Effects of Proteasome Inhibition on the Kidney in Experimental Hypercholesterolemia

Alejandro R. Chade,* Joerg Herrmann,† Xiyangang Zhu,* James D. Krier,* Amir Lerman,† and Lilach O. Lerman‡‡

Department of Internal Medicine, Divisions of *Nephrology and Hypertension and †Cardiovascular Diseases, Mayo Clinic College of Medicine, Rochester, Minnesota

Hypercholesterolemia (HC) and atherosclerosis often accompany and aggravate renal disease. Proteasome inhibitors (PSI) can decrease proliferation and inflammation, likely by reducing activation of the proinflammatory NF-κB. However, chronic proteasome inhibition has never been demonstrated in the HC kidney. Four groups of pigs (n = 7 each) were studied after a 12-wk normal (N) or 2% HC diet alone or supplemented (N+PSI and HC+PSI) with MLN-273 (0.08 mg/kg subcutaneously twice weekly). Renal hemodynamics and function were quantified in vivo using electron-beam computed tomography at baseline and after vasodilator challenge using acetylcholine. Renal tissue was studied ex vivo using immunoblotting, PCR, and immunohistochemistry. Serum cholesterol was similarly elevated in HC and HC+PSI. Basal renal blood flow was similar among the groups, whereas GFR was decreased in both N+PSI and HC+PSI. The blunted renovascular and functional responses to acetylcholine in HC were normalized in HC+PSI (suggesting renal endothelial function improvement), which was accompanied by decreased renal endothelin, NF-κB, and augmented endothelial nitric oxide synthase expression. In parallel, HC+PSI animals also showed elevated NAD(P)H oxidase expression and circulating oxidized LDL, suggesting a potential for increased oxidative stress. This study shows that chronic PSI intervention in HC improves renal endothelial functional responses to challenge, possibly by modulating nitric oxide availability and endothelin. Furthermore, PSI may decrease intrarenal inflammation through modulation of the NF-κB pathway but may potentially increase oxidative stress, which warrants further investigation. This study may support a role for the ubiquitin/proteasome system in the kidney in HC and early atherosclerosis.

Dyslipidemia frequently accompanies renal disease, and a growing body of evidence emphasizes its importance in the pathogenesis and progression of renal injury (1,2). We showed previously that hypercholesterolemia (HC), a surrogate of early atherosclerosis, led to renal hemodynamic and functional impairment, increased propensity for formation of oxidized LDL (Ox-LDL), and renal inflammation (3,4). We have also observed that HC induced a substantial increase in renal expression and activity of NF-κB (4,5), a pivotal transcription factor in atherogenesis involved in important biologic processes such as inflammation, proliferation, and cell death (6). The main activation pathway for NF-κB involves the ubiquitin/proteasome system (UPS), a key mediator in this process (7).

The proteasome plays a role in a myriad of intracellular processes from cell-cycle control to antigen presentation (8). The UPS is considered the major pathway for nonlysosomal degradation of intracellular proteins (7,9), including those involved in the immune response, development, and programmed cell death (10). It mediates degradation of regulatory proteins that modulate numerous processes such as cell-cycle progression, signal transduction, transcriptional regulation, receptor downregulation, and endocytosis. Furthermore, recent experimental evidence implicates the UPS in the initiation and progression of atherosclerosis (7,11), a systemic inflammatory process that is associated with increased oxidative stress and constitutes a well-known risk factor for cardiovascular disease (2,12). In line with these observations, emerging evidence supports the notion that proteasome inhibitors (PSI) may be vascular- and tissue-protective (11,13). Notably, the UPS may also contribute to renal injury (14), and we recently observed its activation in the early stage of experimental renovascular disease (15,16). However, the potentially beneficial effects of PSI on the kidney in the early stage of atherogenesis, a proinflammatory condition associated with increased NF-κB and UPS activation, remain unknown. Therefore, this study was designed to investigate the effects of PSI on the kidney in HC, both in vivo and in vitro.

Materials and Methods

The Institutional Animal Care and Use Committee approved all procedures. Twenty-eight domestic pigs (55 to 65 kg), divided in four groups (n = 7 each), were studied after 12 wk of either a normal (N) or HC diet (2% cholesterol, TD-93296; Harlan-Teklad, Madison, WI) (3,4) alone or supplemented (N+PSI and HC+PSI) with the boronic acid-

Received August 16, 2004. Accepted December 29, 2004.

Published online ahead of print. Publication date available at www.jasn.org.

Address correspondence to: Dr. Lilach O. Lerman, Division of Nephrology and Hypertension, Mayo Clinic College of Medicine, 200 First Street SW, Rochester, MN 55905. Phone: 507-266-9376; Fax: 507-266-9316; E-mail: lerman.lilach@mayo.edu

Copyright © 2005 by the American Society of Nephrology

ISSN: 1046-6673/1604-1005
type PSI MLN-273 (0.08 mg/kg subcutaneously twice weekly; Millen-
mium Pharmaceuticals, Cambridge, MA). The drug inhibits the β-sub-
unit of the 20S proteasome, which is the proteolytic core of the 26S
proteasome. The dose used has been shown to be effective and well
tolerated in previous animal studies using PSI (17,18) and was based on
the pharmaceutical company’s recommendations.

On the day of studies, each animal was anesthetized with intramus-
tcular telazol (5 mg/kg) and xylazine (2 mg/kg), intubated, and me-
chanically ventilated with room air. Anesthesia was maintained with a
mixture of ketamine (0.2 mg/kg per min) and xylazine (0.03 mg/kg per
min), as previously detailed (3,4,16,19,20). In vivo electron-beam com-
tomography (EBCT) studies were performed for assessment of
single-kidney renal blood flow (RBF), GFR, and regional renal perfu-
sion during suprarenal intra-aortic infusion of acetylcho-
line (Ach; 5 µg/kg per min) to test endothelium-dependent responses,
as described previously (3,4). The EBCT technique is an extensively
validated tool that allows studying the single kidney in vivo, under
resting conditions and during challenge (3,4,19,20). Briefly, after place-
ment of intravascular catheters, the animals were allowed a 1-h recov-
er period, during which they were positioned in the EBCT (C-150;
Imatron, South San Francisco, CA) scanning gantry. All EBCT studies
were performed during respiratory suspension at end expiration
following local anesthesia of the tomographic levels containing both kidneys. Initially,
two adjacent mid-hilar tomographic levels demonstrating both kidneys
were selected for performance of flow studies and scanned 30 times
over 3 min at variable intervals. Scanning was initiated 3 s after injec-
tion of the low-osmolal nonionic contrast medium iopamidol (Isovue-
370; Squibb Diagnostics, Princeton, NJ). A blood sample was collected in
the middle of this period and placed on ice. Fifteen minutes after the
baseline flow study, a 20-min infusion of Ach was initiated. BP tran-
siently and similarly decreased in all groups during Ach infusion but
during washout, during which they were positioned in the EBCT (C-150;
Imatron, South San Francisco, CA) scanning gantry. Initially,
two adjacent mid-hilar tomographic levels demonstrating both kidneys
were selected for performance of flow studies and scanned 30 times
over 3 min at variable intervals. Scanning was initiated 3 s after injec-
tion of the low-osmolal nonionic contrast medium iopamidol (Isovue-
370; Squibb Diagnostics, Princeton, NJ). A blood sample was collected in
the middle of this period and placed on ice. Fifteen minutes after the
baseline flow study, a 20-min infusion of Ach was initiated. BP tran-
siently and similarly decreased in all groups during Ach infusion but
rapidly (2 to 3 min) returned to baseline level, and EBCT studies then
were repeated. The flow study was followed by a volume study, in
which the kidneys were scanned from pole to pole during right atrial
infusion of iopamidol (0.5 ml/kg over 5 to 6 s) for subsequent mea-
surement of cortical, medullary, and whole-kidney volume (3,4).

Blood samples were collected for measurement of serum cholesterol,
plasma levels of Ox-LDL, endothelin-1 (ET-1), and superoxide dis-
mutase (SOD) activity. After completion of all of the in vivo studies, the
pigs were killed and kidneys were removed, immediately shock-frozen in
liquid nitrogen, and stored at −80°C or preserved in formalin (3,4).
In vitro studies then were performed to characterize redox status, nitric
oxide (NO) pathway, and inflammation. Protein expression of CuZn-
SOD, NAD(P)H-oxide, endothelial NO synthase (eNOS), and NF-κB
(p65 subunit) and IκB were investigated using Western blotting and immu-
nohistochemistry. Furthermore, mRNA expression of ET-1 and its recep-
tors ET-A and ET-B were investigated using quantitative real-
time PCR.

Ox-LDL, ET-1, and SOD Assays

Circulating Ox-LDL (Mercodia, Uppsala, Sweden), ET-1 levels, and
systemic SOD (Cayman Chemical Company, Ann Arbor, MI) activity were
measured in plasma using spectrophotometric enzyme immuno-
assay kits, following detailed vendor instructions (16,21).

Protein Expression

Western Blotting. Renal tissue (including both cortex and me-
dulla) was pulverized and homogenized, and standard immunoblot-
ting protocols were followed, as described previously (4,16,22), using
specific antibodies against the NAD(P)H-oxidease subunits p67phox
and p47phox, eNOS, p65-NF-κB, IκB (Santa Cruz Biotechnology, Santa
Cruz, CA; 1:200 for all), CuZn SOD (Santa Cruz Biotechnology; 1:500),
and ubiquitin (Berkeley Antibody Co., Richmond, CA; 1:1000).

Immunohistochemistry. Immunohistochemistry for p65-NF-κB
(Santa Cruz Biotechnology; 1:50) and ubiquitin (Berkeley Antibody Co.; 1:200) was performed on deparaffinized renal tissue, using monoclonal
primary antibodies. The secondary antibody, IgG Envision Plus (Dako),
was followed by staining with the Vector Novared substrate kit (Vec-
tor-Laboratories, Burlingame, CA), and slides were counterstained with
hematoxylin (3,4). Kidney sections (one per animal) were examined using
a computer-aided image-analysis program (MetaMetaphor, Meta
Imaging Series 4.6), and results are expressed as average percentage of
staining of total surface area, as detailed previously (3,4).

Real-Time Quantitative PCR

Total RNA was isolated from kidney using the TRIzol (Invitrogen)
method. cDNA was synthesized using Invitrogen SuperScript first-
strand synthesis kit as we have recently described (23). To investigate the
mRNA expression of prepro-ET-1, ET-A, and ET-B receptors, real-
time PCR (DNA engine OPTICON; MJ Research, Waltham, MA) was
performed using SYBR Green JumpStart TaqReadyMix kit (Sigma, St.
Louis, MO). Briefly, 12.5 µl of SYBR Green JumpStart TaqReadyMix,
0.25 µl of internal reference, 0.5 µl of primer 5’, 0.5 µl of primer 3’, 1 µl
of cDNA, and 10.25 µl of DEPC water reached 25 µl of final reaction
volume. The gene-specific sequences used were as follows: prepro-ET-1
(human) primer was upper 5’-TCCTCTGCTGTTCTCTGACT-3’ and
lower 5’-CGAAACTCCACCCCTGTGT-3’; ET-A receptor (porcine)
primer was upper 5’-CAACACATTTCTGGAAAC-3’ and lower 5’-
ATGATCCTGACGAGTGTT-3’; and ET-B receptor (porcine)
primer was upper 5’-TTTACCCCCAGTACCGTGTA-3’ and lower 5’-
CTCTGAGTGAAGGGGAA-3’. The temperature profile included
denaturation at 95°C for 3 min followed by 45 cycles of denaturation at
95°C for 40 s and 60 s at 60°C annealing and elongation with optics on
for fluorescence monitoring. The relative amount of mRNA, normal-
ized to an internal control GAPDH and relative to a calibrator (Nor-
mal), was calculated by 2−ΔΔCT(23). The sequence of GAPDH primer
is upper 5’-GGGCAAGAACCAGTGAAAGT-3’ and lower 5’-GTCCTTG-
CCGTCGAGATT-3’. Real-time quantitative PCR results were quanti-
tified and expressed as percentage change in copy numbers relative to
the normal group.

Data Analysis

Manually traced regions of interest were selected in EBCT images in
the aorta, renal cortex, and medulla, and their densities were sampled.
Cortical and medullary volumes were calculated using a point-count-
ing volume estimation program implemented with the software ANA-
LYZE (Biodynamic Imaging Resource, Mayo Clinic, Rochester, MN).
Time-density curves were generated and fitted with extended y-variate
curve-fits, and the area enclosed under each segment of the curve and
its first moment were calculated using the curve-fitting parameters (20).
These were used to calculate cortical and medullary perfusion (ml/min
per g tissue), RBF (ml/min, sum of cortical and medullary blood flows),
and single-kidney GFR (ml/min, calculated from the rate of contrast
media accumulation in the proximal tubule and multiplied by
volume), using previously validated methods (3,4,20).

Statistical Analyses

Results are mean ± SEM. Comparisons within groups were performed
using paired t test and among groups using ANOVA, with the
Bonferroni correction for multiple comparisons, followed by unpaired
t test. Statistical significance was accepted for P ≤ 0.05.
Results

Body weight and mean arterial pressure were similar among the groups. Total and LDL cholesterol were similarly elevated in both HC and HC+PSI compared with the normal diet groups. Ox-LDL was significantly elevated in HC but even higher in HC+PSI (Table 1). HC showed a significant increase in circulating levels of ET-1, which was normalized in HC+PSI (Table 1).

Renal Function

Basal RBF and cortical perfusion were similar among the groups (Table 1). GFR was decreased in N+PSI and HC+PSI compared with normal (Table 1). In response to Ach, normal and N+PSI animals significantly increased RBF, GFR (P < 0.05 versus baseline; Figure 1), and cortical perfusion (to 5.7 ± 0.5 and 4.3 ± 0.5 ml/min per g, respectively; P < 0.05 versus baseline). RBF, GFR, and cortical perfusion responses were blunted in HC. In contrast, these responses all were normalized in HC+PSI (P < 0.05 versus baseline; Figure 1).

Renal Protein and mRNA Expression

Total renal protein ubiquitination as assessed was similar in normal and N+PSI, decreased in HC, but significantly increased in HC+PSI (Figure 2), suggesting attenuated protein degradation. However, the decreased expression of IκB that accompanied the increased nuclear and cytoplasmic (mainly tubular) expression of p65–NF-κB in HC was normalized in HC+PSI kidneys, suggesting a decrease in proinflammatory activity in these animals (Table 1, Figure 3). The NAD(P)H-oxidase p47phox subunit was unchanged, whereas p67phox activity in these animals (Table 1, Figure 3). The NAD(P)H-oxidase p47phox subunit was unchanged, whereas p67phox showed a tendency to increase in HC compared with normal (P = 0.07). However, the intrarenal expression of both subunits was elevated in N+PSI and even higher in HC+PSI compared with the other groups, suggesting increased potential for generation of superoxide (Table 1, Figure 4, a and b). Nevertheless, eNOS expression, which was slightly attenuated in HC animals, was significantly augmented in N+PSI and even further in HC+PSI, suggesting a potential increase in NO generation (Table 1, Figure 4c). Furthermore, the blunted activity and expression of SOD observed in HC were markedly improved in HC+PSI (albeit less than in N+PSI), suggesting an increase in superoxide scavenging as well (Table 1, Figure 4d). In addition, renal mRNA expression of prepro-ET-1 and the ET-A receptor in HC+PSI pigs. Blunted RBF and GFR responses in HC were normalized in HC+PSI. *P < 0.05 versus baseline.

![Figure 1. Changes (ml/min) in renal blood flow (RBF) and GFR in response to acetylcholine (Ach) in normal, hypercholesterolemic (HC), normal + proteosome inhibitor (N+PSI), and HC+PSI pigs. Blunted RBF and GFR responses in HC were normalized in HC+PSI. *P < 0.05 versus baseline.](image)

Table 1. Systemic and single-kidney characteristics (mean ± SEM), in normal, HC, and PSI-treated animals (N+PSI and HC+PSI)*

<table>
<thead>
<tr>
<th></th>
<th>Normal (n = 7)</th>
<th>HC (n = 7)</th>
<th>N + PSI (n = 7)</th>
<th>HC + PSI (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>114.1 ± 3.1</td>
<td>113 ± 2.8</td>
<td>116.2 ± 2.8</td>
<td>115.5 ± 6.7</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>1.8 ± 0.2</td>
<td>9.8 ± 1.1b</td>
<td>1.7 ± 0.2</td>
<td>9.5 ± 1.0b</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>0.8 ± 0.1</td>
<td>7.4 ± 0.9b</td>
<td>0.7 ± 0.1</td>
<td>7.1 ± 0.7b</td>
</tr>
<tr>
<td>Oxidized LDL (U/ml)</td>
<td>10.5 ± 0.8</td>
<td>18.2 ± 2.2b</td>
<td>11.1 ± 0.8</td>
<td>27.9 ± 5.3b,c,d</td>
</tr>
<tr>
<td>Renal blood flow (ml/min)</td>
<td>586.3 ± 44.5</td>
<td>587.4 ± 52.6</td>
<td>497.1 ± 21.9</td>
<td>474.8 ± 59.4</td>
</tr>
<tr>
<td>Cortical perfusion (ml/min per cm³)</td>
<td>3.9 ± 0.3</td>
<td>3.9 ± 0.4</td>
<td>3.1 ± 0.6</td>
<td>3.4 ± 0.3</td>
</tr>
<tr>
<td>Glomerular filtration rate (ml/min)</td>
<td>77.4 ± 4.7</td>
<td>69.8 ± 4.9</td>
<td>56.6 ± 6.2b†</td>
<td>42.4 ± 7.6b,c,e</td>
</tr>
<tr>
<td>Endothelin-1 (pg/ml)</td>
<td>5.3 ± 0.4</td>
<td>7.3 ± 0.6b</td>
<td>6.7 ± 1.3</td>
<td>5.9 ± 0.3e</td>
</tr>
<tr>
<td>Plasma SOD activity (U/ml)</td>
<td>3.5 ± 0.3</td>
<td>2.5 ± 0.3b</td>
<td>6.6 ± 0.5b†</td>
<td>5.1 ± 0.1b,c,d</td>
</tr>
</tbody>
</table>

aN, normal; HC, hypercholesterolemic; PSI, proteosome inhibitor; SOD, superoxide dismutase.
bP < 0.05 versus normal; cP < 0.05 versus HC; dP < 0.05 versus N+PSI.
Discussion

Our study demonstrates that chronic inhibition of the UPS improves renal vascular and glomerular endothelial function in HC, possibly by improving NO bioavailability and modulating systemic and renal ET, and may decrease intrarenal inflammation through inhibition of NF-κB. These findings may suggest the UPS and NF-κB pathways as potential therapeutic targets in HC and atherosclerosis, although the decrease in basal GFR and increased potential for reactive oxygen species generation needs further studies.

The UPS is a tightly regulated and specific multienzymatic complex of utmost significance for cellular function and is considered the major pathway of intracellular protein degradation (9,24). Inhibition of this system has been shown to be beneficial in disease states such as multiple myeloma, lymphoma, solid malignant tumors, HIV, and cerebral and myocardial reperfusion injury (17,25,26), leading to the design of clinical trials using PSI (27,28). However, the impact of PSI on the kidneys is virtually unknown.

We showed previously in both human (29) and animal (15,16,30) studies that activation of the UPS may potentially play a deleterious role in different stages of atherosclerosis. Early atherosclerosis and HC are systemic inflammatory processes, which are considered cardiovascular and renal risk factors (2,12) and show functional alterations of the endothelium that predate the development of more advanced lesions. Indeed, we showed previously that diet-induced HC (a surrogate of early atherosclerosis) led to renal functional and structural impairment, likely through activation of redox-sensitive mechanisms (3,4,19) that also involved the activation of the UPS (15,16). Furthermore, we also showed that the UPS is functionally active in early coronary atherogenesis (30). However, whether the UPS played any role in renal compromise in the early stage of atherosclerosis remained to be explored.

This study shows that the blunted RBF and perfusion responses to the endothelium-dependent vasodilator Ach in HC (3,4) were normalized during chronic inhibition of the UPS, indicating improved renal endothelial function. A recent study showed that UPS inhibition in endothelial cells led to transcriptional upregulation of eNOS (31), a key regulator in endothelium-dependent vasodilation that is constitutively expressed in the normal kidney. Our study demonstrates that chronic upregulation of eNOS protein can also be observed in vivo in kidneys of PSI-treated animals, reflecting a potential increase in

Figure 2. Representative renal expression (cortex and medulla) of ubiquitin in normal, HC, N+PSI, and HC+PSI pigs. Top, immunohistochemistry (red). Middle, representative immunoblots. Bottom, quantification of Western blotting. Inhibition of the proteasome led to accumulation of ubiquitin (8.5 kD) and multiple bands of polyubiquitinated proteins in HC+PSI kidneys. *P < 0.05 versus normal; †P < 0.05 versus HC; #P < 0.05 versus N+PSI. Magnification, ×20 in top.
NO bioavailability. In addition, infusion of Ach may raise intracellular calcium levels and its binding to calmodulin, which is also a UPS-regulated protein (32). The calcium-calmodulin binds eNOS and can increase eNOS-generated NO (33,34), which thereby also may have improved vasodilator responses in the HC/L1101 PSI kidney. Furthermore, we showed previously that HC increases coronary and systemic levels of ET-1 in both humans and pigs (35–37). We also showed that chronic blockade of the ET-A receptor improves renal endothelial function and decreases inflammation and fibrosis in the HC kidney (4). This study extends our previous observations and shows that diet-induced HC also leads to upregulation of renal prepro-ET-1 and its ET-A receptor, which mediates ET-induced vasoconstriction and inflammation. Notably, chronic PSI intervention in HC resulted in a diminished circulating ET and renal mRNA levels of both prepro-ET-1 and the ET-A receptor. Our results are underscored by previous reports that PSI decrease ET-1 expression in vascular endothelial cells (38) and in ischemic acute renal failure (39,40). Furthermore, our study extends those observations by showing for the first time that PSI can decrease ET-A receptor mRNA expression as well. These effect may have facilitated the response to Ach by decreasing the vascular tone and, consequently, correcting the endothelial dysfunction induced by this HC regimen.

Our study also suggests that the use of PSI in HC and other forms of renal disease should be investigated carefully. Indeed, we observed that basal GFR was decreased in N+PSI and HC+PSI, raising some concerns about potential nephrotoxic effect of PSI, which may potentially limit their clinical applications in HC and/or renal disease. However, the mechanisms for these effects in this model can be speculated. PSI-treated animals showed increased expression of the NAD(P)H-oxidase enzyme, possibly mediated by modulation of Rac-1, a pivotal component in NAD(P)H oxidase activation (41). That in turn suggests increased potential for generation of the vasoconstrictor superoxide anion, which has a distinct constrictor effect on the afferent arteriole (22,42,43) and may account for the lower GFR observed in PSI-treated groups (22). Moreover, potential effects of PSI on the efferent arteriole and on the glomerular ultrafiltration coefficient and/or interactions with the renin-angiotensin system (44) (which is locally activated in HC) cannot be excluded either. Nevertheless, PSI can stabilize the superoxide scavenger SOD (45), and the augmented SOD activity and expression observed in N+PSI and HC+PSI animals might

Figure 3. Representative renal expression (cortex and medulla) of p65 NF-κB and IκB in normal, HC, N+PSI, and HC+PSI pigs. Top, immunoreactivity of NF-κB (more evident in cellular nuclei, arrow). Bottom, Western blotting. HC led to a significant increase in NF-κB immunoreactivity accompanied by diminished IκB expression, suggesting elevated proinflammatory activity. This was normalized in HC+PSI kidneys. *P < 0.05 versus normal; †P < 0.05 versus HC. Magnification, ×40 in top.
have partly counterbalanced superoxide abundance. This augmented scavenging in parallel with the increased NO bioavailability might have contributed to the improvement in GFR response to Ach in these groups.

We showed previously that the increased intrarenal inflammation and fibrosis in diet-induced HC were accompanied by enhanced activation of NF-κB (3,4), which has an important role in the atherogenic process by modulating several factors that are involved in inflammation and cell proliferation (6). Exposure of cells to a variety of extracellular stimuli leads to rapid phosphorylation, ubiquitination, and ultimately proteolytic degradation of IκB. This ubiquitination-dependent proteolysis frees NF-κB to translocate to the nucleus, where it regulates gene transcription, as an integral part of a phosphorylation-based signaling cascade (46). Our study provides support to the notion that HC facilitates this cascade in the kidney by showing decreased expression of IκB and p65–NF-κB. This might have been related to increased intrarenal proteasome activity, which is suggested by the decreased accumulation of ubiquitinated proteins in the HC kidney. This study further shows that PSI supplementation in HC normalized the immunoreactivity of IκB and p65–NF-κB, suggesting attenuated renal proinflammatory activity (47). However, the UPS has a wide variety of activities, and the nonspecific PSI (7) likely result in modulation of several pathways involved in cellular function and tissue remodeling in the atherosclerotic kidney, for example, via increased superoxide generation. In addition, PSI may
potentiate Ox-LDL toxicity (48) and prolong the half-life of its receptor (49). Moreover, our study shows that PSI elevate circulating levels of Ox-LDL, possibly consequent to the concurrent increase in LDL abundance and increased superoxide generation in HC+PSI. Ox-LDL is a vasoconstrictor and cytotoxic agent to renal cells and may promote renal fibrosis (50). Therefore, further studies are needed to characterize fully the effects of these agents in the kidney at different stages of HC and atherosclerosis.

In summary, this study shows, for the first time, the effects of chronic UPS inhibition in the kidney in an animal model of early atherosclerosis. The potential increase in oxidative stress and the decrease in basal GFR by PSI warrant investigation and cautious use. However, PSI in HC improved renal endothelial functional responses to Ach, likely by improving NO bioavailability and modulating both systemic and renal ET. In addition, PSI may decrease intrarenal inflammation in HC through downregulation of the NF-κB pathway, underscoring a deleterious involvement of the UPS in the kidney in HC and early atherosclerosis.

Acknowledgments

This study was supported by grants HL-63282, HL77131, HL69840, and HL63911 from the National Institutes of Health and by the American Heart Association. The authors are grateful to Millenium Pharmaceuticals for generously providing MLN-273.

References


42. Ren Y, Carretero OA, Garvin JL: Mechanism by which superoxide potentiates tubuloglomerular feedback. *Hypertension* 39: 624–628, 2002


