ATHEROSCLEROSIS, INFLAMMATION AND OXIDATIVE STRESS

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ABSTRACT

Atherosclerosis is a focal disease of the arterial wall that leads to cardiovascular disease (CVD), the biggest cause of morbidity and mortality in Western societies. CVD, a group of disorders of the heart and the vasculature, includes atherosclerosis, high blood pressure, coronary heart disease, congestive heart failure, stroke and congenital heart defects. The World Health Organization report emphasizes that the cardio vascular diseases to be the leading cause of death and disability in India by 2020. Atherosclerosis with complicated pathogenesis involving oxidative stress, endothelial dysfunction and chronic inflammation. Epidemiological and clinical studies have shown strong and consistent relationships between markers of inflammation and risk of future cardiovascular events. Furthermore during the last decades several studies have examined the potential role of oxidative stress in atherogenesis. Atherosclerosis and cardiovascular disease take a substantial tax on our society. Understanding of all these processes will help to invent a range of new biomarkers and novel treatment modalities targeting various cellular events in acute and chronic inflammation that are accountable for atherosclerosis. Moreover, presently there is an increasing interest worldwide in herbal medicines accompanied by increased laboratory investigation into the pharmacological properties of the bioactive ingredients and their ability to treat various diseases.

Key Words: Atherosclerosis, Inflammation, Oxidative stress, CRP, Antioxidant

INTRODUCTION

More than 25 million persons in the United States have at least one clinical manifestation of atherosclerosis and in many more, atherosclerosis remains an occult but imperative forerunner of significant cardiovascular events. Atherosclerosis is the preliminary lipid disorders that affect large and medium-sized muscular arteries and is characterized by endothelial dysfunction, vascular inflammation, and the buildup of lipids, cholesterol, calcium, and cellular debris within the intima of the vessel wall. This buildup results in plaque formation, vascular remodeling, acute and chronic luminal obstruction, abnormalities of blood flow and diminished oxygen supply to target organs (Bibave et al., 2011). Disease progression can lead eventually to the occurrence of acute cardiovascular events such as myocardial infarction, unstable angina pectoris and sudden cardiac death.

Atherosclerosis (AS), hardening (Sclerosis) of the arteries (athero) is a slowly progressing chronic disorder of medium and large- sized
arteries result in plaque formation in the walls of the vessels. Arterial plaque consists of cholesterol, fat deposits, calcium, cellular waste products and excess fluid, which can block off an artery or decrease the rate of blood flow throughout the entire body (Livingston and Lynm, 2012). AS is recognized as a subacute inflammatory condition of the arterial vessel wall characterized by infiltration of T-cells and macrophages which interact with one another and with arterial wall cells (Wildgruber et al., 2013). AS and CVD initiates with the movement of oxidized low-density lipoproteins (OxLDL) to the intima (Subendothelial space) causing injury to endothelial cells (Melo et al., 2011). The pathogenesis of atherosclerosis can be accelerated when there is an imbalance between oxidative stress and the antioxidant defence mechanisms (Griendling and FitzGerald, 2003). Atherosclerosis with complicated pathogenesis involving oxidative stress, endothelial dysfunction and chronic inflammation.

**INFLAMMATION AND Atherosclerosis**

Atherosclerosis is characterized by a complex multifactorial pathophysiology. Inflammation is widely considered to be an important contributing factor of the pathophysiology of coronary heart disease (CHD), and the inflammatory cascade is particularly important in the atherosclerotic process. Inflammation in the vessel wall is now considered to play an essential role in the initiation, progression and the final steps of atherosclerosis, namely plaque destabilization and eventually plaque rupture. Histologically atheromatous plaques obtained at autopsy have demonstrated the presence of inflammatory mononuclear cells with foci of monocytes, macrophages and T lymphocytes in the arterial wall. From a pathological point of view, all stages of the atherosclerotic process, from its initiation to plaque rupture, might be considered an inflammatory response to injury and endothelial dysfunction. Damage to the endothelial wall triggers a cascade of events that modulates the inflammatory response, leading to the recruitment of white blood cells into the blood vessel wall, where they give rise to abnormal foam cells and initiate the development of atherosclerotic lesions (Pfützner et al., 2010).

**Inflammatory markers**

The notion of atherosclerosis as an inflammatory disease is based on the finding that immune competent cells are abundant in
atherosclerotic lesions, and also are producing cytokines, especially proinflammatory cytokines (Frostegard et al., 1999). C-reactive protein level is a strong predictor of cardiovascular events, even more than LDL cholesterol (Ridker et al., 2002). The inflammatory processes are considered to be critical determinants of pathological alterations of the vasculature such as thickening of vessel wall, fatty streak formation, or promotion of atherosclerotic plaques.

In consideration of the important role that inflammatory processes play in determining plaque stability, recent work has focused on whether biomarkers of inflammation may help to improve risk stratification and identify patient groups who might benefit from particular treatment strategies. Among them, CRP is an excellent biomarker of inflammation and plays a pivotal role in many aspects of atherogenesis including, activation of complement pathway, lipids uptake by macrophage, release of proinflammatory cytokines, induces the expression of tissue factor in monocytes, promotes the endothelial dysfunction and inhibits nitric oxide production. Many large-scale prospective studies demonstrate that CRP strongly and independently predicts adverse cardiovascular events.

**OXIDATIVE STRESS AND Atherosclerosis**

Oxidative stress have been reported to play an important role in the pathogenesis and development of cardiovascular diseases, including atherosclerosis (Rizzo et al., 2009). Free radicals which possess an unpaired electron in their outermost shell and are capable of independent existence. Their half-lives vary from a few nanoseconds for the most reactive compounds to seconds and hours for rather stable radicals. They trigger chain reactions resulting in the oxidation of macromolecules in order to reach a steady state. Indirect evidence via observing biomarkers such as reactive oxygen species, and reactive nitrogen species production, antioxidant defense indicates oxidative damage may be involved in the pathogenesis of atherosclerosis (Yudoh et al., 2005).

**Oxidative modification hypothesis** : The oxidative modification hypothesis, based on the concept that LDL becomes entrapped in the subendothelial space where it is subject to oxidative modification by resident vascular cells such as smooth muscle cells, endothelial cells, and macrophages. Accordingly, oxidized LDL contributes to atherogenesis by
1) Stimulating monocyte chemotaxis- aiding the recruitment of circulating monocytes into the intimal space
2) Prevents monocyte egress- inhibiting the ability of resident macrophages to leave the intima
3) supports foam cell formation enhancing the rate of uptake of the lipoprotein leading to foam cell formation
4) being cytotoxic, leading to loss of endothelial integrity.
Apolipoprotein B-100 lysine groups are also modified as a consequence of LDL oxidation so that the net negative charge of the lipoprotein particle increases. This modification of apolipoprotein B-100 renders LDL susceptible to macrophage uptake via a number of scavenger receptor pathways producing cholesterol ester-laden foam cells (Haberland et al., 1984).

MECHANISM OF ATHEROSCLEROSIS
Atherogenesis refers to the development of atheromatous plaques in the inner lining of the arteries. Under normal conditions, endothelial cells play a role in maintaining vessel wall homeostasis by producing vasoactive anti-inflammatory, anti-thrombotic, and cytostatic agents that help to maintain vessel tone and protect the vessel wall against inflammatory cell and platelet adhesion, thrombus formation, and vascular cell proliferation.

Early lesion formation
The initial steps of atherosclerosis include adhesion of blood leukocytes to the activated endothelial monolayer, maturation of monocytes (the most numerous of the leukocytes recruited) into macrophages, and their uptake of lipid, yielding foam cells.
(a) Endothelial activation
Arterial endothelial cells, which normally resist attachment of the white blood cells streaming past them, express adhesion molecules that capture leukocytes on their surfaces when subjected to irritative stimuli (such as dyslipidaemia, hypertension or pro-inflammatory mediators). Parallel changes in endothelial permeability and the composition of the extracellular matrix beneath the endothelium promote the entry and retention of cholesterol-containing low-density lipoprotein (LDL) particles in the artery wall (Tabas et al., 2007). Biochemically modified components of these particles may induce leukocyte adhesion, and intact but modified particles undergo endocytosis by monocyte-derived macrophages, leading to intracellular cholesterol accumulation. Chemoattractant mediators direct the migration of the bound
leukocytes into the innermost layer of the artery, the tunica intima.

(b) Monocyte infiltration and differentiation
Once resident in the artery wall, infiltrated monocytes — the most numerous white blood cells in plaques — proliferate and differentiate into tissue macrophages. These cells are antigen-presenting cells that scavenge lipoproteins and other extracellular debris, generate and degrade lipoproteins and produce inflammatory mediators like cytokines and extra-cellular matrix degrading enzymes (Glass and Witztum, 2001; Bae et al., 2009). It involves the cellular infiltration of several cell types including monocytes, platelets, T-lymphocytes and mast cells.

(b) Foam cell formation
In the nascent atheroma, these mononuclear phagocytes engulf lipoprotein particles and become foam cells — a term that reflects the microscopic appearance of these lipid-laden macrophages. The transformation of macrophages into foam cells in atherosclerotic lesions can be affected by a variety of factors, including inflammatory mediators and nuclear receptors via enhancing or inhibiting the expression of the genes involved in cholesterol uptake and/or efflux. LDL, which may be modified by oxidation, glycation (in diabetes), aggregation, association with proteoglycans, or incorporation into immune complexes, is a major cause of injury to the endothelium and underlying smooth muscle. When LDL particles become trapped in an artery, they can undergo progressive oxidation and be internalized by macrophages by means of the scavenger receptors on the surfaces of these cells. The internalization leads to the formation of lipid peroxides and facilitates the accumulation of cholesterol esters, resulting in the formation of foam cells (Ross, 1999).

Advanced lesion and rupture
(a) Recruitment of smooth muscle cells
Lesion progression involves the migration of SMCs from the tunica media — the middle layer of the artery wall — into the tunica intima. During atherogenesis, other SMCs migrate from the media into the intima, and proliferate in response to mediators such as platelet-derived growth factor. In the intima, the SMCs produce extracellular matrix molecules, including interstitial collagen and elastin, and form a fibrous cap that covers the plaque. The inefficient clearance of dead cells — a process known as efferocytosis — can promote the accumulation of cellular
debris and extracellular lipids, forming a lipid-rich pool called the necrotic core of the plaque (Tabas, 2010).

**(b) Thrombosis**
The ultimate complication of atherosclerosis often complicates a physical disruption of the atherosclerotic plaque. Plaques generally cause clinical manifestations by producing flow-limiting stenoses that lead to tissue ischaemia, or by provoking thrombi that can interrupt blood flow locally or embolize and lodge in distal arteries. Plaques that rupture typically have thin, collagen-poor fibrous caps with few SMCs but abundant macrophages. The inflammatory cells may hasten plaque disruption by elaborating collagenolytic enzymes that can degrade collagen, and by generating mediators that provoke the death of SMCs, the source of arterial collagen (Libby, 2009). Plaque macrophages also produce the pro-coagulant tissue factor that renders the lipid core thrombogenic. Thus, the infiltrating inflammatory cells interact with the intrinsic arterial cells (smooth muscle and endothelium), promoting lesion formation and complications.

Finally, endothelium-derived NO, a vasoactive molecule that helps to maintain vascular tone, is reduced at the site of vascular injury. Decreased NO production is implicated in the clinical course of all known CVD. NO has a number of intracellular effects that lead to vasorelaxation, endothelial regeneration, inhibition of leukocyte chemotaxis, and platelet adhesion and aggregation. Endothelium damage induced by atherosclerosis leads to the reduction in bioactivity of endothelial NO synthase with subsequent impaired release of NO together with a local enhanced degradation of NO by increased generation of reactive oxygen species with subsequent cascade of oxidation-sensitive mechanisms in the arterial wall. Therefore, a reduction in NO activity contributes to a pro-inflammatory and prothrombotic milieu (Napoli et al., 2006).

**ATHEROSCLEROSIS INFLAMMATION AND OXIDATIVE STRESS**

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). Evidence suggests that common risk factors for atherosclerosis increase the risk of the production of free ROS, not only from the endothelial cells, but also from the smooth muscle cells and the
adventitial cells (Gozin et al., 1998). This hypothesis raises questions about the understanding of the pathways that induce the oxidative process, as well as the molecular events in vasculature (Harrison et al., 2003).

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. It has been proven that the main sources of oxidative substances and ROS in atherosclerotic vessels are macrophages and smooth muscle cells (Antoniades et al., 2007). 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. Furthermore, the reduction of endothelial-produced NO and $O_2^-$ is able to blunt normal endothelial dysfunction as a result of the decreased endothelial NO production. The increased production of ROS reduces the production and consequently the bioavailability of NO, leading to vasoconstriction, platelet aggregation and adhesion of neutrophils to the endothelium (Vepa et al., 1999). 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 (Bourcier et al., 1997). Another mechanism through which oxidative stress (by $H_2O_2$) affects atherogenesis is the production of transcription factors such as nuclear factor κB (NF-κB) and activator protein 1 (AP-1), which participate in the expression of adhesion molecules, such as vascular cellular adhesion molecules (VCAM1), intracellular adhesion molecules (ICAM-1), E-selectin and other cytokines. It is well established that NF-κB acts in smooth muscle cells of atherosclerotic vessels and is inactivated by antioxidants and anti-inflammatory agents such as salicylics and glucocorticoids (Tousoulis et al., 2007). Thus, it seems that atherosclerosis is an inflammatory process strongly affected by oxidative stress.

**THERAPEUTIC OVERVIEW**

Immune and inflammatory mediators have a complex role in the initiation and progression of atherosclerosis. Understanding of all these processes will help to invent a range of new biomarkers and novel treatment modalities targeting various cellular events in acute and chronic inflammation that are accountable for atherosclerosis. Several biochemical
pathways, receptors and enzymes are involved in the development of atherosclerosis that would be possible targets for improving strategies for disease diagnosis and management. As a result, several large scale randomized clinical trials and observational studies have shown intensive risk reduction therapy to be very effective and critical in reducing adverse cardiovascular outcomes in patients with atherosclerosis (Mervi et al., 2012).

The role of immunity, as defined by the role of activated T-cells and B-cells, in atherosclerosis is much less known, especially in humans, although novel data indicate that underlying immunological factors predispose to inflammation in humans and that immune modulation altering atherosclerosis is possible in animal models, especially mice (Libby et al., 2011). Therefore, oxLDL could play a role both in atherogenesis and in plaque complications. Mediators of innate and adaptive immunity are involved at various stages of atherosclerosis, as might be anticipated for a chronic inflammatory process (Nilsson, 2005).

Inflammation can potentially be detected locally by imaging techniques as well as emerging techniques, such as identification of temperature or pH heterogeneity. It can be detected systemically by measurement of inflammatory markers. Of these, the most reliable and accessible for clinical use is currently high-sensitivity C-reactive protein. A combination of methods may provide the best identification of persons at risk for cardiovascular events who would benefit from treatment.

**Plants: antioxidant and anti-inflammatory agents**

The effect of antioxidants or their interference with the systems that generate ROS has been proved in animal models of atherogenesis (Griendling and FitzGerald 2003). Anitoxidants have the ability to protect the body from oxidative damage by scavenging the free radicals and inhibiting peroxidation and other radical mediated processes. Plants and herbs are mines of large number of bioactive phytochemicals that might serve as lead for the development of effective, safe, cheap novel drugs. Various phytochemical components, especially polyphenols (such as flavonoids, phenylen propanoids, phenolic acids, tannins, etc) are known to be responsible for the free radical scavenging and antioxidant activities of plants (Nickavar et al., 2007). Polyphenolic compounds constitute a crucial category of antioxidant metabolites. These effects are
mainly attributed to their antioxidant activities in scavenging free radicals, inhibition of peroxidation and chelating transition metals. Some of the plants being used such as, *Amomum subulatum* (Joshi et al., 2012), *Ocimum sanctum* (Suanarunsawat et al., 2010), *Zingiber officinale* (Ozougwu, et al., 2008) *Helianthus Annu* (Raju et al., 2012), *Cassia auriculata* (Vijayaraj et al., 2013), *Coriandrum sativum* (Joshi et al., 2012), *Hibiscus platanifolius* (Saravanan et al., 2011).

The Phytomedicine are more import in the treatment of inflammation. Many medicinal plants have shown to exhibit potent anti-inflammatory effect in the treatment of inflammation by using various models such as *Terminalia arjuna* (Moulisha et al., 2011), *Punica granatum* (Sarker et al., 2012), *Piper sarmentosum* (Zaria et al., 2010), *Pedilanthus tithymaloides* (Abreu et al., 2006), *Monochoria vaginalis* (Chandran et al., 2012).

**CONCLUSION**

In conclusion, attention has increasingly turned to the role of inflammation, in the development of atherosclerosis and CHD. In an attempt to improve global cardiovascular risk prediction, considerable interest has focused on Inflammatory markes. C-reactive protein (CRP) has emerged as one of the most important novel inflammatory markers plays a pivotal role in many aspects of atherogenesis. CRP levels can be measured inexpensively with available high-sensitivity assays, and have shown specificity in terms of predicting the risk of cardiovascular disease. The oxidation of low density lipoprotein (LDL) has been recognized to play an important role in atherosclerosis. Immune system cells called macrophages recognize and engulf oxidized LDL, a process that leads to the formation of atherosclerotic plaques in the arterial wall. In addition, supplementation with exogenous antioxidants or boosting of endogenous antioxidant defenses of the body has been found to be a promising method of countering the undesirable effects of oxidative stress (Kasote et al., 2013). World is endowed with a rich wealth of medicinal plants. Numerous drugs have entered the international through exploration of ethnopharmacology and traditional medicine. Although scientific studies have been carried out on a large number of Indian botanicals, a considerably smaller number of marketable drugs or phytochemical entities have entered the evidence based therapeutics. Efforts are therefore needed to establish and validate evidence regarding safety and practices of herbal medicines.
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