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Insights into pathophysiology of smoke-related cardiovascular disease

Approfondimenti nella fisiopatologia delle patologie cardiovascolari correlate al fumo

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ABSTRACT: ***Insights into pathophysiology of smoke-related cardiovascular disease. S. De Rosa, M. Pacileo, L. Sasso, V. Di Palma, P. Maietta, A. Paglia, L. Brevetti, P. Cirillo, M. Chiariello.***

The deleterious effects of cigarette smoke (CS) on cardiovascular morbidity and mortality are well established. Both active and passive smoking represent a major health hazard for both men and women. The great concerns related to the deleterious effects of CS on cardiovascular disease have been translated into various kinds of social interventions and targeted health policies since ever.

The high health impact of cigarette smoking has driven a huge number of researches at the epidemiological, clinical and biological level. Nevertheless, even though many progresses have been made in understanding the mechanisms underlying the high disease burden associated to cigarette smoke, the exact components and the mechanisms by means of which it exerts its effects remain to be completely clarified as yet.

The present paper reviews the main observations on the pathophysiology of smoke-related cardiovascular diseases, providing an up-to-date perspective about one of the main cardiovascular killers of our days.

Keywords: cigarette smoke, cardiovascular disease, atherosclerosis, thrombosis, pathophysiology.

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The deleterious effects of cigarette smoke (CS) on cardiovascular morbidity and mortality are widely recognized, as it represents a major health hazard for both men and women. In particular, both active and passive smoke have been associated with significant increase in risk of developing vascular disease and its complications [1-3]. Nevertheless, the challenging question that smokeless tobacco has also been shown to impact CAD risk has raised the interest of physicians worldwide.

The big concerns related to the deleterious effects of CS on cardiovascular disease have been translated into various kinds of social interventions and targeted health policies since ever. In 1987, Europe was the first of WHO's regions to take the initiative of launching a regional action plan on tobacco called for a comprehensive approach: The First European Action Plan on Tobacco 1987-1991 [4]. Still nowadays, all the scientific organizations of the industrialized world are addressing their efforts towards tobacco control [2, 3].

Altogether, many progresses have been made in the field of tobacco related disease, both at scientific and social levels. However, although the evidence linking cigarette smoking with cardiovascular risk is clearly established, yet the exact components of cigarette smoke and the mechanisms by means of which it exerts its effects remain to be completely clarified.

Components of cigarette smoke

It has been assessed that cigarette smoke contains over 5000 chemicals [5, 6] in various degrees. Thus, it has been very difficult to identify which component is responsible for the relevant effects exerted by cigarette smoking on atherosclerosis and cardiovascular disease.

However, CS-derived compounds can be catalogued in two different phases: the particulate or tar phase contains all particulate material bigger than 0,1µm, that can be trapped by a Cambridge glass-fiber filter, while the gas phase represents all the material that passes the filter.

Both phases contain elevated concentrations of reactive oxygen species (ROS) known to exert a wide spectrum of deleterious cellular effects [7]. In addition, the gas phase contains also several stable compounds that have the potential to increase intracellular production of ROS [5], or that have direct effects on cell populations that are actively involved in pathophysiology of cardiovascular diseases such as cells of the vascular wall (endothelial and smooth muscle cells, macrophages) or blood cells (platelets, mononuclear leukocytes).

Among these compounds, unsaturated aldehydes, unsaturated ketones, and nicotine seem to play a detrimental role for human health. Several evidences indicate nicotine as the main active component of ciga-

Table 1. - Some of the toxic substances found in sigarette smoke

Gas Phase	
Carbon dioxide	Carbon monoxide
Nitrogen oxides	Ammonia
Hydrogen cyanide	Hydrazine
Formaldehyde	Acetone
Acrolein	Acetonitrile
Pyridine	3- Vinylpyridine
N-Nitrosodimethylamine	N-Nitrosoethylmethylamine
N-Nitrosodiethylamine	N-Nitrosopyrrolidine
Particulate Phase	
Nicotine	Toluene
Phenol	Catechol
Stigmasterol	Naphthalene
1-Methylnaphthalene	2-Methylnaphthalene
Phenanthrene	Benz(a)anthracene
Pyrene	Benzo(a)pyrene
Quinoline	Methylquinoline
Harmane	Norharmane
Aniline	o-Toluidine
1-Naphthylamine	2-Naphthylamine
4-Aminobiphenyl	N'-Nitrosonornicotine
NNK	N'-Nitrosoanatabine
N'-Nitrosodiathanolamine	

rette smoke [8-10]. A similar important role has been described for cotinine, the main nicotine metabolite in humans [11]. In particular, nicotine is known to have a pivotal role in modulating the hemodynamic effects of CS. Indeed, nicotine causes important changes of different cardiac functions such as heart rate, myocardial contractility, cardiac output, stroke volume and coronary blood flow [12, 13]. Moreover, Ball and Turner have observed that nicotine induces vasoconstriction in systemic, cutaneous and coronary vascular beds [14]. In addition, this smoke derivative substance might exert effects on blood lipid profile, promoting hyperlipidemia, with increased VLDL and decreased HDL serum levels. Finally, it has influence on vascular wall causing endothelial injury and intimal hyperplasia, platelet activation with subsequent thrombosis and increases plasma levels of catecholamines, as well as of fibrinogen [15, 16].

Mechanisms of action

Many efforts have been made to explain how cigarette smoke affects cardiovascular system. Thus, several hypothetical mechanisms of action have been suggested. Some of them have been scientifically well documented, while others still remain speculative.

Nicotine is by far the most studied component of CS. Among the various mechanism proposed for its action, the more investigated and well documented are mediated through direct interaction with a surface receptor. These did not raised much interest at the beginning. In fact, as nicotine was considered just as the "addictive substance" in CS, nicotinic acetylcholine receptors (nAChR) were considered to be responsible only for the psychoactive actions and addiction properties of tobacco smoke [17]. However, several subsequent evidences demonstrated that this receptor was expressed by non neural cells and that nicotine had direct effects on it, giving a big impulse to research focusing on this approach [18, 19]. In fact, the high density of nAChR on endothelial surface has led to speculations about their pathogenic role in cardiovascular disease [20]. Moreover, deleterious cardiovascular effects of nicotine through stimulation of autonomic nervous system have also been proposed. An intriguing perspective brought about by Yun et al, is that intermittent nicotine exposure may paradoxically induce reflex adrenergic over-activity and thereby contribute to tobacco related diseases [21]. The described "sympathetic bias" has been suggested to be responsible for many other diseases or conditions associated with CS-related cardiovascular diseases, such as type II diabetes, lipid dysfunction, endothelial dysfunction, vasoconstriction or hypercoagulability [22-24].

Oxydative stress is a well documented mechanism involved in the pathophysiology of cardiovascular disease [25-27]. Interestingly, this phenomenon has also been proposed to be responsible for the cardiovascular effects of cigarette smoking. In fact, CS contains big amounts of short-lived, highly reactive compounds [7]. In addition, other chemical components of CS have been described as able to induce ROS production in various cell populations [5], or from other endogenous sources [28-30]. Many of the deleterious effects exerted by CS on cardiovascular system, such as endothelial dysfunction, vascular inflammation, hypercoagulability, lipid peroxidation and reduced fibrinolysis could be explained by effects of oxidative stress [7, 28-32]. Finally, this hypothesis is strongly supported by the

Table 2. - Effects of cigarette smoking on atherosclerosis and cardiovascular disease

Pathologic effects

Vascular intimal injury
Smooth muscle cell proliferation
Atherosclerosis

Physiologic effects

Increase in:
- heart rate
- blood pressure
- cardiac output
- myocardial oxygen and nutrient consumption
- peripheral vascular resistance

Lower ventricular fibrilation thresh hold
Arrhythmias
Impaired coronary artery flow

Hematologic effects

Atherosclerosis
Thrombosis
Altered function of:
- prostaglandin, platelets
- fibrinogen
- plasminogen

Reduced oxygen carrying capacity of hemoglobin

Metabolic effects

Increased level of:
- serum free fatty acid
- LDL cholesterol
- growth hormone
- cortisol
- glucose
- antidiuretic hormone
- glycerol
- lactate
- pyruvate

Decreased levels of:
- serum HDL cholesterol
- estrogen

Altered metabolism of medications

observations that drugs with documented antioxidant and/or scavenger effects or able to interfere with the intracellular redox status, might prevent CS effects both in humans and in experimental models [33-36].

Among the huge number of components that have been found in CS, also carbon monoxide (CO) and polycyclic aromatic hydrocarbons have been investigated [37, 38]. However, their role in CS-induced cardiovascular disease is poorly documented and still controversial [39, 40].

Effects of cigarette smoke on the vascular wall

Cigarette smoke has been shown to have several relevant effects on cells of the vascular wall. In particular, CS seems to increase vascular permeability and to activate the pathways of local inflammation [41-44].

Endothelial cells

Cigarette smoke, and particularly nicotine, exerts powerful effects on endothelial cells, changing the pattern of expression of several genes, some of which are directly correlated with the development of cardiovascular disease [45]. In particular, CS promotes the expression of genes encoding for molecules directly involved in the development of athero-thrombotic disease, such as adhesion molecules and Tissue Factor (TF). Adams *et al.* have shown an augmented expression of functionally active ICAM-1 on human umbilical vein endothelial cells (HUVECs) stimulated with serum obtained from smokers [35], while Wang *et al.* reported that nicotine stimulates adhesion molecules expression through an increase of intracellular free Ca^{2+} and consequent activation of MAP kinases [46]. In addition, Albaugh *et al.* reported a marked increase in mononuclear leukocytes (MNL) adhesion to HUVECs, after incubation of this vascular cells with nicotine, which was able to induce adhesion molecules on their surface [47]. Finally, our group has demonstrated, in human coronary artery endothelial cells (HCAECs), that nicotine induces expression of functionally active ICAM-1 and VCAM-1 and that this phenomenon occurs through RhoA-mediated activation of the transcription factor NF-kB [48]. These experimental observations obtained in vitro have been confirmed by in vivo studies demonstrating increased expression of adhesion molecules and of pro-thrombotic molecules in atherosclerotic plaques of smokers [49].

Several clinical findings have demonstrated a higher degree of activation of the coagulation pathway in smokers [16, 51]. Indeed, CS promotes thrombotic events stimulating platelet aggregation, as described below, and altering the balance between pro-thrombotic and anti-thrombotic molecules across the vascular wall and in the bloodstream. Experimental data have shown, for example, that HUVEC exposed to smokers' serum show a significant reduction in both basal and stimulated t-PA release, resulting in an alteration of anti thrombotic proprieties of these cells [50]. This result received also further confirmation from the findings of other groups, that observed reduction of plasma levels of t-PA antigen as well as of t-PA activity in smokers [51, 52]. This reduction of t-PA levels goes with the increased expression of molecules with pro-thrombotic activity such as Tissue Factor (TF). Matetzky *et al.* have demonstrated, with immunohistochemistry study, increased expression of TF in atherosclerotic plaques of smokers [49]. This results has been confirmed in vitro by the recent finding of our group that nicotine induces TF expression both in coronary endothelial and in aortic smooth muscle cells [53].

The reduced bioavailability of NO associated with CS also contribute to the pro-thrombotic action of smoke [54]. In particular, thiol-reactive stable compounds of CS can activate NADPH oxidase in endothelium, reducing nitric oxide (NO) bioactivity [55] and Barua *et al.* observed that smokers' serum reduced NO production both in HUVECs [56] and in HCAECs [57]. In a more recent work, cigarette smoke extract (CSE) was also shown to reduce NO production by endothelial cells [58].

Bernhard *et al.* recently observed that exposure of endothelial cells to CS caused depolymerization of microtubules and cells contraction. This phe-

nomenon led to increased vascular permeability, which finally favoured the spreading of inflammatory cells across the vascular wall [59]. Increased of vascular permeability had been also previously described, although without reporting any pathophysiological mechanism, both in vitro [60] and in vivo models [61].

Smooth muscle cells

Several independent groups have reported various effects of nicotine on vascular smooth muscle cells (VSMCs). Nicotine has mitogenic properties on VSMCs [62]. In Di Luozzo *et al.* have reported that the mechanism beyond this effect of nicotine is the activation of mitogen-activated protein kinases (MAPK) [63], while Pestana *et al.* reported the involvement of nicotinic and PDGF receptors in the determinism of this effect [64]. Similar effects of nicotine have been reported in endothelial cells, even though data regarding this phenomenon remain controversial [65, 66].

Recent findings have demonstrated that VSMCs express the platelet receptor P2Y12 and that activation of this receptor causes cellular contraction increasing vascular permeability [67]. Interestingly, nicotine seems able to up-regulate expression of this receptor in VSMCs and in other cell populations suggesting a role for this smoke derivative substance in promoting platelet adhesion on vascular wall [68].

Effects of cigarette smoke on leukocytes

Cigarette smoke induces about a 20-25% increase in peripheral blood leukocytes and this increase has been described as related to an higher incidence of myocardial infarction [69]. As it has been discussed in the previous paragraph, CS has various effects on the arterial wall that are responsible for increased leukocytes recruitment and trans-endothelial migration. It has been observed that monocytes isolated from smokers present higher than normal expression of CD 11b/CD 18, an integrin which increases adhesiveness of monocytes to cells of the vascular wall [33].

Adhesion of leukocytes to the vascular wall is not just a mechanical process, it rather represents the first step towards trans-endothelial migration and activation of both endothelial and mononuclear cells themselves [70, 71]. It has been reported that cigarette smoke extracts (CSE) induce the expression of different pro-inflammatory cytokines [72-74]. Release of these cytokines could be responsible for augmented trans-endothelial migration of leukocytes and induction of several inflammatory responses [75, 76]. Nevertheless, this mechanism has been questioned after the recent findings, in epithelial cells, macrophages and human peripheral blood mononuclear cells (PBMCs) that CSE could even inhibit the production of some pro.inflammatory cytokines [77-79].

CS has relevant effects on lymphocytes. A recent study has reported that heavy smoke influences gene expression profiles of T lymphocytes [80]. In addition another study has proposed that one possible mechanism for induction of the chronic inflammatory status related to CS may involve peroxynitrite-induced activation of NF-kB in lymphocytes [81].

Nicotine has been shown to affect also macrophages, a cell population with a key role in atherosclerosis. For example, Lau *et al.* reported that nicotine induces pro-inflammatory responses in macrophages and in the aorta of LDL receptor -/- mice [10]. Macrophages activation by nicotine also results in increased release of TNF-α and IL-1β, that in turn up-regulate the expression of adhesion molecules on HUVECs and increase adhesion of monocytes [82]. Furthermore, CS-related macrophage activation induces release of major inflammatory mediators suggesting that they could be responsible for the inflammatory processes associated with smoke habitus [83]. Of interest, it has been shown that CS activates PBMCs, and in particular neutrophils, which in turn induce Toll-like receptor-4 mediated cytokine production by human macrophages. These last findings link cigarette smoke to inflammation and innate immunity, bringing about new insights in the pathogenesis of tobacco-related disease [84].

Another interesting phenomenon is that smoking and, more specifically nicotine, are able to promote the immune shift from T helper (Th)1 to Th 2 [85]. Even though the exact mechanisms by which CS exerts these effects are not definitively understood, it has been recently proposed that intermittent nicotine exposure may lead to receptor desensibilization and paradoxical antagonism of vagal function [21]. Consequently, increased sympathic activity would be responsible for the shift of Th balance to Th2-driven immune response [22].

Effects of cigarette smoke on platelets

It has been reported that platelets from smokers present an increased aggregation, both spontaneously and after stimulation [86, 87]. In addition, Blache described hyperaggregability in platelets that have been exposed to smoker's serum [88]. In line with these data is the finding that nicotine induces expression of P2Y12, the major platelet receptor that mediates ADP-induced aggregation, not only in endothelial (HCAECs, HUVECs) and smooth muscle cells (HASMCs), but also in human megakaryoblastic cells (MEG-01) [68]. Another mechanism proposed for CS effect on platelet aggregation and thrombosis is a reduced availability of platelet-derived NO, as well as a reduced sensitivity of platelets to exogenous NO [89, 90]. As a matter of fact, there is an overall reduction of NO bioavailability in plasma of cigarette smokers [58], while also a significant reduction of platelet eNOS mRNA levels has been observed [91].

Cigarette smoke and peripheral arterial disease (PAD)

Recently, the Edinburgh Artery Study was designed to investigate whether CS-influence on the

development of peripheral or coronary artery disease might be mediated by other cardiovascular risk factors. In its final report, the authors concluded that the combined effect of smoking on other cardiovascular risk factors could partly explain its influence on peripheral and coronary arterial disease, even though the majority of the effect could not be explained at all, appearing to be due to other mechanisms [92].

Another controversial question is the observation that CS seems to correlate much more strongly with peripheral arterial disease [64] than with coronary artery disease (CAD). Various clinical evidences have strongly correlated smoke habit with PAD. In the Atherosclerosis Risk in Communities (ARIC) Study, smokers had a significantly higher risk of developing PAD over non-smokers [93, 94]. Nevertheless, the stronger association of smoking with PAD than with ischemic heart disease has not been explained yet [95].

Effects of cigarette smoke on neoangiogenesis

Angiogenesis is a complex and dynamic process, in which different mechanisms acting in combination and whose output reflects the balance between pro- and anti-angiogenetic stimuli. Although various studies have reported toxic effects of nicotine on endothelium, being in favour of nicotine-mediated impairment of angiogenesis [54, 96], Heeschen *et al.* have demonstrated that nicotine promotes the growth of atherosclerotic plaques at least in part stimulating pathological angiogenesis [8]. Another group also reported nicotine-induced promotion of angiogenesis, through cyclooxygenase-2 (COX-2) and vascular endothelial growth factor receptor-2 (VEGFR-2) [97]. On the contrary, Makers *et al.* have reported an impairment of angiogenesis in female hamsters that have been exposed to cigarette smoke for 30 days [98], while Michaud *et al.* demonstrated that CS reduces HUVEC migration in response to VEGF [96]. Moreover, an abnormal pattern of blood vessels and altered composition of extracellular matrix in chorioallantoic membranes after exposure to tobacco smoke has also been reported [99]. In contrast with the above cited observations, Eid *et al.* described a so called “smoker’s paradox” because they found a beneficial asymmetric dimethylarginine (ADMA) profile in smokers, responsible of a protective effect against obesity-related endothelial dysfunction [100].

The contrast between findings by independent groups, together with the different experimental settings and the presence of various active compounds in cigarette smoking, make difficult to identify the net effect of smoke on neoangiogenesis. Despite the inexorable difficulties mainly due to the complex physicochemical composition of CS, it appears clear that more accurate determination of the pathophysiologic basis of smoke-related disruption of angiogenetic repair needs rigorous clinical and basic research, specifically designed to complement each other.

Closing remarks

Due to constant efforts by the scientific community worldwide, the epidemiologic and causal association of smoke with cardiovascular morbidity have been strongly demonstrated [92, 101]. Furthermore, also low tar cigarettes and passive smoke have shown a clear association with cardiovascular disease [102, 103]. Nevertheless, also at the epidemiologic level some questions remain unsolved, as the lack of a clear linear dose effect of CS [92]. Despite the big number of studies available on cigarette smoke and its relation with cardiovascular disease, many steps are still to be moved towards a better understanding of the mechanisms by which CS determines cardiovascular disease.

Many experimental difficulties are directly related with the physical properties of CS. In fact, because there are numerous known and unknown components of CS whose metabolic fate in human body is unknown, an appropriate in vitro model for CS-exposure remains to be established.

An intriguing issue regarding the relationship between CS and cardiovascular disease is the strong increase in excess of cardiovascular risk observed along with the interaction of smoke and other classical cardiovascular risk factors [92, 104]. Although it is clear that CS increases the risk of cardiovascular diseases in subjects with other risk factors, further analytical and metodological observations are needed to correctly understand the chemical and biological synergism between CS and other cardiovascular risk factors.

Among the various mechanisms studied in the available literature, most of the experimental evidences have been produced with nicotine as stimulus. This parallels with the clinical evidence that also low tar cigarettes smoke is associated to similar negative effects [102], as also smokeless tobacco is [105, 106]. Another property of CS that also has been strongly claimed as significantly responsible for the observed deleterious effects of CS on cardiovascular system is its capacity to increase the levels of oxygen reactive species, even if it remains difficult to verify if this is a direct mechanism or rather it is mediated by established components of CS.

Besides the important achievements reached in the last years, a better understanding of the complex pathophysiology of cigarette smoke and cardiovascular disease is needed. Further research on this topic should be encouraged, particularly aimed at investigating the molecular and cellular mechanisms of CS-induced alterations in cardiovascular physiology.

Riassunto

Gli effetti deleteri del fumo di sigaretta sulla prevalenza delle patologie cardiovascolari e la mortalità a queste legate sono ormai noti. Inoltre sia il fumo attivo che il cosiddetto “fumo passivo” si correlano ad un rischio elevato di sviluppare malattie cardiovascolari sia negli uomini che nelle donne. Il grande peso del fumo nell’ambito dei fattori di rischio cardiovascolari ha ispirato diverse strategie di intervento sociale soprattutto nell’ultimo decennio.

L'elevato impatto del fumo di sigaretta sul rischio cardiovascolare, e più in generale sulla salute, ha determinato la conduzione di innumerevoli ricerche, tanto epidemiologiche quanto cliniche e biologiche. Tuttavia, nonostante gli evidenti progressi raggiunti, numerosi interrogativi restano ancora aperti. In particolare, non vi sono chiare evidenze su quali siano i componenti del fumo di sigarette maggiormente e direttamente responsabili delle patologie correlate, così come manca un modello universalmente accettato circa i meccanismi patogenetici coinvolti.

Il presente articolo rappresenta una revisione delle principali osservazioni disponibili in letteratura sulla fisiopatologia delle malattie cardiovascolari correlate al fumo di sigaretta, offrendo una prospettiva aggiornata sulla comprensione dei meccanismi responsabili dell'azione di una delle prime cause di morte al mondo.

LIST OF ABBREVIATIONS

CS:	cigarette smoke
CAD:	coronary artery disease
WHO:	World Health Organization
ROS:	reactive oxygen species
VLDL:	very low density lipopriteins
HDL:	low density lipoproteins
nAChR:	nicotinic acetylcholine receptor
TF:	tissue factor
ICAM-1:	intercellular adhesion molecule 1
HUVEC:	human umbilical vein endothelial cell
MAP Kinase (MAPK):	mitogen-activated protein kinase
MNL:	mononuclear leukocytes
VCAM-1:	vascular cell adhesion molecule 1
NF-kB:	nuclear factor kappa-B
t-PA:	tissue plasminogen activator
NO:	nitric oxide
NADPH:	nicotinamide adenine dinucleotide phosphate
CSE:	cigarette smoke extract
VSMC:	vascular smooth muscle cell
PDGF:	platelet-derived growth factor
PBMC:	peripheral blood mononuclear cell
LDL:	low density lipoprotein
TNF-α:	tumor necrosis factor alpha
IL-1β:	interleukin 1beta
Th:	T helper
ADP:	adenodine diphosphate
MEG-01:	line of human megakaryoblastic cells
eNOS:	endothelial nitric oxide synthase
mRNA:	messenger ribonucleic acid
PAD:	peripheral arterial disease
ARIC:	acronym of the study "Atherosclerosis Risk in Communities"
COX-2:	cyclooxygenase-2
VEGFR-2:	vascular endothelial growth factor receptor-2
VEGF:	vascular endothelial growth factor
ADMA:	asymmetric dimethylarginine

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