Miller et al 2005

Advances in Biology and Medicine, vol 5 Eds J.A. Centeno, Ph. Collery, C. Vernet, R.B. Finkelman, H. Gibb, J.C. Etienne John Libbey Eurotext, Paris © 2003, pp. 209-211.

Potential health effects of the heavy metals, depleted uranium and tungsten, used in amor-piercing munitions: comparison of neoplastic transformation, mutagenicity, genomic instability, and oncogenesis

Alexandra C. Miller, Jiaquan Xu, Michael Stewart, Christine Emond, Shelly Hodge, Consuelo Matthews, John Kalanich, David McClain

Armed Forces Radiobiology Research Institute (AFRRI), Bethesda, MD 20889

Abstract: The use of the heavy metals, depleted uranium (DU) and tungsten alloys (HMTA) in military applications worldwide could result in soldiers with imbedded heavy metal shrapnel. The acute and long-term health effects of exposure to these heavy metals are unknown. We have used both an *in vitro* human cell-model and rodent studies to examine the potential late health effects of these heavy metals. Data demonstrate that DU and HMTA are transforming and genotoxic agents *in vitro*. The *in vivo* effects of internalized DU include enhancement of mutagenicity and oncogene activation. Further carcinogenesis studies are warranted.

Introduction Limited data exist to permit an accurate assessment of risks for carcinogenesis and mutagenesis from depleted uranium (DU) embedded fragments or inhaled particulates. Ongoing studies are designed to provide information about the carcinogenic potential of DU using *in vitro* and *in vivo* assessments of morphological transformation, cytogenetic, mutagenic, and oncogenic effects (1-5). As a comparison, other military-projectile metals, i.e., tungsten alloys, and the known carcinogen, i.e., nickel, are being examined.

Materials and Methods: Human osteoblast cells (HOS) were cultured and used as previously described (1-5). DU-uranium oxide (50mg powder/ml) was used in all experiments. To mimic the tungsten alloy used in military applications, a pure mixture of W (92%), Ni (5%), and Co (3%) particles (made in the laboratory without extensive milling) was used This mixture is hereafter called HMTA. All other methods are detailed elsewhere (1-5).

Results and Discussion Quantitative and qualitative *in vitro* transformation studies were done to assess the carcinogenic potential of radiation and chemical hazards. Using a human cell model (HOS), we demonstrated that soluble and insoluble DU compounds can transform cells to the tumorigenic phenotype, characterized by morphological, biochemical, and oncogenic changes consistent with tumor cell behavior (Table I and 2,5). Specific changes include altered saturation density and increased clonability in soft-agar. Similarly, tungsten alloys, and nickel were also shown to be neoplastic transforming agents, although at a reduced frequency than DU (Table I). Furthermore, DU and tungsten alloys were shown to be genotoxic using the sister chromatid exchange (SCE), micronuclei, and alkaline filter elution assays. Exposure to a nontoxic, nontransforming dose of DU did induce a small but statistically significant increase in the number of dicentrics formed in cells.

Table I. HEAVY METAL-INDUCED NEOPLASTIC TRANSFORMATION, MUTAGENICITY, CYTOGENICITY, AND ONCOGENESIS : IN VITRO AND IN VIVO STUDIES

	DU	HMTA	Nickel	Tantalum
In vitro studies				
Transformation Frequency	↑	↑	↑	No change
Growth Rate	↑	↑	↑	No change
Tumorigenicity	↑	↑	↑	No change
Mutagenicity	↑	↑	↑	No change
Micronuclei	↑	↑	↑	No change
Chromosome Aberration	↑	↑	↑	No change
DNA Breakage	↑	↑	↑	No change
Dicentric Formation	↑	none	none	Nd[1]
In vivo studies				
Uranium levels	↑	Nd	Nd	No change
Mutagenic Urine	↑	Nd	Nd	Nd
Ras Oncogene	↑	Nd	Nd	Nd
P53 Alteration	↑	Nd	Nd	Nd
BCl2 Alteration	↑	Nd	Nd	Nd

[1]Nd, not done

DU was shown to induce genomic instability, possibly linked to its tumorigenic potential or its ability to induce a short-term increased resistance to oxidative stress.

Studies with animals with embedded DU pellets demonstrated that increased tissue uranium content is associated with aberrant activation of several oncogenes and tumor suppressor genes associated with human carcinogenesis (Table I). Northern blot analysis of mRNA obtained from muscle tissue proximal to the implanted DU, showed an elevation in *kras*, *bcl*-2 , and p53 gene expression. In contrast, tissues from animals with tantalum implants did not show this aberrant oncogene pattern. Using the Ames bacterial reversion assay, the mutagenic activity of DU was evaluated. Mutagenicity tests with animal urine indicate that urine with a high uranium content is mutagenic. This enhanced DU-induced mutagenicity in urine was both DU-dose and time- dependent (1).

The results in this study demonstrate that insoluble DU and HMTA can transform human cells to the tumorigenic phenotype. The mechanism of this transformation involves DNA damage induction and chromosomal damage. DU can induce chromosomal aberrations that are distinctly characteristic of radiation exposure suggesting that the alpha particle component of DU exposure may play a role in the transformation and genotoxic process. Interestingly, the results with DU and HMTA are consistent with those obtained with the known carcinogen, nickel. In contrast, the inert metal, tantalum, did not induce tumorigenic, mutagenic, or cytogenetic effects.

The *in vivo* results demonstrate that 1) DU is a mutagen and 2) that the enhanced urine-mutagenicity may be a useful marker of DU exposure. While the changes in oncogene and tumor suppressor gene status are inconclusive, this altered activity could

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	No change
	No change
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indeed be a preneoplastic marker. Further studies are warranted to fully understand the potential late health effects caused by exposure to DU or tungsten alloys used in armor-piercing munitions. Taken together, these results suggest that long-term exposure to DU or HMTAs could potentially be critical to the development of neoplastic disease in humans.

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Acknowledgements: The authors would like to thank Dr Terry Pellmar, Dr David Livengood, and Dr. John Ejnik for their invaluable assistance. This research was supported in part by the Armed Forces Radiobiology Research Institute. The views presented are those of the authors and do not reflect the official views of the Department of Defense or the U.S. government.