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Atherosclerotic Events: The Role of Air Particulate Matter

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Abstract

Epidemiological studies associate the increase of respiratory and cardiovascular mortality and morbidity with high levels of air pollution particulate matter (PM). However, the underlying mechanisms of actions by which PM induce adverse health effects remain to be clearly elucidated. Evidence from experimental studies suggests that particle composition can play an important role in PM-toxicity; however, little is known about the specific participation of components (individually or acting in groups) present in such a complex mixture that accounts for toxicity. Correlations between exposure to PM with an aerodynamic diameter 2.5 or 10 μm ($PM_{2.5}$ and PM_{10}, respectively) with cardiovascular effects have been demonstrated. Mechanisms of PM at cellular level involve free radical production (by transition metals and organic compounds), oxidative stress, cytokine release, inflammation, endotoxin-mediated damage, stimulation of capsaicin receptors, autonomic nervous system activity, covalent modification of key cellular molecules and increased pro-coagulant activity. The various interaction between particulate matter (e.g carcinogenic polyaromatic hydrocarbon components) and biological molecules trigger cascade events which initiate or aid the progression of disease conditions through cellular responses which could give rise to oxidized and/or mutagenic lesions such as are found within the atherosclerotic plaque and cancers with the most important mechanisms possibly being reactive oxygen species (ROS) generation, oxidative stress and inflammation.

Keywords: air pollution, particulate matter, atherosclerosis, carcinogenesis, PM-toxicity, reactive oxygen species

1. Introduction

Industrialization in the various regions of the world has been greatly associated with the emission of various substances which constitute air pollutants and increase air pollution. These include substances such as metal fragments, wood chippings, dust particles, and much more. Air particulate matter (a-PM) otherwise known as aerosols is a major atmospheric pollutant [considering the vast sources of emission (Table 1)] with a composition mixture of particles (solid, liquid or both) suspended in air. (Seaton et al., 1995). Depending on emission sources (natural or anthropogenic), a-PM contains complex mixtures of chemical and/or biological components (Alfaro-Moreno et al., 2002; Soukup & Becker, 2001). This composition represents a complex mixture of organic, inorganic and biological components including viable or non-viable microorganisms and fragments of microorganisms which could include toxic components such as endotoxin and mycotoxins (Gangamma, 2012) varying in size, composition and origin with properties summarized based on their aerodynamic diameter (Table 1). Particulate matter (PM) is majorly made up of sulphate, nitrates, ammonia, sodium chloride, carbon, mineral dust and water. These particles are classified as primary or secondary depending on the mechanism of formation. Although natural processes emit primary particles into the atmosphere, anthropogenic processes such as combustion from car engines; solid fuel; combustion in households and industrial activities constitute the greater source of primary particles emitted into the atmosphere (Hammond et al., 2008; Watson & Chow, 2001).

Table 1. Summary of PM size, constituents and possible sources

Particulate matter size	Aerodynamic diameter (μm)	Constituents	Sources
coarse fraction ($PM_{2.5-10}$)	2.5 - 10	Dust, Endotoxin, pollen, fungi debris, ground materials, metals.	Agriculture, Soil, road dust, sea spray, suspension in air from grinding and erosion
fine particles $PM_{2.5}$	< 2.5	Organic/elemental carbon, organic compounds, hydrocarbons	Primary from all combustion sources including coal, oil, wood and gas
Ultra-fine particles $PM_{0.1}$	< 0.1	Primary combustion-hydrocarbons, metals, organic carbon	Fresh automobile and combustion emissions. Volatile and Semi- volatile organic carbons Secondary photochemical formation from gases

Source: Brook, 2008.

2. Health Implications

Besides the effect of particulate matter/ aerosols on climate change, the ability of atmospheric aerosols to exhibit chemical heterogenicity, spatial and seasonal variability have raised concerns regarding a variety of health impacts. These include respiratory diseases, cardiovascular (CV) diseases, eye irritation and lots more (Bell & Holloway, 2007). Significant associations have been shown to exist between excess cardiopulmonary/ CV morbidity and mortality following exposure to particulate air pollution especially ambient air particles with a mass median diameter of less than 10 μM (PM_{10}) (Bascom et al., 1996; Dockery et al., 1993; Bates, 1992). PM fractions of air pollution contain constituents that could increase reactive oxygen species (ROS) generation via reactions such as transition metal catalyses, metabolism, redox-cycling of quinones and inflammation (Knaapen et al., 2004).

Although the biological mechanism is yet to be completely understood, postulations are; that the inhalation of fine particles provokes a low-grade inflammatory response in the lung that aggravates lung disease and a change in blood coagulability thus increasing pulmonary and CV deaths (Seaton et al., 1995); alveolar macrophages (AM) are the most likely link between inflammatory processes in the lung and the systemic response due to their responsibility towards the ingestion and elimination of inhaled particles (Lohmann-Matthes et al., 1994); the phagocytic activity, oxidant production and release of inflammatory mediators such as tumor necrosis factor-alpha (TNF-α) by AMs is increased by their interaction with atmospheric particles (Imrich et al., 1998; Becker et al., 1996); PM induces the activation of the c-jun-n-terminal protein kinase (JNK) which possibly enhances DNA methyltransferase - 1 (DMNT1) transcription and p16 promoter methylation (Soberanes et al., 2012; Soberanes et al., 2009; Soberanes et al., 2006); PM alters the expression of tumor protein p53, cyclin-dependent kinase inhibitor1A gene (p21) and cyclin D1 gene (CCND1) which subsequently affects cell proliferation and apoptosis (Rosas Pérez et al., 2007; Bayram et al., 2006; Dagher et al., 2006; Soberanes et al., 2006).

The putative biological mechanism which links air pollution to heart disease involves the direct effects of pollutants on the CV system, blood/ lung receptors and/or the indirect effects mediated through pulmonary oxidative stress and inflammatory responses (Brook et al., 2004). The direct effects may likely occur via a variety of agents that readily cross the pulmonary epithelium into the systemic circulation. Within the systemic circulation, these direct effects represent a plausible explanation for the occurrence of rapid CV responses such as increased myocardial infarctions (Peters et al., 2001). The less acute and chronic indirect effects likely occur via pulmonary oxidative stress and/or inflammation induced by inhaled pollutants and results in health effects such as systemic inflammatory states capable of activating haemostatic pathways, impairing vascular function and accelerating atherosclerosis (Mutlu et al., 2007; Nemmar et al., 2003).

Dating back to the 18^{th} century and earlier, atherosclerosis was considered a disorder due to fatty acid/lipid metabolism (Steinberg, 2005). The vascular disease is majorly characterized by endothelial dysfunction, vascular inflammation and the build-up of lipid, cholesterol, calcium and cellular debris within the intima of the vessel

wall. However, the critical cellular elements of the atherosclerotic lesion are leukocytes, smooth muscle cells, endothelial cells and platelets (Falk, 2006). These components of the atherosclerotic lesions indicate the possibility of an immunologic response to tissue damage. It is therefore acceptable that atherosclerosis is no longer considered a disorder due to lipid metabolism but a chronic immuno-inflammatory, fibro-proliferative disease of large and medium-sized arteries fuelled by lipids (Hansson, 2005; Glass & Witztum, 2001). This review attempts to understand the possible role of PM in the progression of atherosclerotic events. It explores the possible mechanisms by which exposure to PM encourages atherosclerotic events and perhaps other inflammatory disease conditions.

3. The Atherosclerotic Pathway

Low density lipoprotein (LDL) oxidation is predicted as an early event in atherosclerosis. This suggests that oxidized LDL play a major role in atherogenesis (Asmis et al., 2005; Stocker & Keaney Jr, 2004; Heinecke, 2001). LDL are the major cholesterol transporters consisting of a hydrophobic core containing cholesteryl ester molecules, triacylglycerols and a surface monolayer of polar lipids (mainly phospholipids) and Apolipoprotein-B (Catapano et al., 2000). The efflux of LDL from the aterial lumen into the aterial wall and oxidation (mediated by reactive oxygen species (ROS), sphingomyelinase, secretory phospholipase-2, other lipases and myeloperoxidase) of plasma LDL in the extracellular matrix results in the production of oxidized LDL (OxLDL) believed to be the ultimate atherogenic forms of LDL (Perrin-Cocon et al., 2001).

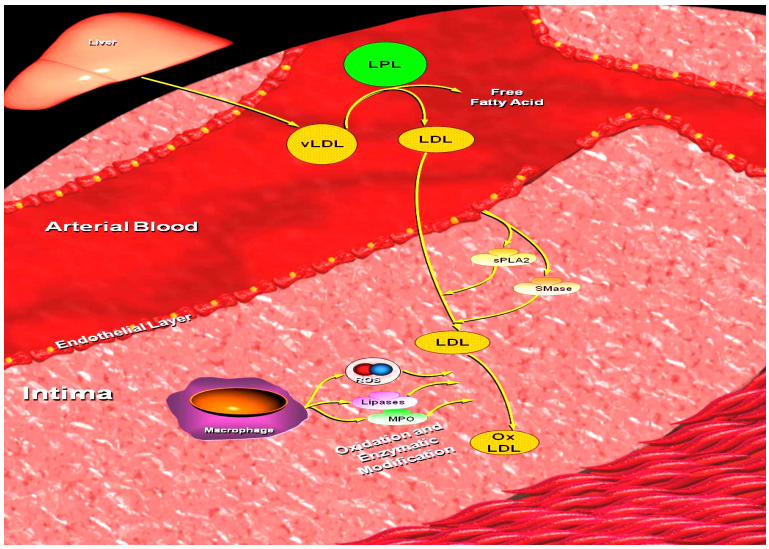

Figure 1. Oxidation of Low density lipoprotein mediated by Reactive Oxygen Species (ROS), the enzymes Sphingomyelinase (SMase), Secretory Phospholipase-2 (sPLA2), and Myeloperoxidase (MPO). Adapted from SABiosciences - Pathway Central

OxLDL induces inflammatory molecules and stimulates inflammatory signalling by endothelial cells. The release of chemotactic proteins and growth factors help the recruitment of monocytes into the arterial wall (Catapano et al., 2000). The OxLDL promotes differentiation of monocytes into macrophages (Figure 2) which engulf the OxLDL and converts them into foam cells. The necrosis of foam cells constitute part of the atherogenic plaque in fatty streak lesions (Meydani, 2001).

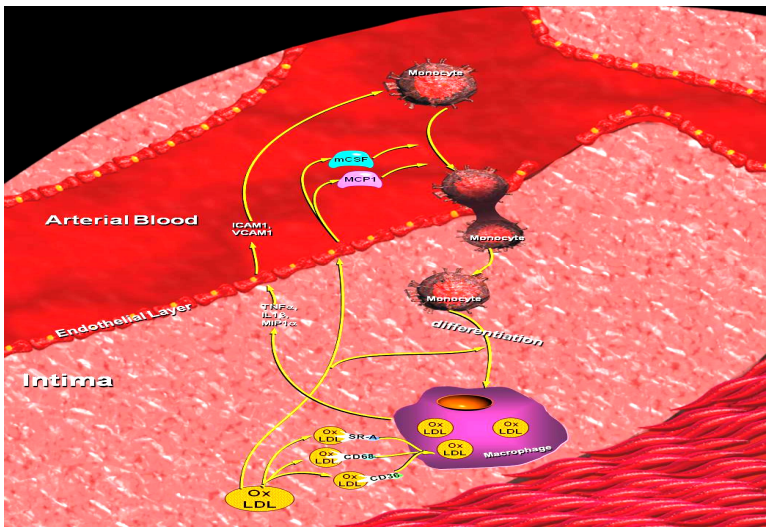

Figure 2. Inflammatory signalling via the release of Monocyte Chemotactic Protein-1 (MCP1) and release of Monocyte Colony Stimulating Factor (mCSF). The recruitment of monocytes into arterial wall, differentiation of monocytes into macrophages and phagocytic action.Adapted from SABiosciences-Pathway Central

OxLDL inhibits the production of NO (Nitric Oxide), an important mediator of vasodilation and expression of endothelial leukocyte adhesion molecules. The OxLDL particles are recognized by Macrophage Scavenger Receptors (Figure 2) Scavenger Receptor-A (SR-A), CD36 Antigen (CD36) and Macrophage Antigen CD68 (CD68). The Macrophages take up the OxLDLs, become enlarged and lipid- filled. These cells accumulate in tissue and are transformed into lipid-laden Foam cells (Figure. 3), dying and forming part of the Atherosclerotic Plaque in the fatty streak lesions (Meydani, 2001). Activation of macrophages result in the expression of cytokines such as Tumor Necrosis Factor-Alpha (TNF-Alpha), Interleukin-1Beta (IL-1Beta), Macrophage Inflammatory Protein-1Alpha (MIP1Alpha) which stimulate the expression of adhesion proteins like Vascular-Cell-Adhesion Molecule-1 (VCAM1) and Intracellular-AdhesionMolecule-1 (ICAM1) by endothelial cells (Figure 2).

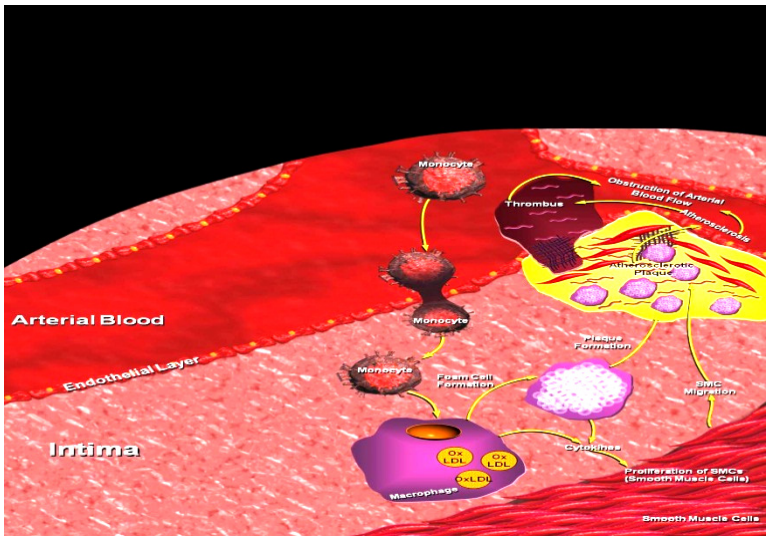

Figure 3. Foam cell formation by lipid-filled macrophages, foam cell necrosis, smooth muscle cell migration and formation of atherosclerotic plaque

Endothelial cells expressing adhesion proteins stimulate the binding of additional blood monocytes to the endothelium and recruitment into the intima. The cytokines released from the Macrophages and Foam cells also

stimulate the migration of smooth muscle cells (SMC) into the Intima, then proliferate and secrete Collagen, Elastin and Proteoglycans to form a fibrous matrix resulting in the formation of Plaques with fibrous caps (Barter et al., 2004). The mature atherosclerotic plaque then consists of a fibrous cap (comprising variable numbers of SMCs, foamy macrophages, lymphocytes, extracellular matrix and a variety of inflammatory mediators) encapsulating an acellular, lipid-rich necrotic core drived partly from dead foam cells. These mature plaques protrude into the arterial lumen causing obstruction of arterial blood flow. Formation of advanced lesions and thrombi in response to rupture or erosion of the plaque results in impeded blood flow and acute occlusion with symptoms such as myocardial infarctions and stroke (Asmis et al., 2005; Catapano et al., 2000).

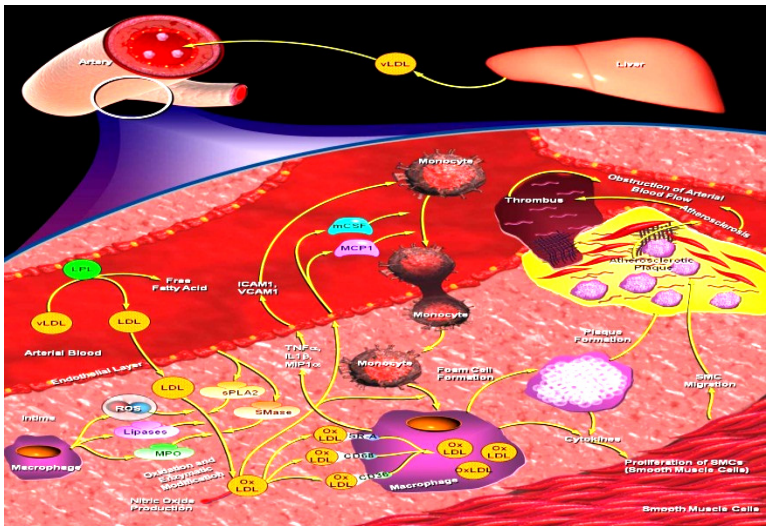

Figure 4. The complete cascade of events from atherogenesis to atherosclerosis.Adapted from SABiosciences-Pathway Central

4. Mechanisms of PM Action

The lung represents an important target tissue in the genotoxicity of pro-oxidant compounds particularly because the bronchial epithelium acts as a physicochemical barrier, playing a crucial role in initiating and augmenting defence mechanisms as well as signalling systemic responses (Vineis et al., 2004; Mills et al., 1999; Ollikainen et al., 1998). Mechanisms of PM at cellular level involve free radical production (by transition metals and organic compounds), oxidative stress, cytokine release, inflammation, endotoxin-mediated damage, stimulation of capsaicin receptors, autonomic nervous system activity, covalent modification of key cellular molecules and increased pro-coagulant activity (Araujo & Nel, 2009; Brook, 2008; Mills et al., 2008; Bhatnagar, 2006; Nel et al., 1998).

The effect of PM on organisms could depend on its chemical composition: a higher content of carcinogenic polyaromatic hydrocarbon (c-PAH) increases the genotoxicity of PM, resulting in the preferential formation of PAH-DNA adducts (Sevastyanova et al., 2008). The presence of other compounds, including o-quinones, or transition metals may lead to ROS formation and the subsequent induction of oxidative stress. Chemical composition however, may not necessarily be informative about the resulting effect of PM on the organism, because it does not take into account the interactions between various components that may cause synergistic, antagonistic, or additive effects (Donnelly et al., 1990). That said the types and sizes of a-PM inhaled may determine their toxicity and relative importance to the various mechanistic pathways. Larger fine or coarse PM cannot be transported into the circulation and would require secondary neural or pro-inflammatory response to mediate extra pulmonary actions while ultra-fine PM (or soluble constituents of larger particles) might directly enter the blood stream as a result of their ability to filter through the various biological barriers (Brook, 2008). Soukup and Becker (2001) report the induction of pro-inflamatory cytokines (IL-6 and TNF-α) in AMs by insoluble $PM_{2.5}$ and PM_{10} with higher induction levels observed in cells exposed to insoluble PM_{10}. It is possible that coarse PM particularly its insoluble components possess the potential to mediate AM functional modulation. As air pollution increases, inadvertently, the amount of particulate matter content within the atmosphere increases too. This relationship and increase in PM content has been shown to increase the incidence of CV

deaths. Various studies document that CV deaths increase by approximately 1% for every 10μg/m^3 short term daily increase in $PM_{2.5}$ (Pope III et al., 2006; Tonne et al., 2007; Analitis et al., 2006; von Klot et al., 2005; Zanobetti & Schwartz, 2005; Peters et al., 2004; D'Ippoliti et al., 2003; Dominici et al., 2003; Zanobetti et al., 2002; Katsouyanni et al., 2001).

Ultrafine particles (< 100nm diameter) are known for marked toxicity and may be held responsible for some of the $PM_{2.5-10}$ adverse effects. MacNee and Donaldson (2003) demonstrated that ultrafine carbon black (ufCB) does not have its effect via transition metal-mediated mechanism. Rather, ufCB and other ultrafine particles generate free radicals at their surface and are able to induce oxidative stress to cells. This ability to induce oxidative stress is likely implicated in the induction of inflammation. The hypothesis that the deposition of ultrafine particles in the lung provokes alveolar inflammation resulting in acute changes in blood coagubility and leads to morbidity and mortality in CV diseases (Seaton et al., 1995) has been supported by studies showing that exposure to ambient PM_{10} promotes inflammation in the lung and is associates with a systemic inflammatory response (Goto et al., 2004; van Eeden et al., 2001; Tan et al., 2000; Terashima et al., 1997; Seaton et al., 1995).

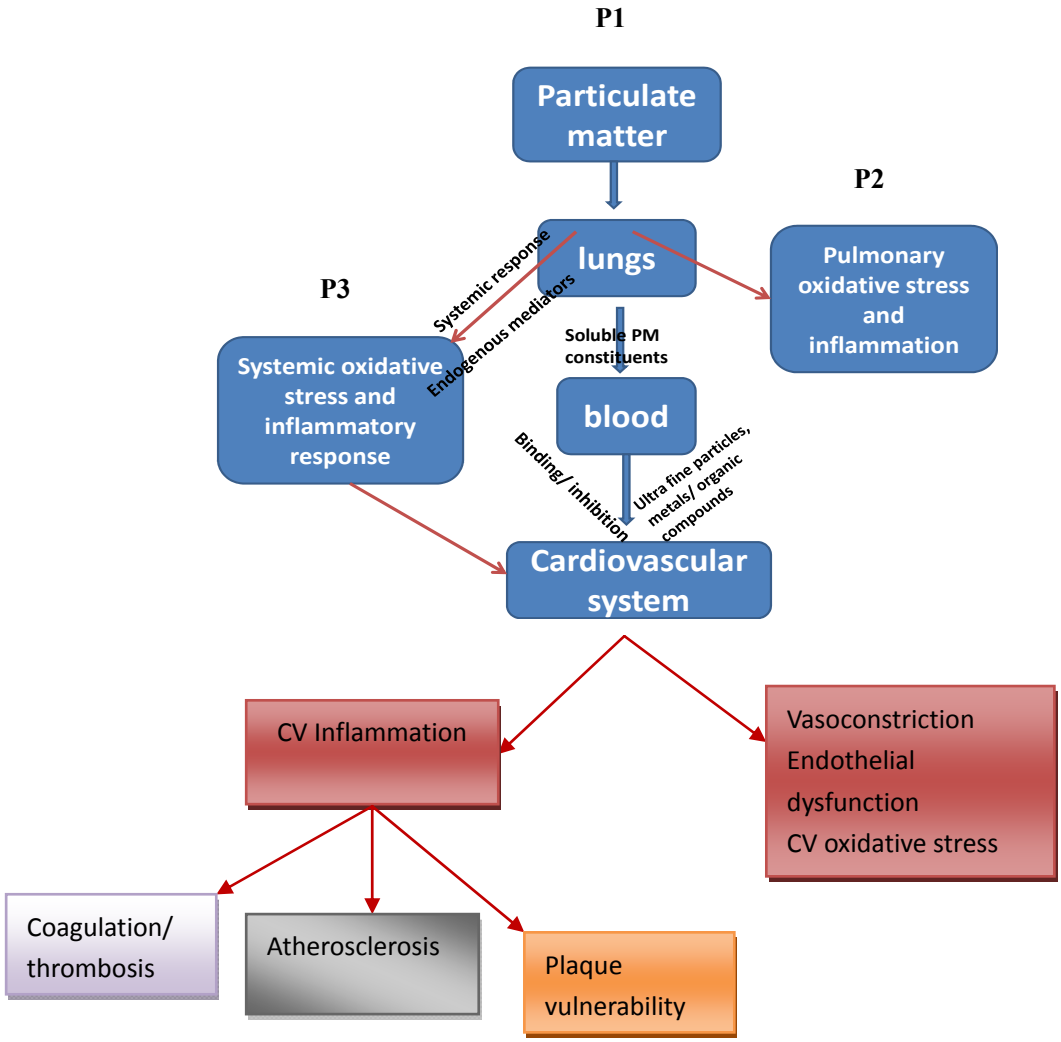

Figure 5. The possible pathways through which particulate matter exerts toxicity leading to atherosclerosis initiation and progression (**P1**: pathway 1, **P2**: pathway 2, **P3**: pathway 3)

Absorption from the lungs is usually rapid and efficient due to the surface area, excellent blood supply and barrier between the air in the alveolus and the blood stream. These properties of the lung make exposure to toxic compounds via the pulmonary vasculature toxicologically important and highly significant (Timbrell, 2000). Soluble compounds and/or Nano meter-sized PM may rapidly enter the pulmonary vasculature and subsequently

be transported throughout the systemic circulation. Following inhalation, the translocated particles could directly interact with the CV system possibly via receptor – binding/ inhibition. (Figure 5. P1.). Pulmonary oxidative stress maybe responsible for instigating CV pro-oxidative (Bräuner et al., 2007; Rhoden et al., 2005; Sørensen et al., 2003; Gurgueira et al., 2002; Sharman et al., 2002) and pro-inflammatory (Behndig et al., 2006) chain reaction observed after PM exposure. Cardiac tissue oxidative stress is shown to increase within hours of $PM_{2.5}$ inhalation (Gurgueira et al., 2002). Elevated free radical generation have been found in remote non-pulmonary animal vessels hours to days following exposure to PMs (Gong et al., 2007; Nurkiewicz et al., 2006; Sun et al., 2005). Pro inflammatory mediators (cytokines and activated immune cells) released from the pulmonary into the system vasculature may then secondarily trigger a variety of adverse CV reactions (Figure 6). However, some studies have reported no signal of a systemic inflammatory response (Diez Roux et al., 2006; Pope III & Dockery, 2006) possibly because specific pollution constituents, co-pollutant levels, the duration of exposure and patient susceptibility play highly important roles in determining the subsequent responses or lack thereof. Due to PM ability to absorb thousands of chemical compounds, it is quite challenging to identify the exact chemical constituents responsible for observed genotoxic effects. As shown in Oh et al. (2011) document that crude extracts of $PM_{2.5}$ fractionated by an acid–base–neutral and silica gel fractionation procedure and divided into chemical classes of increasing polarity showed nonpolar and slightly polar extracts significantly inducing micronuclei formation and DNA breakage at a non-cytotoxic dose. Organic extracts (fractions possibly containing aliphatic chlorinated hydrocarbons, PAHs, nitro-PAHs, ketone and quinones) of $PM_{2.5}$ was observed to have induced significant increase of oxidative DNA damage including oxidized purine and pyrimidine molecules (Oh et al., 2011).

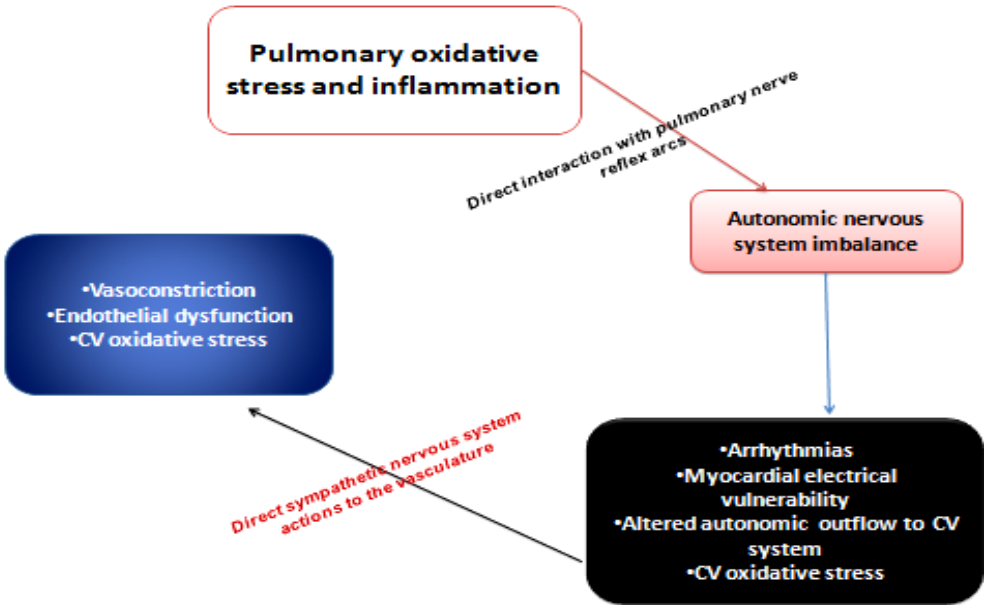

Figure 6. The possible pathways through which particulate matter enhances atherosclerosis progression via the pulmonary oxidative stress pathway

Transition metals may determine the toxic effects of PM through oxidative stress. This could result in injury via increase in airspace epithelial permeability, and inflammation via the activation of transcription factors for pro-inflammatory genes in macrophages and epithelial cells. Seaton et al. (1995) hypothesized that the deposition of ultrafine particles in the lung provokes alveolar inflammation resulting in acute changes in blood coagubility and leads to morbidity and mortality of CV diseases. This hypothesis is supported by studies showing that exposure to ambient PM_{10} promotes inflammation in the lung and is associated with a systemic inflammatory response (Goto et al., 2004; van Eeden et al., 2001; Tan et al., 2000; Terashima et al., 1997). Significantly high amounts of cytokines and chemokines including granulocyte-macrophage colony stimulating factor (GM-CSF), interleukin (IL)-6, IL-8 and chemoattractant protein (MCP)-1 were found in alveolar macrophages and lung epithelial cells incubated with PM_{10} (Fujii et al., 2002; Fujii et al., 2001; van Eeden et al., 2001). Higher levels of circulating cytokines have been observed in cells exposed to PM_{10} particles (Tan et al.,

2000). Studies showing an increase in C-reactive protein (CRP) levels (Pope, 2004; Sandhu et al., 2005) support the concept that exposure to PM_{10} is associated with a systemic inflammatory response. Chronic exposure to PM_{10} is also shown to cause downstream vascular effects resulting in the progression of atherosclerosis (Künzli et al., 2005; Suwa et al., 2002). Soberanes et al. (2012) show that concentrated ambient $PM_{2.5}$ induced oxidative stress within lungs, increased transcription of DNMT1 as well as hypermethylation of the p16 promoter in the lungs of exposed mice.

5. Conclusion

Particulate matter rarely exists by itself within ambient air pollution. However, the particles are constantly changing and in continuous interaction with gaseous, semi-volatile and volatile compounds. A wide variety of these vapour-phase compounds attach to the surface of PM and form secondary aerosol particles. The various interaction between particulate matter and biological molecules trigger cascade events which initiate or aid the progression of disease conditions through cellular responses which could give rise to oxidized and mutagenic lesions such as are found within the atherosclerotic plaque and cancers with the most important mechanisms possibly being ROS generation, oxidative stress and inflammation. Various techniques have been able to detect the effect of particulate matter *in vitro* however; the major research consideration should be the development of protocols and sensing techniques to track the activity of PM *in vivo*. The potential of nanotechnology (Quantum dot) presents an opportunity to achieve enhanced *in vivo* sensing via labelling and conjugates. However, nanoparticles seem to possess adifficult toxicity profile to overcome (Riding et al., 2012) and might limit their *in vivo* applications.

With increased technological advancement, it may be safe to anticipate the emergence of novel a-PM constituents of toxicological concern. Several studies show the need for improved air quality particularly within the urban environment (Laing et al., 2010; Oh et al., 2011) as well as further investigation towards the proper elucidation of PM mechanisms of eliciting cellular damage and possibly initiating/promoting disease conditions. Of interest would be the activity of PM and PM fractions on regulatory proteins/genes and the possible activation or suppression of cell or immune responses.

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