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P-glycoprotein (Mdr1a/1b) and breast cancer resistance protein (Bcrp) decrease the uptake of hydrophobic alkyl triphenylphosphonium cations by the brain

Carolyn M. Porteous [a,b], David K. Menon [c], Franklin I. Aigbirhio [d], Robin A.J. Smith [a], Michael P. Murphy [e,*]

[a] Department of Chemistry, University of Otago, P.O. Box 56, Dunedin 9054, New Zealand
[b] Department of Biochemistry, University of Otago, P.O. Box 56, Dunedin 9054, New Zealand
[c] Division of Anaesthesia, University of Cambridge, Box 93, Addenbrooke's Hospital, Hills Road, Cambridge CB2 0QQ, UK
[d] Wolfson Brain Imaging Centre, Department of Clinical Neurosciences, University of Cambridge, Addenbrooke's Hospital, Hills Road, Cambridge CB2 0QQ, UK
[e] MRC Mitochondrial Biology Unit, Hills Road, Cambridge, CB2 0XY, UK

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ABSTRACT

Background: Mitochondrial dysfunction contributes to degenerative neurological disorders, consequently there is a need for mitochondria-targeted therapies that are effective within the brain. One approach to deliver pharmacophores is by conjugation to the lipophilic triphenylphosphonium (TPP) cation that accumulates in mitochondria driven by the membrane potential. While this approach has delivered TPP-conjugated compounds to the brain, the amounts taken up are lower than by other organs.
Methods: To discover why uptake of hydrophobic TPP compounds by the brain is relatively poor, we assessed the role of the P-glycoprotein (Mdr1a/b) and breast cancer resistance protein (Bcrp) ATP binding cassette (ABC) transporters, which drive the efflux of lipophilic compounds from the brain thereby restricting the uptake of lipophilic drugs. We used a triple transgenic mouse model lacking two isoforms of P-glycoprotein (Mdr1a/1b) and the Bcrp.
Results: There was a significant increase in the uptake into the brain of two hydrophobic TPP compounds, MitoQ and MitoF, in the triple transgenics following intra venous (IV) administration compared to control mice. Greater amounts of the hydrophobic TPP compounds were also retained in the liver of transgenic mice compared to controls. The uptake into the heart, white fat, muscle and kidneys was comparable between the transgenic mice and controls.
Conclusion: Efflux of hydrophobic TPP compounds by ABC transporters contributes to their lowered uptake into the brain and liver.
General significance: These findings suggest that strategies to bypass ABC transporters in the BBB will enhance delivery of mitochondria-targeted antioxidants, probes and pharmacophores to the brain.

1. Introduction

Mitochondrial dysfunction contributes to a wide range of degenerative neurological disorders and to acute brain damage [1–4]. Consequently there is considerable interest in developing therapies that decrease mitochondrial damage and preserve organelle function [1,3]. Mitochondria-targeted therapies based on lipophilic alkyl triphenylphosphonium (TPP) cations have been developed and show promise in vivo and in human trials [1,5,6]. These TPP lipophilic cations pass directly through phospholipid bilayers due to their large hydrophobic surface area lowering the activation energy for uptake [5,7,8], while their positive charge causes their accumulation several-hundred fold within mitochondria inside cells, driven by the plasma and mitochondrial membrane potentials [5,7]. These properties have been used to deliver a range of TPP cations selectively to mitochondria within cells, including: antioxidants [5,9–11], thiol reagents [12,13], spin traps [14–17], fluorescent reactive oxygen species (ROS) probes [18–21], toxins [22,23], DNA alkylating agents [24] and nitric oxide donors [25]. One of these compounds, the mitochondria-targeted antioxidant mitoquinone (MitoQ), has shown efficacy in a number of animal models of pathologies [26–32]. Furthermore, MitoQ has been used in humans and shown to be safe during long-term oral administration [33] and to be effective at decreasing liver damage in a preliminary

Abbreviations: ABC proteins, ATP binding cassette proteins; BBB, blood–brain barrier; Bcrp, breast cancer resistance protein; CsA, cyclosporin A; IP, intra peritoneal; IV, intra venous; Mdr1, multi drug resistance 1; MitoF, 11-fluoroundecyltriphenylphosphonium mesylate; MitoQ, [10-(4,5-dimethoxy-2-methyl-3,6-dioxo-1,4-cyclohexadien-1-yl)decyl] triphenylphosphonium mesylate; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; TPB, tetraphenylborate; TPP, triphenylphosphonium cation; ROS, reactive oxygen species; TPMP, methyltriphenylphosphonium

* Corresponding author. Tel.: +44 1223 252900; fax: +44 1223 252905.
E-mail address: mpm@mrc-mbu.cam.ac.uk (M.P. Murphy).

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phase II trial in hepatitis C patients [34]. Therefore mitochondria-targeted bioactive molecules based on TPP compounds have potential as pharmaceuticals.

A number of animal studies have investigated the distribution of TPP compounds in vivo and these investigations have shown that the uptake of TPP compounds into most tissues was rapid and extensive following long-term oral administration or acute intravenous (IV) or intraperitoneal (IP) delivery [32,35,36]. However, while the TPP compounds were taken up into the brain, the extent of uptake was significantly less than into other tissues [32,35,36]. Supporting the uptake of TPP compounds by the brain, long-term administration of MitoQ by intraperitoneal injection leads to protection against brain mitochondrial damage in the MPTP animal model of Parkinson's disease [37]. Furthermore, oral delivery of MitoQ to the triple transgenic Alzheimer's disease model also protected against oxidative damage and memory impairment [38]. Therefore while TPP compounds are taken up into the brain this uptake is significantly less than by other organs [35,36]. The rate and extent of uptake of TPP compounds into cells and tissues is greatly enhanced by increasing the hydrophobicity of the molecule [36,39,40]. Increasing the hydrophobicity of TPP compounds by incorporating a 10–11 methylene carbon chain enhances the rate of uptake by lowering the activation energy for movement across the plasma membrane and increases the extent of accumulation within mitochondria by increasing adsorption to the matrix-facing surface of the inner membrane [36,39,40]. However, even for hydrophobic TPP cations such as MitoQ and MitoF uptake by the brain was considerably lower than for other organs [36]. This decreased uptake into the brain limits the potency of mitochondria-targeted therapies and the usefulness of probes in the brain. Therefore we set out to understand why the uptake of hydrophobic TPP compounds by the brain is less than for other organs.

A major limitation to the uptake of lipophilic compounds into the brain is the blood–brain barrier (BBB) [41,42]. The BBB comprises the tightly sealed endothelial cells that form the lumen surface of the capillaries perfusing the brain [41,42]. These endothelial cells contain tight junctions, have minimal pinocytosis and very few cell fenestrations so that they seal the brain capillaries [41,42], consequently compounds that enter the brain have to pass through the endothelial cells [41,42]. Restricting this passage are a series of ATP-binding cassette (ABC) transporters that line the lumenal plasma membrane of the endothelial cells and which pump lipophilic compounds out of the cells, thereby limiting their uptake into the brain [41]. The action of ABC-transporters in the BBB is a major factor limiting the delivery of lipophilic drugs to the brain [42].

The archetypal ABC transporter involved in the BBB lipophilic compound efflux is P-glycoprotein (P-gp, multi drug resistance 1 (Mdr1) protein and in humans as ABCB1), which uses ATP to drive the efflux of lipophilic compounds across the plasma membrane and out of cells [43]. The breast cancer resistance protein (Bcrp, ABCG2) performs a similar function in the BBB [41]. Progress on the structure and mechanism of P-gp suggests that BBB ABC-transporters act as "hydrophobic vacuum cleaners" through a binding site within the membrane that allows a wide range of lipophilic compounds to enter the protein from the plasma membrane, followed by efflux from the cell upon ATP hydrolysis [41,43–45]. P-gp is known to actively excrete lipophilic cations [41]. Furthermore, the uptake of MitoQ into cells [46,47] and its excretion into the bile from the liver in vivo are impeded by the action of P-gp [48]. Together these data implicate the activity of ABC-transporters in the BBB as likely contributors to the relatively poor uptake of lipophilic TPP cations into the brain.

Here we set out to test the hypothesis that the lower uptake of hydrophobic TPP compounds into the brain is due to their excretion from BBB endothelial cells by ABC-transporters such as P-gp. To assess this we measured the acute uptake of the hydrophobic TPP cations MitoQ and MitoF, and the more polar TPP cation methyltriphenylphosphonium (TPMP) (Fig. 1) into the brains of mice lacking the major ABC-transporters. In mice there are two isoforms of P-gp, Mdr1a and Mdr1b (also known as ABCB1a/1b) and a mouse model in which both of these are deleted along with Brcp1 (ABCG2) has been developed [49]. The Mdr1a/1b (−/−)(−/−) Brcp1 (−/−) mouse model has been used to assess the contribution of BBB ABC-transporters to exclusion of lipophilic compounds from the brain [50]. We found that the uptake of MitoQ and MitoF into the brain was far greater in mice lacking these three ABC-transporters, compared with control mice. These findings have important implications for the development of mitochondria-specific therapies and probes.

2. Materials and methods

2.1. Chemical syntheses

[^{3}H] TPMP iodide (60 Ci/mmol) was from American Radiolabeled Chemicals. [^{3}H] MitoQ was synthesised and HPLC-purified to >97% radiopurity, as described [39]. [^{3}H] Fluoroundecyltriphenylphosphonium (MitoF) was synthesised and purified as described previously [36]. The published octan-1-ol/PBS partition coefficients are as follows: TPMP, 0.35 [10]; MitoQ, 2760 [51]; MitoF, 740 ± 100 [36].

2.2. Administration of compounds to mice

Mice lacking two isoforms of P-gp (ABCB1a and ABCB1b) and Bcrp1 (ABCG2) were created on the Friend leukaemia virus B strain (FVB) background in the laboratory of Prof. Alfred Schinkel of the Netherlands Cancer Institute [49] and female mice (Mdr1a/1b (−/−)(−/−) Brcp1 (−/−)) lacking all three genes were supplied by Taconic Farms (http://www.taconic.com) Model Number 3998-M (FVB.129P2-Abcb1atm1Bor Abcb1btm1Bor Abcg2tm1Ahs N7). Mice were maintained on a 12 h light/dark cycle with ad libitum access to standard lab chow and water for 8–16 weeks prior to experiments.

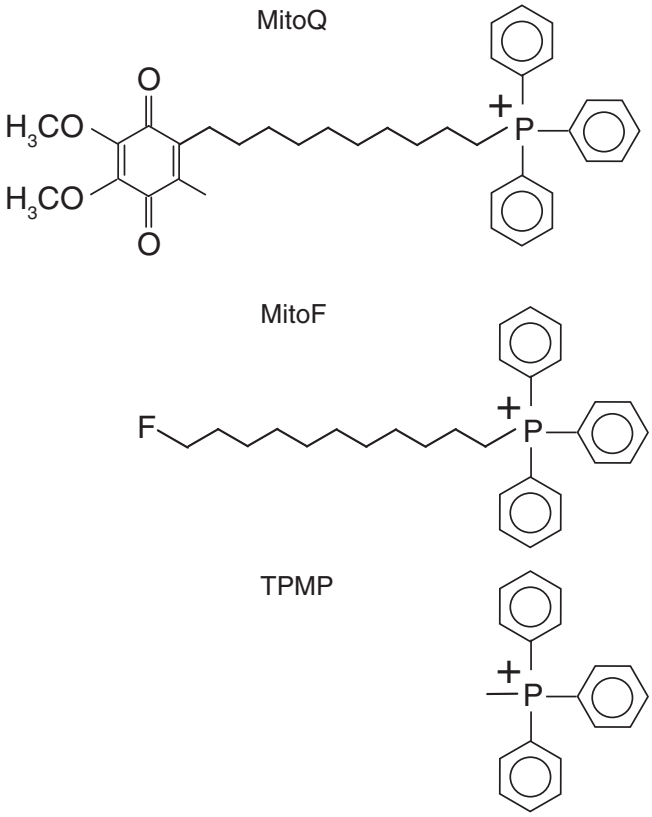

Fig. 1. Structures of the TPP cations investigated.