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The Persian Gulf War resulted in friendly fire casualties among U.S. personnel injured by fragments of depleted uranium (DU) munitions. The demonstrated effectiveness of such weapons makes it likely that they may be used against U.S. forces in future conflicts. Uncertainty about how aggressively to remove fragments of the radioactive, chemically toxic DU has stimulated research into the long-term health consequences of embedded DU fragments. There has been no previous research to determine whether long-term exposure to embedded DU can affect the health of offspring of personnel wounded by DU. This study investigates whether male mice carrying embedded fragments of DU transmit genetic damage to their offspring. We hypothesize that long-term chronic exposure to embedded DU results in paternal transmission of genetic damage to unexposed F1 generation offspring, characterized by increased frequency of in vivo mutations in tissues. During this the second year of this project, we have completed metal (DU and tungsten) implantation surgeries, completed breeding of DU-implanted mice, completed neutron-irradiations, initiated breeding of tungsten-implanted mice, initiated the mutation assay and initiated the DNA damage assay to assess direct DNA damage in germ cells

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INTRODUCTION

The Persian Gulf War resulted in a number of friendly fire casualties among U.S. personnel injured by fragments of depleted uranium (DU) munitions. The demonstrated effectiveness of such weapons makes it likely that they may be used against U.S. forces in future conflicts. Uncertainty about how aggressively to remove fragments of the radioactive, chemically toxic DU has stimulated research into the long-term health consequences of embedded DU fragments. There has been no previous research to determine whether long-term exposure to embedded DU can affect the health of offspring of personnel wounded by DU. This study investigates whether male mice carrying embedded fragments of DU transmit genetic damage to their offspring. Other studies have demonstrated that paternal preconceptional exposure to radiation or metal can induce cancer in unexposed offspring. The transgenic mouse model proposed for this study allows us to estimate overall mutation frequency in tissues from progeny of exposed males by following mutations in lacI gene. We hypothesize that long-term chronic exposure to embedded DU results in paternal transmission of genetic damage to unexposed F1 generation offspring, characterized by increased frequency of in vivo mutations in tissues. Transgenerational transmission of genetic damage may occur because of an induction of germ-line genomic instability and/or direct sperm mutations. Results of this study will provide new information about potential health effects of embedded fragments of "non-traditional" metals such as DU will allow an update of existing fragment removal policies.

BODY

This report summarizes accomplishments for the second year of this. A request has been made to obtain a one-year no-cost extension for this project so that the work can be completed. The approved Statement of Work for year 2 is as follows.

- **Complete implantation surgeries for subgroups**
- **Initiate and complete subgroup analysis for tissue mutation frequency, metal content, and sperm DNA analysis**
- **Complete harvesting of tissues from F1 progeny of implanted fathers**
- **Complete analysis of mutation frequency in bone marrow of F1 progeny**
- **Complete analysis and assessment of tissues from nonsurgical control rats**
- **Complete necropsy and histology for all animals**

While the project was originally planned as a two-year project, several delays that we experienced, mean that it will require three years to complete instead. To accomplish this, I requested and was granted a one-year no-cost extension.

Initiation of the project was delayed for 4-5 months and subsequent initial progress was slower than expected for several reasons. First, there were procurement problems with the tungsten pellets. This procurement problem initially arose because of our decision to choose a different vendor than originally planned. The change, however, allowed us to obtain superior tungsten pellets at less cost, but it also delayed our receiving them. The tungsten pellet procurement problem has been ongoing and was not remedied until October 2003. The supplier indicated that they had competing needs for tungsten from both operational military and military research laboratories. Second, the Institutional Animal Care and Use Committee did not approve the project until June 2002. Once the IACUC approval process was initiated at USAMRMC it was expeditiously completed. Third, the licensing agreement between us and the transgenic mouse provider (Stratagene, Inc., La Jolla, CA) was delayed until all institutional animal use approvals were completed. The licensing agreement was therefore, did not become effective until August 2002. There have also been delays in the measurement of DU and tungsten in vivo, due to mechanical problems with the equipment needed for this measurement (Inductively-coupled plasma mass spectrometer).

We have made significant progress on this project however. While the first year delays meant that we were late initiating implantations of the DU pellets, we have completed the breeding process (Dec 2003) with all control, neutron, tantalum, and DU groups. The implantations of the tungsten pellets have also been completed and the breeding process will be completed in July 2004. We have determined the average litter size for the F1 generation in each treated group and the data are shown in tabular form in the Research Accomplishments section (Table I). The genotyping of the F1 offspring has also been initiated for all groups and data are shown in Table II in the Research Accomplishments section. We have initiated the harvesting of tissues from control-, neutron-irradiated-, and tantalum-implanted- animals and initiated the analysis of the bone marrow in these animals. To increase project productivity, I have trained additional personnel to work on the molecular analyses portion of this study. By adding another technical-molecular biology assistant to help with this project (at no cost to the grant) we expect that we will be able to double the mutagenicity analyses work effort and reduce the time needed to complete this work. Experimental conditions for the DNA damage assay "Comet assay" have also been defined and established; this will allow us to measure the potential DNA damage in testes tissue obtained from control and treated animals. Initial studies have been completed required to calibrate the DNA damage assay.

Table I. Average Number of Offspring per Litter

Time Post Pellet Implantation	Control	Ta	DU-L	DU-H
1 month	5.4	7.2	6	5.2
4 months	6.5	8.3	7.4	7
7 months	5.6	7.6	4.2	4.0

Table I. Transgenic "Big Blue" male mice hemizygous for the *lac*I gene were mated to nontransgenic C57BL/6 female mice to obtain F1 offspring. Breeding occurred at 1, 4, and 7 months post-metal implantation. At birth the F1 offspring were examined and the number of animals per litter was determined. The data are shown in Table I for offspring from males implanted with DU (low dose), DU (high dose), and tantalum (high dose). Controls (no metal implants) are also shown. Data were collected from offspring of Litter sizes of offspring from neutron irradiated males averaged 5.1 animals/litter (not shown).

Table II. Genotyping of Offspring for Transmission of the *lac*I Gene: Paternal Exposure Thirty Days (30 days)

C57BL/6 X Big Blue	Control	Ta	DU-L	DU-H
No. Screened for *Lac*I gene	57	61	48	52
No. *lacI* carriers	26	27	14	27
% *lac*I carriers (observed)	45.6	44.2	29.1	51.9

Table II. Genotyping was performed to determine which offspring carry the transgene (lacI). Genomic DNA was isolated from the tail-tip of offspring and screened by PCR analysis to identify carriers of the lacI gene. Data was compared to DNA from transgenic fathers (+lacI) and nontransgenic mothers (-lacI). The overall transmission rate for the Control, Tantalum, and DU-high groups was comparable to the expected Mendelian rate of 50% transmission for a hemizygous gene. The transmission rate for the DU-low dose group was statistically lower than the expected rate.

REPORTABLE OUTCOMES

None to date.

CONCLUSIONS

Even though the planned initiation of our experiments was delayed by several months, the project is proceeding as planned. I have requested a one-year no-cost extension to this project. Significant progress has been made and we have completed breeding of the DU-exposed mice, initiated key mutagenicity assays, and established the Genotoxicity assay. We have collected data on litter size and completed the majority of the genotyping analysis.