Journal of Human Hypertension (2004) 18, 523–528
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www.nature.com/jhh

ORIGINAL ARTICLE

Oxidative stress in white coat hypertension; role of paraoxonase

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Oxidative stress in sustained hypertension was shown with several biochemical parameters. Oxidized low-density lipoprotein (oxLDL) plays an important role during the atherosclerosis process and paraoxonase (PON1) can significantly inhibit lipid peroxidation. Serum PON1 activity, oxLDL and malondialdehyde (MDA) concentrations and their relationship with serum lipid parameters and systolic and diastolic blood pressures (SBP and DBP) were determined in subjects with white coat hypertension (WCH), sustained hypertension (HT) and normotension (NT). The study group consisted of a total of 86 subjects, 30 with WCH (14 male, 16 female subjects), 30 with HT (13 male, 17 female subjects) and 26 with NT (12 male, 14 female subjects). Both white coat hypertensive and hypertensive subjects had significantly higher levels of MDA than normotensives ($P<0.026$ and $P<0.001$, respectively). The oxLDL level of the HT group was significantly higher than the NT group ($P<0.023$). The WCH group had an oxLDL level similar to both hypertensive and normotensive groups. HT and WCH groups had significantly lower PON1 levels than the normotensive group ($P<0.001$). oxLDL correlated with MDA positively ($P=0.008$), and PON1 negatively ($P=0.008$). A negative correlation between MDA and PON1 ($P=0.014$) was detected. MDA correlated positively with both SBP and DBP ($P=0.001$), while PON1 correlated with both of them negatively ($P=0.01$ and $P=0.008$, respectively). OxLDL correlated with diastolic blood pressure positively ($P=0.008$). Our data demonstrate that oxidative stress increase in WCH is associated with a decrease in PON1 activity. The reduction in PON1 activity may be one of the factors leading to an increase in oxidative status in WCH.

Journal of Human Hypertension (2004) **18,** 523–528. doi:10.1038/sj.jhh.1001697
Published online 26 February 2004

Keywords: white coat hypertension; oxidized low-density lipoprotein (oxLDL); paraoxonase (PON1); malondialdehyde (MDA); oxidative stress; hypertension

Introduction

Atherosclerosis is one of the most common causes of mortality throughout the world. Several lines of evidence suggest that hypertension is associated with enhanced oxidative stress.[1,2] Oxidative modification of low-density lipoprotein (LDL) in the artery wall is currently thought to be central to the development and progression of atherosclerosis.[3] Increased oxidative stress may lead to LDL oxidation. The oxidation of polyunsaturated fatty acids within LDL is followed by their breakdown and the release of aldehydes and ketones, such as malondialdehyde (MDA) and 4-hydroxynonenal, which can modify lysine residues on apolipoprotein B (apo B).[4] Oxidized low-density lipoprotein (oxLDL) plays an important role in endothelial dysfunction. oxLDL induces endothelial injury, inhibits apoptosis, monocyte adhesion, platelet agregation and endothelial nitric oxide synthase (eNOS) expression/activity, all of which contribute to atherosclerotic process and hypertension.[5]

The propagation of LDL oxidation and thus the development of atherosclerotic processes has been shown to be inhibited by some protective properties of high-density lipoprotein (HDL). HDL can prevent

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Received 07 November 2003; revised 05 January 2004; accepted 17 January 2004; published online 26 February 2004

atherogenesis by at least two ways; one is by reverse-cholesterol transport from peripheral tissue to liver, and the other by inhibition of lipid peroxidation. Paraoxonase (PON1) (EC.3.1.8.1) is an HDL-associated esterase that hydrolyses paraoxon.[6] PON1 plays a role in preventing lipid oxidation not only of LDL but also of HDL itself. Aviram *et al*[7] have postulated that both HDL-associated PON and purified PON were potent inhibitors of LDL and HDL oxidation. PON1 deficiency is associated with increased macrophage oxidative stress in PON1-knockout mice, where an effect on LDL oxidation was observed.[8] The PON1 gene has two common polymorphisms at amino acids 55 and 192. The 192 polymorphism leads to a Glu—Arg substitution and results in two different isoforms that have high and low activity towards paraoxon as a substrate.[6] Previous studies have shown that patients with hypertension have reduced serum PON1 activity[9] and concluded that PON1 gene polymorphism is associated with a predisposition to coronary atherosclerosis,[10] but Mackness *et al*[11] have postulated that the quality of the PON1 activity and concentration were more important than the PON1 genotype in coronary heart disease (CHD).

Little is known about the prognosis of the patients with white coat hypertension (WCH). It is said to be an innocent clinical situation by some authors,[12,13] whereas others have found an increased risk for cardiovascular disease.[14,15] It might cause target organ damage such as heart failure, cerebrovascular accident, nephropathy, retinopathy and some metabolic abnormalities like hyperlipidemia, impaired insulin sensitivity, elevated blood glucose and increased serum insulin levels and haemodynamic alterations. The status of oxidation and the antioxidant activity in WCH is not well documentated.

The objective of this study is to assess the status of oxidative stress in patients with WCH and compare it with the oxidative status in normotensives and hypertensives. In order to evaluate the relationship among the oxidative stress markers, antioxidant capacity and blood pressure, correlations among oxLDL, MDA, PON1 and blood pressure values were performed.

Materials and methods

Patients included in the study were selected from our outpatient clinic over the 1-year period of January to December 2002. The subjects in the sustained hypertensive group were referred directly from general practitioners with the aim of participating in a study in hypertension. The main reason for referral to our hospital was to obtain a thorough evaluation before the start of medical therapy. Subjects with a diastolic blood pressure (DBP) >90 mmHg were referred to our outpatient clinic and patients whose ambulatory daytime diastolic pressure was >85 mmHg were enrolled in the HT group, while cases with a clinical diastolic pressure >90 and ambulatory daytime arterial pressure $<135/85$ mmHg were in the WCH group.

Healthy subjects in the same age range as the hypertensive groups were selected as controls for this study. Most subjects in the same age range entered the database by a random selection of employees in the hospital.The normotensive group consisted of subjects with diastolic pressure <90 mmHg measured by a nurse in the clinic.

Patients who fulfilled the inclusion criteria were invited to participate and written consent was requested. The study group consisted of a total of 86 subjects, 30 with WCH (14 male, 16 female subjects) aged 49 ± 11 years, 30 with sustained hypertensives (HT) (13 male, 17 female subjects) aged 47 ± 11 years and 26 normotensive control subjects (NT) (12 male, 14 female subjects) aged 48 ± 10 years. The groups were matched for age and gender (Table 1). Subjects with other risk factors for atherosclerosis (LDL >130 mg, diabetes mellitus, BMI >27 kg/m^2, smoking), and subjects having signs or symptoms of atherosclerotic vascular disease and other endocrine diseases or alcoholism were not included. Patients using drugs that may affect blood pressure and lipid metabolism, and antioxidant substances were also excluded. All patients were free of concomitant vascular disease, malignancy and connective tissue diseases.

Blood pressure measurements

Measurements of brachial arterial pressures in the patients referred to our clinic due to high blood pressure (diastolic pressure >90 mmHg) were obtained in our outpatient department by a nurse with a mercury sphygmomanometer, which was standardized in accordance with the approval of American and British Hypertension Society and World Health Organisation.[16] Measurements were obtained with

Table 1 Characteristics and blood pressures of the groups

	NT (n = *26*)	*WCH* (n = *30*)	*HT* (n = *30*)
Age	48 ± 10	49 ± 11	47 ± 11
Gender (M/F)	12/14	14/16	13/17
BMI (kg/m^2)	24.3 ± 5	25.3 ± 4	25.7 ± 6
Clinical SBP (mmHg)	130 ± 8	156 ± 21	165 ± 24
Clinical DBP (mmHg)	73 ± 8	98 ± 11	102 ± 10
Daytime SBP (mmHg)	128 ± 5	129 ± 5	169 ± 10
Daytime DBP (mmHg)	80 ± 3	81 ± 3	98 ± 6

NT, normotensive; WCH, white coat hypertensive; HT, hypertensive; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; the clinical SBP and DBP of NT are different from WCH and HT ($P<0.001$); the daytime SBP and DBP of HT are different from WCH and NT ($P<0.001$).

the subject in the sitting position after resting for 20–30 min.[17,18] Korotkoff phase I was used to determine the systolic pressure and phase V for diastolic pressure. Measurements were performed on three different days within 5 days. The average of three measurements was taken as the mean systolic and diastolic pressures.

An ambulatory 24-h arterial blood pressure recording was performed in patients whose diastolic pressures were found to be higher than 90 mmHg in the outpatient department, with an instrument (A and D Engineering, TM-2421) that was approved and suggested by European Society of Hypertension.[19] Measurements were performed as British Society of Hypertension suggested on the left arm.[20] According to the results of the ambulatory measurement, patients were classified as white coat *vs* sustained hypertension groups. WCH was defined as clinical hypertension and daytime ambulatory blood pressure of less than 135/85 mmHg. Patients with a daytime ambulatory DBP of more than 85 mmHg were taken into the hypertensive group (Table 1).

Sample collection

Blood was taken after 12–14 h of fasting in the morning. Sera were obtained after at least 30 min clotting by centrifugation at 2500 g for 15 min. Each sample was divided into four aliquots; the first aliquot was used directly for the measurements of cholesterol, triglycerides, HDL-cholesterol and glucose. The second aliquot was kept in -80°C until analysis of serum PON1 activity within 2 weeks; the other two aliquots were stored in -80°C for estimation of serum MDA and oxLDL at a later date (within 1 month). All parameters were analysed in all samples together in a single batch, after we had finished our protocol (control and patient samples were analysed in the same batch).

Assay of TBARS

Lipoperoxidation was ascertained by the formation of MDA, which was estimated by the modified thiobarbituric acid method, described by Buege and Aust.[21] TBARS concentration was calculated using $1.56 \times 10^{-5}\,M^{-1}$/cm as mol/l extinction coefficient. The results were expressed as micromol/l (μmol/l). The coefficients of intra-and interassay variations for MDA assay were 4.1% ($n=12$) and 5.4% ($n=11$), respectively.

Assay of PON1 Activity

PON1 activity was assayed using synthetic paraoxon (diethyl-*p*-nitrophenyl phosphate) as a substrate. PONl activity was determined by measuring the initial rate of substrate hydrolysis to *p*-nitrophenol, whose absorbance was monitored at 412 nm in the assay mixture containing 2.0 mM paraoxon, 2.0 mM $CaCl_2$ and 20 μl of plasma in 100 mM Tris-HCI buffer (pH 8.0). The blank sample containing an incubation mixture without plasma was run simultaneously to correct for spontaneous substrate breakdown. The enzyme activity was calculated from E412 of *p*-nitrophenol (18,290 per M/cm) and was expressed as U/l; 1 U of enzyme hydrolyses 1 nmol of paraoxon/min.[22,23] The coefficients of intra and interassay variations for PON1 activities were 3.6% ($n=12$) and 4.3% ($n=11$), respectively.

Assay of oxLDL

The concentration of oxLDL in serum was measured by a sandwich ELISA procedure using the murine monoclonal antibody, mAb-4E6, as capture antibody bound to microtitration wells, and a peroxidase-conjugated anti-Apo B antibody recognizing oxLDL bound to the solid phase (Mercodia Oxidized LDL ELISA, Uppsala, Sweden). The coefficients of intra and interassay variations for oxLDL were 3.9% ($n=8$) and 7.5% ($n=6$), respectively.

Other serum parameters (total cholesterol, triglycerides, HDL-cholesterol and glucose) were determined by routine laboratory methods using the Hitachi 704 autoanalyzer Boehringer Mannheim. Tokyo, Japan). LDL-cholesterol was calculated.

Statistical analysis

For each variable, values were expressed as mean $\pm$ s.d. The groups were compared with Anova Tukey HSD. A P-value <0.05 was considered to be significant. Correlations were calculated by Pearson's correlation.

Results

The basic characteristics and clinical and ambulatory blood pressure values of the groups are given in Table 1. Age, gender distribution and BMI did not differ among the groups. The clinical systolic and diastolic pressures of the NT group were significantly lower than WCH and HT groups (130/73 *vs* 156/98 and 165/102 mmHg; $P<0.001$), but there was no significant difference between WCH and HT groups.

The ambulatory systolic blood pressure (SBP) and the DBP of the HT group were significantly higher than WCH and NT groups (169/98 *vs* 129/81 and 128/80 mmHg respectively; $P<0.001$), but similar between white coat hypertensives and normotensives.

The biochemical characteristics and plasma MDA, oxLDL and PON1 levels of the three groups are given in Table 2. Glucose, total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides were not

Table 2 Biochemical characteristics, MDA, PON1, oxLDL levels of the study group

	NT (n = 26)	*WCH (n = 30)*	*HT (n = 30)*
Total-C (mmol/l)	4.72 ± 0.54	4.94 ± 0.32	4.91 ± 1.62
LDL-C (mmol/l)	2.91 ± 0.52	3.06 ± 0.78	3.12 ± 1.82
Triglycerides (mmol/l)	1.35 ± 0.00	1.45 ± 0.42	1.37 ± 0.54
HDL-C (mmol/l)	1.54 ± 0.01	1.59 ± 0.02	1.52 ± 0.01
Glucose (mmol/l)	5.28 ± 0.33	5.55 ± 0.39	5.06 ± 1.39
MDA (μmol/l)	4.54 ± 0.67	5.22 ± 0.62	5.9 ± 0.9
oxLDL (U/l)	51.21 ± 16.35	60.15 ± 20.33	70.54 ± 25.75
PON1 (U/l)	94.05 ± 39.17	48.49 ± 16.54	46.81 ± 21.43

NT, normotensive group; WCH, white coat hypertensive group; HT, hypertensive group; MDA, malondialdehide; oxLDL, oxide LDL; PON1, paraoxanase; MDA of NT is different fromWCH. ($P = 0.026$) and HT ($P < 0.001$); oxLDL of NT is different from HT ($P = 0.023$); pppPON1 of NT is different from WCH and NT ($P < 0.001$).

different among the groups. Both white coat hypertensives and hypertensive subjects had significantly higher levels of MDA than the normotensives (5.22 ± 0.62, 5.9 ± 0.9, 4.54 ± 0.67 μmol/l, respectively; $P < 0.001$), but there was no significant difference between the WCH and HT groups. oxLDL levels of the HT group were significantly higher than the NT (70.54 ± 25.75, 51.21 ± 16.35 U/l; $P < 0.023$). The WCH group had an oxLDL level that was similar to both HT and NT groups (60.15 ± 20.33 U/l).

With respect to PON1, the HT and WCH groups had significantly lower levels than the NT group (46.81 ± 21.43, 48.49 ± 16.54, 94.05 ± 39.17 U/l, respectively; $P < 0.001$) and there was no significant difference between the HT and WCH groups. A positive correlation between oxLDL and MDA ($r = 0.379$; $P = 0.008$), a negative correlation between oxLDL and PON1 ($r = -0.379$; $P = 0.008$) and a negative correlation between MDA and PON1 ($r = -0.357$; $P = 0.014$) were detected.

There was a positive correlation between SBP and MDA ($r = 0.453$; $P = 0.001$), a negative correlation between SBP and PON1 ($r = -0.366$; $P = 0.01$), a positive correlation between DBP and MDA ($r = 0.454$; $P = 0.001$), a positive correlation between DBP and oxLDL ($r = 0.376$; $P = 0.008$) and a negative correlation between DBP and PON1 ($r = -0.379$; $P = 0.008$).

Discussion

In the present study, we examined the oxidant and antioxidant status to evaluate the oxidative stress in WCH and compared with that of normotensives and hypertensives. With respect to MDA, which is considered as a marker of lipid peroxidation (reactive oxygen species (ROS)), our findings indicated that oxidative stress increased in both WCH and HT groups similarly, compared with normotensives.

Considering oxLDL, oxidative stress increased significantly in the HT group compared with the WCH and NT groups. The level of oxLDL in white coat hypertensives was not significantly different from hypertensives and normotensives. In other words, it was similar to both groups and it can be interpreted that WCH patients may also have increased risk of oxidative stress, but it was not as high as in the HT group and was not as low as in the NT group. On comparing the PON1 levels, we saw that the antioxidant capacity of the white coat hypertensives decreased as much as that of the hypertensives.

The increase of oxidative stress in HT is consistent with the observations of many authors.[2,24] They reported that the higher oxidative stress in HT was due to the imbalance between oxidants and antioxidants as a result of the increase of oxidants associated with the decrease of antioxidant capacity. Uddin[25] reported that H_2O_2 increased while catalase activity decreased in hypertensive rats. Kumar[26] showed an increase of lipid peroxides with a decrease in that of antioxidants such as catalase and superoxide dismutase. Newaz and Nawal[24] found an increase in lipid peroxidation in the plasma and increase in ROS generation in vascular cells in spontaneously hypertensive rats.

At the present time, many investigators have studied the mechanisms responsible for the increased oxidative stress in hypertension. The results from these studies suggest that the increase in the activity of ROS-generating enzymes, for example, xanthine oxidase and NADH/NADPH oxidase, might be a cause of the increased oxidative stress in certain types of hypertension.[27,28] However, data derived from these studies are inconsistent. Some studies showed that the activities of one or more antioxidant enzymes, for example, superoxide dismutases (SOD), glutathione peroxidases (GPx) and/or catalase, are significantly lower in the blood of hypertensive patients.[2,26] In contrast, other studies have shown that the activities of these antioxidant enzymes are elevated.[29] Although the mechanism underlying the increased oxidative stress in hypertension is not completely understood, it has been generally accepted that an increase in vascular ROS contributes to the genesis of hypertension. It is highly likely that an increase in blood pressure and/or other factors elevates ROS in the arterial wall by reducing the activity of antioxidant enzymes and/or enhancing the activity of the enzyme systems that are capable of producing ROS, for example, xanthine oxidase, NADH/NADPH oxidase and uncoupled eNOS. The increased ROS in turn induce smooth muscle cell (SMC) contraction and proliferation, aggravating hypertension.[30]

Our study suggested that WCH was associated with enhanced oxidative stress like sustained hypertension. Our results were in agreement with those of Redon *et al*[31] who reported that none of the parameters of antioxidant enzymes activity nor the

ROS-derived byproducts in WCH differed from those observed in truly hypertensive subjects. Contrary to our data, no significant difference was observed between the normotensives and white coat hypertensives in Pierdomenico's a study.[32] Pierdomenico used only LDL to investigate the oxidative status. In our study, the level of oxLDL in WCH group was not significantly different from HT and NT groups, demonstrating that it was similar to both groups and it can be interpreted that the white coat hypertensives may also have increased risk of oxidative stress, but it is not as high as in the HT group and is not as low as in the NT group. The difference in oxLDL between the WCH and the NT groups was not significant as in that of Pierdomenico' study, but the similarities with both hypertensives and normotensives associated with the significant increase in ROS (MDA) suggested an increase of oxidative stress.

Gomez *et al*,[33] using a noninvasive method, showed the presence of endothelial dysfunction in WCH like in essential HT by means of endothelium-dependent flow-mediated dilatation. We also observed the presence of endothelial dysfunction in our previous study.[34] In our studies, we found the increasing values of endothelin-1 (ET-1), e-selectin, homocysteine and vascular endothelial growth factor (VEGF) accompanied by a decrease in nitric oxide (NO) in WCH. The relationship of oxidative stress and NO is well known. The low level of NO in WCH may be the result of enhanced oxidative stress.

These data suggest that PON1 enzyme activities are affected by the duration of the disease. This hypothesis was supported by our observation that there was a relationship between the ambulatory blood pressures and oxidative stress parameters; a significant correlation was found. It was seen that due to an increase of blood pressures, oxLDL and MDA increased while PON1 decreased. In other words, oxidative stress increases, while antioxidant activity decreases as the blood pressure rises. We are in agreement with Antonicelli *et al*,[35] who demonstrated a significant correlation between lipoprotein (a) (Lp(a)) levels and the night time systolic and diastolic pressures in essential hypertension. Contrary to us, Redon *et al*,[31] reported that the increase in oxidative stress and reduction in the activity of antioxidant mechanisms appeared to be independent of the blood pressure values.

In conclusion, our data demonstrate an increase in oxidative status and a reduction in the activity of antioxidant mechanisms. It was reported that the higher oxidative stress in HT was due to the imbalance between oxidants and antioxidants as a result of an increase of oxidants associated with a decrease of antioxidant enzymes.[25,26] Reduction in PON1, which is an antioxidant enzyme like SOD, GPx and catalase, may be one of the enzymes increasing oxidative stress in WCH associated with other factors. As the elevated oxidative status was speculated as a strong risk factor for coronary artery disease in sustained hypertension, WCH might not be considered as an innocent trait. Further studies are needed to assess the increased cardiovascular risk in WCH conferred by high oxidative stress.

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