Reduced extracellular phagocyte oxidative activity, antioxidant level changes and increased oxidative damage in healthy human blood as a function of age

Margarita L. Alexandrova · Petyo G. Bochev

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Abstract Age-related changes in the blood antioxidant status, in the prooxidative activity of peripheral phagocytes and in the markers of oxidative injury were simultaneously examined in the circulation of 45 middle-aged and elderly healthy volunteers. The results showed a decrease in the opsonin-dependent and -independent extracellular-phagocyte oxidative activity, evaluated by means of luminol chemiluminescence. An increase in the portion of the mitochondrial superoxide generation within the total oxidative phagocyte response was evaluated by means of lucigenin chemiluminescence. The erythrocyte copper/zinc superoxide dismutase increased with age, while blood catalase and glutathione peroxidase activities remained unchanged. The levels of blood SH-groups decreased with age. An age-related increase in blood concentration of thiobarbituric acid-reactive material, a marker of oxidative damage, was detected. Some data, illustrating the existence of a delicate balance between oxidants and prooxidants, were also obtained. Further studies on the interrelationship between the components determining pro/antioxidative status in an organism may prove useful for developing a complex strategy in combating ageing.

Keywords Reactive oxygen species · Peripheral phagocytes · Lipid peroxidation · CuZn superoxide dismutase · SH-groups · Elderly humans

Introduction

Free radical processes are constantly running in living organisms. Ionizing radiation, environmental pollutants, hyperoxia, hypoxia, side-products of endogenous electron transport processes, phagocyte prooxidative activity, and oxidase-catalyzed reactions produce reactive oxygen species (ROS). Because of the high chemical reactivity of radicals and their intermediates, various components in the organism constantly undergo chemical changes in a more or less random manner.

The intensity of oxidative processes in the organism is controlled by a variety of enzymatic and non-enzymatic antioxidants that can neutralize the produced ROS or prevent their formation. Furthermore, cells are equipped with reparative enzymes that effectively remove oxidative damage. Unfortunately, the antioxidant defense system is not perfect. Under certain conditions, a state of imbalance between the radical-forming processes and the body mechanisms against their deleterious effect may result in a condition known as oxidative stress.

M. L. Alexandrova (✉) · P. G. Bochev
Department of Biophysics, Medical University,
1 Kliment Ohridski str.,
5800 Pleven, Bulgaria
e-mail: margalexandrova@hotmail.com

The senescent organism has been considered to be in a state of oxidative stress (Gilca et al. 2007; Agarwal and Sohal 1994). Oxidative stress causes increased levels of oxidized proteins, lipids and nucleic acids in cells. It induces disturbances in a number of biological functions, such as loss of physical activity, worsened cognitive functioning and reduced metabolic integrity (Stadtman 2006; Sohal and Allen 1990).

Although the relationship between oxidative stress and ageing has been extensively studied, data reported in the literature are inconsistent. Studies on likely causes for the oxidative damage observed in adults have revealed unchanged, increased or even decreased activity of identical antioxidant enzymes (Ho et al. 2005; Rizvi and Maurya 2007; Kasapoglu and Ozben 2001; Casado and López-Fernandez 2003). Data have also been published on increased or unchanged levels of indicators of oxidative damage in elderly subjects. Limited research is available regarding the age-related changes in the extracellular oxidative activity of peripheral phagocytes and their contribution to the prooxidative status of blood. To the best of our knowledge, there are no studies that simultaneously evaluate the rate of extracellular radical generation by peripheral phagocytes, the antioxidant blood potential and the degree of oxidative membrane injury.

The aim of the present study was to simultaneously examine the changes that occur in the opsonin-dependent and -independent extracellular oxidative activity of peripheral phagocytes, in the levels of a number of blood antioxidant components and in the concentration of some markers of oxidative injury in healthy human blood as a function of age. In view of the complexity of pro/antioxidative processes, the aim of the study was also to elucidate the interrelationship between the parameters measured.

Materials and methods

Study group

We studied 45 healthy volunteers (17 men and 28 women; mean age 54.3 years; range 40–80 years). During the study all individuals were in good health with no clinical evidence of acute or chronic infection.

The selection criteria included absence of acute disease, cancer, arterial hypertension, diabetes mellitus or endocrine disease. The individuals did not receive any medication, such as vitamins or minerals, which could influence their antioxidant status. All experiments were conducted in accordance with the rules and regulations approved by the University Research Ethics Committee.

Blood parameters

Blood samples, anticoagulated with heparin (10 U/ml), were collected in a fasting state. Experiments began within 30 min of collection. Each time the number of leukocytes, erythrocytes, platelets and differential cell counts were determined.

Evaluation of ROS generation by peripheral phagocytes

The oxidative activity of peripheral phagocytes to produce ROS was measured in whole blood. The kinetics of luminol (LCL) and lucigenin (LgCL) chemiluminescence reflecting this activity were registered by a computerized chemiluminometer operating in a quanta metric mode (Bochev et al. 1992).

Extracellular luminol chemiluminescence (Extra LCL) by peripheral phagocytes stimulated through opsonin-dependent and independent receptor mechanism

Extra LCL represents the extracellular superoxide production by stimulated phagocytes. Since luminol can easily permeate the cellular membrane, LCL reflects the total myeloperoxidase (MPO)-driven NADPH-oxidase activity. To measure only the extracellular portion of the response, a model system containing sodium azide and exogenous horseradish peroxidase (HRP) was used. Sodium azide inhibits MPO and the exogenous HRP regenerates LCL, and registers the extracellular component of the cellular response. The samples contained: 0.1 ml whole blood (1:10), 0.2 ml luminol (10^{-4} mol/l), 1.0 ml formyl-methionyl-leucyl-phenylalanine (fMLP) (2×10^{-5} mol/l) or 0.5 ml zymosan (1 mg/ml), 0.02 ml NaN_3 (65 mg/ml), 0.2 ml HRP (40 U/ml) and Krebs-Ringer phosphate buffer (KRP) in a total volume of 2 ml (Walan et al. 1992).

Lucigenin chemiluminescence (LgCL)

LgCL has been considered as a specific indicator of the superoxide produced by peripheral phagocytes (Tosi and Hamedani 1992). Lucigenin is able to penetrate into the cells and localize in mitochondria triggering a significant chemiluminescent response through interaction with the intermitochondrial superoxide (Chen et al. 2001). Therefore, LgCL kinetics is determined by two different processes: superoxide production through the respiratory burst activation and superoxide production in mitochondria. The samples contained: 0.1 ml whole blood (1:10), 0.5 ml lucigenin (10^{-4} mol/l), 0.5 ml zymosan (1 mg/ml) and KRP in a total volume of 2 ml.

Two parameters of the chemiluminescent kinetic curves were used to examine the following aspects of phagocyte activity to generate ROS:

- Velocity of phagocyte activation expressed by the reciprocal of the parameter $T_{\max}$ (the time of the peak appearance after the start of chemiluminescent response by phagocyte stimulation).
- Maximum activity achieved by phagocytes expressed by the parameter $I_{\max}$ (the maximum achieved value of chemiluminescence).

To compare chemiluminescent responses of different individuals, the data were normalized with respect to phagocyte number and red blood cell (RBC) absorption in the blood samples (Bochev et al. 1993).

Components of the blood antioxidant capacity

The erythrocyte Cu/Zn superoxide dismutase activity (CuZn SOD) was measured spectrophotometrically according to the method of Maral et al. (1997). It is based on inhibiting the reduction of nitroblue tetrazolium by superoxide generated via photoreduction of riboflavin. A blank in the absence of CuZn SOD was irradiated for 2 min at $\lambda=365$ nm at a distance such as to provide an increase in optical density at $\lambda=560$ nm of 0.20 to 0.21. One unit of activity was defined as the amount of enzyme causing 50% inhibition of the riboflavin reduction to formazan observed in the blank. A standard curve using pure human erythrocyte revealed that inhibition was linear to 40%. The results, normalized with respect to the blood erythrocyte number, were expressed as U/ 10^9 RBC. The reproduc-

ibility evaluated in terms of the coefficient of variation (CV) was calculated to be 3.7% ($n=5$).

The total blood catalase (CTS) activity was assessed spectrophotometrically according to the method of Sigma (1997). To compare activities of different samples, the H_2O_2 concentrations at the beginning and at the end of the assay were accurately defined. The optical density of the substrate solution at $\lambda=240$ nm was adjusted to range between 0.550 and 0.520. The time required for the optical density of the sample to decrease from 0.450 to 0.400 was measured. This corresponded to the decomposition of 3.45 μ mol of H_2O_2 in the 3-ml sample. The results were expressed in kU/ml blood. The CV calculated was 5.4% ($n=5$).

The blood glutathione peroxidase (GSH-Px) activity was evaluated spectrophotometrically according to Pereslegina (1989). The activity was assessed by the degree of oxidized glutathione accumulation in the sample. Data were expressed as μ mol GSSG/min/ml blood. The CV calculated was 4.9% ($n=5$).

The total concentration of blood SH-groups was measured spectrophotometrically according to the method of Ellman (1959). The technique is based on the fact that 5,5'-dithiobis(2-nitrobenzoic acid) reacts with aliphatic thiol compounds to produce a highly colored product. The results were expressed in mmol SH/l blood. The CV was 5.8% ($n=5$).

Degree of oxidative damage

The degree of oxidative damage in peripheral blood was estimated by means of lipid hydroperoxides (ROOH) in plasma and thiobarbituric acid-reactive material (TBARM) in blood.

The plasma ROOH levels were measured according to the method of Yagi (1987). The approach is based on the fact that lipid peroxides react with thiobarbituric acid (TBA) to yield a red pigment. To determine specifically ROOH in plasma, they were precipitated along with plasma proteins to remove water-soluble TBARM. The reaction was carried out at pH 3, where sialic acid could not react with TBA. To increase the sensitivity, the reaction product was determined fluorometrically, where bilirubin, if contained in a sample, did not interfere with the assay. The ROOH levels were expressed in terms of malondialdehyde (MDA) as standard. Since MDA is unstable, tetramethoxypropane, which is converted

quantitatively to MDA in the reaction procedure, was used. The CV calculated was 5.4% ($n=5$).

The blood TBARM concentration was measured spectrophotometrically according to the method of Asakawa and Matsushita (1980). The results were expressed in nmol/ml blood. The CV computed was 4.1% ($n=5$).

To evaluate reliability of the laboratory procedures, we applied the test-retest analysis. Within a single day all the parameter measurements were repeated on a control group of 12 healthy individuals. The values of the obtained correlation coefficients of Pearson (Spearman) and the corresponding levels of significance were as follows: Extra LCL ($R_s=0.61$, $p=0.046$); LgCL ($R_s=0.69$, $p=0.038$); SOD ($r=0.76$, $p=0.026$); CTS ($r=0.94$, $p=0.00002$); GSH-Px ($r=0.74$, $p=0.041$); SH ($r=0.86$, $p=0.00037$), ROOH ($r=0.76$, $p=0.0039$) and TBARM ($r=0.81$, $p=0.0014$).

Statistical analysis

The correlation relationships between the parameters measured were determined applying the *Pearson-product moment correlation*. For maximum intensities of Extra LCL and LgCL, a logarithmic transformation was used to approach normality of distributions. In all further statistical analyses, log-transformed data of these two parameters were used. *Breakdown and one-way ANOVA* was applied to study the effect of the “age” and “sex” factors on the value of the oxidative markers. The study group was classified into four age-subgroups: group 1 (40–50 years); group 2 (51–60 years); group 3 (61–70 years) and group 4 (71–80 years). When the effect of the factors was found to be significant (the F ratio and the corresponding level of significance p are given in the text), the *Newman-Keuls multiple range test* was applied to determine differences among groups. Data are presented as mean $\pm$ SD or median (minimum–maximum value). A p value <0.05 was considered statistically significant.

Results

Peripheral leukocyte count

There were no age-related changes in the total number of peripheral leukocytes ($p=0.13$). No statistically significant differences were observed for the number

of granulocytes ($p=0.49$), monocytes ($p=0.46$) and lymphocytes ($p=0.08$).

Evaluation of ROS production by peripheral phagocytes

Extracellular luminol chemiluminescence

The velocity of phagocyte activation to generate extracellular superoxide did not change with age ($T_{\max}$ of zymosan-stimulated Extra LCL: 14.5 ± 6.3 ; 14.4 ± 5.4 ; 11.7 ± 2.5 and 16.4 ± 6.8 min in the 1st–4th age-subgroups, respectively; $T_{\max}$ of fMLP-stimulated Extra LCL: 1.2 ± 0.5 ; 1.1 ± 0.5 ; 1.8 ± 1.1 and 1.1 ± 0.0 min in the 1st–4th age-subgroups, respectively). The maximum oxidative activity of the cells estimated by the peak intensity of Extra LCL curve in response to opsonized zymosan ($r=-0.67$, $p=0.0000010$) (Fig. 1) and chemotactic agent fMLP ($r=-0.60$, $p=0.000016$) (Fig. 2) was decreased.

Lucigenin chemiluminescence

The velocity of phagocyte activation to produce superoxide decreased with age ($r=0.46$, $p=0.0035$) (Fig. 3). The maximum oxidative activity of the cells, however, did not change [606 (249–1,321); 1,419 (301–3,685); 1225 (600–3,784) and 774 (131–1,417) cps/ 10^4 phagocytes in the 1st–4th age-subgroups, respectively].

Fig. 1 Correlation between the maximum intensity $I_{\max}$ of zymosan-stimulated Extra LCL registered by peripheral phagocytes and age ($n=45$). The statistical analysis was performed on log transformed versus linear data using Pearson product-moment correlation ($r=-0.67$, $p=0.0000010$)

Fig. 2 Correlation between the maximum intensity $I_{\max}$ of fMLP-stimulated Extra LCL registered by peripheral phagocytes and age. The statistical analysis was performed on log transformed versus linear data using Pearson-product moment correlation ($r=-0.60$, $p=0.000016$) ($n=45$)

Components of the blood antioxidant capacity

The activity of erythrocyte CuZn SOD increased with age ($r=0.38$, $p=0.011$) (Fig. 4). This increase, however, was not linear within the whole age-range examined. The analysis of variance ($F=6.09$, $p=0.016$) with a consequent post-hoc comparison showed that the enzyme activity decreased in the oldest subgroup (71–80 years) (Fig. 5). The total blood catalase activity showed only a tendency to increase ($r=0.27$, $p=0.08$) (29.7 ± 5.9 ; 31.7 ± 5.5 ; 32.4 ± 4.1 and 33.8 ± 7.1 kU/ml in the 1st–4th age-subgroups, respectively). No changes with age were established for the blood glutathione-

Fig. 3 Correlation between the time to the peak $T_{\max}$ of zymosan-stimulated LgCL and age ($n=42$). The statistical analysis was performed using Pearson product-moment correlation ($r=0.46$, $p=0.0035$)

Fig. 4 Correlation between erythrocyte CuZn SOD activity and age ($n=30$). The statistical analysis was performed using Pearson-product moment correlation ($r=0.38$, $p=0.011$)

peroxidase activity (9.8 ± 0.8 ; 9.9 ± 0.2 ; 9.4 ± 1.3 and 8.4 ± 0.8 $\mu\text{mol/min/ml}$ in the 1st–4th age-subgroups, respectively). The total SH-groups content in blood decreased with age ($r=-0.31$, $p=0.044$) (Fig. 6).

Degree of oxidative damage

The plasma ROOH levels did not change with age (3.2 ± 1.0 ; 3.7 ± 0.5 ; 3.9 ± 0.7 and 3.7 ± 1.2 nmol/ml in the 1st–4th age-subgroups, respectively). TBARM

Fig. 5 Erythrocyte CuZn SOD activity depending on age. The statistical analysis was performed using breakdown and one-way ANOVA ($F=6.09$, $p=0.016$). The post-hoc Newman-Keuls multiple range test was applied to determine differences among groups

Fig. 6 Correlation between total blood SH groups content and age ($n=42$). The statistical analysis was performed using Pearson-product moment correlation ($r=-0.31$, $p=0.044$)

only showed a statistically significant increase with age ($r=0.45$, $p=0.013$) (Fig. 7).

Correlation interrelationships between the parameters measured

We found that the number of peripheral phagocytes positively correlated with the blood TBARM concentration ($r=0.51$, $p=0.0051$). Surprisingly, $I_{\max}$ of zymosan-stimulated Extra LCL ($r=-0.41$, $p=0.029$) and fMLP-stimulated Extra LCL ($r=-0.39$, $p=0.035$) negatively correlated with the blood TBARM concentration. The time to peak $T_{\max}$ of LgCL ($r=0.45$, $p=0.020$) and the maximum intensity $I_{\max}$ of LgCL ($r=0.49$, $p=0.016$) positively correlated with the plasma ROOH

Fig. 7 Correlation between blood TBARM concentration and age ($n=30$). The statistical analysis was performed using Pearson-product moment correlation ($r=0.45$, $p=0.013$)

concentration. A highly significant positive correlation was found to exist between the normalized CuZn SOD activity in erythrocytes and the plasma ROOH concentration ($r=0.81$, $p=0.0000$). The normalized CuZn SOD activity in erythrocytes correlated with $I_{\max}$ of fMLP-stimulated Extra LCL ($r=-0.39$, $p=0.0087$), $T_{\max}$ of LgCL ($r=0.59$, $p=0.000078$) and $I_{\max}$ of LgCL ($r=-0.37$, $p=0.020$). Both CuZn SOD and GSH-Px activities positively correlated with the number of peripheral monocytes. The total blood SH-groups content positively correlated with the number of erythrocytes ($r=0.34$, $p=0.020$) and hemoglobin concentration ($r=0.43$, $p=0.0032$).

Effect of sex on the oxidative parameters studied

It was found that sex was not a significant factor for the values of the obtained results.

Discussion

The aim of the present investigation was to simultaneously elucidate the age-related changes that occur in the activity of peripheral phagocytes to produce ROS, in the activity of components of the antioxidant defense system and in the levels of markers of oxidative damage in blood.

We established a reduced extracellular LCL by peripheral phagocytes in whole blood in response to two different receptor stimulants: the serum-opsonized zymosan and the opsonin-independent chemotactic tripeptide fMLP. We may, therefore, conclude that the extracellular superoxide generation by phagocyte NADPH oxidase decreases in elderly subjects, which is in accordance with data published (Perskin and Cronstein 1992). The result supports the idea of the age-related immune system remodeling, and not that of the age-dependent leukocyte-mediated potentially destructive extracellular radical overproduction.

We also studied age-related changes in phagocyte oxidative activity by LgCL. We did not find any change in the maximum LgCL intensity with age. We found, however, a positive correlation relationship between the time to the peak of LgCL kinetics and age. Since NADPH-oxidase-driven extracellular superoxide generation is reduced, then the increased time to the peak observed at an unchanged maximum intensity may point out an enhanced portion of the mitochondrial

superoxide generation within the total response. In other words, the increased time to achieve maximum oxidative activity could be an indicator of changed proportions between the NADPH-oxidase mediated extracellular superoxide production and the intracellular mitochondrial superoxide production in favor of the mitochondrial one. Such a conclusion is supported by other authors who have reported that the rate of mitochondrial production of superoxide and hydrogen peroxide significantly increases with age due to the progressive oxidative modification of the mitochondrial enzymes (Passos et al. 2006; Cadenas et al. 2000; Hensley et al. 1999). Our conclusion about the change occurrence in the intracellular redox status may have various physiological consequences, ion imbalance in the cell and changes in the steric properties of chromatin through disulfide bond formation. Some studies have established an inverse relationship between the lifespan potential and the intracellular levels of oxidative damage in different organisms. This is valid irrespectively of the factors that can change the maximum longevity, such as calorie restriction, mutations and levels of antioxidants (Sohal et al. 1995; Sanz et al. 2006).

SOD, CTS and GSH-Px are the three main antioxidant enzymes that control the biological effects of the ROS produced in the organism. The data published on the status of these enzymes in the circulation of elderly subjects are inconsistent and there is no definite opinion on whether their activity increases or decreases with age. Increased (Ho et al. 2005; Ozturk and Gumuslu 2004; Rizvi and Maurya 2007), decreased (Kedziora-Kornatowska et al. 2007; Bolzan et al. 1997) or even normal (Bogdanska et al. 2003; Kasapoglu and Ozben 2001) CuZn SOD activity in erythrocytes with age have been reported. Similar data on increased (Ho et al. 2005; Rizvi and Maurya 2007; Ozturk and Gumuslu 2004; Bolzan et al. 1997), decreased (Volkovova et al. 1996; Kedziora-Kornatowska et al. 2007) or unchanged (Bogdanska et al. 2003; Kasapoglu and Ozben 2001; Bolzan et al. 1997) erythrocyte CTS and GSH-Px activities have also been published. Data have been reported about significantly decreased plasma concentrations of the extracellular SOD with age (Di Massimo et al. 2006). Besides, wide interindividual variations in the activity of the enzymes have been observed (Bogdanska et al. 2003; Volkovova et al. 2005).

We found that the erythrocyte CuZn SOD activity increased with age. The analysis of variance, however, showed that the enzyme activity did not increase linearly

with age but decreased in the oldest subgroup (more than 70 years). Such a result is not unexpected since data have been published about a reduced erythrocyte CuZn SOD activity in very old individuals (Mecocci et al. 2000). At the same time, the blood GSH-Px activity did not change with age. Blood CTS activity showed only a tendency to increase without reaching a statistical significance. We consider that the increased CuZn SOD activity at unchanged blood CTS and GSH-Px activities should not be interpreted as an indicator of increased blood antioxidant capacity with age. Under these conditions, the increased CuZn SOD activity may exhibit prooxidative properties since it leads to an increased concentration of hydrogen peroxide, the latter being more toxic than the superoxide itself.

In the present study, the total blood content of SH-groups as a function of age was measured since they mainly determine the reducing cellular capacity. We found age-related reduction in the total blood SH-groups content to be in agreement with some previously published data (Hack et al. 1998). The functional consequences of reduced blood SH-groups content could be a catalytic inactivation of proteins and a loss of specific cellular functions (Douzou and Maurel 1977; Shacter 2000). The decreased blood SH-groups content could result from reactive oxygen species (Sun and Tower 1999). We did not find, however, any relationship between the total blood SH-groups content and any of the parameters measured. Although it is tempting to consider that age-related changes in the redox status are direct consequences of the increased ROS generation, such a cause-effect relationship remains to be proved.

The plasma levels of ROOH did not change with age, as has been observed before (Block et al. 2002). We found an age-related increased blood TBARM concentration, which is in accordance with the data of Kasapoglu and Ozben (2001). Thus, we may assume an increased level of oxidative damage exists in the blood of elderly subjects.

Besides measuring the levels of oxidative markers in circulation, we also examined their mutual interrelationship. We found that both erythrocyte CuZn SOD and blood GSH-Px activities significantly correlated with the number of monocytes in peripheral blood, a relationship which could be due to the fact that monocytes are a potential source of ROS. The CuZn SOD activity, in turn, correlated negatively with the

maximum intensity of fMLP-stimulated Extra LCL. This result is reasonable in view of the fact that Extra LCL is a measure for the extracellular superoxide produced by the cells, the latter being the enzyme substrate. The CuZn SOD activity highly significantly and positively correlated with the time to the peak and maximum intensity of LgCL. This observation may support the findings about the existence of mitochondria-to-nucleus signaling that regulates the antioxidant protein levels triggered by changes in the mitochondrial production of superoxide (Storz 2006). A highly significant and positive correlation existed between CuZn SOD activity and the levels of plasma lipid hydroperoxides. In this case, the increased CuZn SOD activity at unchanged peroxidase activities could cause increased oxidative injury levels. All these data point out the existence of a delicate balance between prooxidative and antioxidative processes in blood, related interdependently. This assumption is supported by literature data that has shown that oxidants may induce an increase in the activity and capacity of endogenous antioxidant defense system but may also suppress components of this activity (Allen et al. 1984; Allen et al. 1985; Sohal et al. 1985; Blakely et al. 1988; Hill et al. 1987).

The advantage of the present study is that the status of components of the antioxidant defense system was examined along with the activity of peripheral phagocytes to generate ROS, and products of oxidative damage in circulation. The data showed a decrease in the extracellular oxidative activity of phagocytes, an increased mitochondrial superoxide production, differential changes in components of the antioxidant defense system and an indication of an increased level of oxidative damage in blood as a function of age. It is probably incorrect to interpret changes in the radical generation or in the activity of antioxidant components as either a deleterious or useful event occurring in vivo. Detailed studies are needed to determine the interrelationship between the pro- and antioxidative processes in the organism in order to elaborate a complex strategy to delay the ageing process.

References

- Agarwal S, Sohal R (1994) DNA oxidative damage and life expectancy in houseflies. *Proc Natl Acad Sci USA* 91:12332–12335. doi:10.1073/pnas.91.25.12332
- Allen RG, Farmer KJ, Sohal RS (1984) Effect of diamide administration on the longevity, oxygen consumption, superoxide dismutase, catalase, inorganic peroxides and glutathione in the adult housefly, *Musca domestica*. *Comp Biochem Physiol* 78C:31–33
- Allen R, Toy P, Newton R et al (1985) Effects of experimentally altered glutathione levels on life span, metabolic rate, superoxide dismutase, catalase and inorganic peroxides in the adult housefly, *Musca domestica*. *Comp Biochem Physiol* C82(2):399–402
- Asakawa T, Matsushita S (1980) Thiobarbituric acid test for detecting lipid peroxides. *Lipids* 14:401–406. doi:10.1007/BF02533425
- Blakely SR, Slaughter L, Adkins J et al (1988) Effects of beta-carotene and retinyl palmitate on corn oil-induced superoxide dismutase and catalase in rats. *J Nutr* 118(2):152–158
- Block G, Dietrich M, Norkus EP et al (2002) Factors associated with oxidative stress in human populations. *Am J Epidemiol* 156(3):275–285. doi:10.1093/aje/kwf029
- Bogdanska JJ, Korneti P, Todorova B (2003) Erythrocyte superoxide dismutase, glutathione peroxidase and catalase activities in healthy male subjects in Republic of Macedonia. *Bratisl Lek Listy (Tlacene Vyd)* 104(3):108–114
- Bochev P, Bechev B, Magrisso M (1992) Six-sample multiplexing computerized analyzer for integral and spectral luminescence measurements. *Anal Chim Acta* 256:29–32. doi:10.1016/0003-2670(92)85321-V
- Bochev B, Magrisso M, Bochev P et al (1993) Dependence of whole blood luminol chemiluminescence on PMNL and RBC count. *J Biochem Biophys Methods* 27(4):301–309. doi:10.1016/0165-022X(93)90011-C
- Bolzan AD, Bianchi MS, Bianchi NO (1997) Superoxide dismutase, catalase and glutathione peroxidase activities in human blood: influence of sex, age and cigarette smoking. *Clin Biochem* 30(6):449–454. doi:10.1016/S0009-9120(97)00047-7
- Cadenas E, Poderoso J, Antunes F et al (2000) Analysis of the pathways of nitric oxide utilization in mitochondria. *Free Radic Res* 33:747–756. doi:10.1080/10715760000301271
- Casado A, López-Fernandez ME (2003) Age-correlated changes of the erythrocyte catalase activity in the Spanish population. *Gerontology* 49(4):251–254. doi:10.1159/000070406
- Chen H, Cangello D, Benson S et al (2001) Age-related increase in mitochondrial superoxide generation in the testosterone-producing cells of Brown Norway rat testes: relationship to reduced steroidogenic function. *Exp Gerontol* 36(8):1361–1373. doi:10.1016/S0531-5565(01)00118-8
- Di Massimo C, Lo Presti R, Corbacelli C et al (2006) Impairment of plasma nitric oxide availability in senescent healthy individuals: apparent involvement of extracellular superoxide dismutase activity. *Clin Hemorheol Microcirc* 35(1–2):231–237
- Douzou P, Maurel P (1977) Ionic regulation in genetic translation systems. *Proc Natl Acad Sci USA* 74(3):1013–1015. doi:10.1073/pnas.74.3.1013
- Ellman G (1959) Tissue sulphydryl groups. *Arch Biochem Biophys* 82:70–77. doi:10.1016/0003-9861(59)90090-6
- Gilca M, Stoian I, Atanasiu V et al (2007) The oxidative hypothesis of senescence. *J Postgrad Med* 53(3):207–213

- Hack V, Breitkreutz R, Kinscherf R et al (1998) The redox state as a correlate of senescence and wasting and as a target for therapeutic intervention. *Blood* 92:59–67
- Hensley K, Maitt L, Stewart CA et al (1999) Mitochondrial alteration in aging and inflammation: a possible site of action of nitron-based free radical traps. In: Cadenas E (ed) *Understanding the process of aging*. Marcel Dekker, New York, pp 311–326
- Hill KE, Burk RF, Lane JM (1987) Effect of selenium depletion and repletion on plasma glutathione and glutathione-dependent enzymes in the rat. *J Nutr* 117 (1):99–104
- Ho SP, Chan-Yeung M, Chow KK (2005) Antioxidant enzyme activities in healthy Chinese adults: influence of age, gender and smoking. *Respirology* 10(3):305–309. doi:10.1111/j.1440-1843.2005.00702.x
- Kasapoglu M, Ozben T (2001) Alterations of antioxidant enzymes and oxidative stress markers in aging. *Exp Gerontol* 36(2):209–320. doi:10.1016/S0531-5565(00)00198-4
- Kedziora-Komatowska K, Szewczyk-Golec K, Czuczejko J et al (2007) Effect of melatonin on the oxidative stress in erythrocytes of healthy young and elderly subjects. *J Pineal Res* 42(2):153–158. doi:10.1111/j.1600-079X.2006.00394.x
- Maral J, Puget K, Michelson A (1977) Comparative studies of SOD, catalase and glutathione peroxidase in erythrocytes of different animals. *Biochem Biophys Res Commun* 77:1525–1535. doi:10.1016/S0006-291X(77)80151-4
- Mecocci P, Polidori MC, Troiano L et al (2000) Plasma antioxidants and longevity: a study on healthy centenarians. *Free Radic Biol Med* 28(8):1243–1248. doi:10.1016/S0891-5849(00)00246-X
- Ozturk O, Gumuslu S (2004) Changes in glucose-6-phosphate dehydrogenase, copper zinc-superoxide dismutase and catalase activities, glutathione and its metabolizing enzymes, and lipid peroxidation in rat erythrocytes with age. *Exp Gerontol* 39(2):211–216. doi:10.1016/j.exger.2003.10.015
- Passos JF, von Zglinicki T, Saretzki G (2006) Mitochondrial dysfunction and cell senescence: cause or consequence. *Rejuvenation Res* 9(1):64–68. doi:10.1089/rej.2006.9.64
- Pereslegina I (1989) Salivary antioxidant enzymes activity in normal children. *Lab Delo* 11:20–23
- Perskin MH, Cronstein BN (1992) Age-related changes in neutrophil structure and function. *Mech Ageing Dev* 64 (3):303–313. doi:10.1016/0047-6374(92)90086-S
- Rizvi SI, Maurya PK (2007) Alterations in antioxidant enzymes during aging in humans. *Mol Biotechnol* 37(1):58–61. doi:10.1007/s12033-007-0048-7
- Sanz A, Gómez J, Caro P (2006) Carbohydrate restriction does not change mitochondrial free radical generation and oxidative DNA damage. *J Bioenerg Biomembr* 38(5–6):327–333. doi:10.1007/s10863-006-9051-0
- Shacter E (2000) Quantification and significance of protein oxidation in biological samples. *Drug Metab Rev* 32:302–326. doi:10.1081/DMR-100102336
- Sigma (1997) Catalase assay. In: Sigma Chemie Catalog 1997. Sigma-Aldrich, St Louis, p 254
- Sohal RS, Allen RG (1990) Oxidative stress as a causal factor in differentiation and aging: a unifying hypothesis. *Exp Gerontol* 25:499–522. doi:10.1016/0531-5565(90)90017-V
- Sohal RS, Allen RG, Farmer KJ (1985) Effects of exogenous antioxidants on the levels of endogenous antioxidants, lipid-soluble fluorescent material and life span in the housefly, *Musca domestica*. *Mech Ageing Dev* 31(3):329–336. doi:10.1016/0047-6374(85)90098-3
- Sohal RS, Sohal BH, Orr WC (1995) Mitochondrial superoxide and hydrogen peroxide generation, protein oxidative damage, and longevity in different species of flies. *Free Radic Biol Med* 19(4):499–504. doi:10.1016/0891-5849(95)00037-X
- Stadtman ER (2006) Protein oxidation and aging. *Free Radic Res* 40(12):1250–1258. doi:10.1080/10715760600918142
- Storz P (2006) Reactive oxygen species-mediated mitochondria-to-nucleus signaling: a key to aging and radical-caused diseases. *Sci STKE* 332:re3. doi:10.1126/stke.3322006re3
- Sun J, Tower J (1999) FLP recombinase-mediated induction of Cu/Zn-superoxide dismutase transgene expression can extend the life span of adult *Drosophila melanogaster* flies. *Mol Cell Biol* 19:216–228
- Tosi M, Hamedani A (1992) A rapid, specific assay for superoxide release from phagocytes in small volumes of whole blood. *Am J Clin Pathol* 97:566–573
- Volkovova K, Beno I, Staruchova M et al (1996) Antioxidative enzyme activity in the blood of healthy persons. *Bratisl Lek Listy (Tlacene Vyd)* 97(3):134–138
- Volkovova K, Barancokova M, Kazimirova A et al (2005) Antioxidant supplementation reduces inter-individual variation in markers of oxidative damage. *Free Radic Res* 39 (6):659–666. doi:10.1080/10715760500117472
- Walan A, Dahlgren C, Kihlstrom E et al (1992) Phagocyte killing of *Campylobacter jejuni* in relation to oxidative activation. *Path Microbiol Immunol Scand* 100:424–430
- Yagi K (1987) Lipid peroxides and human diseases. *Chem Phys Lipids* 45:337–351. doi:10.1016/0009-3084(87)90071-5