Targeting Proteasome Worsens Atherosclerosis

Tohru Fukai

The ubiquitin–proteasome system is the major pathway (up to 80% to 90%) of nonlysosomal degradation of intracellular and oxidized proteins1–3 (Figure). For a protein to be recognized by the proteasome, a small peptide (ubiquitin) must first be attached to the target protein. The process of ubiquitination transfers polyubiquitin chains to target proteins requires various enzymes such as ubiquitin-activating enzyme (E1), ubiquitin carrier protein (E2), and ubiquitin-protein lipase (E3). The ubiquitinated substrate is rapidly hydrolyzed by the 26S proteasome, an ATP-dependent multiprotein complex containing the proteolytically active 20S proteasome that is capped by 1 or 2 19S regulatory complexes.4 The 20S proteasome has 3 distinct proteolytic activities harbored by β subunits which consist of caspase-like (β1 subunit), trypsin-like (β2 subunit), and chymotrypsin-like (β5 subunit) activities.5 The primary inhibitory effect of overall proteasome proteolytic function is mediated through chymotrypsin-like function of the 20S proteasome.5 Indeed, most of synthetic and natural inhibitors of the proteasome such as MLN-273 and PS-341 act predominantly on the chymotrypsin-like activity.1 In particular, PS-341 (bortezomib [Velcade]) was approved for the treatment of therapy-refractory multiple myeloma in 2003.6

Ubiquitin-proteasome system is responsible not only for maintaining cell quality control by removing damaged and oxidized proteins, but also for regulating cellular mediators involved in atherosclerosis.4 Many of the manifestations of atherosclerosis are dependent on oxidative stress which mediates modification of lipids, induction of proinflammatory genes, and endothelial dysfunction.7 In human atherosclerotic plaques, accumulation of ubiquitinated and oxidized proteins as well as impaired proteasome activity are observed, which is associated with increased oxidative stress.8 Of note, in vitro study shows that proteasome inhibition increases oxidative stress,9 while high levels of oxidative stress can block proteasome function with cytotoxic consequences.10 These findings suggest that impaired proteasome function by oxidative stress may contribute to atherosclerosis. However, little is known about a role of ubiquitin–proteasome system in atherosclerosis in vivo.

In this issue of Circulation Research, Herrmann et al provide direct evidence that ubiquitin-proteasome system prevents atherosclerotic lesion formation in coronary arteries from pigs with a normal and high cholesterol diet.11 These authors have previously reported that ubiquitin-protein conjugates is increased in the presence of unimpaired proteasome proteolytic activity in the coronary artery of experimental hypercholesterolemia.12 In the current study, Herrmann et al obtained several important findings using proteasome inhibitor MLN-273. First, chronic proteasome inhibition by subcutaneous injection of MLN-273 promoted development of early atherosclerotic lesions in coronary arteries from hypercholesterolemic pigs, as defined by intimal thickening, lipid deposition, and macrophage accumulation. Specificity of MLN-273 was shown by selective inhibition of chymotrypsin-like proteasome activity and increased accumulation of ubiquitinated proteins. Consistent with this, Stone et al demonstrated downregulation of genes encoding for components of the ubiquitin-proteasome system in areas of intimal hyperplasia at the anastomosis sites of polytetrafluoroethylene grafts after their implantation into canine carotid arteries.13 Second, MLN-273 increased superoxide production, expression of the NADPH oxidase subunit p47phox, and nitrotyrosine staining in the intimal lesion of atherosclerosis, which was associated with increase in oxidatively-modified proteins by 4-hydroxy-2-nonenal and serum levels of oxidized LDL. Third, MLN-273 impaired endothelial dependent relaxation in coronary arteries from pigs in a normal and high-cholesterol diet, which was associated with increased eNOS protein levels. Although underlying mechanisms have not been assessed, it is possible that MLN-273 treatment induced eNOS uncoupling derived from oxidative degradation of tetrabiopterin (BH4) in pig arteries, thereby inducing impairment of endothelial dependent relaxation. To prove this, additional experiments are required to examine whether BH4 could rescue impairment of endothelial dependent relaxation and eNOS activity. It is unlikely that impairment of endothelial dependent relaxation is attributable to the toxic level of proteasome inhibition, because MLN-273 did not induce either enhanced accumulation of ubiquitinated proteins with normocholesterolemia or an increase in TUNEL-positive (apoptotic) cells in the coronary artery wall including vascular smooth muscle cells (VSMCs). It should be noted that proteasome inhibitor markedly decreased α-actin-positive VSMCs in the intima.

Although the current study supports a detrimental effect of proteasome inhibition, other reports suggest that proteasome inhibition may be beneficial for atherosclerotic plaque progression and complication.14 Stangl et al showed that the proteasome inhibitor MG-132 enhanced endothelial dependent relaxation, which was associated with increased eNOS protein and activity.15 Meiners et al demonstrated that MG-
132 markedly reduced neointima formation in balloon-injured carotid arteries with decreased cell proliferation and suppression of NF kappa B activation and increased apoptosis. In the current study, Herrmann et al showed that chronic proteasome inhibition did not induce apoptosis in VSMCs but only limited with endothelial cell layer, suggesting that results of discrepancy may be attributable to the dose of proteasome inhibitor. This notion is supported by the finding that treatment of proteasome inhibitor at low dose in cultured endothelial cells decreased levels of reactive oxygen species, which was associated with an increase in the expression of antioxidant enzymes and a decrease in the NADPH oxidase family member NOX4. Moreover, the beneficial effect of proteasome inhibitors has been shown in hypertensive rats and in animal models of ischemia-reperfusion injuries through suppressed activation of NF kappa B. Thus, it is tempting to speculate that the ultimate vascular response to proteasome inhibition appears to depend on multiple factors, such as the stage of atherosclerotic lesion, dose, cell type, and the way to administration of proteasome inhibitors. Indeed, one identical dose of proteasome inhibitor has divergent effects on different cell types; proteasome inhibitors induce apoptosis in rapidly proliferating tumor cells, whereas their protective effect from apoptosis has been observed in differentiated and quiescent cells.

The information presented by Herrmann et al strongly suggests that ubiquitin–proteasome system plays an important role in atherosclerosis not only by elimination of intracellular and oxidized proteins, but also by reducing oxidative stress, inflammation, and endothelial function (Figure). Detrimental effect of proteasome inhibitors on atherosclerosis could be attributable to impairment of cell quality control (ie, impairment of removing damaged and oxidized proteins). Indeed, the accumulation of damaged proteins has been causally related to cytotoxicity and to pathologies such as cancers and neurodegenerative diseases. There are many unanswered questions. What are the molecular mechanisms for the increase in endogenous oxidative stress by proteasome inhibitor? Does ubiquitin-proteasome system play a role in regulating BH4 bioavailability, thereby controlling eNOS activity and endothelial function? Is effect of proteasome inhibitor on atherosclerotic lesion dose-dependent (ie, reduce or increase neointimal formation)? Is enhancement of proteasome activity beneficial for reducing atherosclerosis? Addressing these questions will be essential to our understanding the mechanism of atherosclerosis in which ubiquitin–proteasome system plays an essential role.

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References

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