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REVIEW

Potential Geroprotectors – From Bench to Clinic

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Abstract—Geroprotectors are substances that slow down aging process and can be used for prevention of age-related diseases. Geroprotectors can improve functioning of various organ systems and enhance their homeostatic capabilities. We have developed a system of criteria for geroprotectors and proposed their classification based on the mechanisms of their action on the aging processes. Geroprotectors are required to reduce mortality, improve human aging biomarkers, have minimal side effects, and enhance quality of life. Additionally, there are approaches based on combining geroprotectors targeted to different targets and mechanisms of aging to achieve maximum effectiveness. Currently, numerous preclinical studies are being conducted to identify new molecular targets and develop new approaches to extend healthy aging, although the number of clinical trials is limited. Geroprotectors have the potential to become a new class of preventive medicines as they prevent onset of certain diseases or slow down their progression.

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INTRODUCTION

Geroprotectors are compounds affecting the mechanisms of aging and slowing it down. They could be used for prophylactics and delay of onset of age-related diseases and extension of active period of life. Geroprotectors could become and important part of modern prophylactic and personalized medicine [1], and, first of all, of the emerging medicine of healthy longevity [2].

Aging is the main cause of numerous age-related diseases such as cardiovascular diseases, cancer, diabetes, and neurodegenerative diseases. Studying of geroprotectors could shed light on the main biological processes underlying aging. This could help us to understand what molecular and cellular changes occur in an organism with aging, and what mechanisms could be modulated to achieve geroprotection. Investigation of geroprotectors could help us to develop strategies for preventing or slowing down onset of these diseases. This is of tremendous importance for improving quality of life of individuals and to reduce economic burden on healthcare systems. However, it must be mentioned that investigation of geroprotectors is a complicated task. There are multiple factors that could affect the results of such studies, such as differences in experimental conditions, genotypes and species of model organisms, problems with short-lived controls, and irreproducibility of data. This means that further studies and developments are required to establish efficiency and safety of potential geroprotectors for humans.

POTENTIAL GEROPROTECTORS

We have developed a set of criteria for geroprotectors with the goal to describe to the fullest the desired features and properties of a geroprotector and facilitate its introduction into medical practice for prophylactics of aging. We identified main criteria (ensuring effectiveness and safety) and auxiliary criteria (accelerating translation into practice) [3].

Main criteria for geroprotector. *Geroprotector must increase lifespan*. Whether the substance is geroprotector or not is judged by its ability to increase lifespan of the model organism under the used experimental conditions or decrease general human mortality. Our open on-line database (geroprotectors.org) compiled based on 2408 literature sources includes 259 compounds that increase lifespan at least at one concentration in one of 13 model organisms from yeasts to humans [4]. Recently a database within the framework of the DrugAge project was created with our participation that includes more than

1000 compounds effects of which on lifespan were investigated [5].

Geroprotector must reverse processes associated with age-related diseases. Clinical trials or use of geroprotector in modern medical practice is impossible without some symptoms associated with the diseases developed in an individual. Hence, geroprotector should provide certain help with the symptoms of age-related diseases in order to have chance to be accepted in medical practice. Nevertheless, geroprotectors could be potentially assigned to a new class of medicinal drugs, prophylactic agents, because they not only treat the symptoms of certain diseases, but also create background for resistance to the development of certain diseases or slowing down their onset and progression. Vladimir Dilman, expert in gerontology, named aging a universal disease [6]. According to Mikhail Blagosklonnyi, age-related diseases (type 2 diabetes, Alzheimer's disease, cardiovascular diseases) are symptoms of aging, same as smoke is a sign of fire [7]. Hence, effectiveness of a geroprotector should be assessed on its ability to delay onset of several age-related diseases in humans (prevent multimorbidity) and extent healthy lifespan (age before the first chronic disease develops).

Geroprotector should provide positive effect on biomarkers of human aging. Biomarkers of aging are molecular, cellular, physiological, and psychological parameters of an organism, which are known to change reliably, qualitatively, and quantitatively in aging [8]. The geroprotector candidate should change these parameters to the state typical for a younger age. The criteria of geroprotectors associated with aging biomarkers are of special importance for translation of the results into medical practice. Investigation of lifespan of a human treated with geroprotector candidate are very lengthy and costly. Hence, analysis of lifespan could be performed with animals, and effects on the human aging could be elucidated based on the wide spectrum of changes of biomarkers resulting from the geroprotective therapy. Currently there have been many attempts to use estimates of biological age as a complex biomarker of human aging in the randomized placebo-controlled clinical trials. In particular, change in the thickness of the intima-media complex of carotid artery and rigidity of the arterial wall after treatment with the complex terpene preparation was demonstrated [9].

Geroprotector should not exhibit any pronounced side effects and should improve quality of life. Considering that some geroprotectors could exhibit prophylactic effect only at a relatively high concentration, toxic and most effective (from the point of view of slowing down aging) concentrations should differ as much as possible. Some compounds that extent lifespan of animals at certain doses, exhibit side effects in humans. Achieving a geroprotective effect implies long-term (many years) administration of the preparation. Use of such preparation would require to compromise between the expected and side effects. Hence, it is preferably that the number and manifestation of side effects for the geroprotector candidate would be minimal. Efficiency of slowing down aging under the influence of geroprotectors, mostly likely, would be noticeable only after many years of taking the preparation on the regular basis. Hence, it is important that they would be capable of improving quality of life form the very beginning of their administration – facilitate elimination of digestive problems, affect favorably sleeping patterns, prevent development of depression or memory loss.

We applied the criteria for geroprotectors to a wide range of potential geroprotectors. The criteria are met for some natural terpenoids [10] and compounds exhibiting protective effect on the genome [11].

Professor V. N. Anisimov classified the life-extending compounds into three groups [12]. The first group includes geroprotectors that are equally useful for all members of population, because they could delay the onset of aging. The second group includes geroprotectors that reduce mortality of individuals at advanced age. They allow to increase maximum life expectancy by slowing down the process of population aging. The third group are geroprotectors that increase the chances of survival of humans at middle age without increasing maximum life expectancy in the population. This means that they speed up the process of aging, but at the same time increase the probability for the individual to live to old age.

Aging could be defined as gradual reduction of the capability of the organism's systems to maintain stability of inner milieu, which leads to onset of age-related diseases and death. That is why the main strategy in fighting aging and preventing wearing out of the body could be maintenance of homeostatic capabilities at all levels of organization of a live system.

Classification of geroprotectors according to their ability to maintain homeostasis of an organism [13]. *Correction of the consequences of homeostasis disruption.* Disruption of homeostasis with age is manifested by deviation from the healthy level of such vital parameters as blood acid-base balance, arterial blood pressure, level of glucose and cholesterol in blood. Hence, preparations preventing development of such age-related states could be considered as geroprotectors. Geroprotectors that correct consequences of homeostasis disruption could include antidiabetic, antiarrhythmic, hypolipidemic, vascular, and antihypertension preparations. Indeed, such preparations as antidiabetic biguanides (metformin) and sodium glucose cotransporter 2 inhibitors (SGLT2l) are capable of decreasing cardiovascular mortality, beyond the limits of secondary prevention [14, 15]. Bisphosphonates (inhibitors of bone resorption) also decrease overall mortality of the patients [16]. Stable reduction of mortality was demonstrated for the 65 years old and older patients taking statins [17].

Table 1. Mechanisms of action of potential geroprotectors [23]

Mechanism	Group of geroprotectors	Examples
Prevention of free radical oxidation	free radical scavengers or antioxidants; transition metal chelators; fatty acids resistant to lipid peroxidation	vitamins C, A, E, deuterated polyunsaturated fatty acids
Maintenance of mitochondrial functions	mitohormetins and inhibitors of mitochondrial electron transport chain; inducers of mitophagy; activators of PPARγ/PGC-1α and mitoproliferation; NAD^+ precursors	urolithin A, NMN, NR, NA
Maintenance of proteostasis	agent preventing glycation, and inhibitors of crosslinking of advanced glycation end products (AGEs); antagonists of AGEs receptors, RAGE; anti-amyloid compounds; stimulators of extracellular matrix metabolism; inducers of autophagy; proteasome activators; transient translation inhibitors	spermidine
Senolytics/senostatics	compounds facilitating selective elimination of senescent cells or slowing down their emergence	quercetin + dasatinib
Hormetins	compounds inducing moderate damage and stimulating activation of defence mechanisms of stress resistance	sulforaphane, curcumin
Suppressors of genome instability	antimutagen compounds; telomere stabilizers; inhibitors of retrotransposition	inhibitors of reverse transcriptase
Epigenetic regulators	inhibitors of HDACs, HATs; activators of SIRTs	butyrate, beta-hydroxybutyrate, resveratrol
Inhibitors of ageing-associated pathways	inhibitors of mTORC1; inhibitors of PI3K; inhibitors of Ras; inhibitors of Myc; inhibitors of AT1; activators of AMPK; Klotho activators	rapamycin, torin 2, wortmannin, LY294002
Anti-inflammatory preparations	inhibitors of NF-κB; activators of NRF2	ibuprofen, aspirin
Gut microbiota optimizers	prebiotics, metabiotics, and enterosorbents	dietary fibers, lignans
Anti-fibrotic agents	inhibitors of fibrosis-associated signaling pathways	inhibitors of TGF-β→ALK5→ →p-Smad 2,3 pathway
Neurotrophic factors	plat role in development and maintenance of structures in central and peripheral nervous systems	BDNF mimetics
Factors preventing disruption of barrier function	inhibitors of matric metalloproteinases, activators of synthesis of tight junction proteins	MMP9 inhibitors
Immunomodulators	factors facilitating thymus regeneration and preservation of the pool of naïve T-cells	regulators of JAK→STAT pathway

Table 2. Clinical trials of potential geroprotectors

Group	Indication	References
Inhibitor of mTOR, RTB101	immunity to viral infection in elderly	[32]
AMPK modulator, metformin	cardiovascular diseases	[33]
Activator of Nrf2-pathway, sulforaphane	alleviation of symptoms of mild and moderated depression in the patients with cardiological surgeries in anamnesis	[34]
Reduction of NAD^+	supplementing with NAD^+ precursors (nicotinamide, nicotinamide riboside, nicotinamide mononucleotide) positively affect biomarkers of lipid profile in the patients with cardiovascular diseases and dyslipidemia	[35]
Senolytics	quercetin + dasatinib decrease the level of inflammation in adipose tissues and improve systemic metabolic function in elderly	[36]

Enhancement of capabilities of homeostatic systems of an organism. At the cellular level the key homeostatic role is played by the stress response proteins. Nutrient deficiencies, hypoxia, DNA damage, disruptions of homeostasis are perceived by the cell as stress. Activation of the stress-resistance systems could not only alleviate damages, but also could transform the system to the higher level of defence against the new spontaneous errors and damages [18]. Induction of the stress-resistance mechanisms could be realized by the factors causing moderate stress that do not induce damage, but capable of activating defence. This phenomenon was termed "hormesis" [19], and compounds causing it were named hormetins [20].

Neutralization of the agents causing disruptions in homeostasis in external and internal environment. This is a wide group of compounds including, for example, chelators of transition metals Cu and Fe participating in the Fenton, Haber–Weiss, and Maillard reactions; scavengers of reactive oxygen species; compounds cleaving crosslinks; preparations preventing protein aggregation (anti-amyloid compounds).

Suppression of excessive homeostatic reaction causing further loss of homeostasis. Hyperactivation of some homeostatic reaction in response to stress could cause even more severe damage than the initial damage. According to the hypothesis of geroconversion suggested by M. B. Blagosklonny, after the cell cycle arrest the aging cells continue their growth. Geroconversion results in hypersecretion, hypertrophy, and proinflammatory cell phenotype, which depends on activity of the mTOR kinase [21]. Inflammaging postulated by Franceschi [22] could be considered as such excessive homeostatic reaction. Preparations targeting specific targets hyperactivated in aging (mTORC1, NF-κB, PARP1, iNOX, COX2, p38, S6K, TGF-β, AT1, IGFR, HIF-1) could also be also considered as inhibitors of hyperactivation.

At present the number of investigated targets of potential geroprotectors is very limited, and their efficiency in prolonging life leaves much to be desired. One of the potential approaches to expand the list of effective targets is to include in consideration a wider spectrum of molecular, cellular, and systemic processes associated with age-related diseases (Table 1). However, it must be mentioned that each individual compound could affect several pathways, and each geroprotector could belong to several subclasses.

To increase the number of potential effective targets of geroprotectors it has been suggested to use computerized search algorithm based on comparison of transcriptomic signature of old and young cells [24]. Realization of such approach allowed establishing potential geroprotective properties in some FDA approved drugs such as pioglitazone (PPARγ agonist) [25]. New targets for potential drugs with anti-aging properties and associated with treatment of 14 age-related diseases were identified with the help of the specialized computer program, PandaOmics, that uses a set of artificial intelligence algorithms. In particular, several gene targets were found that play an important role in the processes of inflammation and in rigidity of extracellular matrix in the organism tissues. These genes include *CASP3*, *VEGFA*, and *MMP9* [26].

Another way to increase efficiency of geroprotection is combination of several targets associated with aging in one intervention. Combining rapamycin with wortmannin increased drosophila lifespan by 23.4% [27]. Preparations affecting TGF-β and IGF-1 pathways synergistically increased the lifespan of *Caenorhabditis elegans* up to 2-fold [28]. Simultaneous inhibition of TGF-β and treatment with oxytocin enhance neurogenesis, decrease neuroinflammation, improve cognitive functions, rejuvenate liver and muscles, and decrease the number of aging cells expressing p16 in old mice [29].

Year-long treatment of the patients with recombinant human growth hormone together with dehydroepiandrosterone (DHEA) and metformin changes epigenetic age by approximately 1.5 years [30].

Problems associated with application of geroprotectors should be also considered [31]. Majority of geroprotectors investigated in the model organisms increase lifespan only marginally or only in the animals of one sex. Aging is not recognized as a disease or pathological state, which explains low number of clinical trials of pharmaceuticals, practically limiting them to investigation of natural compounds in composition of biologically active supplements. Absence of the generally accepted set of biomarkers of human aging also complicates clinical studies of potential geroprotectors.

The available clinical data are quite limited and provide information only on usefulness of potential geroprotectors with regards to surrogate health markers and mainly for the patients with age-related diseases, but not for the healthy individuals (Table 2).

Considering that the main goal of application of geroprotectors is extension of human healthspan, prospectively treatment should start before the development of the first chronic age-related disease. At present we can only talk about gerosuppressors, because they help to prevent or to slow down some manifestations of aging; so far there is no clinically proven strategies to reverse aging.

CONCLUSIONS

Despite the existence of numerous known mechanisms for prophylactics of aging, many geroprotectors have been poorly investigated even at the preclinical stage. It is difficult to draw any far-reaching conclusions, because there are no sufficient data to examine if potential geroprotectors meet the required criteria. Side effects could be more significant than the potential benefits in the long term. Some potential geroprotectors showed very promising results in the studies with animal models, but their efficiency must be confirmed in the placebo-controlled, blind, multi-center clinical trials using biomarkers of human aging and mortality data.

The goal of using geroprotector is extension of healthy life, and treatment should start before manifestations of chronic diseases in order to delay onset of the first are-related chronic disease. However, it is very difficult to obtain approval for such clinical trial without declaration of any symptoms/indications, especially due to the serious side effects of some preparations with pre-clinically proven geroprotective activity, such as EDTA and rapamycin. It seems more promising, in our opinion, to examine biologically active supplements in clinical setting using biomarkers of aging as check points as well as methods for evaluation of biological age.

At present we can talk not about geroprotectors, but about gerosuppressors, because we can slow down but not prevent development of some manifestations of aging. However, new methods fighting aging could appear in future, and we hope for development of the new branch of medicine, medicine of healthy longevity, in our country, similar to the recently created clinical centers in Singapore, Hong Kong, and USA.

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