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Review Article

ATHEROSCLEROSIS: CRITICAL ROLE OF OXIDATION AND INFLAMMATION

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ABSTRACT

Atherosclerosis - cardiovascular disorder which involves progressive narrowing and degeneration of arteries. The oxidative modification of low density lipoproteins (LDL) in the arterial wall by reactive oxygen species (ROS), elevated levels of homocysteine in blood plasma are the key risk factors for atherosclerosis. Importantly, several processes are triggered by other risk factors, including the expression of adhesion molecules, the proliferation ad migration of smooth muscle cells, the apoptosis of endothelial cells, the oxidation of lipids, the activation of metalloproteinase and the alteration of vasomotor activity. Eventually in its natural progression, calcification of the atheromatous plaque occurs. Depending on the affected artery in the body, the related disorders include coronary heart disease, carotid artery disease peripheral arterial disease and chronic kidney disease.

INTRODUCTION

Atherosclerosis is a chronic inflammatory process affecting large and medium-sized arteries throughout the cardiovascular system [1]. According to the theory of oxidative stress, atherosclerosis is the result of the oxidative modification of low density lipoproteins (LDL) in the arterial wall by reactive oxygen species (ROS) [2]. These processes are triggered by risk factors, including the expression of adhesion molecules, the proliferation and migration of smooth muscle cells, the apoptosis of endothelial cells, the oxidation of lipids, the activation of metalloproteinase and the alteration of vasomotor activity [3]. The early stages of atherosclerosis are similar to the reaction noted in asthma. It consists of infiltration of the affected site by T-lymphocytes and monocytes, which then transforms into macrophages, followed by proliferation of fibrous tissue [3]. Eventually in its natural progression, calcification of the atheromatous plaque occurs. These plaques may partially or totally block the blood's flow in arteries which results in heart attack or stroke.Depending on the affected artery in the body, the related disorders include coronary heart disease, carotid artery disease peripheral arterial disease and chronic kidney disease will occur. 60% of global burden of cardio vascular disorders (CVD) occurs in developing countries results because of increasing longevity, urbanization and life style changes. Studies in developed countries suggest that low income and illiterates are associated with higher incidence of CVD.

Atherosclerosis-Related Diseases

Atherosclerosis can affect any arteries associated with heart, brain, arms, legs and kidneys. Depending on the affected arteries, it may result in Coronary heart disease, Carotid Artery Disease, Peripheral Arterial Disease, Chronic Kidney Disease.

Coronary Heart Disease (CHD), also called as coronary artery disease occurs if plaque builds up in the coronary arteries. These arteries supply oxygen-rich blood to heart. These atherosclerotic plaques narrow the coronary arteries and reduce blood flow to heart muscles. Plaques buildup and also makes it more likely that blood clots will form in arteries. Blood clots can partially or completely block blood flow, which results in angina or myocardial infarction. Plaques may also form in the heart's smallest arteries, results in coronary microvascular disease (MVD). In coronary MVD, plaque doesn't cause blockages in the arteries as it does in CHD. It is estimated that, myocardial infarction occurs every 34 seconds in US and one person dies each minute from a major coronary event.

Carotid Artery Disease occurs if plaque builds up in the carotid arteries. These arteries supply oxygen-rich blood to brain. If blood flow to your brain is reduced or blocked, it results in stroke.

Peripheral Arterial Disease occurs if plaque builds up in the major arteries that supply oxygenated blood to legs, arms, and pelvis. If blood flow to these parts of body is reduced or blocked, it may results in numbness, pain.

Chronic Kidney Disease occurs if plaque builds up in renal arteries. These arteries supply oxygen-rich blood to kidneys. Over time, chronic kidney disease causes a slow loss of kidney function.

Major Risk Factors

Oxidized LDL particles

Oxidative stress leads to oxidation of Low Density Lipoproteins (LDL). Macrophages uptake these oxidized LDL much easier than non-oxidized lipoproteins. The main sources of oxidative substances and ROS in atherosclerotic vessels are macrophages and smooth muscle cells. Reactive oxygen species (ROS) production, in turn, induces endothelial dysfunction. Indeed, hypercholesterolemia stimulates the production of superoxide anion radicals from the smooth muscle cells of vessels, an event that leads to increased oxidation of LDL. [4, 5]

Elevated plasma homocysteine – an inborn error of metabolism

Much of the endothelial dysfunction attributed to homocysteine is proved to occur primarily from oxidative stress. Studies have shown that homocysteine suppresses the vasodilator nitric oxide, perhaps by increasing the levels of asymmetric dimethylarginine (ADMA), a strong inhibitor of endothelial nitric oxide synthase (eNOS) and strong independent risk factor for cardiovascular disease. [6,7]

Smoking

Smoking places a significant physiological stress on the vasculature by acutely decreasing coronary blood flow and myocardial oxygen delivery and by inducing profound, predominantly silent, regional disturbances in myocardial perfusion [7]. Numerous mechanisms contributes to the increased cardiovascular risk in smokers, including increased activation of platelets and leucocytes, and adverse effects on lipids, blood pressure and insulin resistance.. Endothelial cells exposed to tobacco smoke have an irregular appearance with disturbances in membrane architecture, and develop marked functional change, including decreased activity of eNOS, enhanced expression of adhesins and dysregulation of the local thrombotic balance [8]. Cigarette smoke is a complex mixture of chemical compounds containing a high concentration of free radicals. These reactive oxygen species (ROS) damage endothelial cells via several distinct pathways that include direct cellular damage and indirect effects on lipid peroxidation, and scavenging of nitric oxide with generation of the potent oxidant, peroxynitrite [9].

High amounts of fats and cholesterol in blood

High amounts of fats and cholesterol in blood results in obesity which is also associated with chronic inflammatory response [10, 11]. There is indeed evidence that obesity is associated with macrophage accumulation in the adipose tissue. Obesity increases the risk of cardiovascular disorders through 2 pathways; 1) It promotes insulin resistance, which increases the expression of many

inflammatory mediators. 2) It is directly associated with an inflammatory response itself, which affects the production of several proinflammatory cytokines (adipokines) and hormones [12].

Role Of Oxidative Stress

Endothelial function associated in the earlier stages of atherogenesis and is strongly correlated with most of risk factors. Endothelial dysfunction predisposes to long-term atherosclerotic lesions and has been proposed as an important diagnostic and prognostic factor for coronary syndromes. The production of free oxidative radicals is believed to induce endothelial dysfunction, an initial step of atherogenesis. Oxidative stress leads to oxidation of LDL (ox-LDL), whose uptake by macrophages is easier compared to non-oxidized lipoproteins. Indeed, hypercholesterolemia stimulates the production of superoxide anion radicals (O_2^-) from the smooth muscle cells of vessels, an event that leads to increased oxidation of LDL. The increased production of ROS reduces the bioavailability of NO, leading to vasoconstriction, platelet aggregation and adhesion of neutrophils to the endothelium. In fact, oxidative stress by hydrogen peroxide (H_2O_2) increases phosphorylation of tyrosin kinases, which leads to stronger binding of neutrophil cells on endothelium and alteration of vessel permeability. Another mechanism through which oxidative stress (by H_2O_2) affects atherogenesis is the production of transcription factors such as nuclear factor IB (NF-IB) and activator protein 1 (AP-1), which participate in the expression of adhesion molecules, such as vascular cellular adhesion molecules (VCAM- 1), intracellular adhesion molecules (ICAM-1), E-selectin and other cytokines. It is well established that NF-IB acts in smooth muscle cells of atherosclerotic vessels and is inactivated by antioxidants and anti-inflammatory agents such as salicylics and glucocorticoids. Thus, it seems that atherosclerosis is an inflammatory process strongly affected by oxidative stress.

Role of enzyme systems in the oxidative process

Several enzymes sources that use various substrates as sources of electrons for the production of free radicals seem to be important in this process. A subsequent reduction of molecular oxygen (O_2) occurs in favor of a variety of ROS. Specifically, one –electron reduction of molecular oxygen leads to the formation of super oxide, while two-electron reduction leads to hydrogen peroxide.

Nitric oxide synthase

Under normal conditions, binding to tetrahydrobiopterin (BH_4), which acts as cofactor and is used as a substrate for L-arginine, leads to NO formation. However, under certain circumstances, the uncoupling of this enzyme can lead to O_2 reduction and the production of ROS, with consequent production of peroxynitrite and oxidation of lipids and proteins. A series of pathological states is associated with the uncoupling of NO, such as hypercholesterolemia, atherosclerosis, diabetes mellitus (DM) and arterial hypertension. Under certain conditions, this event could be the result of reduced levels of BH_4. Production of NO in endothelial cells needs a certain amount of BH_4 and its administration restores the impaired, endothelium dependent, vasodilation. In addition, L-arginine is also important for NO production and right endothelial function. In patients with hypercholosterolemia or advanced atherosclerosis, L-arginine administration improves NO production and vasodilation. Finally, levels of asymmetric dimethylarginine (ADMA)—a substance which leads to the uncoupling of NO synthase are elevated in various conditions, such as arterial hypertension, and are in a way related to endothelial dysfunction. Administration of L-arginine in patients with elevated levels of ADMA improves endothelial function, suggesting that L-arginine deficiency stimulates nitric oxide synthase to ROS production.

Nicotinamide-adenine dinucleotide phosphate -oxidase

NAD(P)H oxidase has been proved to be an important source of free oxygen species in vascular cells. It is regulated by a variety of pathophysiological stimulations relevant to atherosclerosis, such as angiotensin II, thrombin, platelet-derived growth factor, tumor necrosis factor-alpha, and natural forces such as wall stress. Activation of the type I receptor of angiotensin II leads to the stimulation of protein kinase C, provoking the increase of ROS from NAD(P)H, with a subsequent increase in blood pressure and disturbance of vasodilatation [13] [14].

Xanthine oxidase

This enzyme exists in plasma and endothelial cells but not in smooth muscle cells. In experimental animals with hypercholesterolemia it is capable of producing increased amounts of active radicals leading directly to reduced NO activity. It has been observed that in vessels of hypercholesterolemic patients, vasodilation is improved by the presence of allopurinol or oxypurinol, an inhibitor of the enzyme [15, 16]. Additional facts that support the role of xanthine oxidase in the process of atherogenesis are the following: 1) in patients with coronary syndrome the levels of this enzyme were found to be increased—the same applies to NAD(P)H; and 2) in young asymptomatic patients with familial hypercholesterolemia the increased activity of the enzyme is an early event.

Lipoxygenase

These are enzymes that catalyze the intake of O_2 reaction from the polyunsaturated lipid acids, creating a family of biologically active lipids, such as prostaglandins, thromboxanes and leukotrienes, which participate in inflammatory reactions and increase the permeability of vessels. In experimental models, 15-lipoxygenase induces LDL oxidation by enzymatic and nonenzymatic reactions; 15-lipoxygenase and 5-lipoxygenase are expressed in atherosclerotic lesions in humans and animals with apolipoprotein E deficiency [17]. Experimental animals with an absence of the 15-lipoxygenasegene or reduced expression of 5-lipoxygenase are protected from lesions like those found in animals with apolipoprotein E and LDL-receptor deficiency. Clinical data demonstrate that various genotypes of 5-lipoxygenase promoter are found in patients with atherosclerotic lesions or inflammation.

Role of arachidonic acid metabolism

Stimulation of vascular endothelial cells with agonists such as acetylcholine (ACh) or bradykinin or with shear stress activates phospholipases and releases arachidonic acid (AA). AA is metabolized by cyclooxygenases, cytochrome *P*-450s, and lipoxygenases (LOs) to vasoactive products. In some arteries, a substantial component of the vasodilator response is dependent on LO metabolites of AA. Nitric oxide (NO) - and prostaglandin (PG)-independent vasodilatory responses to ACh and AA are reduced by inhibitors of LO and by antisense oligonucleotides specifically against 15-LO-1. Thus formation of vasodilator eicosanoids derived from LO pathways contributes to the regulation of vascular tone, local blood flow, and blood pressure. In response to chemical or physical stimuli such as acetylcholine (ACh), thrombin, bradykinin, and fluid sheer stress, the vascular endothelium produces several factors that relax the underlying smooth muscle and thus regulate the vascular tone. These relaxing factors are prostacyclin (PGI_2), nitric oxide (NO), and endothelium-derived hyperpolarizing factors (EDHFs). Unlike PGI_2 and NO, numerous endothelium-derived factors function as EDHFs, and no single molecule or pathway has been identified that exhibits all of the characteristics of EDHF in different vascular beds, species, and diseases. Among the compounds and pathways that have been proposed as mediators of EDHF activity are metabolites of arachidonic acid (AA).

Role of inflammation

From a pathological view point, all stages, i.e., initiation, growth, and complication of the atherosclerotic plaque, might be considered as inflammatory response. The risk factors elicit the secretion of both leukocyte soluble adhesion molecules, which facilitate the attachment of monocytes to endothelial cells. The chemotactic factors secreted encourage the monocyte migration into the subintimal space. The transformation of monocytes to macrophages and the uptake of cholesterol lipoproteins initiate the fatty streak. This further attracts macrophages, mast cells, and activated T cells. Oxidized LDL contributes to loss of smooth muscle cells.

Inflammatory Markers

Potential targets for measuring inflammation are proinflammatory risk factors such as proinflammatory cytokines (e.g., interleukin-1, tumor necrosis factor), adhesion molecules (e.g., intercellular

adhesion molecule-1, selectins), inflammatory stimuli with hepatic effects (e.g., interleukin-6) or the products of the hepatic stimulation, such as SAA, C-reactive protein (CRP), and a host of other acute-phase reactants [10]. Finally, other indicators of cellular responses to inflammation, such as elevated leukocyte count, might be evaluated.

Adhesion molecules

Membrane-bound vascular cell adhesion molecule 1 (VCAM-1) allows the tethering of monocytes and lymphocytes as well as firm attachment and transendothelial migration of leukocytes. Soluble forms of VCAM (sVCAM-1) may serve as monitors of increased expression of membrane-bound VCAM-1 and thus may reflect progressive formation of atherosclerotic lesions. Increased sVCAM-1 levels were significantly associated with increased risk of cardiovascular mortality. Levels of sVCAM-1 are independently associated with the risk of cardiovascular mortality in type 2 diabetic subjects and therefore might be useful for identifying subjects at increased cardiovascular risk. Increased plasma sVCAM-1 levels may reflect progressive formation of atherosclerotic lesions, or sVCAM-1 itself may have bioactive properties related to cardiovascular risk. In addition, recent cross-sectional studies showed sVCAM-1 concentration to be positively associated with carotid artery intima-media thickness and with the severity of peripheral arterial disease assessed by angiography.

Cytokines

Obesity-associated TNF-α is primarily secreted from macrophages accumulated in obese adipose tissue, whereas the adipocytes, predominantly produce unsecreted, membrane-bound TNF-α. The secreted adipose tissue TNF-α is specifically increased in visceral adipose depots. The resulting systemic rise in circulating TNF-α has been implicated in causing adipocyte insulin resistance. Circulating TNF-α may also contribute by its induction of CRP production and general systemic inflammation, which, in turn, impacts on the vasculature. In vitro experiments have also shown that TNF-α increases activation of endothelial and smooth muscle NF-κB, which, in turn, induces vascular adhesion molecules and cytokines, resulting in inflammatory and foam cell accumulation [10].

Obesity-associated induction of adipose IL-6 production induces CRP secretion, and there are data that suggest IL-6 decreases lipoprotein lipase activity, which results in increased macrophage uptake of lipids. In primitive atheromatous lesions, macrophage foam cells and smooth muscle cells express IL-6, suggesting a role for this cytokine in the earliest stages of atherosclerosis. Circulating IL-6 stimulates the hypothalamic-pituitary-adrenal axis, activation of which is associated with central obesity, hypertension, and insulin resistance [13].

CONCLUSION

There is a large body of evidence connecting the effects of oxidative stress with atherogenesis. However, specific causative relation between oxidative events in general and oxidative modification of LDL in particular with respect to atherosclerosis was not established. The existing clinical studies of the role of antioxidants according to specific endpoints are quite disappointing. It is therefore important, with the contribution of molecular cardiology and pharmacogenetics, to elucidate the molecules as well as the inhibiting mechanisms that interfere in the oxidative process.

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