Atorvastatin Reduces Plaque Vulnerability in an Atherosclerotic Rabbit Model by Altering the 5-Lipoxygenase Pathway

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Key Words
Atorvastatin • Atherosclerosis • 5-Lipoxygenase-activating protein

Abstract
Objective: The 5-lipoxygenase catalyzed formation of leukotriene lipid mediators is a mediator for inflammatory response in arteries. The present study investigated the relationship between atorvastatin and the 5-lipoxygenase pathway in an atherosclerotic rabbit model. Methods: Thirty male New Zealand White Rabbits were randomized into negative control, positive control and atorvastatin groups. At week 4, the rabbits were subjected to carotid balloon-dilation injury or carotid balloon-dilation injury, followed by treatment with atorvastatin. At week 12, all the animals were sacrificed. Plasma lipids, LTD₄, and 15-epi-lipoxin A₄ were measured using the enzymatic endpoint method and ELISA, respectively. RT-PCR was performed to detect the gene expression of 5-lipoxygenase-activating protein and cysLT₁R in rabbit carotid arteries. Finally, histological analysis was used to evaluate the pathophysiological changes of rabbit carotid arteries. Results: The results showed atorvastatin markedly lowered serum lipids and LTD₄ levels compared with the control group. Similarly, mRNA expression of 5-lipoxygenase-activating protein and cysLT₁R was significantly inhibited by atorvastatin. Decreased carotid plaque instability was evident in atorvastatin-treated animals, as demonstrated by a thickened elastic layer, less neointima hyperplasia and macrophage proliferation. Conclusions: Atorvastatin may stabilize carotid plaque by regulating the 5-lipoxygenase pathway in atherosclerotic rabbits and delay the progression of atherosclerosis by exerting anti-inflammatory effects.

Introduction
Stroke is the third leading cause of morbidity and long-term disability worldwide. Plaque vulnerability, rather than arterial stenosis, plays a crucial role in the pathophysiology of recurrent atherothrombotic stroke [1]. The characteristics of vulnerable plaque include a large lipid core, thin fibrous cap, inflammatory cell aggregation and secretion of metalloproteinases and cytokines. Atorvastatin, a hydroxymethylglutaryl-CoA reductase inhibitor, can reduce plaque destabilization by downregulating matrix metalloproteinase and proinflammatory cytokine levels (e.g. growth-related oncogene, CX3CL1, FasL, etc.) [2].
stimulates activation and chemotaxis of human monocytes and monocyte-derived immature dendritic cells by mediating innate immune reactions [26]. cysLTs have a potent influence on vasopermeability, which may facilitate inflammatory cell or cytokine recruitment into arterial lesions [27]. A recent clinical study suggested that atorvastatin (80 mg/day) caused a nonsignificant decrease in serum LTB₄ levels [28]. It has been reported that fluvalastatin blocked the production of LTC₄ [29]. In accordance with the previous results, our data showed atorvastatin significantly downregulated the level of serum LTC₄. Inhibition of the LTD₄ receptor (cysLT1R) by montelukast abrogated vascular reactive oxygen species production and improves endothelial function and plaque stability [30]. Recently, 15-epi-lipoxin A₄ was proven to be an anti-inflammatory cytokine in the progression of atherosclerosis [31]. Interestingly, atorvastatin had no effect on serum 15-epi-lipoxin A₄ concentration in the study, which probably attributed to the relatively low dose of atorvastatin [32]. Platelet-activating factor lipids derived from oxidized LDL stimulate 5-LO expression in leukocytes, and is accompanied by biosynthesis of MCP-1 and LTB₄. Inhibition of MCP-1 results in a decline of LTB₄ production [33]. The metabolic products derived from the 5-LO pathway aggravates atherosclerosis. Taken together, all these factors contribute to plaque destabilization. Blockade of the 5-LO pathway attributed to the reduction in the number of macrophages in arterial lesions [18, 23]. A recent study by Ye et al. [31] has suggested that atorvastatin increases the phosphorylation of 5-lipoxygenase at Ser-523 by PKA. This phosphorylation prevents the translocation of 5-LO to the membranous fraction, thus inhibiting the interaction between 5-LO and cPLA2.

Instead, P-5-lipoxygenase interacts with COX2 to produce 15-epi-lipoxin A₄, a potent anti-inflammatory mediator. In this study, we found that atorvastatin treatment decreases LTD₄, FLAP and cysLT1R expression in atherosclerotic lesions, as well as attenuates carotid plaque vulnerability. Interestingly, a recent study showed that high dose simvastatin treatment induced over-expression of FLAP in patients’ muscle [34]. There are two probable explanations for the conflicting results: they could be attributable to the dosage forms of statins or species differences.

In conclusion, atorvastatin may attenuate the progression of atherosclerotic lesions by regulating the 5-LO pathway, which may provide a new interpretation of its pleiotropic effects. However, the exact mechanisms of these beneficial effects are unclear, thus warranting further studies.

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**References**


