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Review Article

Bridging the gap: ageing, pharmacokinetics and pharmacodynamics

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Abstract

Changes in pharmacokinetics and pharmacodynamics in elderly patients generally result in an increase in the incidence of drug toxicity and adverse drug reactions. Molecular alterations associated with ageing could bring about biological changes, a consequence of which is an altered response to pharmacological agents. Unfortunately, research in this area has yet to progress beyond the cataloguing of the pharmacokinetic and pharmacodynamic changes observed in the elderly. Therefore, real progress in our understanding of pharmacogerontology could be achieved if it were possible to merge pharmacokinetic and pharmacodynamic studies with recent advances in our understanding of the causal processes bringing about ageing changes at the cellular level. Therefore, this review will focus on the mechanisms of ageing in the hope that the information will be of value to those planning independent studies.

Introduction: why worry about ageing?

During the latter half of the 20th century, average life expectancy throughout the world increased rapidly. Increases in life expectancy are often popularly confused with extensions in maximum lifespan. However life expectancy only actually measures the likelihood of survival to a given age. It should therefore be regarded as an indication of the degree to which a given environment is benign or hazardous and thus is an indirect measure of the quality of life. Life expectancy in the neolithic period is estimated to have been of the order of 25–35 years (Bocquet-Appel & Bacro 1997; Pietrusewsky et al 1997) and that of Soviet infantrymen at the battle of Stalingrad was measured in hours (Beevor 1998). In contrast, the average life expectancy of modern Americans is somewhat in excess of 77 years (Arias et al 2003).

Modern life expectancies are a testament to our ability to deliver concrete healthcare benefits to ordinary people; however, they have not yet been coupled with any significant increase in the maximum human lifespan (the period of time for which the longest surviving member of a given population remains alive). In this regard we differ from our early ancestors only in our increased likelihood of reaching an advanced age.

Increasing life expectancy brings problems as well as benefits. The reduction or elimination of many potential causes of death in the early part of the life course has produced an aged population of unprecedented size. The number of people aged 60 years and older reached nearly 600 million in 1999 and it is expected that this population will increase to approximately 2 billion by 2050 (UN population division, 1999). Human ageing is associated with both an increasing rate of mortality (defined as the likelihood of dying) and an increasing rate of morbidity (defined simply as the period of time spent sick before either death or recovery occurs). This increase in the frequency and duration of morbidity has profound cost and quality of life implications. If we fail to meet this new healthcare challenge we will spend more money than ever to keep more people than ever in a state in which they are more miserable than ever. This is not a reassuring prospect.

Pharmacological changes occur in the elderly

Dealing with the problems posed by a frail elderly population is complicated by the altered responses to pharmacological agents seen in this patient group. These can be due to both pharmacokinetic and pharmacodynamic changes.

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The pharmacokinetic changes observed in elderly patients are well understood and it is easy to make allowance for them. For example, renal decline with age is well documented and it is common practice to reduce the dose of renally excreted drugs in the elderly. This is particularly true for drugs with a low therapeutic index such as digoxin. Indeed age-related renal decline is so well defined that creatinine clearance or glomerular filtration rate (GFR) can be calculated using the following equation:

$$\mathrm{GFR} = [140 - \text{age (years)} \times \text{weight (kg)}]/ [72 \times \text{serum creatinine concentration (mg\%)}]$$

Obviously any patient reaching the (currently unachievable) grand old age of 140 is not expected to have any renal function at all and the use of digoxin would be rather hazardous (Macphee & Brodie 1988)!

Hepatic drug clearance also declines with age and this has been attributed to a decline in hepatic blood flow or metabolic capacity. For example, George et al (1995) have reported a clear negative association between age and total hepatic cytochrome P450 (CYP450) content. In particular, they demonstrated a decline in the 2E1 and 3A isoforms of the enzyme with age, while the 1A2 and 2C isoforms remained constant. They also showed that CYP 2E1 declined by 5%, and CYP 3A declined by 8% with each decade of life. These findings were observed after eliminating confounding factors such as gender, smoking and alcohol consumption. Interestingly the decline in drug metabolism is confined to particular CYP450 isoenzymes and is not a generalized phenomenon. This would suggest a shift in hepatic phenotype rather than simply a change in hepatic viability.

Pharmacodynamic changes have also been extensively studied and are probably best illustrated by studies of adrenoreceptor function in young and elderly subjects. Generally, β-adrenoreceptor function is thought to decline with age, while α-adrenoreceptor function is usually unchanged or even increased. For example, airway β_2-adrenoreceptor responsiveness is diminished in the elderly, suggesting that airway β-adrenoreceptor dysfunction may be implicated in late-onset asthma (Connolly et al 1995). These changes are not just confined to the lung as an age-related decline in β-adrenergic responsiveness, they have also been proposed as a causative factor of reduced bladder compliance in the elderly (Li et al 2003). These authors propose reduced β-adrenoreceptor density and adenylyl cyclase activity as the underlying molecular mechanism for the changes observed.

Reduced β-adrenoreceptor-mediated vascular smooth muscle relaxation in the aged has been demonstrated using the dorsal hand vein technique. Maximal dilatation induced by isoprenaline was reduced in elderly subjects, but maximal dilatation induced by adenosine was not. It is important to note that these results indicate that the age-associated receptor down-regulation is receptor specific and not due to a general loss in the ability to vasodilate (Ford et al 1992).

The decline in β-receptor function just described does not occur with α-adrenoreceptors. Rudner et al (1999) has shown that arterial α_1-adrenoreceptor expression increases with age at the mRNA and protein levels. α_1-Adrenoreceptor expression doubles with age (< 55 versus ≥ 65 years) and is mainly due to an increase in α_{1b}-adrenoreceptor expression. What is more, samples obtained from older subjects are more responsive to α-agonists. Taken together, these studies seem to show that ageing vascular smooth muscle is more responsive to vasoconstricting α-agonists and less responsive to vasodilating β-agonists. In summary, the ageing process produces subtle changes in adrenoreceptor expression, up-regulating some, leaving others unchanged and down-regulating others. What is not happening is a general decline in receptor function due to a general decline in tissue viability.

While these changes themselves have been characterized in some detail, their underlying causes remain unclear. Indeed, it could be fairly said that research in this area has yet to really progress beyond the cataloguing of pharmacodynamic and pharmacokinetic changes and, in some instances, the assignment of a proximal cause. We believe that real progress in our understanding of pharmacogerontology could be achieved if it were possible to mesh pharmacodynamic and pharmacokinetic studies with the recent advances in our understanding of the causal processes bringing about ageing changes at the cellular level. However, insight into these processes has mainly been generated by laboratories focused on the molecular mechanism underlying the ageing process. These groups often have a limited understanding of the pharmacology of the elderly. Since the majority of readers of this article will be familiar with the primary literature in this latter field rather than with the former the rest of this article will focus on the mechanisms of ageing themselves in the hope that the information will be of value to those planning independent studies.

Mechanisms of tissue ageing

Ageing is a universal, intrinsic, progressive and degenerative process that compromises the viability of the organism (Strehlar 1962). The evolutionary rationale for the emergence of this process is well developed and will not be discussed in detail. For our purposes it is sufficient to say here that there is no organismal clock causing ageing. It is an unprogrammed process, in the sense that no genes have evolved specifically to cause it (Partridge & Gems 2002).

In an individual organism, ageing is the sum of the actions of multiple degenerative mechanisms on the various somatic tissues of which it is composed. Somatic tissues comprise a mixture of structural proteins, post-mitotic cellular elements (such as mature neurons and muscle fibre cells) and mitotic cells (such as T cells, endothelial cells and dermal fibroblasts). Although the gradual biological alterations that drive the ageing process cause changes to occur in all the components of this complex mixture, the mechanisms by which each component degenerates appear to be distinct.

Protein modification

The function of structural proteins can be compromised by the time-dependent accumulation of irreversible chemical

modifications (Monnier & Cerami 1981). These include the formation of advanced glycation end-products (AGEs) and the recently described advanced lipoxidation end-products (ALEs) (Baynes 2003), formed by carbonyl amine chemistry between protein and carbonyl compounds derived from autoxidation of carbohydrates and lipids (reviewed in Cloos & Christgau 2004). Nitrosylation of reactive amino acid residues by the activity of reactive nitrogen free radical species can also permanently modify proteins. These modifications occur on both intracellular and extracellular proteins and may result in either disruption to the peptide sequence or secondary structure, or decoration of the protein with additional moieties at the protein surface (Ulrich & Cerami 2001).

Post-mitotic cells are also vulnerable to a wide variety of damaging events (including the action of reactive oxygen and nitrogen free radical species). However, this process is more complicated than the simple accumulation of unrepaired damage as a result of prolonged post-mitotic dwell time within tissue. For example, in skeletal muscle fibre cells, contractile activity generates a variety of oxidants and repeated contractile activity produces an adaptive response, which is marked by the up-regulation of antioxidant enzymes, such as superoxide dismutase, and the increased expression of heatshock proteins (HSPs) (McArdle et al 2002). HSPs act as molecular chaperones, which associate with newly synthesised proteins as a screening system to ensure correct folding and subsequent activity. This screening system is up-regulated during periods of cellular stress to increase the turnover and replacement of damaged proteins. This adaptive response is reduced in muscle fibre cells from aged individuals, resulting in severe oxidative stress and subsequent cell death.

Replicative senescence

Exhaustion of the capacity of mitotic tissue to regenerate was first proposed as an ageing mechanism in the 19th century (Weissman 1891), but until the middle of the 20th century it was widely held that normal somatic tissue could not age in a regenerative sense because it had an indefinite capacity to proliferate. This view had developed for two reasons. Firstly, the Nobel laureate Alexis Carrel appeared to have demonstrated the long-term cultivation of normal chick fibroblasts for periods considerably in excess of the lifetime of the animal (Parker 1938; Witkowski 1990). Secondly, the technical difficulties associated with tissue culture techniques until the 1950s rendered the duplication of Carrel's studies very difficult for all but a few highly specialized laboratories (Parker 1938). Ageing was not the primary research interest of these centres. Thus, it was not until a series of classic experiments by Hayflick & Moorhead in the early 1960s demonstrated that cultures of normal human fibroblasts did not have an infinite capacity to expand that the idea of intrinsically immortal mitotic tissue was seriously questioned. Their work demonstrated that, after a finite period of growth, cultures of normal human fibroblasts became completely composed of viable but non-dividing cells (Hayflick & Moorhead 1961; Hayflick 1965). These initial observations were reproduced in hundreds of studies and since that time virtually all human mitotic cell types subjected to rigorous study have been shown to undergo this cellular senescence in culture. In addition to showing that a limited capacity to divide was an intrinsic property of normal human cells, Hayflick & Moorhead demonstrated that cultures derived from embryonic donors had a considerably higher growth capacity than those derived from adults. Hayflick's subsequent proposal that a relationship existed between cellular senescence in-vitro and the senescence of the intact organism (Hayflick 1965) gave fresh impetus to the idea that the exhaustion of mitotic capacity could play a causal role in ageing. However, the concept was expressed in a necessarily broad sense, which has, in retrospect, given rise to much confusion. A modern view of the hypothetical relationship between replicative senescence and organismal ageing is shown in Figure 1.

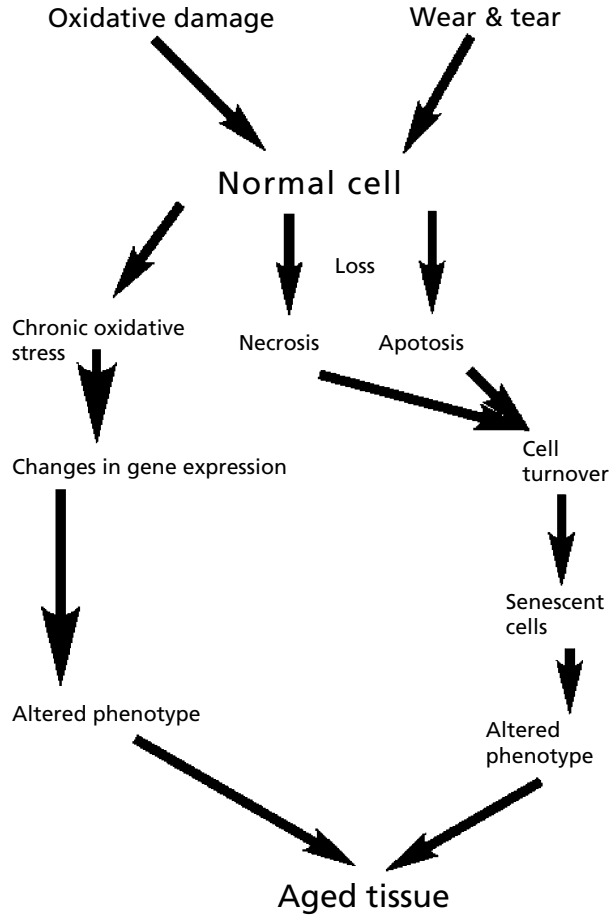

Figure 1 Simple scheme comparing the cell senescence hypothesis of ageing (right hand flow) with the dysdifferentiation hypothesis of ageing (left hand flow). The cell senescence hypothesis postulates that in the normal course of life there is cell loss. That loss is balanced by cell division, which is actively monitored. One or more replicometers act to trigger permanent cell cycle exit (senescence) in individual cells (see text). Cell cycle exit is associated with a broad alteration in gene expression leading to an altered phenotype that affects the microenvironment in which the cell resides and ultimately the entire tissue. In the dysdifferentiation model, chronic oxidative stress leads to a regearing of gene expression generating an altered cellular phenotype, which contributes to tissue ageing. The two models have many essential similarities.

Oxidative damage

Oxidative stress or free radical damage is inextricably linked in the popular imagination with the ageing process. Essentially, this theory proposes that the production of reactive oxygen as a consequence of normal cellular respiration damages proteins, alters lipid membranes and damages DNA leading to the physiological deficits that characterize the ageing process (Arking 1998).

Much of the data on which the oxidative damage theory of ageing is based is necessarily correlative. Many studies have examined antioxidant defence levels in different species to determine whether the ability to defend against oxidative stress correlates with species-specific lifespan (in general the correlation is rather good, see Tolmasoff 1980). Similarly, long- and short-lived strains of the same organism have been compared for antioxidant defence levels (longer-lived animals have more, see Dudas & Arking 1995). However, when interventional tests of the theory have been attempted (typically by feeding ageing rodents dietary antioxidants, such as vitamin E, to try and extend their lifespans), the results have been far less successful. One particularly well-conceived series of studies may stand for the whole. A class of molecules known as the salens were recently shown to act as superoxide dismutase and catalase mimics in-vivo and in-vitro. In particular, these molecules were shown to be able to somewhat extend the lifespan of mice carrying a loss of function mutation in mitochondrial superoxide dismutase (these animals normally have a lifespan of only a few days, the salen was able to increase this to some weeks) (Melov et al 2001). An initial report also appeared to show a clear increase in normal nematode lifespan when treated with the same compounds (Melov et al 2000). However, a subsequent series of nematode studies firstly determined the dose of salen needed to give a protective effect against paraquat-mediated killing (to ensure physiological efficacy) and, secondly, measured the effect of continuous supplementation at that dose on the mean of maximum lifespans of a population of nematodes. No extension of lifespan could be observed (Keaney & Gems 2003; Keaney et al 2004). This is quite a typical pattern in which some studies report a positive effect and others (often published in less commonly read journals) fail to reproduce it.

Replicative senescence and ageing

To date, there is little experimental evidence to directly implicate cellular senescence in the physiological changes associated with ageing. However, critical analysis of the literature on ageing reveals considerable support for this ageing mechanism and therefore will be discussed further in greater detail.

How do cells become senescent?

It was realized relatively rapidly that the most plausible in-vivo role for the Hayflick phenomenon was to limit the expansion of clones of cells carrying mutations predisposing to the development of cancer (Dykhuizen 1974). Thus, research on replicative senescence tended to focus on the mechanisms that gave rise to the senescent state in fibroblasts (e.g. trying to understanding the anti-cancer mechanisms) rather than the effect which senescent cells exerted on tissue (e.g. trying to uncover putative pro-ageing effects). As a consequence of these studies, knowledge of the population dynamics of normal fibroblast cultures increased rapidly. Hayflick's original description of the growth kinetics of primary fibroblast cultures suggested that the population was composed of cells with essentially identical proliferative capacities and that the final failure to expand was the direct result of cell death. The idea that senescence was cell death was disproved very rapidly (Macieira-Coelho et al 1966). However, determining whether the proliferative capacities of the members of a population were the same or different was a considerably more complicated task and required greater time to complete. Initial studies by Cristofalo & Scharf (1973) demonstrated the presence of senescent cells in early passage cultures using long pulse-labelling experiments on embryonic fibroblasts. This observation was difficult to reconcile with the predicted behaviour of a population of cells with nearly identical replicative capacities. A series of clonal isolation and growth studies by Smith & Whitney (1980) directly demonstrated that primary fibroblast cultures are composed of cells that display variation in their proliferative potential. Related experiments also showed that two fibroblasts arising from a single mitosis can differ in their ability to proliferate by as many as eight divisions (Jones et al 1985). This suggested that the anti-tumour properties of replicative senescence were much greater than first thought (because it was likely that any given clone of cells carrying a mutation would become senescent relatively rapidly). However, the mechanism by which individual cells became senescent and thus how the putative anti-cancer mechanism actually worked still remained unclear. With a few far-sighted exceptions, attempts to link replicative senescence and ageing remained in the realm of theory and speculation.

In the mid 1960s it had been proposed that the shortening of chromosomal ends (telomeres) had the potential to explain Hayflick's observations. Alexi Olovnikov, a Soviet researcher, recognized that passage through S-phase would result in the loss of telomeric sequences with each round of division as an inevitable consequence of DNA replication on a linear template (Olovnikov 1996). Olovnikov linked this end-replication problem to the senescence of fibroblasts in culture by postulating that the failure of the population to expand indefinitely was the result of total loss of telomeric sequence followed by additional end-replication losses in essential genes that produced widespread cell death. The existence of end-replication loss as an important biological phenomenon was shown relatively rapidly. However, Olovnikov's explicit linkage of telomere loss with cell death meant that the senescence field largely ignored his ideas (because of the earlier demonstration that senescent cells remained viable for long periods). It was another two decades before experimental support for Olovnikov's linkage of telomere loss and the Hayflick limit emerged. In 1990, using Southern blot analysis, Harley et al (1990) demonstrated that the mean telomere length in five strains of fibroblasts decreased approximately 2 kilobases with cumulative population doublings in-vitro. Although this work established that significant end-replication loss

took place in human fibroblasts, it remained unclear whether telomere loss was the cause of senescence or simply a consequence. Shortly thereafter, mathematical modelling studies suggested that senescence driven by telomere shortening alone could explain almost all of the variation in clonal lifespan first observed by Smith & Whitney (Levy et al 1992). Thus telomere loss appeared to have the potential to explain the kinetics of senescence in human fibroblast cultures but interventional evidence that telomere shortening served as the replicometer in these cells was lacking. Since then, other mathematical models have been developed based on different mechanisms of telomere shortening (recombination, nuclease digestion, oxidative stress), which also fit the kinetics of senescence (Rubelj & Vondracek 1999; Sozou & Kirkwood 2001).

In the mid 1980s the isolation of an enzyme (telomere terminal transferase, or telomerase), which extended chromosome ends in the ciliate *Tetrahymena*, was reported (Greider & Blackburn 1985). Telomerase holoenzyme is composed of an RNA subunit that acts as template (TR) and a catalytic subunit (TERT) that acts as a reverse transcriptase. In 1997, the cloning of the catalytic subunit of human telomerase (hTERT) allowed the effects of the ectopic expression of telomerase on the replicative lifespan of human cells to be determined (Nakamura et al 1997; Bodnar et al 1998). These studies demonstrated that senescence could be prevented by the active maintenance of telomere length. Thus telomeric attrition serves as a primary mechanism controlling replicative senescence. Continual expression of telomerase produces immortalization and is a common finding in many human cancers.

However, subsequent studies demonstrated that the expression of hTERT alone does not always produce immortalization (Kiyono et al 1998). Such cells were found to enter a senescent state with active telomerase and long telomeres. These studies are consistent with the presence of multiple replicometers in human cells, at least one of which does not use telomere length to monitor divisions.

Why is the presence of senescent cells deleterious?

It is proposed that the accumulation of senescent cells can play a role in the ageing of mitotic tissue in two distinct but inter-related ways. Firstly, the presence within a tissue of a substantial fraction of cells that are incapable of dividing is likely to impair the regenerative capacity of that tissue (Faragher & Kipling 1999). Most tissues have an ongoing need for cell replacement to maintain normal function. A good example of this is the vascular endothelium, which regularly loses cells as a consequence of blood flow (Tardy et al 1997; Quilici et al 2004). Secondly, senescent cells have an altered phenotype. This gives them the potential to produce deleterious effects on their immediate microenvironment and ultimately on the tissue within which they reside. The altered phenotype of senescent cells was discovered as a result of a series of studies from the mid 1970s onwards that sought to determine to what extent the phenotype of senescent cells differed from that of their growing counterparts. In fibroblasts, ultra-structural changes and radical shifts in invasiveness, the ability to contract collagen lattices and matrix remodelling capacity were readily apparent (Bell et al 1979; Millis et al 1989; Sandeman et al 2000, 2001). Recently, genomics technology has been used to monitor the gene expression profiles underlying these phenotypic changes and to extend these studies to other cell types. One recent micro-array analysis of primary human lung fibroblasts (IMR-90) and primary skin fibroblasts (Detroit 551) reported that of the 4183 genes analysed, 165 were down-regulated and 191 up-regulated in senescent IMR-90 cells and 154 down-regulated and 76 up-regulated in senescent Detroit 551 cells compared with their growing counterparts (Chen et al 2004). This degree of alteration in the transcriptome is akin to that seen when cells are induced to differentiate (Truckenmiller et al 2001) and produces an equally radical alteration in cellular phenotype. When the senescent state is compared in different cell types, this altered phenotype can be summed up in general terms as a permanent shift to an activated, pro-inflammatory state that mimics the early remodelling phase of wound repair (Shelton et al 1999). The pro-inflammatory phenotype of senescent cells and the chronic inflammation that accompanies normal ageing (so-called inflammaging) is provocative, but as yet little has been done to link these areas of study (Mishto et al 2003).

Perhaps the best known example of senescent cell accumulation-induced impairment of tissue function is that provided by the immune system. In cell culture, it has been shown that when CD8 T cells are subjected to repeated rounds of antigen-driven proliferation, they eventually enter a state of senescence (Effros 2004). One of the senescence-associated alterations that have been observed in these cells is a complete loss of CD28 expression: a CD8 T-cell-specific co-stimulatory molecule that is required for full activation and proliferation. Since CD28 has been implicated in a number of essential T cell functions, there are profound differences in the behaviour of a population of CD28 positive compared with CD28 negative T cells. As senescent CD8 T cells (CD28 negative) persist in the tissue and accumulate with age, there will most likely be an impairment of immunological response with age.

Senescent cells have also been shown to have an impact on the activity of neighbouring non-senescent cells. This has been demonstrated in co-culture experiments in which the production of plasminogen activator inhibitor-1 (PAI-1) by vascular endothelial cells was down-regulated by the presence of low-passage fibroblast cultures, but not by high-passage fibroblast cultures (Zhang et al 1996). This difference is thought to be due to the markedly increased presence of senescent fibroblasts in the latter cultures. Thus, cellular senescence may have both direct and indirect roles in the ageing process.

Unsolved problems in ageing research

Do senescent cells exist in-vivo?

Interestingly, although the idea that random damage caused by oxidative stress or glycation adduction might act as primary ageing mechanisms has seldom been seriously questioned, the notion that senescent cells could contribute to ageing has been subject to serious scrutiny in the last thirty

years. Leaving aside the misconceived assumption that working on a programmed cell-divisional counting system within the context of normal ageing is tantamount to a belief that organismal ageing itself is programmed (Austad 1997, 2004; Bredesen 2004), criticisms of the idea can be broken down into three types: that senescence is simply an artifact produced by poor tissue culture and thus cannot cause organismal ageing; that senescent cells are not artifacts but are incapable of exerting degenerative effects and are thus irrelevant to the ageing process; that they exist in-vivo and can exert degenerative effects but that there are so few of them that for all practical purposes they are irrelevant to ageing.

Establishing that senescent cells are not in-vitro artifacts requires the demonstration that at least a few senescent cells are present in-vivo. Although this appears to be a straightforward experimental proposition, it is more complicated than it sounds. The overwhelming majority of cells in any normal tissue are in a state of quiescence at any given time. Quiescence is a state of transient, rather than permanent growth arrest. Currently, very few markers exist that can be used to histologically distinguish between the two states in-vivo and none could do so before the mid 1990s. As a result, early studies determined the replicative capacity of cultures of cells from donors of different ages as a surrogate for the fraction of senescent cells present in their tissues. Perhaps the best known of these studies (Martin et al 1970) looked at the replicative lifespan of human fibroblasts taken from 100 subjects with an age range from foetus to 90 years. This showed that the growth capacity of human fibroblast cultures declined by approximately 0.2 population doublings per year of donor age.

Studies of this type have been carried out frequently but, because the analysis is indirect (and the number of donors relatively small), they remain open to the criticism that factors other than the presence of senescent cells (e.g. differential resistance to stress or tissue autolysis) are really responsible for the differences in culture lifespans observed. However, in 1995 it was shown that a modified colorimetric assay for β-galactosidase activity could distinguish between senescent, pre-senescent and quiescent fibroblasts, endothelial cells and keratinocytes (Dimri et al 1995). This allowed the direct visualization of senescent cells in skin samples and their frequency in samples from donors of different ages was promptly investigated. An age-dependent increase in the number of β-galactosidase-positive dermal fibroblasts and epidermal keratinocytes was observed. The methodology used in this study has been subjected to some criticism (Severino et al 2000) but it remains the first to provide direct evidence that senescent cells both exist and accumulate with ageing in-vivo. However, establishing the presence of senescent cells in tissues does not also establish that they have harmful effects.

Werner's syndrome and cellular senescence. Probably the strongest evidence that senescent cells can exert degenerative effects in-vivo is provided by a body of work on the human premature ageing disorder Werner's syndrome (Kipling et al 2004). Werner's syndrome first becomes apparent at 15–30 years of age; the patients show short stature, premature greying of the hair, progressive hair loss, mild diabetes, atherosclerosis, arteriosclerosis, osteoporosis and cataract formation. The subcutaneous adipose tissue is also depleted, resulting in tight, shiny scleroderma-like skin on the face and extremities. Poorly healing ulcers develop over pressure points on the limbs (particularly the legs), which also show poor muscular development. Death occurs at an average of 47 years, usually from arteriosclerosis or cancer. Werner's syndrome is caused by loss of function mutations in a member of the RecQ family of DNA helicases. Quite how loss of a DNA unwinding enzyme gives rise to the diverse set of changes seen in Werner's syndrome remained unclear for several years. However, an extensive series of studies involving both human patients and transgenic mouse models of the disease have demonstrated firstly that the loss of the wrn helicase greatly accelerates the rate of senescence in many normal human cell types and, secondly, that wrn knockout mice show no premature ageing features (and no premature senescence) unless they are first engineered to have human-like replicative senescence mechanisms (Faragher et al 1993; Lebel & Leder 1998; James et al 2000; Chang et al 2004). Taken together, these data come as close to formal proof as the scientific enterprise allows that filling a tissue with senescent cells produces a phenotype very similar to ageing.

Although work on Werner's syndrome shows that a tissue with a high proportion of senescent cells can show serious ageing features, it remains a point of legitimate dispute whether there are enough senescent cells present in the tissues of the normal elderly for this to be a normal ageing mechanism. This is currently a difficult question to answer because it requires two things – a histological marker that would allow the ready determination of the frequency and distribution of senescent cells in tissue and an idea of how many such cells is sufficient to cause a defect.

Does oxidative stress really contribute to ageing and, if so, how?

One primary problem with the oxidative damage theory is that an explanation for how damage and alterations to intracellular components causes organismal ageing is often omitted. However, the implicit (and sometimes explicit) thrust of most writers is that oxidative stress is causing cell death. More sophisticated variants on this theory propose that oxidative stress leads to an accumulation of viable but damaged cells with impaired functions that play a causal role in the ageing of tissues.

Given that oxidative damage is often intemperately espoused as an alternative unprogrammed (and by definition more convincing) explanation of ageing than programmed replicative senescence, the invocation of altered phenotypes and cell death as causal players in the oxidative damage theory is ironic. Terminally damaged cells usually remove themselves from the population by programmed cell death (apoptosis); if they do so, the lost cells must be replaced if possible. This requires cell division by the adjacent cells (assuming the tissue in question has mitotic potential). Since cell division is monitored this must lead to an attendant production of senescent cells. Thus, oxidative stress could cause

ageing simply by producing cell turnover and triggering replicative senescence. However, some cells subjected to oxidative stress do not die, they simply enter a non-dividing and transcriptionally altered state so similar to the cellular senescence triggered by short telomeres that it is sometimes called stress-induced premature senescence (SIPS, see Toussaint et al 2002; de Magalhaes et al 2004). SIPS (and normal senescence) are so similar to the viable-but-damaged cells proposed as mediators of the effects of oxidative stress on organisms that the difference between them becomes largely semantic. It is to be hoped that further research will determine the degree of linkage between replicative stress, oxidative stress and organismal ageing. However, in the meantime, simply determining whether or not stressed and senescent cells display an altered responsiveness to pharmacological agents is a valuable area of study that may explain many aspects of the altered pharmacology seen in the elderly.

Conclusion

The administration of pharmacological agents to older persons often results in a higher incidence of drug toxicity and adverse drug reactions compared with the young. This is mainly due to changes in pharmacokinetic and pharmacodynamic properties believed to be the result of biological alterations linked to the ageing process. Therefore, understanding the mechanisms of ageing, the biological alterations they bring about and the biological consequence of such alterations could help answer questions concerning the pharmacokinetic and pharmacodynamic changes observed in the elderly. By bridging the gap between pharmacokinetic and pharmacodynamic studies and molecular gerontology it is hoped that pharmaceutical intervention might one day be more precisely targeted to the age of the patient (and thus, the biological status of the target tissue). It is anticipated that the development of in-vivo and in-vitro models of tissue ageing will facilitate the necessary advances in pharmacogerontology.

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