N-Acetyl-Cysteine Protects Against DNA Damage Associated with Lead Toxicity in $HepG_2$ Cells

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Lead toxicity has been associated with its ability to interact and damage DNA. However, its molecular mechanisms of action are not fully understood. *In vitro* studies in our laboratory indicated that lead nitrate ($PbNO_3$) induces cytotoxicity and oxidative stress to human liver carcinoma ($HepG_2$) cells in a dose-dependent manner. In this research, we hypothesized that n-acetyl-cysteine (NAC), a known antioxidant compound, affords protection against lead-induced cell death associated with genotoxic damage. To test this hypothesis, $HepG_2$ cells were treated either with a physiologic dose of NAC, NAC plus $PbNO_3$, or $PbNO_3$ alone, followed by incubation in humidified 5% CO_2 incubator at 37°C for 48 hr. The cell viability was determined by trypan blue exclusion test. The degree of DNA damage was detected by micro gel electrophoresis (comet) assay. Our results showed that lead exposure induces a substantial cytotoxicity as well as a significant genotoxicity to $HepG_2$ cells. However, co-treatment with a physiologic dose (500μM) of NAC slightly increases cell viability, and significantly reduced ($P<.05$) the degree of DNA damage. Hence, NAC treatment may be a promising therapeutic candidate for chemoprevention against lead toxicity, based on its ability to scavenge free radicals. (*Ethn Dis*. 2010; 20[Suppl 1]:S1-101–S1-103)

Key Words: Lead, Cytotoxicity, Genotoxicity, N-Acetyl-Cysteine, $HepG_2$ Cells

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Introduction

Lead is one of the most toxic heavy metals that have been reported to interact with DNA, but the molecular mechanism of this interaction is not fully understood. The genotoxic potential of lead has been examined in several studies. *In vitro* and *in vivo* studies indicate that lead compounds are not directly genotoxic, but may cause genetic damage through various indirect mechanisms. These include inhibition of DNA synthesis and repair, oxidative damage, and interaction with DNA-binding proteins and tumor suppressor proteins.[1,2] Tests for genotoxicity have indicated that lead compounds cause chromosomal damage, induce chromosomal aberrations, micronuclei, and increased SCE (sister chromatid exchanges).[3,4] N-acetylcysteine (NAC), a potent antioxidant has been used clinically for decades for the treatment of many diseases. It plays an important role in the production of glutathione, which provides intracellular defense against oxidative stress,[5] and it participates in the detoxification of many molecules.[6] During the last decade, numerous *in vitro* and *in vivo* studies have suggested that NAC has beneficial medicinal properties including inhibition of carcinogenesis, tumorigenesis, and mutagenesis, as well as the inhibition of tumor growth and metastasis.[7,8] Although NAC has a long history of therapeutic application, little data are available on the role of NAC in the prevention of cytogenotoxic effects caused by exposure to lead *in vitro*. Hence, our study was designed to evaluate the protective role of NAC against lead-induced cytogenotoxic effects to human liver carcinoma ($HepG_2$) cells.

Materials and Methods

Chemicals and Test Media

Reference solution (1000 ± 10 ppm) of lead nitrate ($PbNO_3$) (CAS No. 10099-74-8, Lot No. 981735-24) with a purity of 100% was purchased from Fisher Scientific (Fair Lawn, New Jersey). Dulbecco's modified eagle's medium (DMEM) was purchased from Life Technologies (Grand Island, New York). Fetal bovine serum (FBS), n-aceltyl-l-cysteine, phosphate buffered saline (PBS) were obtained from Sigma Chemical Company (St. Louis, MO).

Tissue Culture

Human liver carcinoma ($HepG_2$) cells were grown in 96-well format plastic plates in DMEM supplemented with 10% FBS, and 1% penicillin-streptomycin. Cells were maintained in a humidified 5% CO_2 incubator at 37°C for 48 hr according to previous experiments in our laboratory.[9,10]

Cell Treatment and Cell Viability Assay

To assess the cell viability, 1×10^4 cells were plated in each well of 96-well plates. The plates were placed in a humidified 5% CO_2 incubator at 37°C to allow cells to attach to the substratum for 2 to 3 days. From a recently published paper, we reported that $PbNO_3$ is cytotoxic to $HepG_2$ cells, showing a 48 hr-LD_{50} of 37.5 ± 9.2 μg/mL (10). Hence, to examine the effect of NAC on $PbNO_3$-induced cytogenotoxic effects, cells were treated either with a physiologic dose NAC, NAC plus $PbNO_3$, or $PbNO_3$ alone, followed by incubation in a humidified 5% CO_2 incubator for 48 hr. The cells incubated in culture medium alone served as a control for cell viability

Table 1. ***In vitro*** **micro-gel electrophoresis (comet) assay and trypan blue exclusion test results after $HepG_2$ cells exposure to either a physiologic dose of NAC, NAC plus $PbNO_3$, or $PbNO_3$**

Treatment Groups	(%) of DNA Cleavage	Tail Length (μm)	Olive Tail Moment	Cell Viability (%)
Untreated	1.30 ± 1.50	1.1 ± 1.6	0.02 ± 0.06	100.0 ± 6.0
500μM NAC	0.96 ± 1.05	1.0 ± 1.0	0.01 ± 0.02	121.0 ± 6.0
500μM NAC + 30μg/mL $PbNO_3$	1.80 ± 1.87	2.0 ± 2.0	0.04 ± 0.08	109.0 ± 5.6
30μg/mL $PbNO_3$	34.98 ± 23.40	73.0 ± 69.0	4.62 ± 5.67	58.0 ± 4.0

(untreated wells). The cell viability was assessed by the trypan blue exclusion test (Life Technologies) using a hemocytometer to manually count the cells. Briefly, 10 μL of a 0.5% solution of the dye was added to 100 μL of treated cells (1.0×10^5/mL). The suspension was then applied to a hemocytometer. Both viable and nonviable cells were counted. A minimum of 200 cells were counted for each data point in a total of eight microscopic fields.

Micro-gel Electrophoresis (Comet) Assay

After incubation, the cells were centrifuged, washed with phosphate buffered saline (PBS) free calcium and magnesium, and re-suspended in 100μL PBS. In a 2 mL tube, 50 μL of the cells suspension and 500 μL of melted LMAgarose were mixed and 75 μL pipetted onto a pre-warmed cometslide. The slides were placed flat in the dark at 4°C for 10 minutes to allow the mixture to solidify and then immersed in prechilled lysis solution at 4°C for 40 minutes. Slides were removed from lysis solution, tapped, and immersed in alkaline solution for 40 minutes at room temperature in the dark. Slides were washed twice for 5 min with Tris-Borate-EDTA (TBE). Slides were electrophoresed at low voltage (300 mA, 25V, 4°C) for 20 minutes. Slides were placed in 70% ethanol for 5 min, removed, tapped, and air dried overnight. Slides were stained with SYBR Green stain designed for the Comet Assay, and allowed to air dry at room temperature for 6 hours. SYBR Green stained cometslides were viewed with an Olympus fluorescence microscope and analyzed using LAI's Comet Assay Analysis System software (Loates Associates, Inc. Westminster, MD).

Statistical Analysis

Data were presented as means ± SDs. Statistical analysis was done using one way analysis of variance (ANOVA Dunnett's test) for multiple samples and Student's *t*-test for comparing paired sample sets. *P* values $<.05$ were considered statistically significant.

RESULTS

Our study found that NAC treatment increased cell viability and afforded protection of DNA damage in $HepG_2$ cells exposed to $PbNO_3$ (Table 1). The treatment of these cells with 30μg/mL of $PbNO_3$ resulted in a significant decrease of cell viability accompanied by a marked increase in DNA damage compared to the control cells. Interestingly, co-treatment of cells with a physiologic dose (500 μM) of NAC and 30 μg/mL of $PbNO_3$ resulted in a slight increase in cell viability and minimal DNA damage compared to $PbNO_3$ alone. Together, our results indicate that $PbNO_3$ represents a potential cytogenotoxic agent *in vitro*. However, NAC treatment attenuates the cytogenotoxic effects mediated by $PbNO_3$ exposure in human liver carcinoma ($HepG_2$) cells.

DISCUSSION

In this article, we present the novel use of NAC for the prevention of $PbNO_3$-induced cytogenotoxic effects to human liver carcinoma ($HepG_2$) cells. Recent studies in our laboratory showed that low- to high-level of arsenic trioxide exposure induced cytogenotoxic effects to human leukemia (HL-60) cells in a dose-dependent manner.[11] Similar to our previous findings, exposure of $HepG_2$ cells to 30 μg/mL of $PbNO_3$ caused substantial level of cell death associated with a high degree of DNA damage, manifested by an increase in percentage of DNA in the tail and olive tail moment. Interestingly, co-treatment with a physiologic dose (500 μM) of NAC markedly lowered the cytogenotoxic effects of $PbNO_3$ *in vitro*. Consistent with our finding, Dick and his colleagues reported that NAC pretreatment prevents TNF-α production in primary alveolar macrophages treated with ultrafine nickel particles.[12] Other studies indicated that NAC protects macrophage cell line (THP-1) against diesel exhaust particle chemicals.[13] Yang and his co-workers also reported that NAC lowers DNA damage produced by water-soluble cigarette smoke in human lymphoid cells containing Epstein–Barr virus episomes.[14] *In vitro* studies suggest that the toxicity and increase in lipid peroxidation induced by lead in cancer cell lines can be ameliorated by antioxidants such as NAC.[15] Based on this *in vitro* study, we speculate that NAC treatment may be a promising therapeutic candidate for chemoprevention against lead toxicity, probably due to NAC ability to scavenge free radicals. The observed preventive effect of NAC in the present study suggests that treatment with physiologic doses of this antioxidant could reduce the cytogenotoxic effects induced by heavy metals.

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REFERENCES

1. Hartwig A, Schlepegrell R, Beyersmann D. Indirect mechanism of lead-induced genotoxicity in cultured mammalian cells. *Mutat Res*. 1990;241:75–82.
2. Winder C, Bonin T. The genotoxicity of lead. *Mutat. Res*. 1993;285:117–124.
3. Johnson FM. The genetic effects of environmental lead. *Mutat Res*. 1998;410:123–140.
4. Shan XQ, Aw TY, Jones DP. Glutathione-dependent protection against oxidative injury. *Pharmacol Ther*. 1990;47:61–71.
5. Thomas SH. Paracetamol (acetaminophen) poisoning. *Pharmacol Ther*. 1993;60:91–120.
6. De Flora S, Cesarone CF, Balansky RM, Albini A, D'Agostini F, Bennicelli C. Chemopreventive properties and mechanisms of *N*-acetylcysteine: the experimental background. *J Cell Biochem*. 1995;22:33–41.
7. Guo BY, Cheng QK. Chelating capability of tea components with metal ion. *J Tea Science*. 1991;11:139–144.
8. Kumamoto M, Sonda T, Nagayama K, Tabata M. Effects of pH and metal ions on antioxidative activities of catechins. *Biosci. Biotechnol. Biochem*. 2001;65:126–132.
9. Yedjou C, Steverson M, Tchounwou P. Lead nitrate-induced oxidative stress in human liver carcinoma ($HepG_2$) cells. *Metal Ions Biol. Med*. 2006;9:293–297.
10. Tchounwou PB, Yedjou CG, Foxx D, Ishaque A, Shen E. Lead induced cytotoxicity and transcriptional activation of stress genes in human liver carcinoma cells. *Mol Cell Biochem*. 2004;255:161–170.
11. Yedjou CG, Tchounwou PB. *In vitro* cytotoxic and genotoxic effects of arsenic trioxide on human leukemia (HL-60) cells using the MTT and alkaline single cell gel electrophoreis (comet) assays. *Mol Cell Biochem*. 2007;301:123–130.
12. Dick CAJ, Brown DM, Donaldson K, Stone V. The role of free radicals in the toxic and inflammatory effects of four different ultrafine particle types. *Inhal. Toxicol*. 2003;15:39–52.
13. Li N, Wand M, Oberley TD, Sempf JM, Nel AE. Comparison of the pro-oxidant and pro-inflammatory effects of organic diesel exhaust particle chemicals in bronchial epithelial cells and macrophages. *J. Immunol*. 2002;169:4531–4541.
14. Yang Q, Hergenhahn M, Weninger A, Bartsch H. Cigarette smoke induces direct DNA damage in the human B-lymphoid cell line Raji. *Carcinogenesis*. 1999;(20):1769–1775.
15. Yedjou CG, Tchounwou PB. N-acetyl-l-cysteine affords protection against lead-induced cytotoxicity and oxidative stress in human liver carcinoma ($HepG_2$) cells. *Int. J. Environ. Res. Public Health*. 2007;4(2):132–137.