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Effect of Mercuric Nitrate on Repair of Radiation-induced DNA Damage

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1. Introduction

High concentrations of mercury can cause serious damage to the nervous system, immune system, kidneys and liver in humans [1]. And mercury is toxic to developing embryos because mercury ions can penetrate the blood−placenta barrier to reach the embryo [2]. Studies from human monitoring of occupational exposure to mercury vapours have shown that mercury can alter the ability of lymphocytes to repair radiation-induced DNA damage [3]. The aim of this *in vitro* study was to investigate, on the molecular and cytogenetic levels, the effect of exposure to mercury ions on the kinetics of the repair process of DNA damage induced by ionising radiation.

2. Materials and Methods

Whole blood was collected from a non-exposed, non-smoking, healthy man. Part of it was used immediately to cytogenetic studies. From the second part of it, lymphocytes were separated using Histopaque separation medium following the manufacturer's procedure. To investigate the effect of mercury ions on the repair of DNA damage induced by ionizing radiation, the fresh blood or thawed lymphocytes were used.

Challenging exposure to 2 Gy of X-rays was carried out using a Philips MCN 323 machine at 250kV, 10mA (0.5 Gy/min) [3]. Immediately after the exposure, the cell suspension was transferred to the culture medium for chromosome aberration assays. Part of the irradiated cell suspension was transferred into an incubation chamber (37°C) for an incubation time. After the end of post-irradiation incubation cells were immediately transferred to the culture medium as described in the chromosomal aberration assay procedure [4]. Immediately after the completion of post-radiation incubation, the SCGE procedure was performed.

3. Results and Discussions

The time-dependent response curve of DNA damage based on Hg ions uptake in the cells is presented in Fig. 1. Results showed a significant time-dependent increase of DNA damage levels in the range 0−60 minutes of mercury incubation. These results indicate that 60 min incubation time was sufficient and this incubation time was used in further experiments.

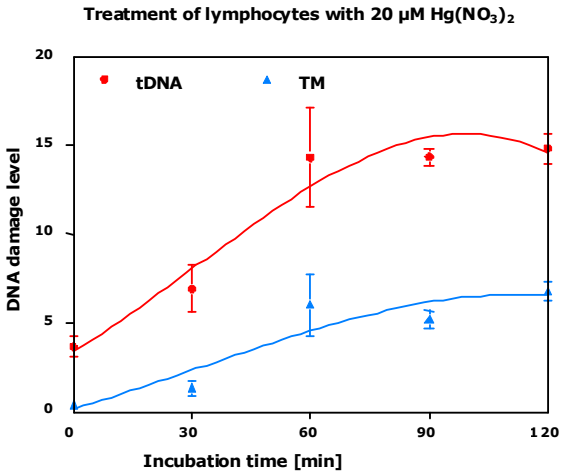

Fig. 1. The effect of incubation with 20 µM $Hg(NO_3)_2$ on DNA damage levels detected in lymphocytes.

Fig. 2 presents the concentration-response relationship of the lymphocytes treated for 1h with various concentrations of Hg ions (0−100 µM). DNA damage levels were evaluated from tDNA or tail moment parameters of the SCGE assay. Results obtained for tail moment parameter indicate a non linear slight dose-dependent increase of DNA

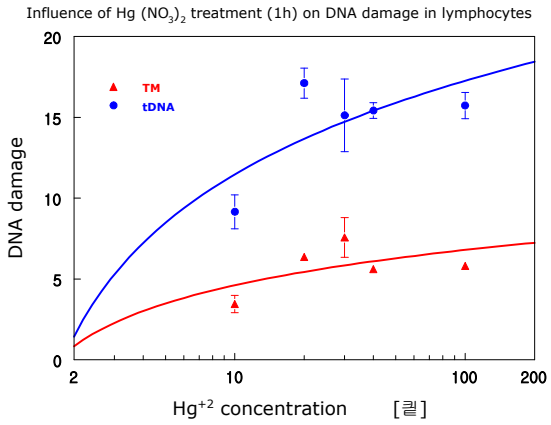

Fig. 2. The concentration-effect relationship for DNA damage levels measured after 1-hour incubation with $Hg(NO_3)_2$.

Fig. 3 presents the residual damage levels after exposure to 2 Gy of X-rays evaluated for tDNA or tail moment parameters in lymphocytes without (upper) and with (bottom) pre-treatment with $Hg(NO_3)_2$. The kinetics of DNA damage repair performed in X-ray-irradiated lymphocytes shows a significant decrease in damage with post-irradiation incubation time. The repair kinetics following irradiation was estimated from comparison of the half life ($t_{1/2}$) time of residual damage. The $t_{1/2}$ for DNA damage repair in 2 Gy irradiated cells was 7.3 min. On the contrary, in the lymphocytes pre-incubated with Hg ions, a strongly linear increase of DNA damage levels with the post-irradiation incubation time was seen (r^2=0.98 for TM). such a change in the kinetics suggests an alteration of the repair processes.

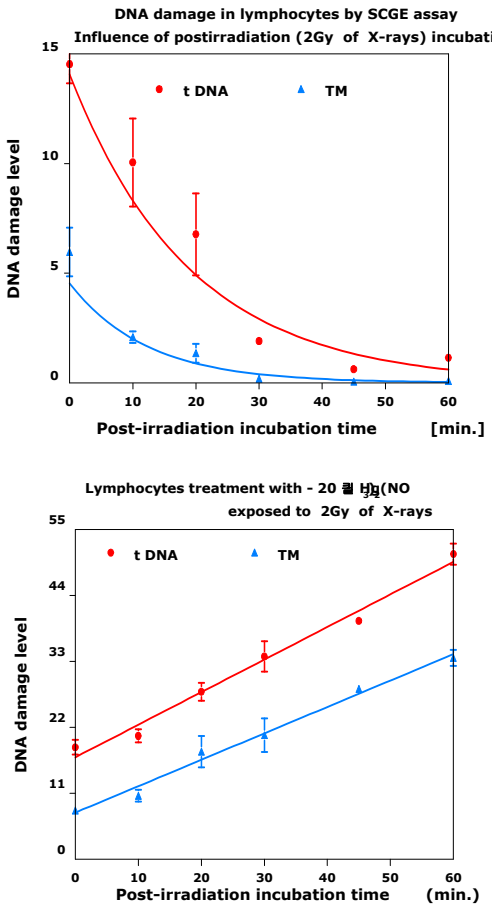

Fig. 3. The time dependence of the residual DNA damage in cells in untreated lymphocytes (upper) and in lymphocytes after $Hg(NO_3)_2$ pre-treatment (bottom). irradiated with (2 Gy) of X rays.

6. DISCUSSION

In this study, the increase in DNA damage levels (measured as tail moment: r^2=0.66, $p<0.049$) linearly correlates with Hg ions concentrations in the range 0−40 μM. Cantoni et al. [5] have also shown that $HgCl_2$ induced DNA single-stand breaks in the range 25−100 μM. A similar analysis of the effect of mercury on DNA damage in lymphocytes pre-incubated with various concentrations of mercury ions and lymphocytes irradiated with 2 Gy of X-rays has not shown any dependence between mercury concentrations and DNA damage.

DNA repair is a system designed to protect the integrity of the genome. Mercury, like heavy metals, is suspected to cause DNA repair deficiencies [6]. This study investigated the effect of mercury on the repair rate of radiation-induced DNA damage comparing the repair kinetics in the irradiated lymphocytes with and without mercury pre-incubation. The statistical analysis conducted for the irradiated lymphocytes has shown a decrease in the X-ray-induced DNA damage with the incubation time. The recorded repair kinetics of X-ray-induced DNA damage is in good agreement with the literature data [7]. In conclusion, the results of this study confirm that Hg ions induced DNA and cytogenetic damage and inhibited the repair of X-ray-induced DNA damage.

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