Zinc Rescue of DNA Repair inhibition by Uranium

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ABSTRACT

Numerous metals, including uranium, are cytotoxic, mutagenic, and carcinogenic. Uranium has radiological and non-radiological impact within biological systems and there is increasing evidence for DNA damaging and carcinogenic properties attributable to depleted uranium through its heavy metal properties. In this study we report that low concentration of uranium (as uranyl acetate, predominantly U^{235}; <10μM) is minimally cytotoxic to human embryonic kidney cells or normal human keratinocytes. However, uranium exacerbates DNA damage and cytotoxicity induced by hydrogen peroxide, suggesting that uranium may inhibit DNA repair processes. Low concentrations of uranyl acetate (≤1 μM) significantly inhibits the zinc finger DNA repair protein poly(ADP-ribose)polymerase (PARP)-1. Exposure to uranyl acetate caused a dose-dependent loss of zinc from PARP-1 and xeroderma pigmentosum, complementation group A (XPA). In keeping with the observed inhibition of PARP activity, exposure to uranyl acetate caused retention of DNA damage. Co-incubation with zinc largely overcame the impact of uranium on PARP-activity and DNA damage. These finding present evidence that low concentration of uranium can inhibit DNA repair through disruption of zinc finger domains of DNA repair proteins. The evidence of short term (24 h) zinc reversing the observed DNA damage retention has led to the question of whether adapting keratinocytes to concentrations of zinc higher than that typically found in culture medium leads to further protection. As normal keratinocytes have relatively few doublings before senescence, an immortalized keratinocyte cell line is required. The NIKS cell line was selected for these zinc adaptation experiments and preliminary characterization of responses to metals with the parental and zinc adapted (2 & 10.7 mM) cells are shown demonstrating their suitability for future studies.

INTRODUCTION

Metal inhibition of proteins involved in DNA repair such as PARP-1 has led to the hypothesis that low concentrations of certain metals may serve as co-carcinogens by preventing effective repair of DNA damage caused by another insult. Experimental evidence supports this hypothesis for metals such as arsenic where co-carcinogenic actions have been well established, leading to questions regarding the co-carcinogenic potential of other metals.

Natural uranium is predominantly comprised of two radioactive isotopes, U^{235} and U^{238}. The radiologic toxicity and carcinogenicity of uranium is established, but the potential for uranium to act as a co-carcinogen through inhibition of DNA repair proteins has not been widely explored. Several studies report that uranium exposure is associated with DNA repair deficiency in exposed populations, but the mechanism is not established. There is limited evidence that uranium may interact with zinc finger (zf) proteins. One study demonstrated that at concentrations equal to or greater than 10 μM, uranyl acetate disrupted the DNA binding activity of two purified zinc finger proteins, Aart and Sp1 *in vitro*. Furthermore, PARP-1 was identified as a uranium binding protein using affinity chromatography and mass spectrometry approaches. Although these studies suggest that uranium may interfere with zinc finger DNA repair protein targets, the experiments did not investigate the impact of uranium on a relevant target protein isolated from exposed cells or direct inhibition of DNA repair activity.

In this study we provide evidence that low, non-cytotoxic concentrations of uranium (as uranyl acetate, UA) enhance the cytotoxicity of another DNA damaging agent, promotes the retention of DNA damage and inhibits cellular PARP activity. In addition, zinc largely overcomes the impact of uranium on PARP-activity and DNA damage. As it is of interest to determine the effects of long term zinc exposures, we have also characterized the metal- and zinc-associated responses of the NIKS, an immortalized keratinocyte cell line. We demonstrate here that the NIKS cell line is suitable for these zinc adaptation investigations.

RESULTS

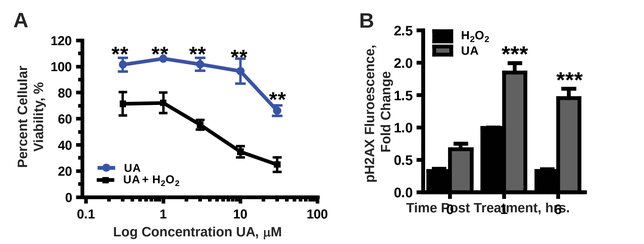


Figure 1: Uranyl Acetate(UA) toxicity and DNA damage retention. Normal human keratinocytes were exposed to UA with and without the induction of oxidative DNA damage (H_2O_2: 100 μM). A) Cellular viability was determined 48 hrs post treatment with PrestoBlue Cell Viability Reagent (Invitrogen). B) Fluorescence intensity of DNA strand breaks (pH2AX) from ICC assays conducted at the indicated times post DNA damage insult. ; ** = significant difference from + H_2O_2 group; n = 3, p<0.001; *** = significant difference between time matched groups; n = 3; p<0.001. Panel A shows that DNA damaged cells are significantly more sensitive to UA. Panel B shows that UA significantly increases the amount of DNA damage induced and that damage is retained.

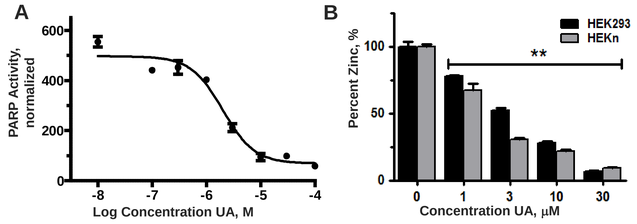


Figure 2: Inhibition of PARP1 activity and loss of zinc by UA. Normal human keratinocytes were exposed to increasing doses of UA. A) Dose response curve. Total protein was collected after 24 hrs for analysis of PARP activity via PAR ELISA (REF). B) PARP1 was immunoprecipitated from 500 μg total protein and zinc content assessed via zinc specific colorimetric assay and normalized to total protein immunoprecipitated. ; ** = significant difference from untreated control; n = 3, p<0.001. Data demonstrates that PARP1 activity is inhibited by UA and that the loss of activity is likely due the loss of zinc from the zf domains of the enzyme.

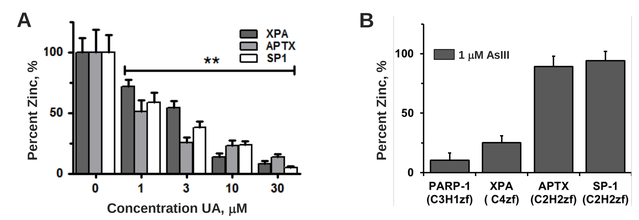


Figure 3: UA is not selective for C3H1 zinc fingers. Normal human keratinocytes were exposed to increasing doses of A) UA or B) a single dose of Arsenite (1 μM) for 24 hrs. Zinc finger (zf) proteins of interest immunoprecipitated from 500 μg total protein and zinc content assessed via zinc specific colorimetric assay and normalized to total protein immunoprecipitated. XPA contains a C4 zf, APTX and SP1 contain C2H2 zfs.. ** = significant difference from untreated control; n = 3, p<0.001 Data demonstrates that C4 and C2H2 zf domains as well as C3H1 (fig 2B) are susceptible to UA-induced zinc loss and inhibition while arsenite is selective for C3 and C4 zf domains.

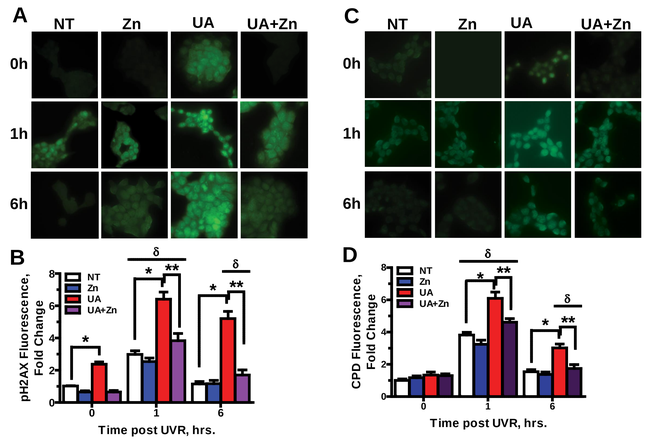


Figure 4: Retention of DNA damage following UA exposure is rescued by zinc. Immunocytochemistry of HEK293 cells was used to illustrate oxidative (A, pH2AX) and direct (B, CPDs) DNA damage. Cells were cultured on 4 chamber slides and treated with UA (10 mM) for 24 hrs with and without the addition of zinc (2 μM), then DNA damage was induced by exposure to UVR (3kJ/m²). Slides were fixed at 0, 1 and 6 hrs post UVR exposure and stained for the DNA damage markers indicated. Images were collected with an Olympus IX70 equipped with a digital camera and CellSense Image Analysis software (Olympus America). Fluorescence intensity was quantified and graphs shown (B, pH2AX and D, CPDs) are mean ± SEM of the intensity per nuclei of at least 10 images from each treatment group from 3 independent experiments. δ = significantly different from t=0; * * = significant difference between marked groups; n = 3, p<0.001.. Data demonstrates that UA enhances UVR induced DNA damage and that supplemental zinc reduces the extent of UA-induced DNA damage retention.

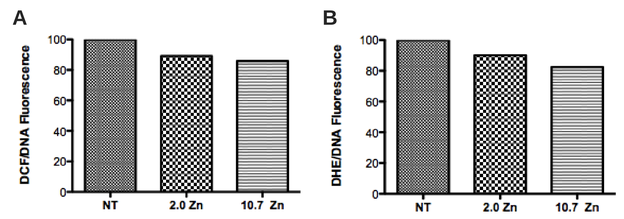


Figure 5: Short-term zinc adaptation lowers basal levels of ROS. Normal human keratinocytes (HEKn) were adapted to 2.0 μM or 10.7 μM of zinc for six days prior assessment of ROS. Cells were stained with either DCF (to assess $^{-}$OH) or DHE (to assess O_2^{-}) and normalized to number of cells per well. Data demonstrates that short-term zinc adaptation of keratinocytes shows a trend towards reduced basal levels of ROS.

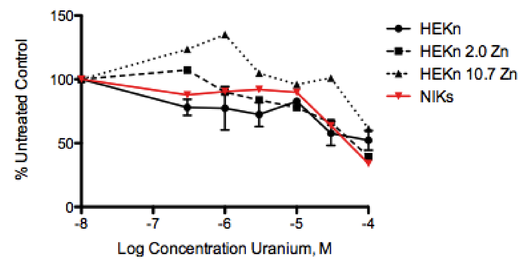


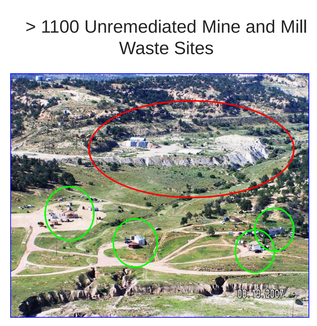
Figure 6: Short-term zinc adaptation slightly attenuates UA induced toxicity. Normal human keratinocytes (HEKn) and normal spontaneously immortalized keratinocytes (NIKs) were exposed to increasing doses of UA for 48 hrs after 6-day zinc adaptation. Relative cell number was assessed using PrestoBlue® cell viability reagent. Data demonstrates that short-term zinc adaptation of keratinocytes reduce metal induced cytotoxicity. In addition, NIKs have a similar response to UA as HEKn and may serve as alternate model for long term zinc adaptation studies.

CONCLUSIONS

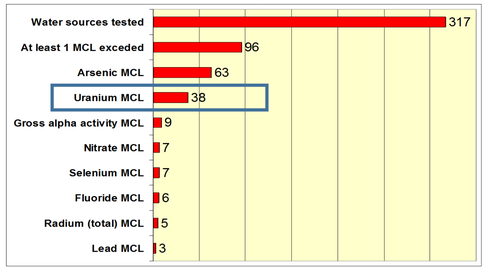
- DNA damaged cells increases sensitivity to UA (Fig. 1A).
- UA enhances DNA damage and damage is retained.(Figs. 1B & 4)
- UA inhibits PARP1 activity via the loss of from the zf domains of the enzyme (Fig. 2).
- In contrast to arsenite, UA is not selective for C3H and C4 zf proteins (Fig. 3).
- supplemental zinc reduces the extent of UA-induced DNA damage retention. (Fig. 4)
- Short-term zinc adaptation reduces basal levels of ROS. (Fig. 5)
- UA induced cytotoxicity is slightly attenuated when cells are adapted to zinc. (Fig. 6)
- NIKS show a similar dose response to UA as HEKn and may serve as an alternate model for long term zinc adaptation studies. (Fig. 6)

SIGNIFICANCE

> 1100 Unremediated Mine and Mill Waste Sites



Navajo Water Sources* Tested Between 2003-2010
DiNEH project, NNEPA, EPA region 9, CDC, Dine College



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