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Oxidation scrutiny in persuaded aging and chronological aging at systemic redox homeostasis level

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ABSTRACT

Background: The effect of the natural aging process on systemic redox homeostasis is previously documented. However, none of the studies specify the effect of experimental aging on systemic redox homeostasis. The purpose of this study is to clarify the ambiguity raised in preliminary reports as to mimetic aging dependency of the type and magnitude of oxidative damage on constituents of plasma.
Methods: In the current study, we investigated the interrelationship among various groups of the systemic oxidative damage markers such as protein oxidation products (*protein carbonyl groups, protein hydroperoxides, advanced oxidation protein products, protein thiol groups*), lipid peroxidation products (*malondialdehyde, lipid hydroperoxides, conjugated dienes*), glycoxidation adducts (*advanced glycation end products*), and antioxidant capacity (*ferric reducing/antioxidant power, Cu,Zn-superoxide dismutase, total thiol, non-protein thiol*). All these markers were measured in plasma of mimetically aged (MA) rats (5-month-old rats subjected to D-galactose-induced experimental aging), naturally aged (NA) rats (24-month-old), and their corresponding young controls (YC) (5 months old).
Results and conclusions: Our current results show that systemic oxidation markers of the MA group share significant similarities in terms of impaired redox homeostasis with the NA rats and may be considered as a reliable experimental aging model for intravascular aging. Additional methodological studies including D-galactose dosage and application time are warranted to clarify the potential involvement of all these systemic redox variations as mechanistic factors in the development of mimetic aging related intravascular deterioration. Reversing or preventing systemic oxidative damage in experimental and natural aging should therefore be considered the primary target for the development of effective therapeutic strategies to prevent or treat age-related vascular disorders.

1. Introduction

Among the various etiological factors contributing to the development of age-related vascular dysfunction, increased production of reactive oxygen species (ROS) seems to play an important role in the occurrence of impaired redox homeostasis (Kayalı et al., 2007; Çakatay et al., 2008, 2010). Important oxidative alterations in the structure and function of endothelial cells accompany advancing age (Çakatay et al., 2008). These age-associated alterations in the endothelial cells of the arterial wall are favorable to the initiation or progression of superimposed vascular diseases (Puca et al., 2013). Although all these changes in vascular structure and function were previously thought to be part of chronological aging, this concept needs to be revisited in view of novel emerging data on D-galactose-induced mimetic aging. These vascular changes are accelerated in the presence of elevated levels of oxidized proteins, lipids and DNA in plasma of naturally aged experimental animals (Çakatay et al., 2010). The aging-dependent decline in the homeostatic redox capacity for plasma is responsible to formation of increased level of oxidized macromolecules. Exact molecular mechanisms are largely unknown and controversial (Çakatay et al., 2008).

Both physiologically and pathologically, the D-galactose-administered animals resemble their aged counterparts of 16–24 months. The underlying mechanism(s) responsible for D-galactose-induced mimetic aging changes have been explained to be due to formation of a high concentration of advanced glycation end products (AGEs) and also due to an

Abbreviations: AGEs, advanced glycation end products; AOPP, advanced oxidation protein products; AU, arbitrary units; CD, conjugated dienes; Cu,Zn-SOD, Cu,Zn-superoxide dismutase; FRAP, ferric reducing/antioxidant power; L-OOH, lipid hydroperoxides; MA, mimetically aged; MDA, malondialdehyde; NA, naturally aged; NP-SH, non-protein thiol; PCO, protein carbonyl groups; P-SH, protein thiol; ROS, reactive oxygen species; T-SH, total thiol; YC, young control.

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increase in osmotic stress resulting from the reduction of galactose to galactitol (Yanar et al., 2011). On the other hand, D-galactose shows its effect by increasing advanced glycation end products (AGEs) as well (Hsieh et al., 2009). The ambiguity arose in preliminary reports as to mimetic aging-dependent variations in the levels of some oxidative-damage parameters in plasma of model subjects (Hsia et al., 2012; Kumar and Rizvi, 2013). The effect of the natural aging process on systemic redox homeostasis of plasma proteins, lipids, DNA and antioxidants is previously documented (Çakatay et al., 2008, 2010; Kayalı et al., 2007). However, none of the studies specifies the effect of experimental aging on systemic redox homeostasis. The purpose of this study is to clarify the ambiguity raised in preliminary reports as to mimetic aging dependency of the type and magnitude of oxidative damage in plasma. We hope that the broad group of oxidative damage markers analyzed in the current study will provide an insight that will clarify the complex issue. All these markers were analyzed in plasma of naturally aged rats (NA), mimetically aged (MA) rats, and their corresponding young controls (YC).

2. Material and methods

2.1. Chemicals and apparatuses

Unless otherwise specified, the chemicals were of the highest analytical grade available. All chemicals and reagents were purchased from Merck (Darmstadt, Germany) and Sigma-Aldrich (St Louis, MO, USA). Deionized water was used in the analytical procedures. All reagents were stored at +4 °C. The reagents were maintained in equilibrium at room temperature for 0.5 h before use. Centrifugation procedures for the analysis of various oxidative stress markers were performed at +4 °C with a Sigma 3–18 KS centrifuge (SIGMA Laborzentrifugen GmbH, Osterode am Harz, Germany). Plasma oxidative damage biomarker profiles were analyzed by spectrophotometric and spectrofluorimetric methods with Biotek Synergy™ H1 Hybrid Multi-Mode Microplate Reader (BioTek US, Winooski, VT, USA). Early phase of lipid peroxidation in plasma samples was assessed using second derivative spectroscopy technique on the same device.

2.2. Animal model and treatment protocol

The study was initially carried out with 24 male Sprague–Dawley rats. All of the experimental studies were conducted in accordance with the national laws of Republic of Turkey. Ethical protocol of the current research was approved by the Ethics Committee of Istanbul University, Istanbul, Turkey: Ethics Committee Issue Number: (2011/162). They were housed in a temperature-controlled room (25 ± 5 °C) with 12-h light–dark cycles. All rats were fed with normal laboratory diet nutrient rich pellets containing total energy such as fat, protein and carbohydrates, and had free access to drinking water. After a 1-week adaptation period, the animals were divided into three groups of animals. Both Group 1: young control (YC) rats (n = 8; 5 months old) and Group II: naturally aged (NA) rats (n = 8; 24 months old) were given daily intraperitoneal injections of NaCl (0.9%) for 6 weeks, Group III: mimetically aged (MA) rats (n = 8; chronologically 5 months old) given daily intraperitoneal injections of D-galactose (60 mg/kg body weight) for 6 weeks. All animals were sacrificed 24 h later following the last galactose injections.

2.3. Blood collection and preparation of plasma samples

When the experimental period was over, rats were sacrificed under anesthesia with ketamine (44 mg/kg)–xylazine (33 mg/kg) combination. Intra-cardiac blood samples were drawn into tubes containing lithium heparin with a gel separator (Vacuette®, Greiner Bio-One, USA). World Health Organization determined that plasma was more feasible than serum in most laboratory tests because plasma, with less interference, was better at reflecting the pathophysiological processes (Banfi et al., 2002). Additionally, plasma has some advantages to serum as a laboratory specimen. First is the prevention of coagulation-induced interferences. In some serum samples with poor coagulability, clot formation can be still ongoing when centrifugation is finished. The gel separator was made up of an organic hydrophobic substance that is inert to blood and forms an impermeable barrier between the plasma and blood cells. Advantages of separator tubes are rapid separation of plasma from blood cells and avoidance of possible interferences derived from blood cells.

During aliquot preparation, plasma was maintained at +4 °C in dim light. The plasma was divided into aliquots (one for each assay) and immediately stored at −80 °C (two-week maximum) for protein carbonyl (PCO), total thiol (T-SH), protein thiol (P-SH), non-protein thiol (NP-SH), advanced oxidation protein products (AOPP), advanced glycation end products (AGEs), malondialdehyde (MDA), Cu,Zn-superoxide dismutase (Cu,Zn-SOD), and ferric reducing/antioxidant power (FRAP) assays. Our preliminary experiments carried out with both fresh and thawed plasma specimens for protein hydroperoxide (P-OOH), lipid hydroperoxide (L-OOH), and conjugated diene (CD) experiments showed that prolonged periods of storage did significantly affect the reliability of test results (data not shown). The assays for the last three aforementioned parameters were carried out with fresh plasma samples.

2.4. Analytical methods

The investigated oxidative stress-related biomarkers are detailed in Table 1.

2.4.1. Assays of protein oxidation markers

2.4.1.1. Protein carbonyl groups. We performed PCO analysis as described by Reznick and Packer (1994) with some slight modifications in order to apply small volumes of plasma samples. PCO groups react with 2,4-dinitrophenylhydrazine (DNPH) (100 μL plasma:400 μL DNPH) to generate chromophoric dinitrophenylhydrazones. DNPH was dissolved in HCl, and after DNPH reaction, proteins were precipitated with an equal amount of 20% (w/v) trichloroacetic acid and washed three times with 400 μL of an ethanol/ethyl acetate mixture (1:1). Washing was done by mechanical disruption of pellets in the washing solution and repelleting by centrifugation at 3000 *g* for 5 min. Finally, the protein precipitates were dissolved in a 200 μL 6 M guanidine-HCl solution and the related absorbance was measured at 360 nm using the molar extinction coefficient of DNPH, $\varepsilon = 22{,}000\ M^{-1}\ cm^{-1}$. The coefficients of intra- and inter-assay variations for modified carbonyl assay were 4.1% (n = 8) and 8.1% (n = 8), respectively. The PCO–BSA positive control and untreated BSA were both prepared according to the method of Lenarczyk et al. (2009) and tested according to the PCO assay protocol.

2.4.1.2. Protein hydroperoxides. P-OOHs were measured by the guanidine–perchloric acid–ferric-xylenol orange method (G–PCA–FOX) (Kayalı et al., 2007) with some modifications. The assay is based on the oxidation of Fe^{2+} by peroxides in the presence of the dye xylenol orange that gives a colored complex with the Fe^{3+} generated. The Fe–XO complex can be measured in the visible absorbance range (560 nm). Proteins were precipitated from 25 μL of plasma by the addition of 125 μL of 0.2 M ice-cold PCA. Samples were kept on ice for 5 min and centrifuged at 6500 *g*. The precipitated proteins were dissolved in 275 μL of 6 M guanidine hydrochloride. The protein solution was then washed twice with chloroform containing 4 mM butylated hydroxytoluene. After mixing of the washed protein solution, xylenol orange, and ferrous solutions, the absorbance at 560 nm was read after 60 min against a blank containing 6 M guanidine-HCl solution instead of the protein solution. The molar concentration of P-OOHs in the final medium was calculated with the equation $c = A_s / \varepsilon$ using the molar absorption coefficient value of 37,000 $M^{-1}\ cm^{-1}$. The coefficients of intra- and