Toxicology Reports 5 (2018) 579–584

Contents lists available at ScienceDirect

Toxicology Reports

journal homepage: www.elsevier.com/locate/toxrep

Methylmercury promotes breast cancer cell proliferation

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ARTICLE INFO

Keywords:
Methylmercury
Mercury
Metalloestrogen
Breast cancer

ABSTRACT

Context: Metalloestrogens are small ionic metals that activate the estrogen receptor (ER). Studies have shown that when metalloestrogens bind to the ER, there is an increase in transcription and expression of estrogen-regulated genes, which induces proliferation of estrogen-dependent breast cancer. Methylmercury (MeHg), a metalloestrogen, is present in the environment and is toxic at moderate to high concentrations. However, at lower concentrations MeHg may promote the proliferation of ER-positive breast cancers and protect cells against pro-apoptotic signals.

Objective: To investigate the effects of MeHg treatment on breast cancer cells in vitro.

Materials and methods: MCF7 breast cancer cells were treated with concentrations of MeHg ranging from 1 nM to 100 mM. Hg analysis was used to quantify intracellular mercury concentrations. Cell proliferation and apoptosis were determined by cell counting and Annexin-V staining, respectively.

Results: We defined a protocol that maximizes cellular exposure to mercury. Treatment of human ER-positive breast cancer cells with 1 nM MeHg promoted proliferation, while treatment with a concentration of 100 nM induced apoptosis.

Discussion and conclusions: Clarifying the effects of MeHg on breast cancer will improve our understanding of how environmental toxins affect tumor progression and may lead to the development of future therapeutic strategies.

1. Introduction

Breast cancer is the second most common cancer diagnosis in women in the United States [1]. It accounts for one in three cancer diagnoses and is the second leading cause of cancer death [1]. Estrogens are a family of steroid hormones that directly control the expression of cell-cycle regulatory genes [2]. Breast cancer is associated with elevated levels of estrogen or estrogen-like substances that bind to the estrogen receptor (ER), causing overstimulation of signaling pathways [3].

The high incidence of breast cancer is likely, in part, due to the presence of environmental estrogens [4,5]. Environmental estrogens such as phytoestrogens or plant-based estrogens (coumestrol and isoflavone genistein) and xenoestrogens or synthetic chemicals (dichlorodiphenyltrichloroethane, bisphenol A, phthalates, dichlorodiphenylethylene, polychlorinated biphenyls, and alkylphenol) have been shown to promote estrogen-like effects [6–8]. These environmental estrogens can be found in plants, pesticides, birth control pills, plastics, auto exhaust, and cigarette smoke [4,9–11]. Recently, several inorganic xenoestrogens—metalloestrogens—have been shown to mimic the effect of estrogens and activate the ER [4,5,11]. Metalloestrogens are small ionic metals and metalloids that fall into two subcategories, oxyanions and bivalent cations [4,12,13]. The oxyanions include arsenite, antimony, nitrite, selenite, and vanadate, while the bivalent cations include cadmium, calcium, colbolt, copper, nickel, chromium, lead, mercury, and tin [4].

Copper, colbolt, nickel, lead, tin, and chromium (II) have been shown to induce the proliferation of ER-positive breast cancer cells [4,5,11,14] and increase the transcription and expression of estrogen-regulated genes [4,5]. These metals have also been shown to bind with high affinity to the ER and block the binding of estradiol [13]. Among the heavy metals, cadmium and mercury are two of the most toxic due to their persistence in the environment [15]. Both have been shown to cause oxidative stress and induce apoptosis [16–19]. Cadmium's role as a metalloestrogen has been extensively studied because it accumulates in the body due to its poor excretion rate, and therefore may be harmful even at low exposures [2]. These studies have shown that cadmium promotes activation of hormone-regulated genes [20,21], proliferation of estrogen-dependent breast cancer cells [20,22–24], premature growth and development of mammary glands, and increased uterine

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https://doi.org/10.1016/j.toxrep.2018.05.002
Received 28 June 2017; Received in revised form 2 May 2018; Accepted 13 May 2018
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weight owing to proliferation of the endometrium [2]. Although the effects of cadmium on breast cancer have been widely studied, investigations into mercury's effects on breast cancer are limited.

Mercury exists in the environment in three forms: elemental mercury, inorganic mercury (mercuric mercury), and organic mercury (ethylmercury and methylmercury) that differ in their metabolism and toxicity [25,26]. These different forms arise from the global cycle of mercury. Elemental mercury, or mercury vapor, is a monatomic gas that evaporates from soil and water. This mercury vapor can also be emitted by volcanoes or coal-burning power stations. After about a year, the mercury vapor is converted into soluble inorganic mercury (Hg^{2+}) and deposited into the earth in rain water. At this point, the inorganic mercury can be converted back into the vapor form by microorganisms or it can attach to aquatic sediments and be converted into methylmercury (MeHg) by microbes. Once in the MeHg form, it enters the aquatic food chain and becomes highly concentrated over time in large predatory fish [25].

Methylmercury (MeHg) is prevalent in the environment. The main sources of possible exposure to MeHg include occupational exposure and eating fish or wild game near the top of the food-chain that have accumulated mercury in their tissues [27]. MeHg exposure can lead to many diseases and disorders due to its liposolubility and its affinity for endogenous sulfur and selenium. When humans digest mercury-contaminated food, MeHg is absorbed in the duodenum, where it binds to thiol (R-SH) and selenol (R-SeH) groups, which are products of digestive breakdown [25].

Several groups have shown that treatment of breast cancer cells with low concentrations of mercuric chloride promotes the proliferation of these estrogen-responsive cells [5,11,14]. One other group has investigated the effects of MeHg on breast cancer cells [28]. Here, we investigated MeHg's proliferative versus toxic effects on MCF7 breast cancer cells. We hypothesized that when breast cancer cells are cultured in the presence of MeHg concentrations comparable to physiological concentrations of estrogen, there will be an increase in cell proliferation. Conversely, we presumed that culturing cells in the presence of elevated concentrations of MeHg would promote apoptosis. These studies will bring us closer to developing therapeutic strategies for treating MeHg-induced breast cancer and will help us to take steps towards preventative interventions.

2. Methods

2.1. Cells and culture conditions

Estrogen receptor-positive MCF7 human epithelial breast cancer cells originating from an invasive ductal carcinoma of the breast, a gift from the Filardo lab at Brown University (Providence, RI), were maintained in phenol red-free Dulbecco's Modified Eagle's Medium (DMEM)/F-12 containing HEPES and L-glutamine with 5% fetal bovine serum. Cultures were maintained in 5% CO_2 at 37 °C.

2.2. MeHg treatment and cell proliferation assay

MCF7 cells (10^4/well) were seeded into 12-well plates in phenol red-free DMEM/F-12 HEPES with 5% FBS and incubated for 24 h. Following incubation, cells were washed 1x with Hank's Balanced Salt Solution (HBBS) and then treated in quadruplicate with various concentrations of MeHg (0, 1 nM, 10 nM, 100 nM, 1 μM, 100 μM, 1 mM, 100 mM) delivered in HBBS for 15 min at 5% CO_2 and 37 °C. The MeHg solution was then removed and DMEM/F-12 HEPES with 5% FBS was added back. Cells were incubated for 5 days. On day 5, cells were washed 1× with HBBS, lifted with 1x trypsin, centrifuged, and re-suspended in DMEM/F-12 HEPES with 5% FBS. Cells were counted using a hemocytometer.

2.3. Annexin-V/PI assays

To determine apoptotic rates of MeHg-treated cells, MCF7 cells were treated with MeHg as described above. On day 5, the apoptotic rates were determined with an Annexin-V-Fluos staining kit according to the manufacturer's instructions (Sigma-Aldrich, United States). In short, the cells were incubated in Annexin-V-FITC (Sigma-Aldrich, United States) for 30 min, washed 3x with HBBS, and analyzed by immunofluorescence [29].

2.4. Mercury partitioning experiment

An additional experiment was performed to examine how the treatment medium influences MeHg partitioning. Cells were treated in triplicate with 0 or 1 μM MeHg delivered in HBBS or in DMEM/F-12 HEPES with 5% FBS for 15 min at 5% CO_2 and 37 °C. The treatment solutions (supernatants) were then removed and saved for Hg analysis. Cells were washed 1x with HBBS, lifted with 1× trypsin, and centrifuged. Cell pellets were re-suspended in 1 ml of HBBS to produce cell suspensions for Hg analysis.

Total mercury concentrations in supernatants and cell suspensions were determined using acid digestion and BrCl oxidation [30]. Briefly, 0.5-mL aliquots were digested overnight in 4 mL of 4.6 M HCl at 60 °C. After digestion, 0.4 mL of BrCl was added to the digestates; a persistent yellow color indicated complete oxidation to Hg^{2+}. Just prior to analysis, excess BrCl was quenched by addition of hydroxylamine hydrochloride. Digestates were analyzed for total mercury via $SnCl_2$ reduction, gold amalgamation, thermal desorption and CVAFS detection [31,32]. Controls were used as procedural blanks.

2.5. Statistical analysis

All of the results are presented as mean +/- standard deviation of biological triplicates or greater. Results were compared by one-way ANOVA and a probability value of $p < 0.05$ is considered significant.

3. Results

3.1. Low concentrations of MeHg induce MCF7 cell proliferation

To determine the effects of MeHg treatment on cell proliferation, MCF7 human breast cancer cells were treated with concentrations of MeHg ranging from 1 nM to 100 μM. We observed significantly reduced growth at MeHg concentrations of 1 μM ($p = 0.01$), 10 μM ($p = 0.005$), and 100 μM ($p = 0.001$) compared to untreated cells (Fig. 1). Increased cell numbers were observed in cells treated with 1 nM MeHg ($p = 0.02$) when compared to untreated cells (Fig. 1). Although not significant ($p = 0.26$), there was a trend towards more proliferation of cells treated with 10 nM MeHg than untreated cells (Fig. 1). These results suggest that at lower concentrations, MeHg promotes proliferation, while at higher concentrations, MeHg promotes cell death.

3.2. High concentrations of MeHg induce MCF7 cell death

To determine whether MeHg treatment of breast cancer cells promotes apoptosis, MCF7 human breast cancer cells were treated with concentrations of MeHg ranging from 1 nM to 100 μM. Apoptosis was detected by immunofluorescence following the use of an Annexin-V-FITC staining kit. We did not observe apoptosis of cells treated with 1 nM and 10 nM MeHg, while we did observed an increase in apoptosis of cells treated with 100 nM and 1 μM. All cells treated with 10 μM and 100 μM MeHg were apoptotic (Fig. 2). These results support the proliferation assay results and suggest that at lower concentrations, MeHg does not induce apoptosis, while at higher concentrations, MeHg promotes cell death.

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