Mechanisms of Transport and Toxicity of Methylmercury in Placental Syncytiotrophoblasts

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Abstract:

Methylmercury (MeHg) is a prevalent environmental toxicant that is present in biological systems as a conjugate of thiol-containing molecules, such as cysteine (Cys). MeHg-Cys is a transportable form of methylmercury that can cross the placenta and cause developmental delays and defects in the fetus. However, the mechanisms by which MeHg crosses the placenta are not well characterized. The purpose of the current project was to characterize the mechanisms by which methylmercury is taken up into placental syncytiotrophoblasts and evaluate the toxicity of MeHg on these cells. BeWo cells, a placental synctiotrophoblast line, were exposed to MeHg-Cys under various conditions and the transport of MeHg-Cys was characterized. Moreover, the toxicity of MeHg-Cys was assessed using the following biochemical assays: TBARS to measure lipid peroxidation, flow cytometry to measure mitochondrial membrane potential and cell viability, Ellman's assay to measure thiol content within the cell, and autophagy to measure the amount of autophagosomes present. Our findings from the transport studies also show that there are sodium-independent and sodium-dependent transporters involved in the uptake of methylmercury-cysteine into BeWo cells. Potential transport proteins is System L, which is a sodium- independent mechanism. Exposure to MeHg-Cys was found to be toxic to placental syncytiotrophoblasts. We found that lipid peroxidation and expression of superoxide dismutase increased, and mitochondrial membrane potential decreased, indicating a decrease in viability. The results of this study provide important insight into the mechanisms of MeHg-Cys transport and toxicity in placental syncytiotrophoblasts.

Introduction:

Mercury (Hg) is a naturally occurring heavy metal. Methylmercury (MeHg) is one of the most dangerous forms of Hg. MeHg readily crosses the placenta and accumulates in tissues. BeWO cells, placental syncytiotrophoblasts, have an apical and basolateral surface, which allows them to mediate the movement of nutrients between maternal and fetal blood. Since there are a large number of amino acid transporters, it is likely that various transport systems are involved in the uptake of MeHg-Cys. Thus, this study investigates the transport systems involved in the apical uptake of MeHg-Cys into the cells. Additionally, MeHg is a serious toxicant that affects enzymes, cell membrane function, and neuronal health. While this explains the effect of MeHg on placental cells, it is also important to identify the mechanisms by which MeHg causes cellular injury and dysfunction in placental syncytiotrophoblasts.

Methods and Analysis:

The uptake of Cystine, MeHg-Cys and Methionine measured radioactivity through liquid scintillation spectrometry. Substrate specificity studies were conducted to determine what compounds block the uptake of MeHg-Cys, thus signaling which transporters are involved. The effects of MeHg-Cys on cell viability were measured by MTT and FACS assays. Additional cell-stress was measured by an Autophagy assay, ATP assay, TBARS, and Quantitative PCR.

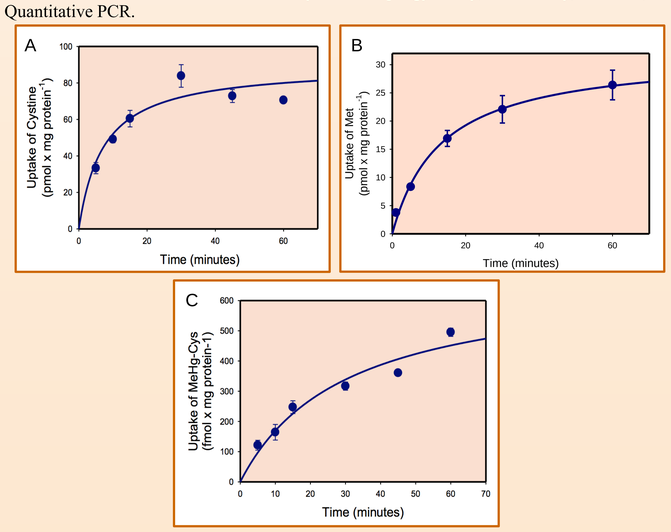

Figure 1: Time-Course Studies

A: Uptake of Cystine is linear up to 15 minutes
B: Sodium-independent uptake of [^{3}H]-methionine began to plateau after 30 minutes.
These data suggest that our cell model is suitable for our studies because it reflects published data.
C: The uptake of MeHg-Cys is much faster compared to the amino acids in 1A and 1B. Since the uptake of MeHg-Cys is linear up to 15 min, we chose 15 min as an incubation time for the remaining experiments.

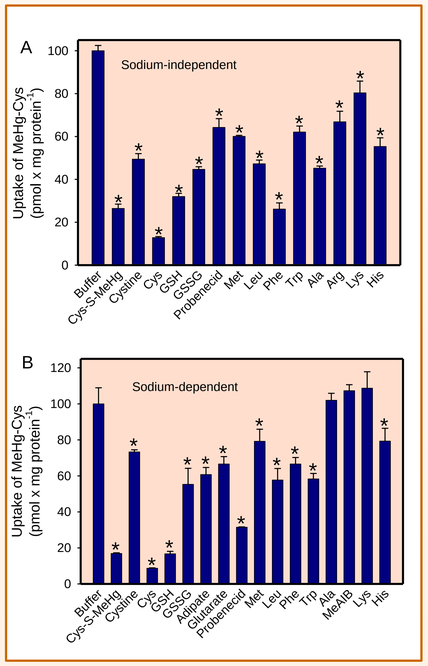

Figure 2: Substrate specificity of MeHg-Cys uptake was carried out under sodium-independent (2A) and Sodium- dependent (2B) conditions.

Na^+-independent carriers appear to play a greater role in the uptake of MeHg than Na^+-dependent carriers. The uptake of MeHg-Cys was partially inhibited by the presence of unlabeled neutral amino acids (Cys, Met, Leu, Phe, Trp, Ala) and acidic amino acids (Arg, Lys, His). This indicates that Lat1, LAT2, Asc-2, y^+LAT1, and System x_c^-, could all be transporters involved in the uptake of MeHg-Cys. Probenecid, GSH and GSSG also inhibited the uptake of MeHg-Cys suggesting that OAT-PE could also be involved.

Under sodium-dependent conditions, Ala and MeAIB did not inhibit uptake of MeHg-Cys, indicating that system A does not play a role in the uptake of MeHg-Cys.

*, significantly different ($p<0.05$) than cells treated with buffer.

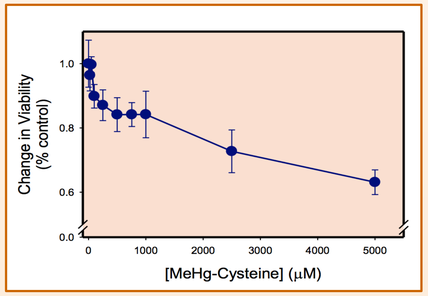

Figure 3: Studies of cell viability via the MTT assay. This assay measures mitochondrial activity and cell energetics. The results demonstrate a decline in mitochondrial viability, which indicates a decline in cell viability.

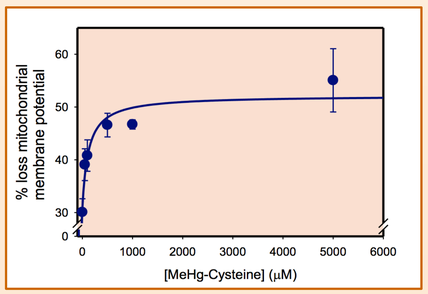

Figure 4: Loss of mitochondrial membrane potential. This is another measure of mitochondrial viability. We used fluorescence activated cell sorting (FACS) to measure DiOC6, which is a measure of mitochondrial membrane potential.

The loss in mitochondrial membrane potential increases quickly, indicating that very low doses of MeHg-Cys can decrease cell viability.

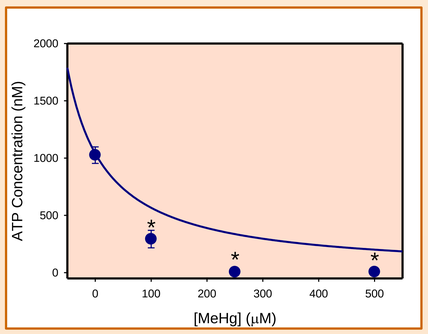

Figure 5: As the concentration of MeHg increases, the concentration of ATP decreases, indicating that exposure to MeHg reduces the production of ATP.

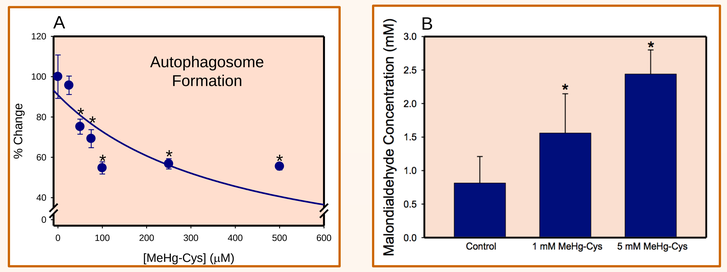

Figure 6: Studies describing the toxicity of MeHg-Cys on BeWo cells
A: Autophagy study: Exposure to MeHg-Cys reduces the number of autophagosomes formed. Fewer autophagosomes leads to excess cell debris in the cytoplasm. This debris can lead to oxidative stress.
B: Malondialdehyde (MDA) is an indicator of lipid peroxidation, which is a sign of oxidative stress and cell injury. MDA concentration increases when cells are treated with MeHg-Cys indicating that MeHg leads to oxidative stress.

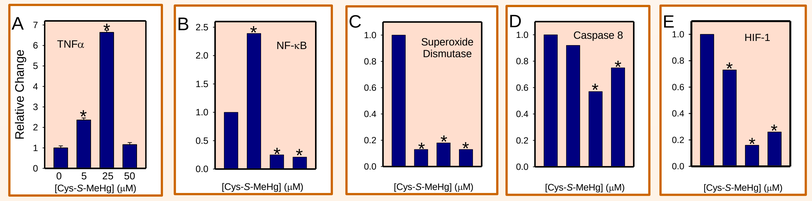

Figure 7: Quantitative PCR. The expression of mRNA encoding tumor necrosis factor alpha (TNFα; 7A), nuclear factor kappa B (NF-κB; 7B), superoxide dismutase 1 (SOD1; 7C), caspase 8 (7D), and hypoxia inducible factor 1 (HIF-1; 7E) was measured in BeWo cells treated with buffer or various concentrations of MeHg-Cys for 16 h.

In 7A, expression of TNFα increased after cells were exposed to 5 and 25 μM MeHg-Cys, while expression following exposure to 50 μM was similar to buffer. In 8B expression of NF-κB increased after exposure to 5 μM and decreased after 25 and 50 μM. This demonstrates an initial inflammatory response followed by a damaged response mechanism that can no longer function. In C, D, and E, the expression of SOD1, Caspase 8, and HIF-1, respectively, decreased after exposure to MeHg-Cys, suggesting that the cell response mechanism is damaged and that oxidative stress is occurring.

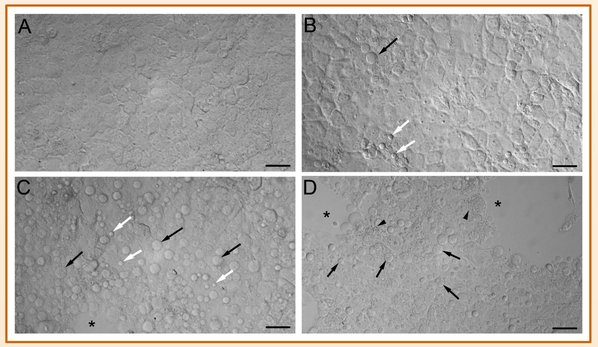

Figure 8: Images of BeWo cell injury when exposed to varying concentrations of MeHg-Cys for 30 min
A: Control cells showing no signs of cellular injury.
B: Cells treated with 100 μM MeHg-Cys. Cytoplasmic blebs are visible (white arrows) and few cells were rounded up and detached from the monolayer (black arrows).
C: Cells treated with 250 μM MeHg-Cys. More cytoplasmic blebs and detached cells were present.
D: Cells treated with 500 μM MeHg-Cys. In addition to the cytoplasmic blebs and detached cells, there are now many areas with missing cells (*) and dark intracellular structures, which may be lysosomes (arrowheads).

Conclusions:

In summary, the current data show that MeHg-Cys is taken up into syncytiotrophoblasts using Na^+-dependent and Na^+-independent mechanisms such as OATPE, LAT1, LAT2, y^+LAT1, asc2, and $b^{0,+}$AT. Once inside the cell, MeHg-Cys is directly toxic to syncytiotrophoblasts. Hg leads to oxidative stress, inflammation, mitochondrial dysfunction, and eventual cell death

Acknowledgements:

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