EMBO reports

science & society

Anti-ageing strategies: prevention or therapy?

Slowing ageing from within

Suresh I. S. Rattan

Biogerontology—the study of the biological basis of ageing—is now a mature research field that could eventually offer effective applications to ease the burden of old age. Unlike other research fields that focus predominantly on producing information, a central characteristic of biogerontology is that it searches for means of preventing the onset of age-related diseases and improving quality of life in old age. Biogerontologists have already produced a large body of descriptive and mechanistic data that allows them to define some general principles of ageing. Although their accumulated research has not yet uncovered any universal markers of ageing, the principles that have been derived could lead to the development of practical and effective anti-ageing strategies based on rational approaches.

First, the 'life-history principle' describes ageing as an emergent phenomenon that takes place primarily in protected environments, and which allows survival beyond the natural lifespan that would occur in the wild. By contrast, the natural lifespan of a species, also termed the 'essential lifespan' (ELS; Rattan, 2000) or 'warranty period' (Carnes *et al*, 2003), is the time required to fulfil the Darwinian purpose of life; that is, successful reproduction. Species that undergo fast maturation and have an early onset of reproduction with high reproductive potential generally have a short ELS, whereas slow maturation with late onset and low potential is concurrent with a long ELS. For example, the ELS for *Drosophila* is less than a week but that for *Homo sapiens* is about 50 years, even though both species can and do live for much longer periods in protected laboratory environments and modern societies, respectively. Consequently, the period of extended survival beyond the ELS is defined as the period of ageing.

Second, the 'non-genetic principle' asserts that there is neither a genetic cause for, nor a programme that controls, the ageing process. Its proponents argue that, unlike development, which is a tightly regulated genetic and epigenetic process, ageing, and therefore the exact lifespan of an organism, is not determined by any specific genes, termed gerontogenes (Rattan, 1995; Partridge, 2001; Kirkwood, 2002). Those genes that do influence ageing and longevity have evolved in accordance with the evolutionary history of a species to assure its ELS, but they do not necessarily control the further progression of the organism beyond reproduction. These arguments follow evolutionary theory in asserting that evolution selects genes that allow us to reach the ELS so we can reproduce, but everything after that age falls into the evolutionary shadow as it does not determine the survival of the species as such. Instead, ample evidence now supports the theory that survival and longevity of a species are a function of the ability of its maintenance and repair mechanisms to keep up with daily wear and tear. There are positive correlations between lifespan and the ability to repair DNA, detoxify reactive oxygen molecules, respond to and counteract stress, and replace worn-out cells. In addition, there is a negative correlation between longevity and the rate of damage accumulation, including mutations, epimutations, macromolecular oxidation and aggregation of metabolic byproducts (Holliday, 2000).

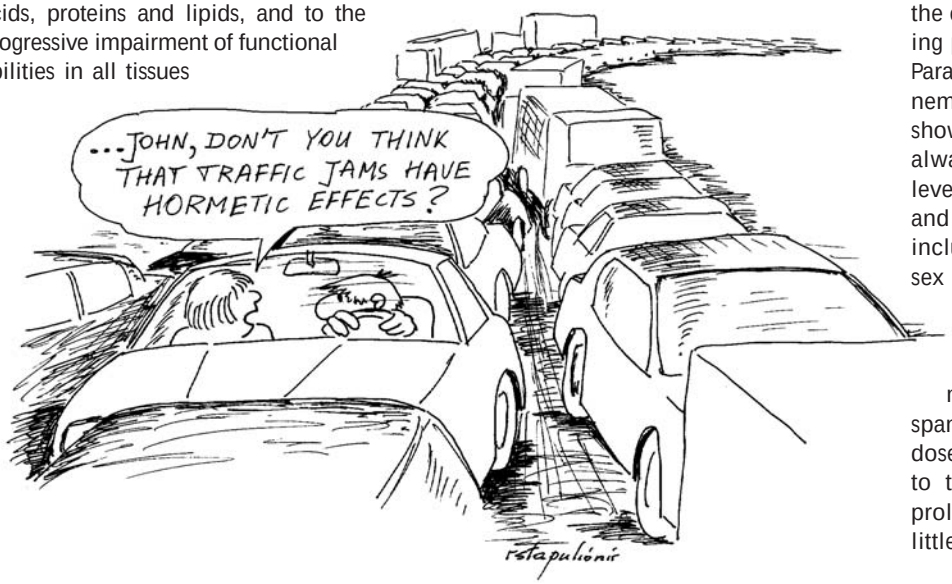
> … the "life-history principle" describes ageing as an emergent phenomenon that takes place primarily in protected environments …

> … our bodies have evolved over millions of years and have been optimized through evolution to ensure proliferation and survival of the species, rather than a long lifespan for the individual

However, a lack of specific gerontogenes does not imply that genes have no influence on survival, longevity and ageing. There is ample evidence from studies performed on yeast, fungi, nematodes, insects, rodents and humans that mutations in certain genes can either prolong or shorten lifespan and cause premature ageing syndromes. These genes are involved in a wide range of biochemical pathways and agents, such as insulin metabolism, kinases and kinase receptors, transcription factors, DNA helicases, telomerase, membrane glucosidases, GTP-binding protein-coupled receptors, cholesterol metabolism, heat-shock protein genes, cell-cycle arrest pathways and others (Rattan, 2003). Nevertheless, regardless of their normal function and mechanism of action, these genes did not evolve specifically to accumulate damage, cause age-related changes and eventually kill the organism. Since their influence on ageing and longevity is only indirect, they have been termed 'virtual gerontogenes' (Rattan, 1995).

Third, the 'mechanistic principle' explains ageing as a progressive failure of homeostasis or homeodynamics—the ability of our bodies to maintain a steady balanced state. This malfunction eventually leads to the accumulation of damage to nucleic acids, proteins and lipids, and to the progressive impairment of functional abilities in all tissues and cells. Since homeostasis or homeodynamics primarily depend on an organism's maintenance and repair processes, it is the progressive failure of these mechanisms that causes ageing and age-related diseases (Holliday, 1995). The first two principles of ageing and longevity explain why these processes have not evolved to function accurately and efficiently for indefinite periods. Together, the three principles state that our bodies have evolved over millions of years and have been optimized through evolution to ensure proliferation and survival of the species, rather than a long lifespan for the individual. The occurrence of ageing beyond the ELS, the onset of age-related diseases and eventual death thus appear to be the 'normal' sequence of events after we have reproduced and therefore fulfilled our evolutionary purpose.

These principles and the underlying knowledge will soon allow researchers to develop reasonable therapies that intervene in the ageing process and treat various age-related diseases and frailties. It is an ironic twist, therefore, that while research in biogerontology has made huge advances over the past decade, it still has a bad reputation. In fact, the history of anti-ageing research is replete with fraud, pseudoscience, quackery and charlatanism, which together have given it a bad name. Even today, in our supposedly enlightened age, claims of miraculous remedies and therapies to overcome ageing run rampant, despite many of these products having no effects whatsoever. Most of these anti-ageing therapies promise a total elimination of ageing, disease and death, or aim to slow down the ageing process, thus delaying the onset of diseases and extending our healthy lifespan. Few of them actually work.



Another, more rational, approach involves targeting specific age-related diseases. Although this is usually effective in curing or halting a specific disease, it does not address ageing itself. Cancer therapy, for instance, will ideally eliminate cancerous cells and restore the affected organ or tissue to its original disease-free state. Nevertheless, although it reduces the risk of dying from cancer, it does not address other age-related diseases and disorders such as Parkinson's or Alzheimer's diseases, dementia, progressive organ failure or cardiovascular diseases. It also means that a 'cure for ageing' will most likely not succeed. How would such a 'treatment' look and to what original 'age-free' stage should one be restored—to day 1, year 1, 10, 30 or 50? Defining ageing as a disease and then trying to cure it is unscientific and misguided.

Instead, rational anti-ageing strategies based on scientific evidence aim to slow down the ageing process by preventing and/or delaying physiological decline and regaining lost functional abilities. Some approaches include supplementation with hormones, including the growth hormone dehydroepiandrosterone (DHEA), melatonin and oestrogen, and nutritional supplements that contain synthetic and natural antioxidants in purified form or in plant extracts. Although some of these therapies have demonstrated various clinical benefits in the treatment of the elderly, none really modulate the ageing process itself (Olshansky *et al*, 2002). Paradoxically, studies performed on nematodes, insects and rodents have shown that lifespan extension is almost always associated with a reduction in the levels and activities of several hormones and hormone-signalling pathways, including insulin, growth hormone and sex steroids (Gems & Partridge, 2001; Bartke *et al*, 2003; Tatar *et al*, 2003). Increasing the levels of various hormones through supplementation may therefore actually shorten lifespan. Similarly, therapies that use high doses of vitamins and antioxidants due to their supposed anti-ageing and life-prolonging effects are backed by very little scientific evidence (Le Bourg, 2005).

Cosmetic treatments of ageing are at best only superficial and temporary, and cosmetics are not legally allowed to affect or modulate underlying cellular and biochemical processes. Yet research has discovered various natural and synthetic compounds that have a much greater potential as gerontomodulatory molecules than in their limited use in cosmetics (Rattan, 2002). For example, N^6-furfuryladenine or kinetin, a cytokinin growth factor, has been shown to provide anti-ageing and other beneficial effects, such as maintaining the youthful morphology of human cells, protecting DNA and proteins from oxidative damage, stimulating antioxidant enzymes in fruit flies, inhibiting platelet aggregation and pulmonary thrombosis in mice, and rescuing mis-splicing of RNA in familial dysautonomia genetic disorder (Rattan, 2002; Hsiao *et al*, 2003; Slaugenhaupt *et al*, 2004). Similarly, carnosine, a β-alanyl-L-histidine dipeptide, has beneficial effects such as the prevention and reversal of the ageing phenotype in human cells, prevention of oxidative damage to proteins and scavenging of free radicals (McFarland & Holliday, 1994, 1999; Hipkiss & Brownson, 2000).

Another preventive approach calls for altering our bodies in order to improve the basic molecular and genetic processes of maintenance and repair so that they either work more efficiently or for longer. These so-called 'strategies for engineered negligible senescence' (de Grey, 2000) require redesigning functional units of the body at the level of genes, gene products, macromolecular interactions, molecular-milieu interactions, and so on. Considering how little information and knowledge we have at present about these interacting factors, it is not clear how such an approach would work in practical terms. Similarly, although piecemeal replacement of nonfunctional or damaged body parts with natural or synthetic parts may provide a temporary solution to the problems of age-related impairments, it does not modulate the underlying ageing process *per se*.

> Even today, in our supposedly enlightened age, claims of miraculous remedies and therapies to overcome ageing run rampant, despite many of these products having no effects whatsoever

Table 1 | Hormetic effects of repeated mild heat shock on ageing human fibroblasts *in vitro*

Characteristic	Hormetic effect	Reference
Cell size	Reduced enlargement	(Rattan, 1998)
Cellular morphology	Reduced irregularization	
Glycation, furasine level	50–80% reduction	(Verbeke *et al*, 2001)
Glycoxidation level	10–30% reduction	
CML-rich protein level	20–85% reduction	
Lipofuscin pigment level	6–29% reduction	
Protein carbonyl levels	5–40% reduction	
Reduced glutathione level	Threefold increase	
Oxidized glutathione level	Twofold reduction	
Induction of sugar-induced protein damage	Tenfold reduction	
H_2O_2 decomposing ability	50–140% increase	(Fonager *et al*, 2002)
Survival after H_2O_2 exposure	10–18% increase	
Survival after ethanol exposure	10–40% increase	
Survival after UVA exposure	5–17% increase	
Hsp27 level	20–40% increase	
Hsc70 level	20% increase	
Hsp70 level	7–20-fold increase	
Hsp90 level	50–80% reduction	
Proteasome activities	40–90% increase	(Beedholm et al, 2004)
20S proteasome content	No change	
19S activator content	No change	
11S activator content	Increase	
11S activator binding	Increase	

A more realistic and promising approach in ageing intervention and prevention makes use of the body's intrinsic capacity for self-maintenance and repair. It is based on observations that exposure to low levels of otherwise harmful conditions can stimulate homeodynamic adaptive responses that benefit individual cells as well as the whole organism. The theory behind the approach—that low doses of toxic or harmful substances have a protective effect—is known as hormesis. Although the hormesis concept has been defined in different contexts such as pharmacology and toxicology (Calabrese & Baldwin, 2000; Parsons, 2000), hormesis in ageing is characterized by the beneficial effects that result from cellular responses to mild repeated stress (Rattan, 2001). Exposing cells and organisms to brief periods of stress should therefore slow down ageing, since the hormetic response to the stressor not only defends the organism against the stress but also over-reacts to remove other accumulated damage in cells and tissues. The paradigm for hormesis is exercise, an activity that is both stressful and damaging due to the production of free radicals, acids, stress hormones and cell and tissue breakage. But as an inducer of repair and maintenance processes, the hormetic effect of this strenuous activity has a wide range of health-promoting effects.

We have demonstrated the hormetic effects of repeated mild heat stress (RMHS) on cultures of human cells that undergo ageing. A mild stress regimen that exposed human skin fibroblasts *in vitro* to 41°C for 1 hour twice a week throughout their replicative lifespan caused several beneficial and anti-ageing effects (Table 1). It is important to note that while RMHS affected several age-related phenomena, it did not modify the proliferative capacity of these cells. This has implications in differentiating the phenomenon of ageing from longevity. It appears that the progression of cellular ageing *in vitro*—increasing molecular disorder—can be slowed down without upsetting the regulatory mechanisms of the cell cycle. Thus, the cells' quality of life in terms of their structural and functional integrity can be improved by mild stress without pushing these cells into a potentially carcinogenic hyperproliferative mode.

Some other mild stresses have been reported to delay ageing and prolong longevity on the level of the whole organism in various experimental animals. These include temperature shock, irradiation, heavy metals, pro-oxidants, acetaldehyde, alcohols, resveratrol, hypergravity, exercise and caloric restriction (Minois, 2000; Hercus *et al*, 2003; Rattan, 2004; Wood *et al*, 2004). Similar

> Defining ageing as a disease and then trying to cure it is unscientific and misguided

responses to mild under-nutrition in human beings have also been reported. For example, peripheral blood lymphocytes that were isolated from people with low body-mass index, such as those who live on a calorie-restricted diet, had higher DNA repair capacity and higher levels of DNA polymerase β, which were also maintained during ageing (Raji *et al*, 1998). Intermittent fasting also has beneficial effects on glucose metabolism and neuronal resistance to injury in mice (Anson *et al*, 2003). In addition, various nutraceutical and pharmacological agents that mimic hormetic stress responses are now under development. For example, bimoclomol, a nontoxic hydroxylamine derivative (Vigh *et al*, 1997), and celastrol, a quinone methide triterpene (Westerheide *et al*, 2004), both induce heat-shock proteins with chaperoning and protein-protective effects and are now being tested for their long-term cytoprotective action. Curcumin, an Indian yellow spice, has also been shown to have cytoprotective effects through its hormetic action in stimulating the synthesis of heat-shock proteins (Kato *et al*, 1998). Other chemical mimetics of caloric restriction, such as 2-deoxy-D-glucose and its analogues, and resveratrol—a polyphenol found in red wine—are being investigated for their use as anti-ageing hormetic agents (Wood *et al*, 2004).

However, before hormesis can be used as an effective way to modulate ageing and prevent the onset of age-related impairments and pathologies, there are several questions and problems that need to be resolved. Research has to establish biochemical and molecular criteria for quantifying the hormetic responses to different stresses, to identify the differences and similarities in stress response pathways that are initiated by these stressors, and to determine the interactive and pleiotropic effects of these pathways. In addition, clinical research is required to adjust the level and duration of mild stress for age-related changes in the sensitivity to stress. Resolving these issues requires a better understanding of the genetic and epigenetic interactive pathways of maintenance and repair networks, and how small changes in one component of the network can bring about equal or larger effects elsewhere in the organism. Furthermore, we need to understand the biochemical basis of age-related alterations and their responses to different kinds of stresses, and how this can be used to define an effective hormetic level of mild stress.

> … hormesis amplifies adaptive responses to stress, which in turn improves overall cellular functions and performance

Since hormetic effects are usually moderate, the biological significance of hormesis in terms of its application to human ageing is not yet clear. However, it should be pointed out that, although the hormetic effects may be relatively small when studied at the biochemical or molecular level, the final outcome—such as overall stress tolerance, functional improvement and survival—is often much more significant. This suggests that hormesis amplifies adaptive responses to stress, which in turn improves overall cellular functions and performance. Exercise is a good example because it is not only the specific target muscles that benefit, but also the immune system, cardiovascular system, sex hormones, libido and mood. Nevertheless, we still need to learn more about the interactive biochemical pathways which, through a process of biological amplification, cause maintenance and/or improvement of physiological functions. In the case of humans, research has just begun to investigate the role of the mental state and psychological stress in modulating various physiological functions, such as the immune response, stress hormone synthesis, gene expression, cardiac output and muscle strength (Bierhaus *et al*, 2003; Padgett & Glaser, 2003).

Applying hormesis to slow down ageing from within, prevent the onset of age-related diseases, and maintain physical and mental abilities in old age is a real possibility. But much more research on the molecular and physiological effects of mild stresses on the human body is needed before we can develop effective means of ageing intervention and prevention.

ACKNOWLEDGEMENTS

Research in the Laboratory of Cellular Ageing is supported by grants from the Danish Medical and Science councils SSVF and SNF; from shared cost action under the EU-Biomed & Health Programme and Quality of Life Projects; and research grants from Senetek PLC. My special thanks go to Dr. Romualdas Stapulionis for drawing the cartoons to illustrate this article.

REFERENCES

Anson RM, Guo Z, de Cabo R, Iyun T, Rios M, Hagepanos A, Ingram DK, Lane MA, Mattson MP (2003) Intermittent fasting dissociates beneficial effects of dietary restriction on glucose metabolism and neuronal resistance to injury from calorie intake. *Proc Natl Acad Sci USA* **100:** 6216–6220

Bartke A, Chandrashekar V, Dominici F, Turyn D, Kinney B, Steger R, Kopchick JJ (2003) Insulin-like growth factor 1 (IGF-1) and aging: controversies and new insights. *Biogerontology* **4:** 1–8

Beedholm R, Clark BF, Rattan SI (2004) Mild heat stress stimulates 20S proteasome and its 11S activator in human fibroblasts undergoing aging in vitro. *Cell Stress Chaperones* **9:** 49–57

Bierhaus A *et al* (2003) A mechanism converting psychosocial stress into mononuclear cell activation. *Proc Natl Acad Sci USA* **100:** 1920–1925

Calabrese EJ, Baldwin LA (2000) Tales of two similar hypotheses: the rise and fall of chemical and radiation hormesis. *Hum Exp Toxicol* **19:** 85–97

Carnes BA, Olshansky SJ, Grahn D (2003) Biological evidence for limits to the duration of life. *Biogerontology* **4:** 31–45

de Grey AD (2000) Gerontologists and the media: the dangers of over-pessimism. *Biogerontology* **1:** 369–370

Fonager J, Beedholm R, Clark BF, Rattan SI (2002) Mild stress-induced stimulation of heat shock protein synthesis and improved functional ability of human fibroblasts undergoing aging *in vitro*. *Exp Gerontol* **37:** 1223–1238

Gems D, Partridge L (2001) Insulin/IGF signalling and ageing: seeing the bigger picture. *Curr Opin Genet Dev* **11:** 287–292

Hercus MJ, Loeschcke V, Rattan SI (2003) Lifespan extension of *Drosophila melanogaster* through hormesis by repeated mild heat stress. *Biogerontology* **4:** 149–156

Hipkiss AR, Brownson C (2000) Carnosine reacts with protein carbonyl groups: another possible role for the anti-ageing peptide? *Biogerontology* **1:** 217–223

Holliday R (1995) *Understanding Ageing*. Cambridge, UK: Cambridge University Press

Holliday R (2000) Ageing research in the next century. *Biogerontology* **1:** 97–101

Hsiao G, Shen MY, Lin KH, Chou CY, Tzu NH, Lin CH, Chou DS, Chen TF, Sheu JR (2003) Inhibitory activity of kinetin on free radical formation of activated platelets *in vitro* and on thrombus formation *in vivo*. *Eur J Pharmacol* **465:** 281–287

Kato K, Ito H, Kamei K, Iwamoto I (1998) Stimulation of the stress-induced expression of stress proteins by curcumin in cultured cells and in rat tissues in vivo. *Cell Stress Chaperones* **3:** 152–160

Kirkwood TB (2002) Evolution of ageing. *Mech Ageing Dev* **123:** 737–745

Le Bourg E (2005) Antioxidants and aging in human beings. In Rattan SIS (ed), *Aging Interventions and Therapies* pp85–107. World Scientific Publishers: Singapore

McFarland GA, Holliday R (1994) Retardation of the senescence of cultured human diploid fibroblasts by carnosine. *Exp Cell Res* **212:** 167–175

McFarland GA, Holliday R (1999) Further evidence for the rejuvenating effects of the dipeptide L-carnosine on cultured human diploid fibroblasts. *Exp Gerontol* **34:** 35–45

Minois N (2000) Longevity and aging: beneficial effects of exposure to mild stress. *Biogerontology* **1:** 15–29

Olshansky SJ, Hayflick L, Carnes BA (2002) No truth to the fountain of youth. *Sci Amer* **286:** 92–95

Padgett DA, Glaser R (2003) How stress influences the immune response. *Trends Immunol* **24:** 444–448

Parsons PA (2000) Hormesis: an adaptive expectation with emphasis on ionizing radiation. *J Appl Toxicol* **20:** 103–112

Partridge L (2001) Evolutionary theories of ageing applied to long-lived organisms. *Exp Gerontol* **36:** 641–650

Raji NS, Surekha A, Rao KS (1998) Improved DNA-repair parameters in PHA-stimulated peripheral blood lymphocytes of human subjects with low body mass index. *Mech Ageing Dev* **104:** 133–148

Rattan SI (1995) Gerontogenes: real or virtual? *FASEB J* **9:** 284–286

Rattan SI (1998) Repeated mild heat shock delays ageing in cultured human skin fibroblasts. *Biochem Mol Biol Int* **45:** 753–759

Rattan SI (2000) Ageing, gerontogenes, and hormesis. *Indian J Exp Biol* **38:** 1–5

Rattan SI (2001) Applying hormesis in aging research and therapy. *Hum Exp Toxicol* **20:** 281–285

Rattan SI (2002) N^6-furfuryladenine (kinetin) as a potential anti-aging molecule. *J Anti-aging Med* **5:** 113–116

Rattan SI (2003) Biology of aging and possibilities of gerontomodulation. *Proc Indian Nat Sci Acad* **B69:** 157–164

Rattan SI (2004) Aging intervention, prevention, and therapy through hormesis. *J Gerontol A Biol Sci Med Sci* **59:** 705–709

Slaugenhaupt SA, Mull J, Leyne M, Cuajungco MP, Gill SP, Hims MM, Quintero F, Axelrod FB, Gusella JF (2004) Rescue of a human mRNA splicing defect by the plant cytokinin kinetin. *Hum Mol Genet* **13:** 429–436

Tatar M, Bartke A, Antebi A (2003) The endocrine regulation of aging by insulin-like signals. *Science* **299:** 1346–1351

Verbeke P, Clark BF, Rattan SI (2001) Reduced levels of oxidized and glycoxidized proteins in human fibroblasts exposed to repeated mild heat shock during serial passaging in vitro. *Free Radic Biol Med* **31:** 1593–1602

Verbeke P, Deries M, Clark BF, Rattan SI (2002) Hormetic action of mild heat stress decreases the inducibility of protein oxidation and glycoxidation in human fibroblasts. *Biogerontology* **3:** 117–120

Vigh L *et al* (1997) Bimoclomol: a nontoxic, hydroxylamine derivative with stress protein-inducing activity and cytoprotective effects. *Nat Med* **3:** 1150–1154

Westerheide SD, Bosman JD, Mbadugha BN, Kawahara TL, Matsumoto G, Kim S, Gu W, Devlin JP, Silverman RB, Morimoto RI (2004) Celastrols as inducers of the heat shock response and cytoprotection. *J Biol Chem* **279:** 56053–56060

Wood JG, Rogina B, Lavu S, Howitz K, Helfand SL, Tatar M, Sinclair D (2004) Sirtuin activators mimic caloric restricition and delay ageing in metazoans. *Nature* **430:** 686–689

Suresh I. S. Rattan is in the Laboratory of Cellular Ageing, Department of Molecular Biology, at the University of Aarhus, Denmark.
E-mail: rattan@mb.au.dk

doi:10.1038/sj.embor.7400401