OXIDATIVE STRESS MAY BE A CAUSAL FACTOR IN SENESCENCE

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One of the most fundamental current questions in gerontology concerns the nature of the underlying cause(s) of the structural and functional changes, associated with senescence, which render the organism progressively maladapted to withstand various endogenous or exogenous stresses. In particular, what needs to be understood is the nature of the events which *initiate* the deleterious changes and thus bring about the various manifestations of aging. Accordingly, a causal hypothesis should explain not only why physiological attritions occur during the latter part of the life span of an organism but also why different members of a cohort, or different species differ in their life spans and, implicitly, have different rates of aging. Furthermore, any purported causal hypothesis should explain why certain experimental regimens such as induced hibernation, lowered ambient temperature in cold-blooded animals, and calorie-restriction in laboratory rodents, which in common, induce hypometabolic states, prolong life span.

The oxidative stress hypothesis postulates the causal involvement of reactive oxygen species, generated as by-products of oxygen utilization. This hypothesis, derived from the free radical hypothesis and ultimately from the rate of living theory of Pearl, subscribes to the view that the rate of progression of the aging process is determined by the rate of accrual of molecular oxidative damage, which in turn is dependent upon the balance between the rate of generation of reactive oxygen species and the antioxidant defenses, or the level of oxidative stress. The hypothesis predicts that molecular oxidative damage increases during aging especially in the latter part of life and that life span potential in different species is inversely related to the rate of accrual of such damage.

Although considerably more work is needed, the evidence amassed thus far seems to be sufficient to implicate oxidative stress as a possible causal factor in the aging process. Studies in laboratory mammals such as mouse, gerbil and rat, and insects such as the housefly and *Drosophila* clearly indicate that the amount of molecular oxidative damage to DNA, proteins and lipids tends to increase exponentially during aging (Sohal and Weindrich, 1996). Damage to nuclear and mitochondria DNA, manifested as β -hydroxydeoxyguanosine, has been found to increase with age in mammals and insects. This type of DNA damage is potentially mutagenic due to base mispairing during repair. Age-related increase in protein oxidative damage, determined as carbonyl concentration and loss of sulfhydryl groups has been reported in insects and mammalian

tissues (Stadtman, 1992; Sohal *et al.*, 1993). Damage to proteins can lead to proteolysis or loss of function with a plethora of potentially deleterious secondary functional consequences. Further indication of the *in vivo* increase in the level of oxidative stress during aging is provided by the increased exhalation of alkanes by insects and mammals. Altogether, there is now ample evidence that the amount of oxidative damage increases during aging. The pattern of increase tends to be exponential implying that the age-associated physiological attrition and the rate of aging may also proceed exponentially with time.

Studies on the underlying causes of the age-associated increase in molecular oxidative damage have indicated that the rate of mitochondrial oxidant generation increases in virtually all the tissues examined (Sohal *et al.*, 1994). Whether the antioxidative defenses decline during aging is difficult to infer because there are a variety of enzymatic and non-enzymatic mechanisms comprising the antioxidative system. Although age-related alterations in various antioxidants do not seem to follow a uniform pattern, several studies have indicated that damage sustained by tissues in response to experimentally-induced oxidative stress is greater in the old than the young animals; suggesting an overall attenuation in the efficiency of antioxidative defenses during aging.

Other factors may also be involved in the age-associated increase in the amounts of oxidatively modified macromolecules. For example, a decreased ability to degrade or repair the oxidized macromolecules can also contribute to the accumulation of oxidized products. The increased availability of redox-active iron, may be another possible causal factor in the age-associated increase in the amount of oxidative damage, however, we have detected no uniform relationship between age and the concentration of bleomycin-chelatable iron in the mouse tissues. In summary, the results of the available studies tend to favor the view that aging is associated with a progressively increasing disturbance in the balance between prooxidants and antioxidants, i. e., the steady-state level of oxidative stress seems to get progressively worse during aging.

Experimental evidence supporting the role of oxidative stress in the aging process was provided by the studies on transgenic *Drosophila melanogaster* simultaneously overexpressing Cu, Zn-superoxide dismutase and catalase (Orr and Sohal, 1994; Sohal *et al.*, 1995a). The experimental flies exhibited an attenuation of the age-related increase in amounts of protein and DNA

oxidative damage and rates of mitochondrial superoxide and hydrogen peroxide generation. The life span of the overexpressing flies was 34% longer and the average amount of oxygen consumed during life or the metabolic potential, was 30% higher than the controls. At all ages, the speed of walking was relatively faster in the transgenic flies.

Experimental regimens, which extend life span, such as caloric restriction in mammals and decrease in ambient temperature or physical activity in cold-blooded animals, also seem to lower the level of oxidative stress. At comparable ages rates of mitochondrial superoxide and hydrogen peroxide generation, and amounts of protein and DNA oxidative were higher in the longer-lived experimental animals than the controls (Sohal *et al.*, 1994; Sohal and Weindruch, 1996). Although such correlative studies cannot establish causality, they do provide a test of the predictions of the hypothesis. Thus, results of such studies indicate that all the known life extension experimental regimens tend to lower the steady-state level of oxidative stress.

Variations in the life spans of different mammalian and insect species have also been found to be correlated with rates of mitochondrial O_2^- and H_2O_2 generation (Ku *et al.*, 1993; Sohal *et al.*, 1995b). At a comparable age the concentration of protein carbonyls was found to be inversely related to the average life span of a sample of dipteran insects. As compared to the mammals of the same body weight, the extended life span of the birds was also found to be inversely correlated with relatively low rates of mitochondrial superoxide and hydrogen peroxide generation.

In summary, the results of the available studies suggest that oxidative damage contributes to the aging process. Future studies are, however, needed to determine the extent to which oxidative damage is responsible for the senescent changes.

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