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Cadmium induced DNA damage in human hepatoma (Hep G2) and Chinese hamster ovary (CHO) cells

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Introduction

Cadmuim (Cd) is one of the most important heavy metal environmental toxicants. It accumulates in human tissues, particularly in kidney and liver. Cd is classified as probable human carcinogen by IARC.[1] The genotoxic potential of Cd is rather weak and restricted to high cytotoxic concentrations. However, at low concentrations Cd enhances genotoxicity of other DNA damaging agents.[2] It was shown that Cd interferes with nucleotide excision repair (NER) by inhibiting DNA damage recognition and incision step of NER.[3] The aim of the study was explore the DNA damaging potential of Cd and its interference with the repair of UV induced DNA damage *in vitro*, using Comet assay. We studied the induction of DNA single strand breaks (ssb) by low nanomolar concentrations of $CdCl_2$ after different duration of exposure on human hepatoma cell line (Hep G2). The effect of $CdCl_2$ on the repair of UV induced DNA damage was studied in Chinese hamster ovary cells (CHO). The results of the formation and disappearance of DNA ssb after different periods of recovery after the UV irradiation, reflecting the nucleotide excision repair (NER) kinetics, are presented.

Materials and methods

Cell lines

Human hepatoma cells (HepG2) cells were cultured in Williams Medium containing 10 % FBS at 37 °C in humidified atmosphere with 5 % CO_2. Chinese hamster ovarian (CHO) cells were cultured in F-12 medium containing 10 % FBS at 37°C in humidified atmosphere with 5 % CO_2.

Treatment of cells

HepG2 cells were incubated with low, non-toxic concentrations of CdCl2 (10 nM, 100 nM and 1000 nM) in complete growth medium for 3, 6, 9, 12, 24 and 72 h. After the incubation period the cells were harvested and subjected to alkaline single cell electrophoresis (Comet assay). CHO cells were seeded in 25 cm^2 tissue culture flasks one day prior to treatment with Cd. Five hours prior to the UV irradiation the cells were treated with 1 μM and 10 μM $CdCl_2$ in complete growth medium. After the treatment the cells were washed with PBS, harvested, suspended in PBS and UV irradiated (20'', 50-cm distance). The irradiated cells were subjected to Comet assay after the recovery period of 0, 10, 20, 30 and 60 minutes at 37 °C.

Comet assay

The cells (HepG2 or CHO) were embedded in 1 % LMP agarose on pre-coated microscope slide and lysed at 4 °C for 1 h (2.5M NaCl, 100mM EDTA, 10 mM Tris, 1 % Triton X-100, pH10). The slides were then placed in the electrophoresis solution (300 mM NaOH, 1 mM $EDTANa_2$, pH13) for 20 min at 4 °C to

allow DNA unwinding and electrophoresed for 20 min at 25V (300 mA). After electrophoresis slides were neutralized (0,4 M Tris, pH 7,5) and stained with EtBr. 50-100 cell nuclei per each experimental point were examined at 400 magnification using a fluorescence microscope (Olympus) and analyzed with the software VisCOMET. The DNA damage is expressed as comet tail moment, which is defined as product of the comet length and percentage of DNA in tail.

Results and discussion

Figure 1 shows the effects of low non-toxic concentrations of $CdCl_2$ on DNA damage in HepG2 cells. Level of DNA damage was assessed after different periods of incubation with $CdCl_2$ by measuring the increase of comet tail moments. Incubation with 10 nM, 100 nM and 1000 nM $CdCl_2$ caused a dose-dependent increase of DNA damage. The DNA damage increased also with the increasing time of exposure to $CdCl_2$ up to 12 h. However, when the cells were incubated for 24 h the DNA damage was lower than after 12 h incubation. After 72 h incubation DNA damage was detected only in cells treated with 1000 nM $CdCl_2$. This time dependent decrease of DNA damage can be due to the Cd mediated induction of the synthesis of metallothioneins, which are known to play role in cellular defence mechanism against Cd toxicity.[4]

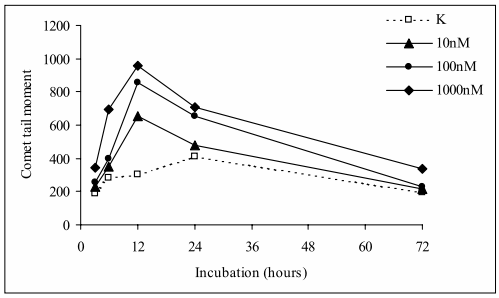

Figure 1. Comet tail moment of HepG2 cells incubated with CdCl2 for 3, 6, 12, 24, and 72 h. 100 comets per experimental point were analysed by image analysis system.

UV irradiation induces pyrimidine dimers and 6-4 photoproducts that are repaired predominantly by NER. With the Comet assay ssb are detected, which reflect the incision step of the NER. In $CdCl_2$ pretreated cells the tail moment was lower than in control cells (Figure 2) indicating that $CdCl_2$ prevented the incision step of NER. After 60 minutes of recovery the residual ssb were higher in $CdCl_2$ treated cells compared to the control, which might reflect either slower NER or inhibition of ligation step of NER. This result confirms the interference of Cd with NER.

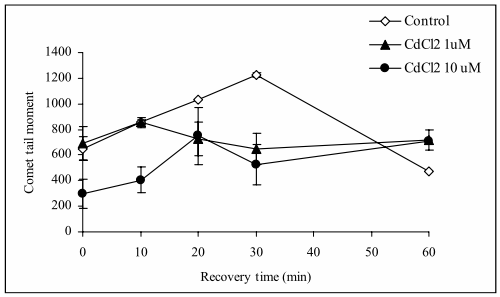

Figure 2. DNA repair kinetic of UV induced DNA damage in CHO cells. Non-treated or CdCl2 pre-treated cells were exposed to UV irradiation and than incubated at 37oC. At different intervals samples were taken for comet assay. 100 comets per experimental point were analysed by image analysis system.

Conclusion

In conclusion, our results in Hep2G cells showed that ssb were induced at 10 nM of $CdCl_2$, which is the concentration that corresponds to the concentrations of Cd found in blood of environmentally exposed population.[5] This damage was detected only after short time of exposure. In CHO cells, we demonstrated, that when cells are exposed to $CdCl_2$ for a short time, the repair of UV induced DNA damage was inhibited. Further experiments are in progress to explore the role of metallothioneins in protection against genotoxic and cogenotoxic effects of Cd.

References

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