Novel Mitochondria-Targeted Compounds Composed of Natural Constituents: Conjugates of Plant Alkaloids Berberine and Palmatine with Plastoquinone

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Abstract—Novel mitochondria-targeted compounds composed entirely of natural constituents have been synthesized and tested in model lipid membranes, in isolated mitochondria, and in living human cells in culture. Berberine and palmatine, penetrating cations of plant origin, were conjugated by nonylxoycarbonylmethyl residue with the plant electron carrier and antioxidant plastoquinone. These conjugates (SkQBerb, SkQPalm) and their analogs lacking the plastoquinol moiety (C10Berb and C10Palm) penetrated across planar bilayer phospholipid membrane in their cationic forms and accumulated in isolated mitochondria or in mitochondria in living human cells in culture. Reduced forms of SkQBerb and SkQPalm inhibited lipid peroxidation in isolated mitochondria at nanomolar concentrations. In isolated mitochondria and in living cells, the berberine and palmatine moieties were not reduced, so antioxidant activity belonged exclusively to the plastoquinol moiety. In human fibroblasts, nanomolar SkQBerb and SkQPalm prevented fragmentation of mitochondria and apoptosis induced by exogenous hydrogen peroxide. At higher concentrations, conjugates of berberine and palmatine induced proton transport mediated by free fatty acids both in model and in mitochondrial membrane. In mitochondria this process was facilitated by the adenine nucleotide carrier. As an example of application of the novel mitochondria-targeted antioxidants SkQBerb and SkQPalm to studies of signal transduction, we discuss induction of cell cycle arrest, differentiation, and morphological normalization of some tumor cells. We suggest that production of oxygen radicals in mitochondria is necessary for growth factors–MAP-kinase signaling, which supports proliferation and transformed phenotype.

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The idea of Peter Mitchell [1] on a key role of transmembrane electric potential (ΔΨ) in coupling of respiration and ATP synthesis has revolutionized the field of bioenergetics since the 1960s. After almost a decade, the idea of E. A. Liberman and V. P. Skulachev [2] to apply membrane penetrating artificial ions for measurement of ΔΨ in mitochondria resulted in experimental evidence of Mitchell’s hypothesis. Applications of penetrating ions in

Abbreviations: ANT, adenine nucleotide translocase; AMVN, 2,2′-azodi(2,4′-dimethylvaleronitride); BLM, bilayer phospholipid membrane; C10Berb, 13-(decyloxycarbonylmethyl)berberine; CM-DCF-DA, 5-(-6)-chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate; C10Palm, 13-(decyloxycarbonylmethyl)palmatine; C12TPP, dodecyltriphenylphosphonium; FA, fatty acid; FCCP, trifluoromethoxybenzoylcyanide phenylhydrazone; ROS, reactive oxygen species; SkQ, 10-(6′-plastoquinonyl)decyltriphenylphosphonium; SkQBerb, 13-[9-(6′-plastoquinonyl)nonyloxycarbonylmethyl]berberine; SkQPalm, 13-[9-(6′-plastoquinonyl)nonyloxycarbonylmethyl]palmatine; SkQR1, 10-(6′-plastoquinonyl)decyrrhodamine 19; TPP+, triphenylphosphonium; ΔΨ, transmembrane electric potential difference.

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# Deceased.
the new millennium flourished with the development of a new class of compounds that selectively accumulate in mitochondria of living cells (for review see [3]). Rapid progress in this field allows us to hope that the new mitochondria-targeted drugs will be effective in treatment of a variety of human pathologies including diseases of the elderly and aging in particular [4].

The most popular representative of the family of penetrating ions has been and remains the tetraphenylphosphonium cation. Its main advantages: (i) high permeability through lipid bilayer in combination with high solubility in water; and (ii) low reactivity and low sorption the cell components. Tetraphenylphosphonium and its derivatives – conjugates of triphenylphosphonium (TPP+) – are widely used to measure membrane potential and for delivery to mitochondria of various compounds. Among the “cargo” to be delivered to the mitochondria the most often used are various antioxidants and indicators of reactive oxygen species (ROS). This focus is based on the idea that mitochondria are a major source of ROS in the cell, and excessive production of ROS causes the development of various pathologies and aging. The mitochondria-targeted antioxidants, TPP+ conjugates to ubiquinone (MitoQ) [5], plastoquinone (SkQ) [6], tocopherol (MitoVitE) [7], lipoic acid (MitoL) [8], ebselen (MitoPeroxidase) [9], and to spin traps (MitoPBN, MitoCP, Mito-TEMPO) [10-12] have been studied in various models. Mitochondria-targeted ROS indicators are represented by TPP+ conjugates with dihydroethidine (MitoSOX) [13] and boronate derivatives (MitoPY) [14].

In the second place in popularity are derivatives of rhodamines – fluorescent penetrating cations introduced into the practice of bioenergetics by Lan Bo Chen [15] and by V. P. Skulachev [16]. Various rhodamines and their esters are widely used for visualization of mitochondria and measurement of ∆ψ in cells. Rhodamine-19 conjugated decylplastoquinone (SkQ1) is one of the most effective antioxidants in vivo [17]. Dihydrorhodamine is used to measure mitochondrial ROS, but it acquires a positive charge and accumulates in the mitochondria only after oxidation, making difficult interpretation of its effects [18]. In 2005, compounds bearing two or more guanidine groups were proposed as mitochondria-targeted cations [19]. The mechanism of accumulation of these substances in the mitochondria is not clear, and probably some carrier proteins are involved in their transport since the permeability of multicharged ions through the lipid bilayer is extremely small [20].

In conclusion of this historical overview, it should be noted that the family of penetrating cations includes: fuscin, Janus green, Cristal violet, and other histological dyes that were used for visualization of mitochondria from the middle of the XIX century. Their direct descendants are the various MitoTracker dyes, which are widely used (for the same purpose) in modern studies.

Recently the ability of TPP+ and rhodamine conjugates to catalyze transfer of protons across the lipid bilayer was described [21]. In the case of TPP+ derivatives, this effect was related to acceleration of transport of fatty acid (FA) anions, which limited protonophorous activity of FA in phospholipid membranes. A similar effect of tetraphenylphosphonium (at much higher concentrations) has been known for almost 20 years [22]. Protonophorous activity of rhodamine-19 derivatives did not depend on FA and was related to protonation-deprotonation of the rhodamine residue [23]. Cations in use as mitochondria-targeted uncouplers of oxidative phosphorylation are of great interest as a possible tool to treat obesity. The main advantage of the cations over conventional protonophores is dependence of their activity on ∆ψ. It could be expected that the decrease in ∆ψ by the cationic uncouplers will be limited since at the same time the driving force for their accumulation in mitochondria will be decreased.

Neither the derivatives of TPP+ nor rhodamines are natural compounds. This fact was important for their application for measurements of ∆ψ, as it excluded participation of specific mechanisms of transport through the mitochondrial membrane. Now, when the possibility of long-term use of derivatives of these cations becomes real, their artificial nature is a matter of concern. In search of natural penetrating cations our attention was attracted by berberine and palmatine, which are isoquinoline alkaloids derived from plants of the Berberidaceae family. Extracts containing berberine and palmatine have been used in traditional Chinese medicine for many centuries. Pharmacological studies of these alkaloids revealed their therapeutic effects against diabetes, inflammatory disorders, arrhythmia, hypertension, etc. Anticancer activity of berberine mediated by its antiproliferative and antiangiogenic properties was also reported [24]. The mechanisms of therapeutic action of the alkaloids are not known, but in vivo studies have shown a pronounced antioxidant effect of berberine [25] or its metabolites [26]. In cell-free models, berberine inhibits lipid peroxidation, but only in the reduced form [27]. It was shown that berberine can accumulate in mitochondria of living cells. Experiments on bilayer phospholipid membranes (BLM) have shown that berberine and palmatine penetrate the bilayer in the cationic form [28].

In 2010, we synthesized conjugates of berberine and palmatine with decylplastoquinone (SkQBerb and SkQPalm), as well as their analogs lacking the quinone residue, C10Berb and C10Palm (Fig. 1). The conjugates with different length of aliphatic chain -(CH2)n, where n ranged from 4 to 10, were also synthesized. Lipophilicity of these compounds was estimated using reversed-phase chromatography (HPLC). Previously it was shown that this analysis gave results corresponding to the partition coefficients in the octanol–water system for conjugates of TPP+ (SkQ1 and MitoQ) [30]. SkQBerb and SkQPalm,