EDITORIAL

Aging delay: of mice and men

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Abstract. The evaluation of the safety of a drug in rodents that may be used as geroprotectors is a challenge of current times. In the paper, we discuss approaches to long-term assays for selection of potent aging delay drugs for humans. Priority is given to methods combining evaluation of carcinogenic safety and life-spanning potential. The use of such methods will be time-efficient and economically feasible. (www.actabiomedica.it)

Keywords: Geroprotectors, testing, rodents, humans

People today live longer than a hundred years ago. The increase of life expectancy is due to the latest inventions, such as refrigerators, improvement of hygiene, and prevention of deaths from age-associated diseases. Thus, the number of elderly people has risen, which, in the final analysis, places a heavy load on the state and society as a whole. Anti-aging medicine aims to slow down aging and the onset of age-associated diseases (1, 2). The experience of the leading national and international institutions that provide medical and social assistance to people of older populations makes it possible to determine the main priorities in the field of aging for the next decade (3):

- Healthy aging to increase life expectancy;
- Maintaining and restoring mental health;
- Inclusion and participation of the elderly in the community and the labor market;
- Quality assurance and maintenance of social protection systems;
- Safe aging at home and in the community;
- Unequal aging and age-related inequalities;
- Biogerontology: from mechanisms to impacts.

Therefore, based on the similarity of the fundamental mechanisms of aging, there is a reason to believe that it is possible to slow down the aging process in humans. Expansion of our knowledge on aging will allow us to better confront organism-exhausting pathologies related to aging, such as cancer, cardiovascular diseases, type 2 diabetes and Alzheimer's disease. There is a consensus about sufficient evidence that aging interventions will delay and prevent the onset of many chronic conditions of adult and old age. The essential pathways have been identified, and behavioral, dietary, and pharmacologic approaches have emerged (4, 5).

Therapy, based on the knowledge of the fundamental mechanisms of aging, will contribute to better counteracting these age-associated pathologies. More extensive research into the fundamental mechanisms of aging and the search for the ways to slow it down can help us achieve much better results in confronting age-related pathologies. As the mechanisms of aging become clearer, effective interventions in the aging process can be created. This will allow a significant number of people to have a longer healthy and productive life.

Many leading researchers share the opinion that it is time not only to consider the therapeutic modalities of treating age-associated diseases, but also to initiate clinical studies with the ultimate goal of increasing the life expectancy (and, of course, longevity) of people,

respecting the medical principle *primum non nocere* (1,4,6). The most promising areas are:

- Pharmacological suppression of the growth hormone axis / IGF-1;
- Long-term use of metformin;
- Restriction of protein intake and starving diets;
- Pharmacological suppression of the mTOR-S6K pathway;
- Pharmacological regulation of some sirtuin proteins and use of spermidine and other epigenetic regulators;
- Pharmacological suppression of inflammation.

The Interventions Testing Program (ITP) established by the National Institute of Aging (NIH) that was started in 2003. ITP focused on testing compounds with the potential to extend lifespan and to delay age-associated diseases and dysfunctions (7). ITP evaluated aspirin, nitroflurodiprophen, rapamycin, resveratrol and some other drugs. Priority consideration was paid to preparations which were easily available, had a reasonable price and could be administered with food (preferably) or with drinking water. The ITP protocol included two phases. During the first phase, the capability of a drug to extend the lifespan was studied. In addition, other parameters, such as the animal's activity in the young and old age, their metabolic hormone levels and T-lymphocyte levels were studied. During the second phase, drugs showing that had shown promising results were studied more comprehensively to select candidates for further clinical studies. Behavioral and cognitive studies, estimation of the parameters of the oxidation stress and post-mortem autopsies of dead animals took place during the second phase.

In Western Europe, few studies of this kind have been performed in recent years, perhaps because of their complexity, duration and high cost. In Russia, a very limited number of laboratories are currently engaged in evaluation of the carcinogenicity and geroprotective activity of drugs. A standard protocol for testing potential geroprotectors was developed (8) and biological activity of more than 30 pharmacological agents was tested (9).

Experimental studies have demonstrated that medications targeting aging (antioxidants, calorie restriction mimetics, autophagy inductors, etc.) can substantially improve health and extend the lifespan (1, 2, 4, 10, 11). Pharmacological intervention in aging appears to be more effective in preventing age-associated pathologies as compared with treatments targeting specific pathologies. Creation of new anti-aging drugs offers a great opportunity for the pharmaceutical and healthcare industries. However, if people live longer, the increase of incidence of age-associated diseases, including cardiovascular diseases, cancer, type 2 diabetes, Parkinson's disease, and Alzheimer's, will pose a great challenge for the humankind. The search for adequate models for selecting the most effective and safest methods of life extension has become the hot spot in aging research.

There are at least two accepted definitions of compounds relating to intervention into aging: a) *anti-aging drugs*, which presumably are able to reverse the aging process (rejuvenation) and b) *geroprotectors*, which lead to prevention of premature aging and/or slow down or delay aging. Spindler (11) introduced the term "longevity therapeutics" to describe drugs that intervene in the process of aging to extend the mean and/or maximum lifespan, maintain physiological function, and mitigate the onset and severity of a broad spectrum of age-associated diseases in mammals. Vaiserman et al. (2) subdivided potential geroprotective agents into several groups: drugs with anti-aging effect, but without any evidence of lifespan increase; drugs which increase the lifespan by reducing the incidence of age-associated pathologies: and drugs which extend the lifespan because they purportedly have a rejuvenation effect. While laboratory animals are similar to humans in some respects (patterns of aging at the molecular, cellular/tissue, and physiological levels, responses to hazardous exposures), there is a growing pool of evidence demonstrating some differences (genetic, metabolic, ontogenetic etc.) among mammalian species that complicate valid interpretation and extrapolation of the results of animal pre-clinical studies to humans. Issues of concordance of responses among rodent species and between rodents and humans – as well as repeatability and site-specificity – are important considerations in evaluating laboratory animal results (2, 12).

In 2000, an international program on the assessment of the efficacy and safety of geroprotectors was

proposed (13). It was suggested that the United Nations Program on Aging, World Health Organization and the International Association of Gerontology and Geriatrics could supervise this program (14). The main aim of this program was to prepare critical reviews by an international working group of experts that would provide guidance on how to obtain evidence relating to the activity and efficacy of geroprotective drugs. The experts could recommend additional studies, if required. An agent could be categorized based on a matter of scientific judgment that considered the strengths and/or limitations of the results of clinical studies in humans and pre-clinical studies in animals. Mechanistic and other relevant data could also be considered.

Group 1: The drug is a geroprotector for *humans*. This category will include drugs with *sufficient evidence of lifespan increase* in humans. Evidence is confirmed by meta-analysis of epidemiological multicenter randomized studies;

Group 2: This category includes drugs for which, on one hand, the degree of evidence of their geroprotective activity in humans is almost *sufficient*, as well as those for which, on the other hand, there is no human data, but for which there is sufficient evidence of lifespan extension in model animals.

Group 3: A drug is *not classifiable as to its geroprotective effect in humans*. This category includes agents for which the evidence of their geroprotective effect is *inadequate* in humans and *inadequate* or *limited* in experimental animals.

Group 4: A drug is probably *not a geroprotector* in humans. This category is assigned to drugs for which there is *evidence suggesting lack of lifespan extension in* humans and in experimental animals.

The publication of the conclusions of the international working group would facilitate national and international health institutions to design and implement their own programs of rehabilitation and prevention of premature aging, as well as to assess the risk-benefit ratio of such programs. Experts in the working group would make a scientific report on the evidence of the geroprotective efficacy and safety of drugs.

Currently, there is no substance which could be classified as a group 1 agent (i.e. the 5 geroprotector activity of the drug has been proved in humans). Drugs that could be in group 2 are probably metformin, rapamycin, melatonin, peptide Ala-Glu-Asp-Gly (epitalon), resveratrol and some others. There is extensive data confirming the geroprotective effect of these drugs in animal experiments and, in some cases, in clinical studies (Table 1). These drugs are probably the most plausible candidates for testing in multicenter randomized clinical trials (10, 13, 15, 16).

The evaluation of the safety of a drug in rodents is a crucial aspect of its preclinical trials. Long-term assays for carcinogenicity in rodents are an integral method, which evaluates toxicity and some adverse effects of the drug being tested (17, 18). Combination of both safety and geroprotective potential of drugs in one study significantly decreases its cost. GeroScope is an *in silico* project that can aid prediction of a

Table 1. Summary on some of the most significant effects of promising geroprotectors observed in rodents

Parameters	Metformin	Rapamycin	Melatonin	Epitalon*
Life span	↑	↑	↑	↑
Antioxidant potential	↑	↑	↑	↑
Susceptibility to insulin	↑	↑	↑	↑
Low-density lipids	↓	↓	↓	↓
Resistance to stress	↑	↑	↑	↑
Reproductive function	↑	↑	↑	↑
Cognitive and learning capacity	↑	↑	↑	↑
Physical endurance	↑	↑	↑	↑
Age-related pathology	↓	↓	↓	↓
Cancer risk	↓	↓	↓	↓

↑- increase; ↓- decrease; *Ala-Glu-Asp-Gly.

novel anti-aging drug from existing human gene expression data (19). It is worth to note that the design of most studies in this field has been found to have limitations. Accordingly, there is a need to create standard guidelines for testing such drugs and for evaluating their lifespan extension potential as well as other late effects, including tumor development. Guidelines for the testing should include animal models (species, strain and sex), design of testing (dose, mode of administration, and constant or course treatment). Preclinical studies of such drugs should include a study of their effects on the biomarkers of aging, the lifespan and the development of various age-associated pathologies, especially tumors. The study should be conducted in rats and mice (inbred, outbreed or genetically modified animals) that will be given drugs in different doses for their whole life (8, 9). The ultimate goal in this field is the choice of geroprotectors for studies in humans. To achieve this goal, international standards for preclinical and clinical studies of agents that are plausible candidate for intervening into aging, as well as for evaluation of the results of such studies, should be developed. In years to come, the perspective direction could be the development of new biomarkers, based mostly on biochemical and genetic methods, for short-term screening of such drugs. At present, cooperative studies on anti-aging drugs and geroprotectors conducted in various laboratories could be promising.

References

1. Blagosklonny MV. Koschei the immortal and anti-aging drugs. Cell Death Dis. 2014; 5: e1552. doi: 10.1038/cddis.2014.520.
2. Vaiserman AM, Lushchak OV, Koliada AV. Anti-aging pharmacology: Promises and pitfalls Ageing Res Rev. 2016;31:9–35. doi: 10.1016/j.arr.2016.08.004.
3. World Report on Ageing and Health. Geneva: WHO. 2015.
4. Longo VD, Antebi A, Bartke A, et al. Interventions to Slow Aging in Humans: Are We Ready? Aging Cell. 2015; 14: 497–510.
5. Kennedy BK, Pennypack JK. Aging interventions get human. Oncotarget. 2015; 69:590-1. doi: 10.18632/oncotarget.3173.
6. Kennedy BK, Partridge L. 2nd interventions in aging conference. Aging (Albany NY). 201; 9: 1090–5.
7. Nadon NL, Strong R, Miller RA, Harrison DE. NIA Interventions Testing Program: Investigating Putative Aging Intervention Agents in a Genetically Heterogeneous Mouse Model. EBioMedicine. 2016; doi: 10.1016/j.ebiom.2016.11.038.
8. Anisimov VN, Popovich IG, Zabezhinski MA. Methods of testing pharmacological drugs effects on aging and life-span in mice. Methods in Molecular Biology 2013; 1048:145–160.
9. Anisimov VN, Zabezhinski MA, Popovich IG, et al. Rodent models for the preclinical evaluation of drugs suitable for pharmacological intervention in aging. Expert Opinion Drug Discovery. 2012; 7:85–95.
10. Anisimov V.N. Premature ageing prevention: Limitations and perspectives of pharmacological interventions. Current Drugs Targets, 2006;7(11):1485–1503.
11. Spindler SR. Review of the literature and suggestions for the design of rodent survival studies for the identification of compounds that increase health and life span. Age (Dordr). 2012; 34: 111–20. DOI: 10.1007/s11357-011-9224-6
12. Anisimov VN, Ukraintseva SV, Yashin AI. Cancer in rodents: Does it tell us about cancer in humans? Nature Rev. Cancer. 2005; 5 (10): 807–819.
13. Anisimov VN. Life span extension and cancer risk: myths and reality. Exp.Gerontol.2001; 26 (7):1101–1137.
14. Anisimov VN, Sidorenko AV. It is necessary to establish an International Agency for Research on Aging. Aging (Albany NY) 2018; 10: 863–867; doi: 10.18632/aging.101451.1.
15. Anisimov VN. Metfomin for cancer and aging prevention: is it a time to make the long story short? Oncotarget, 2015; 6:39398–407.
16. Anisimov VN, Popovich IG, Zabezhinski MA, Anisimov SV, Vesnushkin GM, Vinogradova IA. Melatonin as antioxidant, geroprotector and anticarcinogen. Biochim. Biophys. Acta. 2006; 1757: 573–589.
17. Neff F, Flores-Dominguez D, Ryan DP et al. Rapamycin extends murine lifespan but has limited effects on aging. J Clin Invest. 2013; 123: 3272–91.
18. Montesano R, Bartsch H, Vainio H, et al. IARC Sci Publ No 83. Lyon: IARC, 1986.
19. Aliper A, Belikov AV, Garazha A et al. In search for geroprotectors: *in silico* screening and in vitro validation of signalome-level mimetics of young healthy state. Aging (Albany NY). 2016;8: 2127–52.

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