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Apoptogenic and necrogenic effects of mercuric acetate on the chromatin structure of K562 human erythroleukemia cells

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ABSTRACT

Time lapse video photography was used to follow the movement of individual cells after *in vitro* treatment with Hg(II) acetate. Cellular changes of mercuric ions were characterized by their properties of causing reduced cellular mobility (10–50 μM), and complete lack of cellular movement at higher concentrations (100–1000 μM). Results show that after mercury treatment at subtoxic levels (⩽1 μM): (a) chromatin changes were the earliest signs of cytotoxicity, (b) two major parts in nuclear material of K562 erythroleukemia cells could be distinguished, highly condensed supercoiled and decondensed veil-like chromatin, (c) decondensed chromosomes were rejected as clustered puffs and (d) often the nuclear material was broken down to apoptotic bodies. Nuclear changes caused by Hg(II) acetate in the concentration range between 10 and 50 μM were characterized by apoptosis seen as broken nuclei and apoptotic bodies. High concentration of Hg^{2+} ions (100 μM) initiated necrotic nuclear changes, with enlarged leaky or opened nuclei.

1. Introduction

Mercuric acetate (mercury diacetate) is a highly toxic compound employed as a reagent to generate organic mercurials from unsaturated organic precursors. The toxicity of organomercury compounds is useful in fungicides such as ethylmercury chloride and phenylmercury acetate, and antiseptics. e.g. thiomersal and merbromin. Among the toxic effects of high levels of the mercury burden are damages to the brain, kidney, and lungs (Clifton, 2007). Mercury poisoning (hydrargaria or mercurialism) results in diseases such as acrodynia, Hunter-Russell syndrome or Minamata disease (Davidson et al., 2004). To the contrary low levels of mercury, within WHO industrial guidelines, do not show gross abnormalities, but may exhibit immunological abnormalities. Although, rodent models of mercury-induced toxicity have been characterized by a number of laboratories (Queiroz et al., 1994; Hultman et al., 1994; Queiroz and Dantas, 1997; Dantas and Queiroz, 1997), little is understood regarding the effects of low-level mercury at the cellular or biochemical level. It was found in WEHI-231 cells derived from a B-cell lymphoma cell line that at low, but environmentally relevant exposure levels (0.1 μM) that mercury was not toxic. In the WEHI-231 cells the functional consequences of biochemical events were the upregulation of protein tyrosine kinase activity, increased intracellular calcium and activation of protein kinase C (McCabe et al., 1999), downregulation of DNA synthesis (Boyd and Schrader, 1981; Mizuguchi et al., 1986), followed by the stimulation of apoptotic cell death (Hasbold and Klaus, 1990).

Inorganic mercury ions and organomercury compounds are known to affect membrane transport by binding to the sulfhydryl group of membrane proteins (Atchinson and Hare, 1994; Zalups, 2000) which in turn alters the permeability of inorganic ions (Aschner et al., 1990, 1998; Rothstein and Mack, 1991; Ballatori and Boyer, 1996), osmolytes, and water (Albrecht et al., 1993; Barone et al., 1997; Aschner et al., 1998; Yasui et al., 1999; Hazama et al., 2002). The altered permeability also affects the mitochondrial transport (Weinberg et al., 1982; Chavez and Holguin, 1988; Lund et al., 1993), the cytoskeleton (Stoiber et al., 2004), the cytoplasmic enzymes and the cell volume due to the loss of the volume regulatory function of the cells (Mel and Reed, 1981; Jensen et al., 1993).

Mercury substitution of nucleotides (Hg-dCTP, Hg-UTP) have been incorporated into nascent DNA and RNA to study DNA replication and transcription after mercury substituted nucleic acid was purified by thiol agarose affinity chromatography (Dale et al., 1973; Dale and Ward, 1975; Crouse et al., 1976; Banfalvi and Sarkar, 1983; Banfalvi et al., 1984). The synthesis of mercurated agarose magnetic beads with high capacity for SH groups was applied to the recovery of chromatin restriction fragments by mercury affinity chromatography (Chen-Cleland et al., 1993).

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When mercury-substituted UTP was used to study the transcription of duck reticulocyte chromatin *in vitro* by *Escherichia coli* RNA polymerase (Zasloff and Felsenfeld, 1977a,b) it led to artefacts in the analysis of *in vitro* chromatin transcripts. Mouse liver nuclei were fractionated into condensed heterochromatin and noncondensed euchromatin by differential centrifugation of sonicated nuclei. The partitioning of mercury in euchromatin over heterochromatin was 10:1 indicating that mercury preferentially binds to euchromatin (Bryan et al., 1976). There was a 12- to 15-fold enrichment of mercury in the euchromatin fraction of mice challenged with nonlethal levels of mercury chloride (10^{-3} M) in drinking water leading to the conclusion that mercury can be incorporated into chromatin as a metal–protein complex (Bryan et al., 1974). In rats exposed to $^{203}HgCl_2$ high mercury content was recorded in chromatin preparations. The nonhistone chromatin proteins were mainly responsible for deposition of mercury in the chromatin (Różalski and Wierzbicki, 1979).

As there are no recent data available related to the structural changes of chromatin caused by mercury, the relatively old observations warranted further study. We have used reversible permeabilization to isolate chromatin structures from exponentially growing K562 cells to ascertain the structural alterations in the nuclear material elicited by the *in vitro* treatment of mercury acetate.

2. Materials and methods

2.1. Chemicals and reagents

A stock solution of mercuric acetate (i.e. $Hg(CH_3COO)_2$), obtained from Sigma–Aldrich (Budapest, Hungary) was prepared in

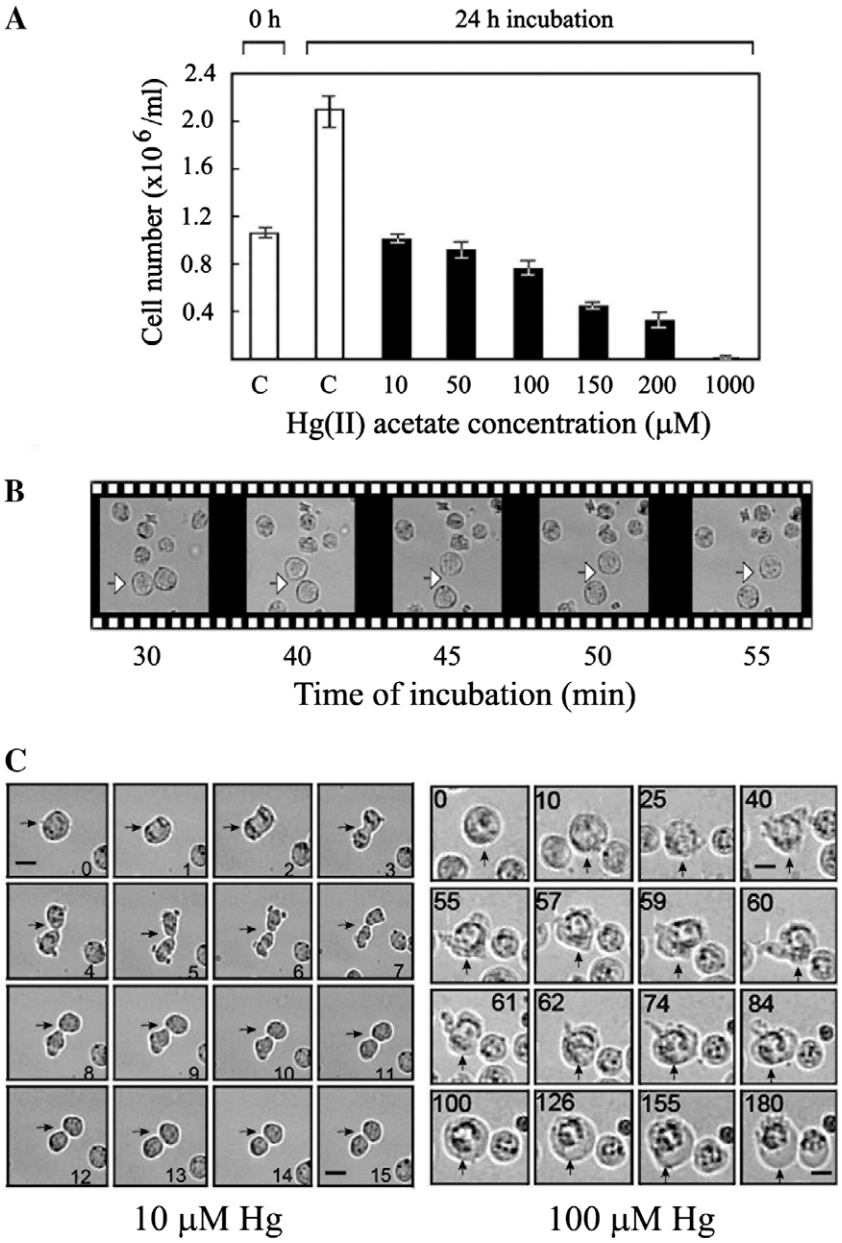

Fig. 1. Inhibition of cell growth at different mercuric concentration. (A) The growth of seven cell cultures was started at 10^6 cells/ml in the presence of 10, 50, 100, 150, 200 and 1000 μM, respectively. The control sample (C) was not treated. After treatment the cell number was counted in each population. In the representation the abscissa is showing the mercury concentration and the ordinate the cell number. (B) Cellular movement after 10 μM Hg(II) treatment. The movement of two cells indicated by the white arrow. Unchanged position of the two cells (after 45 min) indicate cell death. (C) Apoptotic decrease after 10 μM (left panel) and necrotic increase (right panel) of nuclear size after 100 μM mercury treatment. Bar 5 μm each.