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KLOTHO PROTEIN PROTECTS AGAINST AGING AND NICOTINE-INDUCED CHRONIC CELLULAR STRESS

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Summary

From 1997, when Klotho protein was first discovered and named "the anti-aging hormone", scientists have been thoroughly investigating its biological properties. This protein plays an important role in numerous metabolic pathways that contribute to slower ageing processes. The prevalence of neurodegenerative diseases, impairment of cognitive functions and chronic stress increases with age. Additional factors, such as nicotine, rosiglitazone, statins, blood-pressure lowering drugs, calcitriol, magnesium, chronic psychosocial stress and aerobic exercises have an influence on Klotho protein plasma concentration. The research on proteins that increase Klotho plasma levels is important because of broad range of biological processes in which Klotho is engaged. Further studies on this substance could result in new possibilities of treatment for several common diseases.

Keywords: ageing, Klotho protein, chronic stress

INTRODUCTION

The KL gene is located on the long arm of human chromosome 13. Its product protein was discovered in 1997 and it immediately became of interest to many scientists due to its involvement in the mechanisms of ageing. The name of the protein is a reference to the Greek mythology, as Clotho was the youngest of the Three Moirai. She was responsible for spinning the thread of life from her distaff onto her spindle. By naming the protein after her, scientists wanted to emphasize the unique role of the protein. Soon after the discovery of the involvement of Klotho in ageing processes, it occurred that it is also involved in other vital processes and its effects are truly pleiotropic. From a biochemical point of view, Klotho is a novel β-glucuronidase, capable of activating the TRPV5 ion channel (1, 2). Thanks to the use of specific antibodies, it became possible to detect the secreted form of Klotho in the cerebrospinal fluid and serum (1). There are two forms of this protein: transmembrane (130kDa) and secretory (65kDa). They are synthesized in varying organs, but both have an ability to create oligomer complexes (1, 2). Transmembrane form is mainly produced by the brain (specifically, by the choroid plexus) and by the kidneys. The highest quantities of the secretory form are observed in the plasma and in the cerebrospinal fluid (3). The two forms have different functions. The transmembrane form is the co-receptor for fibroblast growth factor (FGF23) and the secretory form interacts with ion channels, transporters and receptors for growth factors (2). Among others, Klotho regulates the functions of the endothelium and the production of nitric oxide by the endothelium. It also affects many intracellular signaling pathways, including p53/p21 pathway, Wnt pathway, cAMP and protein kinase C pathway (2).

KLOTHO PROTEIN AS AGEING HORMONE

A mutation in the KL gene may cause faster progression of symptoms associated with senility, such as shorter life expectancy, infertility, arteriosclerosis, skin atrophy, osteoporosis and emphysema (4). As only some organs are able to produce the Klotho protein, researchers aimed to find target structures for Klotho in different human tissues. The results confirmed that Klotho protein functions as a humoral factor in cerebrospinal fluid and is engaged in hormonal regulation network (5). Recent studies have also underlined the role of the insulin and insulin-like growth factor (IGF) signaling pathway as one of the most important metabolic routes for the lifespan regulation. It has been discovered that even slight alterations weakening the insulin/IGF-1 signaling pathway can extend the lifespan of *Caenorhabditis elegans* even over two times (6). The Klotho protein suppresses intracellular pathways induced by insulin/IGF1 and finally enables the animals to remain active and young for a longer time (7). A relationship between Klotho serum levels and activities of daily living (ADL) in older people has been investigated. Lower levels of Klotho protein have been associated with ADL disability in elderly people (8). Furthermore, it has been

suggested that the Klotho protein can be used as predictor of all-cause mortality (8, 9). Because the study has been conducted on a relatively small group, further research is required to verify if the concentration of Klotho protein may indeed be used as prognostic factor of ADL disability or mortality. The ageing process is strictly related to the overproduction of free radicals and impairment of cytoprotective mechanisms. It is probable that the Klotho protein owns the ability to prolong lifespan due to its capability of reducing oxidative stress (10). Some studies have indicated an increased expression of manganese superoxide dismutase as a result of activation FoxO – forkhead box transcription factors – in mice with high Klotho protein levels (10). Dismutase degrades free radicals and protects against small cellular damages, consequently, increasing the lifespan.

KLOTHO PROTEIN IN NEUROLOGICAL DISEASES

As Klotho protein is involved in multiple processes in the brain, it is extremely important for the normal functioning of the central nervous system (CNS). It has been discovered that there is an allele of the Klotho gene named KL-VS. It includes six unstable sequence variants with two point mutations: F352V and C370S. Inheritance of one copy of KL-VS predisposes to increased lifespan (11). Moreover, two copies of KL-VS do not have the same beneficial effect on longevity and metabolism as one copy (11-13). As a correlation between better cognitive performance and Klotho protein serum concentration had been discovered, a study was conducted in order to establish a relationship between the concentration of the "ageing hormone" and the prevalence of brain atrophy. It has been hypothesized that carrying one copy of KL-VS allele may result in greater gray matter (GM) volume in comparison to the non-carriers (14). The obtained results showed that KL-VS heterozygosity was indeed associated with greater GM volume in the frontal cortex, including right dorsolateral prefrontal cortex (rDLPFC) and left supplemental motor area (ISMA), as well as with better executive functions. On the contrary, the presence of two KL-VS gene copies was related to lower GM volume and poorer executive functions (14). The Klotho protein is responsible for the *in vitro* maturation of primary oligodendrocyte progenitor cells (OPCs) of the rat as well as for the myelination process (15). Furthermore, Klotho knock-out mice exhibit lower total number of oligodendrocytes, predisposing them to defective myelination (15). Demyelination is a process associated with advanced age. Scientists are determined to create a model for brain ageing, which would include alterations in grey matter as well as in white matter, which lead to the impairment of learning processes, memory and cognitive skills (16). When analyzing the differences between monkey brains of young and old specimens, one of the main differences found was that the Klotho protein was decreased in the white matter of older monkeys (16). Therefore, inducers of Klotho may be used in the future as an effective treatment for neurodegenerative diseases, such as Parkinson's disease, Alzheimer's disease and Huntington's disease (17). The pathological mechanisms responsible for neuronal damages have not been studied in detail yet. However, it is evident that advanced age is the most common cause of neurodegenerative diseases. The correlation between plasma concentration of Klotho protein and increased predisposition to neuronal damage was investigated. Abnormal activation of vitamin D led to Klotho insufficiency in mice (18). As a result, a decrease in dopamine levels in the pars compacta of substantia nigra and the ventral tegmental area was noted. It has been suggested that Klotho protein deficiency triggers a signaling cascade, the activation of which results in the dysfunction of dopaminergic system (18). The knowledge of Klotho-mediated metabolic pathways may also shed more light on the pathogenesis of the most common age-related dementia, Alzheimer's disease. As the number of people suffering from Alzheimer's is high and still growing, especially in ageing societies of the well-developed countries, it is clear that this disease requires thorough research (19). The pathogenesis of Alzheimer's disease depends on multiple factors – according to our current knowledge, processes such as accumulation of cytokines, free radicals and nitric oxide contribute to chronic inflammation and to the progression of the disease. These substances also activate microglia and astrocytes, which, in turn, are responsible for inducing neuronal apoptosis, resulting in the damage of the blood-brain barrier.

THE INFLUENCE OF NICOTINE AND KLOTHO PROTEIN ON CHRONIC STRESS AND COGNITIVE FUNCTIONS

Chronic stress results in induction of numerous negative processes in every living organism. It impairs homeostasis and significantly increases morbidity and mortality. Affecting multiple organs, it is also influenced by different internal and external factors. Stress influences production and degradation of many substances, including Klotho. A study on the effect of psychosocial stress on Klotho concentration revealed a causal relationship between ageing and stress with decreased Klotho levels and the prevalence of neurodegenerative diseases (20).

Chronic cellular stress may be caused by smoking. The main active substance of the cigarette smoke, nicotine, was initially thought to be the main culprit. A study was conducted that concentrated on the differences in the perception of stress between groups of smokers, persons after smoking cessation and never-smokers. Smoking led to an increased stress level and more frequent mood fluctuations, which could potentially lead to psychological diseases (21). Those observations

are incoherent with smokers' observations. There is a widespread belief that cigarettes reduce stress and help their users avoid bursts of anger. People that feel stressed often smoke more as they feel more relaxed then. It is a very subjective impression, because it has been revealed that nicotine activates the hypothalamus-pituitary-adrenal (HPA) axis as it stimulates the release of the adrenocorticotropic hormone (ACTH), therefore, increasing the plasma levels of corticosteroids, epinephrine, norepinephrine, and glucose (22). A study performed on mice revealed that animals exposed to chronic unpredictable stress (CUMS) exhibited anxiety disorders, disturbances in memory and depression, while acute or subchronic administration of nicotine decreased all these stress-induced behavioral changes as well as memory deficits (23). Moreover, the study also indicated that CUMS, as well as nicotine administration, caused cellular oxidative stress in the brain, that, if chronic, may cause neurodegeneration and therefore cognitive deficits (23). Another study described similar neuroendocrine effects, moreover, aptitude to greater opioid consumption in nicotine-exposed rats was detected (24). Excessive production of stress hormones results in brain function impairment (25). In particular, brain areas responsible for recording and retrieving new information, like prefrontal cortex or hippocampus, are affected (25). On the other hand, chronic psychosocial stress in rats was found not to impair all the memory-associated capabilities, as it affected only short-term and spatial memory, whereas long-term memory was not affected (25). Furthermore, nicotine treatment of the stressed rats reversed the unfavorable affects (25). Study on the chronic stress in the rat model of Alzheimer's disease revealed that nicotine also plays an important role in protecting the memory skills by decreasing the Aβ amyloid levels and by opposing the pro-inflammatory effect of the disease (26).

When considering the potential neuroprotective effect of Klotho protein, its involvement in the oxidative processes seems to be of great importance (27). Klotho gene knock-out mutants exhibit an impairment of cognitive functions in appropriate tests, as well as increased level of lipids and DNA peroxidation in hippocampus, a brain region particularly important for the learning and memory processes. As it was found, oxidative stress was the most important factor promoting the aging-associated cognition deficits in klotho mutant mice (27). The Klotho protein is probably involved in antioxidant protection within brain (27). Another study proved that antioxidant effect is not the only one of the positive effects of Klotho protein on cognition (28). Transgenic mice with systemic Klotho overexpression performed better in behavioral learning and memory tests comparing to the animals with normal Klotho levels. Moreover, high level of Klotho in mice was also associated with enhanced synaptic plasticity and higher level of GluN2B, a N-methyl-D-aspartate receptor (NMDAR) subunit, which are crucial for the learning and memory processes. Blockade of GluN2B by appropriate inhibitors causes cognitive deficits, which was observed both in young and old mice. This confirms the crucial role of Klotho protein in maintenance of proper brain functions independent from the aging process (28).

WAYS OF MOLECULAR AND NON-MOLECULAR INFLUENCE ON THE KLOTHO LEVELS

There are many molecular and non-molecular mechanisms that help boost Klotho production. It is well known that physical activity has beneficial effects on human body and may delay ageing processes. Aerobic exercise increases the Klotho plasma concentration (29). Apart from that, it correlates positively with carotid artery compliance and ventilatory threshold (VT) (29). The effects listed may partly explain the benefits from daily and systematic physical activity. Recent studies reported on the growing number of the pharmacological modulators of the Klotho plasma levels. The strongest evidence is provided by studies on rosiglitazone, statins, blood-pressure lowering drugs and calcitriol (30). Special attention needs to be drawn to the active form of vitamin D. The Klotho protein participates in maintaining the delicate mineral homeostasis. Vitamin D induces Klotho and FGF23 synthesis, but the latter is unable to regulate phosphate metabolism when the organism is deficient in the Klotho protein. The mechanisms leading to the majority of positive effects of the vitamin D, e.g. in chronic kidney disease, remain unknown. Higher vitamin D intake is related to lower cardiovascular mortality index. Also, one study showed that vitamin D therapy was related to aortic calcifications two times smaller than those in the control group (30). Other effects of the therapy included increase in the serum and urine Klotho levels, increased phosphaturia and decrease in serum fibroblast growth factor-23 level (30). Vitamin D, fibroblast growth factor 23 (FGF-23) and Klotho protein create an endocrine axis responsible for proper renal functioning. Its deregulation may contribute to the development of chronic kidney disease. The immediate effect of the deregulation is the activation of the renin-angiotensin-aldosterone system (RAA), which, in turn, leads to the renal Klotho deficiency (31). Cyclosporine, which is an immunosuppressive medication frequently used in contemporary medicine, induces oxidative stress in human organs. The accumulation of the free radicals results in a decrease of Klotho plasma level. Statins seem to protect against this harmful effect. Scientists revealed that this effect is dose-dependent. In patients treated with high-dosed statins, the Klotho level increased significantly due to the activity of antioxidant enzymes, such as heme oxygenase 1 (32). A study on magnesium, which is a commonly consumed supplement, also revealed its influence on the Klotho plasma levels.

It occurs that it is able to upregulate cellular receptors for FGF23/Klotho, calcium receptors (CaR) and vitamin D receptors (VDR) (32). Therefore, it may be possible in the future to use magnesium to modulate ageing processes or to slow down the progression of some neurological disorders (33).

CONCLUSIONS

The Klotho protein exhibits pleiotropic effects in human body. Its increased expression slows ageing process. In the last years, observations have shown that smoking, as well as reduced serum Klotho levels, predisposes to age-related neurological diseases. Further research is needed in order to determine whether nicotine can induce the development of neurological disorders by decreasing Klotho levels or in the contrary, if low Klotho level and nicotine consumption are two independent risk factors.

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Conflict of interest
None

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