Chapter

Environmental Particulate Air Pollution Exposure and the Oxidative Stress Responses: A Brief Review of the Impact on the Organism and Animal Models of Research

Pauline Brendler Goettems Fiorin, Mirna Stela Ludwig, Matias Nunes Frizzo and Thiago Gomes Heck

Abstract

Particulate matter (PM) is a mixture of solid particles and liquid droplets found in the air, and it is one of the most harmful air pollutants. When inhaled, it affects the pulmonary system, cardiovascular systems, and other tissues. The size, composition, and deposition of PM, mainly related to fine and ultrafine particulate matter, are factors that determine the harmful effects of exposure to particles. Among the main effects is the inducer of ROS production, and consequently oxidative tissue damage in target organs and other responses, mediated by inflammatory cytokines and cellular stress response. The main pathway through which particles are potent mediators of oxidative stress is the damage caused to DNA and lipid molecules, whereas the pro-inflammatory response involves an immune response against PM, which in turn, it is related to cell stress responses observed by heat shock proteins (HSPs) expression and release. Thus, the ability of an organism to respond to PM inhalation requires anti-oxidative, anti-inflammatory, and cellular stress defenses that can be impaired in susceptible subjects as people with chronic diseases as diabetes and obesity. In this chapter, we discuss the mechanistic aspects of PM effects on health and present some animal research models in particle inhalation studies.

Keywords: air pollution, fine particulate matter, oxidative stress, animal models

1. Introduction

Pollutant refers to chemical substances, particles, or toxic gases, introduced into the environment from various sources and that cause adverse effects to living beings and the ecosystem, compromising the soil, water, and atmospheric air. Atmospheric

pollution, in turn, can be defined as any form of matter in quantity, concentration, time, or other characteristics, which make or may make the air unsuitable or harmful to health, inconvenient to public welfare, harmful to fauna and flora, or environmental safety. Air pollution is one of the biggest environmental threats to human health, a condition related to human development activities and climate change agenda. In 2021, the World Health Organization (WHO) listed the improvement of air quality within the top 10 health challenges of the year.

The primary sources of air pollutants are anthropogenic, which originate from processes carried out by industries, mining, transport, and construction. Also, pollutants are classified as mobile sources, which include most forms of transport, such as automobiles, trucks, and planes, or fixed sources, such as industrial and housing facilities. Another form of classification is to determine the pollutants that are emitted directly by polluting sources, called primary pollutants, such as particulate matter (PM), sulfur dioxide (SO_2), nitrogen dioxide (NO_2), carbon monoxide (CO), or those that result from interactions with the environment, called secondary pollutants. Among the air pollutants mentioned, PM is the pollutant that has received greater emphasis in scientific research, both in epidemiological and experimental studies.

The term PM refers to a mixture of solids and liquid droplets suspended in the air [1] classified as large "coarse" particles (PM_{10}; particles with a diameter less than 10 μm). PM_{10} represents mainly the overall mass of PM and can be derived from numerous sources, such as silica-based crustal particles (e.g., soil, sand, and volcanic ash), burning of natural materials (e.g., woodsmoke), or wear of machinery (e.g., vehicle braking and tire erosion). "Fine" PM ($PM_{2.5}$: particles with a diameter less than 2.5 μm) and "ultrafine" PM ($PM_{0.1}$: particles with a diameter less than 100 nm) are generally derived from industrial burning of fossil fuels and traffic-related sources [2]. PM is primarily generated by fuel combustion in different sectors, including transport, energy, households, industry, and agriculture. In 2013, the outdoor air pollution and PM as carcinogenic by the WHO International Agency for Research on Cancer (IARC) [3].

The size of inhaled particles is inversely correlated to their potential to cause health problems. Small particles, less than 10 μm in diameter, are the most significant problems because they can penetrate deeply into the lungs, and some particles can reach the bloodstream [4]. The health risks associated with PM equal or smaller than 10 and 2·5μm in diameter (PM_{10} and $PM_{2.5}$, respectively) represent a particular public health relevance. Both $PM_{2.5}$ and PM_{10} can penetrate deep into the lungs, but $PM_{2.5}$ can even enter the bloodstream, primarily resulting in cardiovascular and respiratory impacts and affecting other organs.

In 2005, the guidelines established by the WHO for $PM_{2.5}$ concentrations went from 65 to 25 μg/m^3 during the 24-h period and from 15 to 10 μg/m^3 during the period one year [5]. However, guidelines may be different in each country. For example, in Brazil, the final air quality guidelines were established by CONAMA resolution No. 0^3/90, updated in Resolution No. 491, of November 19, 2018, which provides for air quality standards. Considering the levels of fine particulate matter ($PM_{2.5}$) of 25 μg/m^3 for the 24-h period and 10 μg/m^3 for the annual average, the same was indicated by the WHO [6].

In 2021, the WHO released the update of air quality guidelines based on the extensive scientific evidence currently available identifying the levels of air quality necessary to protect public health worldwide. The updated WHO Global Air Quality Guidelines (AQGs) provide recommendations on air quality guideline levels and interim targets for six key air pollutants. Moreover, for PM, there was a significant

reduction in the recommended levels. For $PM_{2.5,}$ the standard established for the annual average 15 years ago was reduced by half, from 10 to 5 μg/m^3, and concerning the exposure in the 24 h, the parameter was reduced from 25 to 15 μg/m^3. About PM_{10}, in the annual average, it reduced from 20 to 15 μg/m^3 and the 24-h period values were reduced from 50 to 45 μg/m^3 [7].

To achieve these goals, the WHO has set interim targets that serve as incremental steps in the progressive reduction of air pollution toward the air quality guideline levels and are intended for use in areas where air pollution is high. These levels could be used by authorities in highly polluted areas to develop pollution reduction policies that are achievable within realistic time frames [7].

According to the IQAIR website [8], the 15 most polluted cities in the world, considering only $PM_{2.5}$, are located in China or India (87% are Indian cities). The city of Hotan in China was the "champion" in 2020, with an annual average of 110.6ug/m^3 of pollution by $PM_{2.5}$. China and India are part of the BRICS, a group formed by five large emerging countries—Brazil, Russia, India, China, and South Africa—representing about 42% of the population, 30% of the territory, and 18% of trade worldwide. In the air pollution ranking, South Africa is classified in 49th place, Brazil in 68th and Russia in 86th, with an annual average of 18.0, 14.2, and 9.3 respectively in 2020, which is considered moderate (South Africa and Brazil) and good air quality for Russia, considering the new WHO guidelines [8].

The assessment of environmental data allows us to understand the economic, social, and environmental impact of pollution in both developed or developing countries, and it is crucial for planning to reduce air pollution activities. Since air pollution can affect our health in various ways, depending on the exposure time (short or long term), type of pollutant, concentration of polluting agents, age group, and prior health condition. In this way, a study published in 2017 described ambient $PM_{2.5}$ as the fifth-ranking mortality risk factor in 2015. Exposure to $PM_{2.5}$ caused 4·2 million deaths and 103·1 million -adjusted life-years (DALYs) in 2015, representing 7·6% of total global deaths and 4·2% of global DALYs, 59% of these in the east and south Asia. Deaths attributable to ambient $PM_{2.5}$ increased from 3·5 million in 1990 to 4·2 million in 2015. These deaths occur mainly due to stroke, heart disease, lung cancer, and chronic respiratory diseases [9]. Liu et al. [10], demonstrated in a study that evaluated particulate air pollution and daily mortality in 652 cities that an increase of 10 μg/m^3 in the 2-day moving average of PM_{10} concentration was associated with increases of 0.44% in daily all-cause mortality, 0.36% in daily cardiovascular mortality, and 0.47% in daily respiratory mortality. The corresponding increases in daily mortality for the exact change in $PM_{2.5}$ concentration were 0.68, 0.55, and 0.74%, respectively. Evidencing an independent association between short-term exposure to PM_{10} and $PM_{2.5}$ and daily all-cause, cardiovascular, and respiratory mortality in more than 600 cities across the globe, reinforcing the evidence of a link between mortality and PM concentration established in regional and local studies [10].

Studies have described particulate pollution exposure to various problems, including premature death in people with heart or lung disease; non-fatal heart attacks; cardiac arrhythmia; asthma; decreased lung function; increased respiratory symptoms such as airway irritation, coughing, or difficulty breathing. People with heart or lung disease, children, and older adults are most likely to be affected by exposure to particulate pollution [11]. In addition, sensitive groups (also called at-risk populations)—A term used for a category of people at increased risk of experiencing adverse health effects related to exposure to air pollution, may be at increased risk due to intrinsic (biological) factors, extrinsic factors (external, non-biological), increased

exposure and increased levels of air pollutants. Thus, the severity of the health effects experienced by the sensitive groups may be greater than in the general population [12]. Therefore, the establishment of air quality standards in large cities has contributed to estimates of data that helps environmental management and community health. In parallel, many laboratories are carrying out research that emphasizes the mechanisms by which exposure to air pollution impacts human health, thus alerting the need to develop public health policies and stricter air quality standards.

2. Particulate matter inhalation: characteristics and associated diseases

Among air pollutants, the one that has received greater emphasis in current research is fine particulate matter ($PM_{2.5}$), consisting of pollutants formed from combustion processes [13]. Particulate matter$<_{2.5}$ μm ($PM_{2.5}$) is the most commonly implicated constituent that causes a disproportionate number of global deaths and contributes significantly to global disability. The global burden of disease study report indicated that ambient outdoor air pollution, particularly $PM_{2.5}$, was the fifth leading risk factor for global mortality in 2015 [14].

The source of PM can be explained as either direct emission into the air or as conversion from gaseous precursors (such as sulfur dioxide, oxides of nitrogen, ammonia, and non-methane volatile organic compounds) released from both anthropogenic and natural sources. The chemical constituents in PM are commonly found to include inorganic ions (e.g., sulfates, nitrates, ammonium, sodium, potassium, calcium, magnesium, and chloride) and can be expanded further to include all varieties of constituents such as metals (including cadmium, copper, nickel, vanadium, and zinc), and polycyclic aromatic hydrocarbons (PAH) [15]. The harmful effects of $PM_{2.5}$ on health depend on the exposure time and its concentration in the environment.

Ambient particles contain many soluble metals, including transition metals that are capable of redox cycling [16]. Transition metals are thought to be very important in PM cellular toxicity. The bioavailability of transition metals in PM and their redox properties, which are considered very important for the toxic effects and the oxidative damage [17]. $PM_{2.5}$ can invade the respiratory tract and vascular system [18] and thus cause a systemic effect, acting as a potent inducer of ROS production and the release of pro-inflammatory cytokines (TNF- α, IL-6, IL-8) for circulation [19, 20].

Exposure to PM can occur in many forms, as described by Thompson [21]. As with any chemical contaminant, components of PM may enter the human body by three mechanisms: inhalation, dermal absorption, and ingestion. PM can be ingested by the direct consumption of contaminated beverages and food and during the clearance of particles removed from the airways via mucociliary transport. Another possible route of exposure to airborne PM is impaction or deposition to the skin. Deposition velocities are influenced by substrate surface properties (e.g., roughness, wetness, temperature, surface charge) and particle size-dependent. Inhalation is a rather obvious route of exposure, but to better understand the effects, it is crucial to consider the anatomy of the human airway, patterns of particle size-dependent deposition, and the fate of particles that have been inhaled.

Nevertheless, mainly, the size is determinant for the deposition characteristics and effects of the PM. During a pollution episode, each lung acinus could receive on average 30-million particles and each alveolus about 1500 particles (for 24 h exposure), of which 50% are being deposited. Lung airways and alveoli retain mostly $PM_{2.5}$ rather than PM_{10} since the last one is frequently stopped in upper airway anatomic

structures. Also, analytical electron microscopy measurements showed that 96% of effectively retained particles in the lung parenchyma were $PM_{2.5,}$ and only 5% were ultrafine particles (0.1 μm), which means translocation to the pulmonary and systemic circulation, affecting other organs [17]. Thus, the size of PM and their retention play an essential role in the PM cytotoxic effects, including the site of deposition (upper or lower airways), bio-persistence solubility, and its composition [14].

In the USA, studies involving residual oil fly ash (ROFA), one of the components of $PM_{2.5}$, have helped develop and refine this theory. ROFA, or residual oil fly ash, is the term used to refer to the primarily inorganic residues that remain after the incomplete oxidation of carbon compounds. ROFA contains about 10% by weight of water-soluble Fe, Ni, and V, and its intratracheal instillation in rats leads to aldehyde generation [16, 22].

ROFA particles are generally smaller than 2.5 μm in size and are chemically considered complex compared to other air pollution particles, as they contain sulfates, silicates, carbon, and nitrogen. In addition to the elements mentioned, it also has a large number of metals that are naturally present in fuels (petroleum, paraffin, and diesel oil) and remain when the volatile fraction is distilled [23]. This pollutant has been used in experimental studies as a surrogate particle to investigate the mechanisms of responses to PM inhalation in animals [24, 25]; as it is mainly made up of different metals, it promotes Fenton reactions, producing reactive oxygen species [22].

The harmful effects of $PM_{2.5}$ on human health depend on the time of exposure and particles concentration in the environment. As expected, repetitive and long-duration exposure to higher doses of $PM_{2.5}$ induces cumulative and persistent effects. However, subchronic exposure to low doses (5 μg/day, intranasally) of $PM_{2.5}$ in animal models is sufficient to potentiate metabolic dysfunction in high-fat diet-fed mice, promoting glucose intolerance and increasing fasting glycemia and triglyceride levels [26].

3. The role of oxidative stress induced by PM inhalation in cells and tissues

Oxidative stress is described as a biochemical imbalance, which occurs when the production of pro-oxidants such as free radicals or reactive oxygen species exceeds the body's natural antioxidant capacity, resulting in oxidative damage [27, 28] and consequently leading to the development of a broad spectrum of human diseases.

The main pro-oxidant agents are the reactive oxygen species (ROS), such as radicals, superoxide ($O2^{-}$) and hydroxyl (OH^{-}) radicals, and also some non-radical species derived from O_2, such as the hydrogen peroxide (H_2O_2) [27]. Antioxidants can be classified as exogenous, obtained mainly through the diet, or endogenous, being produced by our body to avoid oxidative stress and consequent tissue destruction. Antioxidant defense systems can inhibit the oxidation of other molecules in the organism, transferring electrons from a substance to an oxidizing agent (ROS), acting in two lines: enzymatic antioxidant defenses [e.g., superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx)] and non-enzymatic antioxidant defenses (e.g., vitamins A, C, and E; glutathione, α-lipoic acid; carotenoids, ubiquinone or coenzyme Q10).

ROS production is inherent in all aerobic species, primarily as a product of mitochondrial electron transport. At physiological levels, ROS are essential for the regulation of critical signaling pathways involved in cell growth, proliferation, differentiation, and survival, but excess ROS, mainly resulting from imbalanced antioxidant defense and detoxification, can lead to harmful (i.e., pathological) oxidative stress [29].

The most important pathophysiological mechanism that has been proposed to explain the association of PM exposure and the occurrence of respiratory infections, lung cancer, and chronic cardiopulmonary diseases is oxidative stress through the generation of ROS [17]. Recent studies using different methods have consistently demonstrated that peroxides and ROS are the critical mediators of particle toxicity [29].

The molecular events by which pulmonary oxidative stress occurs in response to particle inhalation involve the inflammatory process. ROS (e.g., superoxide, hydroxyl radical, nitric oxide, and peroxynitrite) generated in air pollution exposure induces oxidative tissue damage in target organs, with contributions of non-immune and immune cells in the inflammatory response. The role of protective proteins (e.g., surfactant, proteins, and antioxidants) in this process is highly complex and may differ depending on experimental models, especially in concomitant disease states [30].

The pieces of evidence, to date, suggest that ROS generation in response to $PM_{2.5}$ could either involve disruption of cellular redox signaling and upregulation of endogenous ROS production resulting in exaggerated responses, as described in details in some reviews [14, 30].

Wang et al. [31] evaluated two classical mechanisms of oxidative stress and intracellular calcium overload to explore their roles in PM-induced endothelial cell apoptosis from the perspective of subcellular levels in endothelial cells. They showed that internalization of particles induces oxidative stress, followed by the disorder of subcellular structures, including endoplasmic reticulum (ER) stress, mitochondrial dysfunction, activated caspase pathways, which cause endothelial cell apoptosis. They also highlighted that antioxidants and calcium inhibitors confer protective effects. Also, ROS are generated during phagocytosis of the particles, leading to enhancement of oxidative stress and triggering the inflammatory response. Consequently, the activation of inflammatory signaling pathways results in the release of cytokines and other mediators that can further induce ROS production by activating endogenous enzymes, leading to a positive feedback loop, which can aggravate the effects triggered by PM exposure [32].

In summary, it is well known that in the lungs, exposure to PM triggers inflammation, endothelial activation, and oxidative stress, caused by the deposition of PM into the alveolar space in the lung, inducing the release of cytokines from alveolar macrophages. The probable sequence of events for PM-induced lung inflammation involves the following: injury to epithelial cells by ROS, possibly enhanced in the presence of metals via Haber-Weiss and Fenton chemistry; and activation of vascular endothelium and circulating leukocytes, circulating leukocytes; emigration of inflammatory cells from blood to tissue sites, promoting pro-inflammatory condition [32]. Moreover, even at low doses, it is also able to demonstrate early changes in the elastic and viscoelastic pulmonary mechanical components, such as worsening impedance, alveolar collapse, and histological changes, in addition to oxidative stress and inflammation [33].

An epidemiological study conducted by Hu et al. [34] exposed 768 participants to environmental levels of $PM_{2.5}$, and assessed urinary levels of PAH metabolites and metals, and evaluated urinary 8-OHdG, a biomarker of endogenous oxidative damage to DNA, 8-iso-PGF2α a biomarker of excessive chemical lipid peroxidation in humans, and MDA for determination of lipid peroxidation levels. They discuss that particulate matter alone, its bound polycyclic aromatic hydrocarbons, and heavy metals induce increased oxidative stress on DNA and lipid [34].

Since PM can invade the bloodstream, particles exposure may also impact cardiovascular health. Air pollution increases the risk of myocardial infarction, stroke,

and acute heart failure. Since ROS act mainly inducing endothelial dysfunction, monocyte activation, and some proatherogenic changes in lipoproteins, which initiate plaque formation, this context favors thrombus formation because of an increase in coagulation factors and platelet activation [2, 35]. In this way, animal models of acute exposure to higher levels of particles in chambers showed that PM oxidative effects are associated with autonomic nervous system imbalance, which is avoided by the pre-treatment with drugs acting as sympathetic or parasympathetic blockers by antioxidant pre-treatment [36]. In addition, exposure to particles may induce electric alterations on the heart [37], suggesting an explanation of the increased risk of myocardial infarct under inadequate air quality for susceptible subjects [38].

In the lungs and heart, but also in other organs, oxidative stress may induce a cell stress response, characterized by the increased expression and release to the bloodstream of heat shock proteins (HSPs). High plasma levels of these proteins are simultaneously correlated with impaired energy balance, with an alteration in the pro/anti-inflammatory status and with an imbalance in the body's pro/antioxidant systems, and also has been used as a biomarker of cell stress response in diseases as obesity, hypertension, and diabetes [26, 39, 40].

HSPs are highly conserved proteins during species evolution and are found in eukaryotic and prokaryotic organisms. HSPs can be grouped according to their molecular weights into families (HSP110, HSP100, HSP90, HSP70, HSP60, and HSP30). Specifically, proteins from the 70 kDa HSPs family (HSP70) are highly conserved [41] and have a cytoprotective role in cells, as well as present anti-apoptotic and anti-inflammatory proprieties in various stressful conditions [42]. The increase in the synthesis of HSPs may show an increase in stress tolerance, preventing protein damage related to oxidative stress [43].

In this way, a study carried out by Kido et al. [44] suggested that inhalation of air pollution induces an increase in HSP70 in lung macrophages and also a systemic increase in blood HSP70 levels (a.k.a eHSP70 since it is located in extracellular fluids). These stress responses marked by alterations in both intracellular (iHSP70) and extracellular (eHSP70) suggest these proteins as a relevant immunological mediator that contributes to other aggravating factors (vascular dysfunction and cardiovascular events). For example, exposure to ROFA suspension for three consecutive days (750 μg) promoted an increase in plasma levels of eHSP70 associated with plasma oxidative stress, showing that HSP70 represents a potential inflammatory and indirectly an oxidative biomarker [45].

Furthermore, a study conducted by Goettems-Fiorin et al. [26] highlights that exposure to the $PM_{2.5}$ potentiates metabolic dysfunction in mice treated with HFD, associated with altered cellular stress response, assessed by the ratio [eHSP70]/[iHSP70], called H-index, a biomarker of the low grade chronic inflammatory state, increasing the risk of type 2 diabetes development. The study shows a positive correlation between adiposity, increased body weight and glucose intolerance, and increased glucose and triacylglycerol plasma levels. And when evaluating the H-index in the pancreas, demonstrated that the pancreas exhibited lower iHSP70 expression, accompanied by a 3.7-fold increase in the plasma to pancreas [eHSP72]/[iHSP70] ratio, highlighting that exposure to PM2.5 markedly enhances metabolic dysfunction in HFD-treated mice.

Thus, at least in animal models, HSP70 has been used recently as a biomarker for early assessments of harmful health effects, such as those caused by exposure to air pollution [26, 45, 46]. In humans, the study of Chao et al. [47] with drivers showed an increase in pro-inflammatory activity, with an increase in plasma levels of TNF-α related to the level of pollution exposure. In terms of inflammation, exposure to air pollutants

also promotes responses by cytokine signaling, and PM exposure is associated with the expression of IL1, IL6, and TNF-α [48], and the imbalance pro-inflammatory response in immune cells is also associated with unbalanced eHSP70/iHSP70 ratio [49, 50].

In this way, the intensity of $PM_{2.5}$ exposure and its association with other risk factors might reduce HSP70 levels [51–53]. Independently, metabolic impaired condition and PM exposure may increase the eHSP70/iHSP70 ratio [26, 50, 53–55]. Since eHSP70 is related to increase pro-inflammatory signaling, while iHSP70 is had anti-inflammatory roles by inhibiting NF-kB signaling for inflammatory mediators [42], the unbalance in eHSP70/iHSP70 ratio (increased values in favor of eHSP70) indicates an organism under stress condition without an effective stress response [39, 50, 56].

4. Animal models of PM studies

Experimental studies to evaluate air pollution-induced oxidative stress in humans are challenging to perform for ethical and population heterogeneity reasons [29]. Thus, several experimental studies have shown the effects of exposure to air pollution [18, 20, 57–60], for which many models are used to assess the degree of exposure versus the effect produced. Also, the studies of exposure to pollutants in animal models, which mimic exposure to air pollution in the human population, enable the study of pathophysiological mechanisms by which the body develops numerous diseases related to air pollution.

In this way, many in vivo exposure studies models in animals have been published, with different exposure models to atmospheric pollution. Inhalation methods are closer to mimicking the reality of human exposure, such as inhalation of concentrated PM (CAP) or inhalation of environmental air particulates.

In the CAP method, the exposure system uses the principle of the condensational growth of the ambient particles followed by virtual impaction to concentrate the aerosol and allows the particles larger than 2.5 μm in aerodynamic diameter removed at the concentrator inlet, and the remaining aerosol to be concentrated by inertial separation techniques that dispose of most of the carrier air, which enables delivery of concentrated streams of real-world particles to human subjects or laboratory animals via whole-body exposure [60].

The environmental air particles propose, the exposure of the animals to PM in a chamber, where the air is propelled from the environment to the inner area of the chamber, is frequently used [61–63]. In this model, the experimental groups are divided into filtered air (with a series of filters to prevent pollution from entering) or unfiltered (exposed/polluted group). Alternatively, this model can be performed by uptake urban air using a concentrator of atmospheric pollution particles [64–66]. The last one was used early in toxicological studies, using an atmospheric pollution particle concentrator developed by Harvard University [66]. The advantage of the chamber protocols is to submit the organism to a "real word" air pollution context. On the other hand, since the urban pollutants may present a significant variability between the days of the exposure, at least in terms of concentration and composition, the animals are exposed to days of higher levels and days with low levels of PM in the chamber [61, 62]. As a result, the biochemical and molecular outcomes in this model may also present a significant variability and the interpretation of the results can be specific for urban areas.

Other studies used intratracheal and intranasal instillation methods [26, 67, 68]. Intratracheal instillation should generally be considered a method for a single exposure of the lungs to characterize potential toxicity [69]. The intratracheal instillation

method is often used to expose animals to both soluble and insoluble particles, and it is a relatively inexpensive method that allows instantaneous administration directly into the trachea of known concentration of the pollutant under test directly to the lung [70]. In the intranasal instillation model, the handler restrained the animal (suspended by the cervical region, held with the hand on the back). Then the suspension in liquid (with a known concentration of pollutant) is administered directly into the animal's nostril, that by apnea reflex, inhales the suspension [70, 71]. Thus, the advantage of the intratracheal or intranasal protocols is to submit the organism to a controlled pollution context in terms of dose and frequency of exposure, adequate for toxicological studies and mechanistic studies. Also, since the particles may present a significant variability in terms of the composition, dependent on the source of particles used in the study (e.g., metal composition variability), intratracheal and intranasal protocols allow that, even if the animals are exposed to the same dose, but with different source particles, the studies may reveal different responses. However, as limitations, these procedures may not represent a "real world condition".

When compared to inhalation procedures, instillation is also easier, less expensive, and incurs less health risks to the lab staff. Nonetheless, inhalation is a more realistic physiological approach since it better represents the natural route for PM exposure, and CAP inhalation remains the main one, as it is closer to simulating reality environmental conditions and physiological animal responses in laboratory PM administration [72].

A study by Curbani et al. [72] highlights that studies with intratracheal or intranasal instillation models PM concentrations were three orders of magnitude higher than the environmental ones found in megacities through acute and sudden exposures. And inhalation exposure protocols were closer to ambient PM concentrations, being one or two orders of magnitude higher than the PM concentrations found in megacities. Researchers must be aware, since, the discrepancy is a result of the experimental conditions, where most protocols are planned to reduce the exposure time and increase the PM concentration, thereby attempting to achieve the same result as long time of exposure and low PM concentrations [72].

The models described above are complementary in the information about environmental pollution's impact on health. The development of models to assess exposure to air pollution within cities, to attribute the health risks produced is a priority for future research. The mechanisms of harmful health effects related to exposure to pollution are not fully elucidated in clinical and epidemiological studies, raising questions about how pathologies develop and why exposed individuals become more susceptible to certain conditions. The integrative view of epidemiological, clinal, and animal studies is required to elucidate the fundamental problem of air pollution. To reach this goal, animal models that mimic exposure to air pollution effects are appropriate to elucidate the pathophysiological mechanisms by which the body develops numerous diseases. These studies are complemented by in vitro, in silico models to explain toxicological aspects. Studies using these exposure models have effectively related the damage caused by air pollution to the development of chronic diseases, whether respiratory, cardiometabolic, or neurodegenerative, highlighting mainly the physiological effects produced for the aggravation of these conditions [73].

5. Conclusion

Experimental and epidemiological studies have shown evidence of the harmful effects of exposure to air pollution, especially by PM. The effects caused by such

particles depend on their composition, the exposure time, and the particle size, with emphasis on fine and ultrafine particles due to their multisystemic action. Animal models of PM exposure revealed clearly the pathophysiological mechanisms at the systemic and cellular levels, and the choice of the experimental protocol needs to be clear in terms of the source of the pollutant, dose, and representability of toxicological or real word aspects. Complementarily, animal studies highlighted that the increase of air pollution levels induces responses by oxidative stress, which is an initial mediator of inflammatory processes and helps to elucidate such mechanisms and contribute to the establishment of stricter policies to control emissions and air quality.

Author details

Pauline Brendler Goettems Fiorin, Mirna Stela Ludwig, Matias Nunes Frizzo and Thiago Gomes Heck*
Research Group in Physiology, Postgraduate Program in Integral Attention to Health, Regional University of Northwestern Rio Grande do Sul State (UNIJUI), Ijuí, RS, Brazil

*Address all correspondence to: gomesheck@yahoo.com

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