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Review
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SENSITIVITY TO OXIDATIVE STRESS: SEX MATTERS

Tatjana Marotti, Sandra Sobočanec, Željka Mačak-Šafranko, Ana Šarić, Borka Kušić, Tihomir Balog

Division of molecular medicine, Institute «Ruđer Bošković», Zagreb, Croatia

Summary

The excessive production of free radicals in organism and the imbalance between the concentration of these and the antioxidant defences has been related to the process of aging. It has been postulated that oxidative processes and antioxidant defence can bee sex related. Besides, we have noticed that at old age 60% of male mice developed hepatocellular tumors which were absent in females. Thus, it is of interest to determine oxidative and antioxidative status of aging male and female mice under conventional oxygen conditions and in 100-percet oxygen with possible mechanisms involved. The process of lipid peroxidation and antioxidant enzyme activity was age and sex-related, favouring males over females throughout the lifespan. The sensitivity of a cell to free radical attack apparently depends on the relationship among antioxidant enzymes rather than on absolute activities of individual antioxidant enzymes. Indeed, our results imply stronger correlative links in old female than male mice, which might explain why old females are better protected from oxidative stress than males. In the liver of hyperoxia treated mice sex-related difference was found at the physiological level observing malondialdehyde (MDA) increment (one of the end products of lipid peroxidation) and increased catalase (CAT) activity only in male mice. Hyperoxia did upregulate a stress responsive enzyme heme-oxygenase-1 (HO-1), but only in female mice. Also, stress related izoenzymes of the cytochrome P450 family were changed. In female mice Cyp1A1 and Cyp1A2 were downregulated and Cyp2A5 was upregulated. The results of our study suggest that females are less susceptible to oxidative stress by two major mechanisms: upregulated expression of HO-1 genes and different expression of certain P450 enzymes.

Keywords: oxidative stress; gender; hem-oxygenase; cytochrome P450

Corresponding author: Tatjana Marotti
E-mail: marotti@irb.hr

INTRODUCTION

Heightened interest in the aging process has been stimulated by a number of factors; one of the key observations being the impressive increase in the average life expectancy in humans. According to Harman [1, 2] aging is characterized as a progressive decline in biological functions with time and decreased resistance to multiple forms of stress and susceptibility to numerous diseases. In the 1950s Herman [3] proposed the «free radical theory of aging» which implies that the inherent aging process due to damage to cellular macromolecules via free radicals production in aerobic organism is the major risk factor for disease and death. This theory was extended in 1970s by the fact that mitochondria as the major source of free radical production in aerobic organisms [4] are also the most damaged ones by free radicals and that life span is determined by the rate of this damage [5].

Reactive oxidant species

All organisms living in aerobic conditions are exposed to free radicals. Among them the most examined and harmful ones are reactive oxygen species (ROS). ROS are metabolites of molecular oxygen (O_2), highly unstable because of unpaired electrons. ROS can include superoxide radical (O_2^-) and hydroxyl radical (HO) and a nonradical molecules like hydrogen peroxide (H_2O_2). Besides being a byproduct of normal aerobic metabolism, ROS can be also produced under stress and pathological conditions. ROS production also occurs during oxidative phosphorylation in reactions involving peroxisomal oxidases, cytochrome P450 enzymes and NAD(P)H oxidases. Mitochondria consume about 90% of the body's oxygen to generate ATP by oxidative phosphorylation and 1-2% of the oxygen molecules consumed are converted to superoxide anions in mitochondria. In vivo ROS production can be also induced by a number of exogenous stimuli such as radiation, pathogen infection, and exposure to xenobiotics, ultraviolet light exposure, herbicide/insecticide contamination and chemotherapeutic treatment of cancer patients. As such «bad guys» ROS induce oxidative damage to macromolecules such as lipids, nucleic acids and proteins [6]**.** These changes lead to various alterations in cell structures, aberrant damage in cell division and proliferation, functional changes in proteins, and insufficient cell repair which ultimatively leads to loss of homeostasis and finally results in oxidative stress. Lipids are one of the most sensitive oxidation targets to ROS and once initiated, a propagation of chain reactions will occur. During this period end products of lipid peroxidation such as malondialdehyde (MDA),

4-hydroxy-nonenal (4-HNE) or F2-isoprostanes are accumulated in biological systems. Detectable oxidation products of DNA bases oxidation are 8-hydroxy-2-deoxyguanosine. Such oxidased DNA bases can cause mutational deletions in nuclear and mitochondrial DNA. ROS can oxidize almost all amino acid residues forming cysteine residues, carbonyl derivates and methionine sulfoxide. On the other hand, ROS molecules can act (specially in low concentrations) as «good guys» in modulating kinases, phosphatases and transcription factors and during the process of phagocytosis [7].

Antioxidants

Due to potentially deleterious effects of ROS protective mechanisms have evolved to limit the production or release of ROS mainly by converting ROS to stable and innoxious end products such as oxygen and water. The system includes enzymatic scavengers such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (Gpx). Two SOD enzymes exist in the cell; one primarily localized in cytoplasm which is a zinc-copper containing enzyme (CuZnSOD) and the other is a manganese-dependent enzyme in the mitochondrial matrix (MnSOD). In addition to these enzymes a number of small non-enzymatic molecules are scavenging ROS such as glutathione, E and C vitamins, urate, flavonoids carotinoides and others. Glutathione is likely to be the most important scavenger of the non-enzymatic low mass molecules.

Oxidative stress

The state of stress named oxidative stress is the state of imbalance between oxidants and antioxidants due either to excessive production of oxidants or reduced antioxidans production/response. Oxidative stress is a double-edged sword; within physiological range it is necessary for proliferative stimulation and removal of death cells (apoptosis) whereas extensive oxidative stress damages structure and functions of cells.

The majority of studies have been supportive of the oxidative stress hypothesis of aging. The process has been related to excessive production of free radicals in organism and the imbalance between the concentration of these and antioxidant defense [8]. While Sohal [9] hypothesized that rates of prooxidant species generation are closely associated with the rate of aging Ehrenbrink et al. [10] postulated that alteration in antioxidant enzyme activities can be sex specific and related to longevity. Transgenic mice that overexpress CuZnSOD did not live longer than control animals and heterozygous mice with reduced MnSOD activity lived as

long as the wild type mice [11]. Experimentally, a progressive change to a more prooxidant environment has been noted in many species during aging [12]. In addition to disturbed redox balance in aged organisms one of the most common types of evidence is the strong correlation between aging and oxidative damage to tissues such as intracellular macromolecules, primarily lipids. The products of lipid peroxidation (MDA, 4-HNE and F2-isoprostanes) have been found elevated in liver, brain, kidney, lung and muscle of aged organisms [7].

Oxidant/antioxidant status (basal conditions)

In our study we noticed that lipid peroxidation (LPO) changed with age in both sexes, increasing during ageing in female mice while in male mice the increase persisted until the 10^{th} month only. After that period in male mice LPO decreased probably because at that age 60% of males developed hepatocellular tumors which were absent in females. The increase of LPO in male mice was paralleled with altered antioxidant response as measured by increased CAT and Gpx activity as a sign of oxidative stress [13]. To demonstrate that the decline of LPO concentration in 18 months old male CBA mice was associated with the presence of tumor we measured the same parameter in AKR mice which spontaneously develop tumors in both sexes already when 12 months old. Indeed, LPO concentration was decreased in both sexes at that age as the result of tumor presence [14]. Several studies have shown that oxidative stress limits the mitotic capacity of the cells [15]. It might be assumed that oxidative stress conditions found in males, associated with increased antioxidative enzymes, can initiate cell division favoring the clonal expansion. Such phenomenon may finally contribute to the ultimate stages of carcinogenesis. Further question was whether oxidant status and antioxidant enzyme activities during ageing of mouse brain are regulated in a sex-dependent manner. LPO was age and sex-related, favoring males over females throughout the lifespan with the peak in both sexes at 10 months of age. Higher LPO concentration in male brain compared to female brain was associated with higher antioxidant capacity of CAT and Gpx activity of female brain during aging. Taken together, the present findings indicate that brains of female mice are more efficiently protected from oxidative stress than brains of male mice [16]. The reasons for the diversity between male and female oxidative/antioxidative status might be associated with a) well-documented sexual dimorphism of the nervous system ascribed to steroid hormones b) sex-differences in stress response associated with diverse activation of the HPA axis, c) effect of gonadal steroids and d) induction of LPO via nitric oxide system reported higher in males than in females.

Although it is reasonable to assume that the increased damage of oxidative stress in aging is due to a decline in antioxidant defense system in many systems examined, the pattern of age-related changes has been inconsistent. The reason for that may lie in the fact that separately examined enzymes did not correlate to each other during the process of aging may not be relevant to the whole picture.

Sensitivity of a cell to free radical attack apparently depends on the relationship among SOD, CAT and Gpx rather than on absolute activities of individual antioxidant enzymes [17]. We have demonstrated in our study that the cooperation between antioxidant enzymes becomes more coherent with increased lipid peroxidation in liver and brain of old female mice. On the contrary, in older male mice the link among three antioxidant enzymes becomes weaker, regardless of lipid peroxidation. The results imply stronger correlative links in old female than male mice, which might explain why old females are better protected from oxidative stress than males [18].

Oxidant/antioxidant status (hyperoxic conditions)

If males and females have different oxidant/antioxidant capacity in basal conditions do they preserve this sex-related diversity also in conditions of oxidative stress induced by hyperoxia (100% oxygen) in normobaric conditions? The beneficial effects of hyperoxia have been noted in treatment of several diseases such as acute carbon monoxide poisoning, hypoxia, treatment of anaerobic infections, decompression sickness and as supplemental therapy in bronchopulmonary displasia. However, exposure to hyperoxia causes inflammatory response which aggravates oxygen toxicity; the higher the concentration of oxygen the substantial the damage [19]. In the liver of hyperoxia treated CBA mice sex-related difference was found at the physiological level observing MDA (one of the end products of lipid peroxidation) increment and increased CAT protein and activity only in male mice. The observed CAT increase implies the presence of oxidative stress in males because CAT plays the important role in organism◎s coping with the increased ROS as opposed to Gpx which has a major antioxidant role in physiological conditions [20].

Hyperoxia, hem-oxygenase 1 and isoforms of cytochrome P450 family

Previous studies have shown that heme-oxygenase-1 (HO-1) a stress responsive enzyme can be induced upon hyperoxia [21] and may mediate protection against oxidant insults [22]. HO-1 is the rate limiting enzyme in heme degrada-

tion to biliverdin which is rapidly converted to bilirubin with potent antioxidant properties [23]. Hyperoxia did upregulate HO-1 expression, but only in female mice. Beside hyperoxia induced gender-related HO-1 alteration, stress related izoenzymes of the cytochrome P450 family were changed. In female mice Cyp1A1 and Cyp1A2 were downregulated and Cyp2A5 was upregulated. This regulation seems to be connected to HO-1 upregulation in female mice. Namely, as demonstrated by Moorty et al. [24] HO-1 upregulation could decrease Cyp1A1 and Cyp1A2 by increased rates of degradation of the prosthetic heme group of Cyp enzyme. On the other hand, Abu –Bakar et al [25] showed that Cyp2A5 is concurrently induced with HO-1 in oxidative stress induced with cadmium and participates in bilirubin degradation. This seems to be contradictory with no "signs" (LPO and catalase upregulation) in females but Nichols et al. [26] have demonstrated that upregulation of Cyp2A5 after induction with typical Cyp2A5 inducer pyrazole was not paralleled with markers of oxidative stress such as MDA and catalase. The results of our study suggest that females are less susceptible to oxidative stress by two major mechanisms: upregulated expression of HO-1 genes and different expression of certain P450 enzymes [27]. On the contrary, hyperoxia had no effect on MDA and antioxidant enzyme activities in lunges of both sexes. Since this was paralleled with up-regulation of HO-1 in both sexes it seems that HO-1 might have a major role in protection from hyperoxia. We also found sex-dependent pattern of Cyp4A14 expression in regulation of oxidant status upon hyperoxia: males exhibited the reduction of Cyp4A14 on both RNA and protein level which resulted in increased LPO and susceptibility to oxidative stress, while unchanged LPO in females could be the consequence of strong (9 fold compared to control) upregulation of Cyp4A14. Cyp4A14 is involved in hepatic fatty acid disposal and its upregulation depletes the liver of substrate for LPO [28]. Cyp4A14 is modulated via peroxisome proliferator-activated receptor (PPAR) isoforms (PPARα, PPARß/δ, PPARγ). In our study PPARß/δ was found to be down regulated, with marked decrease in females. The over expression of Cyp4A14 gene and protein and lack of LPO-protein adducts in females indicates their greater resistance to hyperoxia compared to males [29].

Gender-related oxidant/antioxidant status in renal cell carcinoma

Considerable evidence has linked LPO and etiology of renal clear cell carcinoma (RCC) of the kidney. It is believed that RCC belongs to tumors with significant changes in cellular redox balance [30] and oxidant alteration of lipids, proteins and DNA [31]. Also, there has been much speculation about the role of various Cyp proteins as causes of cancer since some Cyps may activate pro-car-

cinogens into carcinogens while others are involved in removal of carcinogens from the body. Besides, RCC rates in males are about twice as high as the rates in females [32]. The results of our study (to be published) of oxidant/antioxidant profile of male and female patients with RCC revealed that only tumors from male patients had increased MDA concentration. This increment was measurable also in plasma of male patients. Most of the earlier studies have postulated a low antioxidant enzyme activity in cancer [33]. This was also the case in our study with decreased CAT and Gpx activity in tumor tissue of male and female patients. However, the most interesting result was that of gender-related inversion of SOD izoenzymes. In tumor tissue of male patients elevated CuZnSOD and decreased MnSOD activity was observed. In female patients the increased MnSOD activity of tumor tissue was upregulated already at the transcriptional level. Jansen et al. [34] have demonstrated that the level of MnSOD is high in many human tumors while Manna et al. [35] showed that MnSOD overexpression in tumors could offer a survival advantage to tumor cells. The results of our study support the hypothesis that oxidative stress and the accompanying antioxidant defense might play an important role in RCC growth and progression.

Conclusion

So far, the «free radical theory» of aging has been supported by numerous data. Lately the data have been extended by the fact of gender-related effects of free radicals due to the fact that susceptibility to oxidative stress in various organs is different in males and females. Namely, females seem to be more resistant to oxidative stress than males primarily due to lower oxidation and higher antioxidant enzyme upregulation with more coherent cooperation of antioxidative enzymes at old age. Finally, besides antioxidative enzymes in general view of gender-related susceptibility to oxidative stress factors like heme-oxygenase-1 (HO-1) and several oxidative stress related isoforms of the cytochrome P450 family (particularly Cyp1A1, Cyp1A2, Cyp4A14 and Cyp2A5) have to be considered.

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Sažetak

Uloga spola u osjetljivosti na oksidacijski stres

Proces starenja povezuje se s poremećajem u ravnoteži stvaranja slobodnih radikala i odgovarajuće «obrane» koju omogućuju antioksidansi. Smatra se da su oksidacijski procesi i antioksidacijska obrana spolno vezane kategorije. U svojim istraživanjima utvrdili smo da 60% miševa muškog spola u starijoj dobi spontano obolijeva od hepatocelularnih karcinoma, dok ženke iste dobi od toga ne obolijevaju. S obzirom na povezanost oksidacijsko-antioksidacijskih procesa sa spolom ispitali smo navedene parametre u mužjaka i ženki miševa u uvjetima hiperoksije (100%-tni kisik). Tijekom starenja oksidacijski procesi i aktivnost antioksidacijskih enzima mijenjaju se, ali je njihov odnos uvijek povoljniji u ženki nego u mužjaka. Čini se da je osjetljivost na oksidacijski stres većim dijelom rezultat međudjelovanja različitih antioksidacijskih enzima, a manjim razine pojedinog antioksidacijskog enzima. U prilog tome govori i činjenica da upravo u ženki (ali ne i u mužjaka) u starijoj dobi postoji visok stupanj korelacije između pojedinih antioksidacijskih enzima, zbog čega su ženke bolje od mužjaka zaštićene od oksidacijskog stresa. Naime, mužjaci starije dobi imaju povišene pokazatelje oksidacijskog stresa kao što su razina malondialdehida (MDA) i katalazna aktivnost. Nasuprot tome, ženke u hiperoksiji aktiviraju zaštitni enzim hem oksigenazu (HO-1) i citokrome sustava P450 kao što su CYP1A1 i CYP1A2, koji su regulirani prema dolje, i CYP2A5, koji je reguliran prema gore. Iz navedenih podataka možemo zaključiti da su ženke manje osjetljive na oksidacijski stres i da se ta zaštita ostvaruje zahvaljujući aktivaciji HO-1 i regulaciji enzima sustava P450.

Ključne riječi: oksidativi stres; spol; hem-oksigenaza; citokrom P450