INTRODUCTION

Nutritional factors, especially vitamin D and selenium (Se), have long been considered to play an important role in Osteoarthritis (OA). Profound Se deficiency is associated with the severe form of OA known as Kashin-Beck Disease (KBD) (1). Several lines of evidence suggest Se is a chondroprotective micronutrient, however, its underlying mechanisms have not been fully elucidated. An imbalance in production of reactive oxygen species (ROS) in the joints has been considered as part of the pathogenesis of OA (2). The antioxidant effects of Se in neutralizing reactive oxygen and nitrogen species is one potential chondroprotective mechanism against OA progression. Gene expression of the antioxidant Glutathione Peroxidases (GPXs), a subfamily of selenoproteins, is down regulated in OA cartilage (3). We hypothesize that Se regulates antioxidant enzyme expression as one of the mechanisms for its potential chondroprotective effects. Our preliminary data demonstrated that selenomethionine (SeMet) supplementation could induce both GPX gene expression and enzyme activity in a dose-dependent manner in SW1353 cells, a chondrosarcoma cell line frequently used for OA related studies.

METHODS

Cell Line and Selenium Treatment

SW1353 cells were obtained from the American Type Culture Collection and were cultured in DMEM/F12 (Invitrogen, Carlsbad, CA) with 10% fetal bovine serum (37°C in 5% CO2) (Invitrogen, Carlsbad, CA). Monolayer cells were washed in phosphate buffered saline three times and treated with different doses of SeMet in DMEM with 1μg/ml recombinant human insulin and 0.55μg/ml transferrin for 48 hrs.

RNA Isolation and Real Time RT-PCR

Total RNA was extracted and reverse transcribed into cDNA for Real Time RT-PCR analysis. The ABI Prism 7000 sequence detection system and relative quantification software (Applied Biosystems, Foster City, CA) were used for the real-time analyses. Expression levels of the five GPXs were analyzed for SeMet treated compared to untreated SW1353 cells. Transcript levels were determined by Real Time RT-PCR, using the following Applied Biosystems primer and probe sets: 18S-PDAR (18S rRNA), Hs00173566_m1 (GPX1), Hs00702173_m1 (GPX2), Hs00173566_m1 (GPX3), Hs00157812_m1 (GPX4) and Hs00699698_m1 (GPX6).

Immunoblotting Analysis

Whole cell lysates from SW1353 cells were evaluated for GPX1 and GPX4 protein expression in each tested condition. Proteins were separated by electrophoresis on 4-20% reducing gradient gels, transferred to nitrocellulose membrane and subjected to immunoblotting. Membranes were blocked overnight with 5% nonfat dry milk in TBS/0.1% Tween 20 (TBS-T). Polyclonal primary antibodies against GPX1 (ab22604; Abcam, Cambridge, MA) and GPX4 (ab16737; Abcam Cambridge, MA), were used at 1:1,000 dilution. A monoclonal antibody against tubulin (Sigma) was used as a normalization control at 1:10,000 dilution. Anti-rabbit and anti-mouse IgG-HRP (Jackson ImmunoResearch, West Grove, PA) secondary antibodies were diluted 1:50,000. Antibody-antibody complexes were visualized using ECL Plus Western Blotting Detection Reagents (Amersham Biosciences, Piscataway, NJ). The resulting films were scanned and the images were analyzed using Adobe Photoshop CS (Adobe Systems, San Jose, CA) and image analysis software (ImageJ, National Institutes of Health, Bethesda, MD).

Glutathione Peroxidase Activity Assay

Total cell lysates were extracted from each tested condition according to the manufacturer’s protocol using the HT Glutathione Peroxidase Assay Kit (Treviron, Gaithersburg, Maryland). Cellular GPX activity was determined by following the rate of NADPH oxidation at 340nm in the presence of substrate cumene peroxide. One unit of activity equaled one nmole of NADPH oxidized per min. All results were expressed in units/mg protein.

Statistical Analyses

Statistical computations were performed using GraphPad Prism version 4.0 (GraphPad Software, San Diego, CA). Descriptive statistics, sample means, and standard errors for all values were calculated for the subgroups of interest. For descriptive purposes, nonpairwise comparisons between subgroups of interest were performed using one-way ANOVA and the Dunnett post hoc test. P values less than 0.05 were considered significant.

RESULT

Quantification by Real Time RT-PCR indicated that 48 hrs exposure of cells to SeMet induced mRNA expression of all Se containing peroxidases in a dose-dependent manner except GPX6, which was undetectable in SW1353 cells (data not shown). The effect of SeMet on mRNA expression of the GPXs at 0.5μM was as follows: GPX1-3, upregulated ~ 2-2.5-fold; GPX4 upregulated ~ 1.5-fold. The upregulation of GPX1 and GPX4 protein by SeMet was further confirmed by immunoblot analysis. Protein expression of GPX1 and GPX4 was found to increase up to 4-fold and 2.5-fold respectively even at the lowest tested dose of SeMet of 0.25μM (Fig.1). GPX activity was assayed to examine the overall functional change in the intracellular antioxidant GPX activity that could be affected through the SeMet treatment. Cellular GPX activity was induced significantly and more than 2-fold by SeMet at all tested doses (Fig.2).

DISCUSSION

Taken together, these result demonstrated that expression and activity of the GPXs could be induced by SeMet in our in vitro system. Glutathione peroxidase plays a major antioxidant role to counteract the oxidative stress induced by hydrogen peroxides and lipid peroxides. These results support a chondroprotective mechanism of Se mediated through upregulation of the antioxidant GPX at transcriptional and post-transcriptional levels. More detailed study on the effect of SeMet on other antioxidant enzymes will further be explored in future experiments.

REFERENCE