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Aging Biology and Novel Targets for Drug Discovery

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Despite remarkable technological advances in genetics and drug screening, the discovery of new pharmacotherapies has slowed and new approaches to drug development are needed. Research into the biology of aging is generating many novel targets for drug development that may delay all age-related diseases and be used long term by the entire population. Drugs that successfully delay the aging process will clearly become "blockbusters." To date, the most promising leads have come from studies of the cellular pathways mediating the longevity effects of caloric restriction (CR), particularly target of rapamycin and the sirtuins. Similar research into pathways governing other hormetic responses that influence aging is likely to yield even more targets. As aging becomes a more attractive target for drug development, there will be increasing demand to develop biomarkers of aging as surrogate outcomes for the testing of the effects of new agents on the aging process.

DRUG DISCOVERY

There have been 1,222 new chemical entities (NCEs) approved by the Food and Drug Administration for use as pharmaceutical therapies since 1950 (1). Only 8% of candidate's NCEs developed at the bench are eventually approved and enter the market place after surviving a process of drug development that takes an average of 13.5 years (2). Despite the promise of genetic technologies, proteomics, and high throughput screening, there has not been any increase in the rate of NCEs gaining marketing approval. Therefore, there are growing concerns about the future viability of the current model of drug development (1). The therapeutics industry is searching for new biological targets and new approaches for generating NCEs and novel pharmacotherapy. Aging biology is a largely untapped field for the development of effective new pharmacotherapies. Aging also represents a "blockbuster" market because the target patient group includes potentially every person. Furthermore, profits currently generated by so-called antiaging products show that humans are very willing to pay for chronic medical therapy in order to delay the aging process. Thus, there are many compelling reasons why biogerontology should be a major focus for drug discovery.

The first step in drug development is the selection of a "druggable" and validated target (3). Having a gene target where there are known genetic variations associated with loss-of-function and gain-of-function phenotypes is particularly useful because the targets can be considered to have been validated (2). There are many genes that are known to influence the aging phenotype (4–6), and all these represent potential drug targets. Once a target has been developed, drug discovery in recent decades has relied on extensive screening of chemical libraries to detect compounds with activity against the target. Commercial libraries including either combinatorial or natural products can now exceed one million different compounds. Furthermore, it has been estimated that there might be as many as 10^{40}–10^{100} possible small compounds that are potential drugs (7). Until now, the major enzyme targets that have generated pharmacotherapies include kinases, proteases, phosphatases, oxidoreductases, and transferases, whereas cellular targets include G protein–coupled receptors, nuclear hormone receptors, and some ion channels. Although there are 20,000 genes and around 100,000 proteins in humans, only 324 targets have yet yielded approved drugs (8). Of these, only 266 are human genome–derived proteins,

whereas the rest are microbial targets (9). Academic screening technology is likely to be more innovative. Screening libraries of commercially available compounds are readily available to academics, whereas proprietary libraries are not and mechanisms for access will need to be developed if the industry wishes academia to play a significant role in discovery. Natural product diversity is not captured by industry, and the best natural product collections are within well-organized academic collections. For those rare and fortunate academic researchers who discover a new therapy, deals involving drugs taken to phase I clinical trials averaged US$16 million, whereas those taken to successful phase II trials averaged US$50 million in upfront payments (10). Despite the potential profits and the extraordinary capacity of drug discovery technology, there is a paucity of new drugs in the development pipeline, particularly for those medications that are likely to be highly profitable because they are used long term and by a large proportion of the population. Aging and the "longevity dividend" provide an opportunity to revitalize the drug development pipeline.

THE "LONGEVITY DIVIDEND"

There is accruing evidence for compression of morbidity (11). This means that more people are achieving older ages with less functional impairment and disease burden. This is presumably the result of better health care, preventative medicine, and healthier lifestyles. For example, it has been reported that over the last few decades, every 1% reduction in mortality of older people has been associated with a 2% reduction in disability (11). Old age and the underlying aging process are now considered to be the major independent risk factors for diseases, such as atherosclerosis, arthritis, cancer, and dementia in the Western world (12,13). A consequence of these two trends is that there are now many competing risks for death and disability in very elderly people. Therefore, any benefits from the treatment and diagnosis of a single illness on life expectancy in older people are marginal (14). The typical older person has many comorbidities and chronic illnesses, and treatment or prevention of one illness simply leaves them immediately susceptible to the effects of other illnesses.

The alternative approach to the prevention of disease in older people promoted by Olshansky and colleagues (12,13) has been termed the "longevity dividend." By delaying the aging process, it should be possible to delay all age-related diseases and disabilities rather than attacking them one by one, which is the approach of the current disease-based process of drug development. The development of new drug targets based on aging biology represents a priority for humans and a major opportunity for the pharmaceutical industry (15). However, the issue is whether this can be achieved in a timely fashion, given that evaluation of longevity in humans requires decades and the development of pharmacotherapies for standard diseases already takes more than a decade. The solution will probably emerge from improved access to short-lived animal models for aging interventions coupled with the development of measurable biomarkers of aging in humans.

THERAPIES BASED ON REVERSING AGE-RELATED CHANGES

To date, most "antiaging" therapies have been based on the simple but compelling assumption that all the biological processes that accompany aging are harmful and somehow mechanistically linked to aging. The therapeutic corollary of this assumption is that the treatment and prevention of any biological changes of aging will generate beneficial outcomes (16).

Age-related oxidative stress and the use of antioxidants are a prototypical example of this approach. The free radical theory of aging was first postulated by Denham Harman in 1956 (17). It has been since well established that old age is associated with increased generation of free radicals, mostly from mitochondria, and with increased markers of oxidative injury, such as lipoperoxides, carbonylated proteins, and oxidized DNA (18–20). From as early as 1961, Harman (21) and many others have attempted to delay aging using antioxidants or manipulating antioxidant genes (22,23). Results have been variable; however, recent meta-analyses of antioxidant clinical trials in humans (none of which were specifically designed to delay aging) show that antioxidants do not improve life expectancy and might even increase the risk of premature death (24,25).

There are reductions in many hormones in old age (26), and the use of hormone supplements to delay aging is extensively used and advocated as an antiaging therapy. Growth hormone (GH) declines with old age, and some features of aging, particularly the changes in body composition and sarcopenia, are similar to those seen in people with GH deficiency (27). Yet supplementation of GH in older people has had few beneficial effects and none on the aging process (28–30). Moreover, mice and humans with defective GH receptors have an increased life expectancy (31), which suggests that GH deficiency rather than GH supplementation might extend longevity.

Similarly, serum testosterone concentrations decline in old age in men, and supplementation with testosterone is widespread (32,33). However, clinical trials have failed to provide convincing evidence for health benefits or delaying aging (32,34), and a recent study was terminated early because of increased cardiovascular events in the older men receiving testosterone supplementation (35). Supplementation with dehydroepiandrosterone has not generated any health benefits in old age despite the age-related decline in its levels (34,36). Although there are declines in many hormones with old age, it should be noted that Everitt showed long ago that hormonal depletion in rats by hypophysectomy paradoxically increased life span and delayed age-related changes (37).

The rationale that every biological (or medical) change with old age is necessarily deleterious and contributes to the harmful aspects of aging has been questioned recently. Indeed, there are evolutionary selection pressures to extend postreproductive life span mediated by the so-called "grandmother effect." Therefore, some age-related changes might actually represent useful adaptations in terms of increasing life span. For example, both GH depletion (31) and oxidative

stress (38) that occur with aging potentially increase life expectancy. This controversial concept has been called "adaptive senectitude." The therapeutic consequence of adaptive senectitude is that rather than attempting to reverse some age-related biological changes, there may be benefit in enhancing such changes (16). Apart from GH depletion, this therapeutic approach has not been explored from the interventional point of view. It should also be acknowledged that failure of some of these therapeutic strategies might also be due to poor evidence that any of the targeted physiological declines are actually causally related to aging.

THERAPIES BASED ON CALORIC RESTRICTION

Reduction of food intake by 20%–50% (CR) increases longevity in many species from yeast to nonhuman primates (39,40). It is generally concluded that this effect represents a genuine effect of CR on the aging process because most of the phenotypic and pathological features of aging are delayed. Some studies on the effects of CR on aging have given conflicting results, and there are concerns about the possible proaging effects of control diets in experimental animals and also the extent to which calories per se rather than nutrient balance play a role (41–43). Even so, CR has become the main focus of experimental therapies to delay aging (39,44). Because of the difficulty in maintaining a CR diet for humans, there have been attempts to develop drugs that replicate the effects of CR, the caloric restriction mimetics (CRM) (39,44,45). One of the important issues in the assessment of CRMs is to determine an effect on food intake in order to confirm that the CRM is having an effect on the biological effectors of CR rather than simply suppressing appetite.

The cellular mechanisms for the beneficial effects of CR are gradually being unravelled. In the original studies on CR by Clive McCay in the 1920s and 1930s, it was hypothesized that CR worked by slowing development (46–48). With Harman's free radical theory of aging came the concept that CR worked by reducing oxidative stress generated by food intake (19). Subsequent studies confirmed that CR is associated with reduced markers of oxidative stress and reduced production of free radicals. In 1989, Robin Holliday proposed that the effects of CR might represent an evolutionary response to survive periods of famine. Maintained over the long term, this response will become manifest as delayed aging and increased longevity (49). This changed the mechanistic view of CR from that of a passive response to an active one where there is a metabolic sensor activated by changes in nutritional intake. If drugs could be developed that activate such a switch, then the health benefits of CR could be generated without the need for reduced dietary intake (50). It is likely that CR operates via the same tangle of pathways that also control appetite, thermoregulation, immunity, tissue repair, and metabolism. Even so, there are four main pathways that are thought to be potential targets for CRM drug development because they act as key switches between nutritional status and the beneficial effects of CR: insulin/insulin-like growth factor-1 (IGF-1), sirtuins, target of rapamycin (TOR or mTOR in mammals), and 5' adenosine monophosphate-activated protein kinase (4,50–53).

Insulin, Glucose, and IGF-1

Hyperglycemia and hyperinsulinemia are common in old age. Conversely, CR leads to reduced glucose, insulin, and IGF-1 concentrations, with an increase in insulin sensitivity (54). Moreover, genetic manipulation of the insulin/IGF-1 pathways in *Caenorhabditi elegans* and mice has been found to alter longevity (4,52). Therefore, initial attempts to develop CRM focused on these pathways.

The first CRM, and indeed the concept of CRM, was published in 1998 (55). 2-Deoxyglucose was chosen as a candidate CRM because it is an inhibitor of glycolysis, and there had been some earlier reports showing that it reduced body temperature and tumors. Rats fed 0.4% 2-deoxyglucose had reductions in body weight and insulin concentrations, consistent with the effects of long-term CR. However, a long-term study showed the 0.4% 2-deoxyglucose ingestion increased mortality by 45% in rats secondary to cardiac toxicity and adrenal tumors, despite replicating many of the metabolic features of CR (56).

Insulin sensitizers including the antidiabetic drugs, biguanides (metformin and phenformin), and thiazolidinediones (rosiglitazone and pioglitazone) have also been proposed as CRMs (39,54). Microarray profiling has shown similarity between the gene expression pattern in CR and that generated by treatment with rosiglitazone and metformin (57). The biguanides are also 5' adenosine monophosphate-activated protein kinase agonists, which is an additional advantage in terms of CR activity (58). Phenformin increases life span in rodent studies; however, toxicity related to lactic acidosis precludes its use in humans (54). Although metformin was beneficial in various rodent disease models, a recent study did not find any longevity benefits from metformin in F344 rats (59).

Although 2-deoxyglucose and phenformin showed promise in animal studies, both have revealed significant toxicity with long-term therapy. Such an outcome is unfortunately typical in drug development where toxicology and clinical safety are the major reason for attrition of NCEs during the drug development process (60). Given that any therapy acting on aging will undoubtedly need to be administered chronically, evaluation of toxicity will be especially important for drug development. Adverse drug reactions will be a major hurdle for any effective aging drug that is intended for lifelong primary prevention in the entire population.

Sirtuins

The role of the sirtuin pathway in CR was originally established as part of aging studies in yeast (53). It was found that CR generated by diluting the glucose in

the growth media increased longevity of yeast. Life-span extension from CR was not observed in yeast strains mutant for *SIR2* or *NPT1* (involved in the synthesis of NAD). It was concluded that the increased longevity induced by CR requires the activation of Sir2 by NAD, where NAD directly reflects energy availability (61). Subsequently, a high throughput screen was performed against the mammalian sir2 homolog, SIRT1. A number of compounds including the naturally occurring phytoconstituents quercetin, piceatannol, and the red wine polyphenol resveratrol were reported to be SIRT1 agonists (62). Resveratrol was subsequently shown in some, but not all studies, to increase longevity in yeast (62), *C. elegans* and Drosophila (63). Resveratrol administration was found to prevent insulin resistance, improve mitochondrial function, prevent fatty liver, replicate transcriptional changes seen in CR, and restore normal longevity in obese mice (64). In nonobese mice, resveratrol delayed many age-related physiological changes and diseases but did not increase longevity (65). Recently, it has been reported that resveratrol improved insulin resistance and fatty liver but not life span in a mouse model for the premature aging syndrome, Werner syndrome (66). Further high throughput screening has identified a number of other SIRT1 agonists. One of these, SRT1720, improved insulin resistance and reduced glucose concentrations in rodent models of diabetes mellitus (67). It should be noted that there is some uncertainty about the specificity of the assay used in the high throughput screening and whether the sirtuin pathway is essential for the effects of CR in all species (53). Even so, recently, it has been reported that *sirt1* null mice have reduced benefits from CR (68) and resveratrol (69).

mTOR

Target of rapamycin (TOR and mTOR in mammals) is a nutrient sensor and regulator of growth found across taxa from yeast to humans (70). In the presence of sufficient nutrient and energy availability (such as amino acids, oxygen, glucose) and growth factors, TOR switches on cell growth pathways, such as translation and ribosomal biogenesis, and switches off autophagy and stress resistance (51,71,72). The observation that amino acids activate TOR may provide a mechanism for the recent findings that reducing the proportion of protein in the diet, rather than CR per se, has a longevity-enhancing effect, at least in drosophila (41) and some other experimental animal models (42,43).

5' adenosine monophosphate-activated protein kinase, sirtuins, and insulin/IGF-1 may all act at least in part through the mTOR pathway, and increasingly, it seems that mTOR is a central hub for cell signalling that matches nutrient availability with cell growth. Downregulation of TOR in yeast, *C. elegans* and Drosophila, increases life expectancy and diminishes any further responses to CR (51,71,72).

Downstream from mTOR is a signalling pathway that includes regulators of translation, S6 Kinase and 4E-BP,

which have also been found to be important for the CR response and as such are potential targets for CRM (71). Inactivation of the TOR pathway reduces protein synthesis. Overall, it seems that reduction of protein synthesis leads to an increase in life expectancy in experimental models, yet protein synthesis declines with age (71). In passing, it should be noted that this may be another example of adaptive senectitude (16)

The main antagonist of mTOR is rapamycin (sirolimus). Mice orally administered rapamycin from 20 months of age had increased life span (14% females and 9% males), and a similar trend was seen when administered from 270 days (73). With rapamycin blood concentration of 60–70 ng/mL, there was effective inhibition of phosphorylated rpS6, which is a downstream substrate of the S6 Kinase. A recent study of resveratrol, simvastatin, or rapamycin administered to mice from 9 months of age reported that only rapamycin was associated with an increase in life expectancy (10% in males and 18% in females) (74). Given that rapamycin is a potent immunosuppressant (inhibiting the response to interleukin-2 and thereby blocking activation of T- and B-lymphocytes (75)), it is unlikely that it will enter the human market place as an aging drug; however, rapamycin does provide the proof in principle that targeting CR pathways influences life expectancy. Rapamycin is currently the most promising CRM, and new analogues of rapamycin, rapalogs (76), are being developed but at this stage mostly as chemotherapies. Whether rapamycin influences aging in rodents by influencing CR pathways, the development of hematological malignancies or inflammaging remains to be resolved (72–74).

THERAPIES BASED ON THE BROADER CONCEPT OF HORMESIS

Hormetic agents are those that are unexpectedly beneficial at low doses, whereas toxic at higher doses. The concept has recently been applied to aging and longevity by Masoro (77) and Rattan (78) among others. CR can be considered to be hormetic in that low "doses" (30%–50% CR) increase longevity, whereas high "doses" cause starvation and death (and indeed, the same applies to overnutrition). It seems that animals have evolved to respond to many environmental stressors by increasing their resilience against numerous other stressors and toxic insults. Although these responses have no doubt evolved to survive short periods of environmental stress, it appears that when they are maintained long term or applied at responsive periods of life that there is an effect on delaying aging and increasing life span. Hormetic factors that have been shown to have a positive impact on longevity in some experimental animals include heat shock, oxidative stress, irradiation, alcohol, and some phytochemicals (38,78–82). By determining the biological pathways involved in mediating these hormetic responses, it might be possible to discover new targets for drugs that act on aging (15).

DRUGS WITH MODIFIED OR MULTIPLE TARGETS

The traditional drug discovery approach has been based on a "one-target one-disease" model that searches for agents with high specificity and high affinity. However, systems biology is showing that many cell processes are based on multiple low-affinity/low-selectivity reactions that generate flexibility and redundancy (83). Such complex processes are likely to be involved in the pathogenesis of aging and many diseases too. Therefore, it might be useful to screen for agents that modulate multiple targets simultaneously, that is, "dirty" or "promiscuous" drugs. For example, many effective psychotropic drugs are low-affinity agents that act on multiple receptors, including those for serotonin, dopamine, and acetylcholine (83). Similarly, the beneficial clinical effects of resveratrol might be mediated by its multiple actions (84) rather than the debated effect on a single target, SIRT1. Alternatively, it has been proposed that delaying aging might require treatment with multiple medications that act on several different targets (39), a sort of aging "polypill." However, if aging pathways are involved in many processes, such as immunity, tissue repair, growth, control of appetite, and metabolism, then manipulating any specific target might have multiple unexpected side effects, making it difficult or perhaps impossible to generate specific antiaging outcomes.

Aging is associated many posttranslational changes in proteins, and this is considered to contribute to the aging phenotype. Nonenzymatic glycosylation of proteins is typical of aging across the taxa (85), and it is possible that such posttranslational modifications might influence interaction with drugs. Thus, high throughput screening against modified proteins might yield new therapies too.

What screening strategies could be employed to identify such "dirty" targets? One option could be to perform multiple parallel screens against range of targets in order to identify compounds with broad activity. In addition, "black box" screens against longevity in model organisms such as yeast and *C elegans* (86,87) or other surrogate phenotypes in cells such as stress resistance might be considered.

EVALUATING NOVEL AGENTS THAT ACT ON AGING

Evaluating the effect of any drug that influences aging requires an endpoint to be measured, which must either be life span, health span, or biomarkers of aging. These endpoints are challenging. Life-span studies of course take a long time, particularly in higher animals and humans. Early proof of principle studies have been greatly enhanced by the development of the Intervention Testing Program by the National Institute on Aging. This was initiated 7 years ago to rigorously assess the effects of compounds on longevity using three colonies of genetically heterogeneous mice (88). Clinical studies in humans are unlikely to use longevity as an initial primary outcome; therefore, some sort of surrogate outcome or biomarker of aging is necessary (89). As yet, there is no established set of biological or clinical biomarkers of aging (90). It has been proposed that any useful biomarker of aging should fulfill a variety of criteria, including the following:

1. it must predict the rate of aging and be a better predictor of life span that chronological age alone,
2. it must monitor a basic process that underlies the aging process, not the effects of disease,
3. it must be able to be tested repeatedly without harming the person, and
4. it must be something that works in human and in laboratory animals (90–92).

It is possible that a suite a parameters including clinical and blood measurements will be developed as a surrogate outcome for aging that can be utilized in clinical trials. Composite outcomes and clinical scales are not uncommon in human trials and have been accepted by regulatory bodies, such as the Food and Drug Administration. On the other hand, regulatory bodies and industry might prefer to use outcomes such as delay in the onset of an age-related diseases or frailty rather than aging per se because these types of clinical outcomes are well accepted (93).

It is most likely that drugs that influence aging will be commenced in middle age before the onset of irreversible aging changes. However, it is probable that such therapy will be continued into old age. Old age is associated with increased risk of adverse drug reactions secondary to age-related changes in pharmacokinetics and pharmacodynamics (94,95). Therefore, clinical trials on aging therapies will need to focus closely on collecting data on adverse drug reactions as well as assessing efficacy.

Given the importance of functional outcomes and independence for older people, it is likely that clinical outcomes in trials of drugs that influence aging will need to include functional geriatric outcomes, such as onset of frailty and dependency.

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