

PROTEIN OXIDATION IN MILD ESSENTIAL hypertension

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An intensified oxidative stress has been associated with aging and many disease, including essential hypertension (EH) [6, 11, 16]. In EH an increased level of reactive oxygen and nitrogen species (RONS) may impair the bioavailability of nitric oxide (NO), by inducing its accelerated transformation in peroxynitrite [4]. Beside this, many other pathophysiological processes in EH may be influenced by RONS, considering their harmful effects on the structural and functional properties of lipids, proteins and nucleic acids.

Some points need to be further investigated, such as the relationship between oxidative stress and the degree of hypertension, and identification of the most useful marker of enhanced oxidative stress in this clinical condition.

In EH several papers showed an increase in lipid peroxidation [1, 7, 13]; this datum was also present in juvenile essential hypertension [17] and in newly diagnosed hypertensives [12]. In other reports, however, normal levels of lipid peroxidation were observed [5, 8].

Fewer papers [3, 7, 10, 14] have examined protein oxidation in EH up to now. Among the indicators of protein oxidation, the most widely used is the concentration of protein carbonyl groups (C=O). These groups are generated by oxidation of protein side chains, and some aminoacidic residues, such as lysine, proline, arginine and threonine are the most involved [15].

In this study we examined protein oxidation expressed as plasma protein carbonyl groups in untreated subjects with mild essential hypertension.

We enrolled 23 subjects (17 men and 6 women; mean age 45.05 ± 6.14 years; range 31–53 years). The diagnosis of hypertension was based on blood pressure (BP) measurements taken on two separate occasions with the patient in a seated position after 15 minutes of rest. The mean values of these measurements were 144/87 mmHg. Only a minority of the patients showed a simultaneous increase of systolic and diastolic BP above 140/90 mmHg, the great majority having only either systolic or diastolic values within the range of hypertension. In all patients an ambulatory BP monitoring was performed: day-time systolic BP and diastolic BP were respectively 134.2 ± 8.4 and 86.7 ± 6.1 mmHg, night-time SBP and DBP were respectively 120.5 ± 11.1 and 75.7 ± 7.5 mmHg and 24-h SBP and DBP were respectively 128.9 ± 8.7 and 82.4 ± 5.7 mmHg. In this group the hypertension duration was 13.4 ± 11.9 months. The

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basal glucose level was 91.95 ± 11.04 mg/dl, total cholesterol was 213.3 ± 36.9 mg/dl, LDL-cholesterol was 155.2 ± 32.3 mg/dl, HDL-cholesterol was 44.00 ± 8.04 mg/dl, triglycerides were 100.5 ± 80.6 mg/dl, serum uric acid was 5.85 ± 1.65 mg/dl. In hypertensives the body mass index (BMI) was 27.04 ± 3.38 , the waist to hip ratio (WHR) was 0.913 ± 0.049 .

The control group included 26 healthy subjects (17 men and 9 women; mean age 43.54 ± 6.92 years) recruited from hospital staff members and students. The basal glucose level was 87.54 ± 8.79 mg/dl, total cholesterol level was 207.5 ± 33.0 mg/dl, LDL-cholesterol was 145.5 ± 28.01 mg/dl, HDL-cholesterol was 44.65 ± 7.43 mg/dl, triglycerides were 86.77 ± 35.22 mg/dl, serum uric acid was 4.55 ± 0.98 mg/dl. The mean values of BP in these subjects were 120/71 mmHg. In this group BMI was 25.58 ± 1.45 and WHR was 0.917 ± 0.041 .

The protein carbonyl group concentration was measured by an enzyme-linked immunosorbent assay (ELISA) kit (BioCell PC test kit, Enzo Life Sciences AG, Switzerland), which uses the reagent 2,4-dinitrophenylhydrazine (DNP). Plasma samples were incubated with DNP, and then plasma proteins were nonspecifically adsorbed to an ELISA plate. Unconjugated DNP and non-protein constituents were washed away. The adsorbed proteins were probed with biotinylated anti-DNP antibody, followed by streptavidin-linked horseradish peroxidase. A chromatin reagent was added, and the reaction was stopped by adding an acid solution. Absorbance for each well was measured at 450 nm and related to a standard curve prepared for serum albumin containing increasing proportions of hypochlorous acid-oxidized protein, calibrated colourimetrically. Total protein concentration in plasma samples was evaluated by the method of Lowry et al. [9].

Our data showed no statistical difference between normal controls and hypertensives regarding plasma protein carbonyl groups ($N = 0.440 \pm 0.134$ nmol/mg prot; Hypertensives = 0.459 ± 0.136 nmol/mg prot). In hypertensive and control subjects the carbonyl group concentrations were not related to age, BMI, WHR, metabolic parameters or blood pressure values.

Our datum is different from those observed by other authors who found an increase in plasma carbonyl groups in hypertensives, even though in a study hypertensive patients were much older [7] and in other studies patients had higher pressure values [3, 10, 14]. However, in the paper by Simic et al. [14], an increase of the carbonyl derivatives was found also in a few patients with grade 1 hypertension.

In a previous study [2], we observed an increase in lipid peroxidation, expressed as thiobarbituric acid reactive substance (TBARS), in a group of hypertensives including all the subjects evaluated in the present study. Although our study group is small, our data suggest that, in comparison with indices of lipid peroxidation, protein oxidation markers may appear later during the clinical course of EH, and their evaluation at the early stages of the disease may underestimate the impact of oxidative stress in hypertensive patients.

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