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Minireview

Why females live longer than males? Importance of the upregulation of longevity-associated genes by oestrogenic compounds

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Abstract Females live longer than males in many mammalian species, including humans. Mitochondria from females produce approximately half the amount of H_2O_2 than males. We have found that females behave as double transgenics overexpressing both superoxide dismutase and glutathione peroxidase. This is due to oestrogens that act by binding to the estrogen receptors and subsequently activating the mitogen activated protein (MAP) kinase and nuclear factor kappa B (NF-κB) signalling pathways. Phytoestrogens mimic the protective effect of oestradiol using the same signalling pathway. The critical importance of upregulating antioxidant genes, by hormonal and dietary manipulations, in order to increase longevity is discussed.

Keywords: Gender; Ageing; Oestrogens; Phytoestrogens; Antioxidant enzymes; Free radicals

1. Introduction: Females live longer than males in many species including humans

Females live longer than males in many mammalian species. For instance, male Wistar rats, in our laboratory, have an average life span of 24 months whereas females median life span is 29 months, i.e., 14% more than in males (Table 1). Of the most importance, the same happens in humans. In Europe, the average life span is 73.7 years for males and 83.8 years for females [1]. The fact that this difference occurs in animals as well as in humans, indicates that the difference cannot be attributed to sociological differences but rather to specific biological characteristics of both genders.

2. The mitochondrial theory of ageing: mitochondria are key organelles for the cellular production of oxidants in ageing

The free radical theory of ageing was first introduced by Gerschmann et al. [2] and by Harman [3] in the 1950s. An important feature of this theory is that it provides a rationale for intervention, i.e., administration of antioxidants may decrease the damage associated with ageing. In 1980, Miquel [4] introduced a further development of this theory, pointing to the role of mitochondria as source of free radicals and as a target of oxidative damage in the ageing cell. We reported that mitochondria are damaged inside the ageing cells [5] and that administration of antioxidants partially prevents age-associated oxidative damage [6,7]. Thus, mitochondria are key organelles to study the possible reasons for the different longevity between genders.

3. H_2O_2 production by mitochondria from females is significantly lower than from males

The importance of the rate of H_2O_2 production in determining life span has been highlighted by Barja [8]. The intramitochondrial steady-state concentrations of O_2^- and H_2O_2 are directly related to the rates of O_2^- and H_2O_2 production and inversely related to the enzymatic activity of manganese-superoxide dismutase (Mn-SOD) and glutathione peroxidase (GPx), which constitute the mitochondrial utilization pathways for O_2^- and H_2O_2. We measured the rate of H_2O_2 production in the presence of succinate or malate plus pyruvate. In both cases, mitochondria from females produced approximately half the amount of H_2O_2 than those from males. Mitochondrial H_2O_2, whose stoichiometric precursor is O_2^-, exerts a considerable part of the O_2^-/H_2O_2 toxicity through a Fe-catalysed Fenton chemistry [9], then, it is clear that the lower H_2O_2 production in females should be associated with a lower oxidative damage. In the following section, we describe the different oxidative damage in males and females [10,11].

4. Oxidative damage to key mitochondrial components is significantly higher in males than in females

Glutathione is a major intracellular antioxidant, whose concentration is similar to that of glucose [12] and in fact, it constitutes the major low molecular weight thiol in cells [13]. The levels of intracellular glutathione have been considered a biological marker of ageing [14]. We found that mitochondrial glutathione is related to the damage associated with ageing [15].

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Abbreviations: MAP, mitogen activated protein; NF-κB, nuclear factor kappa B; 8-oxo-dG, 8-oxo-deoxyguanosine; Mn-SOD, manganese-superoxide dismutase; GPx, glutathione peroxidase; 16S rRNA, 16S ribosomal RNA; PDTC, pyrrolidine dithiocarbamate

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Table 1
Inverse relationship between longevity and oxidative stress and damage in male and in female rats

	Males	Females	Females/males (%)
Average life span (months)	24 ± 0.5	29 ± 0.6	121
Mitochondrial H_2O_2 production (nmol/min mg prot)			
•Liver	0.10 ± 0.03**	0.07 ± 0.02	70
•Brain (non-synaptic)	0.08 ± 0.02*	0.04 ± 0.02	50
•Brain (synaptic)	0.29 ± 0.04*	0.17 ± 0.06	59
Reduced glutathione (GSH) (nmol/mg prot)	6.4 ± 0.9*	9.8 ± 1.8	153
Mitochondrial DNA damage (8-oxo-dG/100 000 dG)	55 ± 5**	15 ± 8	27
Mn-superoxide dismutase			
•Expression (arbitrary units)	3.1 ± 0.9*	6.5 ± 1.8	210
•Activity (U.I./mg prot)	34 ± 8**	74 ± 18	218
Glutathione peroxidase			
•Expression (arbitrary units)	2.6 ± 0.3**	5.4 ± 0.3	208
•Activity (U.I./mg prot)	0.18 ± 0.06**	0.51 ± 0.01	283
16S ribosomal RNA			
Expression (arbitrary units)	1.7 ± 0.4**	6.4 ± 0.3	376

Data are expressed as means ± S.D. for 8–10 different experiments. The statistical significance (ANOVA) is expressed as $^{*}P < 0.05$; $^{**}P < 0.01$ vs. females.

Table 1 shows that mitochondrial glutathione levels in males are approximately half than those found in females. DNA is a key component of the mitochondrial machinery [16]. We, and other groups, found that its degree of oxidation increases with ageing [7,17]. We have found (Table 1) that the levels of 8-oxo-deoxyguanosine (8-oxo-dG) (an excellent indicator of oxidative damage to DNA) are fourfold higher in males than in females [10]. This is the highest change we have observed in mitochondrial DNA oxidation in any physiological situation and shows that the chronic, continuous, increase in free radical production in males results in a marked oxidative and mutagenic lesion in mitochondrial DNA [18].

5. Females behave as double transgenics overexpressing mitochondrial superoxide dismutase and GPx

We searched for an explanation of the remarkable difference in free radical production between genders. Since the mitochondrial steady-state concentrations of $O_2^{\cdot-}$ and H_2O_2 are defined by the ratio of the rates of production and utilization of these species, we determined the mitochondrial activity and expression of Mn-SOD and GPx [19]. Table 1 shows that the expression of Mn-SOD, i.e., the mitochondrial SOD isoenzyme, is approximately double in females than in males. Its activity follows a parallel pattern of change.

In a similar fashion, GPx expression and activity is markedly increased in females when compared with males. The fact that females have a higher GPx activity than males was already observed in the 1960s [20] but this was not then related to the different longevity between genders. A few years ago Orr and Sohal [21] observed that *Drosophila* that overexpress either SOD or catalase (they lack GPx) did not increase their average life span. However, when they overexpressed both, the life span was increased. We have found that females overexpress both superoxide dismutase and GPx (both of them mitochondrial enzymes, Table 1). Moreover, this increase can be attributed to oestrogens (see below).

6. Expression of 16S ribosomal RNA (16S rRNA) and glutathione levels, both biological markers of ageing, show that females are younger than males of the same chronological age

The search for reliable biomarkers of ageing is an important issue in gerontology. Hazelton and Lang [14] have shown that glutathione can be considered one such biomarker. A few years ago Marco and his group reported that 16S rRNA expression progressively decreases with ageing in *Drosophila* [22]. Moreover, in an independent study Davies and co-workers [23] reported that the same molecule, i.e., 16S rRNA decreases under conditions of oxidative stress.

Thus, we tested [10] the hypothesis that if females are biologically younger than males of the same chronological age, they ought to express more 16S rRNA than males. This is indeed the case and the expression of 16S rRNA is more than threefold higher in females than in males of the same age (Table 1).

7. Oestrogens do not act as chemical antioxidants in vivo: they exert their antioxidant effect by upregulation of the expression of antioxidant genes

Oestrogens are antioxidants in vitro [24]. However, at physiological concentration it is very unlikely that they may act as such, especially due to their low concentration in plasma. A simple calculation indicates that if the recommended dose of oestradiol in oestrogen replacement therapy is 50 μg/day and the recommended dose of vitamin E as supplement is 500 mg/day; oestrogen ought to be 10 000 times more potent than vitamin E to have a similar antioxidant capacity and this is obviously not the case. Yet biological experiments indicate that oestrogens have a powerful antioxidant effect in vivo: mitochondrial H_2O_2 production is significantly increased (by more than 50%) after ovariectomy and this is completely prevented when ovariectomised rats are treated with oestradiol at doses similar to those used in oestrogen replacement therapy

Table 2
Physiological concentrations of oestradiol decrease H_2O_2 levels in human MCF-7 cells mediated by estrogen receptors/MAPK/NF-κB signalling pathway

	nmol H_2O_2/mg prot
Control	1.50 ± 0.55
Oestradiol 0.2 nM	0.66 ± 0.14**
Oestradiol 0.2 nM + tamoxifen 15 μM	1.72 ± 0.12
Oestradiol 0.2 nM + UO126 1 μM	1.06 ± 0.18
Oestradiol 0.2 nM + PDTC 200 μM	1.31 ± 0.15

Data are expressed as means ± S.D. for 8–10 different experiments. The statistical significance is expressed as $^{**}P < 0.01$ vs. control.

(for details see [9]). We then tested if the antioxidant effect of oestradiol is exerted through the interaction of the hormone with the oestrogen receptors in MCF 7 cells, the human mammary cell line. When these cells were incubated with oestradiol, the rate of H_2O_2 production was significantly decreased. However, when the cells were co-incubated with oestradiol and tamoxifen (an oestrogen receptor modulator) the rate of H_2O_2 production was similar to controls. This indicates that the antioxidant effect of oestrogen is mediated by the interaction of oestradiol with the oestrogen receptor.

We next wanted to elucidate the mechanism by which oestradiol might act to increase the expression of mitochondrial antioxidant enzymes. A direct genomic effect of oestradiol was unlikely because neither superoxide dismutase nor GPx have oestrogen responsive elements in their promoter region. Thus, it was likely that the action of estradiol could be mediated via intracellular signalling cascades. We tested the effect of mitogen activated protein (MAP) kinases by using an inhibitor of the phosphorylation of these kinases, i.e., UO126. Our experiments show that UO126 completely inhibited the lowering effect of estradiol on the level of H_2O_2 in cells (Table 2).

MAP kinases are known to activate nuclear factor kappa B (NF-κB). Thus, we tested whether oestradiol acts by activating it. NF-κB would then be able to upregulate the expression of both SOD and GPx genes, whose promoters contain putative NF-κB-binding motifs. This is indeed the case: when cells were incubated with pyrrolidine dithiocarbamate (PDTC), an

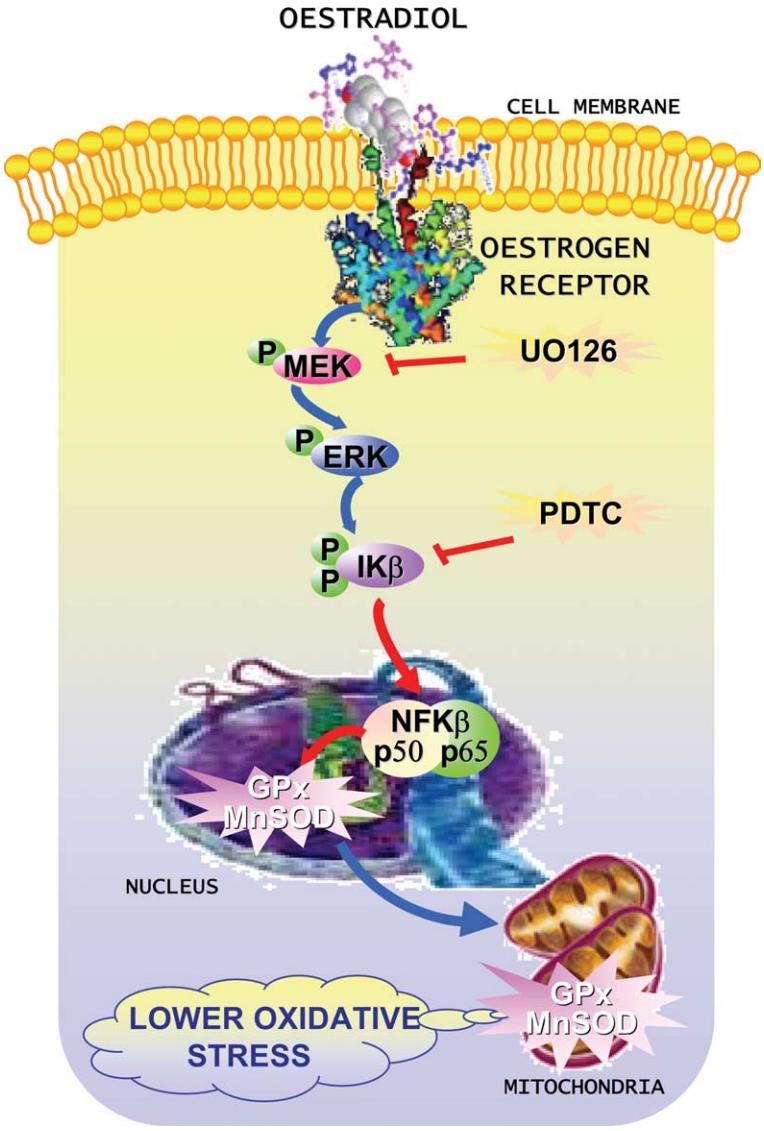

Fig. 1. Proposed mechanism for the action of oestradiol on the expression of antioxidant, longevity-related genes.

inhibitor of the IKB degradation, and therefore an inhibitor of the NF-κB translocation to the nucleus, the effect of oestradiol on the upregulation of antioxidant enzyme expression was prevented (Table 2). Using these pharmacological inhibitors of the signalling pathways, we demonstrate that oestradiol upregulates the expression of Mn-SOD and GPx mediated by the following pathway: interaction with membrane oestrogen receptor → activation of MAP kinases → activation of NF-κB → upregulation of gene expression (Table 2).

8. Phytoestrogens mimic the beneficial effects of oestrogens on the upregulation of antioxidant, longevity-related genes

The effect of oestradiol as an upregulator of antioxidant, longevity-related genes indicates that its administration might be beneficial to increase longevity, particularly of males, to reach a life span similar to females. However, considerable evidence has shown that oestrogen replacement therapy after menopause may have set backs [25]. Phytoestrogens constitute an interesting alternative. Their beneficial effects have been reported repeatedly [26] and, to our knowledge very few, if any, serious reports have shown detrimental effects. Thus, we tested the effect of 0.5 μM genistein, one of the major phytoestrogens in soya [27] on the H_2O_2 levels in MCF 7 cells. This can be considered as nutritionally relevant as it is the concentration normally found in the blood of people in the Far East who eat relatively large quantities of soya in their normal diet. This concentration is, however, significantly higher than the one found in people living in the Western world. We found that genistein significantly decreases H_2O_2 levels in cells and that, just as with oestradiol, this effect is mediated by oestrogen receptors.

We then studied if the signalling pathway that we had found to explain the antioxidant effects of oestradiol also acted for genistein and found that indeed this is the case and that genistein increases MAP kinases and activates NF-κB resulting in an upregulation of the antioxidant gene superoxide dismutase.

9. Concluding remarks

In a series of studies, we have attempted to elucidate the reasons for the different life span between males and females. In vivo experiments showed that oestrogens are responsible for the higher mitochondrial free radical production in males than in females.

Oestradiol does not act as a chemical antioxidant but rather it upregulates the expression of genes encoding for antioxidant enzymes such as Mn-SOD and GPx, both mitochondrial enzymes.

In vitro experiments (mainly using a human mammary gland cellular line) have shown that oestradiol acts through the interaction with oestrogen receptors. The cell-signalling pathway involved is oestrogen → binding to oestrogen receptor → MAPK phosphorylation → NF-κB activation → upregulation of antioxidant genes. Fig. 1 summarizes these findings.

Phytoestrogens are an interesting alternative to oestradiol to decrease free radical production by mitochondria and, thus, to increase life span of males. We have recently shown that, at least, in vitro this is the case and that they bind to oestrogen receptors and activate the same signalling pathway as oestradiol does. The effect of dietary supplementation with phytoestrogens on longevity, particularly to elucidate if they can increase the life span of males to a similar longevity as that of females, remains to be studied in the near future.

The possible importance of these studies lies in the fact that half of the population (males) live ≈10% less than the other half (females). An understanding of the reasons for this difference of longevity may help us to increase the longevity of males and to understand the basic phenomenon of ageing, and to search for safe ways to increase life span of males.

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