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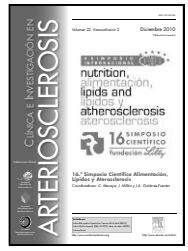

16.º SIMPOSIO CIENTÍFICO ALIMENTACIÓN, LÍPIDOS Y ATEROSCLEROSIS

Air particulate pollutants, systemic oxidative stress and atherosclerosis

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Air pollution has been associated with significant adverse health effects leading to increased cardiovascular morbidity and mortality. Epidemiological studies support the association of exposure to air particulate matter with human atherosclerosis and its designation as a novel cardiovascular risk factor. Experimental animal work has demonstrated that this association is possibly causal. It is likely that the proatherogenic potential of ambient particles depends on their ability to elicit systemic prooxidative and proinflammatory effects. Particle size and chemical composition appear to be important in determining their prooxidative properties. Thus, we have reported that ultrafine particles (<0.18 µm) enhance early atherosclerosis, partly due to their high content in redox cycling chemicals and their ability to synergize with known proatherogenic mediators in the promotion of tissue oxidative stress. These changes take place in parallel with increased evidence of phase 2 enzymes expression, via the electrophile-sensitive transcription factor, p45-NFE2 related transcription factor 2 (Nrf2). Exposure to ultrafine particles also results in alterations of the plasma HDL anti-inflammatory function that could be indicative of systemic proatherogenic effects. This article reviews in a concise fashion, the epidemiological, clinical and experimental animal evidence that support the association of particulate matter with systemic oxidative stress and atherosclerosis.

Air pollution is associated with increased cardiovascular mortality

Extensive epidemiological evidence supports the association of air pollution with adverse health effects leading to increased morbidity and mortality of worldwide significance[1,2]. It appears that most of the air pollution-related mortality is due to cardiovascular diseases and predominantly those of ischemic character[3], reason why exposure to air pollution has been designated as a novel cardiovascular risk factor of great importance since it is modifiable and with the potential to affect large numbers of people around the globe[2,4,5]. While air pollution is a complex mixture of compounds in gaseous (ozone, CO and nitrogen oxides) and particle phases, the cardiovascular effects are mostly ascribed to its particulate components[2,4,5]. Ambient particles can be classified according to their aerodynamic diameter into size fractions such as PM_{10} ("thoracic" particles, < 10 µm), $PM_{2.5-10}$ ("coarse" particles, 2.5 to 10 µm), $PM_{2.5}$ (fine particles, < 2.5 ìm) and UFP (ultrafine particles, < 0.1 µm)[6] that are derived from various sources and by a variety of processes that are characteristic of each size fraction. For instance, UFP are mostly generated through tailpipe emission from mobile sources (motor vehicles, aircrafts, etc.), major sources of $PM_{2.5}$ include power plants, oil refinery, wildfires, tailpipe and brake emissions from mobile sources while coarse particles are typically derived from soil, agricultural and road dust, construction debris, among others.

Various mechanisms have been proposed to explain how inhalation of ambient particulate could result in systemic cardiovascular effects as shown in the figure 1, which include: *a)* activation of pulmonary receptors resulting in autonomic nervous system imbalance and the development of dysrhythmias (pathway 1, Fig. 1); *b)* induction of pulmonary and systemic inflammation (pathway 2, Fig. 1); *c)* access of particles or chemical constituents to the systemic circulation (pathway 3, Fig. 1). Both pathways 2 and 3 can lead to the induction of atherothrombotic effects responsible for acute coronary syndromes and ischemic heart disease. Indeed, several studies support the association between air pollution and atherosclerosis (Table 1). Thus, long-term exposure to

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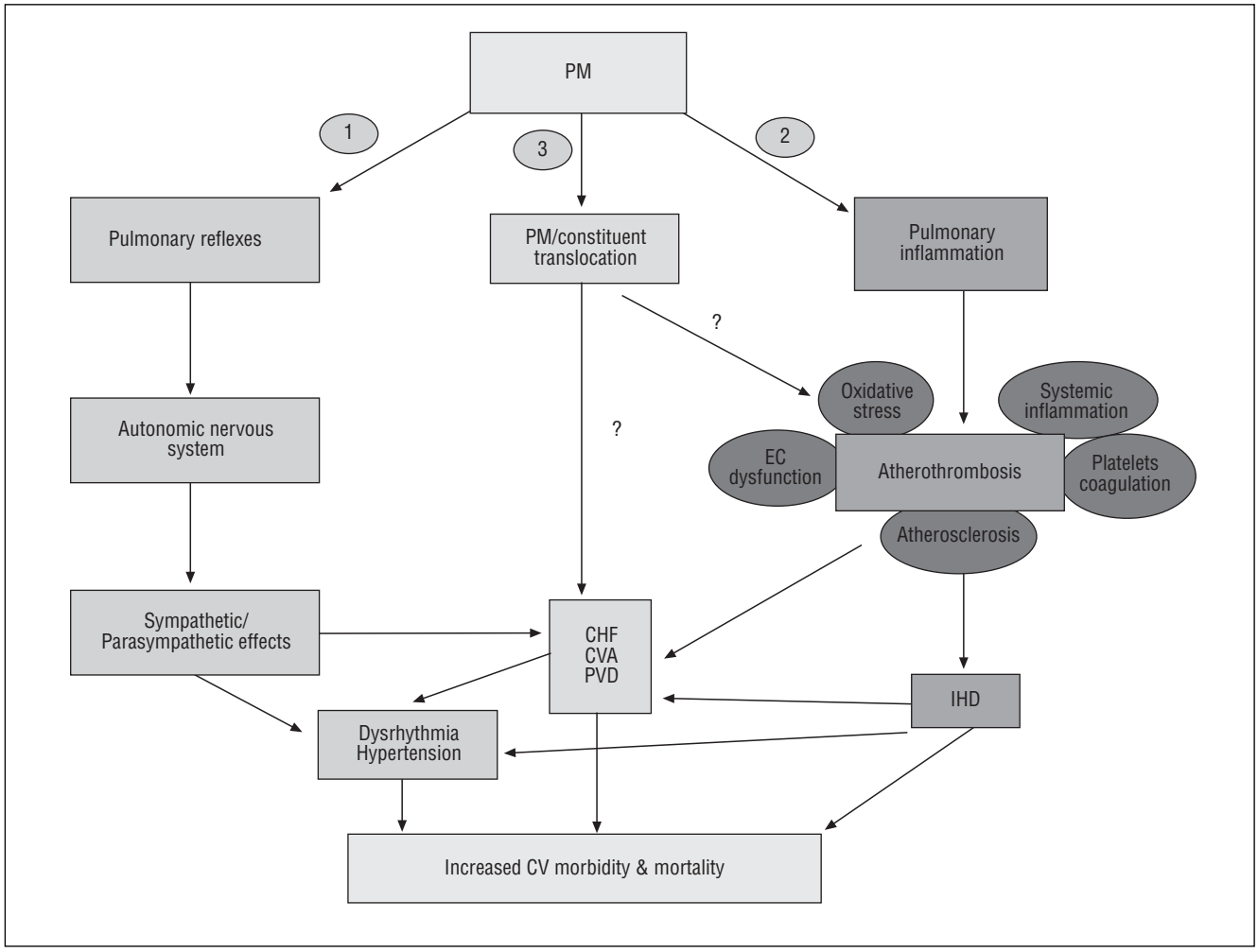

Figure 1 Potential mechanisms how exposure to PM lead to cardiovascular diseases.
Three main pathways are proposed: *a)* induction of autonomic nervous system (ANS) imbalance; *b)* development of pulmonary oxidative stress and inflammation with systemic "spill-over" of inflammatory mediators (e.g. cytokines, activated cells); *c)* translocation of particles and/or chemical constituents to the systemic circulation. Various factors such as the course and length of exposures, particle size, chemical composition, physical properties, redox potential, interaction with vapor and other co-pollutants, among others, may determine the specific pathway(s) that is/are to be activated, degree of overlap and timing (hyperacute vs. acute vs. chronic effects). CHF: congestive heart failure; CV: cardiovascular; CVA: cerebrovascular accident; EC: endothelial cells; IHD: ischemic heart disease; PVD: peripheral vascular disease. Modified from Araujo et al[5].

both PM_{10}[7] and $PM_{2.5}$[8] have been associated with increase in carotid intima-medial thickness. Likewise, exposure to $PM_{2.5}$[9] and living in close proximity to a major roadway[10] has been associated with increased aortic[7] and coronary calcification[10], respectively. Furthermore, epidemiological studies support the notion that the smaller the size, the larger the cardiovascular effect. However, while this is plausible, no human studies have tested the association of UFP exposure with atherosclerosis yet.

Exposure to particulate matter enhances atherosclerosis

Animal experimental work supports the notion of a causal association between PM and atherosclerosis (Table 2). Indeed, exposures to PM_{10}, $PM_{2.5}$ and UFP have been shown to promote atherosclerosis in a variety of experimental designs. Suwa et al have shown that Watanabe heritable hyperlipidemic rabbits to biweekly intrapharingeal instillations of urban air PM_{10} resulted in increased atherosclerotic lesions in the coronary arteries and that the burden of those lesions correlated with the percentage of alveolar macrophages positive for particles[11]. Chen and Nadziejko as well as Sun et al demonstrated that long-term inhalations over 5-6 months to concentrated ambient $PM_{2.5}$ in Tuxedo, New York, led to increased atherosclerotic lesions in the aorta of $apoE^{-/-}$ mice[12-14], which was accompanied by evidence of increased reactive oxygen species (ROS)[13] and increased expression of tissue factor in the aorta[14]. Likewise, Araujo et al reported that exposure inhalation to concentrated ambient UFP in Los Angeles, California over 5 weeks, also resulted in enhanced

Table 1 Human studies linking air pollution exposure with atherosclerosis

Study	Air pollutant	Evaluation of atherosclerosis	Major findings	Reference
Kunzli et al	$PM_{2.5}$ Ozone	CIMT	5.9%increased in CIMT per every 10 µg $PM_{2.5}$/ m^3	8
Hoffman et al	$PM_{2.5}$ Distance to major road	CACS	Increased CAC scores with shorter distances to a major road	10
Diez Roux et al	PM_{10} $PM_{2.5}$	CIMT CACS BAI	1-3%increase in CIMT per every 2 1 and 12.5 µg/ m^3 in PM_{10} and $PM_{2.5}$ respectively	9
Allen et al	$PM_{2.5}$ Distance to major road	Aortic calcification	6%increase in the risk of aortic calcification with a 10 µg/ m^3 contrast in $PM_{2.5}$	7

CIMT: carotid intima-media thickness; CACS: coronary artery calcium score; BAI: brachial artery index.
Modified from reference 5.

Table 2 Animal studies evaluating the effect of particulate air pollution on atherosclerosis

Study	PM fraction (mode of administration)	Animal model	Diet	Assessment of aterosclerosis (method)	Effect on atherosclerosis	Reference
Suwa et al 2002	PM_{10} (IT)	Watanabe rabbits	Chow	%lesional volume in coronary arteries and aorta (histology)	Increase	11
Chen & Nadziejko 2005	$PM_{2.5}$ (inhaled CAPs)	$apoE^{-/-}$, $LDL^{-/-}$ mice	Chow	%lesional area in whole aorta (histology)	No change	12
		$apoE^{-/-}$ mice	Chow		Increase	
Sun et al 2005	$PM_{2.5}$ (inhaled CAPs)	$apoE^{-/-}$ mice	Chow HFD	%lesional area in cross-sections of aorta (histology)	NS increase Increase	13
Sun et al 2008	$PM_{2.5}$ (inhaled CAPs)	$apoE^{-/-}$ mice	Chow HFD	%lesional area in aorta (ultrasound)	NS increase Increase	14
Araujo et al 2008	$PM_{2.5}$ & UFP (inhaled CAPs)	$apoE^{-/-}$ mice	Chow Chow	Mean lesional area in aortic root (histology)	NS increase Increase	15

IT: intratraqueal; CAPs: concentrated ambient particles; HFD: high fat diet; NS: not significant.
Taken from reference 5.

aortic atherosclerosis, accompanied by evidence of increased tissue systemic oxidative stress in the liver and the development of HDL dysfunction[15]. Importantly, UFP inhalations led to a greater degree of promotion of atherogenesis than exposures to $PM_{2.5}$, suggesting that the smaller particle size has a larger proatherogenic potential[5].

Role of particle size and composition

The greater proatherogenic potential exhibited by particles of smaller size in-vivo correlates with their greater prooxidative effects in-vitro, which appears to be a key element in their ability to induce cardiovascular toxicity. Indeed, PM from different size fractions exhibit significant differences that go beyond their size and number, including their source and mode of generation that determine their different chemical composition and physical properties. In addition, ambient PM can trigger Nrf2-regulated antioxidant genes in response to its content of prooxidative and electrophilic chemicals that differ markedly among the various particle sizes. For instance, urban UFP contain greater relative amounts of elemental carbon, organic carbon and polycyclic aromatic hydrocarbons (PAH) than the bigger fine and coarse particles[15]. This greater PAH content could be one of the reasons that explain UFP's greater redox potential. Indeed, UFP samples from the Los Angeles basin are more potent than $PM_{2.5}$ or coarse PM to induce *a)* a greater degree of oxidative stress as measured by the dithiothreitol (DTT) assay; *b)* a larger induction of Heme oxygenase-1 (HO-1) which is an important antioxidant enzyme; *c)* reduced glutathione to oxidized glutathione

(GSH/GSSG) ratio; and *d)* larger ability to cause mitochondrial damage[16]. It is possible then that different PM size and composition responsible for their different prooxidative potential could determine UFP greater ability to induce cardiovascular proatherogenic effects in-vivo, which remains to be studied in human populations.

PM prooxidative properties could lead to the activation of systemic vascular inflammatory pathways[17], a cardinal element of atherosclerosis. Thus, inhalation exposures to concentrated PM has led to increased ROS generation in aortic plaques and increased formation of 3-nitrotyrosine residues[13] as well as increased hepatic oxidative stress[15]. In addition, both $PM_{2.5}$ and UFP exposures led to the development of dysfunctional HDL in apoE$^{-/-}$ mice[15], a condition in which circulating HDL particles lose their antioxidant, anti-inflammatory and/or cholesterol-reverse transport capacity. Furthermore, UFP exposures led to a larger degree of HDL dysfunction than $PM_{2.5}$ did as determined by a monocyte chemotactic assay that allowed estimating the HDL anti-inflammatory function by its ability to abrogate LDL-induced monocyte migration in a co-culture of vascular cells. Thus, plasma HDL collected from $PM_{2.5}$ or UFP-exposed mice either failed to inhibit LDL-induced inflammation or even promoted greater monocyte migration, indicating that UFP greater prooxidative potential was in association with a stronger alteration of the HDL anti-inflammatory function and a larger degree of aortic atherosclerosis[15]. Dysfunctional HDL can be the result of systemic prooxidant and/or proinflammatory effects, it could mediate some of these effects per se or it could at least function as a marker of systemic inflammation[5]. Regardless of its specific mode of generation, it may play a role in disease pathogenesis. It is well established that plasma HDL cholesterol and apoA1 levels are inversely correlated with the risk for coronary artery disease, due to the well-characterized ability to promote reverse cholesterol transport and protect against oxidation, inflammation and thrombotic activities. However, high levels are not always protective in subjects, suggesting that not all HDLs prevent atherosclerosis. Dysfunctional proinflammatory HDL may serve as an useful marker for predicting susceptibility for atherosclerosis in humans and in rabbits, where it has been found to be a better predictor than total or LDL cholesterol levels[18]. It will be important to characterize the type of alterations that UFP exposures induce in HDL particles and confirm whether those occur in human subjects also.

It is unclear however how that is the inhalation of ambient particulate results in the induction of systemic prooxidative and proinflammatory effects, as recently reviewed by us[5]. One possibility is that inhaled particles induce some degree of pulmonary inflammation that can lead to spill-over of inflammatory mediators into the systemic circulation (pathway 2, Fig. 1). While particle deposition has been amply studied in animals and humans as well as their ability to induce free radical reactions in pulmonary cells, this potential mechanism awaits confirmation. An alternative possibility is the induction of particle or particle chemicals translocation (pathway 3, Fig. 1). Once particles get deposited in the lungs, they could undergo transcytosis across epithelia of the respiratory tract into the interstitium and access the blood circulation directly or via lymphatics, or could be taken up by sensory nerve endings embedded in airway epithelia, followed by axonal translocation to ganglionic and CNS structures. While there are multiple reports that support the notion of systemic translocation of synthetic nanoparticles in experimental models and humans, no convincing demonstration has been provided about this route for inhaled ambient particles[5].

Table 3 Summary

Air pollution is a novel cardiovascular risk factor
Particulate matter enhances atherosclerosis both in human and experimental animal models
Particle size and chemical composition are important in determining particle prooxidative and proinflammatory properties. A smaller particle size (e.g. ultrafine particles) correlates with a larger prooxidative potential
Inhalation of particulate matter appears to lead to increased vascular oxidative stress and increased expression of tissue factor
Exposure to particulate matter result in alteration of the HDL antiininflammatory capacity
The smaller the particles, the larger the systemic proinflammatory and proatherogenic effects

Conclusions and perspectives

Cumulative epidemiological data supports the association of exposure to air pollution with cardiovascular morbidity and mortality, including atherosclerosis and ischemic heart disease, mostly in relation to its particulate matter components. This has led to the designation of exposure to air pollution as a cardiovascular risk factor and the establishment of a novel research discipline named Environmental Cardiology. Our work and others have allowed us to establish some points, summarized in Table 3. Experimental animal work using hypercholesterolemic rabbits and apoE null mice have demonstrated that ambient PM exposure enhances atherogenesis, likely by a promotion of systemic prooxidative and proinflammatory effects. Air pollutant chemicals are able to synergize with oxidized phospholipids in the induction of a large number of genes that belong to pathways relevant in vascular inflammation. In addition, it does appear that the smaller the particles, the greater the proatherogenic effects.

UFP particles may be more toxic based on their greater number, larger content of redox active compounds such as PAHs, greater surface-to-mass ratio and larger bioavailability of chemically active constituents. Various possible mechanisms how ambient particles could promote atherogenesis include the induction of dysfunctional HDL, the systemic translocation of either whole particles or their chemical constituents into the circulating blood resulting in interaction with cellular components in the vascular wall, and/or the induction of pulmonary inflammation with

the subsequent release of inflammatory mediators into the systemic circulation (Fig. 1). Although there is no epidemiological or clinical data supporting the association of UFP with atherosclerosis, it is possible that exposure to UFP could result in stronger associations than with the larger particles.

Much work is still needed to better characterize the main toxic compounds, mechanism(s) of pathogenesis, types of genetic susceptibility that exposed individuals may exhibit and degree of associations of UFP with cardiovascular mortality, cardiovascular morbidity and human atherosclerosis.

Conflict of interest

The author declares he has not any conflict of interest.

Acknowledgments

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