

Zinc salts differentially modulate DNA damage in normal and cancer cells

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Received 8 October 2008; revised 28 January 2009; accepted 20 February 2009

Abstract

Zinc plays an essential role in a wide range of cellular processes, including defense against free radicals and maintaining genomic stability. The presence of zinc in some proteins is fundamental for their functioning as transcription factors. Little is known about interaction between zinc and DNA, which can be important in light of reports on the role of zinc in cancer transformation and sometimes contradictory character of these reports. In the present study we studied cyto- and genotoxicity of zinc sulfate (ZnSO₄) in normal human lymphocytes and human myelogenous leukemia K562 cancer cells in the presence of zinc and hydrogen peroxide (H₂O₂). Zinc at concentrations from the range 10–1000 μM decreased the viability of cancer cells and this effect, especially for low concentrations of the element, was much more pronounced than in normal cells. Zinc did not induce DNA damage in normal cells, but did so in cancer cells. We observed a key difference between the action of zinc in normal and cancer cells in the presence of H₂O₂, since the element exerted a protective effect against cyto- and geno-toxic action of H₂O₂ in the former, whereas it increased such action in the latter. Zinc inhibited the repair of DNA damage induced by H₂O₂ in cancer cells. The results suggest that zinc may protect normal cells against DNA-damaging action and increase this action in cancer cells, which indicates the dual action of this element in dependency of target cells and can be useful in cancer therapy.

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Keywords: Zinc; DNA damage; DNA repair; Lymphocytes; K562 cells; Cancer

1. Introduction

Zinc plays a central role in cellular growth and differentiation and the effects of its deficiency are especially pronounced in tissues and organs with a rapid turnover, including immune system and during period of rapid growth both pre- and post-natally (Varin et al., 2008). Zinc may be an increasing environmental problem in this sense that it is found in most vitamin and mineral supplements and various easily accessible products, including cold lozenges and an increasing number of humans using dietary supplements on a regular basis (Haase et al., 2008).

The role of zinc in cancer has received increasing attention, with a link between zinc deficiency and cancer having been established in human, animal and cell culture studies. Zinc deficiency caused oxidative DNA damage and chromosome breaks in animals fed with a zinc-deficient diet (Golub et al., 1985; Oteiza et al., 2000). In rats, dietary zinc deficiency led to an increased susceptibility to tumor development when exposed to carcinogenic compounds (Fong and Magee, 1999; Fong et al., 1978, 1996, 1997, 2001). Cell culture studies showed that zinc deficiency led to an increased oxidative damage to testicular cell DNA (Oteiza et al., 1995). Zinc also plays an important role in maintaining proper functions of prostate. Several studies have implicated changes in zinc accumulation in the development and progression of prostate malignancy (Costello and Franklin, 1998, 2000). Moreover, an increase in dietary zinc was associated with a decrease in the incidence of prostate cancer (Kristal et al., 1999). However,

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the precise role of zinc in the prostate health is largely unknown.

Dietary zinc is generally considered to reduce the risk of cancer, but zinc supplements have been correlated with increasing risk of cancer (Grant, 2008). The reason for this discrepancy may lie in the essential difference between studies on zinc singly, i.e. zinc supplementation with zinc salts and research on zinc-rich and poor dietary components, containing a variety of agents, which may influence carcinogenesis. Moreover, several *in vitro* studies have often different targets, usually normal and cancer cells. To add information to clarify the dual role of zinc on human homeostasis, we compared its cyto- and genotoxicity in normal and cancer cells. Genotoxicity of a compound, usually expressed as its ability to induce DNA damage, is often essential for mutagenesis and cancer transformation (McKinnon and Caldecott, 2007). We investigated DNA damage and repair in human normal lymphocytes and human myelogenous leukemia K562 cells exposed to zinc sulfate in the presence or absence of a DNA challenging factor, hydrogen peroxide (H_2O_2).

2. Material and methods

2.1. Cell lines

Human myeloid leukemic K562 cells, expressing *BCR/ABL* oncogene, were obtained from Dr Malgorzata Czyz of Department of Medicinal Chemistry, Medical University of Lodz, Lodz, Poland. The cells were grown in RPMI, 1640 medium with L-glutamine and Hepes supplemented with 10% FBS, 100 IU/ml penicillin and 100 μ g/ml streptomycin in a 5% CO_2 in air atmosphere at 37 °C.

2.2. Chemicals

Zinc salts: $ZnSO_4$, RPMI 1640 medium with and without L-glutamine, fetal bovine serum (FBS), streptomycin, penicillin, phytohemagglutinin PHA-P from *Phaseolus vulgaris* (red kidney bean), low melting point (LMP) and normal melting point (NMP) agarose, phosphate buffered saline (PBS), 4',6-diamidino-2-phenylindole (DAPI), dimethyl sulfoxide (DMSO), H_2O_2 were purchased from Sigma–Aldrich (St. Louis, MO, USA). All other chemicals were of the highest commercial grade available.

2.3. Lymphocyte isolation

Lymphocytes were isolated from 5 ml of fresh peripheral blood obtained from young (20–31 years old), male, healthy, non-smoking donors by centrifugation in a density gradient of Gradisol L (15 min, 280g, 4 °C) and used immediately. Each experiment was performed on blood obtained from individual donor. The viability of the cells was measured by trypan blue exclusion assay at ~99%. The final density of the lymphocytes was adjusted to 10^6 cells/ml by adding RPMI 1640 to the single cell suspension.

2.4. Viability test

Zinc sulfate was taken from stock (10 mM) RPMI 1640 solution and added to the suspension of the lymphocytes and K562 cells (10^6 ml⁻¹) in the growth medium to give final concentrations in the range 10–1000 μ M. The medium for the lymphocytes was supplemented with 30 μ g/ml lectin. After 4 days of incubation with zinc, the viability of cells was measured by the MTT assay (Siu et al., 1999). Briefly, cells (10^5 ml⁻¹) were plated onto 96-well plates in 100 μ l of growth medium and after treatment with a salt, 10 μ l of 5 mg/ml MTT was added to each well. After a 4 h incubation at 37 °C, 100 μ l of a solution containing 10% of SDS and 0.04 M HCl was added to dissolve the water-insoluble formazan salt. Next day, the difference of optical density at 650 nm and 570 nm was measured with an ELISA microplate reader (BioRad, Hercules, CA, USA). The viability was represented as the percentage of viable cells in suspension culture after pre-incubation with zinc salts, as compared to the untreated control group. Each experiment was repeated 3 times.

2.5. Cell treatment

$ZnSO_4$ was added to the suspension of cells to give final concentrations of 10 and 40 μ M, controls receiving only RPMI 1640 medium. The cells were pretreated with $ZnSO_4$ for 1 h at 37 °C, following which they were washed and incubated with 10 μ M H_2O_2 for 10 min at 4 °C.

2.6. Comet assay

Comet assay was performed under alkaline conditions essentially according to the procedure of Singh et al. (1988) with modifications (Collins et al., 1993; Evans et al., 1995; Klaude et al., 1996), as described previously (Blasiak and Kowalik, 2000). A freshly prepared 10 μ l aliquot of the suspension of the cells in 40 μ l of the 0.75% of LMP agarose dissolved in PBS was spread onto microscope slides (Superior, Germany) precoated with 0.5% of NMP agarose. The cells were lysed for 1 h at 4 °C in a buffer consisting of 2.5 M NaCl, 100 mM EDTA, 1% Triton X-100, 10 mM Tris, pH 10. After the lysis, the slides were placed in an electrophoresis unit, DNA was allowed to unwind for 20 min in a solution consisting of 300 mM NaOH and 1 mM EDTA, pH > 13. Electrophoresis was conducted in a solution consisting of 300 mM NaOH and 1 mM EDTA, pH > 13, at an electric field strength of 0.73 V/cm. The slides were washed in water, drained and stained with DAPI at 2 μ g/ml and covered with cover slips. Comets were observed at 200 \times magnification in an Eclipse fluorescence microscope (Nikon, Tokyo, Japan) attached to COHU 4910 video camera (Cohu, San Diego, CA, USA) equipped with UV-1 filter block (an excitation filter of 359 nm and a barrier filter of 461 nm) and connected to a personal computer-based image analysis system Lucia-Comet v. 4.51 (Laboratory Imaging, Prague, Czech Republic). Fifty images were randomly selected from each sample. Two parallel tests with aliquots of the same sample of cells were performed for

a total of 100 cells. The mean percentage comet tail DNA, is positively correlated with the level of DNA breakage and/or alkali labile sites in the cell and is negatively correlated with the level of DNA crosslinks, was analyzed (Ashby et al., 1995; Tice et al., 2000).

2.7. Data analysis

Values were expressed as mean \pm S.E.M. from at least 3 separate experiments, i.e. data from 3 experiments were pooled and the statistical parameters were calculated. The data were analyzed using Statistica package (StatSoft, Tulsa, OK). If no significant differences between variations were found, as assessed by Snedecor–Fisher test, the differences between means were evaluated by applying the Student *t* test. Otherwise, the Cochran–Cox test was used.

3. Results

3.1. Cell viability

Fig. 1 shows the survival of human lymphocytes and K562 cancer cells after 4 days of incubation with zinc sulfate evaluated by MTT assay. Zinc evoked a concentration-dependent decrease in the viability of the cells. Apart from the highest concentration of zinc, 1000 μ M, lymphocytes much better survived zinc treatment than the cancer cells. For example, at 40 μ M ZnSO₄ the viability of the lymphocytes was 98%, whereas only 42% of K562 cells were viable.

3.2. DNA damage

Fig. 2 displays the extent of DNA damage in the cells incubated with ZnSO₄ evaluated by the alkaline comet assay. Zinc did not induce damage to DNA in normal lymphocytes, whereas damage was pronounced in K562 cells. The damaging

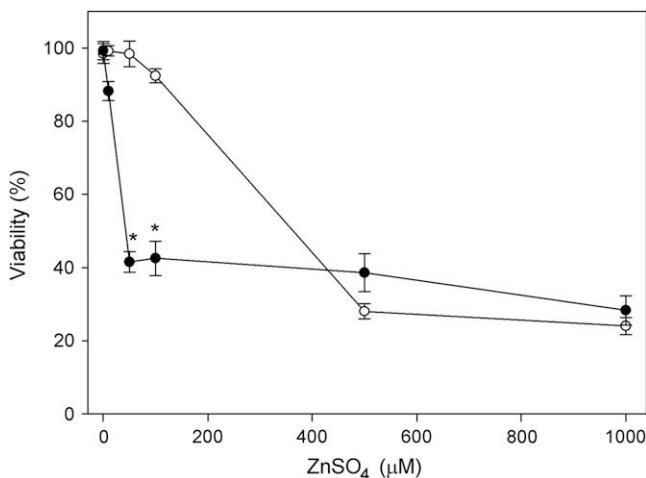


Fig. 1. Viability of normal human lymphocytes (white symbols) and human myelogenous leukemia K562 cells (black symbols) incubated for 4 days at 37 °C with ZnSO₄ measured by MTT assay. Each point is the mean of 3 independent experiments. Error bars denote S.E.M. **P* < 0.001 relative to lymphocytes. *P* value was evaluated by applying the Student *t* test.

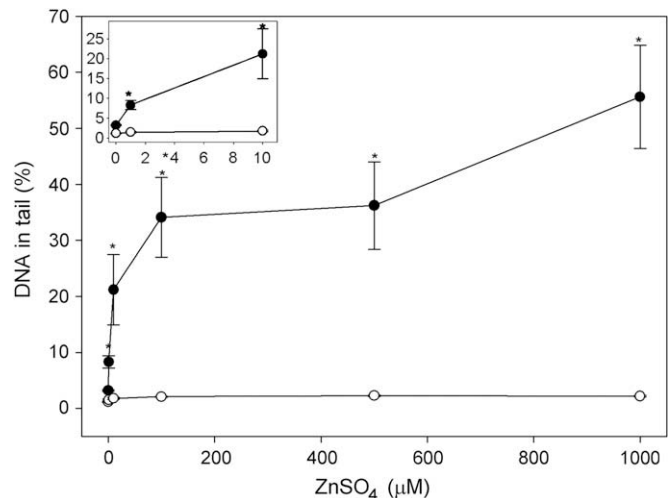


Fig. 2. DNA damage measured as mean comet percentage tail DNA in normal human lymphocytes (white symbols) and human myelogenous leukemia K562 cells (black symbols) exposed for 1 h at 37 °C to ZnSO₄. The number of cells analyzed in each treatment was 100. The figure shows mean results from 3 independent experiments. Error bars denote S.E.M. **P* < 0.001 relative to lymphocytes. *P* value was evaluated by applying the Student *t* test. The inset displays DNA damage in the zinc concentration range 0–10 μ M.

effect in K562 cells increased strongly with the concentration of zinc up to 100 μ M. A mild increase was observed at higher concentrations. Comparing these results with those obtained in the cell viability studies (Fig. 1), presented in the preceding section, we conclude that such a biphasic character of the DNA-damaging effect of zinc can be attributed to its cytotoxicity. A rapid decrease in the viability up to about 100 μ M of ZnSO₄ was seen, and above 100 μ M the decrease became much slower. This suggests that, up to 100 μ M, the genotoxicity of zinc competed with its cytotoxic effect and at higher concentration the cytotoxicity of zinc was so high that a relatively small population of the cells reacted to the DNA-damaging action of Zn.

H₂O₂ applied for 10 min on ice at 10 μ M evoked similar pronounced DNA damage in both kinds of cells (Fig. 3). However, preincubation with 10 and 40 μ M ZnSO₄ decreased that damage in lymphocytes, while it resulted in an increased tail DNA in K562 cancer cells.

3.3. DNA repair

Fig. 4 shows the time-course of the repair of DNA of lymphocytes and K562 cells exposed to 10 μ M H₂O₂ and incubated in the presence or absence of zinc. In all cases, the percentage of DNA in the tail of comet in the control cells was constant, indicating that preparation and subsequent processing of the cells had not introduces significant damage to their DNA. The lymphocytes exposed to H₂O₂ at 10 μ M were able to remove damage of their DNA within 60 min, much faster than in K562 cells. The time-course of DNA repair in lymphocytes challenged with 10 μ M H₂O₂ and incubated with ZnSO₄ was similar to that in lymphocytes without zinc in the repair medium. On the other hand, the extent of DNA damage in K562 cells after 120 min of incubation in the presence of

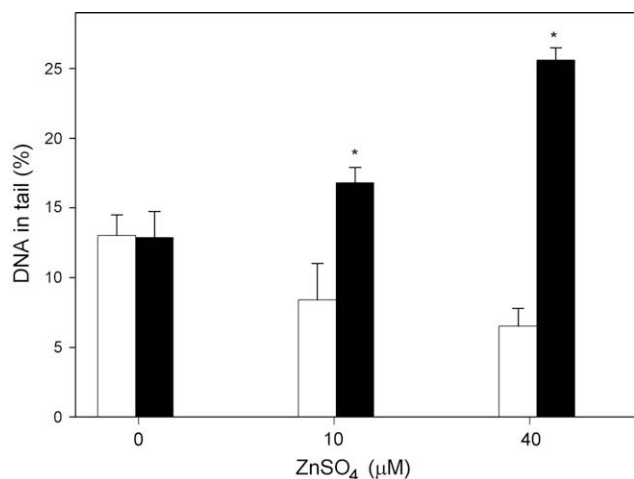


Fig. 3. DNA damage measured as mean comet percentage tail DNA in normal human lymphocytes (white bars) and human myelogenous leukemia K562 cells (black bars) preincubated with 10 or 40 μM ZnSO_4 and exposed to 10 μM H_2O_2 for 10 min at 4 °C. The number of cells in each treatment was 100. The figure shows mean results from 3 independent experiments. Error bars denote S.E.M. * $P < 0.001$ relative to cells not pretreated with zinc. P value was evaluated by applying the Student t test.

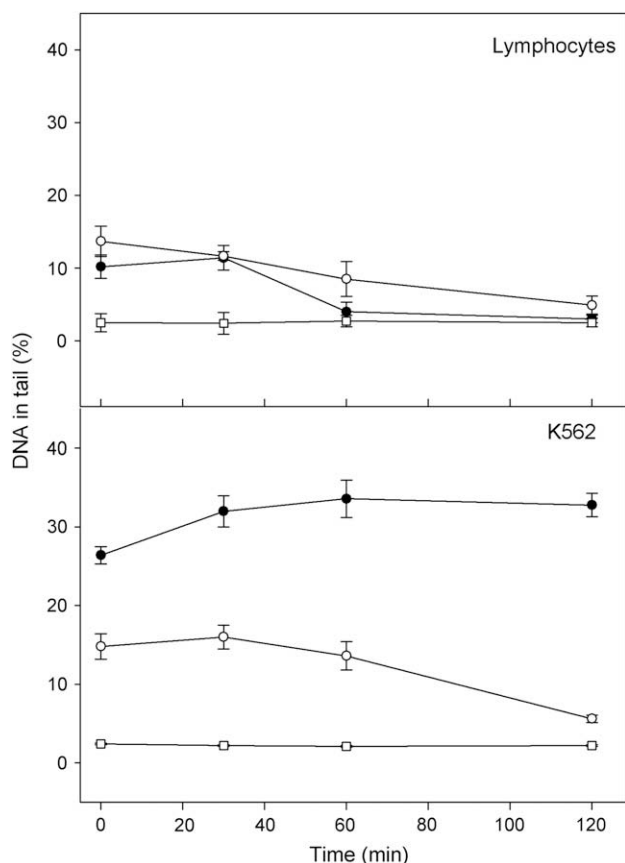


Fig. 4. Time-course of the repair of DNA damage in normal human lymphocytes and human myelogenous leukemia K562 cells exposed to 10 μM H_2O_2 for 10 min on ice, washed and incubated for 120 min at 37 °C in the presence (black circles) or absence (white circles) of 10 μM ZnSO_4 . A negative control with no hydrogen peroxide or zinc treatment is displayed for either cell (white squares). The figure shows mean results from 3 independent experiments. Error bars denote S.E.M.

zinc sulfate was higher than that observed at the beginning of repair incubation. The repair incubation started with the addition of ZnSO_4 to the repair medium. During the short time between the zinc addition and fixing the sample designated $t = 0$, zinc induced DNA damage which can be seen in Fig. 4. This did not affect the kinetics of DNA damage.

4. Discussion

We previously showed that BCR/ABL-positive cells might display an enhanced ability to survive a genotoxic stress due to their increased efficacy to repair DNA lesions, prolonged activation of the G_2/M checkpoint to provide more time for repair, and inhibited proapoptotic mechanisms (Slupianek et al., 2002).

We have now presented the results of 4 groups of experiments, each related to different phenomenon: cell viability, DNA damage, protective action against DNA-damaging action and DNA repair. In each experiment the focus was on the effects of different zinc concentrations of zinc, which differed from its concentration in blood of normal individuals ($\sim 15 \mu\text{M}$; Ver-sieck and Cornelis, 1980). However, a significant effect of zinc on cancer cells at 40 and 100 μM was seen, while normal cells remained unaffected. However, we cannot exclude the possibility that these concentrations of zinc would not exert an effect on normal cells *in vivo*. Zinc at high concentrations can be toxic (Welkell et al., 1986; Llobet et al., 1988; Calesnick and Dinan, 1988). We postulate that zinc at concentrations up to 100 μM could be used in therapy selectively directed at cancer cells, i.e. in such therapeutic regime where the drug is applied directly to the tumor mass, although such treatment does not exclude the use of zinc being administered to the main bloodstream.

We found that zinc at concentrations up to 100 μM did not affect the viability of lymphocytes or introduce damage in their DNA. Moreover, zinc reduced the extent of DNA damage induced by H_2O_2 in normal lymphocytes. In contrast to normal cells, cancer cells were prone to zinc, which induced cyto- and geno-toxic effects. The viability of cancer cells decreased significantly (40–50%) after incubation with low concentrations of zinc compounds. Higher sensitivity of cancer cells to the element may be explained by their active proliferation making chromatin loose and more accessible for genotoxic agents. It is suggested that the genotoxic activity of zinc is caused by linking a histone deacetylase hydroxamic acid group with part of the phenyl amino group of suberoylanilide hydroxamic acid, which is inserted into the pocket-like catalytic site of the enzyme, with a zinc molecule at its base (Marks et al., 2004). This process may inhibit deacetylases activity and change gene expression and increased acetylation of histones may exert an antiproliferating and proapoptotic effects.

Zinc can sensitize cancer cells for physical agents; disulfonated diphtalimidomethyl phthalocyanine zinc (ZnPcS_2P_2) has been used to induce mitochondria-dependent apoptosis in K562 and HL60 cells as a novel amphipathic photosensitizer (Huang et al., 2005a,b). Exposure to H_2O_2 increased DNA fragmentation in K562 cells, preincubated with ZnSO_4 ,

indicating that zinc sensitized cancer cells to genotoxic agents in contrary to normal cells.

The precise role of zinc in cancer cells is unknown and the results of some experimental findings suggest that it can play an important role in the inducing and progression of prostate cancer and sensitize malignant cells to apoptosis induced by cytotoxic agents (Costello and Franklin, 1998, 2000). In general, zinc seems to have a special significance in maintaining DNA integrity in normal prostate cells, first by the interaction with some of the responsible proteins, including those of the cell cycle, apoptosis, transcription and DNA damage response and repair (Yan et al., 2008). Such actions of zinc can play an important role in anticancer therapy, especially that it can protect normal cells against harmful effect of anticancer drugs and radiation. This is confirmed by the results of research indicating that chelating Zn^{2+} , either pharmacologically or by overexpression of Zn^{2+} -binding protein in endothelial cells conferred significant protection from apoptosis and cell death associated with the effects of acute exposure to H_2O_2 . These results suggest that regulation of zinc levels may represent a potential therapeutic target for cardiovascular disease associated with acute oxidative stress (Wiseman et al., 2007). Lastly, it was shown that zinc might stimulate proliferation of Hep-2 tumor cells (Rudolf and Cervinka, 2008). The proposed mechanism of such action of zinc involved enhanced expression of anti-apoptotic Bcl-2 and Bcl-XL proteins and increased expression of proteins of mitogenic signalling pathways, including p53, AKT and MAP kinases. Our results showing a high extent of DNA damage in cancer cells, suggest that apoptosis and cell cycle disturbance may be involved in the interaction between zinc and the cell. We think that taking data on DNA damage and repair as a starting point, apoptosis and cell cycle distribution, as well as the expression of pro- and anti-apoptotic proteins and proteins controlling the cell cycle, might be investigated in searching for the mechanism of the interaction of zinc with the cell.

We incubated cells with zinc at several different concentrations. These concentrations expressed the presence of zinc per volume of a buffer, but the actual concentration of the element in the cell and its distribution within the cell was unknown. This is an important issue, which should be addressed in the future research, since the dependence between observed effect and extra- and intra-cellular concentration of zinc would help to distinguish biological affects from simple chemical inactivation and the difference in zinc accommodation by normal and cancer cells, requiring further analysis.

Acknowledgements

The work was supported by the grant 505/0378 (TS) and 505/376 (JB) from the University of Lodz.

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