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Rules for the use of model organisms in antiaging pharmacology

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Summary

The use of animal models for initially screening antiaging drugs is a promising approach for drug discovery. However, there a number of potential artifacts, confounds and errors that can arise in such research programs. The following rules are intended to minimize such problems: (1) since aging occupies an increasing proportion of human adulthood, data that conflate aging and late life should not be extrapolated to human aging; (2) the response to candidate medications should show a normal dose-response pattern, although not necessarily a linear response; (3) medicated animal models should not be hypometabolic; (4) medicated animal models should not show pronounced reductions in fertility; (5) medicated animal models should not exhibit general nervous system depression; (6) the effect of the medication should not be highly sensitive to the culture environment; (7) the effect of the medication should not be highly dependent on the genetic ancestry of the stock employed, leaving aside inbreeding, which should be avoided because humans are not generally inbred. While these rules do not guarantee successful extrapolation of successful drug results from the animal model to humans in a clinical setting, the failure to adhere to these rules should raise doubts about such extrapolation.
Key words: antiaging; *Drosophila*; drug discovery; mortality; pharmaceuticals; pharmacology.

Introduction

We need appropriate methodologies to study the pharmacology of aging in model species if experimental findings with such systems are to be extrapolated to the treatment of human aging. While testing drugs affecting aging has already started using the established model organisms *Drosophila melanogaster* and *Caenorhabditis elegans*, the fundamental biological issues involved in such screening have not been systematically formulated. We attempt this task here.

Some of our concerns are illustrated by *Drosophila* and *Caenorhabditis* mutants that show increased longevity (Maynard Smith, 1959; Lin *et al*., 1998; Rogina *et al*., 2000; Tatar *et al*., 2001; Marden *et al*., 2003). The biological interpretation of the longevity increases exhibited by these mutants has not been settled. For example, there has been a controversy concerning the status of *C. elegans* mutants with increased lifespan. In some laboratories, long-lived *C. elegans* mutants maintained their metabolic rate (Braeckman *et al*., 2002), while in others these mutants show increased longevity in conjunction with reduced metabolic rate (Van Voorhies & Ward, 1999). These observed differences in metabolic rates could be due to differences in measurement techniques and environmental conditions. If, however, the genetic mutations that increase longevity in *C. elegans* reduce metabolic rate, then these mutants may modulate lifespan by tuning metabolic activity, a discovery well known in research with poikilotherms since 1917 and of limited interest for the purposes of drug development (Finch, 1990; Rose, 1991). By contrast, *Drosophila* bred for postponed aging have no reduction in metabolic rate and a substantial increase in their lifelong metabolism (Rose, 1984; Djawdan *et al*., 1996; Rose *et al*., 2004). This kind of issue is important in establishing rules for the use of model systems in screening candidate antiaging medications, as we will discuss below. However, our present concern is only to point out that longevity data, in and of themselves, are not to be taken at face value.

Fruit flies and humans share a vast number of key metabolic pathways such as superoxide metabolism, DNA repair and insulin-like signaling. Many of these pathways are already considered candidates for pharmaceutical modulation (Melov *et al*., 2000, 2001; Lonn *et al*., 2002; Evason *et al*., 2005). However, the treatment of aging is highly likely to involve secondary and nonadditive effects, given the multifold pathways that affect aging (Fleming *et al*., 1993; Pletcher *et al*., 2002; Rose & Long, 2002). Therefore, the most reasonable expectation is that particular pharmaceuticals that affect aging may do so through multiple pathways, not just the pathway that is of a priori interest. Most importantly, it should not be presumed at the outset of pharmacological research that a drug that is known to affect a particular biochemical pathway of interest *only* has such effects.

Given the potential complexities of antiaging pharmacology in model systems, particularly from the standpoint of appropriate interpretation, we will bring to bear concerns that come from evolutionary biology, quantitative genetics, and clinical pharmacology, all of which routinely deal with multiple effects, including problems of unanticipated side-effects. This knowledge-base will suggest important caveats that have

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not traditionally been the concern of biochemists, molecular geneticists or cell biologists, among the disciplines that have routinely studied aging in model systems in the past.

We formulate our thoughts in terms of rules of investigation. We do not propose that these rules are exhaustive or absolutely preemptive. Instead, we offer them as a starting point for discussions of in the development of appropriate pharmacological protocols for the study of medications that might ameliorate human aging. Here we refer to such medications as 'antiaging compounds' for the sake of brevity.

Rule 1: Experimental trials should study the effects on mortality rates during aging only

It is perhaps obvious that studies of antiaging compounds should not incorporate effects on the duration of life that arise from the prolongation of development. Humans are extremely unlikely to give drugs to children that prolong their preadolescent life, for a host of ethical and practical reasons.

But this is not the only important proscription for the development of antiaging compounds. It is now known that there are at least three phases in life histories: development, aging and late life (Rose *et al*., 2005). In some organisms such as fissile sea anemones and *Hydra*, life cycles involve development proceeding to an act of reproduction, fission that then immediately initiates a new phase of development. Aging does not occur (Martinez, 1998), nor late life. In some other organisms, such as Pacific salmon and the marsupial *Antechinus*, there is a spectacular period of aging which proceeds so rapidly that no individuals survive after the aging phase. But in many animals, we now know, there is a period after aging during which mortality and fecundity plateau (Carey *et al*., 1992; Curtsinger *et al*., 1992; Vaupel *et al*., 1998; Rauser *et al*., 2003). That is, aging stops, and a new phase of life begins (Rose *et al*., 2005; Rauser *et al*., 2006).

The practical importance of this observation for pharmaceutical intervention in aging is that model organisms like fruit flies and nematodes have late-life periods that may be attained by 30–50% of the animals in an experimental cohort. Calculating mean or maximum longevity differences arising from a drug trial confounds effects on aging with effects on late life. This might not seem like a particularly important problem, since ideally one would like to ameliorate the deficits associated with both human aging and late life, as both phases are well known to occur in humans too (Greenwood & Irwin, 1939; Vaupel *et al*., 1998).

The problem is that late life arises very late in the human life cycle, starting only in the 90s, at ages when very few people are left alive (Vaupel *et al*., 1998). This situation is radically different from that affecting *Drosophila*, for example, for which a large proportion of the individuals in a laboratory cohort may survive past the end of the aging phase (e.g. Rose *et al*., 2002).

The solution to this problem is simple: collect data on age-specific mortality effects during the aging phase of the particular model species. We now have objective statistical procedures for estimating the age at which late life starts (Drapeau *et al*., 2000; Rose *et al*., 2002). These techniques can be used to delimit the period during which aging occurs, and statistical estimation of age-specific death rates from such periods can be used to estimate rates of aging without the collection of data from late life (Mueller *et al*., 1995). As a fringe benefit, this restriction of drug trials to the aging phase allows an acceleration in the speed with which antiaging compounds can be screened, because the termination of a drug-screening experiment does not have to wait for the death of the last organism to survive in a cohort.

Rule 2: The dose-response pattern should be estimated

Dose-response relationships are very important in experimental pharmacology. In an ideal setting, the measured therapeutic effect or response should reflect the dose and the response should be quantified at the level of the appropriate molecular, cellular, or organismal phenotype. Unfortunately, clinical pharmacology does not often conform to this ideal. For the majority of drugs, a dosing range that results in a therapeutic concentration is defined. Doses below this range are inefficacious and doses above this range are toxic. But even establishment of a dosing regimen and a therapeutic window can be challenging. For instance, digoxin, a commonly used drug for the symptomatic treatment of congestive heart failure, has a very narrow therapeutic window. Until recently, 0.5 to 2.0 ng mL^{-1} was considered the effective serum drug concentration for digoxin. After a number of clinical trials with thousands of patients, clinicians realized that the optimum drug concentration for digoxin is 0.5 to 0.8 ng mL^{-1}. Investigators observed that serum concentrations above 0.8 ng mL^{-1} can result in increased mortality (Rathore *et al*., 2003). Although dose-response relationship is an important concept, clinical pharmacology still lacks reliable quantitative data about the effects of antiaging drugs on humans. In the absence of such data, it is possible that a bigger dose of a particular antiaging compound may not necessarily mean a bigger phenotypic response. Dose-response data at best should allow reliable interpolation for doses that have never been tested.

Drug trials should aim to determine a robust dose-response relationship between the antiaging compound and a measurable aging phenotype. Unfortunately, the pharmacology of candidate antiaging compounds, even in *Caenorhabditis* and *Drosophila*, is not yet well defined. There are a few studies in the literature where these organisms were fed such compounds and the pharmacological properties of these compounds were assessed by the phenotypic response elicited (Kang *et al*., 2002; Wood *et al*., 2004; Evason *et al*., 2005). However, dose-response relationships have not been established for antiaging compounds in these animal models.

As in human pharmacology, the dose-response relationship that we should determine when screening for antiaging compounds will not necessarily show that a higher dose results in a better response. Our rule simply says that we should determine the dosing range within which a therapeutic response occurs.

Rule 3: Medicated animals should not be hypometabolic

The controversy surrounding the metabolic rates of longer-lived *C. elegans* mutants has already been mentioned. We are not concerned with the resolution of this controversy here. Our concern instead is that compounds which substantially lower metabolism in the course of increasing longevity should be identified as such, and normally excluded as potential human medications. It is well established that the lifespan of poikilotherms can be extended or curtailed as metabolic rates are decreased or increased, respectively (Finch, 1990; Rose, 1991). As humans are homeotherms with fairly stable metabolic rates, drugs that act via gross lowering of metabolic rates, producing hypometabolic syndromes, are not appropriate candidates for antiaging interventions. In addition, metabolic rate could be useful as a surrogate measure for a number of physiological and behavioral phenomena that might be affected by medications.

Thus this rule requires the estimation of metabolic rates, preferably over multiple ages during the aging phase, among test cohorts and their controls. Animals showing a significant decrease in age-specific death rates during aging as a result of treatment should not show a gross lowering of metabolic rate over a range of ages (see Djawdan *et al*., 1996). However, minor reductions in metabolic rate, especially when such reductions are of short duration, are not of particular concern. They could arise from shifts in the allocation of metabolic energy that do not interfere with most functional activities (see Service, 1987).

Rule 4: Candidate antiaging compounds should not greatly curtail fertility

Almost as well established as the relation between metabolic rate and lifespan is the principle that lowering fertility can increase longevity (Finch, 1990; Rose, 1991). For example, this trade-off is a key element in the beneficial effect of caloric restriction (Weindruch & Walford, 1988; Graves, 1993; Phelan & Rose, 2005). It is a very general, though not strictly universal, finding that castration and similar interventions that curtail or greatly limit reproduction can significantly extend longevity.

Compounds that substantially lower fecundity may increase longevity from such 'cost of reproduction' effects alone. That is, reproduction can be such a great physiological burden for an organism, particularly among females, that reducing or eliminating it is generally expected to yield an increase in adult survival rates.

Compounds that act via reproductive impairment, in whole or in part, are not promising candidates as treatments for human aging. Generally, medications can impair reproduction via either sexual dysfunction or infertility. Patients who wish to have children are not likely to accept such effects before the end of their reproductive years.

One of the major adverse effects associated with antidepressants is sexual dysfunction. Based on a National Ambulatory Medical Care Survey (NAMCS), the use of new antidepressants such as SSRI (selective serotonin reuptake inhibitor) and newer non-SSRI has increased from 2.7% in 1989 to 7.1% in 2000 (Pirraglia *et al*., 2003). A cross-sectional and observational study that used a validated scale, the 'Changes in Sexual Function Questionnaire', was performed on 4534 women and 1763 men to assess the prevalence of sexual dysfunction among patients taking the newer antidepressants. The overall prevalence of sexual dysfunction was 37% (Clayton *et al*., 2002). This side-effect not only influences the patient's adherence to the therapy, but it also complicates the course of treatment.

Infertility is also a common side-effect of many drugs. In humans, fertility is largely regulated by the hypothalamus-pituitary-gonadal axis. The secretion of a number of reproductive and sex hormones, such as follicle stimulating hormone, luteinizing hormone, and testosterone, is controlled by this axis. Infertility could also be due to direct cytotoxic effects of drugs on ovaries, uterus, or testes. For instance, a number of antineoplastic drugs, steroids, and hormones (e.g. estrogen products) could impair reproductive capacity. Although, medication-induced infertility is often reversible, there are reports of irreversible drug-induced infertility in the literature arising from the aforementioned drugs (Buchanan & Davis, 1984).

Female fecundity is one of the easiest characters to assay, where reproductive function is concerned. Gross depression of fecundity is not associated with evolutionarily postponed aging (Rose, 1984; Leroi *et al*., 1994; Rose *et al*., 2004), but it *is* associated with dietary restriction sufficient to significantly increase *Drosophila* longevity (Chippindale *et al*., 1993). If we consider the case of antiaging compounds that act by the same pathway(s) as dietary restriction, they may act, in whole or in part, through reduced fecundity. This should be easy to ascertain by comparing the fecundity of treated females with that of untreated control females. When generally lower fecundity arises, the prospects for the development of usable human antiaging compounds may be dim.

Rule 5: Medicated animals should not exhibit general nervous system impairment

The impairment of nervous system function is a common side-effect associated with pharmaceutical agents and will often result in noncompliance to medications. For instance, a number of drugs that are used for pain management cause oversedation which hinders daily activities, especially in the workplace. Drugs that decrease intellectual and physical performance ultimately may result in injuries due to falls among the elderly. In addition, these drugs may also result in cognitive disorders such as delirium, confusion, and memory impairment. Antihistamines are a class of drugs that are highly prescribed for the treatment of rhinitis associated with allergies. Drug-induced sedation and its effects on cognition are considered major limiting factors for the use of first- and second-generation antihistamine (Ng *et al*., 2004). Drugs that result in nervous system

depression often display this side-effect in a dose-dependent fashion. The higher doses that are often needed to achieve a therapeutic response can result in more nervous system depression.

It is often difficult to test for nervous system function in invertebrate models. Mere motion is not enough to establish good neurological function, as limbs may move in an uncoordinated fashion. Particularly with animals as simple as nematodes, this rule may be difficult to follow.

In *Drosophila*, two tests of nervous system function are fairly obvious. A reduction in the ability of medicated flies to learn is a reasonable indicator of nervous system impairment. Another possible test is male mating function. Male *Drosophila* have to perform a fairly elaborate series of behaviors before females will mate with them; failure to accomplish these behaviors individually, or in the correct sequence, normally results in a failure to mate. An excellent test of general nervous system depression in fruit flies thus would be the mating success of medicated males compared with unmedicated males, within the same cohort, in competition as pairs.

Rule 6: The effect of the medication should not be highly sensitive to the culture environment

One of the important findings in the evolution and genetics of aging in fruit flies is the dependence of longevity effects on the culture environment (see Khazaeli *et al.*, 2005). Both evolved and mutant stocks do not necessarily exhibit particular phenotypic differences in all environments.

If similar effects arise with a drug in animal model trials, the question of the robustness of the therapeutic effect becomes important. A medication that does not have a consistent effect in a model species may be less trustworthy as a candidate for the treatment of human aging.

Fortunately, it is easy to vary the culture environment imposed on cohorts of model species like fruit flies and nematodes. It turns out that some phenotypic differences, such as overall longevity, are fairly robust as the environment is varied, while others, such as fecundity, are not (Chippindale *et al.*, 1993; Leroi *et al.*, 1994). This raises the possibility that some antiaging medications may have a consistently beneficial direct effect on adult survival, but inconsistent, potentially severe, deleterious side-effects. In particular, pharmacological effects that are not reliable in model species trials that employ a range of environmental conditions suggest that such drug treatments may not have the consistency that would warrant their further study for the purpose of medical applications.

Rule 7: The effect of a candidate medication should not be overly dependent on the genetic ancestry of the cohort(s) undergoing pharmacological trials

One of the major problems in the literature on the use of mutation or transgenic insertions in the postponement of aging in fruit flies is the stability of the effect on aging. It is a well-established principle of epistasis that some genetic backgrounds will respond differently to the introduction of the same mutation.

In the case of antiaging drug trials, it is possible that a particular compound might increase lifespan in a stock that has accidentally fixed a particular gene, or set of genes, yet the same compound given to a different stock of fruit flies might have no such effect. Fortunately, model species like *D. melanogaster* have a wide range of evolved and mutated stocks. Antiaging drugs that have effects on a cohort with one genetic background can easily be tested in another genetic background. It is possible that the impact and the extent of the antiaging compound might depend on the genetic makeup of the *D. melanogaster*.

In clinical practice, for diseases such as hypertension and lipid disorders, there are multiple drugs that might benefit patients. The differences in the medical use of these drugs are often based on clinical trials and healthcare provider preferences. The use of a particular drug may be discontinued by patients due to either inadequate treatment of the disease or the development of adverse effects. For instance, based on the second National Health and Nutrition Examination Survey data, only 27% of patients with high blood pressure have adequate control of their blood pressure (Chobanian *et al.*, 2003). Through the use of pharmacogenomic inferences derived from the genetic makeup of the patient, it is possible to choose a drug that is likely to be effective with the least potential for adverse effects.

Conclusion

The rules that we have presented here can be reasonably implemented in well-known model species like *Drosophila* and *Caenorhabditis.* These rules constitute a general testing protocol for antiaging drugs. The results obtained from trials that adhere to this protocol should not be immediately extrapolated to humans, but they would help us to narrow the library of compounds that could be considered for further testing in vertebrate animal models, such as mice, and eventually humans.

We also suggest that adherence to drug-testing rules such as those provided here would improve the testing of candidate antiaging treatments in mice and clinical patients as well. In the development of an antiaging pharmacopeia, it will be very important to monitor long-term side-effects of medication, since these medications are not primarily intended for the treatment of specific life-threatening diseases, and therefore may not have any value in conventional medicine. However, it is also possible that medications identified from their antiaging benefits in model species may have value in the treatment of acute disease as well. In either event, we wish to emphasize that screening these drugs for their dose-dependence, side-effects, and other limitations will be of value throughout the process of drug research and development.

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