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# Depleted uranium-catalyzed oxidative DNA damage: absence of significant alpha particle decay

Alexandra C. Miller*, Michael Stewart, Kia Brooks, Lin Shi, Natalie Page

*Applied Cellular Radiobiology Department, Armed Forces Radiobiology Research Institute, 8901 Wisconsin Ave., Bethesda, MD 20889-5603, USA*

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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. DU possesses both a radiological (alpha particle) and a 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. In the current study we demonstrate that DU can generate oxidative DNA damage and can also catalyze reactions that induce hydroxyl radicals in the absence of significant alpha particle decay. Experiments were conducted under conditions in which chemical generation of hydroxyl radicals was calculated to exceed the radiolytic generation by $10^6$-fold. The data showed that markers of oxidative DNA base damage, thymine glycol and 8-deoxyguanosine could be induced from DU-catalyzed reactions of hydrogen peroxide and ascorbate similarly to those occurring in the presence of iron catalysts. DU was 6-fold more efficient than iron at catalyzing the oxidation of ascorbate at pH 7. These data not only demonstrate that DU at pH 7 can induced oxidative DNA damage in the absence of significant alpha particle decay, but also suggest that DU can induce carcinogenic lesions, e.g. oxidative DNA lesions, through interaction with a cellular oxygen species. Published by Elsevier Science Inc.

*Keywords:* Depleted uranium; Oxidative; DNA damage; Human osteoblast; 8-Hydroxydeoxyguanosine; Thymine glycol

## 1. Introduction

Several US military personnel participating in Operation Desert Storm were wounded in friendly fire accidents and currently have retained large fragments (~2–20 mm) of depleted uranium (DU) in their bodies. The use of DU 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 ~1.7-times that of lead (19 vs. 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 vs. 0.7 $\mu Ci/g$, 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 internalized 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 [1], 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 ~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]. While these findings might suggest that the chemical component of DU could be primarily responsible for the transforming effects, recent cellular transformation and cytogenetic findings from our laboratory have shown that alpha particles are involved in the neoplastic transformation process [13]. Furthermore, the involvement of 'bystander effects' cannot be ruled out. Bystander effects, whereby cells that are not directly

*Corresponding author. Tel.: +1-301-295-9232; fax: +1-301-295-0292.

*E-mail address:* millera@mx.afrri.usuhs.mil (A.C. Miller).

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Table 1
Summary of findings on DU and heavy metal tungsten alloy

| Metal, type of study | Parameters examined | Outcome | Reference |
|---|---|---|---|
| DU, in vivo | Oncogenes, uranium levels | ↑ Oncogene expression with DU | [3] |
| DU, in vitro | Neoplastic transformation | DU-induced neoplastic transformation | [4] |
| DU, in vivo | Urine mutagenicity | ↑ Urine mutagenicity with DU level | [5] |
| DU, in vivo | DU tissue distribution | DU redistributed to multiple sites | [6] |
| DU, in vivo | Neurotoxicity | Electrophysiological brain effects | [7] |
| DU, in vitro/in vivo | Genotoxicity | ↑ Genotoxicity/genomic instability | [8] |
| DU, in vitro | Transformation | ↑ Transformation/mutagenicity | [9] |
| Tungsten, in vitro | Transformation/genotoxicity | HMTA induces transformation | [10] |
| DU, in vitro | Genomic instability | ↑ Genotoxicity/genomic instability | [11–13] |

exposed to radiation exhibit adverse biological effects, have been observed in a number of experimental systems. Using uranium isotopes with differing specific activity, our laboratory has provided the first evidence that alpha particle radiation is involved in DU-induced effects [13], although chemical effects cannot be ruled out. EPR spin trapping studies from our institute [14] have shown that uranium can react with $H_2O_2$ generating hydroxyl radicals, therefore, DU may be able to act as a mediator of reactive oxygen species (ROS) similar to nickel (Ni) [15,16]. It could be that DU-induced effects, e.g. transformation, involve both chemical and radiation mechanisms.

To address this question we propose a theory and suggest several simple tests to assess the role of DU as a mediator of hydroxyl radical-generating reactions. While our recent in vitro data have demonstrated that alpha particles are involved in DU-induced cellular effects [13], we also propose that a portion of the transforming mechanisms of DU might be related to its chemical properties. Specifically, DU could participate in cellular biochemistry that generates reactive oxygen species, $OH^{\cdot}$, $O_2^{\cdot}$, and $H_2O_2$ similar to the heavy metal nickel (Ni) [15,16]. Alpha particles are high-LET radiations and can cause DNA damage initiated from either direct ionization by the alpha particles or the indirect action of water radiolysis products including ROS. There could be however another DU-induced mechanism to generate ROS. Since uranium chemistry is similar to that of the transitions metals such as iron (Fe) and Ni, we suggest that DU might also catalyze chemical reactions yielding ROS. Specifically, if DU can mimic Fe in the so-called Fenton reaction [17,18],

$$H_2O_2 + M^N\text{-complex} \rightarrow M^{N+1}\text{-complex} + OH^- + OH^{\cdot}$$

(M is a metal species), it is likely that hydroxyl radicals from chemical transitions could exceed those from radioactive decay, especially since DU has such a low specific activity [1,4]. For example, a typical experimental design might require ~10 pmol of catalyst in 1 ml of reaction buffer. Given the decay constant for DU of $1.8\times10^{-16}$/s ($t_{1/2}=4.47\times10^9$ years), then ~13 radioactive transitions/h would be expected to occur. If the radiochemical yield for $OH^{\cdot}$ is 0.5 then the alpha particles from the radioactive transitions might generate $\sim3\times10^3$ $OH^{\cdot}$/min. If one however assumes that the second-order rate constant for DU in the Fenton reaction (above) is similar to that of Fe (III) (~100 M/s) then the same 10 pmol of DU might catalyze formation of $3.6\times10^{+10}$ $OH^{\cdot}$/min. Therefore, the chemically generated $OH^{\cdot}$ might exceed radioactively generated $OH^{\cdot}$ by a factor of $10^7$.

DU may be able to exert its effects involving both radiation and chemically generated $OH^{\cdot}$ radicals. A determination of the dual contribution of DU's radiation and chemical components is key to understanding the potential of DU as a carcinogen. While it is well known that alpha radiation is involved in the *initiation* of carcinogenesis [1], we speculate that chemical transitions of DU could be involved in *promotion* of the initiated cell. As has been suggested for plutonium [19], the same atom of DU can recycle indefinitely in vivo given ample cellular pools of reductants such as glutathione. The involvement of processes generating oxidative stress and the tumor promotion phase in carcinogenesis is well known [20].

While our recent data demonstrate that alpha particle emissions are involved in the process of DU-induced neoplastic transformation, chemically generated species may also be important to that process. In the present study we propose to examine two questions. First, does DU induce DNA lesions generated by oxidative damage and second, can DU induce this oxidative damage in the *absence* of significant alpha particle decay. The formation of 8-hydroxydeoxyguanosine (8-OHdG) in DNA by DU was assessed first. We then used small quantities of DU as a catalyst of putative Fenton reactions in vitro. Finally, two markers of oxidative DNA base damage are measured following the metal-catalyzed oxidation of ascorbate at pH 7. The metals, Fe and Ni were used for comparison. These tests will enable us to address the question of whether DU can cause carcinogenic lesions, e.g. oxidative DNA damage, in the absence of significant radioactive decay similar to what has been shown for plutonium [19].

## 2. Materials and methods

### 2.1. DNA and glassware preparation

Acid-washed glassware was used and aqueous solutions

were treated extensively with chelating resin to remove trace metal contaminants. DNA from calf thymus (Sigma, St. Louis, MO) was dissolved to 100–200 μg/ml and then dialyzed extensively against potassium phosphate buffer (50 mM, pH 7) that had been de-metaled using Chelex 100 resin (Sigma). This type of preparation was previously described [19]. The DNA stocks were used in acid-washed glassware throughout the experiments.

### 2.2. Depleted uranium and nickel preparation

The DU-$UO_2(NO_3)_2$ stock solution used was a New Brunswick Laboratory (Argonne, IL) standard solution in 5 M nitric acid calibrated as having a specific activity of 3.3 Bq/g. Dilutions of the stock were performed using routine gravimetric methods. The pH was adjusted to pH 7 using equimolar dilutions of $NH_4OH$ immediately prior to the experiments. The $NiSO_4$ stock solution was obtained from Sigma (St. Louis, MO) and similarly prepared. The metals will be designated as follows: DU-$UO_2(NO_3)_2$: DU; $NiSO_4$: Ni; and iron III: Fe.

### 2.3. Ascorbic acid experiments

It has been previously shown that ascorbic acid at pH 7 does not oxidize in the absence of catalytic metals and that this property can be used to detected adventitious metal ions in aqueous solutions [21]. We adapted this procedure to determine if DU or Ni at pH 7 behaved similarly to Fe as a catalytic species. For our experiments a graded concentration series of DU, Ni, or Fe was added to 50 mM potassium phosphate buffer. Each sample was prepared singly in a 1-ml UV cuvette immediately before adding 3.5 μl of a stock solution (100 mM) of ascorbic acid in high purity water (18 MΩ/cm). The oxidation of ascorbic acid was monitored at 265 nm for 15 min using a digital spectrophotometer. For the metal-catalyzed Fenton reaction with DNA, the DNA solutions were diluted to 100 μg/ml in phosphate buffer, after which the solution was brought to 15 nM in DU, Ni, or Fe and 1 mM ascorbic acid. The solutions ranged from 0 to 10 mM in $H_2O_2$ and the subsequent reactions were allowed to proceed for 30 min at 37 °C. The reactions were halted with the addition of 100 U of bovine catalase (Sigma). All materials were freshly prepared in Chelex 100-treated water and 1 μM DTPA was included in the reaction mixture. Thus, trace metals were removed, thereby eliminating the effect of ascorbate auto-oxidation. Triplicate samples were analyzed and each experiment was conducted three times. Data are shown as the means±S.E.M. for three experiments.

### 2.4. Assessment of metal-induced oxidative DNA base damage

To assess the relative levels of oxidative DNA base damage from DU-, Ni-, or Fe-catalyzed $H_2O_2$ reactions, two independent assays were used. First, the yield of 8-oxo-7-hydro-2′-deoxyguanosine (8-OHdG) was measured in calf thymus DNA treated with solutions of ascorbate, $H_2O_2$ and trace quantities of DU, Ni, or Fe. Secondly, an aliquot of the treated DNA was removed for thymine glycol analysis in intact DNA using an enzyme-linked immunosorbent assay (ELISA).

For the 8-OHdG analyses, DNA samples were digested to 2′-deoxynucleosides for analysis by HPLC with electrochemical and UV detectors. The enzymatic DNA digestion procedure used was as described [11]. Briefly, both DNase I at pH 7 (40 units, 30 min) and nuclease PI digestions at pH 5.1 (2.5 units, 60 min) were used. Following incubation with alkaline phosphatase at 37 °C for 30 min, proteins were removed by precipitation. The supernatant was dried, dissolved in 0.4 ml $H_2O$ and passed through a 0.22-μM nylon filter before analyzing the amounts of dG and 8-OHdG. The efficiency of the enzymatic DNA digestion was monitored in each sample by quantifying all four major 2′-deoxyribonucleosides by their UV absorbance at 260 nm. In most cases 10 nmol of total 2′-deoxyribonucleosides was injected in each sample volume of 50 μl. The 8-OHdG level is expressed as 8-OHdG molecules per $10^4$ dG. Triplicate samples were analyzed and each experiment was conducted three times. Data are shown as the means±S.E.M. for three experiments.

For the thymine glycol lesion analysis, a monoclonal antibody-based ELISA procedure was used as previously described [11]. For the ELISA procedure, 0.2 μg of DNA was bound to wells of a 96-well dish treated with sulfact. The wells were rinsed and binding sites were blocked with 1% horse serum. To detect DNA-bound primary mouse IgG antibody, goat antimouse IgG conjugated with alkaline phosphatase was used. The assay for alkaline phosphatase activity was in glycine buffer (100 mM, pH 10.4) for 1 h at 37 °C. The resulting yellow color was measured at 405 nm using a multiwell plate reader. All DNA samples were measured in triplicate wells within each experiment. The experiments were conducted in triplicate; the data were averaged, and are expressed as a net increase above the control DNA background signal.

## 3. Results and discussion

### 3.1. Formation of 8-OHdG in DNA: effect of DU, Ni, and Fe

Fig. 1 shows 8-OHdG formation in DNA obtained with DU (0–1000 μM) plus $H_2O_2$ (0.5 mM) in 10 mM potassium phosphate buffer (pH 7.0) at 37 °C for 1 h. The amount of 8-OHdG in DNA markedly increased when DU concentration was increased from 1 μM ($7.1/10^5$ dG) to 1 mM ($83/10^5$ dG) exhibiting a dose-dependent response. For Fe, the magnitude of the response was similar to DU and resulted in a dose-dependent increase in the amount of

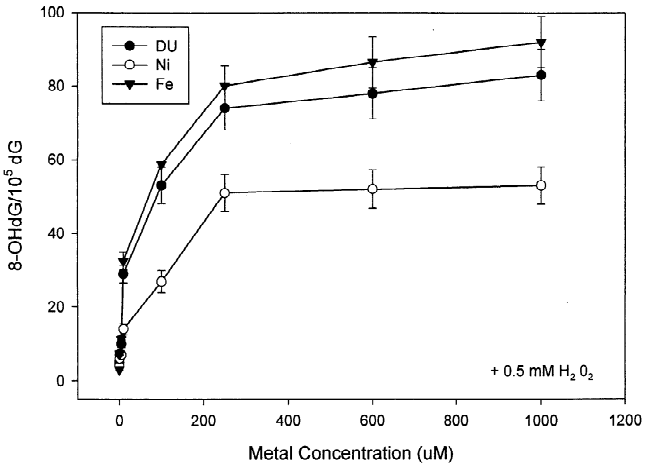


Fig. 1. Formation of 8-OHdG in DNA by metal + $H_2O_2$. Calf thymus DNA was reacted with 1–1000 μM of metal + 0.5 mM $H_2O_2$ in 10 mM potassium phosphate buffer, pH 7 at 37 °C for 1 h. Data are shown for DU (●), Ni (○), and Fe (♦) and are the means±S.E.M. from three independent experiments.

8-OHdG in DNA. In contrast, Ni demonstrated a slight increase in 8-OHdG production in DNA above background levels. Control experiments with DU (1 mM) or $H_2O_2$ (0.5 mM) alone did not enhance 8-OHdG formation in DNA above the background level. The calf thymus DNA (type XV) used in these experiments has an 8-OHdG background level of $5/10^5$ dG. Use of this calf thymus DNA prevented any artifacts from the use of other commercially available DNA that routinely has a background 8-OHdG level of $10–50/10^5$ dG (unpublished data). Our results also indicate that trace metals did not affect the induction of 8-OHdG by the metals tested in our study. The time-course- and pH-dependent formation of 8-OHdG by DU, Ni, or Fe were also determined (data not shown). Those data demonstrate that the formation of 8-OHdG by DU and Ni is both time- and pH-dependent. Again, Fe did not significantly enhance 8-OHdG formation in DNA above background in pH- and time-dependence studies.

These data show for the first time that DU plus $H_2O_2$ is capable of inducing 8-OHdG in DNA. The level of 8-OHdG generated increases in both a dose- and time-dependent manner. These data support the hypothesis that DU may cause DNA damage through the oxidative pathway and confirm earlier EPR-spin trapping data demonstrating that uranium reacts with $H_2O_2$ generating hydroxyl radicals [14]. The next question is whether under conditions where the chemically generated $OH^{\cdot}$ from DU is significantly greater ($>10^7$-fold) than the radioactively generated $OH^{\cdot}$ can DU induce oxidative DNA damage. To address this question, we will use much smaller amounts (nanomolar amounts) of DU than were used in the results shown in Fig. 1 (micromolar amounts). Nanomolar amounts of DU will be used as a catalyst of putative Fenton reactions in vitro.

### *3.2. Oxidation of ascorbic acid: effect of DU, Ni, and Fe*

Results of the ascorbate test using DU, Ni, or Fe as the catalyst are shown in Fig. 2. The data demonstrate a DU-concentration-dependent oxidation of ascorbic acid. Results for Fe were similar. A ratio of the slopes was done for comparison. DU was ~6-fold more effective at catalyzing the oxidation of ascorbate than was Ni. In comparison, Fe was ~3.2-fold more effective than Ni. Control experiments using dilute nitric acid and/or buffers treated with Chelex 100 did not demonstrate significant ascorbate oxidation under experimental conditions (data not shown). DU apparently participates in reactions similar to those of iron in the ascorbate test confirming earlier EPR-spin trapping experiments from our laboratory demonstrating that uranium reacts with hydrogen peroxide to generate hydroxyl radicals [14]. Most importantly, this test shows that DU can catalyze biochemical reactions inducing oxidative stress in the absence of significant radioactive decay.

### *3.3. Formation of 8-OHdG and thymine glycol in DNA: chemically generated OH significantly greater than radioactively generated OH*

The data in Fig. 2 demonstrate that DU can catalyze chemical reactions yielding ROS and that DU can mimic Fe in the Fenton reaction. It is important for a better mechanistic understanding of DU to determine if DU can induce oxidative damage under those circumstances in which chemically generated $OH^{\cdot}$ exceed radioactively generated $OH^{\cdot}$ from DU. To do so we will examine two markers of oxidative damage. Both 8-OHdG and thymine

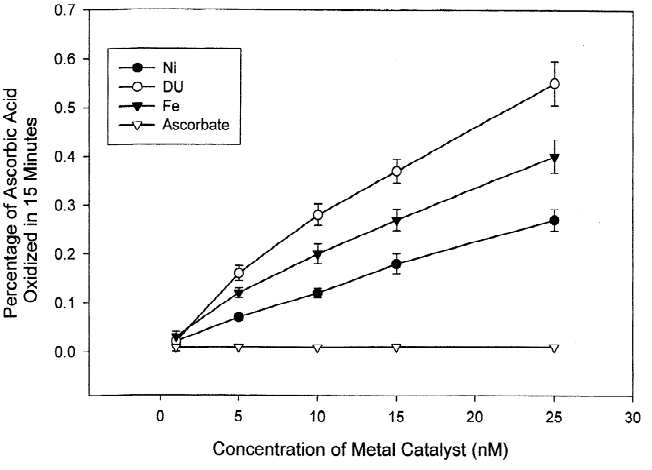


Fig. 2. Oxidation of ascorbate at pH 7 as a function of metal concentration. The metals were tested for their ability to catalyze the oxidation of ascorbate. The initial concentration of ascorbate was 1 mM for all reactions. Data are shown for DU (○), Ni (♦), Ascb (◇) and Fe (●) and are the means±S.E.M. from three independent experiments.

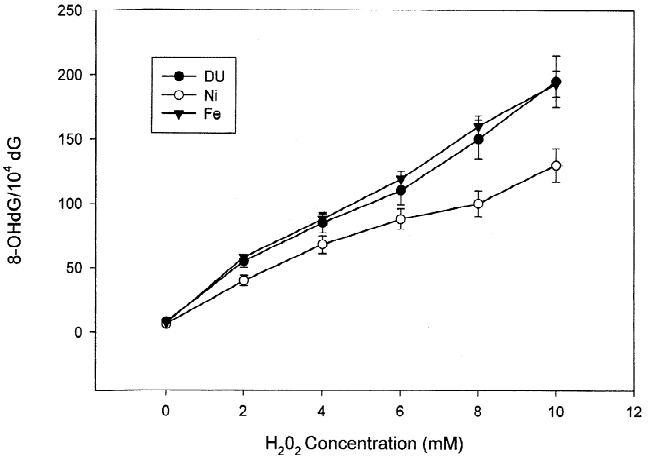


Fig. 3. Metal-catalyzed $H_2O_2$-induced formation of 8-OHdG in DNA: chemically generated OH greater than radioactively generated OH. Duplex DNA was reacted with 1 mM ascorbate, 15 nM DU (●), Ni (○) or Fe (♦) and graded concentrations of $H_2O_2$. Data shown are the means±S.E.M. from three independent experiments.

glycol have been clearly established as molecular biomarkers of oxidative DNA damage from ionizing radiation and oxidative stress [20]. The results demonstrate that DU is more effective than Ni in catalyzing reactions that induce oxidative DNA base damage (Figs. 3 and 4) while DU is at least as effective as Fe. Both Figs. 3 and 4 show that a nanomolar amount of DU was able to catalyze a $H_2O_2$-concentration dependent formation of 8-OHdG and thymine glycol in DNA. The similarity of the response of the two elements suggests that DU might participate in 'site-specific' hydroxylations like those hypothesized to occur in iron-mediated reactions of hydrogen peroxide [22]. The involvement of site-specific mechanisms in the formation of 8-OHdG by DU is currently being studied.

### *3.4. Mechanism of the formation of 8-OHdG in DNA: participation of reactive oxygen species*

The involvement of ROS in the formation of 8-OHdG by DU/$H_2O_2$ was investigated by examining the effect of ROS scavengers, i.e. sodium azide, D-mannitol, methional, and Tris–HCl.

The average amount of 8-OHdG generated was $83/10^5$ dG when DNA was incubated with 1 mM DU plus 0.5 mM $H_2O_2$ at pH 7.0 at 37 °C for 1 h. Additions of sodium azide, D-mannitol, methional, or Tris–HCl to those reaction mixtures caused a marked decrease in the generation of 8-OHdG in DNA (Fig. 5). At equal concentrations of these ROS scavengers, the inhibitory effect was sodium azide>methional>D-mannitol>Tris–HCl. At 0.3 mM, sodium azide reduced 82% of 8-OHdG formation by DU/$H_2O_2$; at 30 mM there was almost a complete inhibition of 8-OHdG formation. Similar results were observed with methional. These results strongly suggest that ROS like hydroxyl radicals, singlet oxygen, and/or superoxide radicals, are involved in the formation of 8-OHdG by DU plus $H_2O_2$. The involvement of reactive species other than $OH^{\cdot}$ is strongly supported by the data with sodium azide and methional; methional scavenges not only $OH^{\cdot}$ but also a variety of reactive species and sodium azide is a well-known singlet oxygen scavenger.

In order to further clarify what kind of reactive oxygen species caused the DU-induced oxidative damage, the effects of catalase and SOD were examined. DNA was

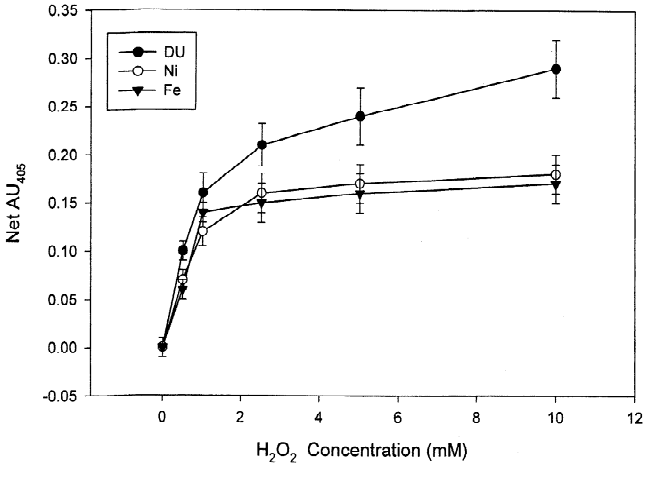


Fig. 4. Metal-catalyzed $H_2O_2$-induced formation of thymine glycol in DNA: chemically generated OH greater than radioactively generated OH. Data shown are thymine glycol in DNA from DU (●), Ni (○), or Fe (♦)-catalyzed $H_2O_2$ reacted with ascorbate. The metal concentration was 15 nM for all reactions. The alkaline phosphate activity assayed at 405 nm is expressed in arbitrary units. The final yield of thymine glycol is dependent upon the amount of metal and the $H_2O_2$ concentration. Data shown are the means±S.E.M. from three independent experiments.

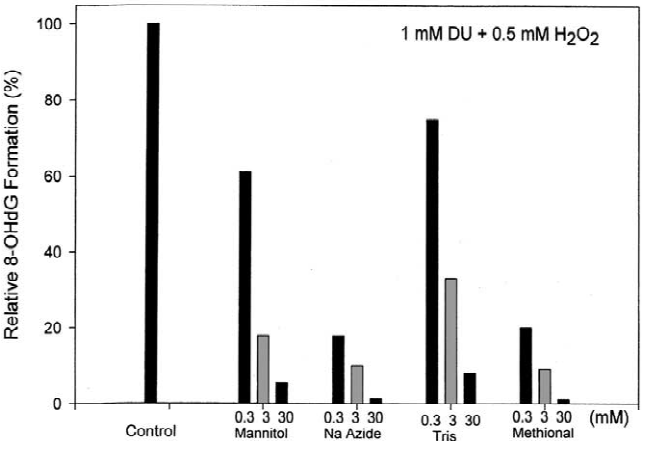


Fig. 5. Effect of ROS scavengers on the formation of 8-OHdG in DNA by DU/$H_2O_2$. Increasing concentrations (0.3–3 mM) of D-mannitol, sodium azide, Tris–HCl, or methional were added to the DNA solutions prior to the addition of 1 mM DU and 0.5 mM $H_2O_2$. The reactions were conducted similarly to that described in Fig. 1. The relative 8-OHdG formation was determined by dividing the amount of 8-OHdG obtained in each experiment containing ROS scavengers by that obtained in DU/$H_2O_2$.

treated with DU plus $H_2O_2$ and the reaction proceeded for 30 min, followed by the addition of enzyme with the reaction continuing for another hour. DU plus $H_2O_2$ treatments for 30 and 90 min were performed simultaneously. As in previous experiments, the average amount of 8-OHdG generated was $83/10^5$ dG when DNA was incubated with 1 mM DU plus 0.5 mM/$H_2O_2$ at pH 7.0 at 37 °C for 1 h. The data in Fig. 6 demonstrate that catalase (2 U/ml) completely inhibited the formation of 8-OHdG by DU plus $H_2O_2$ and that heat-denatured catalase (dnCAT) (2 U/ml) did not influence those formations. This result confirmed that $H_2O_2$ is required for the formation of 8-OHdG. In contrast 12 U/ml of heat-denatured catalase reduced 8-OHdG formation by 34%, possibly due to the non-specific scavenger activity of proteins. Further evaluation of the types of ROS was conducted using an inhibitor of superoxide ion. SOD (12 U/ml) reduced 38% of 8-OHdG formation by DU plus $H_2O_2$, while heat inactivated SOD did not affect the formation of 8-OHdG (Fig. 6). These results indicate that SOD might scavenge some of the superoxide ions and partially inhibit 8-OHdG formation.

The studies described in Figs. 5 and 6 demonstrate that the hydroxyl radicals, superoxides, and singlet oxygen are involved in the formation of 8-OHdG by DU. Furthermore, it appears that $H_2O_2$ is necessary for the DU-induced DNA damage. The question of site-specificity of oxidative DNA damage induced by DU is currently being investigated.

These relatively simple yet standard tests in free radical biology research are extremely helpful in gaining a better understanding of the carcinogenic potential of DU. Our results strongly suggest that DU can participate in chemical reactions generating oxidative stress at physiological pH. Although the concentration of $H_2O_2$ used in the in vitro assay was much higher (100-fold) than the physiological concentration, in an in vivo scenario bound DU ions might undergo repeated oxidations and reduction reactions while catalyzing the formation of ROS causing a gradual accumulation of DNA damage. Our results suggest that DU may cause DNA damage through the oxidative pathway.

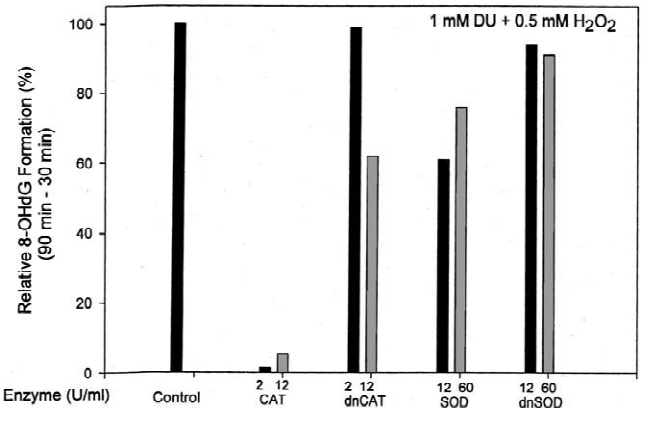


Fig. 6. Effect of catalase and SOD on the formation of 8-OHdG in DNA by DU/$H_2O_2$. DNA was incubated with 1 mM DU and 0.5 mM $H_2O_2$ as described in Fig. 1 for 30 min. Various amounts of catalase or SOD were added to the reactions and incubated for another 60 min. The increased amounts of 8-OHdG formation between 90- and 30-min incubation in DU/$H_2O_2$ treatment of DNA were defined as 100%. The relative amounts of 8-OHdG formation were calculated as a ratio of increased amounts in +enzyme experiments/−enzyme experiments.

The data presented here are significant because they support the hypothesis that DU can induce the formation of oxidative DNA damage in the absence of significant radioactive decay. Since our laboratory recently demonstrated in transformation studies that alpha particle radiation (1% of cells are traversed by an alpha particle, 17 cGy/nucleus) is involved in DU-induced neoplastic transformation (10 μM, 24-h exposure) and genotoxicity [13], it is tempting to speculate that DU might exhibit both a tumor 'initiation' and 'promotion' component. It has been hypothesized that alpha particle radiation can initiate cancer [1,23,24] and the data presented here suggest that DU complexes might contribute to a gradual accumulation of oxidative damage that is important in tumor promotion. To test this theory our laboratory is comparing uranium radionuclides with stable isotopes using both cellular and animal models.

While the data presented here do not fully and definitively answer the question as to the contribution of chemically- versus radiation-induced damage in DU cellular effects, they do provide the first evidence of oxidative chemical 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, results demonstrating that both radiation and chemical effects are involved in DU-induced cellular damage, could have a significant impact on DU risk assessments.

## 4. Abbreviations

| | |
|---|---|
| Ascb | ascorbate |
| DU | depleted uranium |
| DU-$UO_2(NO_3)_2$ | depleted uranium-uranyl nitrate |
| HOS | human osteosarcoma cells |
| $NiSO_4$ | nickel sulfate |
| 8-OHdG | 8-hydroxydeoxyguanosine |
| ROS | reactive oxygen species |

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