Enhanced Plasma Soluble CD40 Ligand Levels in Essential Hypertensive Patients With Blunted Nocturnal Blood Pressure Decrease

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Background: Hypertensives with a blunted nocturnal blood pressure (BP) decrease have increased risk of developing atherosclerotic disease. Soluble CD40 ligand (sCD40L) is involved in the pathogenesis of risk factor-related vascular damage. Therefore, we evaluated the relationship between circulating sCD40L levels, circadian BP profile, and early carotid atherosclerosis in essential hypertensives.

Methods: Plasma sCD40L concentrations were assessed in two groups of 25 never-treated hypertensives, without additional cardiovascular risk factors, differentiated on the basis of a nocturnal decrease of BP either of >10% (dippers) or <10% (nondippers) of daytime values, and in 25 matched normotensives. Carotid intima–media thickness (IMT) was also measured in all participants.

Results: Plasma sCD40L concentrations were higher in nondippers (4.9 ± 1.2 ng/mL) than in dippers (3.7 ± 0.7, P = .0005) and controls (1.6 ± 0.6, P < .0001). These latter had lower sCD40L concentrations than dippers (P < .0001). The IMT was higher in both hypertensive groups than in normotensives (P < .0001). In the entire hypertensive population IMT directly correlated with circulating levels of sCD40L (r = 0.365, P = .01) and inversely correlated with nocturnal systolic BP decreases (r = −0.286, P = .043). In a multivariate regression analysis sCD40L was the main determinant of IMT (r² = 0.157, P = .004).

Conclusions: Nondippers have enhanced plasma sCD40L levels, which may contribute to their increased susceptibility to develop vascular damage. Am J Hypertens 2007;20:70–76 © 2007 American Journal of Hypertension, Ltd.

Key Words: CD40L, blood pressure measurement/monitoring, hypertension, atherosclerosis, isoprostanes.
tion between CD40 ligand and its cognate receptor CD40, a 50-kDa integral membrane protein of the tumor necrosis factor receptor family expressed by several cells of the vasculature, including endothelial cells, smooth muscle cells, and macrophages, is critically involved in multiple ways in the pathophysiology of risk factor-related vascular damage.1,12

The CD40 ligand also occurs in a soluble form, primarily derived from activated platelets and, to a lesser extent, shed from stimulated lymphocytes.9,13 The sCD40L is considered to possess full biological activity.13 Increased sCD40L levels have been observed in obesity,14 hypercholesterolemia,15 and unstable angina.16 In addition, circulating sCD40L has a strong independent prognostic value among apparently healthy individuals17 and patients with acute coronary syndromes.18 The clinical association between sCD40L and cardiovascular events suggests that CD40 signaling function spans from early atherogenesis to late thrombotic complications.

Thus, it is tempting to hypothesize that CD40/CD40L interaction may contribute to the development of vascular damage in hypertensive patients with a blunted nocturnal BP decrease. To address this topic, we measured plasma concentrations of sCD40L in never-treated hypertensive patients with either normal or blunted nocturnal decrease in BP. Circulating levels of isoprostanes were also measured as a potential determinant of platelet activation and sCD40L release in hypertensive patients.19

Methods

Patients

The study population consisted of 50 never-treated white hypertensive patients who were referred for the first time to our Outpatient Unit. Patients were selected as hypertensives (systolic BP ≥140 mm Hg or diastolic BP ≥90 mm Hg, or both) with either a nondipping (n = 25) or a dipping (n = 25) pattern of BP as evaluated on two concordant 24-h ambulatory BP monitorings (ABPMs) performed at 1-week intervals. The ABPMs were prescribed to these patients by the staff of our Outpatient Unit when the following inclusion criteria were met: no smoking habit, absence of diabetes mellitus, body mass index lower than 26 kg/m², normal serum total cholesterol (<5.2 mmol/L) and triglyceride levels (<1.7 mmol/L), no concomitant use of any kind of drugs including aspirin, fish oil and vitamins, normal renal function (serum creatinine <100 μmol/L, absence of macroproteinuria, ie, urinary albumin excretion [UAE] <300 mg/24 h), normal 12-lead electrocardiogram at rest, no personal history of cerebrovascular or cardiovascular disease, no concomitant diseases including an allergic diathesis, no referred inflammatory diseases during the past 3 months, and no evidence of atherosclerotic lesions of the neck and limb vessels (as evaluated by clinical and ultrasound studies). Patients having known conditions interfering with technically adequate ABPM registration (atrial fibrillation and other major arrhythmias) were excluded. The screening for secondary causes of hypertension was performed by appropriate clinic and laboratory tests. Twenty-five matched healthy controls were studied as the control group. The study was been conducted on male patients to avoid the well-known influence of estrogen fluctuation on platelet function.20

All participants gave their informed consent.

Clinic BP Measurement

Clinic systolic and diastolic BP levels were recorded on three different visits (performed at 1-week intervals) during the morning (between 9 AM and noon) in our Outpatient Unit by a physician using an Omron 705 CP automatic device (Omron Matsusaka Co. Ltd., Matsukaka-City, Japan). Measurements taken made in sitting position, after 5 to 10 min of rest. Four measurements were made at 2-min intervals and the average of the last three measurements was used to define clinic systolic and diastolic BP. In each patient, assessment of clinic BP preceded ABPM studies and verification of inclusion criteria.

Ambulatory BP Monitoring

One week after clinic BP levels were assessed and inclusion criteria verified, 24-h ABPM was carried out in hypertensive patients on the nondominant arm using SpaceLabs 90207 devices (SpaceLabs Inc., Richmond, WA). Because the classification of hypertensive patients into dippers and nondippers performed on a single ABPM measure, which has a low reproducibility over time,21 hypertensive patients were identified as dippers or nondippers on the basis of concordance of two ABPMs performed at 1-week intervals.1 The time of application (±1 h) and the type of device were the same for the two ABPM measurements. In all patients, the first BP reading of the device was confirmed by a mercury sphygmomanometer and the use of a Y tube. The device was set to obtain BP readings at 15-min intervals during the day (7 AM to 11 PM) and at 30-min intervals during the night (11 PM to 7 AM). In all cases, ABPMs were performed on the days of typical working activity (ie, avoiding Saturdays and Sundays).

The patients were instructed to record on a diary their activity, the quality of sleep, and the occurrence of poor sleep. In addition, they were asked to remain motionless during measurements, to go to bed no later than 11 PM, and to stay in bed until 7 AM. The nocturnal dipping was defined as a reduction in average systolic and diastolic BP at night more than 10% compared with average systolic and diastolic BP daytime values.1 All evaluated patients had technically adequate ABPMs.

Blood Samplings and Laboratory Procedures

Blood samples were drawn from each participant after a fasting period of 12 h. For this purpose, participants were
admitted to our Outpatient Unit at 8 AM (in the case of hypertensives after both ABPMs have been performed). A forearm vein was cannulated, and after 1 h in a supine position, venous blood samples were collected into pyrogen-free blood collection tubes with no additive and allowed to clot for 1 h before centrifugation at 3000 rpm for 10 min. For plasma sampling, blood was drawn and collected into prechilled pyrogen-free blood collection tubes containing EDTA and immediately immersed in melting ice. To avoid platelet activation during the postharvesting sample procedure,22 platelet-free plasma for sCD40L analysis was carefully prepared by sample centrifugation at 3000 rpm for 10 min. Plasma and serum samples were stored at −80°C and thawed only once.

Circulating sCD40L concentrations were assessed by immunoenzymathic methods (R&D systems, Minneapolis, MN). Plasma total 8-iso-prostaglandin F$_{2\alpha}$ (8-iso-PGF$_{2\alpha}$) levels were assessed by enzyme immunoassay (Assay Design Inc, Ann Arbor, MI). The coefficient of variation was 4.9% for sCD40L and 3.7% for total 8-iso-PGF$_{2\alpha}$ measurement. Circulating C-reactive protein (CRP) levels were measured by highly sensitive (hs) technique using the Integra-Immunoturbidimetric method (Cobas Integra 700, Roche Diagnostics, Milan, Italy). The method is sensitive to 0.3 mg/L. Twenty-four-hour urine samples were also collected in all patients for UAE quantification. The immunochemical measurement of albumin in urine was performed using a Behring Nephelometer II analyzer (Dade Behring Ltd, Milton Keines, UK), which detects albumin in urine at the level of 0.12 mg/dL. Microalbuminuria was defined as an UAE ≥30 and <300 mg/24 h.

**Carotid Ultrasonography**

High resolution B-mode ultrasonography was obtained using a Logic 500 ultrasound imaging system (General Electric Medical Systems-Ultrasound, Milwaukee, WI) equipped with a 7.5-MHz imaging transducer. The carotid arteries were explored with longitudinal and transverse scans, with the patient supine and the neck in slight hyperextension. Measurements of the intima–media thickness (IMT) were performed in both right and left common carotid arteries at least 1 cm below the carotid bifurcation. The IMT was defined as the distance from the leading edge of the lumen–intima interface of the far wall to the leading edge of the media–adventitia interface of the far wall.$^{23}$ The mean of six measurements was performed to derive an estimate of the overall IMT of the common carotid arteries. A normal wall was considered when IMT was <0.9 mm and wall thickening when IMT was ≥0.9 mm and <1.3 mm. A plaque was considered in the presence of IMT ≥1.3 mm. All studies were performed by a physician who was blinded with regard to subject characteristics.

**Statistical Analysis**

Statistical analysis was performed by PC and the SAS statistical software (version 8.12, 2000, SAS Institute, Inc., Cary, NC).

The size of the sample necessary to achieve 80% statistical power at a two-sided significance level of 0.05 was calculated on the basis of the assumption that a difference of at least 25% of circulating sCD40L levels would occur in nondipper compared to dipper hypertensive patients.

The distribution of values for each variable studied was analyzed by Shapiro-Wilk normality test. Comparisons between groups were analyzed by one-way ANOVA for variables that were normally distributed (cholesterol, glucose, insulin, 8-iso-PGF$_{2\alpha}$, IMT). Post hoc comparisons were performed by Tukey’s Studentized range–honestly significant difference test. Kruskal Wallis nonparametric ANOVA was used for variables that were not normally distributed. Post hoc comparisons were performed by Wilcoxon rank sum test with a downward adjustment of α level to compensate for multiple comparisons.

Spearman nonparametric correlation was used to evaluate correlations between variables. Multiple linear regression with stepwise procedure after logarithmic transformation of variables was used to evaluate the relative weight and persistence of the relationship among variables. Unless otherwise stated, data are given as mean ± 1 SD.

**Results**

**Clinical Characteristics**

Clinical and laboratory characteristics of the study population are given in Table 1. The three study groups were matched for age and body mass index. Metabolic parameters, white blood cell count, and erythrocyte sedimentation rate were almost identical in the three study groups (Table 1). Occupational status and education level were also not significantly different between controls, dippers, and nondippers. The majority of participants had sedentary jobs and usually exerted no significant physical activities.

The average of UAE was higher in nondippers (21.6 ± 10.7 mg/24 h) and dippers (15.4 ± 7.11 mg/24 h) than in control subjects (11.0 ± 3.2 mg/24 h; $P < .0001$ v nondippers and $P = .0132$ v dippers). Two dipper (8%) and five nondipper (20%) hypertensive patients were microalbuminuric (UAE ≥30 and <300 mg/24 h). An inverse correlation between UAE and nocturnal systolic BP decrements was found in the entire hypertensive population ($r = -0.300$, $P = .034$).

**BP Measurements**

According to the selection criteria, clinic systolic and diastolic BP values were higher in dipper (153.8 ± 6.2 and 100.9 ± 4.0 mm Hg, respectively) and nondipper (154.1 ± 4.8 and 101.1 ± 4.6 mm Hg, respectively) hypertensives than in normotensive subjects (130.9 ± 6.8 and 82.4 ± 3.9 mm Hg, respectively; $P < .0001$). Clinic BP values were
not significantly different between dippers and nondippers. Ambulatory BP measurements in dippers and nondippers are reported in Table 2. By definition, night-time systolic and diastolic BP levels were significantly higher in nondippers than in dippers, whereas nocturnal BP decrements were higher in dippers than in nondippers. Average 48-h systolic and diastolic BP values were quite similar in dippers and nondippers. Daytime systolic and diastolic BP values tended to be higher in dippers, but only the difference in systolic BP level achieved statistical significance.

Circulating Levels of sCD40L, Isoprostanes, and hs-CRP

Circulating levels of sCD40L and total 8-iso-PGF$_{2\alpha}$ were significantly higher in nondippers than in dippers and normotensives (Fig. 1). These latter manifested with lower sCD40L and total 8-iso-PGF$_{2\alpha}$ concentrations than dippers (Fig. 1). Similarly, although to a lower degree of significance, circulating levels of hs-CRP were higher in nondippers (3.1 ± 0.8 mg/L) than in dippers (2.4 ± 1.1 mg/L; $P = .0129$) and controls (1.6 ± 0.5 mg/L; $P < .0001$). These latter manifested with lower hs-CRP levels than dippers ($P = .0083$).

Circulating levels of sCD40L were directly correlated with nocturnal systolic ($r = 0.473$, $P = .0005$) and diastolic ($r = 0.487$, $P = .0003$) BP levels and inversely correlated with nocturnal systolic ($r = -0.362$, $P = .01$) and diastolic ($r = -0.420$, $P = .002$) BP decrements in the entire hypertensive population. Similarly, plasma total 8-iso-PGF$_{2\alpha}$ concentrations were directly correlated with nocturnal systolic ($r = 0.422$, $P = .002$) and diastolic ($r = 0.389$, $P = .005$) BP values and inversely correlated with nocturnal systolic ($r = -0.287$, $P = .043$) and diastolic ($r = -0.353$, $P = .012$) BP decrements.

Circulating levels of sCD40L were directly correlated with plasma total 8-iso-PGF$_{2\alpha}$ levels ($r = 0.502$, $P = .0002$) and UAE ($r = 0.288$, $P = .05$) in the entire hypertensive population.

Carotid Intima–Media Thickness

The average IMT of the common carotid artery was significantly higher in nondippers (0.80 ± 0.10 mm) and dippers (0.73 ± 0.09 mm) than in controls (0.48 ± 0.11 mm; $P < .05$). One dipper (4%) and four nondipper (16%) hypertensives had intima–media thickening (IMT ≥ 0.9 mm). According to the selection criteria, none of dipper

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<th>Table 1. Clinical and laboratory characteristics in dipper and nondipper hypertensive patients</th>
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ESR = erythrocyte sedimentation rate.

Data are presented as mean ± SD.

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<th>Table 2. Ambulatory blood pressure values in dipper and nondipper hypertensive patients</th>
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DBP = diastolic blood pressure; n.s. = not significant; SBP = systolic blood pressure.

Each value represents the mean of two 24-h recording periods. Data are presented as mean ± SD.
and nondipper hypertensive patients had evidence of atherosclerotic plaque of carotid arteries (IMT ≥ 1.3 mm). Spearman nonparametric correlation found a weak but significant direct relationship between IMT and nocturnal systolic BP ($r = 0.286$, $P = .044$) and an inverse correlation between IMT and nocturnal systolic BP decrement ($r = -0.286$, $P = .043$) in the entire hypertensive population. The IMT was also directly correlated with circulating levels of sCD40L in hypertensives ($r = 0.365$, $P = .01$; Fig. 2). In a multivariate regression analysis with a stepwise approach, sCD40L was the main determinant of IMT, explaining about 16% of its variance ($r^2 = 0.157$, $P = .004$).

**Discussion**

It has been postulated that the lack of a nocturnal BP decrease is associated with serious vascular disease$^{1,2}$ and adverse cardiovascular outcomes$^{4,5}$ than occur in hypertensive patients whose BP decreases during the night. Increased proatherogenic activation of vascular endothelial cells$^6$ and platelets,$^7$ as well as a procoagulant status,$^6,7$ have been recently proposed as candidate pathophysiologic mechanisms linking nondipping BP profile with cardiovascular disease. Our study sheds new light on this topic, providing the first evidence of a possible pathogenic role of sCD40L in the development of vascular damage in nondipper hypertensives.

The relationship between hypertension and the CD40/CD40L system has been recently explored in a few studies that described increased sCD40L levels in middle-aged$^{24,25}$ but not in young hypertensives.$^{26}$ Our study further confirms these suggestions of CD40/CD40L system overactivity in essential hypertension, demonstrating increased sCD40L levels in a well-characterized population of hypertensive patients selected for having no additional cardiovascular risk factors. Even more interesting, our study is the first that relates sCD40L to nondipping hypertension. The highest plasma sCD40L levels were found in hypertensive patients who failed to dip their BP at night. This finding seems to be particularly strong, as dippers and nondippers were quite similar with regard to clinical and laboratory characteristics and all potential confounders were carefully excluded or minimized. In a pathophysiologic perspective our results might have relevant implications as enhanced sCD40L release has all the biological potential to explain the increased susceptibility of nondipper hypertensives to develop vascular damage.$^{11,12}$ In this regard, it appears particularly relevant the evidence of a direct relationship between circulating sCD40L levels and the extent of IMT in the entire hypertensive population. Adjustment for BP levels and metabolic parameters revealed an independent effect of circulating sCD40L on the extent of IMT. Although association does not necessarily imply any causal relationship, our data suggest a role of sCD40L in the early phases of development of vascular damage in hypertensive patients; this effect is likely more relevant in the presence of a nondipping profile of BP. Interestingly, we also found a direct relationship between plasma sCD40L concentrations and UAE in the entire hypertensive population, which represents a manifestation of vascular damage that is not confined to the renal arterial bed.$^{27}$

**FIG. 1.** Circulating levels of soluble CD40 ligand (top) and total 8-iso-prostaglandin $F_2\alpha$ (bottom) in 25 normotensive subjects and in 25 dipper and 25 nondipper hypertensive patients distinguished according to the presence or absence, respectively, of nocturnal blood pressure decrements >10% of daily blood pressure levels. Boxes represent interquartile ranges with the median value shown as a horizontal bar within each box. Bars outside each box show minimum and maximum values. a: $P < .0001$ vs controls; b: $P < .0001$ vs controls and $P = .0005$ vs dippers; c: $P < .05$ vs controls; d: $P < .05$ vs controls and dippers.

**FIG. 2.** Relationships between circulating levels of soluble CD40 ligand and intima–media thickness in the entire hypertensive population. White circles indicate dippers and black circles indicate nondipper hypertensive patients.
With regard to the mechanisms potentially responsible for the enhanced sCD40L release in nondippers, it is known that activated platelets represent the main source of circulating sCD40L. Thus, the increased plasma sCD40L concentration in nondippers likely reflects an enhanced platelet activation in these patients. Accordingly, Lee et al recently described increased circulating levels of soluble P-selectin, an accepted marker of platelet activation, in nondippers. In this regard, it is tempting to speculate that biochemical or mechanical stimuli might have contributed to activate platelets in our patients. We found higher circulating levels of 8-iso-PGF$_2\alpha$ in nondippers than in dippers and a direct correlation between these lipid peroxidation products and plasma sCD40L levels in the entire hypertensive population. Because oxidative stress represents a main determinant of platelet activation in essential hypertension, it is intriguing to hypothesize a role for increased lipid peroxidation in promoting sCD40L release from platelets. In addition, the evidence of an inverse relationship between circulating levels of sCD40L and the degree of nocturnal systolic and diastolic BP decrements in hypertensive patients suggests that hemodynamic forces might have cooperated in enhancing sCD40L release in nondippers. The increased shear stress that platelets are exposed to as a result of the high BP load could, in itself, lead to platelet activation.

We cannot exclude that other activating stimuli, including a low-grade inflammatory state revealed by increased hs-CRP levels in hypertensives, especially in nondippers, might have participated in enhancing the sCD40L release from platelets or other immune and nonimmune cell lines in these patients.

**Potential Limitations**

The patient selection, as well as the relatively small sample size, might limit the generability of our data to the hypertensive population as a whole. In addition, we minimized but not completely excluded the selection bias due to the low reproducibility of the classification of hypertensives into dippers and nondippers.

**Conclusions and Clinical Implications**

In conclusion, our study provides the first evidence that circulating levels of sCD40L are increased in essential hypertensive patients who fail to dip their BP at night. Due to the proatherogenic properties of sCD40L, our data contribute to explain the increased susceptibility of nondippers to develop vascular disease.

**References**


