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Available in: http://www.redalyc.org/articulo.oa?id=37038028004
EVALUATION OF THE OXIDATIVE POTENTIAL OF URBAN PM AND ITS RELATION TO in vitro INDUCED DNA DAMAGE: A SPATIAL AND TEMPORAL COMPARISON

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(Recibido abril 2014; aceptado noviembre 2014)

Key words: PM10, PM2.5, electron paramagnetic resonance, oxidative potential, DNA damage

ABSTRACT

Some toxic effects of particulate matter (PM) are related to the oxidative potential (OP) of the particles. The electron paramagnetic resonance (EPR) technique was used to evaluate the intensity of paramagnetic species (PS) and EPR plus spin trapping, to evaluate the OP of PM. We evaluated, in parallel, the DNA degradation potential of PM10 and PM2.5 collected from three regions of Mexico City in 1991 and 2003. Each region had different sources of pollution: industrial, commercial or residential. Both techniques evaluated Fenton-type reactions in the presence and absence of deferoxamine (DFO). PM10 samples from the industrial region presented similar high OP, independently of sampling year. PM10 and PM2.5 collected in the commercial and residential regions in 2003 had similarly low OP. The OP induced by PM10 from the industrial region was completely inhibited by DFO, and DFO partially inhibited the OP induced by PM10 from other regions. PM2.5 OP was not inhibited by DFO. PM from the industrial region was the most potent inductor of DNA degradation, while PM from residential region was the least potent, correlating with the OP. DFO inhibited the degradation of DNA induced by PM. The OP of PM collected in the industrial and residential region correlated with the DNA degradation. The region, size and year of PM collection are linked to observed OP variations and DNA degradation induced by PM.

Palabras clave: PM10, PM2.5, resonancia paramagnética electrónica, potencial oxidativo, daño ADN
RESUMEN

Algunos de los efectos tóxicos atribuibles a las partículas atmosféricas (PM) están relacionados con su potencial oxidante (OP). La resonancia paramagnética electrónica (EPR) es una técnica que se utilizó para evaluar la intensidad de las especies paramagnéticas (PS), y la EPR más un atrapador de espín, para evaluar el OP de las PM. Se evaluó en paralelo el potencial de degradación de ADN por PM$_{10}$ y PM$_{2.5}$ muestreadas en tres regiones de la Ciudad de México en 1991 y 2003. Cada región tenía diferentes fuentes de contaminación: industrial, comercial o residencial. Ambas técnicas fueron evaluadas en reacciones de tipo Fenton en presencia y ausencia de deferoxamina (DFO). Las PM$_{10}$ de la región industrial presentan alto OP, independiente del año de muestreo. Las PM$_{10}$ y PM$_{2.5}$ muestreadas en las regiones comercial y residencial durante 2003 tuvieron bajo OP. El OP inducido por las PM$_{10}$ de la región industrial fue completamente inhibido por la DFO, el OP inducido por las PM$_{10}$ procedentes de las otras dos regiones fue inhibido parcialmente. El OP de las PM$_{2.5}$ no fue inhibido por la DFO. Las PM$_{10}$ de la región industrial fueron el inductor más potente de la degradación del ADN, mientras que las PM$_{10}$ de la región residencial fueron las menos potentes, lo que se correlaciona con el OP. La DFO inhibe la degradación del ADN inducida por las PM. El OP de las PM de la región industrial y residencial se correlaciona con la degradación del ADN. La región, el tamaño y el año de muestreo parecen estar vinculados a las variaciones del OP y a la degradación del ADN inducido por las PM.

INTRODUCTION

It has been widely demonstrated that particulate matter (PM) is linked to biological effects such as lung inflammation, blood clotting, and various cardiovascular effects (Alfaro-Moreno et al. 2007). Some of these effects (such as endothelial dysfunction) have been related to the ability of the particles to induce oxidative stress (Montiel-Dávalos et al. 2010). Several studies indicate that different physical and chemical properties of the particles are related to the intensity of the biological effects (Veranth et al. 2006, Rosas-Pérez et al. 2007, Mugica et al. 2009). Differences in composition seem to be critical, it has also been shown that PM collected in different cities and even in different locations within the same city, show different toxic potentials (Alfaro-Moreno et al. 2002, Gerlofs-Nijland et al. 2009, Osornio-Vargas et al. 2011).

The toxicological evaluation of PM is a time-consuming effort. Considering the wide range of PM composition and size to which humans can be exposed, there is a need to predict potential toxic effects of PM. For this purpose, electron paramagnetic resonance (EPR) has been used to evaluate the oxidative potential (OP) of PM (Valavanidis et al. 2005b). Source-related variations in chemical components and composition may lead to different oxidative characteristics (Briede et al. 2005). Therefore, PM generated by different sources may have a different pattern of oxidative potential. The relationship between the oxidative potential’s ability to induce an effect at a biological level and the oxidation of biomolecules can help to predict toxicity. EPR can be employed as an alternative method of predicting the toxic potential of PM in a quick, sensitive and reliable way.

We have already mentioned that PM represents a complex mixture and some components, such as metals, can cause inflammatory processes and increased reactive oxygen species (ROS), related to Fenton reactions (Osornio-Vargas et al. 2011).

It has been shown that different biological processes induced by the particles of Mexico City are associated with PM chemical composition (Alfaro-Moreno et al. 2002). One of these effects is DNA damage and is suggested that DNA degradation occurs as a result of metal content, and the generation of ROS involving the Fenton reaction (Lloyd 1997, Lloyd and Phillips 1999).

Naked DNA and the chelating Deferoxamine (DFO) have been used as a simplified method for evaluating the participation of metals present in PM and the induction of DNA damage (Garcia-Cuéllar et al. 2002).

Oxidative stress has been identified as a key factor in causing biological effects induced by PM. Although, it is known that cells exposed to PM show a dramatic increase in oxidative activity (Soukup et al. 2000, Becker et al. 2005), the intrinsic oxidative potential of PM is not well understood. Furthermore, it has been shown that PM with a high oxidative potential is more toxic (Wessels et al. 2010). In the present study, we evaluated the signal intensity of
paramagnetic species and the oxidative potential of urban PM from Mexico City collected in different regions and years using EPR.

**MATERIALS AND METHODS**

**PM$\textsubscript{10}$ and PM$\textsubscript{2.5}$ sampling**

Particulate matter with an aerodynamic diameter ≤10 µm (PM$\textsubscript{10}$) was obtained from industrial (IR), commercial (CR), and residential (RR) regions of Mexico City using a high-volume sampler (GMW model 1200 VFC HV PM$\textsubscript{10}$; Sierra Andersen, Smyrna, GA, USA) for particles with an aerodynamic diameter ≤10 µm (PM$\textsubscript{10}$). In 1991, 24 h PM$\textsubscript{10}$ samples were collected using fiberglass filters (type A/E glass 61638; Gelman Sciences, Ann Arbor, MI, USA; 1.13 m$^3$/min), three days per week during each week of the year. PM was recovered from the membranes after dry sonication for 45 min and subsequently smoothly swept with a brush into an endotoxin-free flask. All PM samples were pooled by region and stored dry in endotoxin-free glass vials, which were kept in a dryer at 4 ºC until use (Alfaro-Moreno et al. 2002). In 2003, PM$\textsubscript{10}$ and particulate matter with an aerodynamic diameter g ≤ 2.5 µm (PM$\textsubscript{2.5}$) were collected using the same instrument, but we introduced a modification involving the use of cellulose nitrate membranes with a nominal pore size of 3 µm. Membranes were cut from rolls (11302-131, Sartorius, Goettingen, Germany) and modified to preserve the airflow rate and particle size sampling performance, as previously described (Alfaro-Moreno et al. 2009).

**Determination of the relative intensity of paramagnetic species in the PM**

To evaluate the linearity of the method, we evaluated the relative intensity of the paramagnetic species using PM$\textsubscript{10}$ (1, 2, 3, 4 and 5 mg) samples from 2003 with an EPR spectrometer (Jeol JES TE-300, Tokyo, Japan) under the following experimental conditions: center field 335 mT; microwave frequency 9.4 GHz; microwave power 1 mW; sweep width +/- 4 mT; sweep time 2 min; modulation width 0.16 mT; amplitude 63; time constant 0.3 s and accumulation 1 (Fig. 1a).

The relative intensity was obtained in relation to a standard curve of Tempol by double integration of each spectrum (Shinji et al. 2004, Dos Santos et al. 2009), using Esprit 382 series V 1.916 Jeol software. The results show the signal intensity and are expressed in arbitrary units.

**Relative intensity in the solid PM**

To determine the relative intensity of paramagnetic species in the solid PM samples, 3 mg of each sample was weighted and evaluated under the conditions previously described. Two independent measurements were taken for each PM sample. The final intensity was calculated based on the standard curve described above.

**Evaluation of the oxidative potential of PM**

The production of the hydroxyl radical (•OH) by PM$\textsubscript{10}$ and PM$\textsubscript{2.5}$ was evaluated in the presence of H$\textsubscript{2}$O$\textsubscript{2}$. For this purpose, a suspension of PM was prepared (3 mg/mL). A total of 100 µL of the suspension was mixed with 200 µL of 5,5-Dimethyl-1-Pyrrole N-oxide (DMPO) (final concentration 0.1 M, Dojindo, Rockville, MD), for use as a spin trap, and 100 µL of H$\textsubscript{2}$O$\textsubscript{2}$ (final concentration 0.125 M, Fluka-Aldrich, St. Louis, MO). The mixture was incubated for 15 min at 37 ºC with continuous shaking. The sample was filtered (0.2 µm; Ministar-RC Syringe, Sartorius, Goettingen Germany) and transferred to a quartz flat cell for the EPR measurements. The formation of hydroxyl radical (•OH) was detected as a well-characterized 1:2:2:1 pattern of DMPO-OH adduct (Fig. 1b; Rinalducci et al. 2004) with aN=aH= 1.49 mT, g = 2.0056 under the following experimental parameters: center field = 335 mT, microwave frequency = 9.4 GHz, microwave power = 1 mW, sweep width +/- 5 mT, sweep time 0.5 min, modulation width 0.04 mT, amplitude 100, time constant 0.1 s and accumulation 3. A mixture of DMPO (0.1 M in water), phosphate buffered saline (PBS) and H$\textsubscript{2}$O$\textsubscript{2}$ (0.125 M in PBS) was used as a blank (Knaapen et al. 2000, Shi et al. 2003).

**Inhibition of the oxidative potential of PM**

Under the same experimental conditions described above we added DFO to the samples as an iron-copper chelating agent. A total of 100 µL of the PM suspension was mixed with 100 µL of DFO (final concentration 2.5 mM in water). The samples were incubated for 3 h with continuous shaking.

**Evaluation of DNA degradation**

The degradation of “naked” DNA was evaluated in DNA isolated from Balb/c 3T3 cells with a commercial kit (DNA isolation kit for cells and tissues; Boehringer, Mannheim, Germany) as previously described (García-Cuéllar et al. 2002). A total of 400 ng of isolated DNA was exposed to 40, 80 or 160 µg/mL of PM$\textsubscript{10}$ in the presence of 1 mM H$\textsubscript{2}$O$\textsubscript{2}$ (Fenton-type reaction). To inhibit the DNA degradation related to transition
metals 1 mM of DFO was added. DNA exposed to CuSO\(_4\) as well as H\(_2\)O\(_2\) was used as a positive control for a Fenton-type reaction. DNA was exposed to the particles for 24 h with constant shaking. The samples were evaluated by electrophoretic mobility (H5 Horizontal Gel Electrophoresis Apparatus; GIBCO-BRL Gaithersburg, MD) in 1.5 % agarose gels at 100 V for 3 hours, then stained with ethidium bromide (1.2 mg/mL) and photographed under UV light using a Kodak Gel logic 200 imaging system (New Haven, CT). All gels included DNA size markers and the following DNA degradation positive and negative controls: 1) \(\lambda\) Hind III (1 µg), 2) 400 ng of DNA alone, 3) 400 ng of DNA with 1 mM H\(_2\)O\(_2\), 4) a “Fenton-type reaction” control using 400 ng of DNA plus 5 mM CuSO\(_4\) with 1 mM H\(_2\)O\(_2\), and 5) 400 ng of DNA plus 1 mM of DFO and 1 mM H\(_2\)O\(_2\).

Statistical analysis

A linear regression analysis was performed for the spin intensity vs. PM mass. The slope and R-value were calculated for each curve. These curves were used to calculate the intensity of spin in all evaluated samples. The data obtained for the different regions, years and sizes of PM were analyzed using an ANOVA followed by a Bonferroni test (Stata 7.0, Windows XP, College Station, TX). Differences were considered significant when p < 0.05.

RESULTS

Determination of the intensity of paramagnetic species (spin) in PM

Dry PM samples showed an pseudoisotropich broad signal (\(g = 2.2\) and \(\Delta H = 68\) mT) indicating a high concentration of paramagnetic species (PS), potentially explained by the presence of previously identified levels of Fe\(^{3+}\), Cu\(^{2+}\) and VO\(^{2+}\) in the PM samples (Fig. 1a). These metals are found in all three regions in different proportions, though the IR was found to have the highest concentrations of metals’ related signal. The intensity evaluation of paramagnetic species in PM\(_{10}\) from 2003 showed a linear correlation between PM mass and intensity under the conditions used for the measurement. When testing the PM from different regions the correlations yielded similar slopes and R-values above 0.99 for all samples (Fig. 2).

Fig. 1. EPR experimental spectra at room temperature for: A) solid PM samples, B) Typical EPR spectrum of short-lived adduct of hydroxyl radical, the DMPO-OH generated in aqueous solutions of PM samples in the presence of DMPO and H\(_2\)O\(_2\).

PS intensity of PM from different years and regions

Fig. 2. Relative intensity correlation to PM mass. A linear correlation between PM mass and intensity was observed for all PM10 samples collected in 2003. The R-value for all samples was above 0.99, with a similar slope.
PM-related PS intensity varied depending on the sampling region, the PM size and the year of collection (p < 0.05). PM$_{10}$ collected in the IR during 1991 presented the highest intensity, followed by samples from the CR (p > 0.05) and the RR (p < 0.05; Fig. 3a). PM$_{10}$ collected during 2003 did not yield significant differences among the sampling sites (Fig. 3b). Nevertheless, a reduction in spin intensity was observed in the IR upon comparison of samples from 1991 and 2003 (p = 0.07). In the IR and the CR, PM$_{2.5}$ had lower PS intensities than PM$_{10}$ samples, ~ 20 % of the PM$_{10}$ value (Fig. 3c).

**Fig. 3.** Intensity of PM collected in different years and regions. A) PM$_{10}$ collected in 1991 showed a significant difference in the intensity depending on the region of collection. Particles from the residential region (RR) had a smaller intensity than those collected in the industrial (IR) and commercial (CR) regions. B) PM$_{10}$ collected in 2003 did not show significant differences between regions of collection. C) PM$_{2.5}$ collected in 2003 did not show significant differences between regions of collection. Differences observed regarding sampling region, PM size and year of collection were statistically significant. Values represent means of three independent experiments ± standard error (SE), ANOVA-Bonferroni test (*, + p < 0.05)

**Oxidative potential of PM by EPR**

PM suspensions in the presence of H$_2$O$_2$ produced a characteristic 1:2:2:1 DMPO-OH adduct pattern (hfcc $a_N$=$a_H$ =1.49 mT, $g$=2.0056; Fig. 1b), indicative of hydroxyl radical (•OH) generation (Rinalducci et al. 2004) and indicative of OP. The OP of PM$_{10}$ collected during 1991 was similar for the IR and the CR and slightly lower for the RR (Fig. 4a; p > 0.05). In contrast, the PM$_{10}$ collected in the IR during 2003 demonstrated a higher oxida-

**Fig. 4.** Oxidative potential of PM. A) PM$_{10}$ samples from 1991 have no significant difference in oxidative potential regardless of the region of sampling. Nevertheless, DFO inhibited the OP of samples collected in the industrial region (IR), but only partially inhibited the OP from the commercial (CR) and residential (RR) regions. B) PM$_{10}$ samples from 2003 collected in the IR had a stronger OP than those collected in the CR and RR. DFO almost completely inhibited the OP of the samples from the IR and only partially inhibited the OP from the CR and RR. C) PM$_{2.5}$ samples from 2003 yielded no differences among the regions of collection, and the addition of DFO did not inhibit the OP of any sample. Values represent means of four independent experiments ± standard error (SE), ANOVA-Bonferroni test (*, + p < 0.05)
Inhibition of PM oxidative potential by DFO

The OP induced by PM\textsubscript{10} from the IR in 1991 and 2003 was strongly inhibited by the presence of DFO ($p < 0.05$; Fig. 4a and b). In the case of the PM\textsubscript{10} collected in the CR and RR, no significant reductions in the OP were observed. The OP of PM\textsubscript{2.5} was not inhibited by DFO (Fig. 4c).

DNA degradation

All samples of PM were capable of inducing DNA degradation under the experimental conditions (Fig. 5). In the case of the PM\textsubscript{10} collected in 1991, the particles from the industrial and the commercial regions were stronger inducers of DNA degradation than those collected in the residential region (Fig. 5a). PM\textsubscript{10} samples from 2003 presented a different pattern; those collected in the CR seemed to induce weaker responses than those from the IR and RR (Fig. 5b). In the case of PM\textsubscript{2.5}, similar effects were observed independent of the region (Fig. 5c). In all cases, DNA degradation was completely inhibited by the presence of DFO.

**DISCUSSION**

In the present study, we observed that the intensity of the paramagnetic species and oxidative potential of urban PM collected in Mexico City varied by year and site of collection. These parameters correlate with the ability of the PM to induce DNA degradation in vitro.

The broad signal induced by the PM dry samples is the result of the dipolar coupling of a high concentration of paramagnetic species, including metals such as Fe$^{3+}$, Cu$^{2+}$ and VO$_2^+$ (Valavanidis et al. 2005a). In the center field, we observed an isotropic fine signal with $\Delta H = 0.4$ mT and $g = 2.0026$ that was attributable to stable organic species that could be semiquinones (data not shown). Regarding the intensity of the paramagnetic species, we observed that the PM from the IR of Mexico City collected in 1991 had a higher content of this intensity than that observed by PM from the RR. In contrast, the PM collected during 2003 did not differ depending on the sampling region. When comparing PM from both years (1991 vs. 2003) a significant reduction in the paramagnetic species content was observed for the PM from 2003 of the IR ($p < 0.05$), whereas the PM from the RR showed an increase ($p < 0.05$). The paramagnetic species in PM from the CR remained similar in both years of sampling. It has been reported that the concentration of different components in PM from Mexico City varied depending on the year of collection (Vega et al. 2004, Bae et al. 2010). The rapid growth of both, population and vehicle fleet (about 2 million units) during the period 1991-2003 has increased the production of organic compounds, PM\textsubscript{10} and PM\textsubscript{2.5} in Mexico City. Besides the growth of the industry, because about 70\% of non-metallic mineral industries, primary metals industry, food and beverage production, are important sources of PM (SMA-GDF 2008). For example, the content of total carbon and elemental carbon increased from 1997 to 2002, and these changes were related to increases in traffic, as well as industrial and commercial activities (Vega et al. 2011).

Previous evaluation of metal content by inductively coupled plasma-atomic emission (ICP-AES) and carbon content (TOR) in the PM\textsubscript{10} from 1991 and 2003 revealed a higher concentration of metals and carbon in samples collected in 2003 (Alfaro-Moreno et al. 2009). Particles from 1991 had a gradient of metal content in which IR$>$CR$>$RR, while samples from 2003 had the pattern IR$>$RR$>$CR. In 1991, we observed that the carbon content had a pattern of IR$=$CR$>$RR, while in 2003, the pattern observed was IR$>$CR$>$RR (Vega et al. 2011).

When the oxidative potential of PM was evaluated, we observed that the samples collected in 1991 did not show significant differences among regions, while the PM collected in 2003 demonstrated that the PM from the IR region had a larger OP than that of the other two regions. If we compare the samples from 1991 and 2003, it is notable that the PM\textsubscript{10} from the IR showed a significant increase in oxidative potential in 2003, while the PM from the other two regions did not significantly vary between the different years. These differences in oxidative potential could be related to the two following phenomena: 1) changes in the content of metals, mainly Fe and Cu, or 2) the oxidation of the sample due to storage time. This oxidation could increase the signal intensity in dry samples but still contribute to the OP, as with the less oxidized species. The second hypothesis could be supported by the observation that the amount of paramagnetic species is larger in the 1991 samples than in the 2003 samples. It makes sense that iron would be a significant contributor to the intensity of paramagnetic species, considering that Fe$^{2+}$ is an inducer of the Fenton reaction (Granados-Oliveros
Fig. 5. DNA degradation by PM\textsubscript{10} and PM\textsubscript{2.5}. A) PM\textsubscript{10} collected in 1991 in the industrial (IR) and commercial (CR) regions were stronger inductors of DNA degradation than those collected in the residential region (RR). B) PM\textsubscript{10} from 2003 collected in the CR appeared to be weaker than those from the IR and the RR. C) PM\textsubscript{2.5} had similar degradation patterns for particles collected in different regions. For all cases, DNA degradation was fully inhibited by the presence of DFO.
Fe\(^{2+}\) is not a paramagnetic species, in contrast to Fe\(^{3+}\) (Valavanidis et al. 2009, Gilch et al. 2010). Given this, it is likely that the formation of the hydroxyl radicals detected comes from both oxidation states of Fe. In addition to Fe, Cu\(^{2+}\) is a good promoter of Fenton-like reactions (Valenzuela et al. 2008) and has shown an even higher rate of oxidizing ability than Fe (Strlič et al. 2003).

The roles of Fe\(^{2+}\) and Fe\(^{3+}\) were highlighted by the suppression of the oxidative potential when DFO was added to the PM-DMPO suspension, considering that DFO is primarily an iron chelator (Valgimigli et al. 2001, Karlsson et al. 2005, Shi et al. 2006).

Various authors have shown the relationship between the oxidative potential of PM and biological effects by measuring lipid peroxidation (Shi et al. 2006), DNA damage (García-Cuéllar et al. 2002, Sánchez-Pérez et al. 2009, Wei et al. 2009) and cellular death (Chirino et al. 2010). In the present study, we observed that the intensity of DNA degradation by the Fenton-type reaction correlated with the oxidative potential. Interestingly, we observed that the addition of DFO to the mixture of DNA and PM abolished the degradation of DNA, while the oxidative potential was still measured with EPR. However, metal synergisms exist and the participation of organics has been described as key issues in PM induced biological effects (Cooper et al. 2009).

Due to the design of this study, no full effect of the organic fraction can be explained, as is in the case of PM\(_{2.5}\). Thus, this result could be explained by differences in resolution between the two methods used (i.e., DNA electrophoresis did not detect minor DNA alterations; Sánchez-Pérez et al. 2009).

It has been shown that the OP of PM, as measured by EPR analysis (•OH radicals), correlates with DNA damage (Shi et al. 2006). The present study supports this previous observation. The reason for this correlation appears to be the high presence of metals. Considering that Fe\(^{3+}\) and Cu\(^{2+}\) are capable of inducing a Fenton-type reaction (Veranth et al. 2006, Gilch et al. 2010), it seems logical to conclude that the oxidative potential of PM, which can be inhibited by DFO, is mainly related to the predominant transition metals. Wessels et al. (2010) have suggested that increases in PS could be related to larger OP. As has been previously discussed, this correlation depends on the chemical species.

## CONCLUSION

In conclusion, the evaluation of paramagnetic species and oxidative potential by means of EPR provides information supporting the understanding of the damage induced by the oxidative potential of PM. In some cases, the evaluation of the oxidative potential of PM could help to predict potential toxicity of these materials. However, PM is a complex mixture composed of different transition metals, organic and biological compounds that interact and can produce additive or synergistic effects. But for this study, metals are the most important sources of OP in PM samples. The EPR spin trapping technique allows us to directly monitor the formation of the •OH free radical and to conduct DNA degradation analysis. Together, these approaches can be used as primary methods for evaluating the toxicity potential of PM.

## ACKNOWLEDGMENTS

This study was partially supported by the CONACyT (project 106057). We would like to thank MSc. Yazmín Segura for her technical support during the 2003 PM sampling, Dr. Yesennia Sánchez-Pérez, MSc. Eva Salinas Cortés and MSc. Leticia Martínez Romero for technical advice in standardizing the DNA degradation assay.

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