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Journal of Neuroimmunology 171 (2006) 17 – 28

Journal of Neuroimmunology

www.elsevier.com/locate/jneuroim

Methylmercury causes oxidative stress and cytotoxicity in microglia: Attenuation by 15-deoxy-delta 12, 14-Prostaglandin J_2

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Received 1 June 2005; accepted 13 September 2005

Abstract

Methylmercury (MeHg) causes severe neurological disorders in the central nervous system. This study focused on the effects of MeHg on microglia, macrophage-like cells that reside in the CNS important in neuro-immune interactions. The murine N9 microglial cell line was used in this set of study. MeHg caused reactive oxygen species generation, mitochondrial depolarization and aconitase inactivation, all of which were signs of cellular oxidative stress. MeHg greatly increased microglial IL-6 secretion despite the fact that it severely inhibited protein synthesis. The concentration that caused 50% cell death in 24 h was ~9 μM. Pretreatment of microglia with the prostaglandin derivative, 15-deoxy-delta 12, 14-Prostaglandin J_2 attenuated MeHg induced cell death. The saving effect did not appear to be mediated through activation of peroxisome proliferator activated receptors (PPAR) since other agonists of these receptors did not prevent MeHg induced microglial death.

Keywords: 15d-PGJ_2; Aconitase; Methylmercury; MMP (mitochondrial membrane potential); N9 microglia; Oxidative stress

1. Introduction

Mercury is found naturally in cinnabar ore. Contamination resulting from mining process is an important environmental concern in some parts of the world and in the United States, such as Nevada (Bonzongo et al., 1996), Alaska (Gray et al., 2000) and California (Domagalski et al., 2004). Emitting mercury through volcanoes is also a process that occurs naturally. For example, eruption of Mt. St. Helens in Washington State contributed significantly to local mercury accumulation (Schuster et al., 2002). However, mercury found in the environment results largely from coal-burning industries, power plants, or from medical wastes. Analysis of the mercury content in glacial ice cores from the Upper Fremont Glacier (Wyoming, USA) indicated that anthropogenic sources contributed 70% of the total mercury input in the environment (Schuster et al., 2002). Bacteria in wetlands, lakes, and oceans convert mercury to various compounds of organic mercury; among these, methylmercury (MeHg) is the most prevalent and the most toxic (Baldwin and Marshall, 1999; Risher et al., 2002). In contaminated water, fish accumulate MeHg in their tissues (Harris et al., 2003). Through bioaccumulation, large fishes at the top of the food chain (e.g., sharks and swordfish) accrue the highest amounts of MeHg (Baeyens et al., 2003; Risher et al., 2002). Humans, being at the very top of the food chain, are in great risk of MeHg poisoning.

Abbreviations: 15d-PGJ_2, 15-deoxy-delta 12, 14-Prostaglandin J_2; FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone; FU, fluorescence units; H_2DCF-DA, 2′, 7′-dichlorodihydrofluorescein diacetate; HBSS, Hank's Balanced Salt Solution; IL-6, interleukin-6; JC-1, 5,5′, 6,6′-tetrachloro-1, 1′, 3,3′-tetraethylbenzimidazolocarbocyanine iodide; MeHg, methylmercury; MMP, mitochondrial membrane potential ($\Delta\Psi m$), MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide); $NADP^+$, β-nicotinamide adenine dinucleotide phosphate disodium salt; OD, optical density; PBS, phosphate buffered saline; PPARs, peroxisome proliferator-activated receptors; ROS, reactive oxygen species.

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doi:10.1016/j.jneuroim.2005.09.007

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. While it is recognized that glia in the CNS are subjected to MeHg toxicity and that malfunction of glial activities when poisoned by MeHg is an important factor contributing to MeHg-induced neuronal death (Aschner et al., 1999), most studies in this area focus on astrocytes. The interactions between MeHg and another type of glia, microglia, are not well studied.

Microglia are macrophage-like cells that reside in the CNS, and are ~15% of the CNS cell population (Barron, 1995; Hickey et al., 1992; Ling and Wong, 1993). They participate in immune activities in the CNS, and are responsible for removal of invading pathogens. These cells secrete a number of proteins, which can be beneficial or toxic to neuronal cells depending on the experimental conditions and the microenvironment involved (Kempermann and Neumann, 2003). For example, microglia are known to secrete interleukin-6 (IL-6) under various experimental conditions. IL-6 is known to be beneficial (Carlson et al., 1999; Loddick et al., 1998; Sanchez et al., 2003) or detrimental (Conroy et al., 2004; Qiu and Gruol, 2003) to neuronal survival dependent of experimental conditions.

Reactive oxygen species (ROS) generation has been linked to MeHg induced toxicity both in vivo and in vitro. For example, brain synaptosomes prepared from animals injected with MeHg showed high levels of ROS content (Ali et al., 1992). Cultured neurons (Mundy and Freudenrich, 2000) and glia (Shanker and Aschner, 2003) respond to MeHg with ROS production. Mercury also causes ROS generation in immune cells, such as T cells, B cells (Kim and Sharma, 2003) and macrophages (Kim and Sharma, 2004). Generation of ROS in microglia resulting from MeHg treatment has not been reported in literature.

Mitochondria are believed to be a major target of MeHg. For example, Yee and Choi reported that cultured CNS cells ceased respiration in ~30 min after MeHg treatment, and presented evidence that MeHg inhibited the mitochondrial electron transport chain. This might be the reason for the ROS generation (Yee and Choi, 1996). Allen et al. also reported that MeHg had a selective effect on mitochondria in cultures of astrocytes (Allen et al., 2001). A decrease of mitochondrial membrane potential resulting from MeHg treatment has been reported in cells derived from the nervous (Limke and Atchison, 2002) and immune (InSug et al., 1997; Shenker et al., 1998) systems. Similar observations were made in other cell types. For example, MeHg was reported to bind to mitochondria, leading to K^+ influx into mitochondria and causing mitochondrial membrane depolarization in liver cells (Sone et al., 1977). The effects of MeHg on microglial ROS generation and mitochondrial membrane are subjects of this current study.

Mitochondrial aconitase is a tricarboxylic acid cycle (TCA cycle) enzyme involved in the conversion of citrate to isocitrate. This enzyme and its cytoplasmic counterpart (cytoplasmic aconitase, also termed iron-regulatory protein 1) are very sensitive to oxidative stress (Gardner et al., 1994, 1995). While MeHg is reported to cause cellular oxidative stress, the effect of MeHg on aconitase activity has not been studied.

By using the murine N9 microglial cell line (Corradin et al., 1993) as a model, we demonstrated that MeHg caused cellular oxidative stress and cytokine production in microglia. Furthermore, we tested the effect of some pharmacological agents known to prevent oxidative stress to determine if they could prevent MeHg induced cytotoxicity.

2. Materials and methods

2.1. Materials

15d-PGJ_2, Ciglitazone, Azelaoyl PAF and WY14643 were purchased from Cayman (Ann Arbor, MI). H_2DCF-DA (2′, 7′-dichlorodihydrofluorescein diacetate) and JC-1 (5,5′, 6,6′-tetrachloro-1, 1′, 3,3′-tetraethylbenzimidazolocarbocyanine iodide) were from Molecular Probes (Eugene, OR). FCCP (carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone), hydrogen peroxide, Hank's Balanced Salt Solution (HBSS), $NADP^+$ (β-nicotinamide adenine dinucleotide phosphate disodium salt), isocitric dehydrogenase, aconitase and other general biochemical reagents were purchased from Sigma (St. Louis, MO) unless otherwise stated.

2.2. Cell culture

The N9 murine microglia cell line was a gift kindly provided by Dr. P. Ricciardi-Castagnoli (Corradin et al., 1993). This cell line has been used extensively as a model for microglia (Barger and Harmon, 1997; Meda et al., 1995). The cultures were maintained in Minimum Essential Medium supplemented with 5% newborn calf serum and 1.4 mM glutamine. In order to maintain a comparable growth condition with regard to cell density and medium volume, cells were plated at 20,000 cells/well in 96-well plates (100 μl medium/well, for MTT cell viability determination and ROS assay) or 140,000 cells/well in 24-well plates (700 μl medium/well, for trypan blue exclusion assay, mitochondrial membrane potential measurement and for IL-6 detection) or 4.7×10^6/75 cm^2 flasks (23.5 ml medium/flask, for aconitase assay).

2.3. Cell viability

As a general protocol, cells were plated in 96-well plates (20,000 cells/well) overnight, then were subjected to MeHg treatment (in medium containing 1% newborn calf serum)