Hormetic Prevention of Molecular Damage during Cellular Aging of Human Skin Fibroblasts and Keratinocytes

SURESH I. S. RATTAN AND REHAB E. ALI

Department of Molecular Biology, Laboratory of Cellular Ageing, University of Aarhus, DK8000 Aarhus–C, Denmark

ABSTRACT: Progressive accumulation of molecular damage is a hallmark of cellular aging, which is amenable to intervention and prevention by hormesis through mild stress. Our studies have shown that repeated mild heat stress (RMHS) has antiaging effects on growth and various other cellular and biochemical characteristics of normal human skin fibroblasts undergoing aging *in vitro*. RMHS at 41°C, for 1 h twice a week, increased the basal levels of various chaperones, reduced the accumulation of oxidatively and glycoxidatively damaged proteins, stimulated proteasomal activities for the degradation of abnormal proteins, improved cellular resistance to ethanol, hydrogen peroxide, and UV-B rays, enhanced the levels of various antioxidant enzymes, and increased the phosphorylation-mediated activities of various stress kinases. RMHS-exposed human fibroblasts are also better protected against glucose- and glyoxal-induced growth inhibition and apoptosis. We have also observed various hormetic effects of RMHS on normal human epidermal keratinocytes, which include increased replicative life span, increased proteasomal activity, and enhanced levels of Na/K-ATPase pump. We are also testing the above effects of RMHS in combination with potential hormetic molecules, such as curcumin, on aging, longevity, and differentiation of human cells in culture.

KEYWORDS: hormesis; hormetin; heat shock; sodium pump; Na/K-ATPase; stress

INTRODUCTION

Aging is characterized by a progressive accumulation of molecular damage in nucleic acids, proteins, and lipids. The inefficiency and failure of

Address for correspondence: Dr. Suresh I. S. Rattan, Department of Molecular Biology, University of Aarhus, Gustav Wieds Vej 10C, DK8000 Aarhus–C, Denmark. Voice: +4589425034; fax: +4586123178.
rattan@mb.au.dk

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maintenance, repair, and turnover pathways are the main causes of age-related accumulation of damage.[1] Therefore, it has been hypothesized that if cells and organisms are exposed to brief periods of stress so that their stress response-induced gene expression is enhanced and the related pathways of maintenance and repair are stimulated, various antiaging and longevity-promoting effects could be observed. Such a phenomenon in which stimulatory responses to low doses of otherwise harmful conditions improve health and enhance life span is known as hormesis.[2] Application of hormesis as an antiaging approach is gaining wide recognition and acceptance.[3]

Various chemical, physical, and biological treatments have been used to unravel the pathways of maintenance and repair whose sustained activities can improve the physiological performance and survival of cells and organisms. Stresses that have been reported to delay aging and prolong longevity in various systems (for example, yeast, *Drosophila,* nematodes, rodents, and human cells) include temperature shock, irradiation (UV-, gamma-, and X-rays), heavy metals, pro-oxidants, acetaldehyde, alcohols, hypergravity, caloric restriction (CR), and exercise.[3] Hormesis-like beneficial effects of chronic but mild undernutrition and intermittent fasting have also been reported.[4,5] Some other compounds, including various components of herbs, medicinal plants, and spices, such as curcumin, have also been used as hormesis-inducing molecules, termed *hormetins*.[6]

HEAT SHOCK (HS) HORMESIS IN HUMAN CELLS

High temperature stress is a widely used hormetic agent, not only because it is easy to implement and gives consistent results, but also because heat stress mainly acts through an evolutionarily highly conserved stress response pathway known as the HS response. HS response is one of the primordial intracellular defense mechanisms against stressful conditions in which extracellular stress (and intracellular stress from denatured proteins) initiates a series of events starting with signal transduction, activation, and nuclear translocation of heat shock factors (HSF), DNA binding of HSF, initiation of HS gene transcription, and preferential translation of heat shock proteins (HSP), which then perform various biological functions.[7] Recently, some hormetic effects of cold shock have also been reported for rat mesenchymal stem cells.[8]

In a series of publications, we have demonstrated the hormetic effects of mild HS on human skin fibroblasts. Using a mild stress regimen of exposing serially passaged human fibroblasts to 41°C for 1 h twice a week throughout their replicative life span *in vitro*, we have reported several beneficial antiaging effects. These effects included the maintenance of youthful morphology, reduced accumulation of damaged proteins, increased levels

of various HSP, increased proteasomal activities, increased antioxidative abilities, increased resistance to ethanol, hydrogen peroxide, and UV-A irradiation, and maintenance of the stress kinase responses.[9–11] Additionally, we have also observed that vitamin D-induced differentiation of bone marrow stem cells into osteoblasts can be enhanced by preexposure to 1 h HS at 41°C and 42.5°C.[12]

HORMESIS IN KERATINOCYTES

Recently, we have undertaken studies on the hormetic effects of repeated mild heat stress (RMHS) on human epidermal keratinocytes, and the results obtained are very much similar to those for dermal fibroblasts. In these studies, normal human epidermal keratinocyte (NHEK) cultures were serially passaged at 37°C in keratinocyte-specific culture medium as described before.[6,13] Three parallel cell lines were maintained until the end of their replicative life span *in vitro,* and these were: (*a*) 37°C control line; (*b*) 41°C line where NHEK were exposed to 41°C in a water bath for 1 h HS twice a week; and (*c*) 43°C where NHEK were exposed to 43°C in a water bath for 1 h HS twice a week. Replicative life span was determined by serially passaging NHEK until their irreversible growth arrest in accordance with the Hayflick system of cellular aging.[14] Levels of HSP, HSP27, HSP70, and HSP90 were determined by Western blots, and chymotrypsin-like activity of the proteasome was determined by methods described before.[6,15,16] Furthermore, Na/K-ATPase or sodium pump content and ouabain-dependent ATPase activity were determined by methods described earlier.[17]

As previously observed for human skin fibroblasts, NHEK also showed a variety of cellular and biochemical hormetic antiaging effects on repeated exposure to mild HS only. These effects included maintenance of youthful cellular morphology, enhanced replicative life span, enhanced proteasomal activity, and increased levels of HSP (Ali, Mahmmoud, and Rattan, personal obs.). In addition, we have also studied the effects of HS on Na/K-ATPase or the sodium pump. Mild HS significantly increases the content and activity of the pump in NHEK. Increased Na/K-ATPase activity is consistent with an overall increase in the metabolic rate of the cell. However, the molecular mechanisms and interactions that bring about the mild HS-induced increase in the amounts and activity of Na/K-ATPase, and its consequences on other biochemical pathways, in NHEK during aging, are yet to be elucidated. Notably, comparable hormetic effects could not be seen in NHEK repeatedly exposed to 43°C, which underlines the differences between the beneficial effects of mild stress and the harmful effects of severe stress. A summary of the main results on the effects of mild and severe HS on NHEK cells is given in TABLE 1.

TABLE 1. Effects of mild and severe repeated HS on NHEK undergoing serial passaging *in vitro*

Biological characteristics	Effect of mild HS at 41°C	Effect of severe HS at 43°C
Replicative life span	Up to 26% increase	No difference
Proteasomal activity	Up to 60% increase	10–20% decrease
Na/K-ATPase content/activity	Up to 90% increase	No difference
HSP	Up to twofold increase	Slight increase

CURCUMIN AND OTHER HORMETIC TREATMENTS

Another hormetic agent (hormetin) that we have tested on human skin cells is curcumin. Previously, we have reported the biphasic hormetic effects of curcumin, which is an active constituent of a widely used spice turmeric, on the stimulation of proteasome activity in NHEK.[6] We have now extended these studies to determine the effects of curcumin, in combination with HS, on the activity of Na/K-ATPase. FIGURE 1 shows the effects of various concentrations of curcumin on Na/K-ATPase activity in isolated cell membranes from mid passage (40% life span completed) and late passage (90% life span completed) NHEK cells from three cell lines (37°C, 41°C, and 43°C) as described above.

Curcumin had a biphasic hormetic effect on Na/K-ATPase activity in 37°C and 41°C cell lines, but not in 43°C severe HS cell line (FIG. 1). There was a significant increase (three- to sevenfold) in Na/K-ATPase activity in mid passage and late passage NHEK treated for 24 h with 0.3, 1.0, and 3.0 μM curcumin. This effect was more pronounced in 41°C cell line than in 37°C cell line, indicating the synergistic effects of curcumin with mild HS. Higher concentration of curcumin (10 μM) was inhibitory for Na/K-ATPase, although the effect was more drastic in 37°C cell line than in 41°C cell line, which appeared

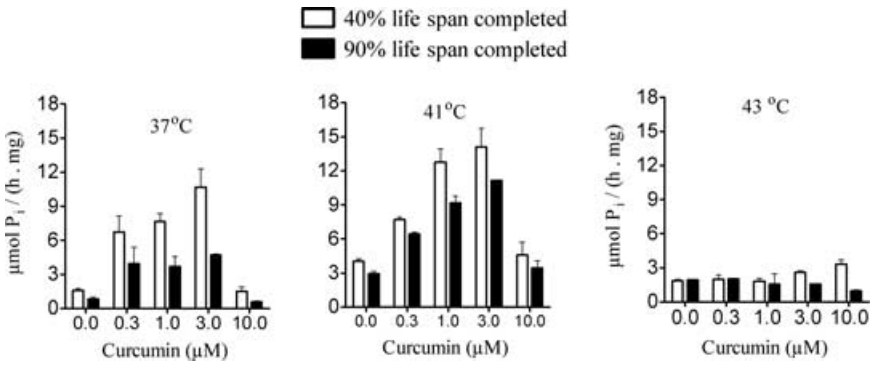

FIGURE 1. Effect of curcumin on the activity of Na/K-ATPase pump in NHEK pre-exposed to mild or severe HS.

to be more resistant due to the beneficial hormetic effects of mild HS. It is interesting to note that severe HS-treated 43°C NHEK cell line had lower activity of Na/K-ATPase, which did not respond hormetically to curcumin treatment (FIG. 1).

We have also observed similar synergistic effects of curcumin on the induction of HSP in late passage normal human skin fibroblasts and in telomerase-immortalized bone marrow stem cells. In this case, although curcumin did not induce the synthesis of HSP27, HSP70, and HSP90 in NHEK on its own, a pretreatment of cells for 24 h with 0.3 μm and 1.0 μM curcumin, followed by a 1-h HS at 41°C or 43°C, increased the levels of HSP up to 5.8-fold, depending on the dose and cell type (Ali, Mahmmoud, and Rattan, personal obs.). Although further studies are required to elucidate other biological effects and mechanisms of action of curcumin, it is clear that curcumin is potentially useful hormetin the may have wide ranging beneficial and antiaging effects brought about by hormetic pathways.

Recently, we have also initiated studies on the hormetic effects of other conditions, such as mechanical stress in osteoblasts, and intermittent fasting of fibroblasts. Whereas studies are in progress with respect to the effects of mild repeated mechanical stress on normal and immortalized bone marrow stem cells (Kraft, Melson, Kassem, Rattan, personal obs.), pilot studies have been performed on the effects of intermittent fasting on the life span and lysosomal activities of human skin fibroblasts. In this series of experiments, human adult skin fibroblasts ASF-2, which were normally cultured at 37°C in a culture medium containing 10% fetal bovine serum,[18] were transferred either to 2% serum-containing medium (2% medium) or to 5% serum-containing medium (5% medium) for 24 h once a week. After 24 h of this 80% or 50% "fasting," cells were stained with the lysosomal stain, neutral red, in order to monitor their autophagic activities. Microscopic observations and semiquantitative measurements of extractable color showed that cells kept in 5% medium and 2% medium for 24 h had about 20% and 50% increased levels of lysosomal staining, respectively. Long-term experiments with ASF2 cells showed that cells cultured in 10% serum medium underwent a total of 43 cumulative population doublings (CPD) during serial passaging, whereas those exposed to once a week 50% and 80% fasting had about 10% and 15% extension in their proliferative life span, respectively (Rattan, personal obs.). These results give further support in favor of the antiaging effects of food restriction as being hormetic.[9,19]

CONCLUSIONS

Our studies performed on normal human skin fibroblasts,[9,10] keratinocytes,[6] telomerase-immortalized human bone marrow stem cells,[12] and on *Drosophila*[20] have provided significant evidence in support of the applicability

of hormesis in aging research and interventions. Although there are several unanswered questions, especially with respect to determining the optimal levels of stress, which are hormetic at various ages, combined action of multiple stresses, and the cell-type specificity of various stresses, our studies have opened up several new lines of investigation. Applying concepts of hormesis in the modulation of aging and differentiation is a powerful new tool for discovering efficient physical, chemical, and biological means of strengthening the homeodynamic machinery of cells and organisms.[1]

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