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Review

In search of novel highly active mitochondria-targeted antioxidants: Thymoquinone and its cationic derivatives

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ABSTRACT

Since the times of the Bible, an extract of black cumin seeds was used as a medicine to treat many human pathologies. Thymoquinone (2-demethylplastoquinone derivative) was identified as an active antioxidant component of this extract. Recently, it was shown that conjugates of plastoquinone and penetrating cations are potent mitochondria-targeted antioxidants effective in treating a large number of age-related pathologies. This review summarizes new data on the antioxidant and some other properties of membrane-penetrating cationic compounds where 2-demethylplastoquinone substitutes for plastoquinone. It was found that such a substitution significantly increases a window between anti- and prooxidant concentrations of the conjugates. Like the original plastoquinone derivatives, the novel compounds are easily reduced by the respiratory chain, penetrate through model and natural membranes, specifically accumulate in mitochondria in an electrophoretic fashion, and strongly inhibit H_2O_2-induced apoptosis at pico- and nanomolar concentrations in cell cultures. At present, cationic demethylplastoquinone derivatives appear to be the most promising mitochondria-targeted drugs of the quinone series.

1. Quinones as mitochondria-targeted antioxidants

Mitochondria are the only intracellular organelles whose interior is negatively charged relatively to the exterior [1,2]. This fact can be used to specifically address various compounds to mitochondria. To this end, it was suggested to combine the transported compound with a positively charged ion easily penetrating through biomembranes [3,4]. To make an ion permeable for membranes, its ionized atom should be surrounded by bulky hydrophobic residues that delocalize the electric charge of this atom [1,2,5]. Such a principle was employed to construct mitochondria-targeted antioxidants [5–18]. Among them, some quinone derivatives proved to be the most active (Fig. 1). As was found in our group, the antioxidant activity measured in isolated mitochondria treated with Fe^{2+} and ascorbate increases in the series: 10-(6′-ubiquinonyl)decyltriphenylphosphonium (MitoQ) < 3′-demethoxyMitoQ (DMMQ) = (6′-methylplastoquinonyl) decyltriphenylphosphonium

Abbreviations: $\Delta\psi$, transmembrane electric potential difference; BLM, bilayer planar phospholipid membrane; C_{12}R1, dodecyl rhodamine 19; C_{12}TPP, dodecyltriphenylphosphonium; DMMQ, 3′-demethoxyMitoQ; MDA, malondialdehyde; MDR, multidrug resistance; MitoQ, 10-(6′-ubiquinonyl)decyltriphenylphosphonium; ROS, reactive oxygen species; SkQ1, 10-(6′-plastoquinonyl)decyltriphenylphosphonium; SkQ3 , (6′-methylplastoquinonyl) decyltriphenylphosphonium; SkQR1, 10-(6′-plastoquinonyl) decylrhodamine 19; SkQT(p), 10-(6′-toluquinonyl) decyltriphenylphosphonium; SkQT(m), 10-(5′-toluquinonyl) decyltriphenylphosphonium; SkQT, a mixture of SkQ(p) and SkQ(m) in proportion of 1.4:1; SkQTR1, 10-(6′-toluquinonyl) decylrhodamine 19

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(SkQ3) < 10-(6′-plastoquinonyl)decyltriphenylphosphonium (SkQ1) [15,16]. Thus, substitutions of methoxy group by methyl and methyl group by an H atom seem to be favorable for antioxidant activity. It would be interesting to continue this quinone series by substituting one more methyl group in plastoquinone by an H atom, just as it occurs in 2-demethylplastoquinone, an intermediate of plastoquinone biosynthesis [19] and in so-called *thymoquinone* (Fig. 1A), a plant antioxidant responsible for many favorable pharmacological effects of black cumin (see below, Section 3). We studied thymoquinone-like derivatives conjugated with penetrating cations, namely triphenyldecylphosphonium (in SkQT1) and decylrhodamine 19 (in 10-(6′-toluquinonyl) decylrhodamine 19 (SkQTR1)) (Fig. 1B). In the next section, some results of this study are reviewed.

2. Cationic thymoquinone derivatives: effect on model membranes, isolated mitochondria and cell cultures

SkQT1 and SkQTR1 were synthesized in essentially the same way as their plastoquinone analogs, SkQ1 and 10-(6′-plastoquinonyl) decylrhodamine 19 (SkQR1) [15]. SkQT1 samples were a mixture of *p* and *m* isomers (Fig. 1B) in the proportion of 1.4:1. SkQTR1 was purified as *p* isomer, like other SkQs [15].

In the first series of experiments, generation of diffusion potential of SkQT1 on bilayer planar phospholipid membranes (BLM) was demonstrated. Like other penetrating cations [2,4], the concentration gradient of SkQT1 was found to generate an electric potential difference, the compartment with lower [SkQT1] being positively charged due to downhill transmembrane diffusion of

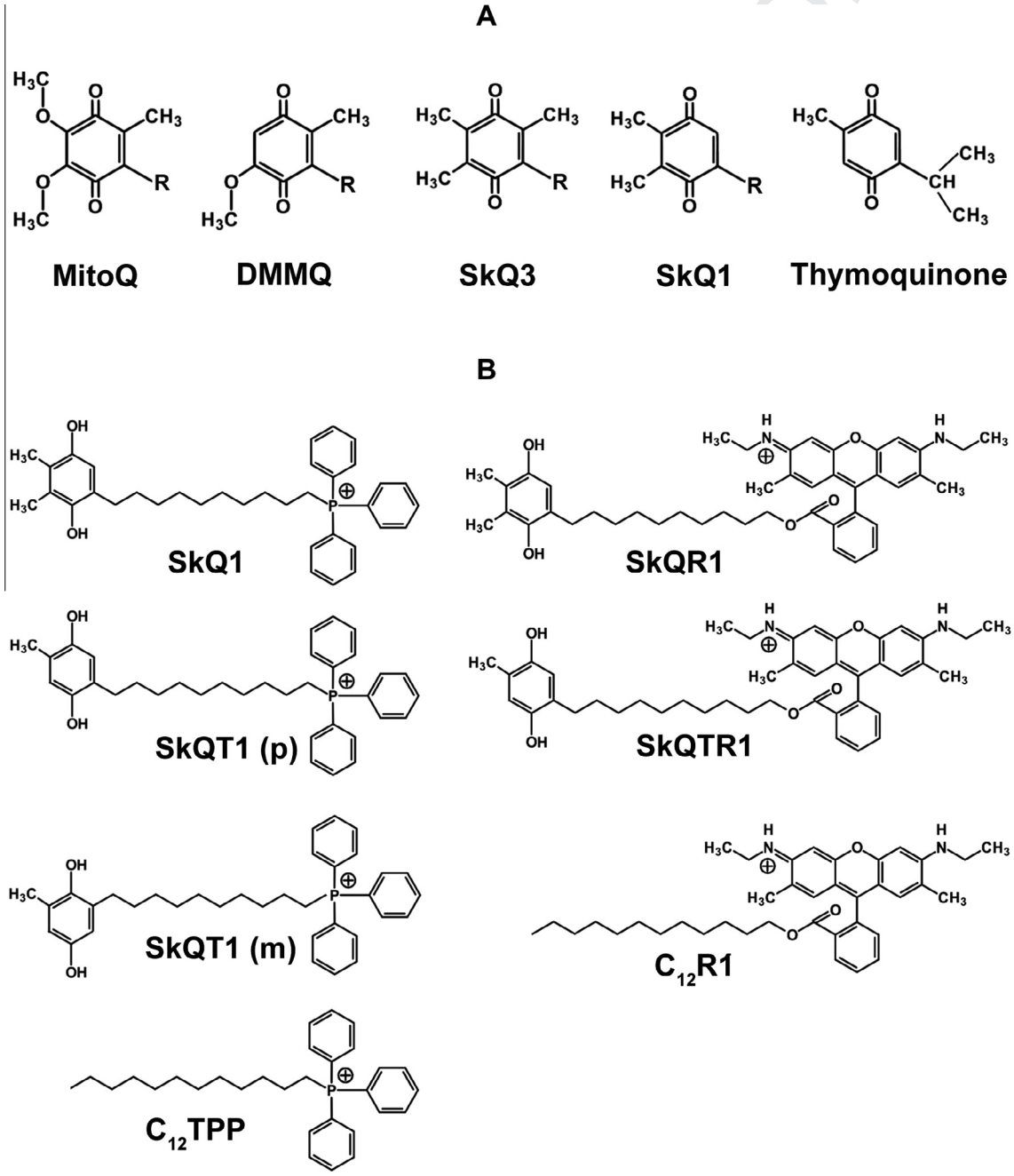

Fig. 1. Formulas of certain compounds considered in this review. (A) Mitochondria-targeted cationic quinone derivatives and thymoquinone. R, decyltriphenylphosphonium. (B) Cationic quinol derivatives of SkQ series and their analogs lacking quinol residue.

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