Morning Blood Pressure Surge as a Destabilizing Factor of Atherosclerotic Plaque
Role of Ubiquitin–Proteasome Activity

Raffaele Marfella, Mario Siniscalchi, Michele Portoghese, Clara Di Filippo, Franca Ferraraccio, Concetta Schiattarella, Basilio Crescenzi, Paolo Sanguinoto, Giuseppe Ferraro, Silvio Siciliano, Francesca Cinone, Gennaro Mazzarella, Simone Martis, Mario Verza, Ludovico Coppola, Francesco Rossi, Michele D’Amico, Giuseppe Paolisso

Abstract—Whether morning blood pressure surge influences the molecular mechanisms of plaque progression toward instability is not known. Recently, we have demonstrated enhanced activity of the ubiquitin–proteasome system in human plaques and evidenced that it is associated with inflammatory-induced plaque rupture. We evaluated the inflammatory infiltration and ubiquitin–proteasome activity in asymptomatic carotid plaques of hypertensive patients with different patterns of morning blood pressure surge. Plaques were obtained from 32 hypertensive patients without morning blood pressure surge and 28 with morning blood pressure surge enlisted to undergo carotid endarterectomy for extracranial high-grade (>70%) internal carotid artery stenosis. Plaques were analyzed for macrophages, T-lymphocytes, human leukocyte antigen–DR+ cells, ubiquitin–proteasome activity, nuclear factor-κB, inhibitor κB-β, tumor necrosis factor-α, nitrotyrosine, matrix metalloproteinase-9, and collagen content (immunohistochemistry and ELISA). Compared with plaques obtained from hypertensive patients without morning blood pressure surge, plaques from with morning blood pressure surge had more macrophages, T-lymphocytes, human leukocyte antigen–DR+ cells (P<0.001), ubiquitin-proteasome activity, tumor necrosis factor-α, nuclear factor-κB (P<0.001), nitrotyrosine, and matrix metalloproteinase-9 (P<0.01), along with a lesser collagen content and IkB-β levels (P<0.001). Enhanced ubiquitin–proteasome activity in atherosclerotic lesions of patients with morning blood pressure surge is associated with inflammatory-dependent unstable plaque phenotype. These data suggest a potential interplay between morning blood pressure surge and ubiquitin–proteasome activity in atherosclerosis pathophysiology. (Hypertension. 2007;49:784-791.)

Key Words: morning blood pressure ■ atherosclerotic plaque ■ inflammation ■ ubiquitin–proteasome activity

Many studies in the past decade have demonstrated diurnal variation in the onset of acute cardiovascular disorders in hypertensive patients, such as acute coronary syndrome, and ischemic and hemorrhagic stroke occurring in the morning (6:00 AM to noon), after a nadir in these events during the night.1 Blood pressure (BP) falls during the night because of the reduction of sympathetic activity that is brought about by sleep and then increases steeply when in the morning the subject awakes and resumes his/her daily activities.2 This increase occurs together with a peak incidence of cerebral and cardiac events in the morning hours.3 Moreover, a recent prospective study suggests that higher morning BP surge (MBPS) might be an independent risk factor of atherosclerotic events beyond ambulatory BP and nocturnal BP falls.4 The molecular mechanisms associating MBPS peak and vulnerable atherosclerotic plaque are not clear, although inflammation, which plays a central role in the cascade of events that result in plaque erosion and fissuring, also were related to MBPS.5

There is emerging evidence that the ubiquitin–proteasome system (UPS), the major pathway for nonlysosomal intracellular protein degradation in eukaryotic cells, induces inflammation in both the initial stage and progression of atherosclerosis.6 Moreover, the ubiquitin–proteasome pathway is required for activation of nuclear factor κB (NF-κB), a central transcription factor that regulates inflammatory genes, by degradation of its inhibitory inhibitor κB (IκB) proteins.7 Although it has been demonstrated that the ubiquitin–proteasome pathway has a crucial role in the pathogenesis of cardiovascular diseases,8,9 still no evidence exists about the potential role of UPS in the evolution of atherosclerotic plaques of hypertensive patients with MBPS peak.
This study was designed to identify differences in inflammatory infiltration, as well as ubiquitin–proteasome activity, between asymptomatic carotid plaques of hypertensive patients with different patterns of morning BP increase. Moreover, because the upregulation of oxidative stress may activate the UPS, the localization of nitrotyrosine, a modified amino acid produced by reactive \( \dot{O}_2 \), in atherosclerotic lesions was measured quantitatively.

### Methods

#### Study Population

Hypertensive patients were recruited from the outpatient department for hypertension at the Second University of Naples from January 1998 to June 2005. The sample size for the planned study was estimated in 60 patients (32 hypertensive patients without MBPS [MP−] and 28 hypertensive patients with MBPS [MP+]). Among them we selected those with asymptomatic carotid stenosis, enlisted to undergo carotid endarterectomy for extracranial high-grade (>70%) internal carotid artery stenosis. All of the subjects had a clinic systolic BP ≥140 mm Hg and/or diastolic BP ≥90 mm Hg on ≥3 visits at 1-week intervals and fulfilled all of the following inclusion criteria: no clinical or laboratory evidence of heart failure, previous stroke, valvular defects, malignant neoplasms, or secondary causes of hypertension and ≥1 valid BP measurement per hour over 24 hours during ambulatory BP monitoring. Carotid sonography was performed on a single ultrasound machine (Aloka 5500). During the observational period, dietary counseling was added to the previous therapy. The study was approved by the ethics committee of our institution, and informed written consent was obtained for each patient.

#### Laboratory Analysis

Plasma glucose and serum lipids were measured by enzymatic assays in the hospital’s chemistry laboratory. Urinary catecholamines (epinephrine and norepinephrine) were measured with high-pressure liquid chromatography. Separate urine samples were obtained during the morning time (6:00 AM to 10:00 AM), daytime (10:00 AM to 10:00 PM), and nighttime (10:00 PM to 6:00 AM). Samples were centrifuged at 3000 rpm for 15 minutes and urine and plasma were decanted and stored at −80°C until analysis. The 24-hour urine was collected on the same day as the ambulatory BP monitoring.

#### 24-Hour Ambulatory BP Monitoring

After the initial screening visit, ambulatory BP monitoring was performed in all of the hypertensive patients (an expanded Methods section is available in a data supplement available online at http://hyper.ahajournals.org). The MBPS peak was defined as a rise in systolic BP ≥50 mm Hg and/or diastolic BP ≥22 mm Hg (90th percentile of normotensive patients) during the early morning (6:00 AM to 10:00 AM), arbitrarily defined as the morning period, obtained from the mean of BP during the first 2 hours after waking (8 BP readings) minus the mean BP during the night (as the average BP of 3 readings centered on the lowest nighttime reading). The normal values (mean±SD) of the MBPS peak obtained from 14 normotensive patients 67±6 years of age were as follows: systolic, 19±6 mm Hg, and diastolic, 10±5 mm Hg. Subjects without an MBPS peak were defined as the MP− group and the others as the MP+ group. Subjects with a nocturnal reduction of systolic and/or diastolic BP ≥10% were defined as dippers and the others as nondippers. Nighttime workers and subjects going to bed later than 1:00 AM were excluded from the study. The 28 MP+ hypertensive patients enrolled in the study were compared with 32 MP− hypertensive patients matched for age, sex, and body mass index before the carotid endarterectomy.

#### Atherectomy Specimens

After surgery, the specimens were cut perpendicular to the long axis into 2 halves. The first half was frozen in liquid nitrogen for the following ELISA analysis. A portion of the other half specimen was immediately immersion fixed in 10% buffered formalin. Sections were serially cut at 5 μm, mounted on lysine-coated slides, and stained with hematoxylin/eosin and with the trichrome method. Carotid artery specimens were analyzed by light microscopy.

#### Immunohistochemistry

After the surgical procedure, samples were immediately frozen in isopentane and cooled in liquid nitrogen. Similar regions of the plaque were analyzed. Serial sections were incubated with specific antibodies anti-ubiquitin, anti-proteasome 20S, antibodies α-smooth muscle actin, anti–human leukocyte antigen (HLA)-DR locus, anti–CD68, and anti–CD3 (Dako); anti–ΙκB-β and anti–matrix metalloproteinase (MMP)-9 (Santa Cruz); anti–tumor necrosis factor (TNF)-α (R&D). Specific antibodies that selectively recognize the activated form of nuclear factor-κB (p65 and p50 subunits, Santa Cruz) were used. Analysis of immunohistochemistry was performed with a personal computer–based quantitative 24-bit color image analysis system (IM500, Leica Microsystems AG).

#### Sirius Red Staining for Collagen Content

After dehydration, the sections were observed under polarized light after being placed on coverslips. The sections were photographed with identical exposure settings for each section.

#### Biochemical Plaque Assays

Plaques were lysed and centrifuged for 10 minutes at 10 000g at 4°C. After centrifugation, 20 μg of each sample were loaded, electrophoresed in polyacrylamide gel, and electroblotted onto a nitrocellulose membrane. Each determination was repeated ≥3 times. Ubiquitin, IκB-β, MMP-9, TNF-α, and nitrotyrosine levels were quantified in plaques using a specific ELISA kits (Santa Cruz, R&D Systems, and Imgenex). Nuclear extracts from plaque specimens were obtained as described by Ohlsson et al. We used a specific antibody that selectively recognizes the activated form of the NF-κB p65 and p50 subunits. NF-κB binding to κB sites was assessed using the Trans-AM NF-κB p65 and p50 transcription factor assay kits (Active Motif Europe, Rixensart). For the quantitative measurement of the protease-20S activity, a specific SDS-activation kit (Boston Biochem) was used. Nitrotyrosine was assayed into the plaque tissue with a kit supplied by Hycult Biotech.

#### Macrophages Extraction From Atherosclerotic Plaques

Macrophages were selectively extracted from plaques as described by de Vries et al. Biochemical assays on cell homogenates for ubiquitin and proteasome 20S determinations were performed as illustrated earlier.

#### Measurement of \( \dot{O}_2^- \)

Production of \( \dot{O}_2^- \) was measured as the superoxide dismutase–inhibitable reduction of cytochrome c, as described previously.

#### Isolation and Culture of Blood Monocytes

Peripheral blood monocytes from 20 MP+ and 20 MP− patients were purified and cultured as described previously (see the online data supplement).

#### Statistical Analysis

Data are presented as mean±SD. Continuous variables were compared among the groups of patients with 1-way ANOVA for normally distributed data and Kruskal–Wallis test for nonnormally distributed data. When differences were found among the groups, Bonferroni correction was used to make pairwise comparisons. Multivariate regression analysis tested the independent association...
Daytime and Nighttime BP Profile of Study Patients

<table>
<thead>
<tr>
<th>Study Patients</th>
<th>MP− Group (N=32)</th>
<th>MP+ Group (N=28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morning (mean: 6:00 AM to 10:00 AM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>146±14*</td>
<td>173±17</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>93±7*</td>
<td>112±10</td>
</tr>
<tr>
<td>Epinephrine urinary output, µg</td>
<td>5.5±0.8*</td>
<td>8.1±1.0</td>
</tr>
<tr>
<td>Norepinephrine urinary output, µg</td>
<td>29.1±2.9*</td>
<td>41.4±4.5</td>
</tr>
<tr>
<td>Day (mean: 10:00 AM to 10:00 PM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>144±13</td>
<td>148±15</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>89±7</td>
<td>92±8</td>
</tr>
<tr>
<td>Epinephrine urinary output, µg</td>
<td>5.8±1.2</td>
<td>5.5±1.3</td>
</tr>
<tr>
<td>Norepinephrine urinary output, µg</td>
<td>26.6±4.7</td>
<td>28±5.2</td>
</tr>
<tr>
<td>Night (mean: 10:00 PM to 6:00 AM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>130±17</td>
<td>129±17</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>82±7</td>
<td>81±7</td>
</tr>
<tr>
<td>Epinephrine urinary output, µg</td>
<td>2.1±0.6</td>
<td>2.4±0.7</td>
</tr>
<tr>
<td>Norepinephrine urinary output, µg</td>
<td>11.2±2.3</td>
<td>11.5±2.4</td>
</tr>
<tr>
<td>Systolic morning BP peak, mm Hg</td>
<td>16±6*</td>
<td>62±10</td>
</tr>
<tr>
<td>Diastolic morning BP peak, mm Hg</td>
<td>11±3*</td>
<td>30±7</td>
</tr>
</tbody>
</table>

Data are presented as mean±SD or No. (%).

*P<0.05 vs MP+ group.

and contribution of changes in MBPS and norepinephrine and epinephrine urinary output in the morning period with the dependent variables (indexes of plaque components). P=0.05 was considered statistically significant. All of the calculations were performed using the computer program SPSS 12.

Results

Preoperative Characteristics of the Patients

Anthropometric characteristics, routine blood chemical analyses, sleep duration, and clinic BP were similar in all of the patients (an expanded Results section and Table S1 are available in the online data supplement). The percentage of carotid diameter reduction, risk factors, and concomitant nonantihypertensive therapy did not differ among the groups (data supplement, Table S1). BP rose steeply after waking up in both groups; however, patients of the MP+ group had a significant increase in both systolic and diastolic BP compared with the MP− group (P<0.01) during the whole morning period. The daytime and nighttime profile did not significantly differ between the 2 groups (Table); there were 18% nondippers in the MP+ groups and 19% in the MP− group (P value was not significant). In the MP− patients, catecholamine urinary output was significantly lower (P<0.02) during the morning period compared with MP+ patients (Table), whereas diurnal and nocturnal catecholamine output was not different (Table).

Plaque Composition of the Study Patients: Is MBPS Increase Associated With Unstable Plaque Phenotype?

Compared with MP− patients, MP+ patients had a significantly greater portion of plaque area occupied by macrophages (P<0.01), T-lymphocytes (P<0.01), and greater expression of HLA-DR antigen (P<0.01), as well as smaller content of vascular smooth muscle cell (VSMC; P<0.01) and interstitial collagen (please see the expanded Results section, Table S2). MMP-9 levels were more abundant in MP+ that in MP− lesions (P<0.001; data supplement, Table S2). Notably, both systolic and diastolic morning BP increases were positively correlated with plaque expression of macrophages (r=0.49 and r=0.43, respectively; P<0.001) and plaque expression of HLA-DR antigen (r=0.38 and r=0.36, respectively; P<0.001) and were negatively correlated with plaque interstitial collagen content (r=−0.45 and r=−0.41, respectively; P<0.001) and VSMC content (r=−0.44 and r=−0.37, respectively; P<0.005). Moreover, both norepinephrine and epinephrine urinary output in the morning period were positively correlated with plaque expression of macrophages (r=0.37 and r=0.41, respectively; P<0.001) and plaque expression of HLA-DR antigen (r=0.39 and r=0.36, respectively; P<0.01) and were negatively correlated with plaque interstitial collagen content (r=−0.38 and r=−0.36, respectively; P<0.001) and VSMC content (r=−0.40 and r=−0.41, respectively; P<0.005). To investigate which variables might account for the association among plaque phenotype, sympathetic activity, and MBPS, multiple regression analysis was performed. Only systolic (P<0.01) and diastolic (P<0.05) morning BP increases were independently associated with plaque expression of macrophages and HLA-DR antigen, as well as plaque interstitial collagen and VSMC content.

Oxidative Stress in the Plaque: Is MBPS Increase Associated With the Upregulation of Nitrotyrosine Levels?

Higher levels and higher staining of nitrotyrosine were found in MP+ plaques as compared with MP− plaques (P<0.001; Figure 1). A similar pattern was found for O2− production (MP+: 7.11±1.3 pmol/L; MP−: 2.56±1.28 pmol/L; P<0.001). Notably, oxidative stress was strongly dependent from morning BP increase, as also reflected by the statistically significant correlation between both systolic and diastolic BP increase and nitrotyrosine concentrations (r=0.58 and r=0.46, respectively; P<0.001).

Ubiquitin–Proteasome Activity in the Plaque: Does MBPS Increase Modulate Ubiquitin-Proteasome Activity in the Plaque via Oxidative Stress?

Immunohistochemistry revealed higher staining of ubiquitin and proteasome-20S in MP+ as compared with MP− inflammatory cells (P<0.001). A similar pattern of response was seen for ubiquitin plaque levels (P<0.001) and proteasome 20S plaque activity (P<0.001; Figure 2). Notably, both ubiquitin levels and proteasome-20S activity were positively correlated with plaque nitrotyrosine (r=0.49 and r=0.43, respectively; P<0.001) and both systolic (r=0.38 and r=0.36, respectively; P<0.001) and diastolic morning BP increase (r=0.44 and r=0.37, respectively; P<0.005).

Ubiquitin and Proteasome 20S in Macrophages Extracted From Plaques

To identify whether the higher ubiquitin–proteasome levels observed in MP+ were produced by macrophages, we repeated
quantitative analyses on macrophages selectively extracted from 14 plaques randomly selected from each group. We observed that the MP+ group had the highest of both ubiquitin levels (488.5 ± 102 ng/mg) and proteasome 20S activity (88.7 ± 29 pmol/mg), and MP− group had the lowest (ubiquitin: 212 ± 68 ng/mg; proteasome 20S: 29.7 ± 9 pmol/mg; ubiquitin, *P* < 0.05; proteasome 20S, *P* < 0.05).

**Inflammation in the Plaque: Does MBPS Increase Upregulate NF-κB Activity in the Plaque via UPS?**

NF-κB activation, as reflected by the selective analysis of activated form of both p50 and p65, was significantly higher in MP+ plaques (*P* < 0.01) as compared with MP− plaques. Immunohistochemistry and quantitative analyses revealed lower staining and levels for IκB-β in MP+ as compared with MP− plaques (*P* < 0.001; Figure 3). Both immunohistochemistry and ELISA revealed markedly higher staining and levels of NF-κB-α in MP+ that in the MP− lesions (*P* < 0.001).

**In Vitro Study**

Higher levels of ubiquitin, p50, p65, and O2− production, as well as higher proteasome 20S along with a lesser IκB-β levels, were evidenced in peripheral blood monocytes from 20 MP+ patients compared with monocytes from MP− patients (*P* < 0.01). Levels of proteasome 20S, p50, and p65 levels and O2− production were significantly lower in monocytes from MP+ patients incubated with than in MP+ patient monocytes incubated without MG132 (p50, *P* < 0.01; p65, *P* < 0.01), whereas IκB-β was significantly higher in the MP+ group monocytes incubated with MG132 than in monocytes incubated without it (*P* < 0.01). Although ubiquitin levels were higher in monocytes incubated with MG132, there was not a statistically significant difference among the groups (*P* = 0.3; data supplement, Figure S1).

**Discussion**

In the present report, we show evidence that MBPS increases are associated with an unstable plaque phenotype, suggesting...
that surges of BP after arising convey a more relevant risk for cardiovascular events. As for the background for this association, we provide evidence for the functional involvement of UPS in the NF-κB–dependent inflammation upregulation in human atherosclerotic plaque of hypertensive patients with morning BP peak. In particular, the present findings are the first, to the best of our knowledge, to do the following: (1) clearly identify differences for ubiquitin–proteasome activity in MP+ versus MP− human atherosclerotic plaques; (2) recognize the oxidative stress and the inflammatory upregulation, as evidenced by the higher levels nitrotyrosine, as well as higher NF-κB–dependent TNF-α levels, in MP+ versus MP− asymptomatic plaques; and (3) relate the presence of ubiquitin–proteasome overactivity to higher MMP-9 levels along with a lesser interstitial collagen content, as well as a lesser VSMC content. These alterations might increase the risk of future acute ischemic events precipitated by inflammatory-dependent rupture of atherosclerotic plaques.

Because an epidemiological study suggests that both ischemic and hemorrhagic strokes showed a greater tendency to cluster in the morning period in the MP+ group than in the MP− group, it is reasonable to suppose that an excessive MBPS increase might trigger strokes through some hemodynamic mechanism, such as increased shear stress, that, in turn, may activate the molecular pathway favoring plaque instability, such as increased oxidative stress, ubiquitin proteasome activity, and inflammation, on the atherosclerotic vessels. In lights of such evidence, the observation that plaques from MP+ patients had higher nitrotyrosine and TNF-α levels along with a lesser interstitial collagen content and a lesser VSMC content compared with plaques in MP− patients suggests that surges of BP after arising may influence the plaque progression toward instability. As for the background for this association, the present study provides the evidence of an association among MBPS peak, higher oxidative stress, and inflammatory markers, as well as morning sympathetic overactivity. Although a direct link between MBPS peak and acute cardiovascular events has not yet been established, it might be hypothesized that morning sympathetic overactivity influencing the surge in BP and oxidative

Figure 2. A, Representative sections show immunochemistry for ubiquitin (×400) and proteasome (×400) in MP− and MP+ plaques. Similar regions of plaque are shown. Results are typical of MP− and MP+ plaques. B, ELISA for ubiquitin levels and specific SDS-activation kit for proteasome-20S in MP− and MP+ plaques. *P<0.05 vs MP+ plaques.
stress may be a factor predisposing to vulnerable atherosclerotic plaques via an increase of UPS overactivity.

A previous study has reported enhanced ubiquitin–proteasome expression in unstable carotid plaques. However, the study did not provide any evidence about ubiquitin–proteasome expression in subgroups of high-risk plaques, such as those found in hypertensive patients with higher MBPS. In our study, macrophages, T-lymphocytes, and HDLA-DR+ inflammatory cells were more abundant in MP+ plaques and represented the major source of ubiquitin–proteasome activity, suggesting the presence of an active inflammatory reaction in MP+ plaques. Concomitantly higher expression of ubiquitin and proteasome was found in human plaque macrophages obtained from the carotid lesions of MP+ patients compared with specimens obtained from MP− patients. In agreement with the difference in the ubiquitin–proteasome staining pattern, the histological milieu of the lesions appears different with regard to cellularity but not in the degree of vessel stenosis, suggesting that MP+ and MP− lesions are only different with regard to inflammatory burden. Hence, the differences in plaque behavior likely stem from differences in the presence of stimuli (ie, oxidative stress, as evidenced by high nitrotyrosine levels and high O$_2^−$ production) for selective expression of ubiquitin proteasome, capable of disrupting plaque stability via NF-κB induction.

NF-κB is normally bound to IκB in the cytosol; this binding prevents its movement into the nucleus. Various cellular stimuli, such as oxidative stress, induce ubiquitination of phosphorylated IκBα and subsequent degradation by the proteasome. Degradation of IκBα results in unmasking of the nuclear localization signal of NF-κB dimers, which subsequently translocates to the nucleus, where it induces the transcription of proinflammatory cytokines, like TNF-α, that play a central role in plaque instability progression. Our findings also suggest that the morning BP increase may induce phosphorylation and degradation of IκBα via the ubiquitin–proteasome overactivity, thus enhancing NF-κB activation. According to the response-to-injury theory, plaque progression may be linked to an inflammatory-proliferative response to an injurious stimulus, constituted by morning BP-dependent shear stress that increases the oxidative stress. Previous reports evidenced the involvement of the UPS in NF-κB activation, particularly under conditions of aggravated oxidative stress. Oxidative stress is the common factor underlying sympathetic overactivity, MBPS increases, and cardiovascular events and may explain the presence of inflammation in all of these conditions. Although it is well recognized that inflammation is 1 manifestation of oxidative stress, and the pathways that generate the mediators of inflammation are all induced by oxidative stress, the mech-
anism by which oxidative stress may be involved in the inflammatory process of MP+ plaques is not fully clarified. In this context, our data suggest a mechanism by which oxidative stress, increasing ubiquitin–proteasome activity, may mediate inflammatory activity in MP+ atherosclerotic plaques. Of note, it has been shown that oxidative stress can stimulate the ubiquitin system in macrophages by inducing the expression of components of its enzymatic machinery, such as ubiquitin-binding proteins. Accordingly, in cultured monocytes from MP+ patients, we evidenced that O2− production, as well as ubiquitin–proteasome activity and NF-κB levels, were significantly higher when compared with MP− patients. Thus, we can speculate that increased ubiquitin–proteasome activity in plaque macrophage as a consequence of oxidative stress overexpression may enhance the synthesis of NF-κB in the same cell, possibly representing a crucial step in the pathophysiology of plaque instability. Because of the study design, we cannot exclude whether the ubiquitin–proteasome dysregulation also may influence the atherosclerosis process through other pathways, such as apoptosis. However, the concomitant presence of higher levels of ubiquitin, proteasome 20S, and NF-κB in cultured monocytes of MP+ patients suggests that the ubiquitin–proteasome pathway may have a proinflammatory effect in MP+ lesions. Thus, we can speculate that increased ubiquitin–proteasome activity in plaque macrophage as a consequence of oxidative stress overexpression may enhance the synthesis of NF-κB in the same cell, possibly representing a crucial step in the pathophysiology of MP+ plaque instability. However, higher expression of ubiquitin proteasome and MMP-9 in MP+ lesions, 1 of the most important enzymes in the process of atherosclerotic plaque rupture, along with a lesser interstitial collagen content, suggests an involvement of the UPS in instability of the MP+ lesion by increasing plaque erosion.

In conclusion, this study demonstrates the enhanced ubiquitin–proteasome activity in atherosclerotic lesions of MP+ patients and provides evidence that the activation of this system in inflammatory cells is associated with a NF-κB–dependent increase in inflammation. The increased ubiquitin–proteasome activity in response to oxidative stress, strictly related to MBPS, may interfere with plaque progression toward instability. Whether this plays a role in the poor prognosis of the MP+ patients awaits further elucidations. For the relatively small patient number of our study and the highly selected elderly patients scheduled for carotid endarterectomy, the results of this study should not be extrapolated to other patient groups. Further research in a larger sample of hypertensive patients is needed to confirm the applicability of our new findings.

**Perspectives**

Because epidemiological data have raised the possibility that MBPS may be an important and modifiable risk factor for poor outcome, these findings are potentially important from a fundamental standpoint, because they identify a mechanism by which MBPS may influence the evolution of atherosclerotic plaques. From a practical standpoint, these findings support the hypothesis that control of excessive MBPS excursions may provide clinical benefit in hypertensive patients.

**Source of Funding**

Funding was provided by PRIN, Ministerial Research Program.

**Disclosures**

None.

**References**


15. de Vries HE, Buchner B, van Berkel TJ, Kuiper J. Specific interaction of
oxidized low-density lipoprotein with macrophage-derived foam cells
isolated from rabbit atherosclerotic lesions. Arterioscler Thromb Vasc
17. Herrmann J, Gulati R, Napoli C, Woodrum JE, Lerman LO, Rodríguez-
Porcel M, Sica V, Simari RD, Ciechanover A, Lerman A. Oxidative
stress-related increase in ubiquitination in early coronary atherosclerosis.
18. Tedgui A, Mallat Z. Cytokines in atherosclerosis: pathogenic and regu-
340:115–126.
Increased reactive oxygen species in rostral ventrolateral medulla con-
tribute to neural mechanisms of hypertension in stroke-prone sponta-
21. Roebuck KA. Oxidant stress regulation of IL-8 and ICAM-1 gene
expression: differential activation and binding of the transcription factors
22. Versari D, Herrmann J, Gossi M, Mannheim D, Sattler K, Meyer FB,
Lerman LO, Lerman A. Dysregulation of the ubiquitin-proteasome
2006;26:2132–2139.
23. Gals ZS, Sukhova GK, Lark MW, Libby P. Increased expression of matrix
metalloproteinases and matrix degrading activity in vulnerable regions of
24. Kario K. Morning surge and variability in blood pressure: a new thera-
Morning blood pressure hyperreactivity is an independent predictor for
hypertensive cardiac hypertrophy in a community-dwelling population.
Morning Blood Pressure Surge as a Destabilizing Factor of Atherosclerotic Plaque: Role of Ubiquitin–Proteasome Activity

Raffaele Marfella, Mario Siniscalchi, Michele Portoghese, Clara Di Filippo, Franca Ferraraccio, Concetta Schiattarella, Basilio Crescenzi, Paolo Sanguolo, Giuseppe Ferraro, Silvio Siciliano, Francesca Cinone, Gennaro Mazzarella, Simone Martis, Mario Verza, Ludovico Coppola, Francesco Rossi, Michele D'Amico and Giuseppe Paolisso

Hypertension. 2007;49:784-791; originally published online February 19, 2007;
doi: 10.1161/01.HYP.0000259739.64834.d4

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2007 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/49/4/784

Data Supplement (unedited) at:
http://hyper.ahajournals.org/content/suppl/2007/02/12/01.HYP.0000259739.64834.d4.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/