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Effect of landfill leachate on oxidative stress of brain structures and liver from rodents: Modulation by photoelectrooxidation process

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ABSTRACT

The decomposition of solid waste in landfill is responsible for the formation of leachate, a dark liquid with an unpleasant odor; studies investigating its toxicity on mammals are rare. Oxidative stress has been considered as an important biochemical mechanism of the toxicity of several xenobiotics. The aim of this study was to evaluate the effects of landfill leachate on oxidative parameters in striatum, hippocampus and liver homogenates of mice and rats. In order to propose a clean technology for the treatment of leachate, we also investigated the effects of landfill leachate submitted to photoelectrooxidation process (PEO). The homogenates of cerebral structures and liver of Swiss albino mice and Wistar rats were incubated with different concentrations of non-PEO landfill leachate and PEO-treated landfill leachate. After the incubation, the levels of free radicals, determined by 2',7'-dichlorofluorescein diacetate probe, and the lipoperoxidation, quantified by the thiobarbituric acid reactive substances, were evaluated. There was an increase on the levels of free radicals in striatum of both mice and rats when exposed to non-PEO leachate. Moreover, PEO-treated leachate increased the lipoperoxidation in striatum homogenates from rodents. However, both leachates did not alter any of the parameters evaluated in the hippocampus. In the liver, the incubation with leachates induced an augment on levels of free radicals only in samples of mice. In addition, PEO-treated leachate increased the lipoperoxidation indexes in the liver of mice and rats. These results suggest that the landfill leachate can induce an oxidative stress state in the liver and the striatum of rodents. Additionally, the PEO process was unable to efficiently alter the toxic compounds of landfill leachate.

1. Introduction

The production of solid waste, which is estimated from 0.5 to 4.5 kg per person per day, constitutes an important environmental problem in recent decades in the world (Bakare et al., 2005). According to the Brazilian Institute of Geography and Statistics, in 2000, Brazil produced 125.281 t of solid waste daily (IBGE, 2000). Landfills are considered to be the most widely employed methods of solid waste disposal around the world; however there are several landfill sites known to contaminate nearby aquifers (Li et al., 2006b). The decomposition of solid waste in the landfill, along with rainwater penetration, is responsible for the formation of a dark liquid with unpleasant odor, known as leachate (Christensen et al., 2001; Sánchez-Chardi and Nadal., 2007). Different substances have been found in the landfill leachate, such as toxic and carcinogenic chemicals, organic and inorganic compounds, metals and ammonia (Li et al., 2010; Christensen et al., 2001).

It has been demonstrated that a small amount of landfill leachate may cause severe pollution to the groundwater aquifer and to the adjacent surface waters (Pivato and Gaspari, 2006; Li et al., 2006b). The toxicity of landfill leachate has been reported in several studies on microbial organisms, plants and aquatic animals (Chandra et al., 2004; Sang and Li, 2004; Omura et al., 1992; Koshy et al., 2008), while only a few studies have investigated the toxicity in mammals. Besides, research with mammals would permit the evaluation of other impacts such as

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neurotoxicity and hepatotoxicity. Gajski et al. (2011) suggest that the histologic and biochemical changes induced by exposure to water contaminated with leachate could have public health implications.

Several studies have showed that the oxidative stress may be a central biochemical mechanism of toxicity of several xenobiotics (El-Demerdash, 2011; Santos et al., 2007). Superoxide anion, hydrogen peroxide and hydroxyl radical are reactive oxygen species (ROS) that may attack macromolecules such as lipids, proteins and DNA, contributing to a wide range of diseases including cardiovascular and neurodegenerative diseases (Dean et al., 1997; Muthuswamy et al., 2006; Bergamini et al., 2004). Recently, Li et al. (2006a,b, 2010) have suggested that oxidative stress may be related to the deleterious effects of landfill leachate, since the exposure for seven days increased lipid peroxidation levels in brain, liver, spleen, heart and kidney of male and female mice, as well as altered antioxidant capacity, female being more sensitive to oxidative damage than male mice (Li et al., 2006a, b). Indeed, this exposure increased protein oxidation and DNA–protein crosslinks indexes in several viscera of male mice (Li et al., 2010). Although these authors have demonstrated the effect of leachate on oxidative stress parameters in the whole brain, it is important to evaluate individual brain structures, since different responses to oxidative stress can be found in specific brain regions (Muthuswamy et al., 2006, Siqueira et al., 2005).

In view of the toxicity induced by leachate, the development of new technologies for the recovering and recycling of chemicals is necessary in order to avoid discharging of these products into the environment (Rodrigues et al., 2008). Many reports have emerged, mainly in the last two decades, in a special category of oxidation techniques known as advanced oxidation processes (Sauer et al., 2006), which might promote the degradation of several pollutant complexes within few minutes (Freire et al., 2000). These processes use different reaction systems to generate hydroxyl radical, which has high oxidative power. The photoelectrooxidation process (PEO), an advanced oxidation process, consists of a combination of electrolysis and heterogeneous photocatalyses, being photon and the electron involved in this process (Pinhedo et al., 2005). To our knowledge, there are no publications evaluating the effects of leachate treated by PEO process in mammals.

The aim of this study was to evaluate the in vitro effects of landfill leachate on levels of free radicals and lipid peroxidation in striatum, hippocampus and liver of two mammal species, specifically mice and rats. Hippocampus and striatum were studied in brain areas, since they are vulnerable to oxidative damage and exhibit distinct functions with central role in cognitive and motor functions, while liver is essentially involved with the biodegradation and excretion of substances, including several xenobiotics. The PEO process was studied in order to obtain data that might support its proposed use as a clean technology for the treatment of this wastewater.

2. Material and methods

2.1. Collection and chemical analysis of the landfill leachate

Samples of the leachate were collected from the municipal Centre of residues, located in Novo Hamburgo, RS, Southern Brazil. The following parameters were determined: chloride, conductivity, chemical oxygen demand (COD), total phosphorus, nitrate, nitrite, ammonia nitrogen, pH, total solids, sulfates, calcium, chromium and magnesium. The samples were collected before and after treatment by PEO and the methodology used was based on American Public Health Association (Clesceri et al., 1989).

2.2. Treatment of leachate by photoelectrooxidation

The landfill leachate was submitted to photoelectrooxidation process performed by the authors of this study at the Centro Universitário FEEVALE. The photoelectrooxidative degradation experiments were performed in a compartment with ultraviolet (UV) radiation (125 W mercury lamp), and electrochemical cell with control potential (Gkika et al., 2005). Electrodes were from Denora DSA® (Dimensional Stable Anodes) $Ti/Ru_{0.3}Ti_{0.7}O_2$, connected to CIDEPE EQ030 controlled-potential equipment. The potential used was 3 V (versus Ag/AgCl), and the current in this system was around 23 mA cm^{-2}. Duration of the treatment was of 90 min.

2.3. Animals

Adult male Swiss albino mice (CF1 strain) were obtained at three to five months of age, provided by the Fundação Estadual de Pesquisa e Produção da Saúde (FEPPS) in Porto Alegre, RS and adult male Wistar rats aged three to five months were provided by Centro de Reprodução de Animais de Laboratório (CREAL) at Universidade Federal do Rio Grande do Sul (UFRGS). The animals were housed (five per cage) with food and water *ad libitum* and kept under standard conditions (twelve hour light/dark cycle, 22 ± 2 °C). The recommendations of the "Guide for the Care and Use of Laboratory Animals" (NIH publication no. 80-23, revised 1996) were followed throughout the study and all experiments were approved by the Local Institutional Research Ethics Committee (Comissão de Ética no Uso de Animais/UFRGS # 20878).

2.4. Tissue preparation and incubation

At the day of the experiments the animals were decapitated without anesthesia; brains of the mice and rats were immediately removed and washed using chilled saline solution. The striatum, hippocampus and liver were rapidly dissected out, instantaneously placed in liquid nitrogen and stored at −70 °C. After, the structures were homogenized in ice-cold phosphate buffer (pH 7.4) and the homogenate was centrifuged at $1000 \times g$ for ten minutes at 4 °C. The resultant supernatant was incubated with different concentrations (0; 3; 10 and 30 percent) of non-PEO landfill leachate and PEO-treated landfill leachate at 37 °C for 60 min. The mixture was used to evaluate the levels of free radicals and lipid peroxidation.

2.5. Levels of free radicals

To assess the levels of free radicals we used 2',7'-dichlorofluorescein diacetate (DCFH-DA) as a probe (Lebel et al., 1990). An aliquot of the mixture of tissue supernatant and landfill leachate was incubated with DCFH-DA (100 mM) at 37 °C for 30 min. Formation of the oxidized fluorescent derivative (DCF) was monitored at excitation and emission wavelengths of 488 nm and 525 nm, respectively. All the procedures were performed in the dark and blanks containing DCFH-DA (no supernatant) were processed for measurement of fluorescence (Sriram et al., 1997; Siqueira et al., 2011). The results were expressed as percentage of control, relative to supernatant and DCFH-DA (without leachate) for all samples.

2.6. Thiobarbituric acid reactive substances (TBARS)

Lipid peroxidation (LPO) was evaluated by the thiobarbituric acid reactive substances (TBARS) test (Bromont et al., 1989). An aliquot of the mixture of tissue supernatant and landfill leachate was incubated with ten percent trichloroacetic acid (TCA) and 0.67 percent thiobarbituric acid (TBA). The mixture was heated (30 min) on boiling water bath. Afterwards, n-butane was added and the mixture was centrifuged. Organic phase was collected to measure fluorescence in excitation and emission wavelengths of 515 and 553 nm, respectively. 1,1,3,3-Tetramethoxypropane, which is converted to malondialdehyde (MDA), was used as standard (Siqueira et al., 2011).

2.7. Protein determination

The protein content of the tissue homogenates was measured by the Coomassie blue method using bovine serum albumin as standard (Bradford, 1976).

2.8. Statistical analysis

Kruskal–Wallis non-parametric test followed by Dunn test was employed considering the distribution of the data. Results were expressed as medians (25th/75th of percentiles) and the significance was assumed as $p < 0.05$.