Accumulation and Distribution of Uranium in Rats after Implantation with Depleted Uranium Fragments

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Depleted uranium/Bone/Kidney/Distribution/ICP-MS.

Purpose: The aim of our study was to clarify the accumulation and distribution of uranium in depleted uranium (DU) implanted rats. Materials and Methods: Male Sprague-Dawley rats were surgically implanted in gastrocnemius muscle with DU fragments at 3 dose levels (low, medium and high), and biologically inert tantalum (Ta) fragments were used as controls. At 1 day and 7, 30, 90, 180 and 360 days after implantation, the rats were euthanized and tissue samples including serum and urine were collected to analyze the uranium levels by inductively coupled plasma-mass spectrometry (ICP-MS). Results: At all time points, uranium levels in all the DU implanted groups were higher than that in Ta control group, and uranium concentrations in kidney and bone were significantly greater than that in other tissues. Otherwise, uranium concentrations increased with a close correlation to the implanted DU doses and duration of exposure, with a peak at 90 days post-implantation, after which followed by a decreasing period, but still maintained at a relatively high level even at 360 days post-implantation. The uranium concentrations in bone were 6.92 ± 0.97 μg U/g, 16.35 ± 1.67 μg U/g and 21.64 ± 3.68 μg U/g in the low-, medium- and high-dose group animals, while values in kidney tissues were 10.66 ± 1.10 μg U/g, 14.06 ± 1.28 μg U/g and 17.79 ± 2.87 μg U/g, respectively, at 360 days post-implantation. Conclusion: It was concluded that kidney and bone are the primary reservoirs for uranium redistributed from intramuscularly embedded fragments, and the accumulations in kidney, bone and many other tissues suggest the potential for unanticipated physiological consequences of chronic exposure to DU.

INTRODUCTION

Depleted uranium (DU) is a by-product of uranium enrichment and has roughly 60–75% of the radioactivity of an equivalent amount of natural uranium due to the reduction of the percentage of radioactive isotopes (234U and 235U) from 0.72 and 0.006%, respectively, in natural uranium to 0.2 and 0.001%,1–3) DU has not only military applications, but also a number of civilian applications due to its physical properties (e.g. high density, relatively abundant quantity and appropriate cost). For example, DU is employed as counterweights or ballasts in aircraft, radiation shields in medical equipment, containers for the transport of radioactive materials and chemical catalysts. DU has also been used in glassware, ceramics (as cooking and serving contain-

ers) and dentistry.4–6) At present, the extensive use of DU results in the increase of interest on the possible effects on environment and health consequence.7–9)

As a kind of heavy metal with low level radioactivity, DU may enter the body principally via: (1) Inhalation through the respiratory tract, in a war particularly, DU shell can form a radioactive aerosol (account for about 80% of the shell body) in a high temperature reaction after explosion, and as a result, the war participants and the population around may be injured in the body due to the inhalation of the radioactive aerosol with DU. (2) Ingestion through the digestive tract, the fine particles of DU carried by wind float, scatter and contaminate the air, ground, water source or any object, and finally enter the human body through the food chain. (3) Embedded injury into wounds, a long retention in the body makes the DU fragments dissolved and absorbed, gradually transferred to some critical organs via body fluids, and produce chemical toxicity and/or radiotoxicity.5,6,10,11)

Due to the similarity of chemical properties between DU and natural uranium, DU shows a toxic effect on the body similar to natural uranium. It has been reported in previous researches that after accidental inhalation, ingestion, or
absorption through intact or wounded skin, natural uranium mainly deposited in the skeleton and in the kidneys, and may induce renal damage and inhibition of bone formation.\textsuperscript{12-14} However, the biokinetics of uranium entering the body through DU-embedded wound received little attention and experimental data are needed. The current study was designed to determine the accumulation and distribution of uranium continuously liberated from DU fragments embedded in the gastrocnemius muscle of rats over the course of 360-d test, to simulate the behavior and consequence of injuries with DU fragments, in order to provide information that may be used in a health hazard assessment of DU-embedded injury.

**MATERIALS AND METHODS**

**Chemicals**

DU fragments were provided by China National Nuclear Corp., composed of 99.25% DU and 0.75% titanium by weight, with the uranium isotopes of \textsuperscript{238}U (99.79%), \textsuperscript{235}U (0.20%) and trace levels of \textsuperscript{234}U. Each DU fragment was approximately 8 mm length × 2 mm width × 1 mm thickness in size (about 0.1 g DU), and it was immersed into dilute nitric acid and ethanol before the implantation. Ta fragments (Tantalum, Ta), used as control fragments with a mass simi-

**Sample Collection**

After urine was collected from each rat, individually housed for 24 h in metabolism cages and having continuous access to food and water, rats were euthanized by exsanguinations from carotid artery under anesthesia (22% ethylurethann, 5 mL/kg). Blood samples were obtained from carotid artery and centrifuged for 5 min at 3000 \times g to extract the serum. The following tissues were pooled among their respective treatment groups for analysis: kidney, liver, spleen, thyroid gland, lung, heart, testes, brain, tibia and gastrocnemius muscle (from right leg per animal). All samples including urine, serum and tissues were frozen and stored at −70°C until analysis.

**Sample treatment**

The tissue samples for the determination of uranium level were performed using wet acid digestion. About 1–2 g wet tissue sample was weighted out into a quartz crucible, 5 mL of nitric acid and 1 mL hydrogen peroxide were added to each crucible, which was then placed on a hot plate and set the temperature to about 150°C for several hours. Then, the samples were carefully heated to a higher temperature to evaporate the solution volume to approximately 0.5 mL. More addition of nitric acid and repeat this procedure would be required if retained residue still remained. Finally, an absolutely digested clear solution was transferred to a flask and diluted with deionized water to 10 mL volum. Serum and urine samples were directly diluted 20 times and 10 times, respectively, with 1% nitric acid (V/V) before measurement. While for the bone samples, about 1 g for each specimen, were placed in a muffle furnace at 300°C for 2 h and 600°C for another 4 h for incineration. After a cooling period, the residue was dissolved using about 1mL of nitric acid and 1mL deionized water, and the digested solution was finally transferred to a flask and diluted with deionized water to 25 mL volume. All reagents used were of the high pure grade. Water used for dilution was Milli Q (Millipore Corp., Millford, MA, USA) deionized water.

**Measurement of uranium**

Uranium determinations in wet-ashed tissues were performed by inductively coupled plasma-mass spectrometry (ICP-MS) using U.S.EPA Method 6020.\textsuperscript{16} After proper dilution, a Thermo X-7 ICP-MS (Thermo Elemental Co., Ltd. USA) was employed for the determination of uranium isotopes and levels. The determination was performed by using the normal mode (Table 1). A Spex standard Uranium solution (Spex CertiPrep Co., Ltd., USA) was used for instrumental calibration. The mass bias was evaluated by

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GBWO4220 and GBWO4222. The instrument working conditions of ICP-MS are shown below (Table 1). The limit of detection (LOD) for uranium using ICP-MS was less than 0.001 ng/mL.

Table 2 was the determination values of the $^{235}\text{U}/^{238}\text{U}$ isotope ratio (Table 2). With regards to the DU fragments used in this experiment, the determined value of the $^{235}\text{U}/^{238}\text{U}$ isotope ratio was 0.00294 ± 0.00005, which was far below the value of natural abundance uranium, and was within the scope of isotope ratio from depleted uranium (Table 2).

### RESULTS

#### Deaths and clinical observation

Of the 132 rats undergoing surgery, two animals died minutes after implantation surgery as a result of anesthesia injection with the analgesic ketamine hydrochloride (2 mL/kg). Of the expired animals, one was implanted with 0.1 g DU + 0.2 g Ta fragments, and another was implanted with 0.3 g Ta fragments. No other deaths occurred during surgery.

Three rats died during the 360d post implantation surgery phase. Of the expired animals, one was implanted with 0.3 g Ta fragments, and two were implanted with 0.1 g DU + 0.2 g Ta fragments. No gross evidence of toxicity or inflammatory reaction was observed in any of these animals.

#### Redistribution of depleted uranium in the body

Immediately 1 day after the DU implantation, the ratios of $^{235}\text{U}/^{238}\text{U}$ in serum, kidney and liver samples decreased quickly down to 0.00297 ± 0.00005, 0.00293 ± 0.00003 and 0.00290 ± 0.00004, respectively, which were as the same value of 0.00294 ± 0.00005 for the embedded DU. From the above results, it was clearly confirmed that the embedded fragments of DU could redistribute throughout the body rapidly. Considering that, only the Uranium concentrations in all samples were measured at the following time points.

#### The changes of uranium level in the serum and urine

At 1 day after DU implantation, very low levels of uranium in serum were observed, but levels in the all dose groups significantly greater than that in Ta controls (Ta group, 0.45 ± 0.17 ng U/mL serum; low-dose group, 3.80 ± 0.94 ng U/mL serum; medium-dose group, 4.58 ± 1.74 ng U/mL serum; high-dose group, 6.70 ± 4.50 ng U/mL serum). Uranium levels in the all dose groups increased significantly at the following 7 d and 30 d time points. The highest concentration of uranium in serum reached at 90 days post-implantation in the high-dose group, and at 30 days in the low- and medium-dose group (Fig. 1A).

A significant accumulation was also observed in urine, and as early as 1 day after DU implantation, uranium levels in the urine obviously increased in the DU-implanted rats (Ta group, 1.48 ± 1.14 μg U/g Creatinine; low-dose group, 128.5 ± 27.4 μg U/g Creatinine; medium-dose group, 259.5 ± 88.2 μg U/g Creatinine; high-dose group, 385.4 ± 34.7 μg U/g Creatinine). Furthermore, as the primary means of uranium excretion, the uranium concentrations in the urine increased with dose and duration of exposure, and the highest concentration (about 3833 ± 628 μg U/g Creatinine) was observed at about 90 days post-implantation in the high-dose group; thereafter, the uranium concentration in the urine decreased gradually, but maintained at a relatively high level even at 360 days after implantation (Fig. 1B). Consequently, the uranium concentration in the urine was useful...
as sensitive indicator for measuring the pattern of uranium retention and the radiation dose in the body after DU-embedded injury.

**Uranium levels in tissues**

Within 1 day after DU implantation, rats showed significant distribution of uranium to different tissues, especially in kidney: 3897 ± 780 ng U/g, 5503 ± 553 ng U/g and 6382 ± 768 ng U/g wet weight kidney, respectively, in the low-, medium- and high-dose group. A dose-dependent increase in uranium concentration was also observed for the tibia reaching 649.8 ± 99.3 ng U/g, 1160 ± 186 ng U/g and 1851 ± 276 ng U/g, respectively, in the low-, medium- and high-dose group. Uranium concentrations measured in other organs were also different, to varying degrees, from those measured in tissues from Ta-controls (detailed data not shown).

At 7, 30, 90, 180 and 360 days following implantation, uranium concentrations in all the DU implanted groups were significantly different from those measured in tissues from Ta-controls, and the highest concentration of uranium continued to be observed in kidney and bone. In other words, kidney and bone was main accumulation organ for uranium after DU-embedded injury, and there was high correlation between the implanted DU doses and uranium concentrations in the kidneys and bone (Figs. 2–6). Interestingly, in the early phase post DU implantation, the uranium concentrations in kidney were significantly higher than those in bone, however, great higher distribution was observed in bone at the later time points (Figs. 2–6).

In addition to kidney and bone, significant accumulations were also observed in other tissues, especially in spleen, liver and lung, and the uranium levels of these organs in all DU implanted groups were significantly higher than those in Ta controls (p < 0.01, Figs. 2–6). Meanwhile, at 7 days after DU implantation, uranium concentrations in testes, thyroid gland and muscle also showed significant increase in the high-dose group, compared with those in the Ta controls (p < 0.01, Fig. 2).

**Fig. 1.** Uranium concentration in serum (A) and urine (B) plotted as a function of time after implantation. Time points were 1, 7, 30, 90, 180 and 360 days. Each point is mean ± SEM, N, 5–6.

**Fig. 2.** Uranium concentrations measured in tissues dissected from rats euthanized 7 days after implantation of DU and Ta fragments. Bars represent mean ± SEM, N, 5–6.* indicates significantly different from tantalum controls.
The dynamic change of Uranium levels in target organ

The uranium levels in kidney and bone, the major organ for uranium accumulation after DU implantation, increased with duration of exposure, and uranium concentrations of all dose groups were observed to reach maximum at 90 days post-implantation, thereafter, the uranium concentration decreased gradually but very slowly and still maintained at a relatively high level even at 360 days following DU implantation (Fig. 7). For example, at 360 days post-implantation, the uranium concentrations in tibia were 6.92 ± 0.97 μg U/g, 16.35 ± 1.67 μg U/g and 21.64 ± 3.68 μg U/g in the low-, medium- and high-dose group animals, and the values in kidney tissues were 10.66 ± 1.10 μg U/g, 14.06 ± 1.28 μg U/g and 17.79 ± 2.87 μg U/g, respectively.

The changes of uranium concentration in additional tissues were analyzed. Uranium concentrations in spleen, liver, lung and heart were significantly greater than those in Ta controls. Similar to observations with kidney and bone, relatively high concentrations were observed at 90 days after DU implantation, and then decreased gradually and slowly (Figs. 8A–8D). However, the uranium concentrations in these tissues were far lower than those in kidney and bone. At 90 days after DU implantation, the average concentrations in these tissues of the high-dose group were 44.48 ± 3.33 μg U/g in tibia, 36.50 ± 2.19 μg U/g in kidney, 1.44 ± 0.15 μg U/g in spleen, 0.53 ± 0.07 μg U/g in liver, 0.56 ± 0.04 μg U/g in lung and 0.15 ± 0.01 μg U/g in heart tissue, respectively.
Fig. 5. Uranium concentrations measured in tissues dissected from rats euthanized 180 days after implantation of DU and Ta fragments. Bars represent mean ± SEM, N, 5–6.* indicates significantly different from tantalum controls.

Fig. 6. Uranium concentrations measured in tissues dissected from rats euthanized 360 days after implantation of DU and Ta fragments. Bars represent mean ± SEM, N, 5–6.* indicates significantly different from tantalum controls.

Fig. 7. Uranium concentration in tibia (A) and kidney (B) plotted as a function of time after DU implantation. Time points were 1, 7, 30, 90, 180 and 360 days. Each point is, mean ± SEM, N, 5–6.
DISCUSSION

The result of this study do indicate that the DU fragments used in this study dissolved in rat muscle over the 360-d test period and resulting free uranium transported to several tissues. Kidney and bone were the primary reservoirs for uranium redistributed from intramuscularly DU-embedded fragments, while uranium was detected in the urine with increasing concentration depending on DU fragment burden.

Depleted uranium is the by-product after $^{235}$U is extracted from natural uranium, so its $^{235}$U content is lower and specific activity is approximately 60–75% of that of natural uranium. As well known, uranium emits $\alpha$, $\beta$, and $\gamma$ ionizing radiation, and $\alpha$ particles are the primary radiation hazard, which do pose a potential hazard after internal contamination. It has been reported in previous studies that DU aggregation may cause impairment of systems such as kidney, lung, and CNS. However, the above conclusions were mainly based on the studies from the health effects of DU aerosol contamination by inhalation exposure on the battlefield, or animal experiments mimicking DU aerosol inhalation. For the behavior and consequence of injuries with DU fragments, because the DU residual fragments retaining in the body are highly insoluble and deliver a very small amount of uranium to the blood, the uranium could gradually transfer to and accumulate in target organ (such as kidneys and bone) and thus induce chemical toxicity and/or radiotoxicity, whereas partly excreted through urine.

It is well known that the assessment of toxicity resulting from radionuclide incorporation is partly based on the prediction of their biokinetics in the body. With regard to the occupational exposure of uranium miner, uranium was found in the highest concentrations in lung, kidney, bone, and liver. Animal studies have explored the distribution of uranium following experimental inhalation, injection, and ingestion. Following intravenous injection, uranium was quickly eliminated from the blood and redistributed to kidney, bone, liver and spleen, and excreted in urine. While in the case of DU aerosol inhalation, just like intravenous injection, soluble DU particles were rapidly cleared from the blood and mainly accumulated in kidney and bone after absorbed into the blood, and then excreted through the urine, whereas for inhalation of hardly soluble DU dust, the highest uranium level appeared in the lung because of the local deposition of hardly soluble DU particles, which was significantly higher than other tissues including kidney and bone. In other words, lung was the main target organ of uranium following inhalation of hardly soluble DU aerosol. It has been revealed that after inhalation of DU aerosol with high concentration, rats mostly occurred pulmonary injury resulting in the degenerative change of the lung, pulmonary edema, pneumorrhagia and bronchopneumonia. The result obtained from this study showed a pattern of uranium distribution different from that obtained from inhalation exposure of hardly soluble DU dust or aerosol. In the DU implanted rats, uranium concentrations in kidney and bone were significantly higher than those in other tissues throughout the experiment, that meant, kidney and bone were main reservoirs for the uranium, and other
tissues such as lung, liver, spleen, testes, heart were also
touched by DU fragments, however, they exhibited uranium
levels far lower than those in kidney and bone (Figs. 2–6).

The interesting phenomenon was that, in the early phase
post DU implantation, the uranium accumulation in kidney
was more obvious than that in bone, whereas as the duration
of implantation extended, particularly at 90 d post-implan-
tation, the uranium concentration in bone was obviously
higher than that in kidney (Figs. 2–6), which may be
explained by the decline of kidney accumulation following
constant excretion of uranium through the kidney and urine.
Previous studies have shown that uranium in bone may be
engaged in the calcification of bone, and the uranyl ion can
displace calcium ion in bone matrix and bind to phosphates,
which may cause about 80–90% uranium accumulation in
the bone and blemish the bone structure. Consequently, once
deposited in bone, uranium is hardly excreted from the
body,10,33) and the uranium level in bone would descend at a
slower rate than that in kidney.

As major organs responsible for the uranium accumu-
lation after DU-embedded injury, kidney and bone showed
increasing uranium concentrations as the duration of DU
implantation extended, and the peak appeared at 90 d post-
implantation. The uranium concentration in kidney and bone
then decreased slowly, but still maintained at a high level
even at up to 360 days post-implantation. As major organs
where uranium accumulated in the body, kidney and bone
had the most uranium deposited but hardly excreted after
DU implantation, and accordingly sufficient attention should
be paid to the studies on the harmful effects on bone metab-
olism and renal function induced by DU implantation.

The pattern of uranium excretion via urine at different
time intervals post-implantation was observed. Because
analysis of the urine volume (data not shown) suggested age-
dependent changes, the uranium concentration excreted dur-
ing a 24-h period was adjusted and reassessed by creatinine.
The present study demonstrated that the excretion of ura-
nium in the urine of DU implanted rats significantly
increased within 1 day after implantation, and uranium was
excreted in the urine throughout the 360 days of the exper-
iment (Fig. 1B). Additionally, at different time point post-
implantation, the uranium level in the urine always exhibited
obvious correlation to the implanted DU dose, and there was
a highest level in urinary uranium over the first 90 days, after
which followed by a decreasing period, but the urinary ura-
nium concentrations maintained relatively high even at up to
360 days after implantation (Fig. 1B). In other words, ura-
nium was persistently excreted via urine even at 360 days
post-implantation, and the uranium excretion in the urine
shared consistent pattern with the change of uranium level
in tissues such as kidney and bone. In light of these findings,
the urinary uranium level may be a sensitive indicator for
measuring the pattern of uranium retention and the radiation
dose in the body after DU-embedded injury.

Furthermore, from 7 days after DU implantation, the ura-
nium concentration in the serum of DU-implanted rats had
some increase but to a minimal extent (Fig. 1A). This Phe-
monomenon may be due to accumulation in the plasma rather
than in the serum after entering in the body, for the heavy
metals such as uranium and lead.23,34) Therefore, the uranium
concentration in the plasma or the whole blood may be a
superior indicator for the radioactive heavy metal such as
uranium.

Similar to the other studies,35) high concentrations of ura-
nium were found in the liver of rats in all experimental
groups in our present study (Fig. 8), indicating liver was
indeed a large pool for the accumulation of uranium. It was
also interesting that uranium level in liver was higher than
that in spleen only during the first 7 days after implantation
(Fig. 8), and from 30 days after implantation, uranium levels
in spleen were at least 2 folds higher than those in liver (Fig.
8). Such results may reflect a different route of uranium exposure (i.e., muscle-embedded fragments). Previous
studies have shown that following inhalation of soluble DU
droplets in the intravenous injection, the uranium was quickly
absorbed and cleared via the blood.26,36) However, as for the
hardly soluble uranium compounds, the uranium may be
locally absorbed, distributed and cleared through the lymph
nodes and the muscle also.37) But, with very much regret, the
uranium levels in the lymph nodes of DU-implanted rats
were not analyzed in this study.

In summary, following implantation of rats with DU frag-
ments, uranium is persistently excreted via urine even at 360
days post implantation, and the uranium excretion in the
urine shares consistent pattern with the change of uranium
level in kidney and bone. Otherwise, the propensity for inter-
nalized DU to translocation to several tissues, especially the
kidney, skeletal tissue, spleen, liver, lung and testes, indi-
cates that these tissues should be examined closely to deter-
mine if they are affected or altered as results of DU exposure
in the case of embedded DU fragments.

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REFERENCES

1. Craft, E., Abu-Qare, A., Flaherty, M., Garofolo, M., Rincavage,
chemistry and toxicological effects. J Toxicol Environ Health
B Crit Rev. 7: 297–317.
of the effects of uranium and depleted uranium exposure on
reproduction and fetal development. Toxicol Ind Health. 17:
180–191.
olar macrophages in culture and chemical dissolution. Hum Toxicol. 8: 111–119.