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REVIEW ARTICLE

Cellular and Molecular Mechanisms of Action of Mitochondria-targeted Antioxidants

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Abstract: Reactive oxygen species generated in mitochondria are an important factor contributing to mitochondrial and cellular dysfunction underlying many degenerative diseases, chronic pathologies and aging. The idea of delivering antioxidant molecules to mitochondria *in vivo* to treat these diseases and slow aging intensively developed in the last 20 years. Derivatives of quinones covalently conjugated to a lipophilic cation (*e.g.*, MitoQ and SkQ) were the most extensively studied mitochondria-targeted antioxidants. These compounds have now been used in a wide range of *in vitro* and *in vivo* studies, as well as in clinical trials in humans. Here, we review recent progress in this field with a special attention on molecular mechanisms of rechargeable mitochondria-targeted antioxidants.

A simple hypothesis that aging results from gradual accumulation of occasional damage inflicted by ROS to DNA, proteins and lipids is apparently insufficient. More and more pieces of evidence indicate that the damage in question is programmed. Moreover, the imbalance in ROS-dependent regulatory mechanisms and compromised ROS signaling are underlying many pathologies and aging. Chain reactions of cardiolipin peroxidation initiated by mitochondrial ROS seem to play a key role in these degenerative processes. Such reactions are specifically abolished by mitochondria-targeted antioxidants.

Keywords: Antioxidant, cardiolipin, mitochondria, mitochondria-targeted, oxidative stress, reactive oxygen species, ROS, SkQ.

1. INTRODUCTION

Reactive oxygen species (ROS) is a broad term used to describe a variety of chemically active molecules and free radicals derived from molecular oxygen. ROS drew no significant attention of biologists until Danham Harman in 1956 proposed his hypothesis of aging based on the free radical chemistry [1]. At that time the mainstream of aging research was the «damage accumulation theory» (also known as the «rate of living» theory) [2]. It postulated that metabolic reactions occurring in a living organism have some inevitable harmful side-effects, and that aging is progressive accumulation of diverse, deleterious changes caused by them.

There was a bitter need for an explanation, what exactly this irreversible damage is. Harman suggested that this damage is inflicted by free radicals reacting with cellular constituents (including proteins and nucleic acids). Such reactions are expected to impair the functional efficiency and reproductive ability of the cell, and to stimulate mutagenesis, aging and cancer. Harman also proposed that free radicals arise as by-products of reactions involving molecular oxygen catalyzed in the cell by the oxidative enzymes [1], and that mitochondria, being responsible for cellular respiration, might serve as a biologic clock that determines the rate of aging [3, 4].

Indeed, a significant – if not the major – fraction of reactive oxygen species generated in an animal cell is a by-product of mitochondrial electron transport. Mitochondria also produce reactive nitrogen species (RNS), primarily nitric oxide and peroxynitrite, that are involved in cellular signaling and degenerative processes including aging (see [5-8] for details), but in this review we focus mainly on ROS.

In 1966 Jensen demonstrated that hydrogen peroxide is formed during respiration on succinate and NADH [9]; in 1974 Loschen *et al.* reported that superoxide is also produced by mitochondria [10]. The puzzle seemed to be solved: mitochondria produced ROS as an inevitable respiration by-product; these ROS were gradually damaging lipids, proteins, and DNA; a fraction of this damage was irreparable, and accumulation of such damage resulted in gradual decline of vital functions, *i.e.* in aging. There is also a «mitochondrial vicious cycle» extension of this hypothesis

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assuming that ROS produced as respiration by-products are responsible for damage first of all in mitochondria, causing further increase in ROS production and mitochondrial dysfunction – a positive feedback that eventually can lead to ROS burst and cell death [11].

The link between mitochondrial ROS production and degenerative processes leading to disease and aging is supported by an overwhelming number of experimental evidence (see [12-17] and references therein). However, the mechanism behind this link proved to be more complex than just unspecific chemical reactions damaging biomolecules. Although numerous experimental works indicate that mutations in mitochondrial DNA are involved in the aging process [18-20], there is no sound experimental evidence demonstrating that oxidative damage to DNA is the primary source of mutation accumulation during aging. Numerous attempts to use antioxidants to prevent the accumulation of oxidative damage failed [21]. The «mitochondrial vicious cycle» theory also gained no conclusive experimental support [22, 23]. Nevertheless, experiments on mice with defective, error-prone mitochondrial DNA (mtDNA) polymerase demonstrated that accelerated development of aging phenotypes triggered by mtDNA mutations is linked to mitochondrial dysfunction and oxidative stress and can be prevented by mitochodrially-targeted catalase [24] or synthetic antioxidants (SkQ1). A recent study on *Drosophila melanogaster* revealed that many features associated with mtDNA mutations in vertebrates are conserved in this insect, including an increase in frequency of mutations with age. However, it remained unclear whether the oxidative stress increasing with age is a major factor in the mutagenesis [25]. Ultra-sensitive sequencing experiments revealed that predominantly transition mutations, rather than mutations commonly associated with oxidative damage to mitochondrial DNA, become more frequent with age [26].

The well-established correlation of mitochondrial ROS production and longevity [27, 28], initially used as a strong argument for the free radical theory of aging, is also not universal: the longest-living rodent naked mole rat exhibit strong ROS production [28] and high levels of oxidative stress markers (isoprostanes, malondialdehyde, protein carbonyls, oxydized cysteines and deoxyribonucleic acid 8-OHdG) in multiple tissues at quite early age [29-31]. The naked mole-rat, and probably some other animal species, can tolerate a high level of oxidative damage while having an astonishingly long lifespan [32].

The modern version of free radical theory of aging suggests that the main factor underlying the deleterious process of gradual aging is not the direct damage inflicted by ROS on a living cell, but the imbalance in cellular ROS signalling (see [12-14, 33] for recent reviews). Mitochondria and the ROS generated by them play a central role in the regulation of programmed cell death and other vital processes in organisms ranging from single-cell eukaryotes like yeast to humans. Mitochondria-targeted antioxidants are a powerful tool to investigate the links between mitochondrial ROS and degenerative processes leading to disease and aging. Moreover, these compounds are potential candidates for drug development and anti-aging medicines. This review focuses on recent progress in application of mitochondria-targeted antioxidants to research and therapy.

1.1. Mitochondrial ROS: Generation and Chemistry

It is common in biologic research to refer to ROS as to a family of compounds with similar physico-chemical properties. However, this simplification is misleading. Among the biologically active ROS that can be generated in an animal organism are: superoxide anion ($O^{\cdot}_2^-$), its protonated form – hydroperoxyl radical ($HO^{\cdot}_2$), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\cdot}$) and nitric oxide (NO), hypochlorous acid, singlet oxygen, lipid peroxides. These compounds have different stability, chemical reactivity, mobility and cause different biological responses in a living cell [34].

Since the first evidence that hydrogen peroxide is produced by respiratory enzyme [9], a vast amount of experimental data was obtained, and the process of ROS generation in mitochondria is now documented in great detail (see [16, 35-38] for excellent reviews). The two primary ROS produced in mitochondria are superoxide and hydrogen peroxide. Superoxide is more reactive than H_2O_2, its pK is 4.7, so at normal intracellular pH it is negatively charged and therefore membrane impermeable. Hydrogen peroxide is much more stable, and does not react with most biomolecules directly (exceptions are thiols (including cysteine residues), transition metal centers, and selenoproteins) – a set of properties that makes it an ideal transmitter of redox signals (see [39] for a detailed recent review on H_2O_2 biochemistry).

Neither H_2O_2 nor $O^{\cdot}_2^-$ is chemically capable of damaging DNA directly [40], but they can do so indirectly by participating in the production of much more reactive $HO^{\cdot}_2$ and $OH^{\cdot}$ radicals [41] via Fenton reaction:

$$O^{\cdot}_2^- + H^+ \rightarrow HO^{\cdot}_2$$

$$H_2O_2 + Fe^{2+} \rightarrow OH^{\cdot} + OH^- + Fe^{3+}$$

It was shown that inactivation of *Escherichia coli* dihydroxy-acid dehydratase, fumarase A, fumarase B, and mammalian aconitase by superoxide correlates with release of iron from [4Fe-4S] clusters of these enzymes [42]. It is highly probable that the same process takes place in the mitochondrial matrix, and that iron ions released serve as a catalyst for $OH^{\cdot}$ generation via Fenton reaction.

$OH^{\cdot}$ radical is a short-lived (half-life about few nanoseconds), but highly reactive ROS able to react with almost any type of cellular macromolecules. Of particular importance among the damaging reactions mediated by $OH^{\cdot}$ radical is lipid peroxidation – a free radical chain reaction of oxidative degradation. It mostly affects polyunsaturated fatty acids that possess especially reactive hydrogens. The reaction is initiated by $OH^{\cdot}$ radical attack at a double bond that results in fatty acid radical formation. The latter is not a stable molecule, and readily reacts with molecular oxygen, producing a peroxyl-fatty acid radical. This radical reacts with another unsaturated fatty acid, propagating the reaction, and cycle continues, as the new fatty acid radical reacts with a neighboring lipid. In such a way a single $OH^{\cdot}$ radical can “burn" a significant fraction of unsaturated lipids in the mitochondrial membrane, resulting in the distur-

bance of membrane organization and function loss. At the same time, the products of lipid peroxidation have been shown to act as redox signaling mediators [43].

The rate of mitochondrial ROS generation depends on many factors: oxygen concentration, respiration rate, calcium concentration, to name just a few. Transmembrane electric potential difference ($\Delta\psi$) on the inner mitochondrial membrane is also an important factor that can regulate the rate of mitochondrial ROS formation. It was proposed in our group in1996 that high $\Delta\psi$ could lead to an increase in superoxide generation [44]. Later it was demonstrated that indeed at $\Delta\psi$ values above a certain threshold (slightly exceeding the $\Delta\psi$ level in mitochondria that actively synthesize ATP, so-called state 3 mitochondria) a strong increase in ROS production occurs [45-47]. Such high $\Delta\psi$ values are quite normal for a living cell at rest, when the energy demand is low, and most adenine nucleotides are in the form of ATP, no further ATP synthesis occurs, and the respiratory chain is self-inhibited by $\Delta\psi$ (state 4 mitochondria).

Another physiologically important situation when a strong burst of ROS is generated occurs upon ischemia-reperfusion, *i.e.* when a tissue is deprived of oxygen for a certain time (long enough to achieve the reduction of electron-transporting co-factors in the respiratory chain) and then re-oxigenated. The oxidative stress induced by ischemia-reperfusion is involved in many fatal pathologies, including cardiac and neurodegenerative diseases [48, 49].

1.2. Cellular Defenses Against ROS

Generation of ROS in mitochodria, as well as in other cellular compartments, is an inevitable process involved in many physiological functions [44, 50]. Several lines of defense against ROS are found in a living cell. Peroxiredoxins, enzymes that reduce H_2O_2, are abundant in the mammalian cell cytoplasm and can amount up to 1% of intracellular soluble protein [51]. The antioxidant mechanism involves oxidation of the protein and its subsequent regeneration by thioredoxin. One protein of this family, peroxiredoxin III, is present in the mitochondrial matrix; its depletion triggers increase in intracellular H_2O_2 concentration, oxidative stress and apoptosis [52]. Glutathione peroxidases are another family of antioxidant enzymes that catalyze the reduction of H_2O_2 to H_2O, typically using glutathione as reductant [53]; glutathione peroxidases 1 is present both in the cytosol and mitochondria. Catalases scavenge H_2O_2 in cytoplasm (but not in mitochondria), decomposing it to water and oxygen [54]. Superoxide dismutases (Cu-Zn-SOD, or SOD1 in cytoplasm and Mn-SOD, or SOD2 in mitochondria) are rapidly detoxifying $O^{\cdot}_2^-$ to H_2O_2. inside the cell, while SOD3 operates in the intercellular space [55].

With such an impressive arsenal of antioxidant defenses, the cell possesses all the means to keep the ROS damage at the safe level. The source of oxidative stress is most likely not the inevitable generation of ROS as steady-state respiration by-products, but the imbalance of the antioxidant regulation and/or failure of the antioxidant defenses to cope with acute stress (*e.g.*, ischemia-reperfusion induced ROS burst).

Mitochondrial ROS play an important role in the initiation of programmed death phenomena from organelle to organism [56-58]. Under certain conditions, ROS induce the opening of permeability transition pore (a non-specific protein channel with a molecular cut-off of about 1.5 kDa) in the inner mitochondrial membrane. Consequent dissipation of $\Delta\psi$ serves as a mark that dooms de-energized mitochondria to digestion by autophagosomes (mitophagy) [59]. This mechanism serves as a quality-control tool that enables selective elimination of malfunctioning mitochondria (*e.g.*, organelles with mutated DNA or with severely oxidized lipids). When malfunction involves the majority of mitochondria in a cell, a more radical (mitoptotic) mechanism is employed: the mitochondria are gathered close to the nucleus, get surrounded by a single membrane, and are expelled from the cell as a "mitoptotic body" [60, 61]. Failure of these mechanisms to dispose of malfunctioning mitochondria may be fatal for the cell: the opening of mitochondrial transition pores leads to the swelling of the mitochondrial matrix, that in turn results in the rupture of the outer mitochondrial membrane, entailing release of the mitochondrial intermembrane proteins to the cytosol and subsequent apoptosis. Besides apoptosis, mitochondrial ROS are able to activate starvation-induced and antibacterial autophagy, as well as autophagic cell death [62].

1.3. Mitochondria-targeted Compounds

Targeting small molecules to mitochondria is now a popular strategy for drug delivery, for design of probes useful to follow biological processes and biochemical reactions in real time, and for other lines of mitochondrial research (*e.g.*, the role of mitochondria in aging). Several comprehensive reviews [63-69] were devoted to this topic in the recent 10 years; here we summarize general information and report most novel findings in each sub-area of mitochondria-targeted antioxidant research.

The main two approaches for targeting small molecules to mitochondria consist in their incorporation into mitochondria-targeted peptides and conjugation to lipophilic cations. A less widely used approach to target substances of interest to mitochondria - particulate carriers that can deliver drug molecules selectively to mitochondria and protect the cargo substance from degradation during delivery (*e.g.* liposomes, biodegradable polymer or metal particles) [70].

1.4. Nitroxides Conjugated to Mitochondria-targeted Moieties

One of the approaches to scavenge mitochondrial ROS is based on conjugating nitroxides (*e.g.*, 4-amino-TEMPO) to hemigramicidin (a modified gramicidin S segment) or segments of other natural products with relatively high affinity for mitochondrial membranes [64]. An important advantage of the nitroxide conjugates is the possibility to measure the distribution of spin label by electron spin resonance and thereby monitor oxidative stress in a living cell and in mitochondria [71, 72]. In 2005 the first mitochondria-targeted nitroxide was proven to be effective in preventing ROS generation and cardiolipon (CL) oxidation in mitochondria and in protecting cells against a range of pro-apoptotic triggers [73] (see also [74] for an excellent re-

view on TEMPO and other redox-cycling nitroxides in models of oxidative stress).

1.5. Mitochondria-targeted Cytoprotective Peptides (Szeto-Schiller Peptides)

Szeto-Schiller (SS) peptides are water soluble tetrapeptides that can readily cross cell membranes and, having a special alternating aromatic-cationic amino acids motif, are targeted to mitochondria [75]. Before these peptides were used at mitochondria-targeted antioxidants, it was found that they selectively bind to the μ-opioid receptor and can be used as long-acting and effective analgetics [76, 77].

Contrary to the compounds with a lipophilic cation moiety (see below), the uptake of SS peptides into mitochondria does not depend on $\Delta\psi$. The peptides are readily concentrated even in the depolarized mitochondria, and addition of uncoupler carbonyl cyanide-4-(trifluoromethoxy)phenylhydrazone (FCCP) reduces the uptake by mere ~20% [75]. The specificity of targeting was assessed in experiments with radioactive [^{3}H] SS-03 peptide, and the compound was concentrated in mitochondria in 100-fold excess in the time scale of several minutes [75, 78].

In vitro studies demonstrated that SS-peptides act as H_2O_2 and peroxynitrite scavengers and prevent linoleic acid oxidation; their antioxidant properties were attributed to their dimethyltyrosine residues [79]. This hypothesis was confirmed by experiments with SS-20, a peptide lacking dimethyltyrosine that failed to act as a ROS scavenger [75].

Antioxidant activity of SS peptides is an obvious factor explaining their beneficial effects *in vivo*. The ability of SS-31 (D-Arg-Lys-Phe-NH_2) to selectively bind to cardiolipin on the inner mitochondrial membrane via both electrostatic and hydrophobic interactions is another feature that might explain its protective effect on mitochondria during ischemia [80]. SS-31 has also demonstrated positive effects in preclinical models of heart failure, ameliorating diastolic dysfunction, cardiac hypertrophy induced by overexpression-induced heart failure [81].

Lipophilic Cations Conjugates

Release of protons from the mitochondrial matrix to the intermembrane space performed by respiratory chain proteins results in a large negative $\Delta\psi$ (~180 mV) at the inner mitochondrial membrane. According to the Nernst equation, the concentration of a membrane-permeable monovalent cation inside mitochondria should be about 1000-fold higher than in the cytoplasm (approximately one order of magnitude for each 60 mV at room temperature). Same logic applied to the plasma membrane (assuming $\Delta\psi$ of 60 mV, cell interior negative) gives further increase in concentration by a factor of 10. Hence, the magnification of membrane-permeable cations concentration inside mitochondria compared with the extracellular space will be about 10,000-fold. Moreover, in the inner leaflet of inner mitochondrial membrane bilayer the concentration should be even higher, because the water-membrane distribution of lipophilic cations is significantly shifted towards the membrane.

The idea to use membrane-permeable cations as locomotives to deliver “payload" specifically to mitochondria was suggested in our group as early as 1970 [82]. But the first successful implementation came 25-30 years later, when M. P. Murphy introduced first mitochondria-targeted antioxidants with lipophilic cations bound to thiobutyl [83], vitamin E [84], and ubiquinone [85]. The latter compound, known as MitoQ, a conjugate of ubiquinone with decyl-triphenylphosphonium cation, had an important advantage: its oxidized form can be regenerated by accepting electrons from the respiratory chain, rendering MitoQ a rechargeable mitochondrial antioxidant [63]. Later studies performed in our group on model systems, mitochondria, and cell cultures indicated that conjugates of penetrating cations with plastoquinone (SkQ) are more promising antioxidants than those with ubiquinone (MitoQ). The latter demonstrated anti-oxidant activity at rather high concentrations, close to the level above which the pro-oxidant activity becomes significant, while for SkQ the “antioxidant concentration window" was much larger [67, 86, 87].

Conjugates of lipophilic cations with rechargeable antioxidants and other types of cargo have an important advantage: they could be applied in extremely low concentrations because of their ability to selectively accumulate in the inner mitochondrial membrane. For potential drug candidates this property allows to greatly diminish the risks of unwanted side effects.

It is therefore not surprising that studies of such compounds and their biological effects in vitro and in vivo became a very active area of research. Physico-chemical properties of a broad spectrum of these compounds were studied; the compounds were tested in model systems and in animals, and clinical trials were carried out (covered in detail in [66, 69, 88, 89]). Below we provide information on the most recent advances in this area.

It was found that 2-demethylplastoquinone (a compound abundant in black cumin seeds used in the past as a medicine to treat many human pathologies) conjugated to lipophilic cations has a significantly larger window between anti- and prooxidant concentrations compared to MitoQ and even SkQ1. The novel compound was readily reduced by the respiratory chain, and was able to strongly inhibit the H_2O_2-induced apoptosis at pico- and nanomolar concentrations in cell cultures [90].

It was also found that SkQ1, as well as mitoTEMPO (mitochondria-targeted nitroxide), reduced the expression of inducible nitric oxide synthase in liver, NO levels in blood and plasma, and markers of organ damage, suggesting that the intracellular signaling pathways mediated by NO and ROS are probably linked to each other via mitochondria and that mitochondrial ROS and NO cooperate to regulate the inflammatory intracellular processes [91].

Mitochondria-targeted compounds introduced immediately after reperfusion were found to reduce brain damage and helped to keep a higher neurological status; cationic decylrhodamine derivatives of plastoquinone were the most promising candidates for anti-ischemic mitochondria-targeted drugs [92].

In mice, lifelong treatment with the mitochondria-targeted antioxidant SkQ1 retarded progression of age-related cardiac dysfunction (cardiomyopathy, cardiac hypertrophy, and diffuse myocardial fibrosis), presumably via a reduction in age-related inflammation [93].

In rats, daily intraperitoneal injections of SkQT1, a mitochondria-targeted thymoquinone, applied for 5 days after brain trauma was found to attenuate the trauma-induced neurological deficit [94].

SkQ1 accelerated resolution of the inflammatory phase, formation of granulation tissue, vascularization and epithelization of cutaneous wounds in aged mice, suggesting that mitochondrial ROS play an important role in the pathogenesis of age-related chronic wounds [95].

Recent electron microscopy study of rat mitochondrial ultrastructure revealed that application of SkQ1 prevented the development of age-dependent destructive changes of mitochondrial reticulum during skeletal muscle sarcopenia in both the control Wistar animals and OXYS rats suffering from excessive oxidative stress and accelerated aging [96]. This finding is in line with earlier experiments, where SkQ1 at nanomolar concentrations was shown to prevent or slow down cerebral dysfunctions, and to decrease the pathological accumulation of AbetaPP, Abeta, and hyperphosphorylation of tau-protein in OXYS rats, as well as age-dependent changes in healthy Wistar rats [97].

1.7. The Role of Cardiolipin

Several mitochondrially-targeted antioxidants tested in different groups exerted their protective effect via prevention of cardiolipin peroxidation. It is therefore probable that the latter process plays an important role in the regulation of oxidative stress and in progression of pathologies.

Cardiolipin (CL) is a tetra-acylated anionic phospholipid localized normally in the inner mitochondrial membrane. It is essential for proper functioning of the respiratory chain [98-100]. Mitochondrial ADP/ATP carrier, an integral protein of the inner mitochondrial membrane that is closely associated with the respiratory chain complexes, also requires CL for normal functioning [101] and looses activity upon its oxidation [102, 103].

Unlike other phospholipids, CL has four fatty acid residues instead of two; and the hydrophobic tails of these fatty acids are all polyunsaturated. This makes CL a particularly vulnerable target for ROS attack. Moreover, in some proteins integrated into the inner mitochondrial membrane (*e.g.*, complex III of the respiratory chain) CL forms dimers, so that 8 unsaturated fatty acid rich with double bonds are present in close vicinity to each other. Such dimers, especially when localized near respiratory chain proteins that generate ROS, are ideal starting points for chain reaction of lipid peroxidation.

CL-cytochrome *c* complexes were identified as important components of the mitochondrial apoptotic machinery that are induced by proapoptotic stimuli and trigger the release of proapoptotic factors from intermembrane space and other apoptotic cascade steps [104]. Formation of such complex changes both functions and redox properties of cytochrome *c*, stimulating its peroxidase activity and diminishing its ability to perform electron transport from complex III to complex IV [105].

It was shown recently in neurons that CL is externalized in response to mitochondrial injury to the outer mitochondrial membrane, where it interacts with the autophagy protein LC3 to mediate the elemination of potentially dangerous damaged mitochondria by mitophagy [106]. Moreover, under certain conditions (*e.g.* neutrophil activation, necrosis, stress, and acute trauma) cells release CL-presenting mitochondria and mitochondrial membranes. In such situation, externalized extracellular CL promotes phagocytosis and attenuates inflammatory immune responses [107].

More and more experimental evidence indicates that CL oxidation is one of the key events in the initiation and/or progression of various pathologies, including brain injury [108].

It is experimentally established that under oxidative stress cytochrome *c* acts as a peroxidase that can oxidize cardiolipin [109]. It was recently demonstrated that addition of H_2O_2+cytochrome *c* to cardiolipin-containing liposomes induces membrane permeabilization for molecules up to 3 kDa. Requirement of unsaturated cardiolipin for the permeabilization suggests that cardiolipin oxidation plays a critical role in the formation of membrane defects induced by H_2O_2+cytochrome *c* [110]. Besides membrane permeabilization, cardiolipin oxidation leads to respiratory chain enzymes inactivation, cellular dysfunction and eventually cell death [111].

Mitochondrially-targeted synthetic antioxidants, even being introduced in micro- and submicromolar concentrations, fully protected the liposomal cardiolipin from peroxidation [112]. Previously, similar effect was shown in isolated mitochondria [67].

CONCLUSION

Mitochondrially-targeted antioxidants provide a potent tool for mitochondrial research and for therapy of ROS-related pathologies. Eye drops containing 250 nM SkQ1 developed against dry eye syndrome, cataract and glaucoma have already passed clinical trials (phase I-III in Russia [113-116], phase I-II in the USA [117]), and over 700,000 samples were sold over-the-counter since 2012, with no side effects reported so far. It is probable that active use of such drugs will have unexpected positive effects on a broad spectrum of age-related diseases, including chronic inflammation, atherosclerosis and on aging in general.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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PATIENT'S CONSENT

Declared none.

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