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Morphologic and functional alterations induced by low doses of mercuric chloride in the kidney OK cell line: ultrastructural evidence for an apoptotic mechanism of damage

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Abstract

Mercury produces acute renal failure in experimental animal models, but the mechanism of tubular injury has not completely been clarified. There is an increased interest in the role of apoptosis in the pathogenesis of renal diseases that result primarily from injury to renal tubular epithelial cells. However, detailed studies of morpho-functional alterations induced by mercuric chloride in kidney cell lines are scarce. This work characterizes these alterations in OK cell cultures. Morphological alterations were profiled using light microscopy, transmission electron microscopy, and confocal microscopy, as well as mitochondrial functional assays in the cells exposed to low concentrations of $HgCl_2$. At concentrations of 1 and 10 μM of $HgCl_2$ there were no morphological or ultrastructural alterations, but the mitochondrial function (MTT assay) and intracellular ATP content was increased, especially at longer incubation times (6 and 9 h). At 15 μM $HgCl_2$, both the mitochondrial activity and the endogenous ATP decreased significantly. At this concentration the OK cells rounded up, had increased number of cytoplasmic vacuoles, and detached from the cell monolayer. At 15 μM $HgCl_2$ ultrastructural changes were characterized by dispersion of the ribosomes, dilatation of the cisterns of the rough endoplasmic reticulum, increase of number of cytoplasmic vacuoles, chromatin condensation, invaginations of the nuclear envelope, presence of cytoplasmic inclusion bodies, and alterations in the size and morphology of mitochondria. At 15 μM $HgCl_2$ apoptotic signs included membrane blebbing, chromatin condensation, mitochondrial alterations, apoptotic bodies, and nuclear envelope rupture. Using confocal microscopy and the mitochondrial specific dye MitoTracker Red, it was possible to establish qualitative changes induced by mercury on the mitochondrial membrane potential after incubation of the cells for 6 and 9 h with 15 μM $HgCl_2$. This effect was not observed at short times (1 and 3 h) with this same concentration,

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neither with 1 and 10 μM $HgCl_2$ in all the studied times. Taken together, these findings indicate that low concentrations of $HgCl_2$ induce apoptosis by inhibiting mitochondrial function, and the OK cell line may be considered a useful tool for the study of programmed cell death involving mercurial species and other heavy metals.

Keywords: Mercuric chloride; Apoptosis; Ultrastructure; Nephrotoxicity; OK cells

1. Introduction

All forms of mercury cause toxic effects in a number of tissues and organs, depending on the chemical form of mercury, the level of exposure, the duration of exposure, and the route of exposure. In occupational and environmental settings, the most common form of mercury encountered is the inorganic mercuric form. The kidneys are the primary target organ where inorganic mercury is taken up, accumulated, and expresses toxicity. In various renal systems, a threshold effect is generally observed, in that no cellular death is observed up to a certain dose. Above that dose, however, cellular death progresses rapidly, and in some systems an all-or-none response is observed. This does not mean that subtoxic doses of mercury do not have biochemical or physiological effects (Zalups, 2000). Concern has recently shifted to the potential hazards to develop renal tubular damage (Satoh, 2000) or adverse effects on the human immune system after chronic exposure to low levels of mercury (Shenker et al., 2000).

In the kidneys, the toxicity of inorganic mercury is related to its accumulation in the epithelial cells from the proximal tubules, and with its binding to intracellular sulfhydryl, carboxyl, and phosphoryl groups (Goyer, 1996). The results of these interactions are enzymatic inactivation, inhibition of protein synthesis (Bohets et al., 1995), inhibition of the cellular multiplication, decrease in the uridine and thymidine uptake, DNA fragmentation, and cellular death (Nakazawa et al., 1975). Inorganic mercury alters the content of intracellular thiols and in this manner, it induces oxidative stress, lipid peroxidation, mitochondrial dysfunction, and changes in the metabolism of heme (Zalups and Lash, 1994). Many research groups have associated morphologic and ultrastructural changes within the kidney when cells are intoxicated with heavy metals (Pfaller et al., 1990; Bizarro et al., 2003). The principal target of mercury toxicity is the *pars recta* (segment S3) from the proximal tubule, particularly the portion at the junction of the cortex and the outer medulla (Magos et al., 1984).

By using different approaches, several groups have demonstrated that both, mercuric chloride and methyl mercury, induced apoptosis in different models of study (Goering et al., 1999; Issa et al., 2003; Kim and Sharma, 2004). The presence of tubular morphologic changes induced by $HgCl_2$ has been documented in vivo (Rumbeiha et al., 2000) and in vitro (Fowler, 1972; Duncan-Achanzar et al., 1996). However, there is no detailed ultrastructural evidence on the apoptotic phenomenon, using proximal tubule epithelial cell lines. On the other hand, the involvement of mitochondria in initiating apoptosis has been demonstrated (Guo et al., 1998; Ethell and Green, 2002). According to Shenker et al. (2000), methyl mercury induces apoptosis in human lymphoid cells via translocation of cytochrome c from the mitochondria toward the cytosol, while mercuric chloride does not induce this event, but rather it diminishes the intracellular levels of gluthatione and this turn-on signals pathways to activate caspases, the inducers of apoptosis. However, Araragi et al. (2003) demonstrated that in human leukemia cells, mercuric chloride is a potent inducer of apoptosis through cytochrome c release. These studies clearly establish that, depending on the model, mercury-induced apoptosis depends on the chemical form of the metal.

There are numerous studies related to the pathogenesis of the nephrotoxicity induced by mercury, but the ultrastructural alterations induced in proximal tubule renal cell lines by low doses of mercury, have not been completely established. In the present study, the morphologic and functional response of OK cells exposed to low concentrations of mercuric chloride were assessed during apoptosis induced by this metal. The OK cell is a continuous cell line derived from the proximal tubule of the American opossum (Koyama et al., 1978). When grown to confluence, these cells express most of the characteristics of proximal tubular epithelia, including cell polarization and transport of several