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Ethylmercury-Induced Oxidative and Endoplasmic Reticulum Stress-Mediated Autophagic Cell Death: Involvement of Autophagosome–Lysosome Fusion Arrest

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ABSTRACT

Ethylmercury (EtHg) is derived from the degradation of thimerosal, the most widely used organomercury compound. In this study, EtHg-induced toxicity and autophagy in the mouse kidney was observed and then the mechanism of toxicity was explored *in vitro* in HK-2 cells. Low doses of EtHg induced autophagy without causing any histopathological changes in mouse kidneys. However, mice treated with high doses of EtHg exhibited severe focal tubular cell necrosis of the proximal tubules with autophagy. EtHg dose-dependently increased the production of reactive oxygen species, reduced the mitochondrial membrane potential, activated the unfolded protein response, and increased cytosolic Ca^{2+} levels in HK-2 cells. Cell death induced by EtHg exposure was caused by autophagy and necrosis. N-acetyl cysteine and 4-phenylbutyric acid attenuated EtHg-induced stress and ameliorated the autophagic response in HK-2 cells. Furthermore, EtHg blocked autophagosome fusion with lysosomes, which was demonstrated via treatment with wortmannin and chloroquine. Low doses of EtHg and rapamycin, which resulted in minimal cytotoxicity, increased the levels of the autophagic SNARE complex STX17 (syntaxin 17)-VAMP8-SNAP29 without altering mRNA levels, but high dose of EtHg was cytotoxic. Inhibition of autophagic flux by chloroquin increased autophagosome formation and necrotic cell death in HK-2 cells. Collectively, our results show that EtHg induces autophagy via oxidative and ER stress and blockade of autophagic flux. Autophagy might play a dual role in EtHg-induced renal toxicity, being both protective following treatment with low doses of EtHg and detrimental following treatment with high doses.

Key words: ethylmercury; ER stress; mitochondrial dysfunction; autophagy; blocking autophagic flux

Mercury is a serious environmental pollutant worldwide; it is produced by industrial processes. It is unique among the heavy metals found in the environment because it takes several physical and chemical forms: elemental mercury, inorganic mercury, and organic mercury (Zalups, 2000). Although the distribution, toxicity, and metabolism of mercury are highly dependent on its chemical form, the primary toxic targets of inorganic and organic mercury compounds are the kidneys and central nervous system, respectively, in humans and animals (Clarkson and Magos, 2006). Methylmercury (MeHg) and ethylmercury (EtHg) are short-chain alkyl mercurial compounds with similar chemical properties. MeHg is known to be one of the most toxic forms of Hg and the most common form of mercury exposure in fish-eating populations. EtHg is derived from the metabolism of