The Role of Oxidative Stress and Mutations of Mitochondrial DNA at Aging, Age Pathology, and Apoptosis - Pharmacorrection by Mitochondria-targeted Antioxidant SkQ1

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Abstract

There is presented the outcome analysis of numerous studies on the mechanisms of oxidative stress (OS) associated with increasing of the generation of reactive oxygen intermediate (ROI) in the electronic transport circuit of mitochondria, as well as the effect of ROI on the level of mutations in mitochondrial DNA (mtDNA). It is shown a fine sensibility of mtDNA to oxidative affections and mutations. The relation of age accumulation of mtDNA mutations with aging and life expectancy has been noted. It is also shown the relationship of OS with mtDNA mutations, apoptosis, diseases, and aging. It is considered a pharmacological way for the protection of ROI, aging, diseases, and prolonging life with the help of the antioxidant SkQ1, addressed directly to the mitochondria.

Key words: Age-related pathologies, aging, apoptosis, genes, mitochondria DNA, mitochondria-targeted antioxidant SkQ1, mutations, oxidative stress, reactive oxygen intermediate

THE HISTORY OF THE DEVELOPMENT OF THE FREE-RADICAL THEORY

The free-radical theory of aging appeared in the middle of the 20th century. The foundations of this theory were introduced almost simultaneously by two authors D. Harman in 1956 and M.M. Emanuel in 1958. The basis for its emergence was successful experiments for the continuation of life with the help of antioxidants - phenol derivatives that inhibit free radical (FR) chemical reactions.[1]

For biological systems, the most important is oxygen FRs, in particular, the superoxide anion radical (O_2^-), hydroxyl radical (OH), and nitric oxide (NO), the so-called reactive oxygen intermediate (ROI). FRs are any molecules or atoms that contain one or more unpaired electrons at an external electronic level. By their nature, FR is very reactive and, being formed in a cell as a result of some biological processes, can lead to damages of biological molecules (proteins, lipids, nucleic acids, etc.).[2] The accumulation of a number of such damages in cells leads to disruption of their normal functioning, as well as to an increase in the probability of death and the occurrence of various diseases. Initially, the FR theory of aging was associated only with FR, as, for example, O_2^-, but later the theory was extended to such reactive oxygen intermediate (ROI) as hydrogen peroxide and peroxynitrite.[3]

Under excessive formation of FR, oxidative stress (OS) may occur. As a result of the OS, free-radical theory accumulates damage at the cellular level, which leads to age-related tissue damage, aging, and carcinogenesis.[4]

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THE MITOCHONDRIAL THEORY OF AGING

The modern theory of aging associates the process of aging with the OS in general. It is unlikely that aging is exclusively associated with damages of FR; environmental factors and the genetic background also play a significant role.[4]

Under normal physiological conditions, the oxygen consumed in the body in addition to the main respiratory phase goes to the generation of FR, which occurs in many cell organelles and with the participation of many enzymes. However, the greatest amount of ROI (about 90%) is formed in the mitochondria. This is a consequence of oxidative phosphorylation - the process of energy generation in the form of ADP and ATP, accumulated on the inner membrane of the mitochondria. It is shown that mitochondrial ROI plays an important role in many oxidation-restorative signaling processes.[5-8]

As a separate case of the FR theory, a mitochondrial theory of aging appeared, which is a modification of the free-radical theory of aging, which states that aging in humans and animals is due to the accumulation of damage in mitochondria and mitochondrial DNA (mtDNA).[9,10] As an intracellular source of ROI, mitochondria are the most vulnerable to direct attack by the ROI. It has been shown that the levels of ROI-modified proteins and lipid peroxides in mitochondria increase with age.[11-13] It should be noted that the membrane lipid of mitochondria, especially cardiolipin, which is important for mitochondrial bioenergetics,[14] lends itself to a high risk of oxidative damage due to high levels of unsaturated fatty acids. Aging and OS are also associated with a decrease in the physical properties of internal membrane proteins, which are prone to OS damage.[15,16] The OS, as one of the mechanisms of aging, contributes to the damage of membrane proteins, which leads to a decrease in their physical properties and a violation of barrier properties of the membrane.

Since mtDNA encodes the polypeptides of the electron transmission network, any mtDNA mutations inevitably affect the entire electron transport chain, potentially changing the functions of numerous nuclear genes involved in the formation of the electron transfer chain.[5]

A damage of mtDNA is usually much more extensive and lasts longer than the damage to nuclear DNA.[7,13] The particular vulnerability of mtDNA is because it does not contain histone proteins that are in nuclear DNA and performs a protective function. An unobstructed oxidative attack on mtDNA can slowly disrupt the functioning of mitochondria in the aging organism.[16,17] Many studies have shown that there is the age-dependent nature of accumulation of mtDNA damage in the skeletal muscles, heart muscle, brain, and liver.[18-21] Strengthening the OS with age leads to a decrease in the intensity of the mechanisms of degradation and neutralization of not only the developed mitochondrial structures but also

lipids, proteins, and components of DNA, which contributes to the accumulation of damaged macromolecules and organelles. The speed of organelle renewal decreases, the accumulation of defective mitochondria increases. Aging mitochondria gradually produce less and less ATP and more ROI.[17,22,23] With age, the OS in the mitochondria plays a key role in the development of the internal pathway of apoptosis. At aging and apoptosis, the same changes occur, such as a decrease in the mitochondrial membrane potential, increased levels of lipid peroxidation, oxidation of glutathione, and mitochondrial oxidative damage to DNA, mainly due to an elevation of OS.[25] All of the abovementioned factors contribute to the gradual reduction of mitochondrial respiratory function in laboratory animals and humans.[24,25]

Mitochondria are the main organelles in the cell responsible for most of the FR reactions associated with the aging process. The life duration is determined by the level of FR in the mitochondria. It is believed that, with the age increment of a human due to OS, an imbalance appears between FR production and antioxidant protection.[26] An increase in the level of oxidative damage of mtDNA, proteins, and lipids and a decrease in the activity of the antioxidant system accompany the aging of organisms.[27,28]

The key role of mtDNA is evidently because mutations in mtDNA can accelerate the damage of the FR cell by introducing changes in the level of activity of components in the electron transport chain.

The malfunction of electronic transport chains and the accumulation of FR subsequently lead to even more damage of mtDNA and ultimately stimulate mtDNA mutations. This "vicious circle" of mutations and generation of FR are considered the cause of cell aging.[29] The mitochondria have its own apparatus for repairing mtDNA damages by exogenous and endogenous agents, where FR is the most common. MtDNA includes only 5% of the genetic material of the animal cell, but it contributes much more to cellular physiology than one can think, focusing only on this percentage. MtDNA, being in the immediate vicinity of the site of the appearance of oxygen, is an available target for undesirable effects of oxygen radicals. Oxidative damage to mtDNA causes a change in nucleotide bases and other types of damage. The greatest harm is caused by 8-oxoguanine, which accumulates in the DNA with age. Finally, defects in the electron transport chain can produce a number of pleiotropic effects, since they affect the cellular energy.[30]

The mtDNA mutations accumulate gradually throughout the life and are directly responsible for the deficiency in oxidative phosphorylation chains leading to the appearance of ROI. The primary importance of mitochondria in the aging process and in determining the lifespan is confirmed by the fact that chemical mutagens and lipophilic carcinogens (for example, polycyclic aromatic hydrocarbons) cause much more damage to mtDNA than nuclear mutations.[31] On this basis, in fact, it

is advanced the hypothesis that the accumulation of damage to mtDNA accelerates aging.

For today, FR theory and mitochondrial theory are the two most well-known and discussed theories of aging, which can relatively satisfactorily explain how and why people develop certain disorders of physiology during aging and appear age-related diseases. FR chain reactions can lead to the formation of crosslinks between molecules. In the case when a FR reaction involves pairs of nitrogen bases in DNA molecules, the two strands of DNA can be joined by cross-linkage.[32] Cross-linkage can lead to various clinical manifestations associated with aging.[33]

The development of the body, the differentiation of its tissues, aging, disease, and death itself are all associated with the permanent death of certain cells, genetically planned or accidental, and replacing them with other useful or dangerous cells. There are two mechanisms of cell death: "Accidental" death - necrosis and "programmed" death - apoptosis.

The role of FR in the implementation of these mechanisms of cell death is shown.[34] Both necrosis and apoptosis are a consequence of the violation of the barrier properties of mitochondrial membranes.[35] With a scarcity of oxygen, calcium ions accumulate in the cytosol, activation of endogenous mitochondrial phospholipase, a reduction in the electrical strength of the lipid layer of membranes, the appearance of permeability of the inner mitochondrial membrane for cations, and swelling of the organelles; this leads to the cessation of the synthesis of ATP and the suppression of the ability of mitochondria to retain calcium in the matrix. If this process covers all mitochondria, the cell dies (necrosis). If only a part of the mitochondria in the cell swells, necrosis does not occur, but the mechanisms of apoptosis may turn on. It is connected with the breakage of the outer membrane of the mitochondria or the appearance of giant pores, through which cytochrome C emerges from the mitochondria, triggering a cascade of reactions leading to apoptosis.[35,36]

The disorder of the mitochondrial respiratory chain as a result of the release of cytochrome C, the action of NO·, and other causes lead to the formation of O_2, which interacts with NO· and iron-containing complexes, leading to lipid peroxidation and the oxidation of thiol groups of mitochondrial proteins. The decisive importance has the oxidation of such groups in the ATP/ADP exchanger, which is accompanied by the formation of giant pores in the outer membrane of the mitochondria, the release of cytochrome C, and the development of apoptosis.

Programmed cell death (apoptosis)

As recent studies have shown that rapid cell deaths, the necrosis, are not only not unique but also not even the main cause of cell death in a variety of diseases. More often than

necrosis, there is a programmed cell death or apoptosis.[24,26] Apoptosis is characterized by a number of morphological and biochemical signs, among which one of the most important is the activation of serine proteinases, the so-called caspases, which trigger a whole series of biochemical reactions that result in the destruction of many proteins of the cytoplasm and nucleus, defragmenting nuclear DNA with signaling to phagocytes, that the cell must be eliminated.[35] Apparently, the main link between the activation of CP formation in mitochondria and the induction of apoptosis is an increase in the permeability of membranes and the release of cytochrome C.[35] The loss of cytochrome C with mitochondria during apoptosis enhances O_2 production. In this experiment, the addition of Bcl-2 (the inhibitor of pore formation in mitochondrial membranes), which reduced the yield of cytochrome C, simultaneously led to a decrease in the O_2 release.[35]

The production of ROI mitochondria increases on transition from state 3 to state 4, with polarographic examination, with an increase in the concentration of oxygen in the medium, a decrease in the activity of cytochrome oxidase, while the uncouplers of oxidative phosphorylation processes reduce the formation of ROI. The increase in the formation of ROI in these cases can be explained by an increase in the concentration of the reduced single-electron donor and, in a number of cases, also by an increase in the concentration of oxygen in the medium.[35]

Pore formation inhibitors in mitochondrial membranes, such as bongkrek acid or Bs1-2 proteins, as well as caspase inhibitors, generally prevent apoptosis. For example, apoptosis induced by p53 is due to the induction of this gene transcription of a number of genes that cause OS in the cell. This leads to oxidative damage to mitochondrial components and programmed cell death. Bongcrack acid, which inhibits increased permeability, effectively inhibits apoptosis induced by p53, although it does not affect either transcription or activation of ROI formation.[35]

MITOCHONDRIA-TARGETED ANTIOXIDANTS: DEVELOPMENT AND EFFECTIVENESS IN AGING

OS is one of the universal processes that to some extent take place in the vast majority of pathological conditions and play a particularly important role in the pathogenesis of alternative and inflammatory lesions, as well as in carcinogenesis and the development of a number of age-dependent pathologies.[37-41]

Such a situation led to a logical idea to use antioxidants (substances of different chemical nature that neutralize the formation of FR s) as drugs for the therapy of various diseases and agents that slow the development of signs of aging and prolong life span (geroprotectors).[37-41] However,

all known antioxidant agents (ascorbic acid, α-tocopherol, N-acetylcysteine, etc.) have two significant drawbacks: they do not have selectivity for the cellular compartments that play a leading role in the development of the OS and may have only a "one-time" antioxidant action, losing its antioxidant properties after interaction with free radicals.[42-44] Since there are certain data according to which one of the important places for the formation of free radicals - reactive oxygen intermediate (ROI) - are mitochondria, and it is these organelles that are capable of initiating the activation of signaling pathways leading to cell death, and, consequently, to tissue damage and functional disorders of organs and systems.[45,46] In this regard, it was necessary to increase the effectiveness of drugs that have antioxidant activity, ensuring their directional accumulation in the mitochondria. Efforts of several scientific teams (primarily Skulachev VP. and Murphy M.) created a whole family of such compounds (SkQ), called mitochondria-targeted antioxidants. Among these antioxidants, a special place belongs to a substance that received the designation SkQ1 (10-(6'-plastoquinonidecyl) triphenylphosphonium). Due to the fact that plastoquinone is present as an antioxidant in this substance, this mitochondria-directed antioxidant has the potential for regeneration in the respiratory chain of the mitochondria, that is, it can act as a unique antioxidant of multiple actions.[47]

Since mitochondria-directed antioxidants are already entering clinical practice and the question of their effects is a problem of the present time, comprehensive testing of these drugs on healthy animals and on models of various diseases is extremely urgent. The study of the regularities of action of these drugs on cells, tissues, and organs *in vivo* will allow not only to outline the range of possible application of mitochondrial-directed antioxidants (in particular, SkQ1) in the clinic but also indirectly reveal the real value of FRs formed in mitochondria in the development of signs of aging and pathogenesis of various diseases. Moreover, the effects of mitochondrial antioxidants, which are directly related not only to the elimination of the damaging effect of FRs but also to the interference of these compounds in the functioning of the signaling systems of the cell, may be of exceptional importance.[5,8,47]

Several reports were published on the results of testing mitochondrial-directed antioxidants on the lifespan of a variety of organisms. Regarding mammals, there is evidence that SkQ1 is able to increase the average life span of female mice of three lines - inbred 129/sv, outbred SHR and HER-2/neu transgenic mice.[48] There were made a lot of works on the effect of mitochondrial-directed antioxidants on the age related, including pathological changes in the body. In a number of studies, rats of the OXYS line were examined, in which an elevated level of ROI leads to the development of premature aging (progeria). It was shown that the addition of very small doses of SkQ1 (50 nmol/kg/day) to food prevented age related: (a) Development of cataracts and retinopathies in the eyes, (b) atrophic processes in skeletal

muscle (sarcopenia), lipid peroxidation, and carbonylation of proteins in skeletal muscles, and (c) reduction of bone mineralization (osteoporosis).[49-53]

Significant age changes in humans and animals occur in the organs of hematopoiesis and immunogenesis. The results have been published, showing that the lifetime addition of SkQ1 mice to drinking water does not only affect hematopoietic stem cells and more different hematopoietic progenitors but also significantly slows the age changes in peripheral blood.[54] During the first 13 months, SkQ1 (0.9 or 28.8 nmol/kg/day) prevents age-related myeloid shift (increase in the proportion of granulocytes and a decrease in lymphocyte count) in mice. During the next year the effect disappears, and the hemogram of the 2-year-old mice receiving the preparation does not differ from the control. The number of mesenchymal stem cells in the bone marrow does not change within 2 years of administration of SkQ1; however, the concentration of these cells in the progeny of colony-forming unit of fibroblasts (CFU-F) increases with increasing dose of SkQ1. The concentration of CFU-F after 1 and 2 years of administration of SkQ1 was twice as high as in young mice. In addition, SkQ1 has been shown to prevent the development of the most well-known marker of age-related changes in the hematopoietic system, the thymus involution.[55]

It is known that aging is often accompanied by impairments of various functions of the central nervous system, in particular, the diseases of Alzheimer's and Parkinson's that are common in elderly people in the development, of which a large role is assigned to the mitochondrial ROS.[42,56] It turned out that mitochondrial antioxidants can prevent the development of such disorders. It was shown in a study of Stefanova *et al.*[57] that SkQ1 (250 nmol SkQ1/kg per day during meals) had a beneficial effect on the motor and search functions of the brain of the Wistar rats and the OXYS line with an accelerated rate of aging. However, SkQ1 did not change the learning ability of the OXYS rats in the Morris water maze test and slightly reduced this ability in Wistar rats, which may be due to differences in redox homeostasis.

In another study,[58] the effect of the mitochondrial antioxidant SkQ1 on sexual motivation in 12-month-old male Wistar rats and male OXYS rats with an accelerated rate of aging was studied. A significantly higher degree of the motivational stage of sexual behavior was manifested in OXYS rats after prolonged preventive exposure to SkQ1 at a dose of 50 and 250 nmol/kg. The continued therapeutic administration of the drug to OXYS rats with an accelerated rate of aging for 3 months at a dosage of 250 nmol/kg was also effective. Another evidence of the neuroprotective effect of SkQ1 on aging is information that SkQ1 is able to slow the development of Alzheimer-related lesions in rats.[59,60]

High efficiency of nanomolar concentrations of SkQ1 *in vitro* as an inhibitor of apoptosis and its early stage - the

fragmentation of mitochondria - caused by hydrogen peroxide and other impacts, such as ultraviolet radiation, TNFα, and p66shc protein in cells of various origins, was demonstrated;[61-70] at that, the difference between the concentration of the drug with antioxidant and pro-oxidant (pro-apoptotic) effects was 1000 times. These results served as the basis for a detailed study of the effect of the mitochondrial-directed plastoquinone SkQ1 compound on models of alternative changes *in vivo* and *ex vivo*, especially in the brain, myocardium, and kidneys. Thus, in the work of Bakeeva *et al.*,[71] there were studied the effect of SkQ1 *ex vivo* and *in vivo* on models of the heart and kidney infarction in rats caused by ischemia/reperfusion, as well as ischemic stroke. The effectiveness of the compound for alternative changes was judged by the size of the lesion, the survival rate of animals, and the level of marker enzymes in the blood. On all these models, SkQ1 had a noticeable protective effect. It was shown that the concentration of SkQ1 of the order of 125–250 nmol/kg/day for 2–3 weeks reduces the size of the infarction zone caused by ischemia/reperfusion of the heart *in vivo* and reduces the blood lactate dehydrogenase and cardiac isoform of creatine kinase, the level of which increases as a result ischemia/reperfusion due to the release of enzymes during cell death. The authors of this study found that, in rats with the single kidney, the ischemia/reperfusion of this kidney results in the death of most animals in 4 days, while a single injection of SkQ1 (1 μmol/kg a day before ischemia) saves the lives of almost all rats. At the same time, it has been shown that SkQ1 does not lead to a normalization of the ROI content in the kidney and creatinine cells in the blood.

One possible mechanism of the neuroprotective action of antioxidant derivatives of plastoquinone is the prevention of OS due to soft separation and prevention of mitochondrial hyperpolarization.[72-74]

Except for ischemia, the inhibitory effect of preparations of the SkQ family on damage in the nervous tissue has been demonstrated in different models of Alzheimer's disease (including *ex vivo*),[60,75-78] as well as in the model of age-related retinopathy.[51]

In the work,[79] an oral supplementation of SkQ1 with food for 2–3 weeks on the magnitude of ischemic and reperfusion injury of the heart was studied under male rats. The same doses of sodium bromide (125 nmol/kg/day for 2 weeks and 250 nmol/kg/day for 2 and 3 weeks) were supplemented orally to the control rats (with food), since SkQ1 contains, in addition to the organic part (plastoquinone, linker and triphenylphosphonium), as well as bromide ions. After anesthesia, regional myocardial ischemia was induced in the animals, followed by reperfusion for an hour. The main criterion for assessing the effect was the size of the zone of myocardial infarction, determined after treatment of the heart sections with tetrazolium blue. Oral supplementation of SkQ1 with food at daily doses of 125 and 250 nmol/kg

for 2 or 3 weeks statistically significantly reduced the size of the infarction zone. In parallel, the activity of marker enzymes (lactate dehydrogenase and MB creatine kinase fraction) decreased in the blood compared to their activity after reperfusion in control animals. At the same time, there was an improvement in biochemical indicators of the state of the risk zone: Greater adenine nucleotide pool safety and an expansion in the number of phosphocreatine. In the ischemic part of the heart, the accumulation of lactate significantly decreased and the content of pyruvic acid increased. The authors concluded that the supplementation of SkQ1 with food increases myocardial resistance to ischemia and subsequent reperfusion, as well as the metabolic changes in cardiomyocytes that occur in the post-ischemic period. This conclusion was fully confirmed in the model of ischemic myocardial damage *ex vivo*, and the authors observed the cause of the observed effect in increasing the antioxidant status of cardiomyocytes.[51]

The test result of mitochondrial-directed plastoquinone compounds on other models of alternative lesions is presented in single publications. Thus, it was shown that SkQ1 inhibits age-related lipofuscinosis of pigment epithelium,[50] lenticular degeneration,[80] structural changes in myofibrils in the development of sarcopenia,[81] and reperfusion changes in liver transplantation after its storage under hypothermic conditions.[82] There are indirect reasons to believe that this drug could be effective in preventing the development of hemosiderosis in certain hemolytic states, since it is able to increase the resistance of erythrocytes to hemolysis caused by OS.[83]

AUTHORS' CONCLUSIONS

Summarizing all the material of this work, we can conclude that SkQ1 is a promising drug substance of a group of mitochondria-directed antioxidants, which has a number of positive effects on models of various pathological processes.

The results of the present work allow us to formulate the mechanism of action of SkQ1 differently than it was possible to do on the basis of theoretical concepts. The obtained materials definitely show that the effect of this compound on the studied pathological processes is the ability to prevent the process of alteration caused by FRs (active forms of oxygen of mitochondrial origin) with the help of SkQ1, as well as to prolong the life of living organisms, to prevent age-related diseases, and to inhibit apoptosis and its early stage - breaking of mitochondria - caused by various effects in cells.

The high biological activity of the mitochondria-targeted antioxidant SkQ1 causes significant possibilities for its use for prolonging life and in the capacity of medical agents of a new generation able to treat diseases hard to cure or even separate diseases beyond cure.

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