Landfill leachate ingestion induces protein oxidation and DNA–protein crosslinks in mouse viscera

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ABSTRACT

In the present study, protein oxidation (PCO content) and DNA–protein crosslinks (DPC coefficient) were investigated in the viscera of mice exposed to a municipal landfill leachate at various concentrations for 7 days. The study was designed to investigate the injuries and a possible mechanism of landfill leachate-induced toxicity on mammals. The results indicate that the leachate sample changed the ratio of viscera to body weight in all organs tested, and the effect on the brain was more obvious than that on other organs. The leachate ingestion increased PCO levels in the brain, kidney, liver and spleen in a concentration-dependent manner, but did not affect the content in the heart. Also, the leachate sample enhanced DPC formation in the tested organs in a concentration-dependent manner, and the responses of the liver, kidney and spleen were more sensitive than that of the brain and heart. These findings provide further evidence that landfill leachate-induced toxicity on mammals might involve the formation of free radicals, either via autoxidation or by enzyme-catalyzed oxidation of pollutants in leachate, and then attack of proteins and nucleic acids via generated free radicals.

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1. Introduction

With increasing use of municipal solid waste landfills for waste disposal, the production of leachate from these landfills is of growing concern due to its long-term discharge and potential toxic impact [1]. A review of previous literature on the occurrence and concentration of pollutants in landfill leachates concluded that toxic and carcinogenic chemicals were present in the leachates of all solid waste landfills studied, including metals, ammonia, organics, etc. [2–4]. It is reported that small amounts of landfill leachate could pollute large volume of groundwater, rendering them unusable for domestic and many other purposes [5]. Also, the chemical substances contained in the leachate could be assimilated by any surviving aquatic species, could pass through the food chain, and bioaccumulate over long-term exposure [6,7]. In fact, an increase of adverse health effects, such as low birth weight and increase of birth defects and certain types of cancers, has been reported in people living near landfill sites [8]. Therefore, more attention has been paid to the health effects of landfill leachate and their possible mechanisms.

Studies on mammals show that landfill leachate induced significant increase in the number of sperm with abnormal morphology,

and caused increases of micronucleus (MN) and chromosomal aberration (CA) formation in mouse bone marrow. This suggests that leachate might be a genotoxic agent in mammalian cells, and the possible mechanism involved the formation of free radicals, either via autoxidation or by enzyme-catalyzed oxidation of pollutants in leachate [9–11]. Further studies also indicate that landfill leachate caused lipid peroxidation and disturbed the antioxidant status in mouse organs [12,13]. Proteins and nucleic acids were targets for free radical, however, few information was available on the oxidative injuries of these macromolecules in different tissues and organs of mice exposed to leachate.

Protein carbonylation was an irreversible, nonenzymatic process that resulted from the direct reactions between proteins and reactive oxygen species (ROS), or from the indirect reaction between proteins and other oxidized macromolecules (lipids or sugars) [14,15]. DNA–protein crosslinks (DPC) were another damnification caused by free radicals, which was relatively persistent in the cells, unlike the strand breaks and other DNA lesions that were readily repaired [16,17]. Various studies indicate that exposures of environmental contaminants such as heavy metals, ethylene oxide, aldehydes, caused DPC by stabilizing the covalent intermediates formed between protein and cellular DNA [18–20]. Therefore, protein oxidation, marked by protein carbonyl (PCO) content, and DPC, produced by free radical attacked proteins and nucleic acids, have been used as sensitive indexes for assessing chemical-induced oxidative stress [21–23]. However, landfill leachate-induced protein oxidation and DNA–protein

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crosslinks in tissues and organs of mammals were scantly investigated.

In small mammals, pollutants in landfill leachate were uptaken into the blood and distributed to different tissues and organs after ingestion [6,7]. The brain, heart, kidney, liver and spleen are the five most important organs in mammals, and usually used as models for studying the pathological mechanism due to their involvement in the modulation of assimilation, metabolism, immunity and diseases. In addition, the tissues and organs are also the vulnerable regions of the body to oxidative stress. In the present study, we investigated landfill leachate-induced protein oxidation and DPC in mouse brain, heart, kidney, liver and spleen. This was with the aim of providing evidence for landfill leachate-induced injuries on mammals and possible mechanisms.

2. Materials and methods

2.1. Landfill leachate sample collection

According to the previous reports [12,13], the leachate sample was collected from Xingou MSW landfill of Shanxi in October 2007 and sealed in a clean plastic barrel with a lid-and-ring clamp at 4 °C until testing. Some physico-chemical properties of the leachate sample were analyzed by the methods described by Xi et al. [24]. Also, the chemical composition was analyzed using the data from our previous report [12].

2.2. Preparation of animals

Approximately 6-week-old male Kunming mice were supplied by the Center of Laboratory Animals, Shanxi Medical University, Taiyuan, China. The mice weighed between 20 and 25 g on the day of the experiment. They were housed in the departmental animal facility for 4 days after receipt and prior to each experiment, and fed a standard rodent pellet diet. The animal room was maintained at 24 ± 2 °C with 50% humidity and time controlled lighting (12 h of light per day).

2.3. Animal treatment

Animals were randomly numbered and divided into five equal groups (each group comprised six mice): one control group drank distilled water for 7 days, and four groups drank the leachate sample at different concentrations of 5, 10, 25 and 50% (v/v; leachate sample:distilled water) for the same period of time. At the end of the experimental period, mice were deprived of food for 24 h, and then sacrificed by cervical dislocation. Immediately, the brain, heart, kidney, liver and spleen were removed surgically, weighed, and used for the determination of the biochemical parameters. The study involving experimental animals was conducted in accordance with Chinese and our institutional guidelines for the protection of animal welfare.

2.4. Determination of PCO levels

PCO content was tested by 2,4-dinitrophenylhydrazine (DNPH) spectro-photometry with some modifications [25]. Briefly, 100–200 mg tissue was thawed and homogenized at 10% (w/v) in HEPES buffer (pH 7.4, containing 10 mM HEPES, 137 mM NaCl, 4.6 mM KCl, 1.1 mM KH_2PO_4, 0.6 mM $MgSO_4$, 40 mg/L PMSF, 0.5 mg/L protease inhibitor, 0.7 mg/L pepsin inhibitor and 1.1 mM ethylenediamine tetraacetic acid (EDTA)). The supernatant was used for DNPH-reaction. PCO content was calculated by the absorbance at 370 nm using the extinction coefficient of 22,000/M/cm for aliphatic hydrazones, and the result was expressed as nmol carbonyl/mg protein.

2.5. Measurement of DPC coefficient

Isolated mouse viscera were washed with ice-cold phosphate-buffered saline (PBS), and were forced through a wire-mesh screen to obtain 1.5×10^6/mL single-cell suspension. The suspension was treated with sodium dodecyl sulfate (SDS, 2%) at 65 °C for 10 min, then KCl (pH 7.4, 1 M in 10 mM Tris–HCl) was added into the system by carefully mixing, followed by passing the mixture through a 1 mL polypropylene pipette tip for six times to shear DNA to a uniform length. The sample was cooled on ice for 5 min and centrifuged at 10,000 rpm for 5 min at 4 °C. The supernatant containing the unbound fraction of DNA (free DNA) was collected in the other tube. The SDS-K$^+$ precipitate containing the protein and DPC complexes was re-suspended with wash buffer (pH 7.4, 0.1 M KCl, 0.1 mM EDTA, 20 mM Tris–HCl) at 65 °C for 10 min, cooled on ice for 5 min, and centrifuged as mentioned above. The precipitate was washed three times, and supernatant from each wash step was pooled with previous unbound DNA fraction. The final pellet was re-suspended in wash buffer with proteinase K at 50 °C for 3 h, then the digest was placed on ice for 5 min and centrifuged at 12,000 rpm (4 °C) for 10 min to collect supernatant (protein-bound DNA). Hoechst 33,258 was added to each supernatant (containing free DNA and protein-bound DNA, respectively) obtained from previous steps in the dark for 30 min. Fluorescence was measured at an excitation wavelength of 350 nm and an emission wavelength of 460 nm. The result was expressed as the percentage of protein-bound DNA to total DNA (free DNA plus protein-bound DNA).

2.6. Protein assay

Protein concentration was determined according to the method of Comassie brilliant blue (G250), with bovine serum albumin (BSA) as a standard [26].

2.7. Data analysis

Data are presented as mean ± SE. Unless stated otherwise, analysis of variance (ANOVA) with Student–Newman–Keuls test was applied for between-group statistical comparison using Origin 7.0 software. Differences were considered significant when $p < 0.05$, $p < 0.01$, $p < 0.001$.

3. Results

3.1. Physico-chemical properties of landfill leachate sample

Some physico-chemical parameters of the leachate sample were measured, with the following results: pH, 7.17; COD_{Cr}, 1800 mg/L; BOD_5, 970 mg/L; NH_3-N, 586.9 mg/L; NO_2-N, 175.6 mg/L; NO_3-N, 4.6 mg/L; TOC, 442.85 mg/L; TN, 933 mg/L; TC, 1244 mg/L; IC, 801 mg/L. Compared to integrated wastewater discharge standard of China (GB 8978–1996) [24], the sample contained high concentration of organic compounds, NH_3-N and NO_2-N.

3.2. Landfill leachate affected the ratio of viscera to body weight

The sample affected the ratio of viscera to body weight in all organs tested, and the effect on brain was more obvious than that on other organs (Fig. 1). After 5, 10, 20 and 50% leachate sample exposure, the ratio of viscera to body weight in the brain, heart, kidney and liver was significantly increased in a concentration-dependent manner (2.03-, 2.80-, 3.28-, 3.54-fold of control in the brain; 1.23-, 1.24-, 1.25-, 1.34-fold of control in the heart; 1.08-, 1.09-, 1.13-,
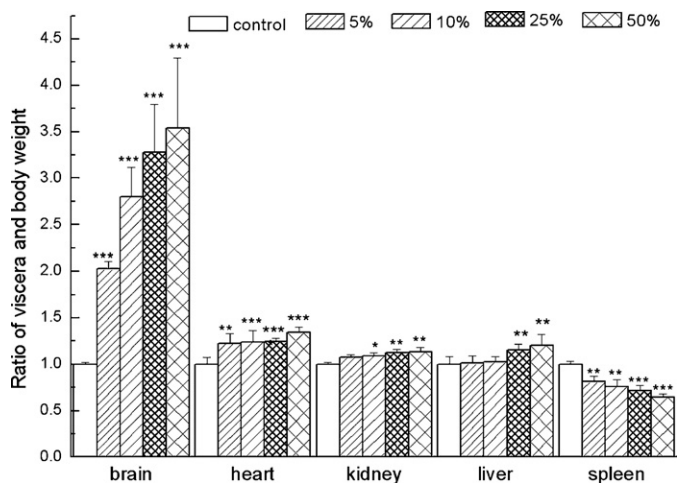
Fig. 1. Effects of Xingou municipal landfill leachate on the ratio of viscera (brain, heart, kidney, liver and spleen) to body weight of mice. Animals were randomly numbered and divided into five equal groups, and each comprised six mice. Value in each treated group was expressed as a fold increase compared to mean value in control group which has been ascribed as an arbitrary value of 1. Data are expressed as mean ± SE, $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

1.13-fold of control in the kidney and 1.01-, 1.03-, 1.15-, 1.20-fold of control in the liver, respectively). In the spleen, the ratio of viscera to body weight decreased after 5 and 10% leachate exposure ($p < 0.01$, $n = 6$), and the statistical difference was enhanced ($p < 0.001$, $n = 6$) with the increase of treatment concentration (20 and 50%).

3.3. Landfill leachate ingestion augmented PCO content

Landfill leachate ingestion increased PCO levels in the brain, kidney, liver and spleen, but did not affect the content in the heart (Fig. 2). For the brain and liver, PCO level was not affected after 5% leachate exposure; the content significantly increased with a concentration-dependent property after treatment with 10 and 25% sample, but decreased at the highest concentration (50%). In the kidney, PCO content tended to increase after leachate exposure, but the statistical difference occurred at high concentrations (25 and 50%). For the spleen, PCO levels were statistically enhanced
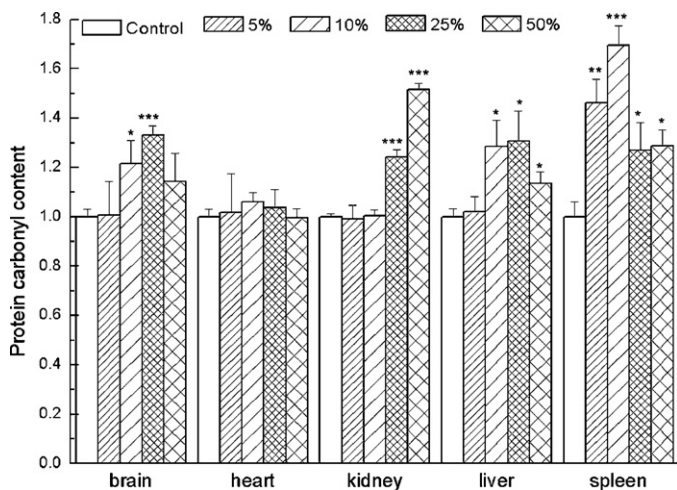

Fig. 2. Effects of Xingou municipal landfill leachate on protein carbonyl (PCO) content in the brain, heart, kidney, liver and spleen from mice. Animals were randomly numbered and divided into five equal groups, and each comprised six mice. Value in each treated group was expressed as a fold increase compared to mean value in control group which has been ascribed as an arbitrary value of 1. Data are expressed as mean ± SE, $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.
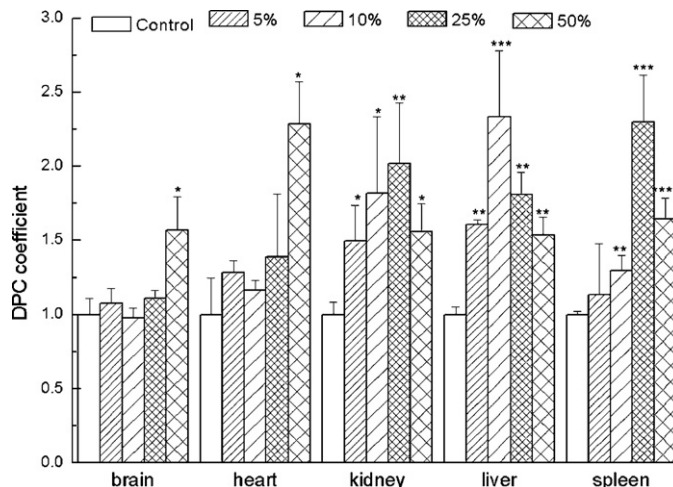

Fig. 3. DNA–protein crosslinks induced by Xingou municipal landfill leachate ingestion in the brain, heart, kidney, liver and spleen from mice. Animals were randomly numbered and divided into five equal groups, and each comprised six mice. Value in each treated group was expressed as a fold increase compared to mean value in control group which has been ascribed as an arbitrary value of 1. Data are expressed as mean ± SE, $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

to 146.17% ($p < 0.01$, $n = 6$) and 169.42% ($p < 0.001$, $n = 6$) of control at the concentration of 5 and 10%, and the enhancement was attenuated to 126.96% and 128.73% of control ($p < 0.05$, $n = 6$) with the increase of concentration (25 and 50%).

3.4. Landfill leachate ingestion increased DPC coefficient

The tested leachate sample caused the increase of DPC formation in the tested organs (Fig. 3). For the brain and heart, DPC coefficient was not affected at low concentrations of the leachate samples (5, 10 and 25%), but was significantly augmented at the highest concentration ($p < 0.01$, $n = 6$). In the kidney and spleen, DPC coefficient increased in a concentration-dependent manner after 5, 10 and 25% leachate exposure, but decreased at the highest concentration. For the liver, DPC coefficient increased and reached peak value at the concentration of 10% (233.43% of control, $p < 0.01$, $n = 6$); with the increase of exposure concentration, the level began to decrease (180.98 and 153.66% of control for 25 and 50% leachate treatment, $p < 0.01$, $n = 6$).

4. Discussion

Landfill leachate is a mixture of organics, inorganics, heavy metals as well as some unidentified toxins [2–4,6,7]. Although the potential damages of these pollutants on mammals have been reported by large amounts of literatures [27,28], it is difficult to assess its toxic mechanisms using single pollutant or several known pollutants due to their synergistic, additive or antagonistic effect [6–13].

Among phospholipids, nucleic acids and proteins [29], proteins were the preferred target for free radicals, and the oxidative modification of proteins might lead to the production of carbonyl compound from amino acids of protein side chains, which caused their structural alteration and functional inactivation, followed by increased aggregation, fragmentation, distortion of secondary and tertiary structure, susceptibility to proteolysis, and diminution of normal function [30]. Carbonyl content is a sensitive indicator of protein oxidative damage [25], and protein oxidation from carbonyls occurs via the $^•OH$ radical, since neither H_2O_2 nor $O_2^{•-}$ is reactive enough to provoke the process [31]. After drinking exposure, the organics, heavy metals and other pollutants in the leachate

could be absorbed into blood or other body fluid and produce free radicals via autoxidation or by enzyme-catalyzed oxidation [6–11]. Under normal condition, generated free radicals could be cleaved by the antioxidant system in the tissue. Because leachate ingestion has been shown to cause the changes of antioxidant status in organs of mice, such as reduction of GSH and decrease of antioxidant enzyme (SOD, GPx) activities [12,13], the excessively produced free radicals, mainly $^\bullet OH$, attacked the amino acids of protein side chains to produce carbonyl [32]. In this way, the proteins were oxidized and the content of the carbonyl in protein was accumulated. The accumulation of carbonyl in organs of mice also indicates that landfill leachate has the potential to induce the formation of free radicals, and their quantity exceeded the defensive capacity of antioxidant system.

DPCs were another damnification due to oxidative mechanisms [33], which was an iron-mediated process, e.g., via the Fenton reaction. Fenton reaction-mediated OH-radicals could attack DNA at the site where iron was bound to produce DPC [34]. There were numerous chemically distinct types of DPC; indeed, proteins could become crosslinked to DNA directly through oxidative free radical mechanisms or indirectly through aldehydes generated by oxidative stress, or they could be crosslinked through a chemical or drug linker or through coordination with a metal atom [35]. Representative pollutants, including chlorinated organics, heavy metals, ammonia–nitrogen and inorganic salts, not only were metabolized to form free radicals, but also served as the bridges between protein and DNA [20,36,37], which contributed to the increased DPC formation in the present study.

5. Conclusion

In the mouse, the tested landfill leachate changed the ratio of viscera to body weight in all organs tested, and the effect on the brain was more obvious than that on other organs. The leachate ingestion increased PCO levels in the brain, kidney, liver and spleen in a concentration-dependent manner, but did not affect the content in the heart. Also, the leachate sample enhanced DPC formation in the tested organs in a concentration-dependent manner, and the response of the liver, kidney and spleen were more sensitive than that of the brain and heart. This provides further evidence that landfill leachate-induced toxicity in mammals might involved the formation of free radicals, and that attack of proteins and nucleic acids occurred via the generated free radicals.

Acknowledgments

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