Atherosclerosis and Oxidative Stress

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Abstract: The “response-to-injury” hypothesis has been proposed as an explanation for the etiology of atherosclerosis. This theory postulates that various biologically active substances are released in response to injury to endothelial cells in the vessel wall, eliciting an inflammatory response from cells composing the wall. When endothelial cells are injured or activated by coronary risk factors, infection, physical stimuli, or oxidative stress, adhesion molecules become expressed in endothelial cells, and peripheral monocytes adhere to the endothelial cell surface. Peripheral monocytes enter the subendothelial area through endothelial intercellular space, where they mature and differentiate into macrophages in the presence of M-CSF. If excessive remnant lipoproteins or low-density lipoprotein (LDL) are present, the remnants and degenerated LDL, having undergone oxidation or other forms of modification, are taken up by macrophages, resulting in the formation of foam cells, which accumulate cholesterol ester and produce plaque. In plaque formation, endothelial cell function, macrophage function, transformation of smooth muscle cells, extracellular matrix function, the lipid storage mechanism of the vessel wall, and the thrombogenic mechanism, as well as oxidative stress, are involved in a complex interaction, and inflammatory cytokines and growth factors play a positive role in the maintenance of homeostasis of the vessel wall.

Key words: Atherosclerosis; Oxidized LDL; Remnant lipoproteins; Plaque

Introduction

The current spectrum of disease in Japan has seen a rapid increase in atherosclerotic diseases, particularly vascular lesions, with changes in the social environment and the aging of society in general. Circulatory diseases mainly involving blood vessels, such as ischemic heart disease and cerebrovascular disorders, are expected to increase further in the future. Given the current environment characterized by a high-calorie, high-fat diet, and lack of physical activity, individuals are likely to accumulate multiple coronary risk factors in the morbid states of energy storage and insulin resistance. This accumulation of coronary risk factors is not accidental; these risk factors are considered to be derived from the common conditions of obesity and...
insulin resistance resulting from a more western lifestyle.

The severer the accumulation of multiple coronary risk factors, the faster the formation of unstable atherosclerotic plaques rich in cholesterol. When thrombus formation, changes in circulatory dynamics, inflammation, or oxidative stress is added, the plaque becomes unstable and disrupted, leading to ischemic heart disease and cerebrovascular disorders that are life-threatening or impair the quality of life.

**Etiology of Atherosclerosis**

Atherosclerotic lesions exhibit morbid reactions by various cells, including the accumulation of cholesterol ester. The response-to-injury hypothesis is a well-known explanation of the etiology of atherosclerosis. It postulates that biologically active substances such as platelet-derived growth factor (PDGF) and macrophage colony stimulating factor (M-CSF) are released in response to injuries to endothelial cells in the vessel wall, eliciting morbid reactions from cells composing the wall.

An atherosclerotic focus consists of various cells, including platelets, endothelial cells, macrophages, smooth muscle cells, and T lymphocytes. These cells actively secrete various cell growth factors and cytokines to regulate the maintenance of homeostasis of the vessel wall and cope with the various risk factors accelerating the development of atherosclerosis (hypercholesterolemia, hypertension, smoking, diabetes mellitus, infection).

**Endothelial Cell Function and Adhesion Molecules**

Vascular endothelial cells function to prevent clotting of blood and adhesion of blood cells to the endothelial cells, in addition to playing the role of a barrier, as a cell monolayer, to prevent blood constituents from invading the vascular wall. When endothelial cells are injured or activated by various coronary risk factors, infection, or physical stimuli, adhesion molecules become expressed in endothelial cells, and peripheral monocytes adhere to the endothelial cell surface.

Adhesion molecules are broadly divided into three molecular families: The integrin family, immunoglobulin family (including ICAM and VCAM), and selectin family (L-selectin, E-selectin, P-selectin).

Following the activation of endothelial cells, the contribution of various biologically active substances and signal transduction causes adhesion molecules to be expressed. Leukocytes first adhere loosely to endothelial cells, i.e., they “roll” on endothelial cells, through the aid of selectin and its ligands. Thereafter, β-integrins (LFA-1, Mac-1) on leukocytes activated by IL-8 and MCP-1 recognize such molecules as ICAM-1 and VCAM-1 on endothelial cells, to produce strong adhesion. The leukocytes, having adhered to endothelial cells, migrate and invade the subendothelial area via endothelial intercellular spaces through the actions of ICAM-1 and PECAM-1 (CD31).

**Foamy Change of Monocytes Due to Oxidized LDL and Remnant Lipoproteins**

Peripheral monocytes enter the subendothelial area via endothelial intercellular space, where they mature and differentiate into macrophages under the action of M-CSF and other factors. If excessive remnant lipoproteins and LDL are present, the remnants and degenerated LDL, having undergone oxidation or other forms of modification, are taken up by macrophages, forming foam cells and accumulating cholesterol ester (Fig. 1). In the initial stage of plaque formation, the main component is macrophage-derived foam cells.

It is considered that remnant lipoproteins and LDL are incorporated through pathways via the LDL receptor family and scavenger receptor family, respectively. Macrophages are characterized by extremely weak expression of LDL receptors. Smooth muscle cells, another
source of foam cells, have sufficient expression of LDL receptors, but accumulation of cholesterol inside these cells inhibits further expression of LDL receptor genes, precluding excessive cholesterol accumulation. Although both sources of foam cells, i.e., macrophages and smooth muscle cells, have mechanisms for preventing excessive incorporation of LDL cholesterol, the accumulation of cholesterol occurs in atherosclerotic foci.

In this context, the view has arisen that, not LDL itself, but degenerated LDL is incorporated by smooth muscle cells, resulting in the accumulation of cholesterol. This is not incorporation of LDL through the LDL receptor, but incorporation of degenerated LDL through the scavenger receptor proposed by Goldstein and Brown et al. Oxidized LDL has been shown to be a type of degenerated LDL present under physiological conditions. Incorporation of oxidized LDL is not ruled by a single mechanism; the presence of a scavenger receptor family including acetyl LDL receptors and CD36 has been described.

It has been reported that remnant lipoproteins seen in patients with insulin resistance, diabetes mellitus, hypertriglyceridemia, or type III hyperlipidemia cause foamy change in macrophages under physiological conditions. Remnant lipoproteins are atherogenic lipoproteins, which have been increasing in the Japanese population as a result of westernization and the overconsumption of food. It has been speculated that apolipoprotein E (ApoE) on remnant lipoproteins causes macrophages to become foam cells because of its strong affinity to the LDL receptor family [LDL receptors, LDL receptor-related protein (LRP), very low-density lipoprotein (VLDL) receptors].

Plaque disruption causes cardiovascular events to take place. Instability and structural vulnerability of cholesterol-rich plaques are largely responsible for this process. The thinness of the fibrous cap covering the plaque is a factor in the easy disruption of plaque. Macrophages in the atherosclerotic focus secrete proteases that digest the extracellular matrix and fibrous components, and thus enhance the

![Fig. 1 Adhesion of monocytes onto endothelial cells and their foamy change](image-url)
vulnerability of plaque.

**Action of Oxidized LDL**

Various reports have discussed the relationship between oxidized LDL and arteriosclerosis. Oxidized LDL has been presumed to be involved in plaque formation in atherosclerosis from the aspects of lipid storage and the inflammation hypothesis. This presumption has been supported by the proposal that the administration of drugs like probucol that have antioxidant activity may inhibit the occurrence of ischemic heart disease, and by the immunohistological evidence of oxidized LDL in the atherosclerotic plaque. In addition, the previously reported finding that administration of the antioxidant probucol to WHHL rabbits, which lack LDL receptors, markedly inhibits the progression of atherosclerosis has led to the presumption that macrophages specifically incorporate oxidized LDL and form foam cells. On the other hand, the effects of probucol in mice are not as remarkable as those in rabbits, with the inhibitory effect rather tending to be correlated with a decrease in cholesterol. Therefore, it is apparent that incorporation of oxidized LDL causes foamy change of cells in vitro, but how and to what extent it is involved in the formation of the initial lesion and subsequent atherogenesis await further investigation.

Oxidized LDL, which is involved in the initiation of atherosclerosis through its various actions, exerts a variety of effects. First, it stimulates the migration of monocytes and macrophages into the subendothelial area in the vessel wall; and, second, it interferes with the outward movement of monocytes, keeping them within the vessel wall, although monocytes and macrophages can migrate outside the vessel wall. Oxidized LDL also plays an important role in the differentiation of macrophages into foam cells.

Another aspect of the action of oxidized LDL recently attracting attention is the lysolecithin present inside oxidized LDL, which induces injury to endothelial cells and takes part in the initiation of atherosclerosis. Thus, oxidized LDL has been implicated in several facets of the inflammatory process, including the invasion of monocytes and macrophages, their differentiation, and the induction of injury to endothelial cells. Oxidized LDL naturally is considered to play the most important role in lipid storage as well.

**Cardiovascular Events Resulting from Plaque Formation**

In the initial stage of plaque formation, the plaque is mainly composed of macrophage-derived foam cells. Along with the progress of atherosclerosis, foam cells characteristic of atherosclerotic foci presumably begin to be derived not only from macrophages but also from smooth muscle cells that have migrated from the tunica media into the intima. Vascular smooth muscle cells originally existing in the tunica media may be altered by some stimulus, such as PDGF, and migrate across the internal elastic membrane into the intima, where they proliferate and phagocytose lipids.

These smooth muscle cells, having migrated from the tunica media, proliferate from the luminal side of the blood vessel to enclose the macrophage-derived plaque, so that the surface layer of the plaque is protected and reinforced by several layers of smooth muscle cells beneath the endothelial cells. This can be considered an attempt to repair the diseased blood vessel undergoing the process of plaque formation. However, if concomitant hyperlipidemia continues to exist, smooth muscle cells also become foam cells, and thus the formation of foam cells progresses from the plaque side toward the luminal surface of the blood vessel, resulting in advanced extension of the plaque. The plaque, formed by the aggregation of foam cells, causes thrombus if disrupted, leading to vascular occlusion and, consequently, a cardiovascular event.

It is thought that disruption of plaque occurs
depending on 1) the size of the lipid core, 2) thickness of the plaque capsule, and 3) degree of inflammation and cell invasion in the plaque capsule. Smooth muscle cells that have migrated to and proliferated in the intima protect against plaque disruption in terms of their effort to reinforce the plaque capsule. However, foamy smooth muscle cells, like foamy macrophages, seem to promote plaque disruption in light of the expansion of the lipid core and weakening of the plaque capsule.

Treatment of Hypercholesterolemia

Among various types of hyperlipidemia, hypercholesterolemia is considered the most important risk factor for ischemic heart disease. The aim of treatment of hypercholesterolemia is to prevent or treat atherosclerotic diseases such as ischemic heart disease.

In recent years, results of large-scale, long-term studies on the primary and secondary prevention of hypercholesterolemia through the use of statins have been reported from Scandinavia (Scandinavian Simvastatin Survival Study, 4S study) and the United Kingdom (WOS study). It is apparent from the reports of these studies that improvement of hypercholesterolemia clearly inhibits the development of cardiovascular events. Both studies demonstrated that the benefit of treatment for hypercholesterolemia would manifest in as short a period as 6 months to 1 year, a far shorter time than had previously been considered. It has become clearer in recent years that this benefit of treatment for hypercholesterolemia is attributable to the inhibition of acute cardiovascular events through the stabilization of plaque, rather than to enlargement of the stenotic vascular lumen.

Treatment of hypercholesterolemia reduces the cholesterol content in plaque and normalizes the function of vascular endothelial cells, thereby stabilizing the plaque. Large-scale prevention trials denote the need for steady correction of hyperlipidemia in high-risk patients through aggressive treatment.

Another important issue related to hypercholesterolemia is that the production of NO, an endothelium-derived relaxing factor, is decreased, resulting in inhibition of the vascular relaxation reaction. The involvement of oxidized LDL has been implicated in the decrease in NO production due to the damage to vascular endothelial cells. Therefore, for the prevention of cardiovascular events, it is important to achieve improved vascular endothelial function and stabilization of plaque, to prevent its disruption. In this regard, antioxidant therapy to suppress LDL oxidation is expected to be effective.

Drugs with Antioxidant Activity

LDL carries vitamin E, an antioxidant important to the living body. In the mechanism of vitamin E transport, vitamin E taken from food is incorporated by the liver in the form of α- and γ-tocopherol, while α-tocopherol alone is forwarded to VLDL, an endogenous lipoprotein, and taken up into the LDL transport system. The action of vitamin E that is transported with LDL becomes important when LDL undergoes oxidation. In fact, this is a reason why vitamin E is used actively in routine medical practice. It is also known that probucol and various other substances possess antioxidant activity.

The effects of vitamin E, in particular, have been examined in several clinical studies. In a recent large-scale epidemiological study, it was found that vitamin E significantly inhibits the development of cardiovascular events. This study, called CHAOS (Cambridge Heart Antioxidant Study), consisted of 2,002 subjects who were randomly assigned to a vitamin E group (1,035 individuals) and a placebo group (967 individuals). The subjects in the vitamin E group were given 400 or 800 IU/day of vitamin E for a mean period of 510 days, and the incidence of cardiovascular events in this group was compared with that in the placebo group. When fatal and nonfatal myocardial infarctions were used
as endpoints, vitamin E was obviously more effective than the placebo.

In the Hypertensive Old People in Edinburgh (HOPE) study, 9,541 high-risk patients were given either vitamin E, 400IU/day (vitamin E group) or a placebo (placebo group) for an average of 4.5 years, and the incidence rates of myocardial infarction, stroke, and death were compared between the two groups. The results indicated no benefit of vitamin E administration. Thus, some reports have documented the benefit of antioxidant therapy using vitamin E, whereas others have indicated no benefit. Results from other, ongoing clinical studies are awaited.

**Conclusion**

Important elements in the stabilization of plaque are considered to include a decrease in lipids in the plaque; a decrease in the component cells, particularly foam cells, of the plaque; reinforcement of the extracellular matrix; stabilization of circulatory dynamics for the prevention of plaque disruption; and prevention of thrombus formation. Large-scale clinical studies have been carried out in regard to lipid storage in the vessel wall, thrombus formation, and circulatory dynamics from the aspects of antihyperlipidemia, anti-platelet, and antihypertensive treatments, and the significance of these treatments has been demonstrated. It is hoped that the value of antioxidant therapy will also be firmly established in the future.

**REFERENCES**