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Prevention of methylmercury-induced mitochondrial depolarization, glutathione depletion and cell death by 15-deoxy-delta-12,14-prostaglandin J_2

Jason Y. Chang [a,b,*], Pao-Feng Tsai [c]

[a] Department of Neurobiology & Developmental Sciences, College of Medicine, University of Arkansas for Medical Sciences, Little Rock, AR 72205, USA
[b] Department of Ophthalmology, College of Medicine, University of Arkansas for Medical Sciences, Little Rock, AR 72205, USA
[c] College of Nursing, University of Arkansas for Medical Sciences, Little Rock, AR 72205, USA

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ABSTRACT

Methylmercury (MeHg) is an environmental toxin that causes severe neurological complications in humans and experimental animals. In addition to neurons, glia in the central nervous system are very susceptible to MeHg toxicity. Pretreatment of glia with the prostaglandin derivative, 15-deoxy-delta-12,14-prostaglandin J_2 (15d-PGJ_2), caused a significant protection against MeHg cytotoxicity. Results with the C6 glioma cells demonstrated that the protection was dependent on the duration of pretreatment, suggesting that time was required for the up-regulation of cellular defenses. Subsequent experiments indicated that 15d-PGJ_2 prevented MeHg induced mitochondrial depolarization. Similar protection against MeHg cytotoxicity was observed in primary cultures of mouse glia. Analysis of cellular glutathione (GSH) levels indicated that 15d-PGJ_2 caused an up-regulation of GSH and prevented MeHg-induced GSH depletion. Buthionine sulfoximine (BSO), a GSH synthesis inhibitor, completely inhibited the GSH induction by 15d-PGJ_2. However, BSO did not prevent the stabilization of mitochondrial potential and only partially prevented the protection caused by 15d-PGJ_2. While induction of heme oxygenase-1 was implicated in the cytoprotection by 15d-PGJ_2 under some experimental conditions, additional experiments indicated that this enzyme was not involved in the cytoprotection observed in this system. Together, these results suggested that while up-regulation of GSH by 15d-PGJ_2 might help cells to defend against MeHg toxicity, there may be other yet unidentified mechanism(s) initiated by 15d-PGJ_2 treatment that contributed to its protection against MeHg cytotoxicity.

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1. Introduction

Methylmercury (MeHg) causes severe neurological disorders in the central nervous system (CNS) in humans and experimental animals (Clarkson et al., 2003). The particularly devastating effects on development of the fetal CNS prompted the Food and Drug Administration and the Environmental Protection Agency to issue a series of Consumer Advisories alerting pregnant women and women of childbearing age of the dangers of MeHg toxicity. Other than direct neurotoxicity, the malfunction of glia when poisoned with MeHg is an important factor contributing to MeHg-induced neuronal death (Aschner, 1996; Aschner et al., 1999).

Mitochondria are believed to be a major target of MeHg. For example, Yee and Choi (1996) reported that cultured CNS cells ceased respiration within 30 min of MeHg treatment, and presented evidence that MeHg inhibited the mitochondrial electron transport chain. Allen et al. (2001) also reported that MeHg had a selective effect on mitochondria of cultured astrocytes. Electrical distribution on the mitochondrial membrane is polarized such that it is negative inside the organelle. MeHg causes mitochondrial dysfunction and depolarization in a variety of cell types (Limke and Atchison, 2002; InSug et al., 1997; Shenker et al., 1998; Garg and Chang, 2006). This could lead to subsequent glutathione (GSH) depletion and reactive oxygen species (ROS) generation (Yee and Choi, 1996; Shenker et al., 1999), and oxidative stress may play a role in MeHg cytotoxicity.

* Corresponding author at: Department of Neurobiology and Developmental Sciences, Slot 510, University of Arkansas for Medical Sciences, 4301 W. Markham Street, Little Rock, AR 72205, USA. Tel.: +1 501 686 7025; fax: +1 501 686 6382.
E-mail address: changjasony@uams.edu (J.Y. Chang).

Abbreviations: 15d-PGJ_2, 15-deoxy-delta-12,14-prostaglandin J_2; BSO, D,L-buthionine-(*S*,*R*)-sulfoximine; HBSS, Hank's balanced salt solution; HO-1, heme oxygenase-1; JC-1, 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolocarbocyanine iodide; MeHg, methylmercury; MEM, minimum essential medium; MMP, mitochondrial membrane potential ($\Delta\Psi_m$); MTT, (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide); OD, optical density; PBS, phosphate-buffered saline; ROS, reactive oxygen species; SnMP, tin-mesoporphyrin.

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The prostaglandin derivative 15-deoxy-delta-12,14-prostaglandin J_2 (15d-PGJ_2) has a wide variety of effects on cellular functions. This compound is normally present in tissues at low levels (<1 nM), but can reach high concentrations during infection and inflammation (Gilroy et al., 1999; Itoh et al., 2004). This agent has a potent inhibitory effect on immune cells. For example, it is a potent inhibitor of macrophage (Jiang et al., 1998; Ricote et al., 1998; Castrillo et al., 2000) and microglia (Petrova et al., 1999; Bernardo et al., 2000; Koppal et al., 2000) activation. Some of its functions are dependent on the activation of the nuclear receptor, peroxisome proliferator activated receptor-gamma (PPARγ), but others are PPARγ independent (see Straus and Glass, 2001 for a review). Our previous studies showed that 15d-PGJ_2 prevented cell death caused by oxidative stress induced by H_2O_2 or *t*-butyl hydroperoxide (Garg and Chang, 2003, 2004) in a PPARγ independent manner. Results from our studies indicate that 15d-PGJ_2 can prevent oxidant-induced mitochondrial depolarization, which should partially account for its cytoprotection (Garg and Chang, 2004). We also demonstrated that MeHg cytotoxicity in the N9 microglia cell line could be partially prevented by 15d-PGJ_2 (Garg and Chang, 2006). The protection of this agent against MeHg was not mediated through PPARγ activation because other PPARγ agonists did not exhibit the protective effect (Garg and Chang, 2006).

Recent studies indicate that the α,β-unsaturated carbonyl group within the cyclopentenone ring of 15d-PGJ_2 is essential for its PPARγ-independent activity (Straus and Glass, 2001). Specifically, it enables the molecule to function as an electrophile which can bind to sulfhydryl groups or cysteine residues of proteins and change their functions. By this mechanism, 15d-PGJ_2 can activate the Keap1-Nrf2-ARE pathway in the following manner (Kensler et al., 2007): binding of 15d-PGJ_2 to Keap1 releases the transcription factor, Nrf-2, which is translocated to the nucleus. Together with other transcription factors and proteins, the transactivation of ARE (Antioxidant Responsive Element, also known as Electrophile Responsive Element)-responsive genes occurs. Many of the genes induced in this manner are cytoprotective.

With respective to MeHg cytotoxicity, the induction of cellular GSH synthesis by 15d-PGJ_2 through the mechanism described above appears to be very important. 15d-PGJ_2 can up-regulate glutamylcysteine synthetase, the rate limiting enzyme for GSH synthesis (Qin et al., 2006; Saito et al., 2007). This property can prepare cells to fight against oxidative stress induced by oxidant (Qin et al., 2006) or by glutamate (Aoun et al., 2003; Saito et al., 2007). Enhanced cellular GSH levels might modulate MeHg cytotoxicity because cells with intrinsic higher GSH levels appear more resistant to MeHg cytotoxicity (Shenker et al., 1993).

Another important gene induced by 15d-PGJ_2 is heme oxygenase-1 (HO-1). Induction of HO-1 in immune cells by 15d-PGJ_2 may modulate inflammation (Gong et al., 2002; Itoh et al., 2004; Alvarez-Maqueda et al., 2004). While up-regulation of HO-1 is neuroprotective under various experimental conditions (Hegazy et al., 2000; Imuta et al., 2007; Le et al., 1999; Satoh et al., 2003), it can be detrimental to cells in other conditions (Song et al., 2006, 2007). Whether HO-1 induction caused by 15d-PGJ_2 can modulate MeHg cytotoxicity is not clear.

Based on the discussion above, it is clear that while MeHg causes mitochondrial dysfunction and GSH depletion, 15d-PGJ_2 can prevent oxidant induced mitochondrial dysfunction and increase cellular GSH levels. The current study tested the protective effect of 15d-PGJ_2 in glia, and tested the hypothesis that up-regulation of GSH by 15d-PGJ_2 was the major mechanism leading to prevention of MeHg-induced mitochondrial depolarization and cell death. The C6 glioma cell line and primary mouse glia were used in this study.

2. Materials and methods

JC-1 (5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolocarbocyanine iodide) was from Molecular Probes (Eugene, OR, USA). 15d-PGJ_2 (1 mg/ml in methyl acetate) was from Cayman (Ann Arbor, MI, USA). Tin-mesoporphyrin was from Frontier Scientific (Logan, UT, USA). BSO (D,L-buthionine-(*S*,*R*)-sulfoximine) was from Acros Organics (distributed by Fisher Scientific (Pittsburgh, PA, USA)). Hank's balanced salt solution (HBSS), MeHgCl (contained ~13% Cl), monochlorobimane and other general biochemical reagents were from Sigma (St. Louis, MO, USA) unless otherwise stated.

2.1. Cell cultures

The C6 glioma cells (from the American Type Culture Collection) were grown in 75 cm^2 tissue culture flasks for routine maintenance. Mixed mouse cerebral glia derived from 1 to 2 days old C57BL mice were plated in 75 cm^2 flasks, expanded once into 150 cm^2 flasks before plating into multi-well culture plates for experiments. Astrocytes constituted the majority of cells in these cultures because more than 90% of culture surface was covered by cells positive for glial fibrillary acidic protein (GFAP) staining. The growth medium, identical for both cell types, was composed of minimum essential medium (MEM) supplemented with 5% newborn calf serum and 2.5 mM glutamine. To prepare for experiments, cells were plated into culture plates in this growth medium overnight at the following density: 20,000 cells/well in 96-well plates with 100 μl medium or 140,000 cells/well in 24-well plates with 700 μl medium. The medium was switched the following day to MEM supplemented with 1% newborn calf serum and 2.5 mM glutamine for MeHg treatment.

2.2. Cell viability

Viability was determined by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay (Garg and Chang, 2004, 2006). The OD of each well was measured by a plate reader (Spectra Max 190, Molecular Devices, Sunnyvale, CA, USA) with a filter setting at 570 nm (reference filter setting was 630 nm). Our previous studies indicated that the MTT assay agreed well with results from the trypan blue exclusion assay (Garg and Chang, 2006).

2.3. Measurement of mitochondrial membrane potential (MMP, $\Delta\Psi_m$)

Cells were plated in 24-well plates overnight before the assay was performed as described previously (Garg and Chang, 2004). Each culture was treated with MeHg (700 μl/well prepared in medium with 1% newborn calf serum) at 37 °C for a period of time indicated in each experiment. MeHg-containing medium was then replaced with 5 μM JC-1 prepared in Hank's balanced salt solution (300 μl/well) for 15 min at 37 °C. After this incubation period, cells in each well were rinsed once with 500 μl HBSS, collected into 300 μl HBSS, and then transferred to a 96-well tissue culture plate. The fluorescence of each well was detected by a fluorescence plate reader (Spectra Max Gemini XS, Molecular Devices, Sunnyvale, CA, USA) with the following settings: excitation 485 nm, emission 545 and 595 nm, cutoff 530 nm. In some experiments, the emission spectra between 500 and 620 nm were obtained. To prepare JC-1, stock solution (5 mM prepared in DMSO) was diluted (1:4) into 5%

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