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Chemical form of selenium differentially influences DNA repair pathways following exposure to lead nitrate

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ABSTRACT

Lead, an environmental toxin is known to induce a broad range of physiological and biochemical dysfunctions in humans through a number of mechanisms including the deactivation of antioxidants thus leading to generation of reactive oxygen species (ROS) and subsequent DNA damage. Selenium on the other hand has been proven to play an important role in the protection of cells from free radical damage and oxidative stress, though its effects are thought to be form and dose dependent. As the liver is the primary organ required for metabolite detoxification, HepG2 cells were chosen to assess the protective effects of various selenium compounds following exposure to the genotoxic agent lead nitrate. Initially DNA damage was quantified using a comet assay, gene expression patterns associated with DNA damage and signalling were also examined using PCR arrays and the biological pathways which were most significantly affected by selenium were identified.

Interestingly, the organic type selenium compounds (selenium yeast and selenomethionine) conferred protection against lead induced DNA damage in HepG2 cells; this is evident by reduction in the quantity of DNA present in the comet tail of cells cultured in their presence with lead. This trend also followed through the gene expression changes noted in DNA damage pathways analysed. These results were in contrast with those of inorganic sodium selenite which promoted lead induced DNA damage evident in both the comet assay results and the gene expression analysis. Over all this study provided valuable insights into the effects which various selenium compounds had on the DNA damage and signalling pathway indicating the potential for using organic forms of selenium such as selenium enriched yeast to protect against DNA damaging agents.

Introduction

DNA is the repository of genetic information in each living cell and its integrity and stability are essential to life [1]. Genomic DNA, however, continually faces the challenge of damage caused by various environmental and endogenous insults such as ultraviolet (UV) and ionising (IR) radiation in addition to oxidative damage from by-products of metabolism such as reactive oxygen species (ROS) [2,3]. Under normal cellular conditions the level of ROS is in equilibrium with the antioxidant capacity, however, when the production of ROS exceeds antioxidant levels, oxidative stress and subsequent DNA damage ensue [4].

The ability of cells to correctly respond to and repair DNA damage and progress through the cell cycle in a regulated manner is critical, failure to do so could interfere in many essential cellular processes and may eventually lead to apoptosis [5,6]. To counteract these insults a complex network of different repair systems has evolved to maintain genetic integrity. To the fore of DNA repair mechanisms is base excision repair (BER), which is the most versatile pathway of choice for repairing oxidative damage, single-strand (SSB) breaks and other small non-helix distorting DNA damage. The two major BER subpathways are detailed in Ref. [7]. Nucleotide excision repair (NER) involves removing bulky DNA lesions i.e. pyrimidine dimers, that distort the double helix. It is divided into two distinct pathways including global genomic repair and transcription-coupled repair [8,9]. Alternative DNA repair mechanisms include DNA damage response (DDR), mismatch repair (MMR) and double-strand breaks (DSB) which are repaired by either of two mechanisms, nonhomologous end joining (NHEJ)

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or homologous recombination repair (HR) [10–12]. These breaks are considered the most detrimental DNA lesions and if unrepaired can lead to cell death or if misrepaired may lead to mutations and chromosomal rearrangements [13]. It is estimated that cells each day sustain approximately 10,000 lesions due to DNA damage and if not sufficiently repaired are potentially mutagenic [14].

Lead (Pb) is a pervasive environmental toxin known to induce a broad range of physiological, biochemical and behavioural dysfunctions in humans [15]. Absorbed Pb is stored mainly in bones but also in blood and soft tissues including the kidneys, liver and brain [16], however from among the soft tissues the liver accounts for the largest repository (33%) [17]. This essential organ plays a primary role in safeguarding other physiological systems in the body by detoxifying and metabolising toxic substances [16,18]; however, it is also susceptible to oxidative damage via the toxic effects of concentrated drugs and xenobiotics that are carried via the portal vein following intestinal absorption [19]. Exposure of the liver to Pb is known to decrease antioxidant levels, increase the concentration of free radicals and induce DNA damage and apoptosis by influencing the synthesis of both DNA and RNA [15,20,21].

Selenium (Se) is an essential trace element with antioxidant properties that protect cells against the damaging effects of ROS. Detailed information regarding Se forms and functions can be found in the following Refs. [22–32]. It has been documented that the transport and storage of Se is exerted via the liver through selenoprotein P (SePP) [31,33]. As such the liver is an appropriate organ to consider when analysing various seleno-compounds in addition to investigating the effects of toxic compounds [34,35]. The molecular mechanisms of Pb-induced liver injury and hepatoprotective effects of selenium are not yet completely understood, and it is therefore of interest to focus on mechanisms whereby DNA repair responses can be induced in liver cells through supplementation with antioxidant compounds such as Se.

The objective of this study was to evaluate possible protective mechanisms of different selenium sources against lead induced oxidative DNA damage in liver cells. Selenium based compounds included sodium selenite (Sel-Ni), selenium yeast (SeY), seleno-methionine (Sel-M), and sodium selenate (Sel-Na). Initial work involved studying DNA damage using a comet (single cell gel electrophoresis) assay. This simple procedure involved embedding cells in agar, lysing them and allowing the DNA to unwind. Using electrophoresis at alkaline pH, loops of DNA around strand breaks are more relaxed and pull toward the anode giving the appearance of a comet tail. Undamaged DNA remains tightly wound in the nucleoid or comet head [36]. Subsequent to comet analysis, gene regulation patterns were determined using RT^2 Profiler^{TM} PCR Arrays. These arrays contain primer sequences for genes belonging to a specific function, pathway or disease. For this research the DNA damage and signalling pathway array were chosen for real-time analysis. Finally iReport^{TM} from Ingenuity® systems was used to identify biological pathways of most significance to the dataset. It was anticipated the data generated from comet analysis would correlate with generated gene expression and iReport^{TM} profiles thus contributing to the overall understanding of the molecular mechanisms by which selenium compounds affect DNA repair.

Materials and methods

Chemicals and kits

Dulbecco's modified eagle medium (DMEM), trypsin-EDTA, phosphate buffered saline (PBS 10X), seleno-L-methionine, sodium selenite, sodium selenate, lead (II) nitrate were purchased from Sigma (St. Louis, MO, USA). Selenium yeast (Sel-Plex) was obtained from Alltech Inc., Nicholasville, Kentucky. Fetal calf serum (FCS), Trypan Blue solution and L-glutamine were purchased from Gibco® (Grand Island, NY, USA). AlarmarBlue® was supplied by Invitrogen, CA, USA. RNAlater® was supplied by Ambion, TX, USA. Comet slides and assay kits were provided by Trevigen, Gaithersburg, USA. RNeasy® Micro Kit was supplied by Qiagen, Germany. RNA Nano 6000 LabChip® kits were provided by Agilent Technologies, CA, USA. The RT^2 Profiler^{TM} Arrays and RT^2 First Strand Kit were supplied by SABioscience, Frederick, MD, USA.

Cell medium and culture conditions

The HepG2 (human liver heptocellular carcinoma) cell line were supplied by the Health Protection Agency Culture Collections (HPACC, cell line number 85011430). Cells were cultured in DMEM medium fortified with 10% FCS, 1 mM sodium pyruvate, 1% non-essential amino acids, 50 μg/mL penicillin/streptomycin to prevent bacterial contamination. The cells were grown in monolayers at 37 °C in an atmosphere of 5% CO_2 in humidified air.

Preparation of selenium compounds

Each selenocompound (SeY, Sel-M, Sel-Ni and Sel-Na) was resuspended in H_2O, shaken for 15 min, centrifuged at 800 × *g* for 5 min and resultant supernatant removed. Se analysis was performed on an Agilent 120 Poroshell EC-C8 3.0 mm × 100 mm 2.7 μm column using an Agilent Technologies 1260 infinity series LC system connected to an Agilent Technologies 7700x series ICP-MS. The mobile phase was water/methanol/trifluoroacetic acid (TFA; 97.9:2.0:0.1) at a flow rate of 0.9 mL/min and the column temperature was set at 30 °C. Each sample was analysed in triplicate using an injection volume of 10 μL and monitored for isotopes 76Se, 77Se, and 78Se.

All compounds were subsequently diluted to give a final Se concentration of 1 μg/mL in cell medium and were sterilised by filtration through a 0.2 μm filter. The Se concentration chosen for analysis had previously been detailed as sufficient to inhibit oxidation (data not presented).

Preparation of lead compound

$Pb(NO_3)_2$ was dissolved at a concentration of 1 mg/mL Pb in MilliQ-H_2O and diluted in fully supplemented growth medium for HepG2 cells to a final concentration of 0, 40 or 80 μg/mL Pb. The solutions were sterilised by filtration through a 0.2 μm filter and stored at 4 °C.

Cell assay

HepG2 cells were seeded at a concentration of 1.5×10^5/cm^2 into 24-well plates overnight. Triplicate plates were set up for each sample of interest. After 24 h incubation cells were screened by phase contrast microscopy to confirm sufficient attachment and even distribution of cells. Spent media was removed and replaced with fresh media containing a 1:1 ratio of test substances and genotoxic agent to give a final Se concentration of 1 μg/mL and Pb $(NO_3)_2$ concentration of either 0, 40 or 80 μg/mL. Cells were returned to incubator for a further 24 h. Subsequent to this cells were washed, trypsinised and counted. Cell viability was then assessed using AlamarBlue®.

Per treatment, 3×10^5 cells were suspended in PBS for comet assay analysis while remaining cells were collected by centrifugation and resuspended in RNAlater (Ambion, TX, USA) prior to RNA