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OBSERVATION OF RADIATION-SPECIFIC DAMAGE IN HUMAN CELLS EXPOSED TO DEPLETED URANIUM: DICENTRIC FREQUENCY AND NEOPLASTIC TRANSFORMATION AS ENDPOINTS

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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 immortalised human osteoblast cells (HOS) is both neoplastically transforming 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 internalised DU exposure. Two approaches were used to address this question. The frequency of dicentrics was measured in HOS cells following DU exposure *in vitro*. Data demonstrated that DU exposure (50 μM, 24 h) induced a significant elevation in dicentric frequency *in vitro* in contrast to incubation with the heavy metals, nickel and tungsten which did not increase dicentric frequency above background levels. Using the same concentration (50 μM) of three uranyl nitrate compounds that have different uranium isotopic concentrations and therefore, different specific activities, the effect on neoplastic transformation *in vitro* was examined. HOS cells were exposed to one of three uranyl nitrate compounds (^{234}U-uranyl nitrate, specific activity 0.33 μCi.g^{-1}; DU-uranyl nitrate, specific activity 0.44 μCi.g^{-1}; and ^{235}U-uranyl nitrate, specific activity 2.2 μCi.g^{-1}) delivered at a concentration of 50 μM for 24 h. Results showed, at equal uranium concentration, there was a specific activity dependent increase in neoplastic transformation frequency. Taken together these data suggest that radiation can play a role in DU-induced biological effects *in vitro*.

INTRODUCTION

Several US military personnel participating in Operation Desert Storm were wounded in friendly fire accidents 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. Chemically similar to natural uranium[1], DU is a low specific activity heavy metal, with a density approximately 1.7-times that of lead (19 g.cm^{-3} as against 11.35 g.cm^{-3}). DU differs from natural uranium in that it has been depleted of ^{235}U and ^{234}U. As a result, the specific activity of DU is significantly less than natural uranium (0.44 μCi g^{-1} rather than 0.7 μCi.g^{-1}, respectively)[2].

The acute and long-term health effects of exposure to these heavy metals are unknown. Our laboratory has used both an *in vitro* human cell-model and rodent studies to examine the potential late health effects of these heavy metals. Data from our laboratory have demonstrated that DU is neoplastically transforming and genotoxic *in vitro*. The *in vivo* effects of internalised DU include enhancement of urine mutagenicity, oncogene activation, and uranium redistribution to multiple organs. A review of our findings is shown in Table 1[3–12].

DU, unlike natural uranium, which is considered to be both a radiological and a chemical (heavy-metal) hazard[13], is not believed to be a significant radiation hazard because of its low specific activity. Studies with DU in our laboratory demonstrated neoplastic transformation of human cells under conditions in which approximately 14% of the DU-exposed cells were transformed but with less than 5% of the DU-exposed cells actually being traversed by an alpha particle[4,8,9]. These findings suggest several possible explanations. First, the chemical effect of DU could be primarily responsible for the transforming effects. Alternatively, alpha particles could be involved in the transformation process because of the involvement of non-targeted effects like the bystander effect or induction of genomic instability. These non-targeted effects can result in damage in cells not traversed by an alpha particle. Lastly, it could be that DU transformation involves chemical, targeted radiation, and non-targeted radiation effects. Whatever the mechanism, the involvement of radiation in DU-induced biological responses could have potential implications for current risk estimates for internalised DU exposure.

Therefore, a study has been undertaken to address the question as to whether radiation plays a role in DU-induced damage. First an examination is carried out to determine whether DU exposure can induce dicentrics, a lesion believed to be primarily induced by radiation or

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radiation-mimetic drugs. Secondly, three uranyl nitrate compounds have been obtained that have different uranium isotopic concentrations and therefore, different specific activities. Using these compounds, that each have equal chemical effect, the contribution of increased specific activity at constant uranium-chemical effect can be assessed.

In this study, it is demonstrated that DU exposure to human cells *in vitro* induces dicentrics in human cells. Uranyl nitrate compounds at the same chemical concentration, but different specific activities, caused a specific activity-dependent increase in neoplastic transformation. Taken together, these data suggest that radiation plays a role in DU effects observed *in vitro*.

MATERIALS AND METHODS

Cell culture

Human osteoblast cells (HOS), an immortalised attached cell line, were cultured and used as previously described[14,8–15]. Exponentially growing HOS were used in all experiments.

Transformation

The standard focus formation assay described by Reznikoff *et al*[15] and others[17] was used. Transformation and growth studies have been previously described in detail by this laboratory[7].

Dicentric analysis

Measurement of dicentrics was previously described by this laboratory[5–6]. Briefly, cells were incubated with colchicines and then fixed onto slides. Chromosomes were stained with giemsa and dicentric chromosomes were counted in 150–500 chromosome spreads.

RESULTS AND DISCUSSION

Induction of dicentrics by DU

Human cells were incubated with DU-uranyl nitrate and then processed for dicentric analysis.. Nickel and tungsten were used as nonradioactive, metal controls. HOS cells were exposed to soluble DU (0–50 μM) for 24 h. In a parallel experiment, cells were incubated with nickel sulphate (0–50 μM, 24 h), a known carcinogen and transforming agent without mutagenic activity *in vitro*. The frequency of dicentrics measured after incubation with DU or nickel is shown in Figure 1. A DU-concentration-dependent increase in the yield of dicentrics was observed. Microdosimtery calculations indicate that there was a yield of approximately 0.30 dicentrics per cell and per Gy. In contrast, there was no significant increase in dicentric frequency in cells exposed to nickel (Figure 1). Since dicentrics are prim-

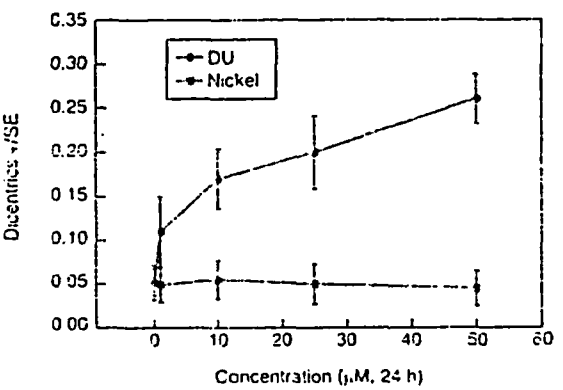

Figure 1. Dicentrics induced in human cells by DU exposure. Dicentrics were scored in exponentially growing human HOS cells following exposure to DU-uranyl nitrate (●) or nickel sulphate (◆). Data are shown as number of dicentrics ± standard error.

Table 1. Summary of findings on DU and HMTA.

Metal; Type of Study	Parameters Examined	Outcome	Authors
DU, *in vivo*	Oncogenes, uranium levels	↑ Oncogene expression with DU	Miller *et al*. (1996)
DU, *in vitro*	Neoplastic transformation	DU induced neoplastic transformation	Miller *et al*. (1998)
DU, *in vivo*	Urine mutagenicity	↑ Urine mutagenicity with DU level	Miller *et al*. (1998)
DU, *in vivo*	DU tissue distribution	DU redistributed to multiple sites	Pellmar *et al*. (1999)
DU, *in vivo*	Neurotoxicity	Electrophysiological brain effects	Pellmar *et al* (1999)
DU, HMTA *in vitro/in vivo*	Genotoxicity	↑ genotoxicity/genomic instability	Miller *et al*. (2000)
DU, *in vitro*	Transformation	↑ transformation/mutagenicity	Miller *et al*. (2001)
HMTA, *in vitro*	Transformation/genotoxicity	HMTA induces transformation	Miller *et al*. (2001)
DU, HMTA, *in vitro*	Genomic instability	↑ genotoxicity/genomic instability	Miller, (in press)

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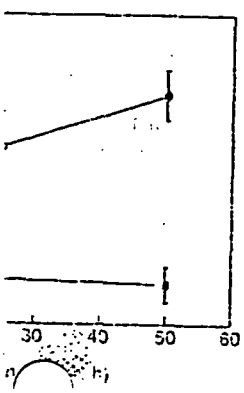

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arily induced by exposure to radiation it can be speculated that DU exposure *in vitro* can cause radiation-specific damage. Our conclusions are supported by the observation that both nickel (Figure 1) and tungsten (data not shown) were unable to increase the dicentric frequency above background levels. The occurrence of dicentrics following DU exposure does not, however, definitively answer the questions as to whether alpha particle radiation is involved in DU-induced biological effects since radiomimetic chemicals like bleomycin can induce dicentrics. These data are the first known evidence of the possibility that radiation contributes to DU effects *in vitro*.

Effect of uranium isotopic concentration on neoplastic transformation

Neoplastic transformation was used as an endpoint to determine the effect of cellular exposure to uranium compounds delivered at equal chemical concentration but with different specific activities. HOS cells were exposed to one of three-uranyl nitrate compounds (^{238}U-uranyl nitrate, specific activity 0.33 μCi.g^{-1}; DU-uranyl nitrate, specific activity 0.44 μCi.g^{-1}; and ^{235}U-uranyl nitrate, specific activity 2.2 μCi.g^{-1}) delivered at a concentration of 50 μM for 24 h. For these uranium compounds microdosimetric calculations have estimated the alpha particle equivalent dose to these cells within 24 h in 50 μM to be, 35 cGy, 46 cGy, and 227.5 cGy, respectively. The spontaneous transformation frequency of HOS cells was also measured. The cells were then processed to determine the number of transformants. The results in Figure 2 demonstrate that there was a specific activity-dependent increase in transformation frequency under experimental conditions where the uranium concentration in each uranyl nitrate compound was the same (50 μM). The transformation frequencies resulting from exposure to ^{238}U-uranyl nitrate, DU-uranyl nitrate, or ^{235}U-uranyl nitrate were (84.5 ± 6.7) × 10^{-4} per surviving cell, (110.5 ± 8.6) × 10^{-4} per surviving cell for DU-uranyl nitrate, and (727.3 ± 55.2) × 10^{-4} per surviving cell, respectively. The spontaneous transformation frequency of HOS cells was (5.2 ± 0.51) × 10^{-4} per surviving cell. The statistically significant difference in transformation frequency observed in cells treated with a DU versus a ^{238}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 the dicentric results, the transformation studies suggest that radiation plays a role in the DU-induced cellular effects.

Although the data indicate that radiation is involved in DU effects *in vitro*, several questions remain unanswered. The extent to which radiation contributes to the effects exerted by DU is not known nor its mechanism (s) understood. Furthermore, one can only speculate as to whether the radiation- and chemical-effects are synergistic. Limited studies have shown that a non-radioactive metal like cadmium combined with gamma radiation can result in a synergistic response *in vivo*[18]. It is intriguing to ask whether radiation actually play a significant role in DU cellular effects perhaps through nontargeted 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[16–23]. 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 the first 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 and similar future results could have a significant impact on DU risk assessments.

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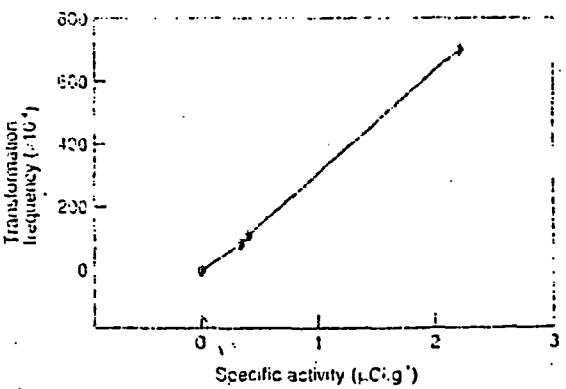

Figure 2 Equal chemical effect with increasing specific activity: neoplastic transformation. Uranyl nitrate compounds that were either pure ^{238}U, DU, or ^{235}U were used. Exponentially growing HOS cells were exposed to uranyl nitrate compounds (50 μM) with specific activities of 0.33 μCi.g^{-1}, 0.44 μCi.g^{-1}, or 2.2 μCi.g^{-1}, respectively for 24 h. The spontaneous transformation frequency for untreated HOS cells (specific activity 0 μCi.g^{-1}) is also shown. Cells were rinsed and prepared for neoplastic transformation assay as described[14].

REFERENCES

1. NRC. *Biological Effects of Ionizing Radiation (BEIR) IV. Health Risks of Radon and Other Internally Deposited Alpha-emitters.* National Research Council Committee on the Biological Effects of Ionizing Radiation. (Washington: U.S. National Research Council) (1988).
2. Danesi, M. E. *Kinetic Energy Penetrator Long Term Strategy Study (Abridged).* (Picatinny, NJ: US Army Armament Munitions and Chemical Command — AMCCOM, Picatinny Arsenal) (1990).
3. Miller, A. C., Whittaker, T., Hogan, J., McBride, S. and Benson, K. *Oncogenes as Biomarkers for Low Dose Radiation-induced Health effects.* Cancer. Detect. Prev 20(5). 235–236 (1996)
4. Miller, A. C. and 10 others. *Depleted Uranium-Uranyl Transformation of Human Osteoblast Cells to the Tumorigenic Phenotype by Depleted Uranium Chloride.* Environ. Health Perspect. 106, 465–471 (1998).
5. Miller, A. C., Fuciarelli, A. F., Jackson, W. E., Ejnik, E. J., Emond, C., Strocko, S., Hogan, J., Page, N. and Pellemar, T. *Urinary and Serum Mutagenicity Studies with Rats Implanted with Depleted Uranium or Tantalum Pellets* Mutagenicity 13, 643–648 (1998).
6. Pellmar, T. C., Fuciarelli, A. F., Ejnik, J. W., Hamilton, M., Hogan, J., Strocko, S., Emond, C., Mottaz, H. M. and Landauer, M. R. *Distribution of Uranium in Rats Implanted with Depleted Uranium Pellets.* Toxicol. Sci. 49, 29–39 (1999).
7. Pellmar, T. C., Kaiser, D. O., Emond, C. and Hogan, J. B. *Electrophysiological Changes in Hippocampal Slices Isolated from Rats Embedded with Depleted Uranium Fragments.* Neurotoxicol. 20, 785–792 (1999).
8. Miller, A. C., Xu, J., Stewart, M., Emond, C., Hodge, S., Matthews, M., Kalanich, J. and McClain, D. *Potential Health Effects of the Heavy Metals, Depleted Uranium and Tungsten, Used in Armor-Piercing Munitions: Comparison of Neoplastic Transformation, Mutagenicity, Genomic Instability, and Oncogenesis.* Metal Ions 6, 209–211 (2000).
9. Miller, A. C., Xu, J., Whittaker, T., Stewart, M. and McClain, D. *Suppression of Depleted Uranium Induced Neoplastic Transformation of Human Cells by the Phenyl Fatty Acid Phenylacetate.* Radiat. Res. 155(1 Pt 2). 163–170 (2001).
10. Miller, A. C. and 10 others. *Neoplastic Transformation of Human Osteoblast Cells to the Tumorigenic Phenotype by Heavy-Metal Tungsten-Alloy Particles: Induction of Genotoxic Effects.* Carcinogenesis 22(1), 115–125 (2001).
11. Miller, A. C., Xu, J., Prasanna, P. G. S. and Page, N. *Potential Late Health Effects of the Heavy Metals, Depleted Uranium and Tungsten, Used in Armor Piercing Munitions: Comparison of Neoplastic Transformation and Genotoxicity Using the Known Carcinogen Nickel.* Milit. Med. (in press) (2001).
12. McClain, D. E. and 17 others. *Health Effects of Embedded Depleted Uranium.* Milit. Med. (in press) (2001).
13. Lin, X. and Costa, M. *Transformation of Human Osteoblasts to Anchorage-independent Growth by Insoluble Nickel Particles.* Environ. Health Perspect. 102. 289–294 (1994).
14. Rhim, J. S., Par, D. K., Arnstein, P., Huebner, R. J., Weisburger, E. K. and Nelson-Rees, W. A. *Transformation of Human Cells in Culture by N-methyl-N'-nitro-N-nitrosoguanidine.* Nature 256, 751–753 (1975).
15. Miller, A. C., Kariko, K., Myers, C. E., Clark, E. P. and Samid, D. *Increased Radioresistance of Elras-transformed Human Osteosarcoma Cells and its Modulation by Lovastatin, an Inhibitor of $p21^{ras}$ Isoprenylation.* Int. J. Cancer 53, 302–307 (1993).
16. Reznikoff, C. A., Bertram, J. S., Brankow, D. W. and Heidelberger, C. *Quantitative and Qualitative Studies of Chemical Transformation of Cloned C3H Mouse Embryo Cells Sensitive to Postconfluence Inhibition of Cell Division.* Cancer Res. 33, 3239–3249 (1973).
17. IARC/NCI/EPA Working Group. *Cellular and Molecular Mechanisms of Cell Transformation and Standardization of Transforming Assays of Established Cell Lines for the Prediction of Carcinogenic Chemicals: Overview and Recommended Protocols.* Cancer Res. 45, 2395–2399 (1985).
18. Prise, K., Belyakov, O. V., Folkard, M. and Michael, B. D. *Studies of Bystander Effects in Human Fibroblasts using a Charged Particle Microbeam.* Int. J. of Radiat. Biol. 74(6), 793–798 (1998).
19. Zhou, H., Randers-Pehrson, G., Waldren, C. A., Vannais, D., Hall, E. J. and Hei, T. K. *Induction of a Bystander Mutagenic Effect of Alpha Particles in Mammalian Cells.* Proc. Natl. Acad. Sci. USA. 97(5): 2099–2104 (2000).
20. Little, J. B. *Radiation Carcinogenesis.* (Review) Carcinogenesis. 21(3), 397–404 (2000).
21. Mothersill, C. and Seymour, C. *Genomic Instability, Bystander Effects and Radiation Risks: Implications for Development of Protection Strategies for Man and the Environment.* (Review) Radiat. Biol. Radioecol. 40(5), 615–620 (2000).
22. Belyakov, O. V., Malcolmson, A. M., Folkard, M., Prise, K. M. and Michael, B. D. *Direct Evidence for a Bystander Effect of Ionizing Radiation in Primary Human Fibroblats.* Br. J. Cancer. 84(5), 674–679 (2001).
23. Sawant, S. G., Randers-Pehrson, G., Geard, C. R., Brenner, D. J. and Hall, E. J. *The Bystander Effect in Radiation Oncogenesis: I. Transformation in C3H 10T1/2 Cells in vitro can be Initiated in the Unirradiated Neighbors of Irradiated Cells.* Radiat. Res. 155(3). 397–401 (2001).

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POSSIBLE BIOLOGIC OF RADIA

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Abstract — Possible dose hypersensitivity acute exposures, whe radiobiological repor survival response at process of carcinoge for some time and th data on the lung car low doses.

INTRODUCTION

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