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# Occupational contamination through emissions of different types of PM promotes systemic oxidative stress that can be attenuated by an antioxidant intervention

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**Abstract**

The emission of several airborne particulate matter (PM) is associated with occupational contamination that affect workers directly exposed to incineration of solid residues of health services (SRHS) (Possamai et al., 2009), coal mining (Ávila Jr. et al., 2009) and also coal burning derived from an electric-power plant (Possamai et al., 2010), as well as the population living in the vicinity of such activities, leading to endogenous overgeneration of reactive oxygen species (ROS), therefore to a systemic oxidative stress (OS). Several OS biomarkers, both enzymatic and non-enzymatic, were measured in blood samples of these workers (n = 20) and also from subjects indirectly exposed (n = 20) living in the vicinities (~0.1 km to 2-15 km) regarding these airborne emissions, before and after an antioxidant intervention of daily oral supplementation of vitamins C (500 mg) and E (400 mg) during six months. Compared to controls, the antioxidant intervention after 6 months was able to confer a consistent protective effect against the systemic oxidative insult irrespective of the dust source of the airborne contaminants (Possamai et al., 2010; Wilhelm Filho et al., 2010). The present review indicates that an antioxidant intervention could be recommended in order to attenuate the related deleterious oxidative consequences in workers directly or indirectly exposed, as well as in subjects (residents) indirectly exposed to such airborne emissions. The main related data available in the literature regarding airborne contamination associated with occupational contamination involving coal mining, coal combustion as well as incineration of solid residues are compared with our own findings.

*Keywords: Coal mining; coal combustion; hospital residues incineration; air contamination; oxidative stress; antioxidant therapy.*

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## 1. Introduction

ROS and also reactive nitrogen species (RNS) have been implicated in the pathogenesis of several airborne occupational contaminations such as coal dust-induced toxicity. When not appropriately counteracted by enhanced antioxidant defenses, ROS overgeneration leads to a systemic oxidative stress (OS) (Sies, 1985; Halliwell & Gutteridge, 2007), which is an imbalance between antioxidants and oxidants in favor of the oxidants leading to cellular damage (Sies, 1985). In the last years several OS biomarkers have been used to monitor environmental contamination associated with different human disorders, reinforcing the concept that they are a very useful tool regarding environment and health assessment (Halliwell & Gutteridge, 2007).

Two important industrial activities are involved in the release of airborne contaminants over the world and particularly in southern Brazil. Mineral coal mining activity gives rise to environmental contamination by generating PM emissions during the extraction, beneficiation, combustion, and processing of minerals, eliciting systemic OS (Ávila et al., 2009). Similarly, SRHS incineration produces distinct contaminants such as heavy metals, dioxins, furans, poliaromathic hydrocarbons, among others, promoting systemic OS as well (Possamai et al., 2009). Inherent to the process of energy generation, emissions from electric power plants also expose humans to several occupational diseases, due to the high content of redox-cycling organic chemicals and heavy metals, which are also implicated in ROS overgeneration and therefore in systemic OS (Possamai et al., 2010). Moreover, prospective studies suggest that air pollution may be responsible for increased risk of developing lung cancer and cardiovascular diseases, linking these risks with PM exposure (Vineis & Husgafvel-Pursiainen, 2005) and with ROS overgeneration (Becker et al., 2002; Li et al., 2003; Chen et al., 2006;.Hatzis et al., 2006; Li et al., 2008).

The wind regime in the studied area prevails in a northeast direction most part of the year, together with the fact that the wind rebounds in a local mountain shield, favor an almost continuous exposure of the local population (Monteiro & Furtado, 1995). Thus a relatively persistent exposure to different airborne contaminants occurs: a) coal extraction at the surface or into the galleries in workers directly exposed, as well as in non-workers (subjects living in the city of Lauro Müller (~15,000 inhabitants, ~15 km away from the mining area); b) coal burning in workers directly exposed at an electric power plant (~0.2 km away from the contamination source), as well as in non-workers living in the city of Tubarão (~30,000 inhabitants living ~2-3 km away); c) and finally, in workers directly or indirectly exposed (~0.1 km away) and also non-workers living in the city of Capivari de Baixo (~20,000 inhabitants ~5 km away) exposed to SRHS incineration. All the subjects here examined were exposed for at least 5 years to the corresponding sources of airborne contamination, and were not diagnosed for pneumoconiosis or any lung inflammatory disease. Due to space limitations, the references from our laboratory mentioned in the abstract, throughout the text and also in the reference list, contain all the other references cited in the present review.

## 2. Coal mining and coal combustion: OS before and after an antioxidant intervention

It is well known that air pollution particles cause increases in morbidity and mortality, which are reported in several epidemiological studies (e.g., Schwartz et al., 1996). In particular, chronic inhalation of coal dust in miners leads to several diseases such as pneumoconiosis, fibrosis, bronchitis, emphysema, cancer and other pathologic-related

events (Sorensen et al., 2005). In this regard, around 30% of the local medical procedures from Lauro Müller (localized ~15 km from the mining area) was caused by respiratory diseases and 4% related to different kinds of cancer in this region (DATASUS 2008). Therefore, occupational exposure to metals particularly in coal mining is considered to be the major cause of metal-exposed cancer (Steenland et al., 1996). Accordingly, atmospheric pollution has been studied and described as a possible cause of increases in morbidity for lung diseases and mortality for tumors in epidemiological data (DATASUS, 2009).

Regarding coal extraction, a systemic OS was detected in the blood or workers directly (mine surface and galleries) or indirectly exposed (subjects living in the city of Lauro Müller) compared to controls (blood donors living 150 km away from the mines) (Ávila Jr. et al., 2009), which were in accordance to the related literature (Nadif et al., 2001; Becker et al., 2002; Altin et al., 2004; Sorensen et al., 2005; Korashy & El-Kadi, 2008).

Furthermore, production of energy with mineral coal through combustion in an electric power plant generates two types of ashes, the ultrafine particles and the fly ashes or PM around 0.1 μm size, which are considered the most harmful because of their high content of redox-cycling organic chemicals (Li et al., 2003, 2008), and also metals capable of producing ROS and therefore OS. Similarly, our group found that coal combustion was also capable to induce a systemic OS in the blood of subjects exposed directly (working closed to the burning area) or indirectly (working at the administration building, *ca.* 0.2 km, or living in the vicinity, *ca.* 2 km away of the electric-power plant) from the PM emissions (Possamai et al., 2010).

It is well known that the chronic exposure to heavy metals leads to metal-induced ROS overgeneration and ultimately to carcinogenesis (Halliwell & Gutteridge, 2007). In our study, determination of heavy metal contents carried out in urine samples through atomic mass spectroscopy, revealed higher contents in exposed coal mining workers compared to controls. Lead contents in underground (159.0%) and surface mine workers (326.4%), and also in subjects living at the vicinity of the mines (151.4%), were much higher compared to controls. Copper, zinc and iron concentrations were also elevated in the urine of both exposed groups of coal extraction workers, surface and underground miners, while only copper was elevated in the urine of subjects living near from mining plant. Surprisingly, manganese showed decreased concentrations in the urine of the exposed mining workers. Similar enhancements in the above heavy metals were also detected in subjects directly or indirectly exposed to coal combustion from the electric power plant.

In our study, all the exposed subjects showed enhanced levels of plasma TBARS and protein carbonyls (OS markers of lipid and protein oxidation, respectively), which were reversed after the antioxidant intervention. Similar results were also found in coal mining workers at early stages of pneumoconiosis (Altin et al., 2004), and also in rats exposed to coal mine ambience (Armutcu et al., 2007). Interestingly, in the late study, after treatment with the antioxidant erdosteine, levels of lipoperoxidation in those rats were similar to those of controls, also indicating the efficacy of an antioxidant intervention.

In addition, several other OS markers were changed compared to controls. GST activity, an important component of phase II enzymes of the biotransformation of xenobiotics, was elevated in both groups, directly and indirectly exposed to mining activity and coal burning, although GSH depletion was detected in all groups examined. The important endogenous antioxidant GSH is a cofactor necessary for GST to conjugate and eliminate xenobiotics (Halliwell & Gutteridge, 2007). It can be inferred that in these subjects GST consists in a better compensatory response to the oxidative insult. Interestingly, subjects living near the mining area showed the highest GST values compared to both worker groups. However, in a related study, decreased GST activity was found in erythrocytes of subjects in early stages

of pneumoconiosis compared with miners not diagnosed for this disease (Evelo et al., 1993), which can be attributable to a severe chronic condition. Anyway, the increased GST activities found in all exposed groups of our study might reflect a response for an adequate maintenance of the biotransformation process, together with the additional capacity of GST to detoxify hydroperoxides derived from lipoperoxidation processes (Halliwell & Gutteridge, 2007). After supplementation, the GST profile was similar to that of GPx activities. In this regard, Korashy & El-Kadi (2006) observed increased GST activity concomitant to GSH depletion in cell cultures exposed to heavy metals that elicit OS.

In accordance to our findings, Evelo and collaborators (1993) also revealed a decreased GPx activity concomitantly to decreased GSH contents in erythrocytes of coal miners at early stages of pneumoconiosis. Catalase activity was increased in directly exposed coal miners, but only underground workers showed decreased GPx activity. In this regard, as found for GST activity (Evelo et al., 1993), GPx inhibition seems to be also consistent with chronic exposure to airborne contaminants (Nadif et al., 2001; Altin et al., 2004), which will consequently render higher hydroperoxide levels inside the cells. In the presence of transition metal ions, such as ferrous or cuprous ions, $H_2O_2$ is converted to the most deleterious radical hydroxyl ($HO^{\bullet}$), *via* the Fenton reaction (Halliwell & Gutteridge, 2007).

SOD activity was also inhibited in all groups examined and a similar response was also found in other studies involving coal mining workers (Nadif et al., 2001; Altin et al., 2004). On the other hand, rats exposed sub-chronically to a mine ambience showed SOD and GPx induction at the first week followed by SOD inhibition at the fourth week of exposure (Armutcu et al., 2007). Accordingly, coal miners showed a progressive loss of an adequate antioxidant compensation regarding SOD and GPx activities in plasma, which were parallel to the tissue damage (Altin et al., 2004). This apparent decrease of an adequate antioxidant response was accompanied by persistent high lipoperoxidation levels in plasma, resembling the general OS profile found in our study involving chronic exposure to mine ambience (Ávila Jr. et al., 2009). The *in vitro* inhibition of GPx, SOD and GR was demonstrated by Hatzis and collaborators (2006) after exposure to three different PM sources.

After the antioxidant supplementation, the decreased values found in TBARS and PC levels are probably due to the quenching ability of the liposoluble antioxidant α-tocopherol, which acts blocking the propagation of lipoperoxidation in cell membranes. The interception of the peroxyl radical (ROO• ) by α-tocopherol results in the formation of the tocopheroxyl radical, which in turn is regenerated back to α-tocopherol by ascorbate, reduced glutathione or ubiquinol (Halliwell & Gutteridge, 2007). Also, vitamin C might act as a possible lead chelator showing a similar capacity of that of EDTA (Flora et al., 2008). Similarly, Pinho and collaborators (2005) showed decreased pulmonary OS biomarkers in rats exposed to coal dust using N-acetylcysteine (NAC) and deferoxamine as antioxidants.

Regarding mining activity, plasma α-tocoferol levels detected in underground workers (≅10 μM) showed around half the values found in controls (≅20 μM). Despite the fact that no significant differences were detected, surface workers and residents living near the mining area showed values (≅15 μM) intermediary to those found in underground workers, revealing that this nutritional antioxidant is being depleted in both groups. The similar profile obtained for vitamin E and total glutathione and also for GSH contents, reinforces the concept that all these subjects are facing a systemic OS, in which both antioxidants are probably counteracting the excess of ROS generation associated with a chronic exposure to coal dust.

In summary, chronic exposure to airborne contaminants associated with coal extraction as well as with coal combustion leads to a progressive decline in the antioxidant capacity (in other words, a systemic OS) present in the blood of workers directly or indirectly

exposed to such PM contamination. Also concerned, the results showed that people indirectly exposed (residents) to the mine ambience living in the vicinity of the PM emissions revealed a very similar profile regarding the systemic OS, being in great health risk associated with such airborne contamination. The antioxidant intervention was able to significantly attenuate such systemic OS, after a six-month period, thus a general improvement was achieved both in enzymatic and non-enzymatic antioxidant defenses present in the blood of subjects exposed to such activities (Possamai et al., 2010; Wilhelm Filho et al., 2010).

## 3. Incineration of SRHS: OS before and after the antioxidant intervention

An important local increase in morbidity and mortality in the city of Capivari de Baixo occurred in the recent years, the mortality numbers were closely related to tumors, different from data of the whole country where tumors are the third cause of mortality (DATASUS, 2008). This anomaly numbers of public health triggered the study related to contamination by fly ashes from SHRS incineration developed by our group (Possamai et al., 2009).

One of the most useful methods for elimination of SRHS is incineration. However, it also provokes the emission of several hazardous air contaminants such as heavy metals, furans and dioxins, among others, which produce ROS overgeneration and therefore a systemic OS. Very similar to the results found in subjects exposed to the activities of coal extraction and coal combustion (Ávila Jr. et al., 2009; Possamai et al., 2010), the same enzymatic and non-enzymatic OS biomarkers present in the blood revealed a profile that resembles the OS associated with coal activities. In short, the results also revealed that subjects directly or indirectly exposed to SRHS are facing an oxidative insult, consequently being in health risk regarding fly ashes from SRHS incineration (Possamai et al., 2009).

Moreover, municipal solid waste incinerators (MSWI) in different countries revealed a very similar pattern of contaminant generation compared to SRHS, implying that such airborne contaminants are produced through similar processes, regardless the composition of solid wastes and the incinerator design (Tong & Karasek, 1986), thus validating comparisons between both processes. Nevertheless, the industrial incinerator seems to be even more harmful compared to MSWI, because the contents of dioxin and malondialdehyde (MDA, the major end-product of lipid oxidation) detected in subjects living near an industrial waste incinerator were higher compared to subjects living near a MSWI (Leem et al., 2003). In addition, people living near an industrial area containing incinerators seem to accumulate more dioxin in their sera compared to subjects residing in a rural area from Belgium (Fierens et al., 2003).

Concerning the contamination of heavy metals, a similar profile for lead concentrations detected in coal mining workers (Ávila Jr. et al., 2009), although not so pronounced, was also found in the urine of workers (45.5%) of incineration of SRHS, as well as in people not directly exposed and living in vicinal areas (98.6%) when compared to controls (Possamai et al., 2009). Accumulation of these metals in humans increases ROS production, causing a severe systemic OS (Gaetke & Chow, 2003; Korashy & El-Kadi, 2008). Iron contents were also significantly increased but only in the blood of workers directly exposed to SRHS incineration when compared to controls (Possamai et al., 2009), very similar to the profile obtained in the urine of coal mining workers and subjects living in the neighborhood (Ávila Jr. et al., 2009). Enhanced zinc concentrations were also found in incineration workers (60.8%) and residents (87.9%) when compared to controls (Possamai et al., 2009). Similarly, Reis and collaborators (2007) revealed increased levels of Cd, Hg and Pb in subjects indirectly exposed to solid waste incineration in Portugal. According to Wang and

Fowler (2008), chronic exposure to Pb, Cd and As induces OS by inhibition of important antioxidant enzymes.

In our study people exposed directly and indirectly exposed to SHRS showed a similar OS profile, suggesting a close correlation between the exposure to the incineration of SRHS and the response obtained from different OS biomarkers in both groups (Possamai et al., 2009). Except for GR activity, all other biomarkers examined were altered both in workers and also in indirectly exposed subjects to SRHS emissions. While GST activity was increased and GPx was decreased in both exposed groups, CAT activity was increased and SOD activity was decreased only in workers. The contents of vitamin E and GSH were depleted and TBARS and GSSG levels (the oxidized form of glutathione, an excellent OS biomarker) showed increased values in plasma of both workers and subjects living near the emissions, when compared to controls (Possamai et al., 2009). This elevated TBARS contents are well in line with the results found in workers and residents exposed to mining activity (Ávila Jr. et al., 2009). A similar profile was also obtained regarding plasma contents of vitamin E and whole blood contents of GSH in both groups exposed to coal extraction (Ávila Jr. et al., 2009). The depletion of these two important non-enzymatic antioxidants together with the persistent high lipoperoxidation levels found in both studies strongly indicates a condition of a chronic and severe systemic OS in all exposed subjects.

Surprisingly, while SOD activity was decreased, CAT activity was enhanced in workers exposed to SHRS incineration, and the same response was also obtained in workers exposed to mining activity (Ávila Jr. et al., 2009). Rats exposed sub-chronically to a mine ambience showed SOD and GPx increases after the first week followed by SOD inhibition after the fourth week of exposure (Armuctu et al., 2007). Accordingly, coal miners showed a progressive loss of an adequate antioxidant compensation regarding SOD and GPx activity measured in plasma (Altin et al., 2004). Taken all these results into consideration, it is suggested that, irrespective to the kind of airborne contamination, time of exposure is determinant, while acute exposures elicit an appropriate antioxidant enzymatic compensation, chronic exposures seems to indicate a loss of adequate compensation.

In a related study, increased lipid peroxidation in workers and residents were also found, and the authors also suggested that this effect was related by hazardous substances emitted by the incinerator (Leem et al., 2003). Under long-term exposure, the oxidative damage may inhibit cell-mediated immunity, cause DNA damage, and induce the development of tumors (Ong et al., 2002). In particular, dioxins are able to directly or indirectly generate ROS and to induce carcinogenesis, and the main route of ROS generation is probably *via* biotransformation of xenobiotics through the induction of the phase I super-family of CYP450 enzymes together with the phase II family of glutathione S-transferases and other related enzymes (Nims & Lubet, 1996; Yoshida & Ogawa, 2000). Accordingly, GST activity was increased both in workers and residents exposed to SRHS emissions. Interestingly, despite GSH depletion verified in all groups examined, it seems that all these subjects are apparently still able to compensate the xenobiotic insult, keeping sustained high GST activities. Again, a similar GST profile was obtained in residents and workers exposed to emissions from a mine plant (Ávila Jr. et al., 2009).

TBARS and PC contents, which were elevated before the antioxidant intervention, were significantly decreased after the antioxidant supplementation. Similarly, the contents of vitamin E and GSH, which were decreased before the antioxidant intervention, reached values near those found in controls, while SOD, CAT and GST activities were reestablished in all groups, showing values similar to those before such intervention. Thus, the systemic OS detected previous to the vitamin supplementation in directly and indirectly subjects exposed to SRHS incineration, were attenuated after the antioxidant intervention (Wilhelm

Filho et al., 2010). In such a way, this compensatory antioxidant response, again resemble the effect obtained in subjects exposed to coal extraction (Ávila Jr. et al., 2009) as well as those exposed to the coal emissions from an electric-power plant (Possamai et al., 2010).

## 4. Main conclusions

The antioxidant therapy with vitamins E and C during six months was effective in attenuating the systemic oxidative insult associated with exposure to PM emissions derived from coal extraction and combustion as well as from SRHS incineration. Therefore, such antioxidant intervention might be recommended for subjects exposed directly or indirectly to these occupational contaminants. It is worth to emphasize here that, irrespective of the type of airborne contamination, a similar systemic OS was found in workers as well as in non-workers, i. e., subjects living near to the corresponding emission source as well, which in turn was also attenuated by the antioxidant intervention.

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