar concentrations and 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 moving to cells from isolated mitochondria the targeted antioxidants will be accumulated 5–10 fold relative to the extra-cellular environment by the plasma membrane potential [10]. Consequently a given concentration of targeted antioxidant added to the incubation will disrupt mitochondria in cells more than for isolated mitochondria. In yeast, toxicity due to mitochondrial disruption can be assessed by observing the effects on cell growth on non-fermentable medium. For mammalian cells in culture we find that concentrations in the range 0.1-1 µM generally avoid short-term toxicity (unpublished observations). However this varies considerably with cell density, type and incubation conditions and should be checked for all new experimental arrangements.

The uptake by MitoQ into cells is far faster than that of TPMP, presumably due to its greater hydrophobicity lowering its activation energy for passage through the plasma membrane [10]. MitoQ uptake into cells is largely blocked by abolishing the mitochondrial membrane potential by the uncoupler carbonylcyanide-*p*-trifluoro-methoxyphenylhydrazone (FCCP), consistent with uptake being primarily into the mitochondria and not to other cell compartments. These data are consistent with rapid equilibration of MitoQ across the plasma membrane followed by accumulation into mitochondria. It is technically difficult to confirm that a lipophilic cation taken up by cells is actually located within mitochondria as the mitochondria depolarise and rapidly release the compounds during cell subfractio-nation. 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 [20]. 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 [20]. This suggests that, in cells in culture, nearly all accumulated lipophilic cations are present within mitochondria. One further indication of the membrane potential-dependent mito-chondrial concentration of TPP-containing molecules within cells was the FCCP-sensitive protection afforded by MitoQ in a cell model of Friedreich's ataxia, while FCCP did not affect the potency of decylQ or idebenone [21]. This is fully consistent with MitoQ protecting against the damage in this cell model due to its $\Delta\psi$-dependent uptake into mitochondria. Therefore on incubation with cells in culture, MitoQ is predominantly accumulated within the mitochondria, but the amount of MitoQ present throughout the cell is currently difficult to quantify.

MitoQ has been used in a large range of mitochondrial and cell models [7], where they show protection against oxidative damage. The interaction of MitoQ with mitochondrial ROS within rotenone-treated fibroblasts has been studied in detail [22]. MitoQ did not decrease superoxide production as measured by dihydroethidium oxidation but it did prevent lipid peroxidation as measured by the fluorescent probe C11-BODIPY [22]. This finding is consistent with the model for MitoQ action developed from studies with isolated mitochondria, namely that the main antioxidant action of MitoQ is to prevent lipid peroxidation. It remains to be seen if this is the major mechanism by which MitoQ acts as a protective agent in all cell types and forms of oxidative stress.

4. Targeting antioxidants to mitochondria in vivo

To function as therapies mitochondria-targeted antioxidants must be delivered to mitochondria within cells in patients, preferably following oral administration. As 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 when simple alkylTPP compounds, MitoE or MitoQ 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 [23]. These experiments clearly indicate that once in the bloodstream alkylTPP compounds rapidly redistribute into organs.

Importantly, TPP-derived compounds are orally bioavailable to mice, as was shown by feeding mice tritiated TPMP, MitoE or MitoQ in their drinking water which led to uptake into the plasma and from there into the heart, brain, liver, kidney and muscle [23]. The TPMP was shown to be cleared from all organs at a similar rate by a first order process with a half life of about 1.5 days [23]. Therefore these studies are consistent with orally-administered alkylTPP compounds distributing to all organs due to their facile permeation through biological membranes.

The non-specific 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 [23] TPMP and MitoE showed no toxicity at 300 nmol intravenous (~4–6 mg/kg) but did show toxicity at 500 nmol (~6–10 mg/kg). MitoQ was marginally better tolerated with no toxicity at 750 nmol (~20 mg/kg) but toxicity evident at 1,000 nmol (~27 mg/kg). Administering 500 M TPMP, MitoE or MitoQ to mice in their drinking water could be maintained for several weeks: no toxic effects were noted for TPMP for at least 43 days, MitoE for at least 14 days or MitoQ for at least 14 days [23]. The similarities in toxicities are consistent with the hypothesis that the toxic effects are largely due to non-specific disruption to mitochondria caused by accumulation of large amounts of the lipophilic cation. 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.

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. When 500 µM MitoQ 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 [24]. 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 [24]. However, this has yet to be confirmed by showing that MitoQ can block an increase in markers of oxidative damage in mitochondria. In another work, MitoQ protected against oxidative damage caused by nitroglycerin in the rat aorta [25]. Therefore there is evidence that mitochondria-targeted antioxidants may be effective in vivo.

The development of MitoQ 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 [26]. 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 is relatively large for a pharmaceutical and it has a high octanol/PBS partition coefficient, it is readily bioavailable and passes easily through biological membranes. A further unusual feature of the TPP-targeted compounds is that they are targeted to an organelle to interfere in a general rather than a specific process, viz oxidative damage. Therefore if lipophilic cations such as MitoQ prove to be effective pharmaceuticals it represents an unusual approach to medicinal chemistry and drug discovery. MitoQ is now being developed as a pharmaceutical by Antipodean Pharmaceuticals Inc. (http://www.antipodeanpharma.com/).

5. Conclusions

The use of the TPP cation to increase the antioxidant defences of mitochondria has been demonstrated to be a viable strategy in vitro. It has also been shown that compounds such as MitoQ can be formed into pharmaceuticals that can be successfully delivered orally to humans. Animal experiments have indicated that MitoQ has anti-oxidant 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 vivo 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. 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. In addition there is considerable potential to use these compounds to act as probes for mitochondrial ROS production and oxidative damage and to manipulate and report on mitochondrial function in a number of ways.

Acknowledgments

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