aptara | NYAS | nyas˙5193-1509013 | Dispatch: 1-16-2010 | CE: N/A
Journal | MSP No. | No. of pages: 5 | PE: Amanda/Carey

Ann. N.Y. Acad. Sci. ISSN 0077-8923

ANNALS OF THE NEW YORK ACADEMY OF SCIENCES

Targeting the age-related occurrence, removal, and accumulation of molecular damage by hormesis

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Strategies for testing and developing effective means of intervention, prevention, and modulation of aging incorporate means to minimize the occurrence and accumulation of molecular damage, to reduce molecular heterogeneity, and to evaluate the relevance of the type and extent of damage with respect to its role in aging and age-related diseases. One such approach is that of mild stress-induced hormesis, which stimulates maintenance and repair systems and strengthens the homeodynamic space of cells and organisms. Hormesis through mild heat shock, natural and synthetic hormetins, and other stressors brings about several antiaging effects in human fibroblasts, keratinocytes, and telomerase-immortalized bone marrow stem cells. Depending on the cell type, these antiaging hormetic effects include extension of replicative life span, enhanced proteasomal activities, increased chaperone levels, and improved wound healing, angiogenesis, and differentiation. The main molecular pathways for achieving such hormetic effects are through targeting the processes for the repair and removal of molecular damage, which can slow aging.

Keywords: antiaging; hormesis; hormetin; stress; molecular heterogeneity

Introduction

Aging at the molecular level is characterized by the progressive accumulation of molecular damage in nucleic acids, proteins, lipids, and carbohydrates.[1,2] Although the action of the damaging agents is mainly stochastic, the result, whether a specific macromolecule will become damaged and whether the damage will persist, depends on the structure, localization, and interactions of the macromolecule with other macromolecules and on the activity and efficiency of a complex series of maintenance and repair systems (*MARS*). The resulting increase in molecular heterogeneity has major biological consequences in terms of interrupted networks and illegitimate networks.[2] More specifically, damage in MARS leads to age-related failure of homeodynamics, altered cellular functioning, reduced stress tolerance, emergence of diseases, and ultimately death.

Strategies for testing and developing effective means of intervention, prevention, and modulation of aging incorporate means to minimize the occurrence and accumulation of molecular damage, to reduce molecular heterogeneity, and to evaluate the relevance of the type and extent of damage with respect to its role in aging and age-related diseases. One such approach is that of mild stress-induced hormesis, which stimulates MARS and strengthens the homeodynamic space of cells and organisms.[3] Hormesis through mild heat shock (*HS*), natural and synthetic hormetins, and other stressors brings about several antiaging effects in human fibroblasts, keratinocytes, and telomerase-immortalized bone marrow stem cells. Depending on the cell type, these antiaging hormetic effects include extension of replicative life span, enhanced proteasomal activties, increased chaperone levels, and improved wound healing, angiogenesis, and differentiation.

Stress and hormesis

A critical component of the homeodynamic (homeostatic) property of living systems is their capacity to respond to stress. In this context, the term "stress" is defined as a signal generated by any physical, chemical, or biological factor (stressor) which in a living system initiates a series of biological events that enable it to counteract, adapt, and survive. Table 1

Table 1. Major molecular-level SRs in human cells

Response	Stressor(s)	Effectors	Reference
DNA repair response	Radiation, oxidants, free radicals	DNA repair enzymes	4
Antioxidant response	Free radicals, reactive oxygen species, pro-oxidants	Nrf-2, heme oxygenase, FOXO	5
HSR	Heat, heavy metals, antibiotics, protein denaturation	HSP, proteasomes, other proteases	6
Unfolded protein response	Unfolded and misfolded proteins in the endoplasmic reticulum	Chaperones, cochaperones	7
Autophagic response	Food starvation, hypoxia, damaged organelles	Lysosomes	8
NF-κB inflammatory response	Pathogens, allergens, damaged macromolecules	Cytokines, nitric oxide synthase	9
Sirtuin response	Energy depletion	Sirtuins	10

gives a list of major molecular pathways of stress response in mammalian cells. These include heat shock response (*HSR*), unfolded protein response, DNA repair response, antioxidant response, and autophagy, which are integral to the organismic property of homeodynamics. Based on the involvement of one or more molecular stress responses (*SR*), higher-order (cellular, organ-level, and body-level) SR are manifested, which include apoptosis, inflammation, and hyperadrenocorticism.

Not all pathways of the SR respond to every stressor, and although there may be some overlap, generally, SR pathways are quite specific. The specificity of the response is mostly determined by the nature of the damage induced by the stressor and the variety of downstream effectors involved. For example, cytoplasmic induction of protein denaturation by heat, heavy metals, and antibiotics will initiate HSR by inducing the synthesis of heat shock proteins (HSP) followed by the activation of proteasome-mediated protein degradation.[6,11] But, unfolded proteins in the endoplasmic reticulum will induce an unfolded protein response and will initiate the induction of synthesis of a totally different set of proteins and their downstream effectors.[7,12]

The consequences of SR can be both harmful and beneficial, depending both on the intensity, duration, and frequency of the stress and on the price paid in terms of energy utilization and other metabolic disturbances. But the most important aspect of SR is that it is not monotonic with respect to the dose of the stressor; rather, it is almost always characterized by a nonlinear biphasic relationship. Several meta-analyses performed on a large number of papers published in the fields of toxicology, pharmacology, medicine, and radiation biology have led to the conclusion that the most fundamental shape of the dose–response curve is neither threshold nor linear but has a U or inverted U shape, depending on the end point being measured.[13,14] This phenomenon of a biphasic dose response is termed hormesis.[15]

Since several terms, such as autoprotection, heteroprotection, adaptive response, preconditioning, hormesis, xenohormesis, and others, have been used to describe the biological responses to various stressors, recommendations have been made for the use of a common terminology which is consistent with the quantitative features of the dose response and underlying molecular foundations (for information on the historical development of the term, see Ref. 16). It has been proposed that a common terminology should include the operational term hormesis, which would be preceded by the type of inducing agent and whether or not conditioning was present.[13] Three main categories of such terms are:

1 physiological conditioning hormesis, in which an exposure to a stressful condition, such as hypoxia, ischemia, radiation, or a toxic chemical, conditions the system to tolerate much higher doses of the same stressor subsequently

2 physiological, chemical, or radiation hormesis, when hormesis occurs without prior conditioning
3 postexposure conditioning hormesis, in which hormesis occurs following exposure to high doses of a stressor, such as radiation for cancer therapy, followed by a repeated low-dose exposure to that stressor.

The key conceptual features of hormesis are the disruption of homeodynamics, a modest overcompensation, the reestablishment of homeodynamics, and the adaptive nature of the process. An example of stress-induced hormesis is the well-documented beneficial effects of moderate exercise as a hormetic agent, which initially increases the production of free radicals, acids, and aldehydes.[17] Another frequent observation in studies of hormesis is that a single hormetic agent, such as heat shock or physical activity, can improve the overall homeodynamics of cells and enhance other activities, such as tolerance to other stresses, by initiating a cascade of processes resulting in a biological amplification and eventual beneficial effects.[18] Hormesis in aging research and antiaging interventions is represented by mild stress-induced stimulation of protective mechanisms in cells and organisms that results in biologically beneficial effects.[3,18]

Hormetic modulation of aging human cells

In a series of papers published since 1998, our labs have reported the hormetic effects of mild HS (41° C, 1 h, 2 times/week) on cultured human skin fibroblasts, keratinocytes, and bone marrow stem cells. Table 2 summarizes the main results obtained so far. Briefly, these effects include (i) a reduction in age-related changes in cell morphology,[19,20] (ii) an increase in cellular replicative life span,[20] (iii) a reduction in the accumulation of damaged proteins,[21–23] (iv) an increase in intracellular antioxidative abilities, and (v) an increase in resistance to ethanol, hydrogen peroxide, and UV-A irradiation.[24] The main mechanisms involved in bringing about the above beneficial effects of mild HS in fibroblasts require increased levels of various HSP,[24] increased proteasomal activities,[25] and efficient stress kinase activation.[20] Similar cellular and biochemical hormetic antiaging effects of repeated exposure to mild HS were observed in normal human epidermal keratinocytes. These effects included maintenance of a relatively youthful cellular morphology, enhanced replicative life span, enhanced proteasomal activity, increased levels of HSP, increased content and Na/K-ATPase activity of the sodium pump, and improved cellular differentiation.[26,27] In the case of telomerase-immortalized human bone marrow stem cells, vitamin D-induced differentiation of bone marrow stem cells into osteoblasts could be enhanced by pre-exposure to a 1-h HS at 41° C or 42.5° C.[28]

Other hormetic effects of mild HS on human cells are improved wound healing and enhanced angiogenesis *in vitro*.[29] We are now analyzing various molecular markers of cell migration, such as paxillin, talin, and focal adhesions, to elucidate the mechanisms of mild HS-induced improvements. Although the general mechanisms of severe HS response are well understood, it is not clear whether there are any significant differences between mild HS, which has hormetic effects, and severe HS, which has deleterious effects.[30] It is likely that the physiological cost of stress in terms of energy utilization, molecular damage overload, and metabolic shift determines the difference between the

Table 2. Summary of antiaging hormetic effects on human cells in culture

Antiaging effects on fibroblasts and keratinocytes
• Extension of replicative life span[13]
• Maintenance of youthful morphology[12,13]
• Reduced extent of accumulation of damaged proteins[14–16]
• Increased ability of intracellular antioxidative defenses[17]
• Increased resistance to ethanol, hydrogen peroxide, and UV-A irradiation[17]
• Increased levels of HSP[17]
• Increased activities of proteasomes[18]
• Maintenance of stress kinase response[13]
• Increased content and activity of sodium pump[19]
Other functional improvements
• Enhanced differentiation of epidermal keratinocytes[20]
• Enhanced differentiation of bone marrow stem cells[21]
• Enhanced wound healing *in vitro*[22,25]
• Enhanced angiogenesis *in vitro* by endothelial cells[22,25]

outcomes of mild and severe stresses. Also, it is yet to be understood how the transient appearance of HSP leads to biologically amplified hormetic effects at various other levels of cellular functioning, such as improved proteasome activity, enhanced resistance to other stresses, and maintenance of cytoskeletal integrity.

Hormetins and future perspectives

Several lines of evidence support the view that hormesis can be applied successfully to aging research and intervention. Hormetic stressors have been also termed hormetins[18] and may be categorized as physical, nutritional, or mental hormetins, depending on the nature of the hormetic stress. At the mechanistic level, the induction of any set of SR pathways with mediators of hormetic effects is only a partial explanation and cannot account for the wide-ranging and long-lasting biological effects. Therefore, it is important to determine how various components of the homeodynamic machinery respond and interact during stress-induced hormesis and how relatively small individual hormetic effects lead to a significant biological amplification that results in an overall improvement of the living system.

The main promise and potential of hormesis as a modulator of aging lie in its mode of action. Since hormetic effects occur by involving a series of molecular and physiological processes, the final target of hormesis is the overall homeodynamic machinery of living systems. The process of aging is primarily characterized by a progressive shrinking of homeodynamic space in terms of increased molecular heterogeneity, which leads to increased vulnerability, onset of diseases, and eventual death.[2] Hormesis appears to be a useful practical approach to target the occurrence and accumulation of molecular damage by strengthening the homeodynamic space and by slowing its rate of shrinkage during aging.

Acknowledgment

A research grant from Eva and Henry Fraenkel Memorial Fund is gratefully acknowledged.

Conflicts of interest

The author declares no conflicts of interest.

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