Toxic and Proarrhythmic Effects of Airborne Particulate Matter Exposure: In-vitro, In-vivo and In-silico Study

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Abstract

Exposure to particulate matter (PM) is related to an increase in cardiovascular diseases and the appearance of cardiac arrhythmias. However, the pathophysiological mechanisms have not been well established. This study aims to evaluate the toxic and proarrhythmic effects of PM, for this, PM was extracted from monitoring station filters using Soxhlet systems. Cardiomyocyte cultures of the HL-1 cell line were exposed to different PM concentrations and MTT assay was performed to assess cell viability, which decreased as the PM concentration increased. The lethal dose (LC50) obtained was adopted as the IC_{50} value for in-silico studies to evaluate the PM effects in 2D ventricular and atrial tissue models. As the concentration of PM increased, major pro-arrhythmic effects were observed with a shortening of the action potential duration and increasing vulnerable windows. To verify the results, we used mice with inhalation exposure to vaporized aerosols, which generate high PM levels. In-vivo programmed electrical stimulation in the exposed mice suggested a trend for a decreased ventricular effective refractory period as well as significantly increased inducible ventricular arrhythmogenesis compared to room air control animals. Overall, the results suggest cytotoxic and proarrhythmic effects of PM in a concentration-dependent manner.

1. Introduction

Air pollution is responsible for millions of global deaths annually [1]. Particulate matter (PM) is a complex mixture of particles suspended in the air and has been related to important health effects [2]. Studies also have shown an increased probability of cardiac arrhythmia generation in the population after exposure to air pollutants [3]–[5], concluding that air pollution is an acute trigger of these arrhythmias [6]. It has also been demonstrated that prolonged exposures reduce the life expectancy of people for several years and increase hospital admissions for cardiovascular diseases due to exposure to high concentrations of pollutants [7], [8]. Cardiac morbidity [5] and mortality [9] have been reported from both short- and long-term exposure to PM. However, the mechanisms underlying PM-induced cardiovascular disease in humans are still poorly understood [10], making clinical management difficult and impeding the development of effective preventive approaches [11]. This study aims to evaluate the toxic and proarrhythmic effects of PM.

2. Methods

2.1. In-vitro methodology

The PM was obtained from a total of 148 PM10 filters from different monitoring stations located in central areas of the city of Medellín, Colombia, during the first half of 2022. The PM was extracted from the filters using Soxhlet systems [12]. To assess the impact of PM on cell viability, HL-1 cells were seeded in multi-well plates and cultured according to the protocol recommended by the supplier. From the PM extract, 15 serial dilutions were made (1:1), starting from a concentration of 25 mg/ml of complete PM and diluted in DMSO until obtaining a concentration of 0.002 mg/ml. A positive control (pure DMSO), a negative control (cells without treatment), a solvent control (DMSO 0.19%), and a medium control (without cells) were adopted.

Cell death was assessed using the MTT assay according to the protocol of Muelas *et al*. [13]. Regression was performed to determine the number of cells suitable for performing the assays (between 8000 and 10,000 cells). 10,000 cells were seeded per well, and treatments were performed 24 hours after seeding by adding 10 µl of each of the 15 dilutions of the PM samples. After 24 hours, the culture medium was discarded, 50 µl of MTT (3-4,5-dimethylthiazol-2,5-diphenyltetrazoliumbromide) solution (1 mg/ml) was added, and the well was incubated in the dark at 37 ºC for 2 hours. Then, the medium was discarded

and 100 µl of isopropanol was added to each well and stirred constantly for 30 min. Finally, the optical density was read with a Multiskan-go spectrophotometer at a wavelength of 570 nm. This test is performed to find the lethal concentration 50 (LC50) after exposure to PM.

2.2. In-silico methodology

The Courtemanche [14] and ten Tusscher [15] models were implemented to simulate the action potential of atrial and ventricular cardiomyocytes, respectively. Based on studies in myocytes indicating that PM affects cardiac electrical activity by blocking the L-type calcium channels (I_{CaL}) [4], we developed a model of the PM effect on I_{CaL} using the steady-state fraction of block (b_{PM}). The Hill equation was adopted to fit the concentration-response relationships for the block, as follows:

$$b_{PM} = \frac{1}{1+\left(\frac{IC_{50}}{D_{PM}}\right)^{h}}, \quad (1)$$

where IC_{50} is the half maximal inhibitory concentration for the I_{CaL} current block by PM, and D_{PM} is the PM concentration with values of 0 nM, 1.5 nM, 3 nM, 4.5 nM, and 6 nM. A Hill coefficient (h) of 1 indicates completely independent binding. The LC50 obtained was adopted as the IC_{50} value for in-silico simulations. The blocking factors were applied to I_{CaL} equations in both cell models.

We developed a 6 x 6 cm square mesh, composed of 192 x 192 elements, with a spatial discretization of 312.5 µm. An isotropic tissue was considered. The following equation describes the propagation of the action potential in 2D domains:

$$K\left(\frac{\partial^2}{\partial x^2}+\frac{\partial^2}{\partial y^2}\right)V_m = C_m\frac{\partial V}{\partial t} + I_{ion} + I_{st}, \quad (2)$$

where V_m is the membrane potential, I_{ion} is the sum of the transmembrane ionic currents corresponding to each model, I_{st} is for the external stimulation and C_m is the membrane capacitance. The diffusion coefficient K was adjusted to obtain a realistic conduction velocity of 43.5 cm/s under control conditions (without PM). The integration time step was 0.01 ms.

At the single cell level S1-S1 stimulation protocol was applied, which consists of a train of 10 rectangular pulses with a duration of 2 ms and amplitude of -2000 pA, at a basic cycle length (BCL) of 1000 ms. At the 2D level, a cross-field S1-S2 stimulation protocol was applied, where S1 is a flat stimulus applied to the left end of the tissue, and S2 is a premature stimulus applied to the top of the tissue when the repolarization wavefront generated by S1 exceeds half the domain. The time elapsed between the application of S1 and S2 is called the coupling interval.

2.3. In-vivo methodology

To verify the results of our simulations, we used mice with inhalation exposure to vaporized aerosols of a humectant mixture containing 70% vegetable glycerin and 30% propylene glycol, which are constituents of electronic cigarette products, and shown to generate high levels of PM. Wild-type C57bl/6J mice were instrumented with telemetric ECG devices. After 2 weeks of recovery, they were put in an inExpose nose-only exposure system, 1 hour per day, for a week, as a training period, to have the mice acclimatize to their presence in the device. Subsequently, we studied the in-vivo inducibility of VT in 5 mice exposed to vaping for 10 weeks versus 7 mice exposed to air control, using in-vivo programmed electrical stimulation.

3. Results

3.1. In-vitro results

Cell viability decreases as PM concentration increases, with a cytotoxic effect observed. The most cytotoxic effect was observed in cells exposed to PM at 25 mg/ml, where viability was reduced to 15.95%. Figure 1 shows the average viability percentage, versus the PM concentration. Cytotoxicity in the cell cultures is expressed as LC50, the concentration of PM that is lethal to 50% of the cells. In this in-vitro study, the LC50 after exposure of HL-1 cultures to PM was 1.44 mg/ml.

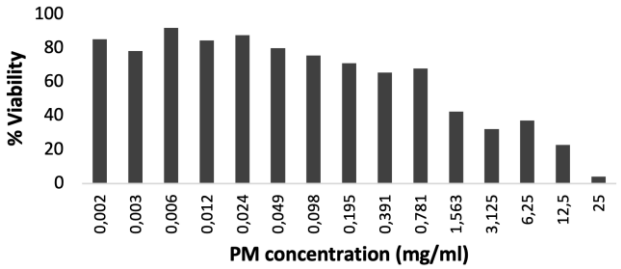

Figure 1. Bar graph of average viability versus PM concentration.

3.2. In-silico results

Under normal physiological conditions, as the PM concentration increases, a reduction in the magnitude of the maximum peak of the I_{CaL} current is observed and consequently a reduction in the action potential duration (APD). This reduction amounted to 53% (144 ms) and 42% (192 ms) at the highest PM concentration, for the Courtemanche and ten Tusscher models, respectively (see Table 1). A loss of the action potential dome in the plateau phase was also observed in both models (Figure 2).

Table 1. APD reductions at different PM concentrations in atrial and ventricular cell models.

[PM]	Atrial cell		Ventricular cell	
	APD_{90} (ms)	% reduction	APD_{90} (ms)	% reduction
0	309	-	331	-
1.5 nM	296	4 %	253	24 %
3.0 nM	205	34 %	223	33 %
4.5 nM	185	40 %	208	37 %
6.0 nM	144	53 %	192	42 %

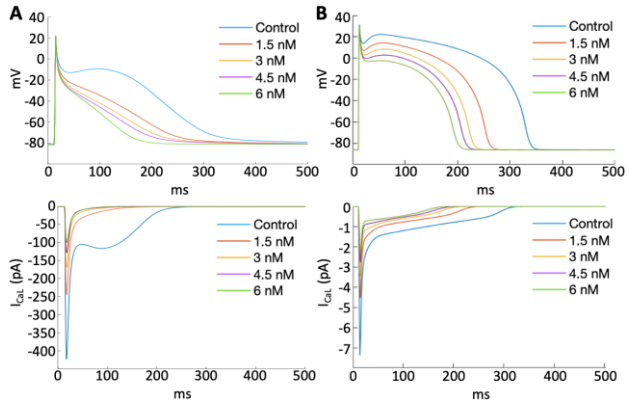

Figure 2. Action potential and I_{CaL} current at different PM concentrations from the a) atrial and b) ventricular cell model.

At high PM concentrations (4 nM and 6 nM) it was possible to generate reentries within vulnerable windows of 23 ms and 44 ms for the atrial and ventricular cell models, respectively. Figure 3 depicts the reentry sequence obtained by applying the 6 nM of PM concentration at a coupling interval (time between S1 and S2) of 160 ms and 400 ms for the atrial (Figure 3A) and ventricular models (Figure 3B), respectively.

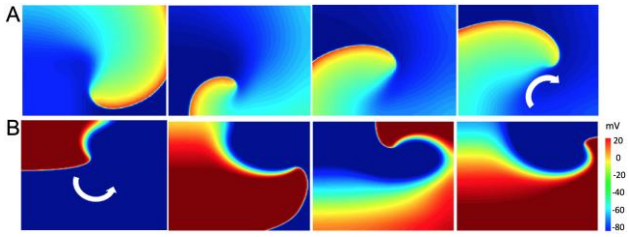

Figure 3. Reentrant propagation dynamics in the 2D model of a) atrial and b) ventricular tissue.

3.3. In-vivo results

In-vivo programmed electrical stimulation in the exposed mice suggested a trend for a decreased ventricular effective refractory period (ERP) as well as significantly increased inducible ventricular arrhythmogenesis compared to room air control animals. The ECGs of Figure 4 show that a 1-second, 36 Hz burst stimulus at 1 mA, induced a short-lived ventricular tachycardia (VT) episode, after which the heart reverted back to sinus rhythm. A similar burst stimulus induced a longer VT episode in the vaped mouse. Six out of 7 air control mice had VT, while 5 out of 5 vaped animals had VT, however, the arrhythmia duration was significantly longer in vaped compared to WT mice (t-test, $p<0.05$).

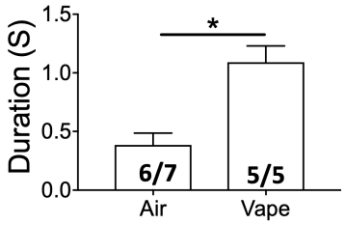

Figure 4. Ventricular tachycardia (VT) duration in control and vaped mice (t-test, $p<0.05$).

4. Discussion

Epidemiological, clinical, and experimental studies have demonstrated the proarrhythmic impact of PM [2], [9], [10], [16], [17]. To explore the possible cardiac health effects associated with exposure to PM, this study employed in-vitro, in-silico, and in-vivo models. Our in-vitro results show that the PM induces loss of cell viability as PM concentration increases. The simulation results indicate that PM may induce a proarrhythmic effect in atrial and ventricular cardiomyocytes, where a greater reduction in APD in a concentration-dependent manner and attributable to I_{CaL} inhibition. The plateau is also reduced, which coincides with the findings of Ferreira de Mattos et al. [18] who found a negative inotropic effect, a reduction in cardiac contractility, early afterdepolarization, and a plateau reduced, in the concentration-dependent way due to a block of L-type Cav1.2 channels. An experimental study [19] suggests that acute PM exposure diminishes myocardial contractility by decreasing calcium influx through the sarcolemma. Our findings in 2D models of atrial and ventricular tissue suggest a proarrhythmic effect characterized by sustained reentry into an increased vulnerable window. On the other hand, our in-vivo results suggest a trend for a decreased ventricular ERP as well as significantly increased inducible arrhythmogenesis in the exposed mice. Has been demonstrated that animals with cardiovascular disease antecedents exposed to high levels of PM have a greater risk of their condition disease worsening [17], [20]. Although the mechanism of the

association between arrhythmias and PM exposure has not been fully understood, arguments include increased heart rate, decreased heart rate variability [16], and inflammation [21].

5. Conclusion

The results indicate that PM has cytotoxic and proarrhythmic effects that increase with concentration. These effects lead to a higher risk of cardiac arrhythmias, directly affecting the electrical stability of the heart. This highlights the need to regulate PM exposure to protect cardiovascular health.

Acknowledgments

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