

# Observation of radiation-specific damage in cells exposed to depleted uranium: *hprt* gene mutation frequency

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## Abstract

Depleted uranium (DU) is a dense heavy metal used primarily in military applications. Published data from our laboratory have demonstrated that DU exposure *in vitro* to immortalized human osteoblast cells (HOS) is both neoplastically transforming and genotoxic. Recent animal studies have also shown that DU is leukemogenic and genotoxic. DU possesses both a radiological (alpha particle) and chemical (metal) component. Since DU has a low specific activity in comparison to natural uranium, it is not considered to be a significant radiological hazard. The potential contribution of radiation to DU-induced biological effects is unknown, and the involvement of radiation in DU-induced biological effects could have significant implications for current risk estimates for internalized DU exposure. The purpose of the current study was to measure the induction of mutagenic damage in V79 cells and to determine if radiation plays a role in the induction of that damage. Mutagenicity at the hypoxanthine (guanine) phosphoribosyltransferase (*hprt*) locus was measured by selection with 6-thioguanine. There was a dose-dependent increase in mutagenic response following DU exposure (10–50  $\mu\text{m}$ ); the average increase in mutagenicity above background ranged from  $2.54 \pm 1.19$  to  $8.75 \pm 1.8$  ( $P < 0.05$ ). Using the same concentration (25  $\mu\text{M}$ ) of two uranyl nitrate compounds that have different uranium isotopic concentrations and, therefore, different specific activities, we examined the effect on *hprt* mutant frequency *in vitro*. V79 cells were exposed to either  $^{238}\text{U}$ -uranyl nitrate, specific activity 0.33  $\mu\text{Ci/g}$ , or DU-uranyl nitrate, specific activity 0.44  $\mu\text{Ci/g}$ , delivered at a concentration of 25  $\mu\text{M}$  for 24 h. Results showed, that at equal uranium concentration, a 1.33-fold increase in specific activity resulted in a  $1.27 \pm 0.11$ -fold ( $P < 0.05$ ) increase in *hprt* mutant frequency. Taken together these data support earlier results showing that radiation can play a role in DU-induced biological effects *in vitro*.

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**Keywords:** Depleted uranium; HPRT; Mutagenicity; V79 cells

## 1. Introduction

Several US military personnel participating in Operation Desert Storm were wounded by friendly fire and currently have retained large fragments (approximately 2–20 mm) of depleted uranium (DU) in their bodies. DU used in military applications worldwide could result in soldiers with imbedded heavy metal shrapnel (The Royal Society Report, 2001). Chemically similar

to natural uranium, DU is a low-specific-activity heavy metal, with a density approximately 1.7 times that of lead ( $19 \text{ g/cm}^3$  versus  $11.35 \text{ g/cm}^3$ ). DU differs from natural uranium in that it has been depleted of  $^{235}\text{U}$  and  $^{234}\text{U}$ . As a result, the specific activity of DU is less than natural uranium (0.44  $\mu\text{Ci/g}$  versus 0.7  $\mu\text{Ci/g}$ , respectively) (The Royal Society Report, 2001).

The acute and long-term health effects of exposure to DU are unknown. Our laboratory has used both an *in vitro* human cell model and rodent studies to examine the potential late health effects of DU. These *in vitro* and *in vivo* investigations have demonstrated the transforming ability (Miller et al., 1998a) and the genotoxicity (Miller et al., 2001) of DU as well as aberrant induction of gene expression

Abbreviations: DU, Depleted uranium; V79, Chinese hamster lung cells; DU-UO<sub>2</sub>NO<sub>3</sub> depleted uranium-uranyl nitrate

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(Miller et al., 1996, 2004) and genomic instability (Miller et al., 2003). Additionally, studies demonstrated that DU can cause radiation-specific damage in a cell model system (Miller et al., 2002a, b). Recent *in vivo* studies using mice chronically exposed to embedded DU have demonstrated the redistribution of uranium throughout the body (Pellmar et al., 1999a, b) and most importantly that DU is leukemogenic in a mouse model system (Miller, 2005). The mutagenic potential of DU *in vivo* was suggested in rodent studies which showed that internalized DU caused an increase in urine mutagenicity (Miller et al., 1998b). Since mutations play an important role in the etiology of cancer, the induction of mutations by DU exposure needs to be further explored.

The purpose of the current study was to measure the potential for DU as uranyl nitrate to induce mutations and cell transformation in Chinese hamster lung fibroblast V79 cells and to determine whether radiation plays a role in DU-induced mutagenicity. Mutagenicity at the hypoxanthine (guanine) phosphoribosyltransferase (*hprt*) locus was measured by selection with 6-thioguanine (13). The *hprt* gene codes for the HPRT enzyme that is involved in purine recycling. The *hprt* gene is located on the X chromosome of mammalian cells. The modified purine, 6-TG, also serves as a substrate for the HPRT enzyme. In the presence of a functional enzyme, 6-TG is phosphorylated and subsequently incorporated into DNA, resulting in cell death. Therefore, incubating treated cells with 6-TG allows for selection of cells carrying mutations to the *hprt* gene. Cells that have lost the functional HPRT enzyme will survive in the presence of 6-TG, and cells with functional HPRT enzyme will die. This HPRT assay selects for any mutation that produces a non-functional enzyme including base substitutions and major deletions.

## 2. Materials and methods

Reagents and chemicals: DV as uranyl nitrate (DU-UN) with a  $^{234}\text{U}/^{238}\text{U}$  activity ratio of 0.12 was obtained from Spectrum Chemical Mfg. Corp. (Gardena, CA). An additional isotope of uranium,  $^{238}\text{U}$ -uranyl nitrate, was also obtained. The specific activities of these two uranyl nitrate isotopes were 0.43 and 0.33 for DU and  $^{238}\text{U}$  respectively. Cell culture: Chinese hamster lung fibroblast V79 cells were grown as monolayers in Dulbecco's Eagles Medium (GIBCO) as previously described. Survival and mutation assay: Clonogenic survival was measured as previously described (Miller et al., 1998a, b, 2001, 2002a, b). The mutagenicity of DU-uranyl nitrate in V79 Chinese hamster cells was measured at the *hprt* locus by selection of cells resistant to 6-TG (13). After a 24 h DU treatment cells were harvested and analyzed for clonogenic survival. From the same population, approximately  $10^6$  cells were seeded and that amount was passaged every 3 days until the 9 day post-treatment. Data are expressed as mutants per  $10^6$  surviving cells calculated from the observed 6-TG resistant colonies and the 10 day clonogenic values. Average induced mutant frequency and average mutant increase above background were calculated from the differences and ratios of individual experiments. Experiments were repeated three times. Radiation: Alpha

particle radiation was conducted at the Columbia University Radiation Accelerator Research Facility (RARAF). Alpha particle radiation was performed using the track-segment alpha particle beam (LET 120 keV/ $\mu\text{m}$ ). Because the beam is vertical, the track-segment facility can irradiate attached cells growing in dishes filled with culture medium. The dishes are 3.5 cm i.d. stainless steel rings with 6  $\mu\text{m}$  thick Mylar epoxied onto them. The vertical beam passes through a thin metal foil into the atmosphere, through the Mylar dish bottom, and irradiates the sample attached to the Mylar. A slot-shaped aperture approximately 6 mm wide defines the beam irradiating the samples. A stepping motor rotates a wheel containing up to 20 dishes at a rate defined by the desired dose and the instantaneous beam current striking the beam-defining aperture. Each point on a dish passes through the beam in about 25 steps. The mono-energetic beam of charged particles passes through the thin sample ( $\sim 10$  mm) so that the same segments of the particle tracks are deposited in all the material of interest.

## 3. Results and discussion

### 3.1. Induction of HPRT mutations by DU

The mutagenicity of DU-uranyl nitrate in V79 Chinese hamster cells was measured at the *hprt* locus by selection of cells resistant to 6-TG. After 24 h treatment and a 9 day recovery time to allow for expression of the mutant phenotype, survival of DU-treated cells recovered to 19–90% of control (Table 1). The data demonstrate that DU was mutagenic to V79 cells. V79 cells treated with DU for 24 h produced *hprt* mutants with a positive dose response for doses 10, 25, and 50  $\mu\text{M}$ . These data demonstrate that DU-uranyl nitrate can cause mutagenic damage in V79 cells. The mutagenicity of the alpha particles in V79 cells was also examined for comparison. DU and alpha particle exposure caused a similar increase in magnitude in the induction of HPRT mutants. This does not mean that the mutagenicity induced by DU was due to its alpha particle capability; the current findings of DU-induced *hprt* mutations in V79 cells are, however, consistent with previous studies showing soluble uranium causing cellular and genetic damage in immortalized human cells (Miller et al., 1998a, b, 2001, 2002a, b, 2004; Miller, 2005). Uranyl chloride caused cell death, micronuclei formation, chromosomal aberrations, neoplastic transformation, and genomic instability. Furthermore, uranyl acetate has been shown to form uranium–DNA adducts and *hprt* mutations in CHO cells (Stearns et al., 2005). These data suggest that uranium is chemically genotoxic and mutagenic through the formation of strand breaks. Our data and the previously published data of others (Stearns et al., 2005), do not definitively indicate whether both the chemical and radiation components of DU are involved in the induction of *hprt* mutations. The involvement of alpha particle radiation in DU-induced mutagenicity cannot be assessed using this approach. Since the health risks for uranium exposure could involve both chemically generated damage and radiation damage, another approach was undertaken in our laboratory. This new approach involves using different uranyl nitrate compounds with

Table 1  
Cell survival and *hprt* mutation induction in V79 cells treated with depleted uranium or alpha particles (broad beam, 10–50 μm, 24 h)

Treatment	Survival <sup>a</sup> (%)	Mean number of mutants (per 10 <sup>6</sup> survivors)	Mean induced mutation frequency <sup>b</sup> (mean ± SE)	Ave. increase above background <sup>c</sup> Treatment MF/control/MF
Control	100 <sup>d</sup> ± 4	3.3 <sup>d</sup> ± 3.1	3.2 <sup>d</sup> ± 3.0	1
DU-UO <sub>2</sub> 10 μg/ml (42.5 cGy; 17% <sup>e</sup> )	90 ± 6	18.4 ± 2.7	15.1 <sup>d</sup> ± 2.1	4.7 <sup>d</sup> ± 1.19
DU-UO <sub>2</sub> 25 μg/ml (46.7 cGy; 31% <sup>e</sup> )	81 ± 6	44.6 ± 5.9	41.3 ± 4.5	13.2 ± 1.36
DU-UO <sub>2</sub> 50 μg/ml (51.5 cGy; 66% <sup>e</sup> )	19 ± 4	88.9 ± 8.2	85.6 ± 7.8	25.71 ± 2.8
Alpha particles 5 cGy	90 ± 7	5.1 ± 2.1	1.8 ± 0.9	1.54 ± 0.77
Alpha particles 10 cGy	82 ± 7	9.9 ± 3.1	6.3 ± 2.1	3.0 ± 1.0
Alpha particles 20 cGy	22 ± 5	38.2 ± 5.9	34.9 ± 5.2	11.58 ± 1.90

<sup>a</sup>Colony formation at 9 days.

<sup>b</sup>Treatment mutant frequency—Control mutant frequency per 10<sup>6</sup> viable cells; mean ± standard error of the mean from 6 individual experiments.

<sup>c</sup>Treatment MF/control/MF mean from ratios in individual experiments.

<sup>d</sup>Mean ± standard error.

<sup>e</sup>Average energy of alpha particles and probability of nuclei being traversed by one alpha particle based on Poisson analysis.

differing specific activities. Using this approach we could hold the uranium concentration constant while varying the specific activity.

### 3.2. Effect of uranium isotopic concentration on mutagenicity

The *hprt* mutation frequency was used as the endpoint to determine the effect of cellular exposure to uranium compounds delivered at equal chemical concentration but with different specific activities. V79 cells were exposed to soluble uranyl nitrate compounds with different isotopic concentrations (<sup>238</sup>U-uranyl nitrate, specific activity 0.33 μCi/g, or DU-uranyl nitrate, specific activity 0.44 μCi/g) delivered at a concentration of 25 μM for 24 h. For these uranium compounds microdosimetric calculations have estimated the alpha particle equivalent dose to these cells within 24 h in 25 μM to be 35 or 46 cGy, respectively. The *hprt* mutation frequency was determined as we showed previously in the current work and using established procedures (Miller et al., in press; Stearns et al., 2005). The results in Fig. 1 demonstrate that there was a specific activity-dependent increase in *hprt* mutagenicity frequency under experimental conditions where the uranium concentration in each uranyl nitrate compound was the same (25 μM). *hprt* mutagenicity measurements indicate that there was also a specific activity-dependent increase in *hprt* mutagenicity ( $P < 0.05$ ). Results showed that, at equal uranium concentration, a 1.33-fold increase in specific activity resulted in a  $1.27 \pm 0.11$ -fold ( $P < 0.05$ ) increase in *hprt* mutant frequency.

The statistically significant difference in both *hprt* mutation frequency observed in cells treated with a DU versus a <sup>238</sup>U compound with equal chemical effect suggests that the difference in the frequency was due to the increased radioactivity in the uranium compound tested. Similar to our results previously published, demonstrating dicentric results in human cells (Miller et al., 2002a, b), the *hprt* studies shown here further suggest that radiation plays a role in the DU-induced cellular effects.

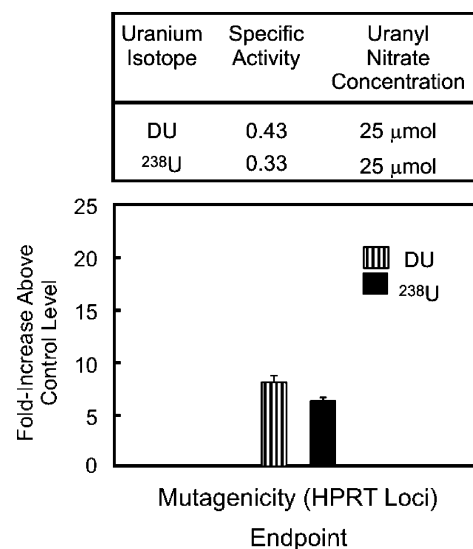


Fig. 1. Equal chemical effect with increasing specific activity *hprt* mutations. Uranyl nitrate compounds that were either pure <sup>238</sup>U or DU were used. Exponentially growing V79 cells were exposed to uranyl nitrate compounds (25 μM) with specific activities of 0.33 or 0.44 μCi/g, respectively, for 24 h. *hprt* mutagenicity was determined as previously described ( $P < 0.05$ ).

Although the data indicate that radiation is involved in DU effects *in vitro*, several questions remain unanswered. We neither know the extent to which radiation contributes to the effects exerted by DU nor understand its mechanism(s). Furthermore, we can only speculate as to whether the radiation and chemical effects are synergistic. Limited studies have shown that a nonradioactive metal like cadmium combined with gamma radiation can result in a synergistic response *in vivo* (Prise et al., 1998). It is intriguing to ask whether radiation actually plays a significant role in DU cellular effects perhaps through non-targeted effects of radiation exposure. Several recent radiation studies have demonstrated the important role that bystander effects have in cellular radiation response by causing damage in

unirradiated neighboring cells (Prise et al., 1998; Zhou et al., 2000; Belyakov et al., 2001; Sawant et al., 2001). In the case of DU, cells not traversed by an alpha particle may be vulnerable to radiation-induced effects as well as chemically induced effects.

While the data presented here do not fully and definitively answer the question as to the contribution of radiation-induced damage in DU cellular effects, they do provide additional evidence of radiation involvement in the cellular effects of DU and, therefore, potentially in DU-associated health effects. Considering that conventional understanding of potential DU health effects assumes that chemical effects are of greatest concern, these results could have a significant impact on DU risk assessments.

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