Oxidized low-density lipoprotein (oxLDL) is considered to be the strongest proatherogenic lipoprotein. OxLDL is rapidly taken up by endothelial cells and macrophages and via the steps of foam cell formation and cell death, oxLDL eventually accumulates in the lipid core of atherosclerotic plaques.1

The interaction of oxLDL with cells is mediated by several receptors.2 Members of the scavenger receptor family predominate in macrophages,3 whereas endothelial cells mainly take up oxLDL by the lectin-like ox-LDL receptor-1 (LOX-1).4 On binding to LOX-1, oxLDL activates a multitude of signaling cascades involving MAP kinases,5,6 protein kinase C (PKC),7,8 and protein kinase B9 in endothelial cells. The consequence of this cellular activation, is apoptosis and superoxide anion production, the latter originating from the NADPH oxidase and uncoupling of the endothelial NO synthase (eNOS). OxLDL therefore is a strong inducer of endothelial dysfunction, a state in which the endothelium, instead of generating NO, produces superoxide anions. Ox-LDL also modulates the specific activity of NO. It increases caveolin I expression and the interaction of NOS with this protein impairs NO production.10 Furthermore, ox-LDL modulates PKC activity and expression and this family of kinases has been shown to acutely attenuate eNOS-dependent NO production and to promote eNOS uncoupling during prolonged exposure times.11

Several observations suggest that the supply of eNOS with the substrate L-arginine is impaired in the presence of oxLDL: In hypercholesterolemia, supplementation of L-arginine improves endothelium-dependent relaxation and NO production;12,13 L-arginine also attenuates the development of atherosclerosis,14 and normalizes leukocyte adhesion in hypercholesterolemia.15 Indeed, hypercholesterolemia favors the accumulation of asymmetrical dimethyl-L-arginine (ADMA), a competitive inhibitor to L-arginine of eNOS. ADMA levels are increased in hypercholesterolemic monkeys16 and oxLDL increases the activity of the S-adenosylmethionine-dependent protein arginine methyltransferases,16 which generate ADMA from L-arginine. Moreover, oxLDL decreases the activity of the ADMA-degrading dimethylarginine dimethylaminohydrolase.17

In this issue of Circulation Research, Ryoo and colleagues identified a new mechanism for oxLDL-induced endothelial dysfunction.18 They demonstrate that oxLDL acutely increases arginase II activity as well as induces arginase II protein expression in human aortic endothelial cells (Figure 1). Arginase catalyzes the hydrolysis of L-arginine to L-ornithin and iso-urea. As iso-urea spontaneously isomerases to urea, this reaction is practically irreversible. Arginine catabolism by arginase is nearly 200-fold greater than that of NOS and thus arginase limits the supply of substrate to NOS. Through this competition for arginine, arginase attenuates NOS-dependent NO production.19 Two arginase isoforms have been identified and differential expression has been noted between cells types and species. In human aortic endothelial cells, arginase II is the predominant isoforms and consequently, the study from Ryoo et al focused on this enzyme.18,19

The authors observed that following stimulation with oxLDL arginase II activity is increased in human aortic endothelial cells and this effect was accompanied by an attenuation of NO production, as determined by nitrite measurements. Small interfering RNA directed against arginase II prevented the oxLDL-induced increase in activity and maintained NO production even in the presence of oxLDL. Moreover, in intact rat aortic segments oxLDL attenuated endothelial NO production and this effect was sensitive to the arginase inhibitor (s)-(2-boronoethyl)-L-cysteine. A further analysis revealed that oxLDL increased cellular arginase II activity via 2 different mechanisms: An acute stimulation of the specific activity of the enzyme was followed by a robust induction of the messenger RNA (mRNA) expression of the enzyme. The mechanism of arginase II activation involved the dissociation of the enzyme from the microtubule cytoskeleton. Accordingly, microtubule disruption by nocodazole increased arginase II activity under basal conditions, whereas stabilization of the microtubule with epothilone B prevented the oxLDL-induced activation of arginase. With these observations, the authors not only discovered a new road to endothelial dysfunction induced by oxLDL, they also identified a new mechanism of arginase activation.

The exact mechanism of interaction of arginase with the microtubule however requires further research. From the study of Ryoo et al it appears that primarily the degree of tubulin polymerization determines the interaction of arginase with the microtubule network. Nevertheless, the interacting domains have not yet been identified and it cannot be
excluded that adapter proteins, which might also be involved in signal transduction are required in the process.

It is currently unknown how oxLDL destabilizes the microtubule and thus facilitates the release of arginase from these filaments. Small GTPases of the Rho family positively as well as negatively affect microtubule stability. It has recently been noted that also thrombin increases the arginase II activity in human umbilical vein endothelial cells via a nontranscriptional mechanism. Intriguingly, in that study it was observed that arginase II activation could be prevented by the inhibition of RhoA and RhoA-dependent kinase (ROCK). Whether thrombin-induced activation of arginase II involved dissociation from microtubule, however, was not studied.

It is remarkable that once again Rho family proteins appear to be the “bad guy” for endothelial function. Indeed, RhoA is activated by oxLDL. It has previously been reported that RhoA is involved in destabilizing eNOS mRNA and another member of the Rho family, Rac, is required for NADPH oxidase activation and thus initiation of oxidative stress. The signaling of Rho GTPases requires the interaction of the proteins with the cell membrane via a geranyl-geranyl anchor. One of the pleiotropic effects of the lipid-lowering HMG-CoA reductase inhibitors (statins) is the prevention of this lipid anchoring of Rho GTPases. It might be speculated that inhibition of arginase II is another pleiotropic effect of statins, but this hypothesis needs to be tested.

In the present study Ryoo et al used oxLDL which was generated by copper oxidation. Although this is certainly the standard procedure to obtain oxLDL, the method has been criticized as trace amounts of copper may have serious effects on cells and as the degree of oxidation often exceeds that observed in vivo. Moreover, oxidative modification of LDL is only one way to render it proatherogenic. Consequently, the data obtained by Ryoo et al require confirmation to some extent. It would be helpful to demonstrate that the effects do not occur with LDL and that the signaling involves LOX-1. Moreover, it would be important to study arginase II activity in hyperlipidemic animals. Ryoo et al already made a first step in this direction by using ex vivo incubation of aortic rings with oxLDL. Moreover, it has been observed previously that arginase activity is increased in aortas of ApoE mice. The hyperlipidemia in this transgenic strain, however, is because of the accumulation of very low density lipoprotein (VLDL) and not LDL. Atherosclerotic tissue also comprises several different cell types with arginase activity such as macrophages and smooth muscle cells and thus it will be difficult to identify endothelium-selective effects in animal models. Further work will be needed to demonstrate that the mechanism proposed by Ryoo et al contributes to LDL-induced endothelial dysfunction in vivo.

Disclosures

None.

References


**Key Words:** statins | arginase | hyperlipidemia | endothelial dysfunction | atherosclerosis | GTPases
Roads to Dysfunction: Arginase II Contributes to Oxidized Low-Density Lipoprotein-Induced Attenuation of Endothelial NO Production

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