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Alterations in antioxidant/oxidant gene expression and proteins following treatment of transformed and normal colon cells with tellurium compounds

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ABSTRACT

The current study evaluated the potential of $TeCl_4$ and DPDT to accumulate within cells and cause oxidative stress. HO-1, antioxidant gene expression and protein alterations were studied.

- Significant Te accumulation was observed in HT-29 cells (500–1000 μM DPDT; 125–1000 μM $TeCl_4$) and CCD-18Co cells (500 μM DPDT and $TeCl_4$).
- Significant increases in HO-1 were observed with 250–1000 μM DPDT and 62.5–1000 μM $TeCl_4$ in HT-29 cells and in 500–1000 μM DPDT and 62.5–1000 μM $TeCl_4$ in CCD-18Co cells.
- In CCD-18Co cells, a significant increase in COX-2 was observed at 500–1000 μM DPDT and 125–1000 μM $TeCl_4$.
- Significant increase in NQO1 was observed with exposure to 500–1000 μM DPDT and $TeCl_4$.
- In HT-29 cells, increased CYGB was noted at concentrations of 500–1000 μM DPDT and $TeCl_4$, while significant increases were noted in NCF-1at 1000 μM DPDT and $TeCl_4$.
- No change in MT-3 and GSR were observed in either cell line.

1. Introduction

Tellurium (Te) is a rare trace element present in the earth's crust at an average concentration of 2 μg/kg or 0.002 ppm (Baesman et al., 2007). Occasionally tellurium is found in its native form, but most commonly as telluride's of gold (calaverite) or combined with other metals. While no physiologic role has been found for Te in mammalian species, Schroeder et al. (1967) reported that humans possess measurable levels of Te of >0.5 g, mostly in bone. In addition, Siddik and Newman (1988) reported human levels of 5 ng/ml in blood and 50 ng/ml in urine. Te is present in soil and seawater where it can be absorbed by both terrestrial and aquatic plants. It is also present in plants of the *Alium* family (Larner, 1995), including onions and garlic. Tellurium is present in the manufacture of semiconductors (Abdel-Aziz, 2006), in the production of quantum dots (Deng et al., 2007) and in the manufacture of blu-ray discs (Nishiuchi et al., 1998; Nishihara et al., 2005) making human occupational exposure likely.

Little work has been done involving tellurium toxicity of the gastrointestinal tract however previous work in our laboratory demonstrated that diphenyl ditelluride (DPDT) and tellurium tetrachloride ($TeCl_4$) were cytotoxic to human colon cell lines. The transformed cell lines, HT-29 and Caco-2 were found to be more sensitive to Te toxicity than the normal cell line. It was also shown that DPDT exposure resulted in apoptosis via the intrinsic pathway in all cells tested, while treatment with $TeCl_4$ resulted in oncosis (Vij and Hardej, 2012). Te may undergo redox cycling leading to the formation of reactive oxygen species (ROS) thus triggering oxidative damage to nucleic acids, proteins and lipids (Sailer et al., 2004). The mechanisms of toxicity by organotellurium compounds may be related to the oxidation of thiol groups of important biomolecules (Nogueira et al., 2001), the replacement of selenium

Abbreviations: Te, Tellurium; ROS, Reactive oxygen species; GPx, Glutathione peroxidase; CAT, Catalase; ICP-OES, Inductively Coupled Plasma-Optical Emission Spectroscopy; $O_2^{\bullet -}$, Superoxide anion; H_2O_2, Hydrogen peroxide; $HO^{\bullet}$, hydroxyl radical; Nrf2, Nuclear factor erythroid 2-related factor 2; GSR, Glutathione reductase; SOD, Superoxide dismutase; PTGS-2, Prostaglandin synthase-2; COX-2, cyclooxygenase-2; HNE, 4-hydroxy-2-nonenal; NQO1, NADPH quinone oxidoreductase 1; MT, Metallothionein; ELISA, Enzyme linked immunosorbent assay; HO-1, Heme oxygenase -1; NCF-1, Neutrophil cytosolic factor-1; CYGB, Cytoglobin.

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in selenoproteins (such as thioredoxin reductase) (Engman et al., 2000), and the capacity of Te compounds to induce the formation of reactive oxygen species (ROS) (Chen et al., 2001; Funchal et al., 2011). On the other hand, pharmacological properties of tellurium compounds have also been reported. These include antitumor and chemoprotective effects (Engman et al., 2000; Cunha et al., 2005), and glutathione peroxidase (GPx) like activity (Engman et al., 1994). The conflicting toxic and beneficial effects of tellurium compounds accentuates the need for further research on toxicological mechanisms of action of these compounds. Oxidative stress occurs when the production of oxidizing agents, free radicals and reactive oxygen species (ROS), exceeds the antioxidant capacity of cellular antioxidants in a biological system, resulting in cell death (Halliwell, 1996). Oxidative stress can also lead to the development of intestinal pathological conditions, which makes it particularly important to study the damaging effects of oxidative stress on the intestinal epithelium and to understand protective mechanisms by which the cells respond to stress.

Antioxidant enzymes are considered to be a primary defense that prevents macromolecules from oxidative damage (Subramanian and James, 2010). Alterations in enzymatic antioxidant systems are frequently used as markers of toxicity caused by a variety of organic compounds and heavy metals (Ling et al., 2011). The nuclear factor erythroid 2-related factor 2 (Nrf2) is a known, well established regulator of cellular resistance to oxidants. Nrf2 controls the basal and induced expression of an array of antioxidant response element-dependent genes to regulate the physiological and pathophysiological outcomes of oxidant exposure. Nrf2, protects normal cells from basal levels of ROS, can be subverted by cancer cells to protect themselves from the cellular stress-inducing conditions of the tumor microenvironment. In order to survive, cancer cells must adapt to this toxic environment, moderating ROS levels below a certain threshold and within a range that permits their growth and survival. In such a situation, an active Nrf2 pathway could maintain a favorable redox balance and upregulate ARE-dependent genes to generate antioxidants in cancer cells to promote their survival. Nrf2 has a dual role in cancer; in cancer prevention and promotion (Lau et al., 2008). Reactive oxidants are counterbalanced by complex antioxidant defense systems regulated by a web of pathways to ensure that the response to oxidants is adequate for the body's needs (Ma, 2013). These defense systems also regulate the expression of several antioxidant proteins such as glutathione S-transferase, glutathione peroxidase, glutathione reductase, superoxide dismutase, catalase, and thioredoxin reductase (Hur and Gray, 2011).

The purpose of this study was to evaluate alterations in selected antioxidant gene expression and proteins following treatment of transformed and normal colon cells with tellurium compounds. The ability of these compounds to induce oxidative stress were also investigated as well as examining the accumulation of Te within these cells and evaluating alterations in antioxidant gene expression and corresponding protein levels.

2. Materials

Diphenyl ditelluride (DPDT, MW: 409.41 g) was purchased from TCI America (Cambridge, MA) and tellurium tetrachloride ($TeCl_4$, MW: 269.6 g) was purchased from Alfa Aesar (Ward Hill, MA). Fetal Bovine Serum (FBS) was purchased from Atlanta Biological (Norcross, GA). McCoy's 5A Medium containing L-glutamine and Dulbecco's Modified Eagle's Medium with high glucose were obtained from Caisson Laboratories (Logan, UT). Human Oxidative Stress and Antioxidant Defense PCR Array were purchased from Qiagen (Valencia, CA). Inductive coupled plasma optical emission spectroscopy (ICP-OES) Te standard was purchased from Perkin Elmer (Waltham, MA). Heme oxygenase-1, glutathione peroxidase, glutathione reductase and cyclooxygenase-II Enzyme-linked Immunosorbent Assay (ELISA) kits were purchased from Enzo (Farmingdale, NY). Cytoglobin, metallothionein-3 and neutrophil cytosolic factor-1 ELISA kits were obtained from Cloud Clone Corp. (Houston, TX). NADPH dehydrogenase quinone 1 was purchased from Abcam (Cambridge, MA). All other reagents were obtained from other reputable chemical suppliers and were of reagent grade. Sterile plastic wares used were manufactured by Corning®, Falcon® and Nunc®.

2.1. Cell culture

Human colon cancer cells HT-29 (ATCC HTB-38) and normal human colon fibroblasts cells CCD-18Co (CRL-1459) were obtained from the American Type Culture Collection (ATCC) (Manassas, VA) as frozen cultures. They were cultured as per ATCC instructions. HT-29 cells were grown in T75 flasks in McCoy's 5A Medium containing L-Glutamine and supplemented with 10% Fetal Bovine serum (FBS) and 0.1% gentamicin. CCD-18Co cells were cultured in Dulbecco's Modified Eagle's Media with high glucose and L-glutamine, and supplemented with 20% Fetal Bovine serum (FBS), 1% non-essential amino acid and 1% sodium pyruvate with 0.1% of penicillin-streptomycin. The flasks were placed in an incubator maintained with 5% CO_2 at 37 °C.

2.2. Treatment criterion

Cells were treated with DPDT and $TeCl_4$ in concentrations ranging from 62.5 μM to 1000 μM. After 24 h exposure, cells were scrapped and centrifuged at 1000 rpm for 5 min at 4 °C. Cells were pooled separately for all treatment groups and cell pellets were stored at −80 °C until further use. The calculated LC_{50} of DPDT and $TeCl_4$ in CCD-18Co cells were 558.77 μM and 589 μM. A 500 μM concentration was used for both the compounds for qPCR. Similiarly, calculated LC_{50} of DPDT and $TeCl_4$ in HT-29 cells were 270.36 μM and 181.86 μM. Concentrations of 250 μM DPDT and 125 μM of $TeCl_4$ were used on HT-29 cells. Serial dilutions of these concentrations were prepared from 1000 μM stock solution and the concentration closest to LC_{50} was used in qPCR.

2.3. Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES)

Concentration of tellurium was determined by using the ICP-OES analysis method. Cells were seeded in T75 flasks at a density of 1.2×10^6 cells and treated with DPDT and $TeCl_4$ in the concentration ranging from 62.5 μM to 1000 μM. Cells were processed for ICP-OES according to the procedure of Roy and Hardej (2011).

2.4. RNA extraction

Cells were seeded in T75 flasks at a density of 1.2×10^6 cells/flask and total cellular RNA was extracted after 24 h exposure from control and treated cells (250 μM DPDT and 125 μM $TeCl_4$ for HT-29 cells; 500 μM DPDT and $TeCl_4$ for CCD-18Co cells) according to the manufacturer's protocol. The RNA elution was stored in −80 °C freezer. RNA was quantified using the Thermo Scientific NanoDrop 2000c Spectrophotometer. Only RNA samples with an A260/A280 ratio of 1.8–2.0 and A260/A230 ratio of >2.0 were used for analysis. RNA integrity was confirmed by using Agilent 2200 TapeStation system and Formaldehyde agarose (FA) gel electrophoresis.