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Mechanisms and Modifiers of Methylmercury-Induced Neurotoxicity

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Abstract

The neurotoxic consequences of methylmercury (MeHg) exposure have long been known, however a complete understanding of the mechanisms underlying this toxicity is elusive. Recent epidemiological and experimental studies have provided many mechanistic insights, particularly into the contribution of genetic and environmental factors that interact with MeHg to modify toxicity. This review will outline cellular processes directly and indirectly affected by MeHg, including oxidative stress, cellular signaling and gene expression, and discuss genetic, environmental and nutritional factors capable of modifying MeHg toxicity.

Introduction

The toxicity of mercury (Hg) is widely accepted, and while several Hg species induce neurotoxicity, methylmercury (MeHg, CH₃Hg⁺) in particular is highly neurotoxic¹. The majority of Hg is naturally found in inorganic forms such as cinnabar ore, inorganic mercuric salts enter the environment naturally through geocycling, and anthropogenically through mining, industrial processes and waste incineration. Microorganisms generate organic mercury by methylation of elemental and inorganic mercury to form MeHg, which is then biomagnified in the food chain, particularly in aquatic fish and mammals. MeHg is the most common form of Hg that humans are exposed to, primarily through consumption of fish containing MeHg.

As early as the mid 1800's the neurotoxic effects of MeHg were evident from poisoning and deaths caused by industrial exposure. In Minamata Japan, industrial wastewater containing MeHg was released into the environment over a number of years and bioaccumulated in fish consumed by the local population. In the 1950's many adults developed Minamata disease, characterized by paresthesia, sensory deficits, slurred speech, unsteady gait, muscle weakness, irritability, memory loss, depression, and sleeping disturbance². Similar symptoms were also observed in Iraq in 1956 and 1960 after consumption of bread made from grain treated with ethylmercury and again in 1971 from grain treated with methylmercury³.

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The first recorded case of prenatal MeHg neurotoxicity in humans occurred in the 1950's, a few years later children in Minamata, Japan presented with severe neurodevelopmental deficits that were later related to maternal consumption of fish contaminated with MeHg. Studies from the Iraq exposures provided further evidence that the developing brain is particularly sensitive to MeHg^{3, 4}. Since these incidences, several large-scale epidemiological studies have examined prenatal MeHg toxicity in humans from Minamata and fish-eating regions in the Seychelles, Faroe Islands and New Zealand.

This review will outline cellular processes directly and indirectly affected by MeHg and discuss genetic, environmental and nutritional factors capable of modifying MeHg toxicity.

Mechanisms of Toxicity

The toxicological properties of MeHg are largely related to its pro-oxidative effects. In the nervous system, MeHg disturbs oxidative balance, disrupts calcium homeostasis and alters glutamate and γ -aminobutyric acid (GABA) signaling. In addition to directly promoting neurotoxicity, these biochemical consequences of MeHg exposure cause indirect dysregulation of cellular signaling and gene expression, which also contributes to toxicity.

Oxidative Stress

MeHg is a soft electrophile and interacts with thiol (-SH) and selenol (-SeH) groups, which are the only biological soft nucleophiles. Thiols and selenols play a fundamental role in MeHg-induced toxicity⁵. Hg compounds react specifically with sulfhydryls and selenols, forming stable complexes with defined stoichiometry. Hg's affinity for these groups is extremely high⁶ and in biological media MeHg is always complexed to -SH-containing ligands⁷⁻¹⁰. Mechanisms of MeHg's membrane transport also invoke -SH-containing amino acids¹¹⁻¹⁷. MeHg conjugates with cysteine (Cys) and is transported via the large amino acid transporter (LAT), which transports methionine¹⁷.

Several important regulators of the antioxidant response are rich in thiol and selenol groups. Glutathione (γ -glutamyl-cysteinyl-glycine; GSH) is a highly abundant tripeptide, which serves as a reducing agent for reactive oxygen species (ROS) and other unstable molecules, a reaction catalyzed by the selenoprotein glutathione peroxidase (GPx). GSH can also be conjugated to toxins to facilitate excretion during detoxification. The majority of GSH is present in the reduced form (GSH) while approximately 10% is present as the oxidized form glutathione disulfide (GSSG), the GSSG:GSH ratio reflects the oxidative state of the cell¹⁸. MeHg interacts with GSH to form an excretable GS-HgCH₃ complex¹⁴, increasing the GSSG:GSH ratio and reducing the antioxidant capacity both in astrocytes and microglia^{19, 20}. Furthermore, thioredoxins (Trxs), a family of proteins with abundant thiol groups, are essential in regulating cellular thiol/disulfide redox status. Trxs contain an invariant active center, -Cys-Gly-Pro-Cys- where the two Cys residues undergo reversible oxidation/reduction in the presence of the selenoprotein Trx reductase (TrxR) and NADPH²¹. The cytosolic Trx (Trx 1) is also implicated in growth stimulation, transcriptional regulation and apoptosis²². The mitochondrial Trx (Trx 2) is a critical component of the mitochondrial antioxidant system and protects against oxidative stress-induced cell death²³. *In vitro*, MeHg inhibits GPx and TrxR activity²⁴. Prior to inducing cell

death, MeHg inhibits GPx activity in cultured cerebellar granule cells, and overexpression of GPx prevents cell death²⁵. These effects also occur *in vivo* in mice and fish^{26, 27}.

In addition to impairing the antioxidant response of the cell, MeHg increases production of ROS. Hydrogen peroxide (H₂O₂) levels are increased concomitant with altered motor reflexes in mouse pups exposed to MeHg through lactation²⁸. H₂O₂ levels are also increased by MeHg in cultured astrocytes²⁹ and in mitochondria isolated from mouse and rat brain^{30, 31}. Decreased H₂O₂ detoxification resulting from reduced GSH and GPx activity contributes to these increases, although Mori and colleagues observed increased H₂O₂ production from MeHg-induced changes in the mitochondrial electron transport chain (ETC) complexes II and III in mitochondria isolated from cerebellum, but not liver or cerebrum^{31, 32}. Superoxide anion (O₂^{•-}) levels also increased due to changes in ETC complexes I and III respiration³³. Nitric oxide (NO) production rises as well following MeHg exposure³⁴⁻³⁶, likely through stimulation of nitric oxide synthase (NOS) by increased Ca²⁺ (discussed below).

The resulting pro-oxidative shift causes lipid peroxidation, protein oxidation and DNA oxidation. Owing to the high content of polyunsaturated fatty acids (PUFAs) in the central nervous system, lipid peroxidation is a major consequence of free radical-mediated injury. H₂O₂ undergoes Fenton chemistry, producing the hydroxyl radical (•OH), which is a primary cause of lipid oxidation³³. Mitochondrial function is also affected by oxidation of thiol rich proteins, such as mitochondrial creatine kinase and respiratory chain complexes^{37, 38}, and Mori et al demonstrated that the ETC is disrupted by MeHg in cerebellar mitochondria³². The oxidative shift caused by MeHg also affects DNA. A study of Chinese mine workers and local residents chronically exposed to various forms of MeHg from Hg mining plants revealed increased urinary 8-oxo-2'-deoxyguanosine (8-OHdG) levels, a metabolite of oxidized DNA, as well as increased serum markers of oxidation in exposed individuals compared to age and gender matched control individuals³⁹.

Neurotransmitters

In addition to increasing oxidative stress, MeHg directly inhibits proteins necessary for calcium homeostasis, glutamate transport and GABA signaling, processes that are both exacerbated by and contribute to oxidative stress. Astrocytes have a central role in maintaining the synapse through regulation of neurotransmitter metabolism and reuptake and supporting neuronal metabolism through the glutamate/glutamine cycle⁴⁰. MeHg preferentially accumulates in astrocytes and increases extracellular glutamate levels by reducing glutamate uptake and increasing glutamate release in astrocytes⁴¹. In cerebellar slices, Manfroi et al. demonstrated a nearly 50% reduction in glutamate uptake in slices from mice exposed to MeHg through milk compared to unexposed controls²⁸. This increase in glutamate results in excitotoxicity, which can be blocked in part by blocking N-methyl-D-aspartate (NMDA) receptors⁴². Mercuric chloride (HgCl₂), which accumulates in the brain following MeHg exposure, decreases glutamine synthetase activity and inhibits glutamine transport in cultured astrocytes^{43, 44}.

Along with glutamate stimulated Ca²⁺ entry through NMDA receptors, MeHg causes release of inositol trisphosphate (IP₃) sensitive endoplasmic reticulum Ca²⁺ stores through direct

inhibition of the thiol rich endoplasmic Ca^{2+} -ATPase^{45, 46}. Disrupted Ca^{2+} homeostasis contributes to toxicity by increasing neurotransmitter release, altering mitochondrial membrane potential, and promoting Ca^{2+} -mediated intracellular signaling^{45, 46}.

In contrast to increased excitatory glutamate signaling, MeHg decreases GABA signaling, the most abundant inhibitory neurotransmitter. In primary cerebellar granule cells, MeHg interacts with thiol residues on GABA_A receptors, which modifies receptor conformation⁴⁷. Chronic low-level MeHg exposure in captive minks reduces the activity of the synthetic enzyme glutamic acid decarboxylase, and significantly decreases of GABA_A receptors and transport activity in the brain stem and basal ganglia⁴⁸. Glutamate and GABA signaling are crucial for establishing equilibrium between excitation and inhibition. Shifts in this equilibrium are associated with regulation of normative critical periods in neurodevelopment⁴⁹ as well as with neurologic disorders such as epilepsy⁵⁰. In combination, these direct effects of MeHg on the regulation of neuronal signaling likely contribute to MeHg-induced neurotoxicity.

Intracellular Signaling

MeHg affects the regulation of several signaling pathways including phospholipase C (PLC), calcium signaling and phosphatidylinositol 3-kinases/protein kinase (PI3K/Akt). MeHg in MDCK cells activates PLC, and PI3K/Akt, blocking PLC activation reduced toxicity⁵¹. PLC activation, in addition to the effects described above, increases intracellular Ca^{2+} . Chang et al demonstrated that a PLC-mediated Ca^{2+} increase mediates interleukin-6 (IL-6) release in cerebellar granule cells exposed to MeHg, which triggers the inflammatory response⁵². This is consistent with upregulation of inflammatory gene expression observed in a microarray from cerebellum of young adult mice injected with MeHg for seven days⁵³.

In pancreatic β -cells, MeHg increases PI3K/Akt activity⁵⁴, in part due to increased ROS production and in part through inactivation of, a thiol-rich PI3K inhibitor⁵⁵. Low concentrations of MeHg trigger ROS production and increase PI3K activity and its downstream effector, phospho-Akt⁵⁶, pharmacological inhibition of PI3K attenuates these changes in astrocytes¹⁹. PI3K is activated by a G-protein-coupled receptor or receptor tyrosine kinase, such as the insulin receptor⁵⁷. Once activated, PI3K phosphorylates phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P₂] to form phosphatidylinositol (3,4,5)-triphosphate [PtdIns(3,4,5)P₃]. Akt functions downstream of PI3K and is activated by phosphorylation⁵⁷. Akt activation has many consequences including regulation of the protective transcription factors forkhead box protein O (FOXO) and nuclear factor (erythroid-derived 2)-like 2 (Nrf2) and additional signaling cascades, regulating cellular growth and morphology. These observations are consistent with MeHg's widespread damage in fetal and neonatal brain, characterized by hypoplastic and symmetrical brain atrophy, and reflective of aberrant cell division, migration, differentiation and synaptogenesis^{2, 58-60}.

Gene Expression

Alterations in the redox status of a cell can activate several pathways. Nrf2 is a basic leucine zipper transcription factor that under unstressed conditions resides in the cytoplasm bound by the Keap1 protein, an adapter protein for Cullin 3-dependent ubiquitination and

proteasomal degradation⁶¹. However, during oxidative stress, such as exposure to MeHg, Nrf2 is released from kelch-like ECH-associated protein1 (Keap1) by disruption of cysteine residues on Keap1 or through oxidant-dependent kinase signaling, and translocates into the nucleus, where it can bind to antioxidant response element (ARE) promoters as a heterodimer with Maf proteins⁶¹. In addition to redox regulation, a crosstalk exists between the protein kinase pathways and the Nrf2-dependent antioxidant system⁶². Nrf2 interacts with p38 mitogen-activated protein kinase^{63, 64}, ER-resident kinase PERK⁶⁵ and a Src family tyrosine kinase, Fyn⁶⁶. The PI3K/Akt pathway controls Nrf2's function upstream.

Nrf2 induces the expression of several cytoprotective proteins including NAD(P)H quinine oxidoreductase (NQO1), glutamate-cysteine ligase catalytic subunit and modifier subunit (GCLC and GCLM), and heme oxygenase-1 (HO-1)⁶¹. MeHg has been shown to activate Nrf2 in both cell lines and primary cells, allowing for an up-regulation of NQO1 and HO-1¹⁹. In addition to oxidative stress derived from MeHg exposure, Toyama et al have shown MeHg's ability to bind to recombinant Keap1 and to activate Nrf2⁶⁷. Nrf2 has been shown to be protective in a *Caenorhabditis elegans* model of dopaminergic neurodegeneration induced by MeHg, where reduction of SKN-1, the Nrf2 homologue, increases the vulnerability for neurodegeneration⁶⁸. Activation of Nrf2 by MeHg is a protective response; however MeHg often alters gene expression resulting in neurotoxic effects.

Recently, several toxicogenomics studies have been performed to characterize the changes in gene expression upon MeHg exposure^{53, 69, 70}. In the cerebellum of eight-week-old mice exposed to 10 mg/kg/day for 7 days, several genes involved in inflammation (such as the chemokines, CCL2 and CCL4), metabolism, anti-apoptosis (Bcl2a1b) and signal transduction were increased above control, while genes involved in cell proliferation, oxidative stress (selenoprotein H) and apoptosis (Ifi27) were decreased⁵³. Increased oxidative stress can cause inflammation through the activation of the nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) transcription factor, and inflammation contributes to several neurologic diseases, such as Alzheimer's disease and Parkinson's disease⁷¹. MeHg has been shown to increase IL-6 release from glial cells⁷² and activation of NF- κ B has been observed in rat cerebrum and cerebellum⁷³. Similarly alterations in metabolism, cell signaling and apoptosis can affect neuronal health.

Changes in gene expression can be more severe if the exposure occurs during neurodevelopment. The adult brain has a complex structure formed from several cell types and numerous connections. Disruption of normal neurodevelopment or death of cells caused by exposure to a xenobiotic can have consequences on the functioning of the adult brain. There are several reports of genome wide analyses of genes that are up-regulated and down-regulated in adults or in various stages of development. In a comparative study, pooling transcriptomics data sets from studies involving mouse embryos exposed *in utero* to MeHg, young mice exposed to MeHg *in utero* and postnatally, adult rats chronically exposed to MeHg, whole embryo cultures exposed to MeHg, embryonic stem cells induced into cardiomyocytes or neurons and mouse embryonic fibroblasts exposed to MeHg, Robinson et al. found that although different models were used, as well as experimental variables of dose and time of exposure, there is a set of genes that are altered by MeHg that effect

development of brain as well as heart and kidney⁶⁹. Using neural cells differentiated from murine embryonic stem cells exposed to 25 nM MeHg for 8 consecutive days, Theunissen et al. found MeHg to increase late differentiation gene and neuroectodermal gene sets, and to decrease early differentiation gene, mesodermal and endodermal derived tissue gene sets, resulting in cultures with retarded development and that deviated from their normal differentiation track⁷⁰. Large studies like these are important in identifying pathways effected by MeHg exposure although exact mechanisms of how an individual gene is up or down regulated may not be clear.

Epigenetic Modifications

Changes in gene expression, whether up regulated or down regulated, can occur not just through inhibiting or activating transcription factors, but also through epigenetic changes. Alterations in gene expression from epigenetics do not occur through changes in DNA sequence but from post-translational modifications of histone proteins that package DNA or through methylation of the DNA. Orishchenko et al investigated the changes in expression of brain-derived neurotrophic factor (BDNF), a gene regulated by epigenetic modifications and associated with long-lasting depression-like behavior in mice. Mice exposed to 0.5 mg MeHg /kg/day via drinking water from gestational day 7 to post natal day 7 show depressed behavior through measures of immobility in forced swim tests⁷⁴. MeHg decreased BDNF expression in the dentate gyrus of the hippocampus⁷⁴.

The BDNF promoter in hippocampus of mice exposed to MeHg showed decreased histone H3 acetylation on K9 and K14, a marker of active chromatin state, and increased trimethylation on histone H3 K27, a marker of repressed gene expression^{74, 75}. Additionally DNA methylation was enriched in four separate CpG sites in the BDNF promoter after exposure to MeHg⁷⁴. DNA that is hypomethylated correlates with increased DNA binding to transcription factors whereas hypermethylation is associated with less transcription factor binding⁷⁶. All of these markers cumulatively suggest that the reduction in BDNF levels in the hippocampus after MeHg exposure is a result of epigenetic modifications, which suggest that MeHg may affect other genes through alteration of epigenetic markers. The mechanisms of how MeHg increases DNA and histone methylation and decreases histone acetylation are not known.

Modifiers of Toxicity

Genetic Polymorphisms

The susceptibility of an individual to a toxic chemical is determined not solely by the toxicant itself but also by variations in alleles and inherited defects. Recent studies suggest that the body's response to Hg may be mediated by polymorphisms in several genes responsible for absorption, distribution, metabolism and excretion.

A study in Austria by Gundacker and colleagues (2009), examined in medical students the potential associations between Hg exposure and the glutathione s-transferases (GSTs), GST theta 1, GSTT1; GST mu 1, GSTM1; GSTA1, GST pi 1, GSTP1; glutamate-cysteine ligase catalytic subunit, GCLC; and metallothioneine (MT) polymorphisms. The levels of Hg

exposure of students were low as determined by analysis of blood, urine, and hair. GSTP1-114 and MT4 allele carriers as well as GSTT1–/– and GSTM1–/– jointly deleted phenotypes were associated with higher hair mercury levels than homozygous wildtypes. GSTP1-114/GSTT1 and GSTP1-105/GCLC combinations showed synergistic effects on hair Hg levels compared to single-gene variants and the authors suggest that the GSTP1 variants are more important in mercury toxicokinetics than the other GST polymorphisms⁷⁷.

In a study of Michigan dental professionals, Goodrich and colleagues (2011) evaluated polymorphisms in key glutathione synthesizing enzymes, glutathione s-transferase, and selenoprotein genes underlying inter-individual differences in Hg body burden. They measured urine and hair Hg levels. Five polymorphisms were significantly associated with urine Hg levels (GSTT1 deletion), hair Hg levels (GSTP1-105, GSTP1-114, GSS 5'), or both (SEPP1 3'UTR). The authors suggest that polymorphisms in these genes may influence elimination of Hg in the urine and hair or retention following exposures to elemental Hg through dental amalgams and MeHg through fish consumption⁷⁸. Similar data was presented by Schlawicke Engstrom and colleagues, demonstrating that carriers of GSTP1-105G and GSTP1-114T accumulated less Hg in erythrocytes after controlling for levels of polyunsaturated fatty acids⁷⁹. Custodio and colleagues reported that the T allele of the GSTP1-114 polymorphism was associated with increased blood mercury levels⁸⁰.

Autism is a neurodevelopmental disorder with genetic and environmental components, and genetic susceptibility to high Hg has been suggested as a potential risk factor. Owens and colleagues tested the hypothesis that Hg could be implicated in the etiology of autism if genetic susceptibility altered Hg's metabolism or intracellular compartmentalization. Genetic sequences of four genes implicated in the transport [LAT1 and divalent metal transporter1 (DMT1)] and response to Hg [metal regulatory transcription factor1 (MTF1) and metallothionein1a (MT1a)] were screened for variation and association with autism. The group identified and characterized 74 variants in MT1a, DMT1, LAT1 and MTF1. Polymorphisms identified were evaluated for differences in allele frequencies. They reported no association of any variant evaluated with autism suggesting that variations in these genes may not play a significant role in the etiology of autism⁸¹. Another group using the *C. elegans* model system to study synapse formation and function as related to autism showed that neuroligin mutants (postsynaptic cell adhesion proteins that bind specifically to presynaptic membrane proteins-neurexins) are hypersensitive to Hg compounds and are defective in sensory behaviors and processing. They suggest that the behavioral deficits are similar to traits frequently associated with autism spectrum disorders and report a possible link between genetic defects in neuroligin, and sensitivity to Hg in the development of autism⁸².

Hg exposure is associated with cardiovascular problems. A common polymorphism of matrix metalloproteinase (MMP)-2 gene is the C(-1306)T which disrupts a promoter site and is associated with lower promoter activity when the T allele is present affecting the expression and activity of the enzyme. A study by Jacob-Ferreira and colleagues (2011) examined how this polymorphism affects the circulating MMP-2 levels and tissue inhibitor of metalloproteinase-2 (TIMP-2) following environmental exposure to Hg. They reported a positive association between plasma Hg concentrations and the ratio of MMP-2/TIMP-2.

The C(-1306)T polymorphism modified MMP-2 concentrations and MMP-2/TIMP-2 ratio in subjects exposed to Hg, with higher MMP-2 levels found in subjects carrying the C allele. Their findings suggest a significant interaction between the C(-1306)T polymorphism and Hg exposure, with possible ramifications for those carrying the C allele⁸³.

Another study examined the role polymorphisms of the endothelial nitric oxide (eNOS) gene (T-786C and Glu298Asp) on nitrite concentrations following Hg exposure in humans. The hypothesis tested was that Hg exposure might decrease circulating nitrite concentrations and these polymorphisms might enhance the effects of Hg resulting in increased risk of cardiovascular disease. Polymorphisms did not seem to influence the decreased nitric oxide (NO) production as it was found to be predominantly due to Hg, age and gender. Their findings suggest that there is not an association between increased risk for cardiovascular disease in MeHg-exposed subjects and eNOS gene polymorphisms (T-786C and Glu298Asp)⁸⁴. The same group investigated the contribution of the 27-nt tandem repeat of intron 4 of the eNOS gene to NO production, which could enhance susceptibility to cardiovascular disease in the MeHg-exposed study population. They found no significant differences in age, arterial blood pressure, body mass index or cardiac frequency between genotype groups, but observed different nitrite concentrations, with lower nitrite levels for the 4a4a genotype carriers. Age, gender and the presence of intron 4 polymorphism contributed to nitrite reduction as a result of blood Hg concentration. Their results suggest increased susceptibility to cardiovascular diseases after MeHg exposure in carriers of the 27nt repeat polymorphism of intron 4 in the eNOS gene⁸⁵.

In a Swedish study, Engström and colleagues (2011) studied whether genetic polymorphisms in glutathione-related genes modify the association between the PUFAs eicosapentaenoic and docosahexaenoic acid (DHA) or MeHg and risk of first ever myocardial infarction. Polymorphisms in glutathione-synthesizing (glutamyl-cysteine ligase catalytic subunit, GCLC and glutamyl-cysteine ligase modifier subunit, GCLM) or glutathione-conjugating (glutathione S-transferase P, GSTP1) genes were assessed. The authors evaluated the impact of these polymorphisms on the association between erythrocyte-Hg and risk of myocardial infarction, as well as between plasma eicosapentaenoic and DHA and risk of myocardial infarction. They report no statistically significant genetic modifying effects for the association between plasma eicosapentaenoic and DHA or erythrocyte-Hg and risk of myocardial infarction but do report a relatively rare GCLM-588 TT genotype that may have an impact⁸⁶.

An association between the Hg resistance merA gene and antibiotic resistance has been described in the literature and has been shown to be widely distributed among bacteria (both *Escherichia coli* and *Staphylococcus aureus*) isolated from healthy adults and children⁸⁷⁻⁹². To study this further, Skurnik and colleagues (2010) analyzed Hg resistance in collections of strains from two populations with different levels of Hg exposure and various levels of antibiotic resistance. Hg-resistant *E. coli* was found significantly more frequently in the population that had the highest antibiotic-resistant *E. coli* and antibiotic resistance was higher in the population living in an environment with a high exposure to Hg⁹³.

Developmental Vulnerability

The increased vulnerability of the developing nervous system to MeHg is not disputed. Developmental exposure to MeHg, even at very low levels, results in more severe and widespread damage than exposure in adults. Development is characterized by rapid progression of developmental processes within defined critical periods including neural tube closure, cellular proliferation and differentiation, and synaptic development. MeHg can alter many of these events, Robinson et al observed delayed neural tube closure in embryos of two different mouse lines exposed to MeHg⁹⁴. In zebrafish embryos, MeHg delayed hatching and reduced cellular proliferation in the neural tube⁹⁵. In neuronally differentiating mouse embryonic stem cell culture, expression of markers of neuronal differentiation, neurotransmitter receptors and transporters as well as growth factors was reduced by MeHg⁹⁶.

Optimal neurodevelopment requires large amounts of energy, and as a result mitochondrial activity and subsequent ROS production is higher in the developing nervous system than in the adult nervous system⁹⁷. Accordingly, in mice the GSH system develops rapidly, with significant increases in GSH, glutathione reductase (GR) and GPx occurring in the first three postnatal weeks⁹⁸. Prenatal MeHg exposure not only prevents these increases in antioxidant capacity, but also continues to suppress the system at postnatal day 21, when MeHg levels were no longer elevated, GSH, GR and GPx remained decreased relative to controls⁹⁸. This suggests that the developing nervous system is vulnerable to the acute pro-oxidative effects of MeHg, effects that may affect the antioxidant response throughout life.

In addition to promoting plasticity and survival throughout life, growth factors such as BDNF and neuronal growth factor influence cell migration, differentiation and survival, axonal and dendrite structure and targeting, and synapse formation during development⁹⁹. Most neurotrophic factors signal through intracellular cascades susceptible to the oxidative effects of MeHg as described above. Together these early changes not only cause immediate developmental abnormalities such as observed in congenital Minamata disease, but may also contribute to latent effects. For example, neurological deficits have emerged months and years following MeHg exposure in Minamata as well as in other instances of human exposure and experimental primate studies^{100, 101}.

Nutrition and Additional Toxicants

In some instances, variation and manipulation of external factors can enhance or attenuate the toxicity of MeHg. For instance the benefits of nutrients found in fish such as Se, and PUFAs including omega-3 fatty acids like DHA may counterbalance some of the toxic effects of MeHg. Inclusion of PUFA levels from maternal blood samples collected during gestation and shortly after birth in the analysis of offspring at 9 and 30 months of age revealed beneficial effects of PUFAs on developmental outcomes, particularly at 9 months of age¹⁰².

Experimental studies have shown that Se supplementation attenuates growth and motor deficits in rats chronically exposed to MeHg following weaning, the severity of deficits was not directly related to MeHg levels in the brain but rather by the Hg:Se ratio¹⁰³ In 15-day-

old mice born to dams fed MeHg with or without Se supplementation through gestation and lactation, combined Se and MeHg regulated expression of 63 genes in whole genome cerebral microarray compared to 8 and 5 genes affected by Se or MeHg alone, respectively¹⁰⁴. This is consistent with a functional Se deficiency resulting from MeHg inhibition of selenoproteins, effects that can be lessened by Se supplementation¹⁰⁵. In a similar study, Jayashankar and colleagues exposed mice to MeHg and/or DHA through maternal diet. DHA decreased accumulation of MeHg in the brain and eliminated reflexive deficits observed in non-DHA supplemented MeHg pups, as well altering gene expression¹⁰⁶.

On the other hand, the contribution of concomitant exposure to additional environmental toxins such as arsenic, pesticides, and polychlorinated biphenyls (PCB) to MeHg toxicity has also been investigated. There is limited support for interactions between MeHg and these additional toxins; however many studies have failed to demonstrate consistent interactive toxicity and more research is needed¹⁰⁷.

Summary

MeHg-induced neurotoxicity is mediated at the cellular level by a complex interplay between direct and indirect consequences of the pro-oxidative nature of MeHg, including oxidative stress, disrupted neuronal signaling, intracellular signaling, and gene expression. However, these effectors alone do not provide a satisfactory understanding of MeHg toxicity. The contribution of gene-environment interactions, such as polymorphisms, developmental stage, and additional nutritional and chemical factors, are significant and may allow the development of more effective treatments to proactively attenuate the effects of MeHg.

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