40

4th INTERNATIONAL SYMPOSIUM ON TRACE ELEMENTS IN HUMAN: NEW PERSPECTIVES

PROCEEDINGS BOOK
PART I

9 - 11 October 2003 - ATHENS Greece

Editors: S. ERMIDOU-POLLET – S. POLLET

BLOOD ELEMENTAL LEVELS AND ANTIOXIDANT MARKERS IN HUMANS WITH ATHEROSCLEROTIC DISEASE

Paula Alexandra Lopes[1], Patrícia Napoleão[1], Teresa Pinheiro[2], Maria Cristina Santos[3], Maria Odete Rodrigues[4], Fátima Ceia[1], Luís Vicente[1], Maria da Luz Mathias[1], Maria Leonor Pavão[5], Jean Nève[6] and Ana Maria Viegas-Crespo[1*]

[1]Centro de Biologia Ambiental and Departamento de Biologia Animal, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal; [2]Departamento de Física, Instituto Tecnológico e Nuclear, Estrada Nacional nº 10, 2685-953 Sacavém, Portugal; [3]Centro de Estudos de Bioquímica e Fisiologia and Departamento de Química e Bioquímica, Faculdade de Ciências de Lisboa, 1749-016 Lisboa, Portugal; [4]Centro de Biopatologia, Química Clínica, Instituto Nacional de Saúde Dr. Ricardo Jorge, Av. Padre Cruz, 1649-016 Lisboa, Portugal; [5]C.I.R.N, Universidade dos Açores, 9500 Ponta Delgada, Portugal; [6]Institut de Pharmacie, Université Libre de Bruxelles, Campus Plaine 205-5, B-1050 Bruxelles, Belgique

**Author to whom correspondence and reprints should be addressed*
Ana Maria Viegas-Crespo
Departamento de Biologia Animal, Faculdade de Ciências, Universidade de Lisboa, Bloco C_2, 3º piso, 1749-016 Lisboa, Portugal.
Telephone: (+) 351217500000; Fax: (+) 351217500028;
Email: amcrespo@fc.ul.pt

Abstract

Although mortality from coronary heart disease has declined recently, atherosclosis and related vascular diseases are still the major cause of death in the western countries. Experimental studies have demonstrated that a disturbed balance between free radical formation and antioxidant defenses, can play a role in the development and progression of various diseases, namely atherosclerosis. In this study trace element levels were measured in plasma and in blood cells of subjects suffering from atherosclerotic disease. Blood activities of antioxidant enzymes, superoxide dismutase (SOD) and glutathione peroxidase (GPx) and plasma total thiols were also evaluated in these patients. Significantly decreased Se and Fe levels were found in blood plasma of the atherosclerotic group. A

tendency for disruption in antioxidant enzyme status was observed in these patients, but plasma total thiols were unchanged.

These are preliminary results and a large atherosclerotic group is required in order to clarify the observations and to contribute for the knowledge on the molecular basis of atherosclerosis.

Key Words: atherosclerosis, oxidative stress, trace elements, antioxidant enzymes, total thiols

Introduction

Atherosclerosis is the leading cause of death in industrialized societies[1]. The etiology of this disease is multifactorial, involving genetic and metabolic factors, as well as dietary habits and life style which are well established risk factors for the development of this pathology[2]. A disturbed balance between formation of free radicals and antioxidant defences can play a role in the progression of various degenerative diseases, including atherosclerosis. Experimental studies suggest that the LDL oxidation in the vessel walls is directely related to the progression of atherosclerotic lesions through several mechanisms[3]. In consequence, fundamental research on redox balance in atherosclerosis and associated pathologies (such as cardiovascular disease and stroke) is an important goal and may help ones to select more appropriate study designs, in terms of preventive measures in populations. In this work preliminary results are presented under the scope of a project aiming to evaluate the redox balance and its relationship with genetic and clinical aspects in subjects with well recognized and established diagnostic of atherosclerosis. The objective of the present study is the measurement of biochemical indicators, comprising the assessment of blood superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities and plasma total thiols as indicators of redox balance. Trace elements levels (Fe, Cu, Zn and Se) related to the oxidant/antioxidant balance in plasma and whole blood were also measured.

Material and Methods

Study design

The protocol of this project was approved by the Human Ethics Committee of the National Health Institute Dr. Ricardo Jorge. The sampling group consisted of 23 volunteers (14 healthy and 9 patients) of Portuguese origin living in the city of

Lisbon with ages between 40 and 70 years. Informed consent forms were obtained from each donor before blood was drawn. All subjects were screened to determine eligibility, which implicated filling up a clinical report stating all relevant information to the study. Atherosclerotic diagnosed subjects constituted the pathological group. Information regarding age, gender, drug intake (oral contraceptives, hormonal replacement therapy and others), smoking habits and general clinical state was obtained. The healthy group was constituted by non smokers subjects without apparent chronic diseases.

Methods

Blood collection: The blood collection took place at the National Health Institute Dr. Ricardo Jorge. The venipuncture of the individuals was carried out in the morning after 12-hour fasting. Peripheral blood samples were obtained by venipuncture and placed into S-Monovette® Z-Gel and Lithium-heparin tubes. Serum and plasma were removed after centrifugation at 1500g for 10 min at 4 ºC and distributed in various aliquots for trace element determinations and antioxidant indicators. The aliquots for trace elements were frozen at –20º C and those for total thiols were storage at –80º C until analysis.

Elemental analysis: The PIXE (Particle Induced X-Ray Emission) analytical technique installed at the ITN Van de Graaff 3MV accelerator, was used for Fe, Cu, Zn and Se concentration assessment in blood plasma and blood cells fractions obtained after centrifugation of total heparinized blood. The plasma and blood cells fractions were freeze-dried and sample water content determined. All samples were subjected to acid digestion with nitric acid together with Y (Alfa 3333) as an internal standard, in closed Teflon™ bombs for microwave (Parr® bombs) or conventional oven as a pre-concentration procedure[4]. From the resulting solution a 10 µl aliquot is analysed. The quality control of the analytical procedure was carried out using Gent 2nd generation Freeze-dried Human Serum[5]. The reference material was analysed in each batch of digestions performed and run together with samples.

Determination of enzymatic activities: SOD activity was determined in erythrocytes using the Randox test (RANSOD), which is based on a method described elsewhere[6,7]. The results are expressed *per* g haemoglobin.

The quantification of the activity of glutathione peroxidase (GSH-Px) was based on Paglia & Valentine method[8] and determined by using a Randox test combination kit (RANSEL). The results are expressed *per* g haemoglobin.

The determination of total haemoglobin was assessed using the Randox kit.

Determination of total thiols: The quantification of total thiols in plasma was achieved through the Ellman's[9] method adapted by Marinho[10]. The results are expressed as μmol/L plasma.

Statistics

The statistical analysis was performed in the software STATISTICA 6.0, while the collumns plots for antioxidant enzymatic activities, as well as total thiols were performed with ORIGIN 6.0.

Differences between groups were tested by Kruskall-Wallis test.

Results

The elemental results, either for plasma as well as for blood cells fractions, are presented in Table 1.

Table 1: Elemental concentrations in plasma (mg/L) and blood cells fractions (mg/kg wet mass) in healthy and atherosclerotic subjects from Lisbon population.

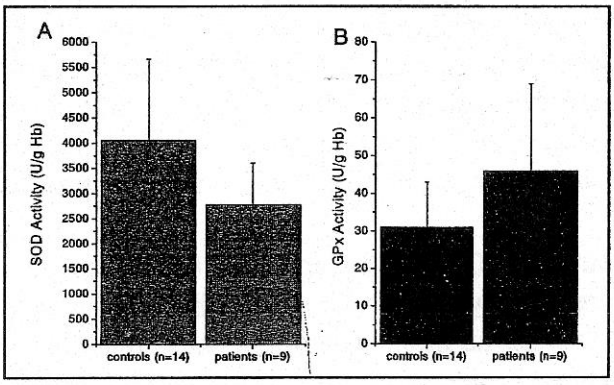
	Control		Atherosclerotic	
Elements	**Plasma**	**Blood Cells**	**Plasma**	**Blood Cells**
Fe	1.18 ± 0.221 (n=14)	942 ± 91.9 (n=14)	0.687 ± 0.297* (n=5)	939 ± 47.8 (n=5)
Cu	1.11 ± 0.392 (n=13)	1.11 ± 0.217 (n=14)	1.14 ± 0.185 (n=5)	1.14 ± 0.217 (n=5)
Zn	1.01 ± 0.228 (n=13)	12.5 ± 1.72 (n=14)	0.825 ± 0.081 (n=5)	13.1 ± 1.74 (n=5)
Se	0.064 ± 0.013 (n=13)	0.091 ± (n=10)	0.037 ± 0.020* (n=4)	0.094 ± 0.054 (n=5)

Values represent the mean ± standard deviation. Asterisks denote statistical significance comparing to the respective control, using Kruskall-Wallis test, *p<0.05.

The analysis of Table 1 revealed that, Fe and Se are significantly decreased in the pathological group in comparison to the healthy one. A similar tendency appeared to occur for Zn (p=0.0682). Cu levels remained within normal range with no variations for both groups studied.

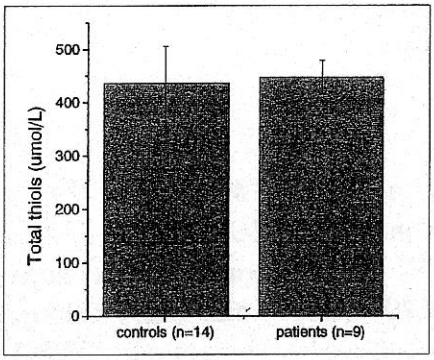
Concerning blood cells fractions, data obtained were similar for both groups.

Figure 1: Activities of superoxide dismutase in erythrocytes (A) and glutathione peroxidase in whole blood (B) of healthy and atherosclerotic subjects from the Lisbon population. Values represent the mean ± standard deviation.



Through the observation of Figure 1, it is possible to verify a slight decrease of superoxide dismutase activity in the atherosclerotic group comparing to control (p=0.0677). In relation to glutathione peroxidase activity, it was observed a slight increase of values in the pathological group, although not statistically significative. As far it concerns to plasma thiols (Figure 2), the results are very similar for both groups considered.

Figure 2: Plasma total thiols in healthy and atherosclerotic subjects from the Lisbon population. Values represent the mean ± standard deviation



As far it concerns to Spearman´s correlations, negative coefficients were observed in relation to health condition for plasma Fe and Se (r=-0.63, r=-0.62 ; p<0.05, respectively).

Discussion

Preliminary data were obtained on trace element levels and redox parameters in plasma and blood cells of humans with established atherosclerotic disease.

Se status was diminished in patients which could be related to the pathological complications from the atherosclerotic process. The reason for decreased plasma Se levels in these subjects is not clear and epidemiological studies are known to be controversial with respect to the association of lower levels of the element and the occurrence of cardiovascular diseases[11]. Plasma Fe levels decreased in the same atherosclerotic patients. In general, this element has been inconsistently associated with cardiovascular disease in spite of plausible hypothesis as to how transition metal might accelerated the progression of atherosclerosis[12].

In the studied patients, SOD activity appeared to decrease in erythrocytes and GPx activity to increase in whole blood. Although the patients group was a very small one, it appeared that an imbalance might occur relative to enzymatic antioxidant defenses in these subjects with atherosclerotic manifestations, which might contribute for oxidant-mediated injury. In fact, SOD is an important antioxidant enzyme, which degrades the superoxide anion-radical. Decreased SOD activity contributes for higher levels of that radical which has been implicated in the patogenesis of many degenerative diseases, including atherosclerosis[13]. Other studies have reported decreased SOD activity in subjects suffering from ischemia and hypoxia[14]. In addition, an extracellular form of SOD has been found in atherosclerotic vessels, however its functional significance is not clear[13].

In conclusion, the preliminary results present in this report, will be in the near future consolidated with a larger number of patients in order to clarify these observations and by this means, to contribute for a better knowledge of the basic mechanisms of atherosclerosis.

Acknowledgements

This work forms part of the project *"Markers of the prooxidant/antioxidant balance and characterization of the allelic profile of Apo E in inhabitants of Lisbon and Ponta Delgada"*. It is supported by Fundação para a Ciência e a Tecnologia (POCTI/ESP/41008/2001) and CBA (Environmental Biological Center). Paula Alexandra Lopes is a PhD grantee (FCT/PRAXIS XXI/BD/21444/99).

References

1. Heinecke JW. Clinical trials of vitamin E in coronary artery disease: Is it time to reconsider the low density lipoprotein oxidation hypothesis? Current Atherosclerosis Reports: 5 (2003): 83-87.
2. Hennig B, Toborek M, McClain CJ. High-energy diets, fatty acids and endothelial cell function: implications for Atherosclerosis. J. Am. Coll. Nutr. 20 (2) (2001): 97-105.
3. Van de Vijver LPL, Kardinnal AFM, Grobbee DE, Prince HMG, Van Poppel G. Lipoprotein oxidation, antioxidants and cardiovascular risk: epidemiological evidence. Prostaglandins, Leukotrienes and Essencial Fatty Acids 57 (1997): 479-487.
4. Pinheiro T, Duflou H, Maenhaut W. Applicability of microwave acid digestion to sample preparation ob biological materials for analysis of particle-induced X-ray emission (PIXE). Biol. Trace Elem. Res. 26/27 (1990):589-597.
5. Versieck J, Van Ballenberghe L, De Kesel A, Hoste J, Wallaeys B, J. Vandenhaute, Baeck N, Steyaert H, Byrne RA, Sunderman FWJr. Certification of a second-generation biological reference material (freeze-dried human serum) for trace element determinations. Anal. Chim. Acta 204 (1988):63-75.
6. McCord JM & Fridovich I. Superoxide dismutase. An enzymatic function for erythrocuprein. (hemocuprein). J. Biol. Chem. 244 (1969): 6049-6055.
7. Misra HP & Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J. Biol. Chem. 247 (1972): 3170-3175.
8. Paglia DE & Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J. Lab. Clin. Med. 70 (1967): 158-168.
9. Sdlak J, Lindsay RH. Estimation of total, protein-bound and nonprotein sulphydryl groups in tissue with Ellman´s reagent. Anal. Biochem. 25 (1968): 192-205.
10. Marinho S. Metabolismo do Glutationo no fígado do rato normal e no fígado de rato hepatomisado. Ph D Thesis (1995), Universidade de Lisboa.
11. Nève J, Palmieri Y. First symposium on human health related aspects of selenium research in Europe. J. Trace Elements Med. Biol. 14 (2000): 116-121.
12. Corti MC, Gaziano M, Hennekens CH. Iron status and risk of cardiovascular disease. Ann. Epidemiol. 7 (1) (1997): 62-68.

13. Fukai T, Folz RJ, Landmesser U, Harrison DG. Extracellular superoxide dismutase and cardiovascular disease. Card. Res. 55 (2002): 239-249.
14. Yucel, D, Aydogdu S, Cehreli S, Saydam G, Canatan H, Senes M, Topkaya BC, Nebioglu S. Increased oxidative stress in dilated cardiomyiopathic heart failure. Clin. Chem. 44 (1998): 148-154.