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DNA-damage induction by eight metal compounds in TK6 human lymphoblastoid cells: Results obtained with the alkaline Comet assay

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ABSTRACT

Metal compounds are long-lived and can react with different macromolecules, producing a wide range of biological effects, including DNA damage. Since their reactivity is associated with their chemical structure, it is important to obtain information on more than one compound from the same metal. In this study, the DNA-damaging potential of two mercury compounds (mercury chloride and methyl mercury chloride), two nickel compounds (nickel chloride and potassium hexafluoronickelate), two palladium compounds (ammonium tetrachloropalladate and ammonium hexachloropalladate), and two tellurium compounds (sodium tellurite and sodium tellurate) was evaluated in human lymphoblastoid TK6 cells by use of the alkaline version of the Comet assay. As the use of computerized image-analysis systems to collect comet data has increased, the metric used for quantifying DNA damage was the Olive tail moment. Treatments lasted for 3 h and the range of concentrations tested was different for each metal compound, depending on its toxicity. Both mercury agents produced DNA damage in TK6 cells, with mercury chloride producing considerably more DNA damage than methyl mercury chloride. Of the two nickel compounds, only nickel chloride (a Ni(II) compound) induced DNA breaks. Similarly, of the two palladium compounds, only the Pd(II) compound (ammonium tetrachloropalladate) was positive in the assay. Sodium tellurite was clearly positive, producing concentration-related increases in DNA damage, while sodium tellurate gave a negative response. In conclusion, the ability of inducing DNA damage by the selected metal compounds in human TK6 cells, when measured with the Comet assay, was dependent on the chemical form and, in general, compounds containing the metal in the lower valence state displayed the greater DNA-damaging ability.

1. Introduction

Metals are widely spread in the environment, from both natural and man-made origin. They are extremely long-lived chemical species, displaying environmental persistence. While some metals are essential for living organisms, others are toxic [1] and, depending on the concentration, essential compounds can also become toxic.

Human exposure to metal derivatives results in a wide range of more or less specific toxicities, from acute toxicity to chronic effects, including cancer. When exogenous metals bind to macromolecules, different from those that detoxify them, cellular functions can be disrupted [2]. The damage induced by metals may include oxidative modifications of the metal–complexion ligands themselves, as well as oxidative damage to neighbouring molecules that are attacked by reactive intermediates produced by the initial reaction. The major oxidative DNA lesions associated with exposure to toxic metals, both in experimental animals and in cultured cells include DNA-base damage, strand breaks, apurinic sites and cross-links [2]. It must be emphasized that over 40 clinical conditions are known in which oxygen radicals play pathogenic roles [3].

In terms of their genotoxic potential, metals can impair the active oxygen scavenging defences in cells, as reported for nickel [4] and mercury [5]. Metals can also inhibit different steps in DNA excision repair. Thus, Ni(II) inhibits the repair of DNA strand-breaks in cultured mammalian cells after UV or X-irradiation [6].

The single-cell gel electrophoresis (SCGE) assay or Comet assay is a rapid, simple, and sensitive technique for measuring DNA damage [7]. In vitro studies have used the Comet assay for detecting the DNA damage induced by different genotoxic agents, such as

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radiation [8], herbicides [9], and heavy metals [10,11], as well as for examining DNA repair under a variety of experimental conditions [7]. With this assay, effects such as DNA single-strand breaks, incomplete excision repair sites, and alkali-labile sites can be detected by analysing the amount of DNA that migrates out of immobilized cell nuclei that are subjected to electrophoresis.

In the present study, we have employed the alkaline version of the Comet assay to evaluate the DNA damage induced by eight metal compounds in human lymphoblastoid TK6 cells. Eight different mercury, nickel, palladium and tellurium compounds were evaluated for their genotoxic potential, as part of a large project developed by the Autonomous University of Barcelona (Bellaterra, Spain) and the Joint Research Centre (Ispra, Italy) aiming to determine the genotoxic risk of a large set of metals. Two different chemical forms of each metal were used to detect differences in genotoxicity.

2. Materials and methods

2.1. Cell culture

The human lymphoblastoid TK6 cell line was obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). This cell line has been used extensively for mutagenicity and genotoxicity studies, including the Comet assay [11–14]. TK6 cells were maintained in suspension culture in RPMI 1640 medium supplemented with 10% heat-inactivated foetal bovine serum, 2 mM L-glutamine, 50 U/mL penicillin, 50 μg/mL streptomycin, and 2.5 U/mL amphotericin B. The cultures were incubated at 37 °C in a humidified atmosphere of 5% CO_2 in air. Asynchronous cultures in the exponential phase of growth were used in all experiments.

2.2. Chemicals

The eight metal compounds tested were the following: mercury chloride ($HgCl_2$), valence II, 99.999% purity; methyl mercury chloride (CH_3HgCl), valence II, >95% purity; nickel chloride monohydrated ($NiCl_2{\cdot}H_2O$), valence II, 99.95% purity; potassium hexafluoronickelate (K_2NiF_6), valence IV, 99% purity; ammonium tetrachloropalladate ($(NH_4)_2PdCl_4$, valence II, >99.995% purity; ammonium hexachloropalladate ($(NH_4)_2PdCl_6$, valence VI, 99.99% purity; sodium tellurite (Na_2TeO_3), valence IV, 99% purity; and sodium tellurate dihydrate ($Na_2TeO_4{\cdot}2H_2O$), valence VI, >95% purity. All were obtained from Sigma (St. Louis, MO, USA) except sodium tellurate dihydrate (Alfa Aesar, Ward Hill, MA, USA). RPMI 1640 medium, foetal bovine serum (FBS), L-glutamine, penicillin–streptomycin, amphotericin B (AMPH), low-melting point agarose (LMA), and normal-melting-point agarose (NMA) were purchased from Gibco BRL (Paisley, UK); while phosphate-buffered saline (PBS), 30% (w/w) hydrogen peroxide, ethylene diaminetetraacetic acid, and the stains, ethidium bromide (EtBr), trypan blue (0.4% solution), and fluorescein diacetate (FDA), were purchased from Sigma. Sodium chloride and dimethyl sulfoxide (DMSO) were from Panreac Química (Barcelona, Spain); *N*-laurosylsarcosine sodium and polyethylene glycol *tert*-octylphenyl ether (Triton X-100) were from Fluka (Buchs, Switzerland); sodium hydroxide and absolute ethanol from Carlo Erba Reagenti (Milan, Italy); and Tris buffer was purchased from US Biochemical (Cleveland, OH, USA).

2.3. Treatments

Cell cultures were centrifuged and the pellet was resuspended in RPMI 1640 medium (10^6 cells/mL). Each metal compound was dissolved in double-distilled water except for the two mercury compounds, which were dissolved in DMSO. All metal solutions were prepared immediately prior to treatment of the cells. Ten microlitres of each of the different concentrations of the metal salt solutions were added to 1 mL volumes of the cell suspensions and incubated for 3 h at 37 °C. The test agents were assayed using five different concentrations, ranging from 0.01 μM for mercury chloride to a maximum of 10,000 μM for nickel chloride, depending on toxicity or solubility. Cell cultures also were treated for 3 h with 10 μL H_2O_2 (final concentration 2000 μM) as a positive control, and with 10 μL of metal vehicle (DMSO or double-distilled H_2O) as a negative control. For both positive and negative controls, the incubation time and the temperature were the same as during treatments with the metal compounds (3 h and 37 °C, respectively). After incubation, the cells were washed with RPMI 1640 medium and resuspended in PBS for the Comet assay. The cell viability was assessed with the fluorescein diacetate/ethidium bromide (FDA/EtBr) viability/cytotoxicity assay based on the simultaneous detection of living and dead cells with the FDA/EtBr dye [15]. Two hundred cells were scored for each treatment. All treatments resulted in a minimum of 70% viable cells, a level sufficient for avoiding cytotoxicity artefacts in the Comet assay [16].

Table 1
Percentage of living cells after treatment with mercury chloride ($HgCl_2$) and methyl mercury chloride (CH_3HgCl), as measured by the FDA/EtBr assay

Mercury chloride		Methyl mercury chloride	
Concentration (μM)	Viability (%)	Concentration (μM)	Viability (%)
0	83.50	0	78.00
0.01	83.75	0.01	84.25
0.1	87.75	0.1	87.25
0.5	81.25	0.5	84.25
1	85.00	1	82.50
2	77.50	3	80.50

2.4. Comet assay

The Comet assay was performed as previously described [17], with minor modifications. Cell samples (approximately 40,000 cells in 20 μL) were carefully re-suspended in 75 μL of 0.5% LMA, layered onto microscope slides that had been pre-coated with 150 μL of 0.5% NMA and dried for 10 min at 65 °C. Cover slips were placed on the slides, and the agarose was allowed to solidify for 15 min at 4 °C. After removal of the covers slips, 75 μL of 0.5% LMA were again spread over the slides, which were then covered with cover slips and kept for 20 min at 4 °C. The covers slips were removed and the slides immersed in cold fresh lysing solution (2.5 M NaCl, 100 mM Na_2EDTA, 10 mM Tris, 10% DMSO, 1% Triton X-100, and 1% laurosylsarcosinate, pH 10) for 2 h at 4 °C in a dark chamber. To avoid additional DNA damage, these procedures were performed under dim light. Afterwards, the slides were placed for 40 min in a horizontal gel electrophoresis tank filled with cold electrophoresis buffer (1 mM Na_2EDTA and 300 mM NaOH, pH 13.5) to allow DNA unwinding. Electrophoresis then was performed in the same buffer for 20 min at 0.73 V/cm and 300 mA. The unwinding and electrophoresis steps were performed in an ice bath. After electrophoresis, the slides were neutralized twice for 5 min with 0.4 M Tris (pH 7.5) and fixed with absolute ethanol for 3 min. The slides were stained with 50 μL of 0.4 μg/mL EtBr just before analysis. Finally, the slides were examined, in a blind study, at 400× magnification with an Olympus BX50 fluorescence microscope equipped with a 480–550-nm wide-band excitation filter and a 590-nm barrier filter, and images of cells were analysed with the Komet 3.1 Image Analysis System (Kinetic Imaging, Liverpool, UK). One hundred randomly selected cells per data point (50 cells from each of the two replicate slides) were analysed per sample. A metric based on the concept of Olive tail moment [18] was computed utilizing the Komet software and used as measure of DNA damage.

2.5. Statistical analysis

According to the current recommendations for in vitro studies [19], the culture was used as the experimental unit. All assays were repeated on a least two separate occasions. The homogeneity of variance between concentration levels was determined using Levene's test. The statistical significance of the results was determined by means of an analysis of variance. When the results were significant, pair-wise comparisons of data from treated cultures with the controls were conducted using the Dunnett's correction for multiple tests. A result was considered statistically significant when $P \leq 0.05$. All analyses were performed using the SAS proc MIXED v8.0 (SAS Institute, Cary, NC, USA).

3. Results

Tables 1–4 indicate the percentage of living cells after treatments with different concentrations of the selected metal compounds. No concentrations producing a percentage of living cells <70% were selected in the current study.

Table 2
Percentage of living cells after the treatment with nickel chloride mono-hydrate ($NiCl_2{\cdot}H_2O$) and potassium hexafluoronickelate (K_2NiF_6), as measured by the FDA/EtBr assay

Nickel chloride monohydrated		Potassium hexafluoronickelate	
Concentration (μM)	Viability (%)	Concentration (μM)	Viability (%)
0	94.00	0	87.67
1	90.00	1	86.67
10	91.00	10	89.67
100	89.00	25	85.00
1000	91.00	75	85.50
10000	81.00	150	83.83