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Review Article

Smoking and Platelet Function

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ABSTRACT

Smoking is considered among the strongest environmental pollutants and represents a recognized high risk factor for cardiovascular diseases. Adverse cardiovascular consequences of cigarette smoking are serious and diverse as coronary heart disease, peripheral vascular disease, aortic aneurysm, myocardial infarction, cerebrovascular disease, worsened outcomes after angioplasty or bypass surgery, increased hypertension complications and sudden death. Of particular importance, there is very strong evidence that smoking actively enhances platelet activity and highly promotes platelet aggregation. This increases greatly the risk of intravascular thrombosis. At the level of coronary arteries, thus, smoking seriously amplifies the risk of coronary heart disease which may be complicated by higher mortality among middle-aged individuals. Smoking-induced platelet aggregation may be effected by several mechanisms for example enhanced inflammatory reactions, reduced monoamine oxidase activity and elevated levels of certain growth factors as platelet activating factor and thrombopoietin. Further, smoking may alter fibrin clot formation and structure encouraging intravascular clotting. On the other hand, chronic smoking may significantly impair the cardio-protective properties of certain drugs thus reduce their effectiveness in management of cardiovascular disease. Although some novel markers may be used to determine enhanced platelet activity and new therapeutic tools could be valuable in reducing adverse consequences of smoking on platelet function, yet physicians should continue to encourage and help all their smoking patients to quit.

KEY WORDS: Smoking, platelets, monoamine oxidase, inflammation, thrombosis, thrombopoietin, serotonin, fibrin clot.

INTRODUCTION

Tobacco use is the leading cause of preventable death and morbidity worldwide. Smoking is a modifiable risk factor mainly for cardiovascular diseases, pulmonary diseases and cancer. This addictive disorder is responsible for about 20% of deaths among which cardiovascular disease (CVD) is the leading cause of mortality with major prevalence among people less than 50 years of both sexes. [1, 2]

Smoking may also enhance platelet aggregation and promote intravascular thrombosis through various pathways. [3] Increased platelet count has been observed in adolescents who recently have started smoking. [4] Smoking influences platelet life time by shortening platelet survival half-life (less than 92 hours) in healthy persons. [5] At the level of coronary arteries, smoking-induced atherosclerosis and thrombosis are active players that mediate the pathogenesis of CVD. This review discusses the adverse effects of smoking on platelet activity as well the mechanistic through which smoking modulates the process of thrombosis.

SMOKING, ENDOTHELIUM AND PLATELET FUNCTION

In addition to effects of smoking on atherosclerosis, smoking promotes coronary artery thrombosis by increasing platelet adherence to endothelium and platelet aggregation. Platelet activation is one of the major factors by which tobacco smoke mediates the pathogenesis of CVD, which may be related to endothelial dysfunction and/or direct effects of oxidant chemicals. [6]

Actually, there is recent evidence that environmental tobacco smoke may adversely affect vascular endothelium both by direct injury and interference with vascular repair.[7] Endothelial dysfunction

significantly decreases basal release of nitric oxide (NO), which normally inhibits platelet activation.[8] Accordingly the serious possibility of intravascular thrombosis is heightened. Further, smoking reduces release of tissue plasminogen activator (tPA), associated with coronary atherothrombosis, while enhances secretion of plasminogen activator inhibitor-1. Thus tobacco smoke markedly reduces chance of intra-coronary fibrinolysis adding another risk element. [9,10]

SMOKING-PLATELET INTERACTION ADVERSELY AFFECTS CORONARY ARTERY

Smoking is an established major risk factor for CVD.[11] Platelet aggregability has been observed to be significantly higher in smokers than in nonsmokers.[12] Smoking-enhanced platelet thrombosis may be an important contributory mechanism to acute coronary events in smokers. Coronary heart disease (CHD) patients who are smokers have worse outcomes of urgent revascularization, myocardial infarction or death, than nonsmokers.[13]

Smoking two unfiltered tobacco cigarettes acutely increased circulating platelet aggregates in healthy volunteers and in patients with CHD as detected by lowering of the platelet aggregate ratio.[14] Biological markers of platelet activity, for example, platelet factor 4 which is regarded as a platelet-specific protein (content of platelet alpha-granules) and thromboxane A_2 and its metabolites were significantly higher in smokers than in nonsmokers. Furthermore, levels of thromboxane A_2 and its metabolites increased with daily cigarette consumption.[12, 15]

Following administration of cigarette smoke, spontaneous reductions of coronary blood flow in a stenosed circumflex artery were greatly exacerbated compared to the flow reduction speed before smoking, and morphometrically-measured platelet thrombus formation on arterial media increased significantly.[16] Thus it is possible that smokers are more susceptible than nonsmokers to develop an acute occlusive platelet thrombus in a diseased and stenotic coronary artery. In fact, quantitative coronary angiographic analysis has suggested that the mechanism of infarction in smokers is more often thrombosis of a less critical atherosclerotic lesion compared with nonsmokers.[17]

SMOKING-INDUCED INFLAMMATORY RESPONSE & PLATELET ACTIVATION

Cigarette smoke and its extracts induce diverse inflammatory events whose cumulative effects could contribute to cigarette smoke–related pathology. Smoking elicits the recruitment and adhesion of circulating leukocytes to the vessel wall, the initial step in inflammation and atherosclerosis.[18-20] Due to smoking-induced chronic inflammation, cigarette smokers have increased serum C-reactive protein (CRP), and appear to have elevated plasma sCD40L concentrations. Smokers also have increased surface expression of CD40 and CD40L on monocytes. Plasma cotinine (a nicotine metabolite) concentration correlates with monocyte CD40 and CD40L expression. [21]

Cigarette smoke could induce the inflammatory response through a direct effect on target cells as cigarette smoke extracts induce expression of adhesion molecules on endothelial cells.[18,22] Cigarette smoking, within minutes, induces leukocyte adhesion to the vascular wall and formation of intravascular leukocyte-platelet aggregates. Additional secondary effects of cigarette smoke may extend this response through induction of fast release and accumulation of platelet activating factor (PAF)-like lipid mediators in the blood.[23, 24] Such mediators are formed by non enzymatic oxidative modification of existing phospholipids that are distinct from biosynthetic PAF.[25]

Cigarette smoke introduces a high burden of radicals into the organism and also stimulates the generation of further radicals and reactive oxygen species from activated leukocytes.[26-29] The increased burden of reactive species in the plasma of smokers creates a high oxidative stress that exhausts particularly the antioxidant vitamin C and GSH.[30,31] This is followed by accumulation of various lipid oxidation products in the blood.[32,33] These products activate inflammatory cells through the PAF receptor, a reaction that induces monocytes and platelets to aggregate resulting in increased secretion of IL-8 and macrophage inflammatory protein-1α. Such events can be blocked by PAF receptor antagonists restoring greatly normal pavement along the vascular wall.[25]

Interestingly, dietary supplementation with the antioxidant vitamin C prevents the accumulation of PAF-like lipids, and it prevents cigarette smoke–induced leukocyte adhesion to the vascular wall and formation of leukocyte-platelet aggregates. After administration of vitamin C, intraplatelet vitamin C levels increase, platelet-derived NO is released and intraplatelet cGMP levels and platelet aggregation in smokers are restored to the levels of nonsmokers.[7]

Thrombopoietin- platelet interaction in smokers:

Enhanced platelet aggregability and subsequent alterations in the clotting cascade have been evoked as main pathogenic factors sustaining the increased risk of coronary artery thrombosis in long-term smokers.[34-36] Besides Inflammation plays a central

role in the pathogenesis of atherosclerosis and its complications.[37]

Thrombopoietin (TPO) is a humoral growth factor originally identified for its ability to stimulate the proliferation and differentiation of megakaryocytes. TPO is constitutively produced in the liver and kidneys and is then cleared from circulation upon binding with its receptors which are expressed by mature platelets.[38-40]

In addition to its actions on thrombopoiesis, TPO directly modulates the homeostatic potential of mature platelets by influencing their response to several stimuli. Chronic smokers have higher circulating TPO levels than nonsmokers and higher platelet activation indicated by higher platelet-monocyte aggregation.[41] Circulating TPO does not induce platelet aggregation *per se* but is able to facilitate platelet activation and enhance platelet aggregation by sensitizing platelets to the action of other agonists ("priming effect").[40] Indeed, TPO stimulates platelet-leukocyte associations in whole blood through expression of platelet P-selectin.[42]

Chronic ex-cigarette smokers show a significant decrease in TPO level associated with reduced platelet-monocyte and platelet-granulocyte bindings.[41] So, TPO may contribute to enhance platelet activation and platelet monocyte cross-talk in cigarette smokers. Actually, increased TPO may represent a novel pathogenic mechanism whereby cigarette smoking promotes atherogenesis and is associated with the development of adverse cardiovascular events.[41]

Platelet/vessel wall interaction in smokers:

Smokers' platelets were shown to be less sensitive to the anti-aggregatory action of exogenous prostaglandin I (PGI), as compared with non-smokers'.[43] Both antigen and activity of tPA and plasmin-inhibitor complex as markers of fibrinolytic activity in vivo markedly increase after smoking, and smokers with vascular disease might be more susceptible to a state of disequilibrium in favor of coagulation.[44] Platelet/vessel wall interaction is stimulated in smokers as indicated by a higher serum Tx-M level in smokers than in nonsmokers.[45]

MONOAMINE OXIDASE-PLATELET INTERACTION IN SMOKERS

Smoking inhibits monoamine oxidase (MAO):

MAO enzymes are integral proteins of outer mitochondrial membranes devoted to the organism homeostasis protection. They constitute major enzymes which occur in various cells, both neuronal and non-neuronal in the central nervous system (CNS) and peripheral organs. Their main function is to proceed an oxidative deamination of biogenic amines, both exogenous (tyramine) and endogenous (norepinephrine (NE), dopamine and 5-HT) in peripheral tissues and brain. Their inhibition, by either exogenous or pharmaceutical substances, leads to the increase of the above monoamines and has major consequences particularly in the CNS.

MAO activity is modified by smoking and is significantly lowered in heavy smokers.[46] Enzyme inhibition occurs both in smokers' peripheral tissues and different organs especially lungs, kidneys, brain and spleen as well as in platelets.[47,48] Large distribution of MAO accounts for the implication of these enzymes in cardiovascular diseases, pulmonary diseases, cancer and affective disorders.[1]

There are two MAO isoenzymes, MAO-A and MAO-B, which differ by their substrate specificity and even more by inhibitor selectivity. High concentrations of MAO-B are found in blood platelets. End products of the MAO action on bioamines are aldehydes and H_2O_2 involved in oxidative processes. Aldehydes are either oxidized into 5-hydroxy indol acetic acid (5-HIAA) for 5-HT catabolism or reduced into 3,4-dihydroxyphenylglycol (DHPG) for norepinephrine catabolism.

Serotonin and platelet activity:

Smoker's platelets are activated, while cigarette smoke may directly cause platelet activation in passive smokers.[21,49,50] Platelet activation is associated with progression of atherosclerosis and precipitation of events that cause stenosed arteries.[51,52] Activated platelets release their granule content which leads to the exposition of new adhesive receptors and secretion of cytokines, RANTES and growth factors.[21,53] Among the released mediators, serotonin is quantitatively the major one considering its millimolar concentration within platelet dense granules. High plasma serotonin contributes to activate lung vascular receptors and mediate pulmonary hypertensive complications.[54-56] Serotonin induces smooth muscle cell proliferation and contributes to inflammatory and thrombotic activation of the vessels.[57-59] In atherosclerotic lesions, platelet serotonin granule secretion plays a critical role in vascular remodelling.[60]

MAO as a novel risk marker of smoking-induced platelet activation:

The MAO-B amount correlates significantly with the number of atherosclerotic plaques and the number of atherosclerotic sites, especially the femoral sites.[61] It also correlates with the duration of smoking.[1] It is well established that smoking inhibits MAO.[1] Interestingly former smokers show lower platelet 5-HT content but higher plasma level of 5-HIAA.[61] In

smokers and former smokers the smoking-induced inhibition of MAO activity is counter-balanced by higher enzyme amounts. After quitting smoking, MAO activity is no longer inhibited but the high amount of protein results in a higher 5-HT degradation into 5-HIAA.

Surprisingly the high smoking-induced platelet MAO-B amount remains elevated several years after quitting smoking (mean 13 years). A persistent increase in the amount of MAO-B lasting over 10 years after quitting smoking suggests a modification at the gene level. Indeed the smoking-induced variations of MAO expression are explained by a reduced methylation level of the MAO-B promoter resulting in a more active transcription of the MAO and hence a higher MAO amount.[61] The higher the MAO-B promoter is methylated, the lower MAO amount is found. Unfortunately, the high formation of 5-HIAA from 5-HT via MAO may be accompanied by the generation of ROS which participate in smooth muscle cell proliferation, hypoxia, respiratory distress and cancer growth .[62-65] A reversal of increased MAO-B protein synthesis may not occur until about 20 years after quitting smoking.[1]

FIBRIN CLOT STRUCTURE AND FUNCTION IN SMOKERS

The formation of fibrin clots that are relatively resistant to lysis represents the final step in blood coagulation. Fibrin clots composed of compact, highly branched networks with thin fibers are resistant to lysis. Smoking cessation increases clot permeability and susceptibility to lysis. Growing evidence indicates that abnormal fibrin properties represent a novel risk factor for arterial and venous thrombotic events, particularly of unknown etiology in young and middle-aged patients.[66]

Platelet activation and fibrin clot formation:

Nicotine stimulates the release of catecholamines that promote platelet aggregability.[67] Proteins released from platelets alter clot properties, particularly at sites of platelet aggregation. Increased amounts of platelet factor 4 are associated with the formation of a compact clot structure.[68] Polyphosphate, a negatively charged polymer of inorganic phosphate secreted from dense granules, also modifies the fibrin network and its plasmin-mediated degradation.[69,70] The effects of polyphosphate on clot structure are calcium dependent and independent from FXIII activation.[69] Polyphosphates lead to the formation of tight fiber aggregates interspaced with large pores.[71] Fibrinolysis is impaired because of reduced binding of plasminogen tPA to partially lysed fibrin.[71] Platelets also release PAI-1 that contributes to impaired fibrin degradation and the role of PAI-1 in clot lysis increases with the number of platelets.[71]

Smoking-fibrinogen interaction and clot formation:

There is strong evidence that cigarette smoking is markedly associated with elevated fibrinogen levels. Fibrin clots that may develop upon acute exposure to cigarette smoke are mostly denser and composed of thinner fibers compared to those observed in non smokers or ex-smoker individuals.[72] Interestingly, acute smoking could be associated with significant lower clot lysis as evidenced by thromboelastography performed in whole blood before and after smoking.[73] Further, apparently healthy individuals who have been smoking for 5 years or more show marked lower clot permeability and longer clot lysis compared with never smokers.[74] Such smoking-related fibrin clot abnormalities may be largely explained by elevated fibrinogen associated with enhanced oxidative stress.[74]

ASPIRIN AND PLATELET ACTIVITY

Aspirin is well recognized as an effective antiplatelet drug for secondary prevention in subjects at high risk of cardiovascular events. The primary effect of aspirin on hemostasis is to acetylate platelet cyclooxygenase-1 (COX-1) and thereby inhibit the synthesis of thromboxane A_2, a powerful platelet agonist.[75] Because platelets lack the biosynthetic machinery necessary to synthesize new protein, acetylation of platelet COX-1 by aspirin is rapid, irreversible, and permanent (for the life of the platelet), and COX-1 is believed to be saturable at low doses.[76] Acetylation of platelet COX-1 does not attain functional relevance until the maximal capacity to generate thromboxane A_2 is reduced by at least 95%.[77] Actually, very small amounts of residual COX-1 activity can generate sufficient amounts of thromboxane to support thromboxane-dependent platelet function. Thus, as much as 99% inhibition of serum thromboxane may be necessary to efficiently inhibit platelets.[78] An association between platelet concentration or aggregability and long-term incidence of fatal CHD has been observed in a population of apparently healthy middle-aged men.[79]

Aspirin anti-platelet effect in smoking patients with CHD:

Some studies have suggested that aspirin can inhibit augmented platelet aggregability in healthy smokers. Aspirin administration (20 mg, twice daily) restores normal Tx-M excretion level that is paralleled to the recovery of platelet cyclooxygenase in healthy smokers.[80] However, smoking-stimulated platelet

aggregation and release of the contents of platelet alpha-granules are hardly affected by pre-administration of aspirin in patients with CHD.[81]
Low-dose of aspirin used in common clinical practice to inhibit platelet thromboxane production and prevent CHD in most non-smokers may not be entirely effective in smoking patients with CHD. While low-dose aspirin can not sufficiently inhibit the platelet aggregability in smokers with CHD; both clopidogrel (adenosine diphosphate [ADP] receptor antagonist) and higher-dose aspirin (325 mg/d) can effectively inhibit platelet activity.[45]

Clinical implications:
Routine low-dose aspirin, that is effective in preventing CHD in most non-smokers, may be unable to inhibit the augmented platelet aggregability in smokers with CHD. This partially explains the higher morbidity and mortality in these patients. Therefore, it is important for patients with CHD to quit smoking. For current smokers with CHD, it may be necessary to take larger-doses of aspirin than normal or take ADP receptor inhibitors along with aspirin to effectively inhibit the augmented platelet activity. Statins might reduce the platelet activity by inhibiting inflammation that results from smoking.

CONCLUSION

Several studies have suggested that smoking can enhance platelet aggregation through various pathways. Smoking may promote coronary artery thrombosis due to endothelial dysfunction that decreases NO release and enhances platelet adherence to endothelium. In addition smoking evokes release of several oxidizing mediators which produce inflammatory reactions that participate in increased susceptibility of platelet aggregation. CRP and TPO show higher levels in smokers. Also PAF-like lipids are highly produced and share in heightening the oxidative stress that consumes particularly Vitamin C as a potent anti-oxidant. Use of PAF receptor antagonists along with high vitamin C supplementation may alleviate smoking-induced oxidative stress.
Platelet MAO-B activity and amount is significantly altered by smoking favoring platelet aggregation in active smokers. Smoking evidently reduces MAO-B activity due to oxygen deprivation. However, this initial effect may be compensated for, at least partly, by enhanced methylation of the MAO-B promote resulting in increased MAO-B protein synthesis. MAO-B could be a risk biomarker of smoking-induced morbidity, due to the role of the bioamines/MAO system in all smoking related diseases. This risk factor can easily be measured from a blood platelet sample. The potential value of MAO-B as a smoking biomarker might therefore be of public health relevance to help in individual susceptibility evaluation and disease diagnosis and prognosis.
Up to ten years after smoking cessation, although platelet aggregation susceptibility is significantly reduced yet increased intraplatelet 5-HT metabolism by higher MAO-B concentrations may produce certain mediators that stimulate smooth muscle proliferation and even cancer growth. Smoking also may be associated with elevated fibrinogen content and higher intravascular clotting chance. All together smoking-induced effects highly increase incidence of intracoronary thrombosis and clot formation creating a high risk factor for CHD and its serious consequences. Smoking also reduces activity of aspirin as a potent antiplatelet drug thus greatly alters its effectiveness as an aid of coronary protection. Continuous efforts are needed both to help smoking cessation and develop better therapeutic strategies to decrease the morbidity and mortality of CHD in cigarette smokers.

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