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Principles of Ageing and the Practice of Anti-ageing Therapies

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Abstract : After years of generating data describing age-related changes in organisms, organs, tissues, cells and macromolecules, biogerontologists are now able to construct general principles of ageing and explore various possibilities of gerontomodulation. There is significant evidence to show that ageing is characterized by a stochastic accumulation of molecular damage and by a progressive failure of maintenance and repair. Several approaches are being tried and tested to modulate ageing in a wide variety of organisms with the ultimate aim of improving the quality of human life in old age, and prolong human health-span. These approaches include gene therapy, hormonal supplementation, nutritional modulation and intervention by antioxidants and other molecules. A recent approach is that of applying hormesis in ageing research and therapy, which is based on the principle of stimulation of maintenance and repair pathways by repeated exposure to mild stress. Our studies on the beneficial effects of repeated mild heat shock on human cells in culture, and other studies on the anti-ageing and life prolonging effects of proxidants, hypergravity, irradiation, and ethanol on cells and organisms have provided the proof of principle that hormesis as an anti-ageing and gerontomodulatory approach has a promising future.

Keywords : Age, Anti-ageing, Homeostasis, Homeodynamics, Repair, Damage, Hormesis, Survival, Longevity

Introduction :

Ageing is a complex biological phenomenon. The phenotype and the progression of ageing is different in different species, in individuals within a species, in different organs and tissues in an individual, in different cell types in an organ, and in different macromolecules in a cell (Rattan, 2000 a, b). Thus, there is neither a single way of defining ageing, nor is there a single cause. These observations have led to the abandonment of the notion of a strict genetic program for ageing. Instead, ageing is now considered as being epigenetic and stochastic in origin.

Although, these views about ageing also imply that there may not be any universal markers of ageing, some general principles can be derived, which can be the basis for developing rational strategies for anti-ageing interventions.

The three main principles of biological ageing and longevity are as follows :

1. Life history principle : According to the life history principle of ageing and longevity, ageing is an emergent phenomenon seen primarily in protected environments which allow survival beyond the natural lifespan in the wild. The natural lifespan of a species has also been termed "essential lifespan" (ELS) (Rattan, 2000a), or the "warranty period" of a species (Carnes *et al.*, 2003), which is defined as the time required to fulfill the Darwinian

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purpose of life, that is successful reproduction for the continuation of generations. Species undergoing fast maturation and early onset of reproduction with large reproductive potential generally have a short ELS. In contrast, slow maturation, late onset of reproduction, and small reproductive potential of a species is concurrent with its long ELS. It is during this period of extended survival beyond ELS when ageing becomes progressively obvious.

2. Mechanistic principle : Biochemcial and molecular basis of ageing reside in the mechanisms of progressive failure of homeostasis or homeodynamics, which leads to the accumulation of damage in nucleic acids, proteins and lipids. This results in the impairment in functional ability at all levels of organization thereby increasing the possibilities of a plethora of diseases and eventual death of the organism. Since homeostasis or homeodynamic ability of a living system is primarily due to its maintenance and repair processes, it is the progressive failure of maintenance and repair mechanisms which is the universal biochemical basis of ageing and age-related diseases (Holliday, 1995). The reasons that these processes have not evolved to keep functioning accurately and efficiently for ever lie in the first two principles of ageing and longevity discussed above.

3. Non-genetic principle : The non-genetic principle of ageing rules out any genetic program for ageing. Based on evolutionary reasons, it has been argued that unlike development which is a highly programmed and well-coordinated genetic and epigenetic process, there are no genes which determine the exact duration of survival of an organism (Holliday, 1995; Kirkwood, 2002). Genes that do influence ageing and longevity are those that have evolved in accordance with the life history of a species for assuring ELS. There is enough evidence to show that natural survival and longevity of a species is a function of its maintenance and repair capacities, which are genetically controlled. For example, positive correlations between species lifespan and the ability to repair DNA, to defend against reactive oxygen species, to respond and to counteract stress, and to proliferate and turnover the cells have been reported. In contrast, there is a negative correlation between longevity and the rate of damage accumulation, including mutations, epimutations, macromolecular oxidation and aggregation (Holliday, 2000).

Virtual gerontogenes and vitagenes :

A lack of specific gerontogenes does not imply that genes cannot influence survival, longevity and the rate of 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 the lifespan, and can be the cause of premature ageing syndromes. These genes cover a wide range of biochemical pathways, 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, 2003a).

Genes that do influence longevity are those that have evolved in accordance with the life history of a species for assuring ELS. Several lines of evidence support the view that natural survival and longevity of a

species is a function of its maintenance and repair capacities. For example, positive correlations between species lifespan and the ability to repair DNA, to defend against reactive oxygen species, to respond and to counteract stress, and to proliferate and facilitate turnover of cells have been reported. In contrast, there is a negative correlation between longevity and the rate of damage accumulation, including mutations, epimutations, macromolecular oxidation and aggregation (Rattan, 1989; Holliday, 1995; Rattan, 1995a).

Additionally, genetic linkage studies for longevity in mice have identified major histocompatibility complex (MHC) regions, and quantitative trait loci on chromosomes 7, 10, 11, 12, 16, 18 and 19 (Gelman *et al.*, 1988; De Haan *et al.*, 1998; Miller *et al.*, 1998) as putative genes for ageing. In human centenarians, certain alleles of HLA locus on chromosome 6 (Gelman *et al.*, 1988), regions of chromosome 4 (Puca *et al.*, 2001), different alleles of APO-E and APO-B, and DD genotype of angiotensin converting enzyme (ACE) have been linked to exceptional longevity. Similarly, several other studies have been published reporting an association between human longevity and single nucleotide polymorphisms in a variety of genes, including heat shock response, immune response, cholesterol metabolism and others (Tan *et al*., 2001; Altomare *et al*., 2003; Bessenyei *et al.*, 2004; Singh *et al*., 2004; Atzmon *et al*., 2005).

What is clear from the identification of the genes influencing ageing and longevity is that whatever their normal function and mechanism of action may be, these gerontogenes did not evolve to accumulate damage specifically, to cause age-related changes and to kill the organism. Since their involvement in influencing ageing and longevity is also a biological fact, such genes have been termed "virtual gerontogenes" or vitagenes (Rattan, 1995b, 1998). "Post-reproductive genetics" is another term used in order to explain different biological roles played at different ages by the same genetic variants (Franceschi *et al*., 2005).

Molecular mechanisms of ageing :

A generalised definition of ageing as the failure of homeodynamics still requires mechanistic explanation(s) as to why such a failure occurs in the first place and what controls the rate of failure in different species. Over the last fifty years, researchers have proposed a large number of hypotheses which attempt to explain how the observed age-related changes in macromolecules, cells, tissues, organs and systems may occur. Main examples of such hypotheses include altered gene regulation somatic mutation accumulation, protein errors and modifications, reactive oxygen species and free radicals, immune-remodeling and neuroendocrine dysfunctioning. At the cellular level, the so-called telomere loss theory, and epimutation theory of progressive loss of DNA methylation are other examples of providing mechanistic explanations for the loss of proliferative potential of normal, differentiated and diploid cells *in vitro* and *in vivo* (Rattan, 2004a).

Several theoretical and mathematical models are being developed in order to understand the interactive nature of the biological networks and trade-offs (Kowald and Kirkwood, 1996; Franceschi *et al*., 2000). Recently, the reliability theory of ageing and longevity about the inevitable failure of complex systems such as cells and

organisms (Gavrilov and Gavrilova, 2001) has reiterated the fundamental law that no process can be one-hundred-percent accurate one-hundred-percent of the time, and it is the interactive nature of genes, milieu and chance that effectively determines how long a system can survive. Therefore, to resolve the issue of widely varying rates of ageing in nature, it is important to undertake comparative studies on various aspects of the ageing process in a variety of organisms with widely differing life-history scenarios. Furthermore, resolving these issues requires a better and deeper understanding of the basic biological processes involving molecular network interactions, emergence and amplification of biological effects, mechanisms of action-at-a-distance and so on. In many cases, new and powerful technologies, such as nanotechnology, bioinformatics, single cell analysis, molecular heterogeneity analysis, and epigenetic regulators of stress response and post-synthetic modifications will be highly desirable.

Anti-ageing : therapy or prevention?

Occurrence of ageing in the period beyond ELS, and the onset of one or more diseases before eventual death appear to be the "normal" sequence of events. This viewpoint makes modulation of ageing different from the treatment of one or more specific diseases. In the case of a disease, such as a cancer of any specific kind, its therapy means the removal and elimination of the cancer cells and restoration of the affected organ/tissue to its original disease-free state. What will be the "treatment" of ageing and to what original "age-free" stage one would hope to be restored? Considering ageing as a disease and then trying to cure that disease is unscientific and misguided. Similarly, although piecemeal replacement of non-functional or half-functional body parts with natural or synthetic parts made of more durable material may provide a temporary solution to the problems of age-related impairments, it does not modulate the underlying ageing process as such (Rattan, 2005).

Scientific and rational anti-ageing strategies aim to slow down ageing, to prevent and/or delay the physiological decline, and to regain lost functional abilities. However, the history of anti-ageing research and therapy is replete with fraud, pseudoscience and charlatanism, and has often given a bad name to the whole field (Boia, 2004). Claims for miraculous remedies and promises for extremely long lifespan are prevalent even today. Recently, highly critical analyses of such approaches have been made by biogerontologists with a view to educate and inform people about the science and non-sense of ageing-intervention research (Olshansky *et al*., 2002a).

While not giving serious consideration to the claims made by charlatans, it cannot be ignored that several researchers are making genuine attempts to test and develop various means of intervention for the prevention and treatment of age-related diseases, for regaining the functional abilities and for prolonging the lifespan of experimental organisms. Some of the main anti-ageing approaches include supplementation with hormones including growth hormone, dehydroepiandrosterone (DHEA), melatonin and estrogen, and nutritional supplementation with synthetic and natural antioxidants in purified form or in extracts prepared from plant and animal sources (Rattan, 2003b; Ferrari, 2004).

Although some of these approaches have been shown to have some clinical benefits in the treatment of some diseases in the elderly, none of these really modulate the ageing process itself (Olshansky *et al*., 2002b). Furthermore, claims for the benefits of intake of high doses of vitamins and various antioxidants and their supposed anti-ageing and life-prolonging effects have very little scientific evidence to back them (Le Bourg, 2005).

In contrast to this, nutritional modulation through caloric restriction (CR) has been shown to be an effective anti-ageing and longevity extending approach in rodents and monkeys, with possible applications to human beings (Roth *et al*., 2004). But, this is a highly debatable issue at present both in terms of the practicalities of defining CR and of applying CR in human beings in physiological and evolutionary contexts (Demetrius, 2004).

Some studies have reported an extension of lifespan of experimental animals by gene manipulation. For example, overexpression of superoxide dismutase and catalase genes and of heat shock protein (hsp) genes have resulted in the increase in average lifespan in *Drosophila* and nematodes, respectively (Orr and Sohal, 1994; Yokoyama *et al*., 2002). Such a gene-therapy approach for gerontomodulation requires redesigning the blueprint for structural and 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 all those interacting variants of genes, molecules, milieu and chance, it is not clear what this approach really means in practical and achievable terms. Similarly, although piecemeal replacement of non-functional or half-functional body parts with natural or synthetic parts made of more durable material may provide a temporary solution to the problems of age-related impairments, it does not modulate the underlying ageing process as such.

Principle of hormesis and its applications :

A promising approach in ageing intervention and prevention is based in making use of an organism's intrinsic homeodynamic property of self maintenance and repair. Since ageing is characterized by a decrease in the adaptive abilities due to progressive failure of homeodynamics, 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 upregulated and the related pathways of maintenance and repair are stimulated, one should observe anti-ageing and longevity-promoting effects. Such a phenomenon in which stimulatory responses to low doses of otherwise harmful conditions improve health and enhance lifespan is known as hormesis.

Although the phenomenon of hormesis has been defined variously in different contexts, for example in toxicology, pharmacology and radiation biology (Calabrese and Baldwin, 2000; Parsons, 2000), hormesis in ageing is characterized by the beneficial effects resulting from the cellular responses to mild repeated stress (Rattan, 2001). The paradigm of hormesis is moderate exercise which is well known to have numerous beneficial effects despite it being a generator of free radicals, acids, and other damageing effects (McArdle *et al*., 2002).

During the last few years, research done in our labs has shown hormetic effects of mild stress. We have demonstrated the hormetic effects of repeated mild stress (RMS) on human cells undergoing ageing in culture. Using a mild stress regime of exposing human skin fibroblasts to 41°C for 1 hr twice a week throughout their replicative lifespan *in vitro*, several beneficial and anti-ageing effects have been observed. These effects include reduced accumulation of oxidized proteins, increased levels of various hsp, increased proteasome activities, and enhanced stress resistance to other stresses (Rattan *et al*., 2003; Rattan *et al*., 2004a, b).

Other chemical, physical and biological treatments can be used to unravel various pathways of maintenance and repair whose sustained activities improve the physiological performance and survival of cells and organisms. Stresses that have been reported to delay ageing 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, exercise and CR (Minois, 2000; Hercus *et al*., 2003; Rattan, 2004a, b). Hormesis-like beneficial effects of chronic but mild undernutrition have been reported for human beings (Raji *et al*., 1998). For example, it was reported that peripheral blood lymphocytes isolated from people with low body mass index, representing a group with natural intake of restricted food calories, had higher DNA repair capacity and higher levels of DNA polymerase β, which were also maintained during ageing (Raji *et al*., 1998). Intermittent fasting has been reported to have beneficial effects on glucose metabolism and neuronal resistance to injury (Anson *et al*., 2003).

Although, at present there are only a few studies performed which utilize mild stress as a modulator of ageing and longevity, hormesis can be a useful experimental approach in biogerontology. However, there are a few issues that remain to be resolved before mild stress can be used as a tool to modulate ageing and prevent the onset of age-related impairments and pathologies. Some of these issues are: (1) to establish biochemical and molecular criteria for determining the hormetic levels for different stresses; (2) to identify differences and similarities in stress response pathways initiated by different stressors; (3) to quantify the extent of various stress responses; (4) to determine the interactive and pleiotropic effects of various stress response pathways; (5) to adjust the levels of mild stress for age-related changes in the sensitivity to stress; (6) to determine the biological and evolutionary costs of repeated exposure to stress; and (7) to determine the biological significance of relatively small hormetic effects, which may or may not have large beneficial effects during the entire lifespan. Resolution of these issues requires much more research on hormesis than being carried out at present.

The proof of the hormetic principle has now been provided by experiments with a wide variety of biological systems and by using a range of physical, chemical and biological stressors. Two of the main lifestyle interventions, exercise and reduced food intake, both of which bring their beneficial and anti-ageing effects through hormesis (Masoro, 1998, 2000; Yu and Chung, 2001; McArdle *et al*., 2002; Singh,

2002), are being widely recognized and increasingly practiced as an effective means of achieving a healthy old age.

One can also expect the availability of certain nutriceutical and pharmacological hormetic agents to mimic the HS response and CR. For example, bimoclomal, a nontoxic, hydroxylamine derivative with hsp-inducing activity and cytoprotective effects is under Phase II clinical trials (Vigh *et al*., 1997, 1998). Celastrol, a quinone methide triterpene (Westerheide *et al*., 2004), and paeoniflorin (Yan *et al*., 2004), which are active components of certain Chinese medicinal herbs are other hsp-inducing hormetic agents under test for their cytoprotective effects. Similarly, curcumin, an Indian yellow spice, has also been shown to have cytoprotective effects through its hormetic action in co-stimulating the synthesis of hsp (Dunsmore *et al*., 2001). Various chemical mimetics of CR, such as 2-deoxy-D-glucose and its analogues (Lane *et al*., 2002), and resveratrol, which is a polyphenol found in red wine, are being tested for their use as anti-ageing hormetic agents (Howitz *et al*., 2003; Lamming *et al*., 2004; Wood *et al*., 2004)

Another small molecule, N^6-furfuryladenine or kinetin, has been shown to have significant anti-ageing (Rattan and Clark, 1994; Rattan, 2002), and anti-thrombotic (Hsiao *et al*., 2003) effects in human cells. Kinetin is considered to work both as a direct antioxidant (Olsen *et al*., 1999; Verbeke *et al*., 2000), and as a hormetic agent by inducing the synthesis of other protective enzymes and hsp (Barciszewski *et al*., 1999; Holmes-Davis *et al*., 2001; Rattan, 2002). Although at present the use of kinetin has been limited to being a cosmeceutical ingredient in a range of cosmetics products, its usefulness as a hormetic nutriceutical agent is under investigation.

Finally, it must be emphasized that the goal of research on ageing is not to increase human longevity regardless of the consequences, but to increase active longevity free from disability and functional dependence. Healthy old age is an achievable goal that however requires significantly more research support and efforts in biogerontology.

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