Tel./fax: +91 484 2575588, +91 484 2576267
E-mail addresses: cspaulose@cusat.ac.in, paulosecs@yahoo.co.in (C.S. Paulose)

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Ultrasound of 10 MHz frequency as a novel strategy for skin anti-aging therapy

Introduction

Aging is believed to be connected with accumulated damaging of cellular macromolecules in non-dividing cells as well as with reduction of the cells capability to repair this damage. Damage of the macromolecules occurs through different physical and chemical factors (e.g. different types of irradiation, heat, toxins, etc.). The fate of the damaged macromolecules is dependent on such processes as degradation of unstable proteins, refolding of misfolded proteins, controlling of regulatory proteins and prevention of protein folding. These processes are essentially regulated through activation of heat shock proteins (HSPs) [1,2]. HSP family includes different proteins classified according to their molecular weights. Among them the HSP70 and HSP90 seems to be responsible for the recognition and refolding of proteins. HSPs are present in all cell types and were found not only inside of the cells where they believed to act cytoprotectively (to prevent the cells against the second stress that would otherwise cause lethal molecular damage) but also in the cell membrane and in the extracellular space where they can show the cytotoxic effect increasing the cell death [3,4]. It is well known that HSPs are over-expressed in cancer cells as well as on outer cellular membranes of damaged cells after some aggressive treatments (e.g. [5]). There is also increasing evidence of HSPs expression in different neurodegenerative disorders, during inflammation, by trauma as well as by cerebral and cardiac ischemia. It is believed that in the most of these diseases induction of a heat shock response might be an effective prophylactic treatment to minimize the severe injuries, making HSPs activation a potential therapeutic modality.

Recently it was proposed that increased protein damage in aging can be connected with decreased production of HSPs and thus with partial loss of protein quality control. Such a reduction of HSPs activity was found in liver [6], neuronal tissue [7] and muscles [8] of aging animals. For example, the muscle contraction during exercise normally induces the HSPs production; this induction shows however strong negative correlation with aging. The aged liver cannot produce HSPs sufficiently which may be important for its ability to respond to hepatic toxins.

Insufficient HSPs production is believed to be responsible at least partly for the cell abnormalities and apoptosis [9,10]. At the same time the over-expression of HSPs can define lifespan of the cells as it was demonstrated for the long-lived strain of *Caenorhabditis elegans* [11]. All together leaves almost no doubt that the protein quality control is tightly coupled to the longevity of the cells and species as well as to the rate of aging. Because of this the stimulation of HSPs actually considered to be an important factor in the new strategies of anti-aging [12]. One can assume that in the next future different chemical and physical pathways of the HSPs activation will be of the primary interest to develop the new strategies of anti-aging pharmaceuticals as well as some new ways of anti-aging physical therapy.

HSPs activation

HSPs can be initiated not only by the elevated temperature as it was assumed initially but also by many chemical and physical stimuli, such as heavy metals, oxidative and osmotic stress, viral infection, ultraviolet and ionizing irradiation [13,14]. Almost all of them can show significant side effects. One of the most investigated and non hazardous treatment methods which already has find some application in the medical praxis is the RMHS (repeated mild heat stress) [15,16]. Recently it was demonstrated that RMHS has a hormetic effect on the human keratinocytes and fibroblasts [17] which can be of essential importance for the skin anti-aging therapies. Different activation pathways show different magnitude and dynamics of HSPs activation. So RMHS has to be applied at least twice a week for 1 h at the temperature of 41–42 °C. Some other activation pathways shows the rapid and dramatic increase of HSPs production which however decreases to the normal value already in several hours which makes such type of treatment less applicable in the medical praxis.

Ultrasound (US) waves are an interesting potential physical agent for HSPs activation because of their local effects and possibility to regulate the depth of their action through the change of US-frequency. However the results were till now very contradictive. It was shown that the US-waves with a frequency of 1 MHz can cause some increase of Hsp72 content in mice cells [18], however no effect was observed for the same conditions in the rat cells [19]. We have recently demonstrated a strong positive correlation between the activation of Hsp72 in HL-60 cells and the applied US-frequency [20]. No HSPs activation could be observed after irradiation with 1 MHz. US-waves of 3 MHz could induce approximately 9-fold increase of Hsp72 which decreased to the normal value after 24 h. US-waves of 10 MHz produced more than 18-fold activation of Hsp72 which was still 8-fold over the control level after 24 h. Very similar effect was also observed by dual application of 3 and 10 MHz US-waves in the so called LDM-modus. It was also demonstrated that this effect is not connected with the heating of the medium through US, which shows its non-thermal nature.

Hypothesis

Chemical stimulation of HSPs synthesis is actually considered to be important for the design of anti-aging pharmaceuticals, which can be used for the prolongation of the lifespan as well as for slowing down the aging of single tissues and organs. Chemicals such as amphetamine, aspirin, radicicol geldanamicin, arachidonic acid, etc. can stimulate HSPs expression but their clinical application is restricted due to their generalized effect and their potential to evoke side effects. These problems can be significantly reduced though application of spatially and temporarily limited physical stimuli which can be applied for the local application such as skin anti-aging treatments. The direct application of the superficial or deep heating is however restricted through the thermotolerance of the cells. Because of this it is of special interest to look for some physical stimuli which can produce the non-thermal activation of HSPs. Our previous work [20] has demonstrated the possibility to stimulate the HSPs production through high frequency US, especially with a frequency of 10 MHz. Such HSPs induction is not only very intensive but also relatively long lasting. We hypothesize that application of US-waves of such a high frequency can be a new strategy in the local anti-aging treatments, e.g. for slowing down of the skin aging.

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