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Chromatin changes induced by Pb and Cd in human cells

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ABSTRACT

Our earlier data on the apoptotic effects of heavy metals (Cd, Hg, Ni, CrVI, and Ag) and irradiation (gamma and UV-B) indicated that cellular effects exerted on chromatin structure differ from one other. These observations suggested that chromatin distortions could be characteristic to the genotoxic agent. This notion raised questions whether or not (a) a specific genotoxic agent causes the same chromatin changes in different cell types, (b) closely related toxic compounds could be distinguished based on their chromatin toxicity. To answer these questions we have compared Cd induced toxicity in different cell types. The same Cd-specific chromatin changes were seen in human K562, Chinese hamster ovary and murine preB cells. K562 cells were chosen for fluorescent microscopy to compare chromatin damages caused by Cd and Pb. Characteristic distortions allowed a clear distinction between Cd and Pb toxicity at micromolar concentrations. The visualization of these changes is relatively easy and fast carrying the potential of early detection of heavy metal poisoning.

1. Introduction

Due to the presence of *d* electrons in their electronic configuration, heavy metals are much more reactive than light bioelements known as the CHNOPS group containing only *s* and *p* electrons. Heavy metals have been defined as those reactive trace elements that contain *d* electrons and have higher than 3 g/cm^3 densities (Banfalvi, 2011). Ionic heavy metals or their compounds contribute to the formation of free radicals with odd number electrons in their outer orbital. They can be divided into two major groups: (a) microelements with essential biological functions and (b) toxic heavy metals.

Among the heavy metals lead and cadmium belong to the most toxic ones. Lead is an ubiquitous industrial and environmental pollutant present in air, water and soil inducing a wide range of physiological, biochemical and behavioral dysfunctions (Needleman, 2004). After absorption lead is distributed mainly in blood, liver, kidney and bone. Lead was reported to induce apoptosis in a variety of cell types through DNA damage, mitochondrial dysfunction caused by the pro-/antiapoptotic imbalance of Bax/Bcl2 and by the activation of caspases (Xu et al., 2006). Genotoxic lead compounds affect the integrity of chromosomes and cause concentration-dependent increase in the frequency of micronucleus formation (Bonacker et al., 2005).

Cadmium increases the level of 8-deoxyguanosine, induces DNA strand breaks and DNA repair, reduces replicative DNA synthesis. The damage can be expressed by a general toxicity ratio of replication/repair synthesis (Banfalvi et al., 2000). Cadmium is known to influence proliferation and differentiation, and causes apoptosis (Bertin and Averbeck, 2006). Oxidative stress is supposed to play a central role in carcinogenesis due to aberrant gene expression, inhibition of DNA repair and apoptosis (Joseph, 2009). Cd- and Pb-driven redox reactions are likely to exhaust the antioxidant defense systems of cells, induce the inhibition of major antioxidant enzymes and increase the levels of endogenous oxidants toxic to DNA (Kasprzak, 1995; Myers et al., 2008). The most significant functional consequences of metal toxicity beside redox reactions have been described as the inhibition of replication, the elevation of repair synthesis, binding to proteins involved in the regulation of gene expression and carcinogenesis through epigenetic mechanisms. There is a general consensus that proteins are the major targets of heavy metal ions (Kasprzak, 2011).

Heavy metal binding to nuclear proteins is likely to have structural and functional consequences. The structural effects of heavy metal binding to nuclear proteins have been poorly characterized. The binding of heavy metals to nuclear proteins is likely to affect chromatin structure in each step of chromosome condensation. This paper describes characteristic chromatin distortions induced by μM concentrations of Cd and Pb. Cd induced genotoxicity manifested as large holes and disruptions in the nuclear membrane. These structural alterations have been contrasted by those chromatin changes that were caused by Pb and became visible as the expulsion of decondensed chromatin, lobular condensation of chromatin around the disrupted nuclear membrane. Expulsion of

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chromatin indicated leakage and moderate swelling of cells rather than apoptotic shrinkage. Time-lapse imaging of Pb treated cells confirmed the absence of apoptosis. Beside the regular cell division an increased frequency of rarely occurring separation into three cells referred to as trivision was registered after Pb treatment.

2. Materials and methods

2.1. Materials

Freshly made stock solutions of lead nitrate ($Pb(NO)_2$), and cadmium chloride ($CdCl_2$) (Sigma–Aldrich, Budapest, Hungary) were used. Growth media and sera were obtained from Invitrogen (Carlsbad, CA, USA), 6-diamidino-2-phenylindole dihydrochloride (DAPI), 1,4-diazabicyclo-(2,2,2)-octane (DABCO) and other reagents were purchased from Sigma–Aldrich (Budapest, Hungary). Antifade medium consisted of 90% glycerol, 2% (w/w) 1,4-diazobicyclo-(2,2,2)-octane, 20 mM Tris–Cl, pH 8.0, 0.02% sodium azide, and 25 ng/ml DAPI. Swelling buffer consisted of 50 mM KCl, 10 mM $MgSO_4$, 3 mM dithiothreitol, and 5 mM $NaPO_4$, pH 8.0. Hypotonic buffer for reversible permeabilization contained 9 mM HEPES, pH 7.8, 5.8 mM dithiothreitol, 4.5% dextran T-150, 1 mM EGTA and 4.5 mM $MgCl_2$. Fixative solution contained methanol:glacial acetic acid (3:1). DAPI Stock Solution (5 mg/ml) was aliquoted (100 μl) and stored at −20 °C. DAPI working solution for staining was diluted with PBS to 0.1 μg/ml.

2.2. Cell cultures

The epithelial-like Chinese hamster ovary cells (CHOK1, ATCC #CCL61) were kept in suspension culture in spinner flasks using F-12 Ham's medium supplemented with 10% heat-inactivated fetal bovine serum at 37 °C and 5% CO_2. The murine pre-B-cell line 70Z/3-M8 (Offer et al., 2001) was grown in suspension culture at 37 °C in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS), 2 μg/ml mycophenolic acid, 150 μg/ml xanthine, and 15 μg/ml hypoxanthine and 2×10^{-5} M β-mercaptoethanol. The K562 cell line is a human immortalized myelogenous leukemia line that has been established as a bcr:abl positive erythroleukemia line derived from the pleural effusion of a 53 year old female chronic myeloid leukemia patient in terminal blast crisis (Lozzio and Lozzio, 1975). K562 cells are round, non-adherent cells, and bear some resemblance to both undifferentiated granulocytes and erythrocytes. Cultured K562 cells exhibit much less clumping than other suspension lines, presumably due to the downregulation of surface adhesion molecules by bcr:abl (Ziegler et al., 1981). Due to these properties, K562 cells are suitable to detect the stickyness of chromatin upon toxic treatment. Human erythroleukemia K562 cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum.

2.3. Exposure to heavy metals

Cultured cells were grown in the presence of either Pb(II) nitrate (1–40 μM) or cadmium chloride (0.2–10 μM). Cells were grown for 24 h before being harvested, unless otherwise noted.

2.4. Synchronization of cells

Synchronization of CHO, murine preB and human K562 cells was performed by centrifugal elutriation as described earlier before and after heavy metal treatment or ultraviolet B irradiation (Banfalvi et al., 2005; Ujvarosi et al., 2007). Each fraction was routinely monitored by light microscopy and fluorescence activated cell sorting (FACS). Cell number, size and volume were determined. Viability (>98%) was determined by trypan blue dye exclusion in each fraction.

2.5. Time-lapse photography

Two inverse microscopes located inside a Sanyo MCO-18AIC CO_2 incubator were equipped with high sensitivity video cameras, connected to a dual image acquisition computer system. Custom-built illumination was developed to minimize heat- and photo-toxicity. Operation of the spectrally warm-white light emitting diodes was synchronized with image acquisition periods. Exposure times were minimized to avoid phototoxicity. Cell cultures in T flasks were placed on inverse microscopes. Transmission light microscopic images of K562 cells were taken every minute using the eTox LTS extended time-lapse imaging system. Image acquisition parameters were tuned for maximal greyscale dynamic range resolution averaging 5 auto-intensity histogram equalized images. Time difference between images taken from each flask was not more than 6 s ±8%. The screen of the computer was divided in two portions showing side-by-side the morphological changes of the control and the treated cells. The time of exposure was indicated in each frame. Exposures were converted to videofilms by speeding up the projection to 30 exposures/s. Individual K562 cells of suspension cultures were selected for analysis. Individual photographs were chosen as panels shown in the figures. Time-lapse photography of individual cells was used to determine the growth profile of individual cells and to distinguish among genotoxic specific growth, density and size profiles of Pb toxicity.

2.6. Image analysis

Cell growth, apoptotic and necrotic cell death can be mathematically converted to objective data, such as cell surface, calculated from cell perimeter, area and their ratio. Edges of the cells were detected using a diamond-shaped pixel pattern. Cell surface was calculated and given as relative arbitrary units. More than 200 dividing necrotic, or apoptotic cells could be analysed at the same time. The graphs presented are characteristic to the observed processes (Nagy et al., 2010).

2.7. Reversible permeabilization

This method was originally developed for the reversible permeabilization of lymphocytes isolated from the murine thymus by using dextran T-150 in a slightly hypotonic buffer (Banfalvi et al., 1984). Reversible permeabilization was adapted to CHO, murine preB and K562 cells. Briefly, 1 ml of hypotonic buffer was added to 10^6 cells in the presence of dextran T-150 as a molecular coat to prevent cells from disruption. Permeabilization lasted for 1 min at 0 °C. For reversal of permeabilization, the slightly hypotonic solution was replaced by medium containing 10% fetal bovine serum, and the cells were regenerated in CO_2 incubator at 37 °C and 5% CO_2 for 3 h. After permeabilization cells were resealed in complete medium, reaching 100% permeability and 80% reversal of permeabilization measured by ^{3}H-thymidine and ^{3}H-thymidine triphosphate incorporation.

2.8. Isolation of nuclei

On account of the cyclic character of chromatin unfolding and chromosome condensation, which is limited to a relatively short time, synchronized cell populations were treated with colcemide to arrest the cycle in metaphase. Cells (10^6) were resuspended in growth medium after reversal of permeabilization and treated with 0.1 μg/ml colcemid for 2 h at 37 °C under 5% CO_2. Cells were washed with 5 ml PBS and centrifuged at 500g for 5 min. Nuclei