Antioxidant capacity in relationship to serum lipid peroxides levels in healthy elderly of Mexico City*

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Summary

The aging is one of the factors that cause decrease in the antioxidant capacity. Likewise, it has been proposed that subjects exposed permanently to air pollution develop deficient antioxidant capacity to oxidative stress (OxS). This study aimed to analyze the antioxidant capacity against elevated lipid peroxides in healthy elderly of Mexico City. 105 adults (44 ± 10.8 years) and 126 elderly subjects were studied (68 ± 7.1 years); residents of Mexico City (clinically healthy, non-smokers, non-vitamin supplement takers) who had lived in the city for >10 years. Plasma lipoperoxides (LPO), total antioxidant status (TAS), the activity of red blood cells superoxide dismutase (SOD), and plasma glutathione peroxidase (GPx), were studied in all subjects. LPO levels were found significantly higher ($p < 0.05$) in the elderly subjects in comparison with the adults; in addition, TAS and GPx were higher in adults than among the elderly people ($p < 0.0001$). Nevertheless, SOD was similar in both groups ($p = 0.346$). These findings reveal that the elderly residents of Mexico City have TAS and GPx lower than adults, and similar SD activity, probably due to the fact that these antioxidants are neutralizing the higher LPO levels of elderly people. Therefore, this mechanism could be considered as an efficient antioxidant capacity in the elderly, as response to high LPO levels, since the health status, mortality prevalence and life span life of the older people of Mexico City are similar or better than other cities of Mexican Republic.

Key words: Adaptation to oxidative stress * total antioxidant status * elderly people * pollution * superoxide dismutase * glutathione peroxidase.

Resumen

CAPACIDAD ANTIOXIDANTE EN RELACIÓN A NIVELES SÉRICOS DE LIPOPERÓXIDOS EN ANCIANOS SANOS DE LA CIUDAD DE MÉXICO

Se ha propuesto que los sujetos expuestos permanentemente a la contaminación ambiental tienen una deficiente capacidad para contrarrestar el estrés oxidativo (EOx) y que el envejecimiento es un factor causante de

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dicha alteración. EL objetivo de este estudio fue analizar la capacidad antioxidante contra el aumento de lipoperóxidos (LPO) en adultos mayores sanos de la ciudad de México. Se estudiaron 105 adultos residentes de la ciudad de México (44 ± 10,8 años) y 126 adultos mayores (68 ± 7,1 años) clínicamente sanos, no fumadores, sin ingesta de vitaminas antioxidantes, con residencia en esta ciudad por más 10 años. Se cuantificó a todos los sujetos los LPO plasmáticos, capacidad sérica antioxidante total (AT), actividad eritrocitaria de superóxido dismutasa (SOD) y plasmática de glutatión peroxidasa (GPx). Se encontró que los niveles de LPO fueron más altos en los adultos mayores comparados con los jóvenes ($p < 0,05$); asimismo, AT y GPx fueron mayores en los jóvenes ($p < 0,0001$). La SOD fue similar en ambos grupos ($p = 0,346$). Estos hallazgos revelan que los ancianos residentes de la ciudad de México tienen concentraciones más bajas de AT y GPx en comparación con los adultos y una actividad similar de la SOD, debido probablemente a que estos antioxidantes están neutralizando los niveles más altos de los LPO de los ancianos. Por lo tanto, este mecanismo podría ser considerado como una capacidad antioxidante eficiente en los ancianos como respuesta a los altos niveles de LPO, ya que el estado de salud, prevalencia de mortalidad y longevidad de los adultos mayores de la ciudad de México es similar o mejor al de los residentes de otros estados de la República Mexicana.

Palabras clave: Adaptación a estrés oxidativo * capacidad antioxidante total * adultos mayores * contaminación ambiental * superóxido dismutasa * glutatión peroxidasa.

Introduction

Oxidative stress (OxS) is a serious imbalance between the reactive oxygen species (ROS) produced and the effective action of the antioxidant system. It is a factor that contributes to aging and the development, among other diseases, of diabetes mellitus, chronic obstructive lung disease, atherosclerosis, Parkinson's disease, Alzheimer's disease, rheumatoid arthritis, and some types of cancer (1). Diverse factors affect the antioxidant status in favor of OxS, such as an antioxidant-deficient diet, strenuous exercise, smoking, alcoholism, exposure to air pollutants, genetic alterations and age (2).

There are abundant experimental and observational evidence that supports the idea that aging is the sum of all free radical reactions throughout all cells and tissues, or at least that they are a major contributor to it (3)(4).

The inhabitants of Mexico City are exposed most of the time to high levels of air pollutants, which have been associated with an increase in the incidence of mortality in children (5). However the health status, mortality prevalence and life span of the elder people in Mexico City is similar or better than others cities of Mexican Republic (6).

In such regard, it has been demonstrated that newly arrived subjects to Mexico City (1-8 days) present greater lipoperoxidation concomitant with a greater production of Cu/Zn-superoxide dismutase (SOD), in comparison with permanent residents. In spite of this, SOD activity decreases by 50% at 16 weeks, accompanied by a lowering in plasma lipoperoxides (LPO) of 30%, probably due to the adaptive capacity or efficient antioxidant activity that the inhabitants of Mexico City develop to air pollution (7).

Therefore, the purpose of this study was to evaluate the antioxidant activity in healthy adults and elderly people, to ascertain the influence of the aging and exposition to air pollution on the capacity of response against lipid peroxides production that occur in the Mexico City elderly population.

Material and Methods

POPULATION UNDER STUDY

The study included free-living subjects: 105 adults aged < 60 years (mean 44 ± 10.8 years) and 126 elder subjects aged 60-85 years (mean 68 ± 7.1 years). All of them had lived in Mexico City for the past 10 years. None of the subjects studied had been taking antioxidant supplementation (vitamins and/or minerals) smoked, had acute or chronic diseases, or was receiving prescribed medication, and were not alcohol heavy drinkers for at least 6 months before the study iniciation.

The subjects were accepted to participate in the study after their informed consent. The Ethics Committee of Universidad Nacional Autónoma de México (UNAM) Zaragoza Campus approved the research protocol for this study.

Weight, height, and body mass index (BMI) were obtained as anthropometric measurements. Weight was measured with the subject in a fasting state and after evacuation, in underwear and a clinical smock. A

Torino' scale (Tecno Lógica Mexicana, Mexico) was used, calibrated prior to each weight measurement. Height was obtained with an aluminum cursor stadimeter graduated in millimeters, with the subject without footwear, with heels, back, and head in contact with the stadimeter in Frankfurt horizontal plane. BMI was calculated by means of the division of weight in kg by height in squared meter (kg/m^2).

ATMOSPHERIC MONITORING

Air-pollution data was collected from the regional quality network. Annual mean of ozone concentration in the atmospheric environment of Mexico City was 0.155 ± 0.46 ppm (8).

BLOOD SAMPLING AND PREPARATION

In the all subjects, blood samples were collected after a 12 hour fasting period by venopuncture and placed in vacutainer/siliconized test tubes containing a separating gel and no additive. EDTA or heparin was employed as the anticoagulant agent. Blood samples containing EDTA were analyzed using a complete blood count (including hemoglobin, hematocrit, and leukocyte counts). The following serum quantifications were conducted: glucose, urea, creatinine, urate, albumin, cholesterol, triglycerides, and high-density lipoproteins (HDL) cholesterol. These tests were used as screening measurements for the diagnosis of the clinically healthy subjects. All reagents employed in biochemical tests were obtained from Randox Laboratories, Ltd. Cut-off points for reference values were determined at the Gerontology Clinical Research Laboratory of the UNAM, Zaragoza Campus, in Mexico City (9).

TOTAL ANTIOXIDANT STATUS

Total antioxidant status was determined using $ABTS^+$ (2,2'-azidodiethylbenzothiazolin sulphonate) radical formation kinetics (Randox Laboratories, Ltd). The presence of antioxidants in plasma suppresses the bluish-green staining of the $ABTS^+$ cation, which is proportional to the antioxidant concentration. Kinetics is measured at 600 nm.

RED BLOOD CELL SUPEROXIDE DISMUTASE (SOD)

The method employs xanthine and xanthine oxidase (XO) to generate superoxide radicals, which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT) to form a red formazan dye. SOD activity was measured by the degree of inhibition of the reaction (Randox Laboratories, Ltd). Kinetics is measured at 505 nm.

PLASMA GLUTATHIONE PEROXIDASE (GPX)

GPx catalysed the oxidation of glutathione (GSH) by cumene hydroperoxide, in the presence of glutathione reductase (GR) and NADPH; the oxidized glutathione (GSSG) was immediately converted to the reduced form with a concomitant oxidation of NADPH to $NADP^+$. The decrease in absorbance at 340 nm was measured (Randox Laboratories, Ltd).

PLASMA LIPOPEROXIDES

The thiobarbituric acid reacting substances (TBARS) assay was used, as described by Jentzch et al. (10). In the TBARS assay, one molecule of malondialdehyde (MDA) reacted with two molecules of thiobarbituric acid (TBA) with the production of a pink pigment with absorption at 535 nm. Amplification of peroxidation during the assay was prevented by the addition of the chain-breaking antioxidant BHT.

Plasma (400 µL) or MDA standard (0.2-4 mmol/L) prepared by hydrolysis of 1,1,3,3-tetramethoxypropane (TMP) (Sigma Chem. Co. St. Louis, MO. USA) was mixed with 400 µL orthophosphoric acid (0.2 mol/L) (Sigma Chem. Co.) and 50 µL BHT (2 mmol/L) (Sigma Chem. Co.), in 12 X 75 mm tubes. Then we added 50 µL TBA reagent (0.11 mol/L in 0.1 mol/L NaOH) (Fluka Chem., Buchs, Switzerland) and mixed; subsequently the contents were incubated at 90 °C for 45 min in a water bath. The tubes were put on ice to stop the reaction. TBARS were extracted once with 1000 µL n-butanol (Sigma Chem. Co.). The upper butanol phase was read at 535 nm and 572 nm to correct for baseline absorption in UV-Spectrophotometer (Shimadzu, Columbia, MD, USA). MDA equivalents (TBARS) were calculated using the difference in absorption at the two wavelengths and quantification was done with calibration curve.

STATISTICAL ANALYSIS

Data were processed through use of standard statistical software SPSS 10.0 (SPSS Inc. Michigan, IL, USA). Descriptive statistics are means ± standard deviation (SD); results were analyzed using Student's *t*-test. A p-value < 0.05 was considered significant.

Results

Biochemical characteristics of the subjects under study showed that the elderly and adults had normal levels of all parameters (Table I).

LPO were found significantly higher ($p < 0.05$) in the elderly as compared with adults (Table II); in the same manner, TAS and GPx activity were observed to

be higher in the adults than among older persons ($p < 0.0001$); nevertheless, SOD activity was similar in both groups ($p = 0.346$).

Discussion and Conclusion

Studies on the molecular biology during the aging process are not entirely consistent, probably due to the biological and social heterogeneity of the populations studied, in addition to the environmental influence (11). For this reason, although some generalizations have been established, such as that DNA oxidative damage increases with age (12), it has been demonstrated that this does not occur in all populations. In this regard, it was reported that 45% of the elderly people in Mexico City have oxidative DNA damage in lymphocytes (13)(14), and at the same time that urban elderly inhabitants have higher LPO levels and lower antioxidant capacity than rural elderly population (15).

In respect with diseases related to aging, it has been demonstrated that oxidative DNA damage is associated with heart disease (16); in the same manner Lerman et al. found higher prevalence of diabetes mellitus among elderly residents of Mexico City in contrast to elderly residents of a rural area (17). Moreover, Leinonen et al. revealed an association between antioxidant capacity and coronary heart disease as well as renal dysfunction in subjects with diabetes mellitus (18).

On the other hand, it has been established that OxS increases with aging; however, King et al. demon-

Table I. *Biochemical characteristics and body mass index (BMI) of the subjects under study.*

	Adults (n = 105)	*Elderly (n = 126)*
Glucose (mmol/L)	5.27 ± 1.72	5.44 ± 2.0
Urea (mmol/L)	11.42 ± 3.21	12.49 ± 3.57
Creatinine (mmol/L)	85.74 ± 22.10	81.33 ± 20.33
Urate (µmol/L)	303.45 ± 95.20	297.50 ±107.10
Cholesterol (mmol/L)	5.28 ± 0.98	5.77 ± 1.45
Triglycerides (mmol/L)	2.08 ± 1.07	2.06 ± 0.92
HDL cholesterol (mmol/L)	1.24 ± 0.33	1.32 ± 0.39
Albumin (mmol/L)	0.66 ± 0.06	0.62 ± 0.07
Hemoglobin (mmol/L)		
Females	8.88 ± 0.74	8.75 ± 0.80
Males	10.24 ± 1.12	9.81 ± 1.12
Hematocrit		
Females	0.44 ± 0.03	0.44 ± 0.04
Males	0.49 ± 0.03	0.46 ± 0.05
Total leukocytes ($X10^9$/L)	6.66 ± 1.51	6.49 ± 1.55
BMI (kg/m^2)	27.5 ± 4.0	27.8 ± 4.3

Table II. *Mean values ± SD of plasma lipoperoxides, total antioxidant status, and antioxidant enzymes (SOD and GPx) in adults and elderly.*

	Adults	*Elderly*
n	105	126
Lipoperoxides (µmol/L)	0.328 ± 0.17	0.399 ± 0.19*
Total antioxidant status (mmol/L)	1.28 ± 0.27	1.16 ± 0.21†
Superoxide dismutase (U/L)	175 ± 11.3	173 ± 17.6
Glutathione peroxidase (U/L)	7525 ± 2030	6281 ± 2166†

*$p < 0.05$, †$p < 0.0001$; Student's *t* test.

strated that antioxidant levels, GPx and catalase (CAT) activities, and ceruloplasmine levels were significantly higher in a group of elderly adults from 75-80 years of age compared with individuals in the age groups from 35-39 years, 50-54 years, and 65-69 years (19). Similar results have been observed in centenarians (20). Also, it has been demonstrated that tolerance or adaptation to OxS increases during the life span, this probably associated to better health (21). In this sense, the results of this study show that SOD activity is similar in elderly and adults ($p > 0.05$), though older subjects have LPO higher as compared to the adults ($p < 0.05$), which could be considered as a response of adaptation to oxidative stress. In this regard, it has been reported in several studies that there exists a progressive increase of LPO age-related associated with a decrease in SOD ($r = –0.83$) (22)(23), however in this study it was not observed decrease in SOD activity age-related. Nevertheless, Mecocci *et al.* observed that SOD activity rises proportionally during aging, though diminishing in centenarians, which can be interpreted as a compensatory response of the organism to elevate in ROS with increasing age, for enjoy a succesful aging (20). This same incremental behavior in SOD activity with higher ages was observed by Okabe et al. (24). However, Medina-Navarro et al. demonstrated that SOD initially increases prior to exposure to air pollution, to later diminish by 50% at 4 months of constant exposure (7). In this sense, the results of this study reveal that the elderly residents of Mexico City have TAS and GPx lower than adults and a similar SOD activity, due probably to the fact that these antioxidants are neutralizing the higher LPO levels. Therefore this mechanism could be considered as an efficient antioxidant capacity against high LPO levels by exposure to air pollution. In this sense, the health status, mortality prevalence and life span of the Mexico City inhabitants are similar or better than other cities of Mexican Republic (6). In such regard, it has been showed that resistance to oxidative stress may be acquired by coordinated changes in multiple antioxidant pathways (25).

With relation to TAS, in this study it was observed a statistically significant decrease in the elderly, in comparison to younger persons ($p < 0.0001$), which contrasts with that reported by Aejmaleus *et al.*, who found that antioxidant capacity increases in relation to age increase (26). This may be due to the fact that the elder subjects, living in Mexico City exposed to a higher OxS from air pollution, show a relative diminution in antioxidant capacity as a consequence of the permanent consume of antioxidant by aggression of free radicals. This mechanism can be a response of an adaptation process, which is necessary to survive in a city with high pollutants levels like Mexico City.

On the other hand, it was found in this study a significantly lower GPx activity in the elderly subjects as compared to young adults ($p < 0.0001$), in contrast with that reported by King *et al.* and Mecocci *et al.*, who concluded that GPx activity increase with age (19)(20). In such regard, the importance of GPx to maintain homeostasis in the light of increase of LPO has been demonstrated by Laaksonen (27). Therefore, the lower levels of GPx in the elder inhabitants of Mexico City, in comparison with those of young adults, could be due to an efficient biological response or adaptative process to the greater production of ROS, due to pollution and aging itself, a response achieved little by little through a process of adaptation to OxS.

Although the results are not conclusive since it is a cross-sectional study, it allows us to infer that the elderly residents of Mexico City have TAS and GPx lower than adults, and similar SOD activity , due to the fact that these antioxidants are neutralizing the higher LPO levels. Therefore this mechanism could be considered as an efficient antioxidant capacity in the elderly, as response to high LPO levels.

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