The putative role of leukotrienes in experimental atherogenesis

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KEY WORDS

atherosclerosis, leukotriene, 5-lipoxygenase activating protein (FLAP)

ABSTRACT

Since inflammation plays an important role in atherogenesis, during recent years it has become apparent that the 5-lipoxygenase (5-LO) pathway may take a significant part in the pathogenesis of atherosclerosis. These data raised the possibility that antileukotriene drugs may be an effective treatment regimen in atherosclerosis. This review describes the research performed on the apolipoprotein E/low-density lipoprotein receptor-double knockout mice as a model of atherosclerosis. The study has shown that 5-LO activating protein inhibitors and leukotriene receptor blockers decrease atherosclerosis in atherosclerotic mice. The article also discusses the importance of these findings for the future use in the clinic.

INTRODUCTION Atherosclerosis is an inflammatory vascular disease[1] of increasing incidence in the developed countries. Mouse has become an excellent experimental model of atherosclerosis[2] since 1992, when apolipoprotein E (apoE)-knockout mice were developed.[3-5] More recently, apoE and low-density lipoprotein receptor-double knockout (apoE/LDLR-DKO) mice have provided numerous possibilities for both studying pathogenesis and testing the effectiveness of treatment in a model of atherosclerosis and hyperlipidemia.[2-4,6] In apoE/LDLR-DKO mice fed with atherogenic Western diet lesion formation is greatly accelerated.[7]

Since inflammation plays an important role in atherogenesis, during recent years it has become apparent that the 5-lipoxygenase (5-LO) pathway may take significant part in modifying the pathogenesis of atherosclerosis. Enzymes associated with the 5-LO pathway are abundantly expressed in arterial walls of patients afflicted with various lesion stages of atherosclerosis of the aorta and of coronary arteries. These data raised the possibility that antileukotriene drugs may be an effective treatment regimen in atherosclerosis.[8]

Lipoxygenases are enzymes that catalyze the stereospecific dehydrogenation and subsequent dioxygenation of polyunsaturated fatty acids with the 1.4-cis-pentadiene structure.[9] Of special interest for atherosclerosis is the arachidonate 5-LO, which was originally identified in polymorphonuclear leukocytes,[10] but which over-expression was recently demonstrated in macrophages, dendritic cells, foam cells, mast cells and neutrophils within atherosclerotic vessels. This enzyme generates an unstable epoxide intermediate compound leukotriene A_4 (LTA_4), which is an important precursor of LTB_4, LTC_4 and other cysteinyl leukotrienes. Initial observations and the use of drugs affecting the 5-LO metabolism were mainly connected with asthma and other inflammatory diseases.[11] However, a growing understanding of the role of inflammation in atherosclerosis has brought attention to the potential role of leukotrienes and their metabolism.

First results of the research on the role of lipoxygenases in atherogenesis were published in 1999, when Cyrus et al. observed that absence of 12/15-lipoxygenase (12/15-LO) decreased atherogenesis in apoE-deficient mice.[12] The confirmed later[13-20] role of this enzyme in the formation of atherosclerotic plaque was explained by oxidation of LDL particles in subendothelial space.

In 2002 Mehrabian et al. identified the 5-LO as a crucial enzyme contributing to atherosclerosis susceptibility in mice.[8,21] This observation, after a long pause,[22] has again focused the attention of researchers on the role of leukotrienes in the pathogenesis of atherosclerotic plaque.[23-33] Therefore, the speculations have been risen that anti-

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asthmatic drugs could have beneficial effects on atherogenesis.[11, 34-37]

Indeed, it has been recently demonstrated that the 5-LO substantially contribute to atherosclerosis in both mouse models and humans.[38,39] Later Aiello et al. showed that LTB_4 receptor antagonism reduced monocytic foam cells in mice.[40] Lotzer et al. pointed that macrophage-derived LTs differentially activate cysLT_2-Rs *via* paracrine stimulation and cysLT_1-Rs *via* autocrine and paracrine stimulation, during inflammation and atherogenesis.[41]

Working hypothesis Therefore, a hypothesis has been formulated that leukotriene-inhibiting drugs developed to treat asthma might protect the heart.[11] There are numerous potential targets that could be useful in the intervention in leukotriene metabolism in atherosclerosis. Interestingly, the 18 kDa microsomal protein termed five lipoxygenase activating protein (FLAP) was found to be critical for the regulation of 5-LO activity and biosynthesis of leukotrienes within certain compartments of plasma membrane. The role of FLAP in atherosclerosis was additionally confirmed in humans by Helgadottir et al.[42] who showed that genetic polymorphisms of FLAP are associated with myocardial infarction and stroke by increasing leukotriene production and inflammation in the arterial wall.

The 5-LO is abundantly expressed in atherosclerotic lesions of apoE and LDLR deficient mice, appearing to co-localize with a subset of macrophages but not with all macrophage-staining regions.[8] Indeed, the results of the current study showed that the inhibition of FLAP by MK-886 or BAYx1005 can significantly prevent the development of atherosclerosis in gene-targeted apoE/LDLR-DKO mice. Moreover, this study showed that cysteinyl leukotriene receptor blocker montelukast decreases atherosclerosis in apoE/LDLR-double knockout mice.[43-45] The findings of the present study concerning MK-886 were soon confirmed by Back et al. on their model of transgenic $apoE^{-/-}$ mice with the dominant-negative transforming growth factor β type II receptor, which displays aggravated atherosclerosis.[46]

MATERIAL AND METHODS The present study used female apoE/LDLR-DKO mice on the mixed C57BL/6J × 129/SvJ background. At the age of 8 weeks mice were put on Western diet (consisting of 21% fat by weight, 0.15% cholesterol by weight) for 4 months. Experimental groups (in each group n = 10) received the same diet, mixed with MK-886 at a dose of 30 mg per kg of body weight daily, BAYx1005 at a dose of 18.75 mg per kg of body weight daily, and montelukast at a dose of 1.25 mg per kg of body weight daily.

At the age of 24 weeks the mice were put down and plasma, hearts and aortas were collected. Total cholesterol and triglyceride levels and cholesterol lipoprotein profile were determined using the fast protein liquid chromatography, and "en face" and "cross-section" methods were used to measure atherosclerotic lesions in the whole aorta and in the root of aorta. Moreover, the compositions of plaques were estimated by measuring the numbers of macrophages and T lymphocytes, smooth muscle cells and collagen contents.

Ten μm-thick cryosections were cut from the aortic root using a standardized protocol.[47] Eight adjacent sections were collected at 100-μm intervals starting at a 100-μm distance from the appearance of aortic valves. After fixation in a 4% paraformaldehyde, sections were stained with hematoxylin and oil red O. Images of the aorta were recorded using the digital camera and stored as TIFF files. The total area of the lesion was measured semi-automatically in each slide using the AnalySIS FIVE software. For each animal the mean lesion area was calculated from eight sections, reflecting the cross-section area covered by atherosclerosis.

The aorta from the arch to the bifurcation was fixed in a 4% formaldehyde, opened longitudinally, pinned onto black wax plates and stained with Sudan IV. The aortic lesion area and total aortic area were calculated using the LSM Image Browser software.

Results were expressed as mean ±SEM. The nonparametric Mann-Whitney U test was used for analysis of the data. $P<0.05$ was considered as statistically significant.

RESULTS Aortas differed in the degree of atherosclerosis between the control group and experimental groups. Measured by the "en face" method, the percentage of occupied by Sudan IV – stained surfaces were 25.5 ±2.% in the control group, whereas in the MK-886 – treated group 11.16 ±0.7%, in the BAYx1005 group 15.16 ±1.4%, and in the montelukast group 17.23 ±1.8%.

The „cross-section" of aortic roots revealed the difference in atherosclerotic lesion areas. Measured in 8 consecutive sections mean surfaces ± SEM occupied by oil red O stained changes were 455,494 ±26,477 μm^2 in the control group vs. 263,042 ±20,736 μm^2 in the MK-886 – treated group, 278,107 ±21,824 μm^2 in the BAYx1005 group, and 299,201 ±20,373 μm^2 in the montelukast group. All these differences were statistically significant.

Finally, it was shown that all studied drugs may increase plaque stability by decreasing the number of macrophages and T lymphocytes and increasing collagen and smooth muscle cell plaque content.

SUMMARY The discussed study showed that montelukast – the antagonist of cysteinyl leukotriene receptor also decreases atherosclerosis in gene-targeted mice, however, to a lesser extent than FLAP inhibitors. This might result from the fact that FLAP inhibitors act "upstream" of the leukotriene cascade, blocking both leukotriene B4 and cysteinyl leukotriene production. In contrast, montelukast inhibits the cascade "downstream"

by blocking only the effect of cysteinyl leukotrienes and leaving LTB_4 untouched. Currently, the important role of LTB_4 in atherogenesis is undisputable.[22,48-50]

These results, together with many previous publications[11,33-37,42,46,48,52] demonstrate the need for further clinical trials. Surprisingly, Colin D. Funk's research team has recently questioned the hypothesis concerning leukotrienes, 5-LO and their role in atherogenesis in gene-targeted mice, stating that in mouse plaques there is no 5-LO over-expression detectable.[51] However, it still does not exclude the role of leukotrienes in human atherogenesis, since in human plaques there is abundant 5-LO expression[52] which even correlates with symptoms of plaque instability. Therefore, further research works, mainly clinical trials, are still needed in this field.[53]

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