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Chapter VII

VASA VASORUM HYPOXIA: A POSSIBLE SOLUTION FOR THE CHOLESTEROL CONTROVERSY

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ABSTRACT

It is unlikely that cholesterol initiates atherosclerosis, because most of the risk factors (smoking/nicotine, low physical activity, high blood pressure, stress and apnea) or protective factors (high physical activity, vitamin D and alcohol consumption) are minimally linked with serum cholesterol levels. Furthermore, the site of the initial development of atherosclerosis cannot be explained on the basis of cholesterol hypothesis. We propose an alternative hypothesis called "vasa vasorum constriction/hypoxia", which logically covers all previous hypotheses and is in good agreement with risk and protective factors. We postulate that a small constriction of peripheral arteries (including external vasa vasorum) will lead to a progressive hypoxia in the branching areas of these end arteries deep in the smooth muscle layer. This leads to a prolonged contraction and increasing oxygen consumption. Hypoxia will develop to a severe anoxia and capillary damage. Macromolecules (HDL-, LDL-cholesterol= "cholesterol hypothesis", microbes= "microbe hypothesis", matrix vesicles = "microvesicle hypothesis" etc) leak into the wall of the artery (extravasation). An inflammation begins ("inflammation hypothesis"). Hypoxia/anoxia will also cause neoangiogenesis and regeneration.

According to our hypothesis, a high physical activity prevents atherosclerosis, because it causes a peripheral vasodilatation. On the other hand, nicotine is known as peripheral vasocontrictor and therefore it is a risk factor. The beneficial effects of statins might be due to their vasodilatatory properties in addition to their anti-inflammatory action. The hypothesis suggests that peripheral arterial vasodilatation could be more suitable for primary prevention of atherosclerosis than statins, which are not as successful in the primary prevention as in the secondary prevention.

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INTRODUCTION

Lipid/cholesterol hypothesis was originally proposed by Rudolf Wirchow in 1856, but Nikolai Anitschkow showed the first experimental evidence for the possible role of cholesterol in atherosclerosis in 1913[1]. Finally, Ancel Keys in 1953 proposed that serum fatty acids and cholesterol cause atherosclerosis [2]. Two Nobel Prices strengthened the significance of cholesterol in atherosclerosis: Konrad Bloch and Feodor Lynen shared the Nobel Price in Physiology and Medicine in 1964 for their discoveries concerning the regulation of cholesterol and fatty acid metabolism and Michael S. Brown and Joseph L. Goldstein in 1985 for the clarification of the signaling pathway. "Framingham study" led to a conclusion that the higher serum cholesterol the highes is risk of coronary disease [3]. The results from the early cholesterol-lowering diet trials were controversial raising doubts about the validity of the lipid hypothesis [4]. This begun a discussion referred to as the "cholesterol controversy". Later some diet studies have demonstrated a positive correlation between a decreased dietary fat consumption and a decreased risk of coronary disease [5]. As a result a worldwide production of low-fat food products was launched, which besides statin drugs is the major consequences of the lipid hypothesis.

It was postulated that clinical trials with statins would finally solve the cholesterol controversy. The Coronary Primary Prevention Trial [6] and Scandinavian 4S study [7] among others supported the cholesterol hypothesis. However several negative studies were ignored [8] and the debate is continuing. Although there seems to be a general consensus on the cholesterol hypothesis as proven [9], from time to time there seems to be criticism against the hypothesis. It has been argued that the hypothesis is based on associations and misrepresented or over-interpreted data, and has not been shown a scientifically validated causal mechanism [10, 11]. In fact, even in the positive studies with statins the decrease of the cardiovascular mortality is not impressive. One would expect more robust decrease in mortality, if cholesterol were the cause of atherosclerosis.

Since clinical trials with statins seem to be important evidence for cholesterol hypothesis, it has been proposed that the pleiotropic effects of statins such as anti-inflammary action and decrease of blood pressure might contribute to the cardioprotection [12]. Statins were developed to inhibit 3-hydroxy-3-methylglutaryl coenzyme A, but they at the therapeutic concentration may interact with vitamin D receptor leading to its activation [13]. This is in good agreement with the result that vitamin D is necessary for nicotine-induced atherosclerosis in rats, a mdel used for atherosclerosis experiments [14]. Also clinical data suggest that vitamin D is involved in the development of atherosclerosis [15]. Here we criticize the logical failures of the cholesterol hypothesis attempting to make a comprehensive synthesis covering the known facts of atherogenesis.

DILEMMAS OF THE CHOLESTEROL HYPOTHESIS

The present cholesterol hypothesis includes the following events: 1. Increased serum (oxidized) LDL cholesterol will lead to an enhanced extravasation of the macromolecules via endothelial inflammation and an increased risk of development of atherosclerosis, whereas serum HDL cholesterol will counteract this development. 2. The lipoprotein particles pass

through the endothelial cells (transcytosis) either via LDL cholesterol receptors [16] or without them. 3. LDL cholesterol accumulates in the intima. 4. (Inflammation) activated macrophages express scavenger receptors and begin phagocytosis of LDL cholesterol forming foam cells. 4. Lipoprotein plaques are formed, and they are gradually calcified. 5. The plaques induce neovascularization. 6. As the plaque grows, it will rupture into the lumen and blood platelets attach to the damaged endothelium forming a blood clot leading to hypoxia.

The above hypothesis includes several critical problems: 1. It is not proven that high serum oxidized LDL cholesterol can cause endothelial inflammation. 2. Endothelium is one of the tightest membranes in the body. No macromolecules are able to pass through endothelium, if it not seriously damaged. If LDL cholesterol utilizes its receptors, it will internalized via clathrin coated pits and destroyed in lysosomes. 3. Familial hypercholesterolemia (FH) is regarded as an evidence for cholesterol hypothesis, because the associated atherosclerosis can be prevented by statins, although sometimes add-on drugs (cholestyramine, nicotinic acid or fibrates) are needed [17]. The disease is usually caused by mutations in the LDL receptor or apolipoprotein B gene, both leading to a deficient binding of LDL to its receptor. In fact, this is against cholesterol hypothesis, if the receptor is needed for the transcytosis [16]. It is more likely, that the high risk of atherosclerosis in FH is associated with the disease, but caused by other factor(s) [18].

Furthermore, the cholesterol hypothesis cannot explain several phenomena of the development of atherosclerosis: 4. The formation of cholesterol plaques occurs deep in the intima (close to the smooth muscle media)[19, 20]. One of the earliest signs of atherosclerosis is smooth muscle damage [21]. If LDL particles penetrate through endothelium or its junctions, they should first appear immediately under the endothelium. How the particles "jump" from the subendothelial intima to their actual site between intima and media, is not known. 5. The plaques are initially formed at the branching areas of the arteries. It has been speculated that the turbulence of the blood stream at the branching areas might be the cause, but this has not been proven. 6. Plaques contain several different microbes and viruses, which are thought to cause atherosclerosis (microbial hypothesis). In fact, plaques contain a history of the infections of a individual [22]. The cholesterol hypothesis contributes no explanation. 7. The plaques are not present in veins, intramural coronary arteries or in pulmonary arteries. High serum cholesterol should be as fatal to all parts of the vasculature, but it is not. Only the differences in blood pressure and vasa vasorum can explain the fact as described below.

VASA VASORUM CONTRICTION/HYPOXIA HYPOTHESIS

We postulated that a functional hypoxia of the most peripheral vasa vasorum (vv) develops gradually in response to a constriction of the peripheral small arteries and hypertension compressing intramural small arteries and capillaries in the wall of large arteries [23](Figure 1). The external vv originate from the branches of the main artery and they run longitudinally along the media-adventitia border [24]. The branches of vv run circumferentially or retrograde towards the branch point. Vasa vasorum are functional end arteries. The oxygen perfusion of the wall of the main artery comes into the intimal layer directly from the lumen (outward diffusion) and into the adventitia and media from the vasa vasorum (inward diffusion). The putative sequential events of atherosclerosis are:

1. Peripheral vasoconstriction
2. Hypoxia in the smooth muscle layer
3. Prologed contractions of smooth muscle
4. Increased oxygen consumption, severe hypoxia or anoxia
5. Capillary damage and increased permeability
6. Extravasation of LDL- and HDL-cholesterol, microbes and inflammatory cells
7. Inflammation, neovascularization
8. Plaque formation and calcification
9. Rupture of the calcified plaque

Our vasa vasorum hypoxia hypothesis includes that not cholesterol or microbes are the initial cause of the atherosclerosis but vasoconstriction (and consequent hypertension) begins the fatal process. Our hypothesis includes the cholesterol, microbial and inflammation hypotheses, since accumulation of cholesterol, microbes and inflammatory cells are consequences of damages of capillaries in the arterial wall allowing free efflux of the macromolecules, microbes and inflammatory cells. The inflammation activates macrophages to express scavenger receptors and the process continues as described by the cholesterol hypothesis. According to our hypothesis, serum cholesterol concentration plays no role in the initiation of atherosclerosis, which is in agreement with the poor clinical result in the primary prevention of the disease by statins [25]. On the other hand, the positive results in the secondary prevention by statins might be explained by our hypothesis, because the further development of plaques could slow down with lower serum cholesterol. However, statins do have actions, which better fit to our hypothesis such as vasodilatation, decrease of blood pressure and anti-inflammation. The recurrent branch of the external vv ends to the concave angle of the arterial bifurcation. This is the most vulnerable part of vv to vasoconstriction and hypertension from two sides compressing the intramural artery. According to Lame's law the oxygen perfusion from the vv is limited leading to hypoxia in the oxygen demanding smooth muscle layer. Since the muscle contraction is prolonged in hypoxia, the situation is progressive, unless peripheral vasodilatation increases the perfusion of the most distant vv.

DISCUSSION

Rhytmical contractions of aterial smooth muscles are crucially important for the blood circulation. The contractions need a lot of oxygen. Oxygen supply for the smooth muscle layer comes entirely from the external vasa vasorum. Even a small constriction of the vasa would lead to hypoxia, because these vessels are end arteries. Contractions will be prolonged and more oxygen is needed, therefore hypoxia gets worse and worse leading to capillary damage. Our vasa vasorum constriction/hypoxia hypothesis can explain the conflicting clinical results with fat restricted diet or statin prevention trials even within Scandinavia [7, 25], since cholesterol is not involved in the initiation of atherosclerosis, but may be moderately involved in the progression of the disease. There are several risk factors associated with atherosclerosis including sedentary lifestyle, hyperlipidemia, elevated serum cholesterol and triglycerides, obesity, smoking, hypertension, stress, male gender, sleep apnea, infections and diabetes mellitus [5, 26-29]. Protective factors include active sports,

female gender (estrogen), vitamin D_3, high HDL cholesterol, caloric restriction, low body weight, moderate alcohol use. All the risk factors seem to be vasoconstrictive, whereas many of the protective factors are vasodilatative, but only few of them affect serum cholesterol levels. Therefore the cholesterol hypothesis cannot explain the situation, whereas our vasa vasorum hypoxia hypothesis is in good agreement [30]. Nicotine is known as high risk factor for atherosclerosis. However it does not affect serum lipid composition [31]. Nicotine is strongly vasoconstrictive for the peripheral vessels [32]. It reduces production and bioavailability of nitric oxide (NO), increases production and release of endothelin. Obesity and metabolic syndrome are well known risk factors of atherosclerosis. Typical lipid profile and hypertension belong to the definition of the metabolic syndrome [33]. Eventhough stress is a clear risk factor for atherosclerosis [34] and it is often associated with changes in serum lipid profile, the mechanism is not known and it is unlikely that stress could directly regulate cholesterol metabolism. In vascular stress, endothelin-1 is released from the endothelium leading to a peripheral vasoconstriction [35]. Endothelin-1 plays an important role in hypertension by increasing peripheral resistance via vasoconstriction [35], but it does not affect serum lipids. Sleep apnea can directly cause hypoxia in vasa vasorum, but it is a high risk factor when it is combined with hypertension [36]. All the protective factors in atherosclerosis seem to be vasodilators. Estrogens may explain the sex difference in serum lipid profile [37], they are also known to dilate peripheral arteries [38]. Vitamin D is proven to be effective in prevention of atherosclerosis [15], it has weak or no effect on serum lipids. Physical exercise seems to lower LDL cholesterol, but it does not affect HDL cholesterol [39], its vasodilatating effects are obvious. Statins were developed to lower serum cholesterol, but their pleiotropic effects include vasodilation [40]. Besides its beneficial effect on HDL, niacin can dilate peripheral vessels causing flush [41]. The effect of alcohol on atherosclerosis is controversial, although its beneficial effects of a moderate alcohol use are well documented [42]. It is clear that acute low doses of EtOH increase both the release of NO and endothelial NOS (eNOS) expression, and augment endothelium-mediated vasodilatation, whereas higher doses impair endothelial functions [43]. It is interesting that the pleiotropic effects of statins include vasodilation and decrease of blood pressure [44-47]. Although the pleiotropic effects of statins have been widely analysed, their significance in the prevention of atherosclerosis has been neglected. The ability of nicotinic acid to strongly increase the plasma concentration of high-density lipoprotein (HDL) cholesterol has in recent years led to an increased interest in the pharmacological potential of nicotinic acid. [41, 48] Flushing is regarded as an adverse effect of niacin, results from GPR109A-mediated production of prostaglandin D2 and E2 in Langerhans' cells which act on DP1 and EP2/4 receptors in dermal capillaries causing their vasodilatation [49]. DP1 receptor antagonist (laropiprant) attenuates the niacin flush in animals and humans. A reformulated preparation of extended-release niacin lowers flushing compared with the extended-release niacin. Aspirin pretreatment seems to attenuate flushing from this preparation. However, these combination drugs prevent most of the peripheral vasodilatation and may, thus, be less effective in decreasing blood pressure, which might be also a beneficial effect of niacin. We propose that it is reasonable to re-evaluate the goals in the primary prevention of atherosclerosis. It seems that vasodilatation might be the most important, whereas lipid-lowering drugs may delay the progression atherosclerosis in the secondary prevention, when combined with vasodilatation. Therefore, statins are optimal in the secondary prevention, but also niacin by increasing HDL should be useful, if its capacity for peripheral vasodilatation is maintained. Since there is a significant comorbidity between

coronary disease and chronic obstructive pulmonary disease (COPD) and a common risk factor (smoking), we assumed that there could be similar changes in vasa nutritia of the terminal bronchioli as in the walls of coronary arteries (Tuohimaa and Järvilehto in press 2012). In fact, smoking is almost the only cause of COPD and constituents of the cigarette smoke are thought to irritate epithelium to begin inflammation and bronchoconstriction. We expected that LDL cholesterol plaques could be found in the bronchial wall, however, there are no visible plaques, but cholesterol is found inside the bronchial lumen [50]. This intraluminal cholesterol cannot come from the lumen, but it comes from the vasa nutritia, which are similarly contracted by nicotine as vasa vasorum leading to hypoxia and extravasation of LDL cholesterol. In COPD, the intraluminal LDL/HDL cholesterol is degraded and the released cholesterol changes the properties of surfactant leading to emphysema. It can be concluded that all risk factors of atherosclerosis are vasoconstrictive and hypertensive and all protective factors are vasodilatative, but not all of them influence cholesterol levels suggesting that cholesterol cannot explain all the development of atherosclerosis, but vasocontriction and blood pressure might be more crucial. According to our "vasa vasorum hypoxia" hypothesis the vasodilatation would be beneficial in the prevention of the atherosclerosis, which fits well with the clinical experience. NO is a critical endothelium-derived vasodilatatory factor with anti-atherosclerotic properties [51].

Peripheral vasodilatators are not available, because they proved to be unsuitable for clinical practice, since orthostatic hypotension was serious side effect, which was difficult to control. It seems that it would be reasonable to begin a new pharmaceutical development of this class of antihypertensive drugs for the prevention of atherosclerosis.

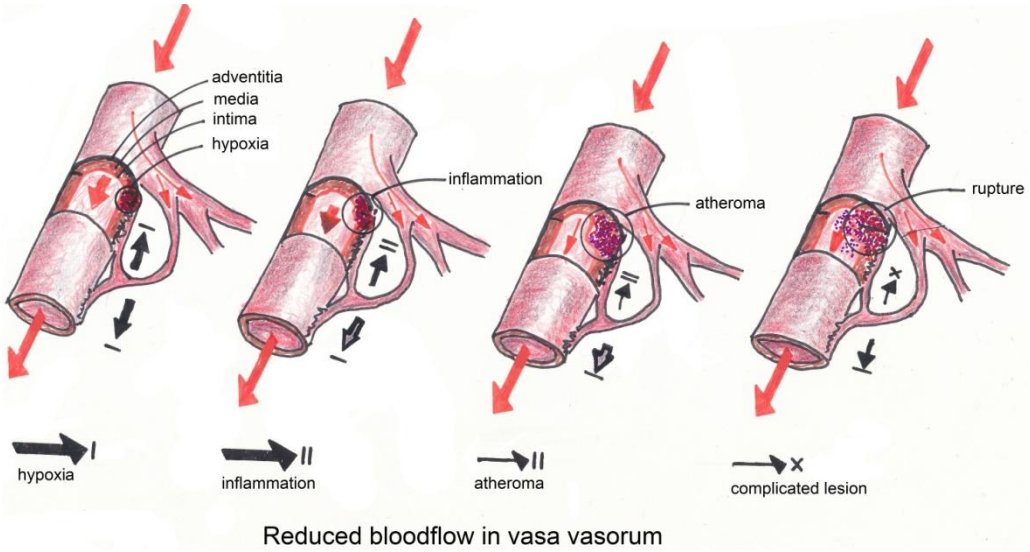

Figure 1. Vasa vasorum constriction/hypoxia hypothesis. Atherosclerosis begins with vasoconstriction of the external vasa followed by hypoxia in the most vulnerable area, intima-media border at the branching area of the artery. Hypoxia leads to a prolonged contraction of smooth muscle and therefore more severe hypoxia and inflammation. After destruction of endothelial barrier, macromolecules extravasate and plaque is formed. Intima= inner layer of artery, media= smooth muscle layer, adventitia= outer loose connective tissue layer containing plexus of the external vasa vasorum.

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