Rationale and methods of discovering hormetins as drugs for healthy ageing

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Introduction: Mild stress-induced hormesis is becoming increasingly attractive as an ageing interventional strategy and is leading to the discovery of hormesis-inducing compounds called hormetins. Almost 50 years of modern biogerontolgical research has established a clear framework regarding the biological basis of ageing and longevity, and it is now generally accepted that ageing occurs in spite of the presence of complex pathways of maintenance, repair and defense, and there is no 'enemy within.' This viewpoint makes modulation of ageing different from the treatment of one or more age-related diseases. A promising strategy to slow down ageing and prevent or delay the onset of age-related diseases is that of mild stress-induced hormesis by using hormetins.

Areas covered: The article presents the rationale and a strategy for discovering novel hormetins as potential drugs for ageing intervention by elucidating multiple stress responses of normal human cells. Furthermore, it discusses the first steps in identifying prospective hormetin drugs and provides a recent example of successful product development, based on the ideas of hormesis and by following the strategy described here.

Expert opinion: As a biomedical issue, the biological process of ageing underlies several major diseases, and although the optimal treatment of every disease, irrespective of age, is a social and moral necessity, preventing the onset of age-related diseases by intervening in the basic process of ageing is the best approach for achieving healthy ageing and for extending the healthspan.

Keywords: ageing, anti-ageing, homeodynamics, homeostasis, stress

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1. Introduction

Although the chances for the emergence of several diseases increase with age, ageing in itself cannot be considered a disease. This is because, firstly, a disease, by definition, is an abnormal and exaggerated situation that does not happen to each and every individual. Second, a disease, at least in theory, is completely treatable and the patient can become disease free. Ageing does not fall in either of these two categories [1]. Therefore, modulation of ageing requires strategies different from those for the treatment of diseases. **Table 1** lists the main strategies for ageing intervention being tested or practiced presently with variable success.

The piecemeal therapies and replenishment approaches often have some short-term benefits in acute situations, but have little or no effect on the ageing process and its long-term consequences. More recently, a promising strategy to slow down ageing and prevent or delay the onset of age-related diseases is that of mild stress-induced hormesis by using hormetins [2-6]. However, in order to fully appreciate hormetin-based ageing interventions, it may be useful to first have a brief

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Article highlights.

- Ageing is a progressive shrinkage of the homeodynamic space and reduced buffering capacity of cells and organisms.
- Occurrence and accumulation of macromolecular damage due to imperfect maintenance and repair systems are the bases of ageing and age-related diseases.
- Seven molecular stress response pathways comprise an integral component of the homeodynamic space and are necessary for adaptation, remodeling and survival.
- Whereas severe and chronic stress exhausts homeodynamics, repeated exposures to mild stress of choice stimulates maintenance and defense processes, slows down ageing and extends healthy longevity.
- The phenomenon of mild stress-induced strengthening of homeodynamics with physiologically beneficial and health-promoting effects is termed as physiological hormesis, and the science and study of hormesis is termed as hormetics.
- A physiological hormetin is a condition that induces hormesis by initially causing some molecular damage that leads to the activation of single or multiple intracellular stress response pathways followed by enhanced activities of maintenance and repair processes.
- Exercise, electromagnetic field, irradiation and hypergravity are well-known examples of physical hormetins. Various components of the food, including spices, polyphenols, flavonoids and micronutrients are potential nutritional hormetins.
- A strategy is presented for testing synthetic and natural single compounds or extracts by using normal human cells in culture and determining their immediate and delayed stress responses and functional biomarkers, for screening and selecting prospective hormetins as ageing-modulatory drugs.

This box summarizes key points contained in the article.

overview of the present understanding of the biological basis of the phenomenon and process of ageing, which forms the rationale for such a strategy.

2. Homeodynamics, ageing and longevity

All living systems have the intrinsic ability to respond, to counteract and to adapt to the external and internal sources of disturbance. The traditional conceptual model to describe this property is homeostasis, which has dominated biology, physiology and medicine since 1930s. However, advances made in our understanding of the processes of biological growth, development, maturation, reproduction and, finally, of ageing, senescence and death have led to the realization that homeostasis as an explanation is incomplete. The main reason for the incompleteness of the homeostasis model is its defining principle of 'stability through constancy,' which does not take into account the themes, such as cybernetics, control theory, catastrophe theory, chaos theory, information and interaction networks, which comprise and underline the modern biology of complexity. Since 1990s, the term 'homeodynamics' is being increasingly used to account for the fact that the internal milieu of complex biological systems is not permanently fixed, is not at equilibrium and is a dynamic regulation and interaction among various levels of organization. Therefore, homeodynamics is the property of living systems that enables them to counteract stress, adapt, survive and maintain health [7-9].

Survival of an organism is a constant struggle between the occurrence of damage and the mechanisms of maintenance and repair [8,10,11]. There are three major sources of damages within a cell: i) reactive oxygen species (ROS) and free radicals (FR) formed both due to external inducers of damage (e.g., ultraviolet rays) and as a consequence of cellular metabolism involving oxygen, metals and other metabolites; ii) nutritional components such as glucose and its metabolites, and their biochemical interactions with FR; and iii) spontaneous errors in biochemical processes, such as DNA duplication, transcription, posttranscriptional processing, translation and posttranslational modifications.

Millions of damaging events occur in cells constantly, but a wide range of molecular, cellular and physiological pathways of repair counteract them and assure survival. The main biochemical and physiological pathways of maintenance, repair and defense are listed in **Box 1**. All these processes and their interactions give rise to a 'homeodynamic space' or the 'buffering capacity,' which is the ultimate determinant of an individual's chance and ability to survive and maintain a healthy state [9,12]. A normal and healthy child is born with intrinsic and genetically variable homeodynamic space, which undergoes expansion during growth, development and maturation. Hundreds of genes are involved in making the homeodynamic space of an individual, and these genes are crucial for its survival. However, this protective survival ability is not perfect and absolute due to the complex and interactive nature of biological processes. There is a potential 'vulnerability zone' around this protective homeodynamic space, the extent of which can vary among individuals depending on factors such as genetic polymorphisms, prenatal exposures, and early growth and developmental conditions. Early life events and other lifestyle factors such as nutrition, infections, mental stimulation and physical activity affect the strength and extent of the homeodynamic space [9,12].

2.1 Causes of ageing

Evolutionary processes have shaped the homeodynamic space in accordance with the need of the species for fulfilling the biological purpose of life, that is, reproduction and continuation of generations. This evolutionary lifespan is called 'essential lifespan' (ELS) [13,14], for which an efficient homeodynamic space is the basic requirement. ELS is generally much shorter than the maximum lifespan potential of a species or the average lifespan of organisms within the species. For example, ELS for rats and mice in nature is less than 1 year, but in the highly protected laboratory conditions,

Box 1. Main biochemical and physiological pathways and processes of maintenance and repair.

- The processes for sensing and responding to intra- and extracellular stressors, such as heat shock response, stress hormones and ionic fluxes
- The multiple pathways of nuclear and mitochondrial DNA repair
- The pathways for protein repair, such as the renaturation of proteins by chaperones, and the enzymatic reversal of the oxidation of amino acids
- The pathways for the removal and turnover of defective proteins by proteasomes and lysosomes
- The antioxidative and enzymatic defenses against reactive oxygen species
- The processes for the detoxification of harmful chemicals in the diet
- The cellular and humoral immune responses against pathogens and parasites, including massive apoptosis (programmed cell death) after the completion of the cellular immune response
- The processes of wound healing, blood clotting and tissue/organ regeneration including angiogenesis

Table 1. Current ageing intervention strategies.

Interventional strategy	Examples of intervention
Piecemeal therapies – fixing one problem at a time	Organ and tissue transplants, and stem cell therapy
Replenishment and corrective measures	Hormones, cells, natural and synthetic compounds and food supplements, including vitamins, antioxidants, biotics, enzymes and specific gene therapies
Strengthening the homeodynamics	Hormesis

these animals can live for 2, 3 or more years. Similarly, ELS for humans is about 40 years, but in modern societies with protected environments and good nutritional and healthcare access, human populations can expect to live more than double ELS [15,16].

What is most important to understand and realize is that after the period of ELS, evolution has not created any specific gerontogenes to cause ageing and death of the organism [7,14,15,17]. Instead, ageing is allowed to happen due to the inefficiency and failure of the homeodynamic processes of maintenance and repair. One way of conceptualizing ageing is the progressive shrinkage of the homeodynamic space that happens during the period of survival beyond ELS [9]. During ageing, the protective homeodynamic space, slowly and steadily, becomes lesser and lesser with corresponding increase in the zone of vulnerability. This leads to the increased chances of the emergence of one or more diseases during ageing and of an eventual death. All major age-related diseases are, therefore, due to the reduced and weakened homeodynamic space. A visual representation of the concept of homeodynamic space and its shrinkage during ageing is given in **Figure 1**.

The molecular basis for the shrinkage of the homeodynamic space is the accumulation of damage in all components of the biochemical machinery [8]. Many kinds of damages have been shown to occur in all macromolecules in the cells and in the extracellular matrix. **Table 2** gives an example of molecular damages known to occur and accumulate during ageing. The biological consequences of increased levels of molecular damage can be wide ranging, including altered gene expression, genomic instability, mutations, loss of cell division potential, cell death, impaired intercellular communication, tissue disorganization, organ dysfunctions and increased vulnerability to stress and other sources of disturbance. At present, it is a challenge for biogerontologists to determine the relevance and consequences of molecular damage in functional terms [8]. For example, at what level a particular damage becomes intolerable and causes functional impairments, and how much damage must be removed in order to maintain or regain function and health. Such studies are essential with respect to establishing biomarkers of health, frailty and longevity, and for developing novel methods for the prevention and reversion of age-related changes.

3. Hormetics, hormesis and hormetins

A promising strategy to slow down ageing and prevent or delay the onset of age-related diseases is that of mild stress-induced hormesis. The consequences of stress can be both harmful and beneficial depending on the intensity, duration and frequency of the stress, and on the price paid in terms of energy utilization and other metabolic disturbances. However, the most important aspect of biological stress response (SR) is that it is not monotonic with respect to the dose of the stressor. SR 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 is neither threshold nor linear, but is U- or inverted U-shaped, depending on the endpoint being measured. This phenomenon of biphasic dose–response was termed as hormesis, and the study and science of hormesis is here proposed to be termed as *hormetics*. The terminology for hormesis has been further refined to specify the nature of the hormetic responses, such as physiological hormesis, preconditioning hormesis and postexposure conditioning hormesis [18].

The key conceptual features of hormesis are the disruption of homeodynamics, the modest overcompensation and the reestablishment of homeodynamics. Hormesis in ageing is characterized by the life-supporting beneficial effects resulting from the cellular responses to single or multiple rounds of mild

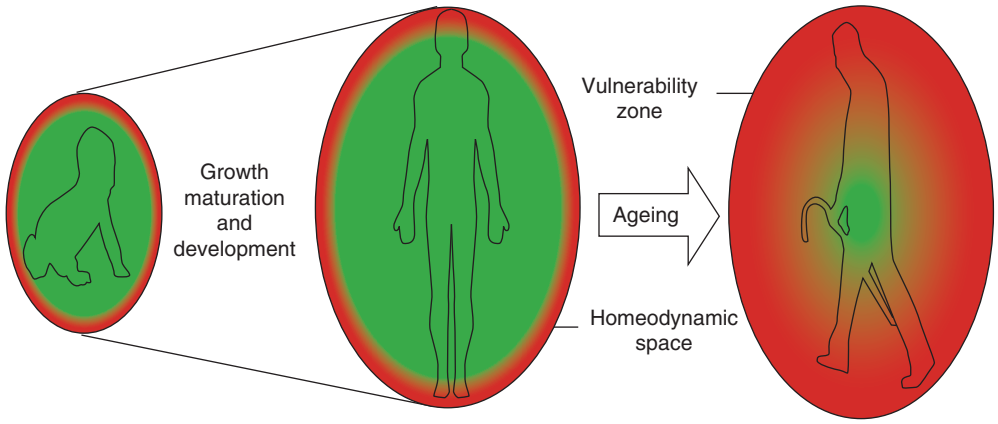

Figure 1. Homeodynamic space is the ultimate determinant of an individual's chance and ability to survive and maintain a healthy state. A normal and healthy child is born with intrinsic and genetically variable homeodynamic space, which undergoes expansion during growth, development and maturation. Ageing is considered as a progressive shrinkage of the homeodynamic space, which leads to increased zone of vulnerability, reduced adaptive ability and increased probability of the emergence of several diseases.

Table 2. Main categories of molecular damages occurring during biological ageing.

Macromolecule	Examples of damage
DNA	Mutations, epimutations, base modifications, deletions and strand breaks
RNA	Base modifications, miscoding and missplicing
Protein	Amino acid modifications, misincorporation, misfolding and aggregation
Carbohydrates, lipids and molecular conjugates	Advanced glycation end-products, lipofuscin and aggrosomes

stress [6]. This is figuratively represented in **Figure 2** that the homeodynamic ability of a biological system is strengthened in a hormetic zone (H) during mild stress, whereas chronic and severe stress results in the progressive weakening of homeodynamics and an increased zone of disruption (D) leading to functional impairments, diseases and eventual death [19]. It is important to note that although the hormetic zone is usually small, with respect to both the dose and the effect, its biological consequences are cumulative, amplified and physiologically significant.

3.1 Hormetins

All such conditions that bring about biologically beneficial effects by initially causing low-level molecular damage, which then leads to the activation of one or more SR pathways and thereby strengthens the homeodynamics, are termed as hormetins [20-22]. These may be further categorized as i) physical hormetins, such as exercise, thermal shock and irradiation; ii) psychological hormetins, such as mental challenge and focused attention or meditation; and iii) biological and nutritional hormetins, such as infections, micronutrients, spices and other sources.

An example of stress-induced hormesis is the well-documented beneficial effects of moderate exercise as a hormetin, which initially increases the production of FR, acids and aldehydes [23-25]. Another frequent observation in hormetics is that a single hormetic agent, such as heat shock (HS) or exercise, can strengthen the overall homeodynamics of cells and enhance other abilities, such as tolerance to other stresses, by initiating a cascade of processes resulting in a biological amplification and eventual beneficial effects [26,27].

Various mild stresses that have been reported to delay ageing and prolong longevity in cells and animals include temperature shock, irradiation, heavy metals, pro-oxidants, acetaldehyde, alcohols, hypergravity, exercise and food restriction. Ageing modulatory and other effects of hormesis have been reported for human cells also. For example, using a regimen of repeated mild HS given to cultured normal human skin fibroblasts, keratinocytes, endothelial cells and telomerase-immortalized bone marrow mesenchymal stem cells, a variety of hormetic effects have been reported. These effects include slowing down of cellular ageing, extension of cellular replicative lifespan, maintenance of youthful morphology, reduction in molecular damage and improvement in differentiation, wound healing and angiogenesis [27]. Other hormetic conditions, which have been shown to have anti-ageing effects in human cells, are irradiation, mechanical stretching and electromagnetic fields shock [21].

Nutritional hormetins, especially those derived from plant sources, have generated much scientific interest for their health beneficial effects. This is because of the realization that not all chemicals found in plants are beneficial for animals in a simple and straight-forward manner. Instead,

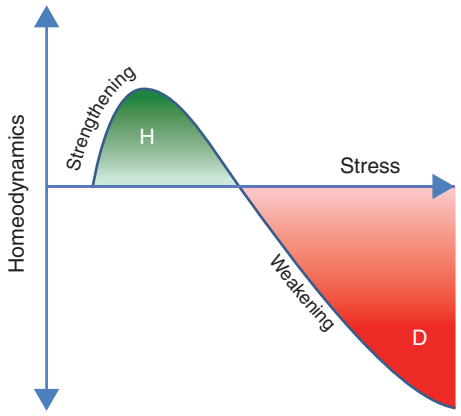

Figure 2. Hormesis: homeodynamic ability of a biological system is strengthened in a hormetic zone (H) at low levels of stress by stimulating maintenance and repair processes, whereas chronic and severe stress results in the progressive weakening of homeodynamics and an increased zone of disruption (D), by exhausting the energy sources. Hormetics is the science and study of hormesis.
Reproduced from [19] with permission from Elsevier.

they often cause molecular damage by virtue of their electrochemical properties and have a typical biphasic hormetic dose–response. Some examples of nutritional hormetins involving heat shock response (HSR) are phenolic acids, polyphenols, flavonoids, ferulic acid geranylgeranyl, rosmarinic acid, kinetin, zinc and the extracts of tea, dark chocolate, saffron and spinach [9,28].

3.2 Screening for novel hormetins

Discovering novel hormetins by putting potential candidates through a screening process for their ability to induce one or more SR pathways in cells and organisms can be a successful strategy. **Figure 3** provides a general scheme and strategy for screening natural and synthetic single compounds or complex extracts for their use as hormetins for human beings. It is suggested that initial screening and testing should be done using normal diploid human cells in culture. The use of normal diploid cells is very important for such studies, since immortal cell lines usually have one or more genetic and metabolic deviations, which are rarely comparable with normal cells. However, the cell type to be used for such a screening will depend on the biological endpoint that one expects to improve by hormetin treatment. For example, screening for hormetins for improving wound healing may be best done by using normal fibroblasts, whereas for improving angiogenesis it will require endothelial cells and for improving bone formation it will require osteoblasts and so on.

Determining a dose- and time-dependent SR profile for the seven main SR pathways [19,21,22] is the first step in discovering novel hormetins. Since most of the early SR markers are transcription factors, which undergo posttranslational modifications and translocation from the cytoplasm to the nucleus, immunofluorescence microscopy and immunodetection of proteins by a combination of ELISA, Western blotting and flow-cytometry may be sufficient at this stage. However, for identifying the late SR effectors, such as induced synthesis of heat shock proteins (HSP), chaperones, cytokines, sirtuins and other antioxidative enzymes, both the gene array analysis and proteomic analysis will be required.

The initial screening of test materials by determining their effects on early and late SR markers has to be followed by performing cell type-specific functional assays. Cellular motility and wound healing assay for fibroblasts; induction of differentiation in stem cells and keratinocytes; blood vessel formation by endothelial cells; osteocalcin and mineralized matrix formation by osteoblasts; and muscle fiber formation by muscle cells are some examples of these functional assays, which can be performed easily. Only after this step, one could take the tested material for its further testing as a prospective hormetin, by appropriate testing at tissue, organ and organismal level, using different model systems, before the ultimate test and use for human beings.

A crucial requirement for determining the physiological usefulness of a potential hormetin is the extent of induction of SR. An induction of maximum SR, at the cost of inhibition of other physiological processes, does not indicate its potential hormetic nature. For example, whereas a maximum HSR can be achieved in cultured human cells by exposing them to severe 42.5°C or above, this condition also inhibits almost all other normal metabolic processes, including the transcription and translation of genes except for HSR genes [29]. Although a single severe stress exposure may be beneficial for the surviving cells with respect to a similar or severe stress in the future, this treatment is physiologically and energetically costly and does not strengthen the homeodynamics over a longer period. In order for a condition to be a hormetin, it is essential that the SR induction is within the hormetic zone (**Figure 1**) with an SR stimulatory range of 15 – 30% [18]. For example, in studies with the hormetic effects of mild HS on human skin fibroblasts, it was reported that an HSR of up to 30% of the maximal response, in terms of the synthesis of HSP, was beneficial and that the human cells could be exposed to this mild stress repeatedly for more than 100 times during their lifespan in culture [6,27]. Another important aspect of hormetic treatment is the interval between the exposures. The physiologically beneficial effects of hormesis are actually achieved not during the period of exposure to mild stress, but during the so-called recovery period, as is well known for exercise. Studies on the beneficial effects of mild HS on human cells have shown that it is important to allow the cells to recover so that the level of HSP reaches to within 5% of the basal level and that the cells do not stay in a high SR status before being exposed to another round of mild stress [6,27]. Therefore, it will be crucial to establish the dose, duration of exposure and the duration of recovery for a potential hormetin.

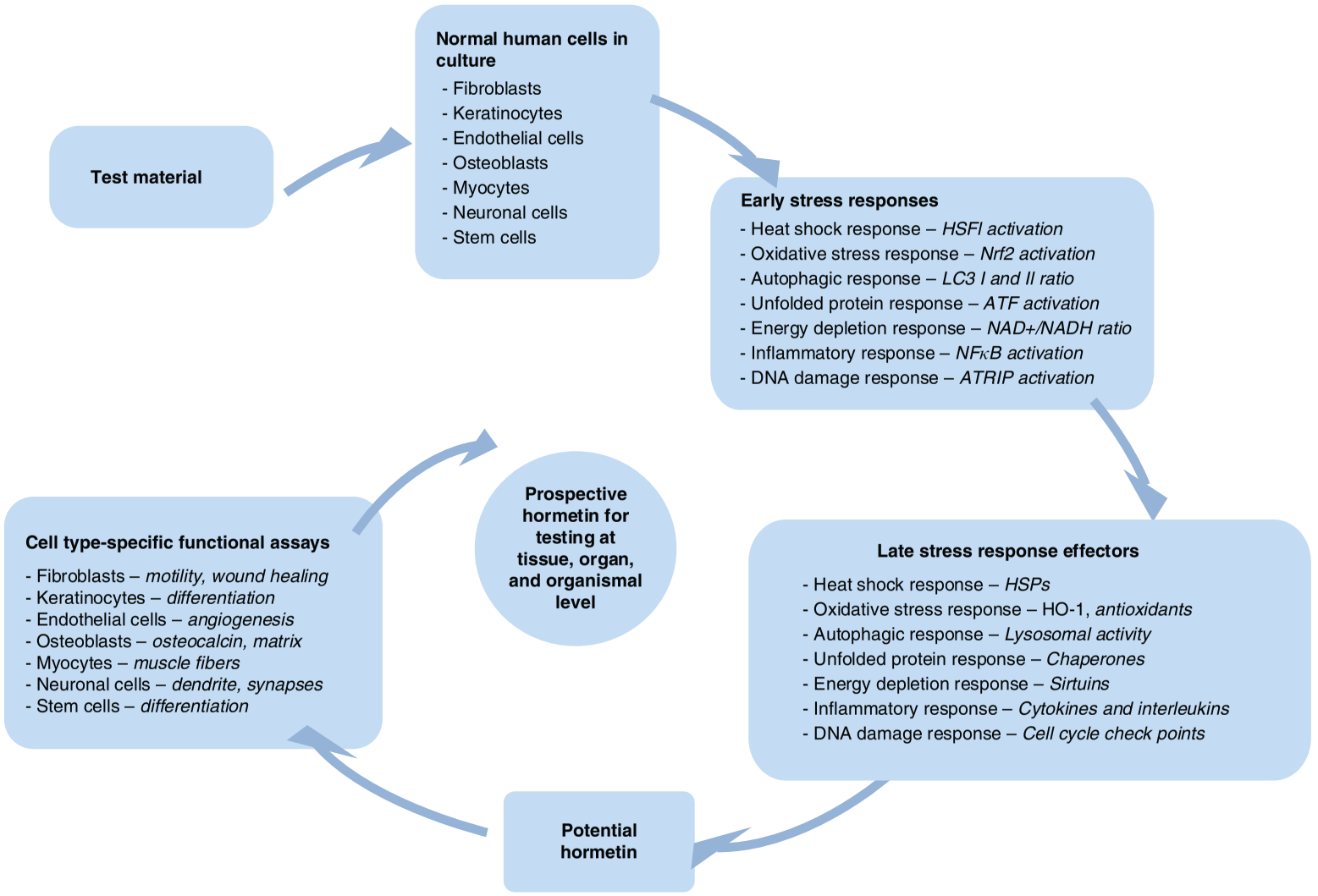

Figure 3. A general scheme and strategy for screening natural and synthetic single compounds or complex extracts using normal human cells and their stress responses, for their eventual use as hormetin drugs for healthy ageing in human beings.

A recent example of a successful product development based in the ideas of hormesis and following the strategy outlined above (**Figure 3**) is a skin care cosmetic [28]. This was achieved by analyzing the stress-inducing effects of active ingredients extracted from the roots of the Chinese herb Sanchi (*Panax notoginseng*) on gene expression at the level of mRNAs and proteins in human skin cells. The results showed that the ginsenosides extracted from Sanchi induced the transcription of stress genes and increased the synthesis of stress proteins, especially the heat shock protein HSP1A1 in normal human keratinocytes and dermal fibroblasts [28]. Thus, it may be possible to screen natural and synthetic compounds and mixtures for their single or multiple SR-inducing effects in human cells in culture, followed by the demonstration of biologically relevant beneficial effects, and then testing them as prospective hormetin drugs that strengthen the homeodynamic space, leading to the slowing down of ageing and prevention and delay of age-related diseases.

3.3 Molecular basis of hormetin action

Although the exact nature of the initial molecular damage caused by a compound may not be easily identified, activation of one or more SR pathways is a good indicator of the primary action of a potential hormetin. For example, the antioxidant response by the activation of Nrf2 transcription factor follows the electrophilic modification/damage of its inhibitor protein Keap1, which then leads to the accumulation, heterodimerization, nuclear translocation and DNA binding of Nrf2 at the antioxidant response element, resulting in the downstream expression of a large number of the so-called antioxidant genes, such as heme oxygenase HO-1, superoxide dismutase, glutathione and catalase [30-34]. Some well-known phytochemicals, which strongly induce Nrf2-mediated SR, include curcumin, quercetin, genistein and eugenol [30,35]. A similar induction of SR involving Nrf2 has also been reported for various food extracts, such as coffee, turmeric, rosemary, broccoli, thyme, clove and oregano [30]. Antioxidant activity of fresh and processed Jalapeno and Serrano peppers may also be due to their hormetic effects [36]. The ketocarotenoid astaxanthin found in the microalgae is another possible hormetin [37].

Another SR pathway, which has been studied in detail and can be the basis for identifying novel hormetins, is HSR. Induction of proteotoxic stress, such as protein misfolding and denaturation, initiates HSR by the intracellular release of the heat shock transcription factor from their captor proteins, followed by its nuclear translocation, trimerization and DNA binding for the expression of several HSP [29,38]. A wide range of biological effects then occur, which involve HSP and include protein repair, refolding and selective degradation of abnormal proteins leading to the cleaning up and an overall improvement in the structure and function of the cells. Various phytochemicals and nutritional components have been shown to induce HSR and have health beneficial effects including anti-ageing and longevity-promoting effects. Some examples of hormetins involving HSR are phenolic acids, polyphenols, flavonoids, ferulic acid [39,40], geranylgeranyl, rosmarinic acid, kinetin, zinc [40-42] and the extracts of tea, dark chocolate, saffron, spinach and a Chinese herb Sanchi [28,43]. Resveratrol and other mimetics of calorie restriction also work by the induction of one or more of these SR pathways [42,44,45].

Other pathways of SR, which are involved in initiating hormetic effects of potential hormetins are the NFkB, FOXO, sirtuins, DNA repair response and autophagy pathways. Of these, the role of FOXO1 is being investigated in much more detail. This is because FOXO1, one of the four isoforms of forkhead transcription factors, is highly expressed in insulin-responsive tissues, influences the transcriptional cascades regulating glucose metabolism and is a major target of insulin, which inhibits its transcriptional activity [46]. For example, in skeletal muscle, FOXO1 maintains energy balance during fasting and provides energy supply through breakdown of carbohydrates [46].

Not all pathways of the SR respond to every stressor, and although there may be some overlap, generally SR pathways are quite specific [19,21,22]. 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 HSP followed by the activation of proteasome-mediated protein degradation [29,38]. But unfolded proteins in the endoplasmic reticulum will induce unfolded protein response and will initiate the induction of synthesis of a totally different set of proteins and their downstream effectors [46-48].

Similarly, whereas oxidative damage to proteins will generally initiate Nrf2-mediated antioxidant response, damage to DNA by FR or other agents will result in the activation of DNA repair enzymes. In the same vein, whereas nutritional deprivation and low energy levels will activate autophagy and FOXO–sirtuin pathways, infections and antigenic challenge will generally initiate pro-inflammatory NFkB response. However, often the source of activation (stressor) cannot be easily identified and may involve more than one stressor and their effectors. Examples of such SR include early inflammatory SR and neuro-endocrinal SR, which lead to the synthesis and release of interleukins and corticoid hormones, respectively. Similarly, pathways involving NFkB, Nrf2, FOXO, sirtuins and HO activation may involve more than one type of stressors and stress signals, including pro-oxidants, FR, ROS and nutritional components.

This novel category of hormetin 'drugs' are not *against* any particular disease, but are *for* maintaining health and for extending healthspan, by maintaining and strengthening homeodynamics and which can prevent or delay the onset of age-related diseases.

4. Expert opinion

If ageing is understood as an emergent phenotype due to the failure of homeodynamics and not due to the action of any harmful and death-causing mechanisms, it transforms our approach towards ageing interventions from 'anti-ageing' to 'healthy ageing.' Ageing occurs in spite of the presence of complex pathways of maintenance, repair and defense, and there is no 'enemy within.' This viewpoint makes modulation of ageing different from the treatment of one or more age-related diseases. Interventions in ageing require a strategy for maintaining health, preserving the homeodynamics and strengthening the homeodynamic space. A promising strategy to slow down ageing, prevent or delay the onset of age-related diseases and extend healthspan is that of mild stress-induced hormesis by hormetins. Physical, nutritional and mental hormetins, which initiate SRs and strengthen the homeodynamics, are potentially effective agents for maintaining health, achieving healthy ageing and for extending the healthspan.

A very important point about hormetins is that observing a particular biological end-result in response to a treatment with a test compound may not imply a straightforward chemical nature and mode of action of that compound. For example, the biological end-result of being antioxidative is often interpreted as an evidence for the compound itself being a chemical antioxidant in terms of being a direct scavenger of ROS, which can be incorrect and misleading. Various polyphenols, flavonoids and spices are examples of such compounds, which have eventual antioxidative effects but without being direct chemical antioxidants [35,49,50]. The concept of physiological hormesis, however, implies that a biologically beneficial antioxidative end-result can be achieved by hormetins, which actually cause some oxidative damage. Since identical biological end-results could be achieved by activating mechanistically very different pathways, it is very important to know the earliest step(s) in the mode of action of a compound for establishing its uniqueness and specificity. Such knowledge can also be useful in claiming the intellectual property rights and to facilitate further modifications and applications of the compound.

Applying the concept of hormesis in testing the effects of natural and synthetic compounds and extracts, by analyzing various SR pathways, can help to screen and select potentially useful compounds with specific targets. Some suggestions for discovering novel hormetins by activating different SR pathways are autophagic response for food-restriction mimetics, DNA repair response for UV protectors, sirtuin and FOXO response for mitochondrial energy production enhancers, Nrf2 response for antioxidants and NFkB response for anti-inflammatory compounds. Finally, a change in the social perception of stress through scientific understanding of the biphasic and hormetic nature of stress will open up possibilities for developing many other lines of healthcare products and technologies using physical, mental and nutritional hormetins.

4.1 Concluding remarks

Novel hormetins as potential drugs for strengthening the body's own mechanisms of maintenance and repair, for slowing down ageing and for extending healthspan can be discovered by applying the principles of hormesis and by utilizing the SR pathways of normal human cells in culture.

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Declaration of interest

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Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Rattan SIS. Anti-ageing strategies: prevention or therapy? EMBO Reports 2005;6:S25-S9
• **A useful opinion article about various approaches for anti-ageing interventions.**
2. Rattan SIS. Ageing, gerontogenes, and hormesis. Indian J Exp Biol 2000;38:1-5
3. Rattan SIS. Hormesis in biogerontology. Crit Rev Toxicol 2001;31:663-4
4. Rattan SIS. Applying hormesis in aging research and therapy. Hum Exp Toxicol 2001;20:281-5
5. Rattan SIS. Mechanisms of hormesis through mild heat stress on human cells. Ann NY Acad Sci 2004;1019:554-8
6. Rattan SIS. Hormesis in aging. Ageing Res Rev 2008;7:63-78
•• **A comprehensive review on the application of hormesis in ageing research and interventions.**
7. Rattan SIS. Theories of biological aging: genes, proteins and free radicals. Free Radic Res 2006;40:1230-8
•• **A very important article on the status of theories of biological ageing.**
8. Rattan SIS. Increased molecular damage and heterogeneity as the basis of aging. Biol Chem 2008;389:267-72
9. Rattan SIS. Biogerontology: from here to where? The lord cohen medal lecture-2011. Biogerontology 2012;13:83-91
•• **A useful review on understanding the present state of knowledge in biogerontology.**
10. Holliday R. Aging is no longer an unsolved problem in biology. Ann NY Acad Sci 2006;1067:1-9
11. Holliday R, Rattan SI. Longevity mutants do not establish any "new science" of ageing. Biogerontology 2010;11:507-11
12. Rattan SIS. Homeostasis, homeodynamics, and aging. In: Birren J, editor. Encyclopedia of Gerontology. 2nd edition. Elsevier, Inc.; UK: 2007. p. 696-9
• **A useful article to understand the differences between homeostasis and homeodynamics.**
13. Rattan SIS. Biogerontology: the next step. Ann NY Acad Sci 2000;908:282-90
14. Carnes BA. What is lifespan regulation and why does it exist? Biogerontology 2011;12:367-74
•• **A very useful article for understanding the evolutionary reasons for ageing and essential lifespan.**
15. Carnes BA, Olshansky SJ, Grahn D. Biological evidence for limits to the duration of life. Biogerontology 2003;4:31-45
16. Olshansky SJ, Biggs S, Achenbaum WA, et al. The global agenda council on the ageing society: policy principles. Global Policy 2011;2:97-105
17. Rattan SIS. Gerontogenes: real or virtual? FASEB J 1995;9:284-6
18. Calabrese EJ, Bachmann KA, Bailer AJ, et al. Biological stress response terminology: integrating the concepts of adaptive response and preconditioning stress within a hormetic dose-response framework. Toxicol Appl Pharmacol 2007;222:122-8
•• **An extremely important article for hormesis terminology and concepts.**
19. Demirovic D, Rattan SIS. Establishing cellular stress response profiles as biomarkers of homeodynamics, health and hormesis. Exp Gerontol 2012; doi:10.1016/j.exger.2012.02.005
20. Rattan SIS, Demirovic D. Hormesis and aging. In: Mattson MP, Calabrese E, editors. Hormesis: a Revolution in Biology, Toxicology and Medicine. Springer; New York: 2009. p. 153-75
21. Rattan SIS, Demirovic D. Hormesis can and does work in humans. Dose Response 2010;8:58-63
• **An article reviewing the successful application of hormesis in humans.**
22. Rattan SIS, Demirovic D. Hormesis as a mechanism for the anti-aging effects of calorie restriction. In: Everitte AV, Rattan SIS, Le Couteur DG, de Cabo R, editors. Calorie Restriction, Aging and Longevity. Springer; Dordrecht: 2010. p. 233-45
23. Sen CK, Packer L, Hanninen O. editors. Handbook of Oxidants and Antioxidants in Exercise. 1st edition. Elsevier; Amsterdam, The Netherlands: 2000
24. Radak Z, Chung HY, Goto S. Exercise and hormesis: oxidative stress-related adaptation for successful aging. Biogerontology 2005;6:71-5
• **A review article providing the hormesis-based explanations for the mode of action of exercise.**
25. Williamson J, Pahor M. Evidence regarding the benefits of physical exercise. Arch Intern Med 2010;170:124-5
26. Mattson MP, Calabrese E. editors. Hormesis - a Revolution in Biology, Toxicology and Medicine. Springer; New York: 2010
•• **An important book highlighting the importance and application of hormesis in biology.**
27. Rattan SIS, Fernandes RA, Demirovic D, et al. Heat stress and hormetin-induced hormesis in human cells: effects on aging, wound healing, angiogenesis and differentiation. Dose Response 2009;7:93-103
28. Rattan SIS, Kryzch V, Schnebert S, et al. Hormesis-based anti-aging products: a case study of a novel cosmetic. Dose Response 2012; [Epub ahead of print]
29. Verbeke P, Fonager J, Clark BFC, Rattan SIS. Heat shock response and ageing: mechanisms and applications. Cell Biol Int 2001;25:845-57
30. Balstad TR, Carlsen H, Myhrstad MC, et al. Coffee, broccoli and spices are strong inducers of electrophile response element-dependent transcription in vitro and in vivo - Studies in electrophile response element transgenic mice. Mol Nutr Food Res 2011;55:185-97
31. Calabrese EJ, Mattson MP, Calabrese V. Dose response biology: the case of resveratrol. Hum Exp Toxicol 2010;29:1034-7
32. Calabrese V, Cornelius C, Dinkova-Kostova AT, et al. Cellular stress responses, the hormesis paradigm, and vitagenes: novel targets for therapeutic intervention in neurodegenerative disorders. Antioxid Redox Signal 2010;13:1763-811
•• **A very useful review on the mechanistic aspects of hormesis and its applications.**
33. Calabrese V, Cornelius C, Mancuso C, et al. Cellular stress response: a novel target for chemoprevention and nutritional neuroprotection in aging,

neurodegenerative disorders and longevity. Neurochem Res 2008;33:2444-71

34. Demirovic D, Rattan SIS. Curcumin induces stress response and hormetically modulates wound healing ability of human skin fibroblasts undergoing ageing in vitro. Biogerontology 2011;12:437-44

35. Lima CF, Pereira-Wilson C, Rattan SI. Curcumin induces heme oxygenase-1 in normal human skin fibroblasts through redox signaling: relevance for anti-aging intervention. Mol Nutr Food Res 2011;55:430-42

36. Alvarez-Parrilla E, de la Rosa LA, Amarowicz R, Shahidi F. Antioxidant activity of fresh and processed Jalapeno and Serrano peppers. J Agric Food Chem 2011;59:163-73

37. Yuan JP, Peng J, Yin K, Wang JH. Potential health-promoting effects of astaxanthin: a high-value carotenoid mostly from microalgae. Mol Nutr Food Res 2011;55:150-65

38. Liberek K, Lewandowska A, Zietkiewicz S. Chaperones in control of protein disaggregation. EMBO J 2008;27:328-35

39. Barone E, Calabrese V, Mancuso C. Ferulic acid and its therapeutic potential as a hormetin for age-related diseases. Biogerontology 2009;10:97-108

40. Son TG, Camandola S, Mattson MP. Hormetic dietary phytochemicals. Neuromolecular Med 2008;10:236-46

• **A useful review on the hormetic nature of food components.**

41. Berge U, Kristensen P, Rattan SIS. Hormetic modulation of differentiation of normal human epidermal keratinocytes undergoing replicative senescence in vitro. Exp Gerontol 2008;43:658-62

42. Sonneborn JS. Mimetics of hormetic agents: stress-resistance triggers. Dose Response 2010;8:97-121

43. Wieten L, van der Zee R, Goedemans R, et al. Hsp70 expression and induction as a readout for detection of immune modulatory components in food. Cell Stress Chaperones 2010;15:25-37

44. Longo VD. Linking sirtuins, IGF-I signaling, and starvation. Exp Gerontol 2009;44:70-4

45. Giovannelli L, Pitozzi V, Jacomelli M, et al. Protective effects of resveratrol against senescence-associated changes in cultured human fibroblasts. J Gerontol A Biol Sci Med Sci 2011;66:9-18

46. Kousteni S. FoxO1: a molecule for all seasons. J Bone Miner Res 2011;26:912-17

• **A very useful review on the multiple roles of the transcription factor FOXO.**

47. Yoshida H. ER stress and diseases. FEBS J 2007;274:630-58

48. Basaiawmoit RV, Rattan SIS. Cellular stress and protein misfolding during aging. Methods Mol Biol 2010;648:107-17

49. Pun PB, Gruber J, Tang SY, et al. Ageing in nematodes: do antioxidants extend lifespan in Caenorhabditis elegans? Biogerontology 2010;11:17-30

•• **An important article about direct and indirect antioxidants, and the role of so-called antioxidants in ageing interventions.**

50. Birringer M. Hormetics: dietary triggers of an adaptive stress response. Pharm Res 2011;28:2680-94

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