Role of Intracellular Defense Factors against Methylmercury Toxicity

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Methylmercury (MeHg) is a causative agent of Minamata disease and an environmental pollutant that comprises a large portion of organically occurring mercury. Many aspects of the biological defense mechanisms against MeHg toxicity remain unclear. Recently, nuclear factor-E2-related factor 2 (Nrf2), heat shock factor protein 1 (Hsf1), and hydrogen sulde were identified as intracellular defense factors against MeHg toxicity. These findings suggest that novel biological defense mechanisms against MeHg toxicity exist in the living organism. In addition, the expression of downstream genes that mediate activation of the transcription factors Nrf2 and Hsf1 was markedly induced by MeHg treatment, suggesting that this action is involved in the reduction of MeHg toxicity. On the other hand, when the gaseous form of hydrogen sulde (H2S) binds directly to MeHg, bismethylmercury sulde (MeHg-S-HgMe) as a low toxicity metabolite is formed. This suggests the involvement of the gaseous form of H2S in the reduction of MeHg toxicity. In this topic, we summarize the roles of factors involved in novel biological defense mechanisms against MeHg toxicity.

Key words methylmercury; nuclear factor-E2-related factor 2; heat shock factor protein 1; hydrogen sulde

1. INTRODUCTION

Methylmercury (MeHg) is well known for causing Minamata disease. MeHg affects various internal organs; however, the nervous system is a major target organ for MeHg toxicity in many animal species. Developing embryos and fetuses are also highly susceptible to MeHg because MeHg can pass not only through the blood–brain barrier but also through the placenta. MeHg in the ocean becomes concentrated in the tissues of fish and shellfish through the aquatic food chain. In recent years, the ingestion of comparatively high amounts of seafood by pregnant women has drawn attention to the damage that can be caused to the brain of an unborn child.

Oxidative stress has been known to contribute to MeHg-induced central nervous system (CNS) damage. Many studies have reported that the activation of antioxidant enzyme system plays a significant role in the reduction of MeHg toxicity. We have elucidated that the ubiquitin–proteasome system, a selective protein degradation system, also functions as a biological defense mechanism against MeHg-induced cytotoxicity. In addition to the abovementioned biological defense mechanisms, other mechanisms against MeHg toxicity are thought to exist within the living organism. Thus, it is very important to elucidate the molecular mechanisms underlying these additional defense systems to evaluate MeHg toxicity in humans.

Intracellular factors that contribute in reducing MeHg toxicity [i.e., transcription factors such as nuclear factor-E2-related factor 2 (Nrf2) and heat shock factor protein 1 (Hsf1) as well as in vivo metabolites such as hydrogen sulde (H2S)] have been identified. This proved the existence of novel biological defense mechanisms against MeHg toxicity. Here we review the roles of the factors underlying the biological defense mechanisms against MeHg toxicity.

2. NRF2

The living organism rapidly senses the various stress and prepares a protection system from such stress. The induced expression of stress response factors regulated by various transcription factors has an important function in such a system.

Recently, Toyama et al. reported that neuroblastoma cells with high expression levels of Nrf2, an important transcription factor in xenobiotic metabolism and in oxidative stress responses, showed MeHg resistance, whereas the cells with suppressed Nrf2 expression by RNA interference were highly sensitive to MeHg. Since the revelation of these findings, Nrf2 has been found to contribute to the reduction of MeHg toxicity in astrocytes and microglial cells. Recent research has also shown that administration of the Nrf2 activator sulforaphane to mice suppressed MeHg neurotoxicity. Therefore, from cultured cells to mice, Nrf2 is thought to function as a protective factor against MeHg toxicity.

Nrf2 is a basic leucine zipper transcription factor involved in the induction of expression of genes encoding many cytoprotective proteins, namely antioxidant enzymes such as heme oxygenase-1 (HO-1), phase II enzymes of drug metabolism such as reduced nicotinamide adenine dinucleotide phosphate (NAD(P)H) dehydrogenase, quinone 1 (NQO1), and phase III excretion transporter proteins involved in drug metabolism, including multidrug resistance-associated proteins (MRPs).

It has been known that during MeHg exposure, the expression of abovementioned downstream genes is induced by the increase of the levels of Nrf2 in the nucleus. Furthermore,
a decline in mercury accumulation mediated by the induced expression of MRP genes has been found in elevated Nrf2 expression neuroblastoma cells and in sulfophosphate administered mice. These findings suggest the potential involvement of Nrf2 in the reduction of MeHg toxicity. On the other hand, Nrf2 induces the expression of genes encoding the antioxidant enzymes such as HO-1, and this result in the reduction of MeHg induced oxidative stress. In any case, because of the ability to induce the expression of various downstream genes, Nrf2 can be considered as a protective factor against MeHg toxicity.

In the absence of stress, Nrf2 binds to Kelch-like ECH-associated protein 1 (Keap1), a cysteine-rich protein, and localizes in the cytoplasm. Keap1 binds to the ubiquitin ligase Cullin-3, which is involved in the ubiquitination of substrates. Consequently, Nrf2 is ubiquitinated by Cullin-3 and degraded by proteasomes in the absence of stress. However, electrophiles induce the covalent modification of the cysteine residue within Keap1, which triggers conformational changes. This causes Nrf2 to dissociate from Keap1 and translocate into the nucleus. Keap1 contains 25 cysteine residues and among these, Cys151, Cys273, and Cys288 are considered to be highly reactive thiol groups. Furthermore, the modified cysteine has been found to differ depending on the type of electrophile (i.e., chemical compound). This is most likely due to the differences in the three-dimensional structure and in the size of the various electrophiles.

As mentioned above, Nrf2 activated by MeHg functions as a protective factor against MeHg toxicity. However, the molecular mechanism underlying this activation remains unclear. Recently, Kumagai et al. found that MeHg binds covalently to Cys151 of Keap1, suggesting that this action may contribute to Nrf2 activation (personal communication). This suggests that the direct action of MeHg on Keap1 is also partially involved in the MeHg-mediated activation of Nrf2. As outlined above, Keap1 directly senses whether cells are exposed to MeHg and activates Nrf2; and this action could be perceived to represent the protection of cells from MeHg toxicity.

3. HSF1

We have recently found that the suppressed expression of the transcription factor Hsf1 leads to high susceptibility to MeHg in human cultured cells. Similar to Nrf2, Hsf1 could function as a protective factor involved in the reduction of MeHg toxicity. Hsf1 is responsive to heat shock and oxidative stress, and thus protects cells from such stress by inducing the expression of downstream genes such as heat shock protein (HSP). In the absence of stress, Hsf1 binds to inhibitory HSP (e.g., Hsp90) and exists in the cytoplasm. However, when subjected to stresses such as heat shock, the levels of denatured proteins within the cell increase, the inhibitory HSP dissociates from Hsf1, and the free Hsf1 protein translocates into the nucleus, thereby inducing the expression of HSP genes encoding proteins associated with the reduction of stress.

Our recent findings have also suggested that subcytotoxic MeHg treatment promotes translocation of Hsf1 into the nucleus (Hwang et al., unpublished data). In addition, Toyama et al. reported that MeHg treatment causes an increase in HSP such as HSPA1A and HSPA6 at the mRNA level. Thus, Hsf1 is activated according to MeHg exposure, and therefore, the resultant induced expression of downstream genes is considered to be potentially involved in the reduction of MeHg toxicity.

Aggregations of denatured proteins have been found in several neurodegenerative diseases, including Alzheimer’s disease and polyglutamine disease. These degenerative proteins are considered to cause damage to the nerve system. Interestingly, it has been reported that mouse models of polyglutamine disease subjected to high expression levels of Hsf1 showed a decreased levels of aggregated proteins, thereby lengthening the lifespan of the mice. Considering these findings, we can conclude that MeHg induced cytotoxicity through degenerative proteins and the accompanying protein aggregates. HSP induced by Hsf1, however, refolds the proteins denatured by MeHg exposure; therefore, it can partially reduce toxicity. By further investigating the molecular mechanisms related to the MeHg-mediated activation of Hsf1, we anticipate revealing a novel biological defense mechanism against MeHg toxicity.

4. HYDROGEN SULFIDE

According to recent findings by Yoshida et al., high expression levels of cystathionine β-synthase (CBS), which is associated with H,S production, provides MeHg resistance to neuroblastoma cells. H,S is now the focus of attention as a novel protective factor against MeHg toxicity. H,S is a gaseous compound synthesized within the living organism from cysteines such as l-cysteine as well as from l-homo-cysteine by CBS and cystathionine γ-lyase (CSE). It has also been reported that cysteine aminotransferase (CAT) produces 3-mercaptopropionic acid using cysteine and α-ketoglutaric acid, and that 3-mercaptopropionate sulfurrtransferase (3-MST) produces H,S using 3-mercaptopropyric acid as a substrate. It is clear that H,S produced within the cell reacts with MeHg to produce bismethylmercury sulfide (MeHg-S-HgMe). It has also been demonstrated that neuroblastoma cells and mice treated with MeHg-S-HgMe have a higher survival than neuroblastoma cells and mice treated with MeHg. The pKₐ of H,S is 6.76, and ca. 80% of H,S is readily converted into HS⁻ anion under physiological conditions. H,S produced within the living organism covalently bonds with a single molecule of MeHg to form MeHg sulfide (MeHg-SH, pKₐ=7.5), which then binds to an additional molecule of MeHg to form MeHg-S-HgMe. This reaction suggests that H,S contributes to the reduction of MeHg toxicity by reacting with MeHg to produce the low toxicity metabolite MeHg-S-HgMe.

On the other hand, H,S has also been found to exhibit a reducing action against glutamate induced neurotoxicity. This action is thought to be involved in the increase of glutathione levels within the cell and is mediated by the increased activation of cysteine transporters and γ-glutamylcysteine synthetase (γ-GCS; a rate-limiting enzyme of glutathione synthesis). Biological substances possessing dissociative thiol groups, e.g., glutathione, have an extremely important role in the reduction of MeHg toxicity. Thus, the formation of the bond between MeHg and glutathione is considered to control toxicity. The increase in glutathione levels caused by H,S might be involved in the reduction of MeHg toxicity.

We have also recently shown that the levels of cystathionine, a byproduct of H,S synthesis, increased within the
brains of MeHg administered mice (Hwang et al., unpublished data). This finding suggests that MeHg exposure causes an increase in the levels of H₂S within the living organism. The living organism, may perhaps, reduce MeHg toxicity by activating the H₂S production system as a defense mechanism.

5. CONCLUSION (FIG. 1)

When exposed to MeHg, the living organism is considered to reduce MeHg toxicity by activating stress response transcription factors such as Nrf2 and Hsf1. So far, it has been reported that the levels of HSP70, a factor downstream of Hsf1, is higher in Nrf2-deficient cells than in normal cells. Genes such as those encoding HO-1 also accelerate transcription activation in accordance with both transcription factors. There is a reasonable probability that biological defense mechanisms mediated by Nrf2 and Hsf1 act complimentarily in response to various stress. However, crosstalk of biological defense mechanisms mediated by both transcription factors in relation to various stress, including that of MeHg, has remained unclear. In the future, the mechanisms underlying novel biological defenses can be clarified by a more detailed examination of the roles of both transcription factors that reduce MeHg toxicity.

On the other hand, it is suggested that H₂S, the biomolecule recently identified as being responsible for reducing MeHg toxicity, could also function defensively against nerve damage caused by, for example, hypoxia. H₂S has been studied as a possible in vivo gaseous signal transmitter similar to nitric monoxide. Henceforth, we eagerly anticipate new functions of H₂S not only as a gaseous mediator but also as a factor involved in biological defense mechanisms against MeHg toxicity.

REFERENCES


