Effects of Chelating Agent CBMIDA on the Toxicity of Depleted Uranium Administered Subcutaneously in Rats

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Abstract- We examined the acute toxicity of depleted uranium (DU) after subcutaneous injection as a simulated wounds model, and the effects of the chelating agent catechol-3,6-bis(methyleiminodiacetic acid) (CBMIDA), by local treatment in rats. First, to examine the initial behavior and toxicity of uranium of different chemical forms, male Wistar rats were subcutaneously injected with 4 and 16 mg/kg DU (pH 1) in a solution of pH 1 and 7, respectively, and were killed 1, 3, 6 and 24 hours later. After the injection of DU(pH1), about 60 % of the uranium was retained for first 1-3 hours at the injected sites, and then decreased to 16% at 24 hours in the 4 mg/kg DU group; however, the uranium did not change significantly in the 16 mg/kg DU group. Urinary excretion rates of uranium increased in a time-independent manner after the injection. Depositions of uranium in the liver, kidneys and femur were found at 1 hour after DU injection, with significant increases in serum and urinary biochemical markers indicating acute and severe damage. The results of the DU (pH 7) injection were useful for estimating the toxicity of uranium by the chemical changes in the body. Second, CBMIDA (480mg/kg) was infused into the DU-injected site at 0, 10, 30, 60 min and 24 hours after the subcutaneous injection of 4 mg/kg DU (pH 1 and 7). When CBMIDA was administered within 120 min after DU (pH 1) injection, the uranium at the injected sites decreased to 4-17% of that in the no-treatment DU (pH 1) group, and was excreted effectively in the urine and feces, with decreased levels in the kidneys and femur. The results indicated that the subcutaneously injected uranium acutely induced severe damage in the DU-injected sites and organs after DU intake, relating to chemical forms of uranium by pH and that local treatment of CBMIDA was effective in decreasing the acute toxicity of uranium if carried out as early as possible (at least within 2 hours) after DU administration.
KEYWORDS: ***Depleted uranium, Wounds, Chelating agent CBMIDA, Chemical toxicity***

1.Introduction

Radiation workers and civilian are always exposed to a risk of contamination of radioactive materials from radiation accident, environment pollution, and nuclear-terrorism. Uranium is a radionuclide that can induce acute and serious toxicity damages by due to its the chemical action toxicity rather than the radiation effects toxicity [1]. In radiation accident uranium may be taken into the body though wounds. In the first stage of radiation emergency medicine, it is important to know primary information such as the contaminated site, doses and chemical forms, to estimate the degrees of uranium-induced damages, and decide the application of the chelating agent. There have been only a handful of studies on the toxicity of uranium after intake in wounds [2-5]. There are several problems to clarify the uranium toxicity and effects of chelating agents; the depth to which uranium reaches in wounds, the chemical structures of uranium [6], and uranium combination with various constituents in the body [4,7]. In the present study, we examined the toxicity of uranium when the different chemical forms and doses of uranium reaches subcutaneously as a model of wounds.

Chelation therapy is a unique method to reduce the toxicity of uranium. Many chelating agents have been examined in animal studies over the years [8-16]. Compared with these agents, catechol-3,6-bis(methyleiminodiacetic acid) (CBMIDA) is more effective than other agents [3,12]. To obtain the optimum effects of chelating agents, the timing of administration and the dose must be adjusted. In addition, the administration route of chelating agents is important in, for example, deciding whether to treat locally and systemically for wounds that are contaminated with uranium

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[2]. Therefore, we examined the effects of CBMIDA by infusion into uranium-injected sites because we expected CBMIDA to combine effectively with uranium in the wounds, and because this method can be applied as a local treatment with fewer side effects than when systematically administered.

2.Materials and methods

Uranyl nitrate (99.696% 238U, 0.299% 235U and 0.005% 234U) was dissolved in distilled water and the pH was adjusted to 1 and 7 with bicarbonate sodium. CBMIDA was dissolved in distilled water with bicarbonate sodium and the pH was adjusted to 6.8. The concentration of CBMIDA was controlled to administer 480 mg/ kg of body rat's weight. The molecular rate of CBMIDA: uranium (4 mg/ kg) was 71.8 times.

Experiment I: To examine the initial behavior and deposition of uranium, and the acute toxicity, one-hundred and twelve male Wistar rats, 8 weeks-old, weighing 180 ± 5g were divided into four groups of twenty-eight rats each. The first and second groups were injected subcutaneously with 4 mg/ kg and 16 mg/kg DU in the pH 1 solution in the center of the middle abdomen of the rats, and the third and fourth groups were injected with 4 mg/kg and 16 mg/kg DU in the pH 7 solution. To compare the influence of nitric acid and the administration volume of solution, twenty rats were injected with the same volume of pH 1 solution as they were injected with dissolved uranium, and ten rats were injected with the same volume of pH 7 solution. Each pH of the solutions was adjusted with bicarbonate sodium. The other intact ten rats was used as a control group. The rats were individually kept in a metabolic glass cage until being killed.

Under anesthesia the rats in the DU administered groups were killed each seven animals at 1, 3, 6 and 24 hours after the injection. The animals administered only the pH 1 solution were each five animals on the same schedule as that of the DU groups, and the rats of only the pH 7 solution were by each five animals at 1 and 24 hours after the injection. The blood, urine in bladder, skin and subcutaneous tissues including the periphery where DU was injected, along with the kidneys, liver, femur and digestive duct were obtained. In addition, urine and feces excreted until the rats were killed were collected.

Experiment II: To examine the effects of CBMIDA administered into DU-injected site, male Wistar rats of the same age and mean body weights as those in experiment I were used. First, to examine the behavior of uranium combined with CBMIDA (DU-CBMIDA) in the body, DU (equivalent to 4 mg/kg) in the pH1 and pH7 solutions and CBMIDA (equivalent to 480 mg/kg) in the solution were mixed 30 min prior to the administration. The DU-CBMIDA in the pH 1 and 7 solutions was injected subcutaneously into the center of the middle abdomen of seven rats. Second, to examine the effective timing of CBMIDA administration after DU injection, thirty-five rats were injected subcutaneously into the center of the middle abdomen with 4 mg/kg DU in the pH 1 solution, and divided into 5 groups of seven rats each. Each group was infused with 480 mg/kg CBMIDA into the DU-injected site at 10, 30, 60, 120 min and 24 hours after DU injection. Another thirty-five rats injected with 4 mg/kg of DU in the pH 7 solution were treated with the same schedules and doses of CBMIDA as the pH 1 group. The rats were kept in a metabolic glass cage and killed 24 hours after CBMIDA administration. The same samples as those in experiment I were collected.

Radioactivity measurement of uranium: The samples were incinerated at 1000 $^{\circ}$C for 24 hr in a crucible. The ash material was dissolved in a 10% nitric acid solution. The solution was subsequently poured into a counting vial with a scintillator. The alpha activity of uranium in the vial was measured for 30 min by spectrometry using an alpha liquid scintillation counter. The recovery and counting efficiency were confirmed based on the value measured in the solution with the uranium by alpha spectrometry.

Serum and urinary biochemical parameters: Glutamic pyruvic transaminase (GPT), glutamic oxalate tran saminase (GOT), alkaline phosphatase (ALP), blood urea nitrogen (BUN), creatinine (Cre), in the serum, and N-acethyl-bD-glucosaminidase (NAG) and creatinine in the urine indicating

the values of NAG/Ccre were measured by an autoanalyzer.

Histological examination: The kidney obtained in experiment I was fixed in 10 % buffered formalin and embedded in paraffin by the routine process. The block was cut into 3 μm slices with a microtome. The specimens were stained in Hematoxylin-Eosin solution and observed histologically under a light microscope.

Statistical analysis:Statistical analyses were performed by a Mann-Whitney and Student's t test using Stat-View 4.5 (Abacus Concepts, Inc., USA). A p value below 0.05 was considered significant.

3.Results
Experiment I:

Uranium in the 4 mg/kg DU-injected site retained 57.6-61.0 % of the injected dose for first 1-3 hours after the injection, and then decreased to 15.7% at 24 hours in the pH 1 group, and retained 34.6% at 1 hour and then decreased to 2.4% at 24 hours in the pH 7 group. The values in the 16 mg/ kg DU group did not change significantly (Fig.1).
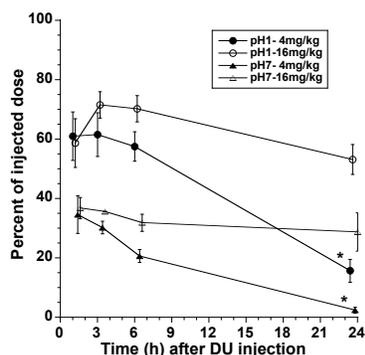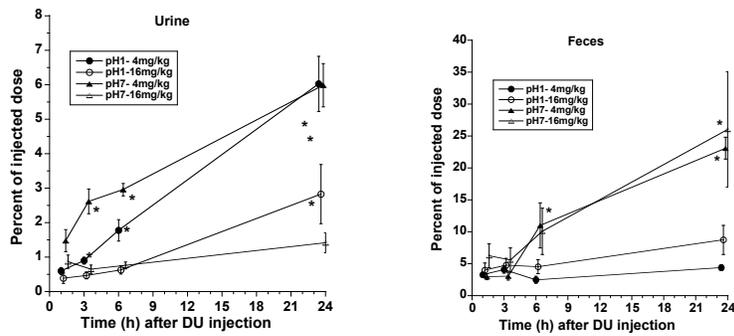

Fig.1 Concentration rates of uranium (of the administered doses) retained in the injected sites after the injection of 4 and 16 mg/kg DU dissolved in the pH 1 or 7 solution in experiment I. Data are presented as the mean ± SE in seven animals; * significantly different from the value at 1 hour after the injection (p<0.05).

The concentration rates of uranium in the serum did not change significantly by the pH of the solution and with time after the DU injection (Table 1). There were differences in the concentration rates of uranium between the 4 and 16 mg/kg DU groups regardless of the pH of the solution.

The urinary excretion of uranium in the 4 mg/kg DU increased rapidly in a time-dependent manner to about 6% at 24 hours (Figs. 2). After16 mg/kg DU injection, the uranium increased slowly to 2.8% (pH 1) and 1.4% (pH 7) at 24 hours. The excretion rates of uranium in the feces and digestive duct were 2.5-4.4% in the 4 mg/kg DU (pH 1) group, but increased siginificantly to 23.1% at 24 hours in the DU (pH 7) group (Figs. 2). Similarly, in the 16 mg/kg DU group the values did not change (pH 1), but increasing significantly to 26.1% (pH 7) at 24 hours.

The uranium concentrations in the liver were highest at 1 hour and decreased significantly after 6 hour post-injection in the 4 mg/kg mg DU (pH 1) group, but did not change in the 4mg /kg DU (pH 7) and the 16 mg/kg DU groups of both pH solutions (Table 1). In the kidney, the uranium concentration rates did not change in the 4 mg/kg DU group of both pH solutions, except that at 3 hours (pH 7) group. In the 16 mg/kg DU (pH 7) group, the values increased significantly to 11.6% at 24 hours. In the femur, there were no significant differences in the values by the differences of doses of DU and the pH of the solution.

Figs.2 Urinary (right) and fecal (left) excretion rates of uranium (of the administered doses) after the injection of 4 and 16 mg/kg DU in the pH1 or 7 solution in experiment I. Data are presented as the mean ± SE in seven animals; *significantly different from the value at 1 hour after the injection (p<0.05).

Table 1 Uranium concentrations in serum and organs after the DU injection in experiment I. Data are presented as the mean ± SE in seven animals. * Significantly different from the value at 1 h after DU injection (p<0.05). Uranium was not detected in the intact group.

| Groups and measured time	Serum	Kidney	Femur	Liver
4 mg/kg DU (pH1)				
1h	0.47±0.10	2.28±1.01	0.97±0.18	2.58±0.49
3h	0.41±0.08	2.65±1.26	0.92±0.16	1.18±0.25
6h	0.58±0.05	3.07±1.05	0.95±0.14	0.92±0.10*
24h	0.47±0.05	4.26±1.50	1.18±0.09	0.84±0.17*
16 mg/kg DU (pH1)				
1h	0.13±0.02	0.24±0.08	0.26±0.09	0.36±0.21
3h	0.12±0.02	0.32±0.10	0.31±0.13	0.68±0.25
6h	0.10±0.01	0.49±0.11	0.26±0.09	0.34±0.10
24h	0.09±0.01	0.16±0.02	0.42±0.15	0.25±0.03
4 mg/kg DU (pH7)				
1h	0.52±0.07	1.63±0.66	1.41±0.15	0.96±0.26
3h	0.53±0.07	5.61±1.79*	1.39±0.11	1.54±0.60
6h	0.49±0.09	1.75±0.80	1.43±0.06	0.84±0.05
24h	0.50±0.04	2.57±1.22	1.74±0.94	1.58±0.39
16 mg/kg ^{DU} (pH7)				
1h	0.11±0.01	1.71±0.88	0.99±0.12	0.72±0.13
3h	0.12±0.02	3.99±1.64	1.18±0.11	0.86±0.34
6h	0.13±0.02	6.40±2.95	1.35±0.15	0.89±0.42
24h	0.12±0.01	11.62±1.76*	1.15±0.14	1.34±0.58

In the results of serum and urinary examinations in the DU (pH 1) group, GPT, ALP, creatinine, and BUN in the serum increased significantly after 1 hour post-injection. ALP and BUN increased also significantly in the no-DU group. GOT in the DU groups increased, although varied. NAG/Cre increased significantly at 24 hours in the 4 mg/kg DU group, and 3 and 24 hours in the 16 mg/kg DU group. In the pH 7 group, ALP and BUN increased significantly after 1 hour post-injection in all of the groups. Creatinine increased significantly at 6 h and 24 hours in the 16 mg/kg and only the pH 7 solution groups. NAG/Cre increased significantly at 1-24 hours in the DU groups.

After the injection of DU in the pH 1 and pH 7 solutions, the center of the DU-injected site of the skin hardened and the surrounding area weakened, even at 1 h after DU injection. The degree of damage to the skin was severer in the DU (pH 1) group, although the same amount of damage and alterations were not seen in the pH 1 solution group. In the histological observations of the kidney, the swelling, vacuolar degeneration and necrosis of epithelial cells of the proximal and distal tubes began at 1 hour. The cells were released from the basement membrane of the renal tube at 3 and 6 hours, and blood cells were also found between the epithelial cells and base membrane of the renal tube. Thereafter, the epithelial cells were lost, and blood and protein were stored in the proximal and distal tubes until 24 hours, regardless of the doses of uranium and pH of the solution; however, these alterations were severe in all of the DU (pH 7) groups.

Experiment II

The concentration rate of uranium retained in the DU injected was 4% in the DU (pH1) group, and decreased significantly to 6-17% when CBMIDA was administered within 120 min after DU injection, but it did not decreased in the pH 7 group (Fig.3). The serum levels of uranium did not change in the pH 1 group, and varied in the pH 7 group.
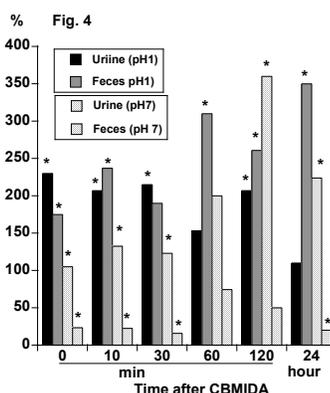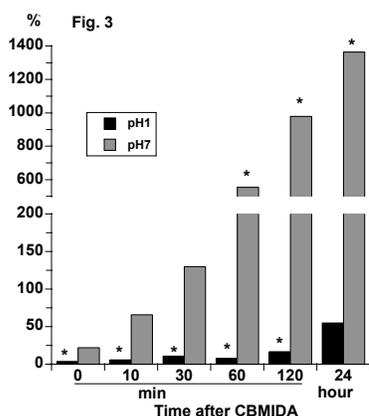
Figs. 3&4 Uranium concentration rate retained in the DU-injected site (Fig.3) and excreted in urine and feces (Fig. 4) in the groups that were treated with CBMIDA in experiment II. Data are presented as percent of the mean value at 24 h and asterisk (*) different significantly (p<0.05) from the value corresponding to measured items in the 4 mg/kg DU (pH1 and 7) groups in experiment I.

The urinary and fecal excretion rates of uranium increased significantly in all of the pH 1 group except in the urine of CBMIDA group administered at 24 hours. In the pH 7 group, the total excretion rates of uranium did not change significantly by CBMIDA treatment.

The concentration rates of uranium in the liver did not change in all of the groups (Table 3). The uranium in the kidneys decreased significantly in the groups of DU-CBMIDA and CBMIDA administered within 30 min after DU (pH 1) injection, but did not change significantly in the DU (pH 7) group. In the femur, the uranium concentration rates decreased significantly in the groups of DU-CBMIDA and CBMIDA administered within 120 min after DU (pH 1) injection, and decreased significantly in the groups of DU-CBMIDA and CBMIDA within 30 min after DU (pH 7) injection.
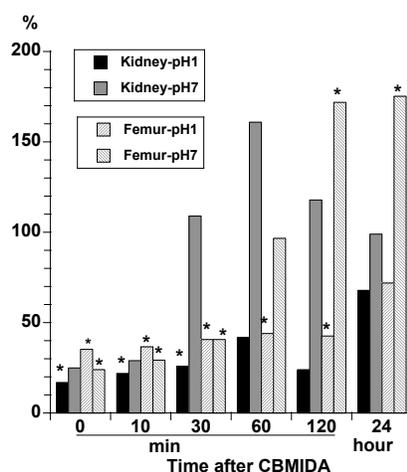
Fig. 5 Uranium concentrations in kidneys and femur of the groups that were treated with CBMIDA in experiment II. Data are presented as percent of the mean value at 24 h and asterisk (*) different significantly ($p<0.05$) from the value corresponding to measured items in the 4 mg/kg DU (pH1 and 7) groups in experiment I.

In the serum and urinary examinations, GPT and GOT did not change in all groups. However, ALP and BUN in the DU (pH 1 and pH 7) groups increased significantly compared with those in the intact group. Creatinine did not change in the DU (pH 1) groups except in the group that was administered CBMIDA at 24 hour post-DU injection, but the values increased significantly in all of the DU-CBMIDA and CBMIDA groups. NAG/Cre in the DU (pH 1) groups did not increase significantly from the values of the intact group, except when administered with CBMIDA at 24 hours post-DU injection, but increased significantly in the groups that were treated with CBMIDA at 1 and 24 h post-DU (pH 7) injection.

Compared with the DU-injected site in experiment I, no alterations in the skin treated with CBMIDA were found, or if found, these alterations were slight, and the skin did not tear, except in the group in which CBMIDA was injected at 24 h after DU (pH 1) injection. In the histological observation of the kidney, the vacuolar degeneration and loss of epithelial cells in the proximal and distal tubes decreased sharply in the groups of DU-CBMIDA and CBMIDA administered within 120 min after DU (pH 1) injection, although the loss of epithelial cells was slight in the group treated with CBMIDA at 24 h after DU injection. The damage in the kidneys of the CBMIDA treated groups was reduced compared with that in the experiment I groups. However, in the DU (pH7) group, there was significant vacuolar degeneration and loss of epithelial cells, and there was also protein and urine stored in the proximal and distal renal tubes, particularly in the groups that CBMIDA was administered within 30 min after DU injection.

4.Discussion

The results that more uranium retained in the DU (pH 1) injected site than in the pH 7 solution at 1 hour after the injection might be due to the following reasons: uranium (UO_2^{2+}) combined with the various constituents in the body fluids [7] and the tissues that altered by the acid burn of the solution. In addition, the uranium itself changed complexly and was agglutinated by the body fluid, and thus the uranium could not move easily from the injected site. Because, it was estimated that some chemical forms of uranium, in which produced in the pH 7 solution, could move rapidly without the disturbances from acid.

The uranium in the DU-injected site of the 4 mg/kg DU group decreased more rapidly than in the 16 mg/kg DU group regardless of the pH of the solution (Fig.1). Also, the concentrations of uranium in the serum and urine in the 4 mg/kg DU group were higher than in the 16 mg/kg DU group (Figs. 2). Based on the differences (4 times) in the administration doses of DU, there were no

differences in the net concentrations of uranium in either group. This indicated that the mass of uranium in the serum might be limited by the capacity of uranium to combine with various constituents [7], and also decreased by the early toxic action of uranium, because BUN, creatinine and NAG/cre increased even at 1 hour after DU injection.

On the other hand, the fecal excretion rates of uranium in the DU (pH 7) group increased in a time-dependent manner, and were higher than in the DU (pH 1) group (Fig. 3). These results indicate that uranium is excreted rapidly in the feces via the liver. The results also indicate that the excretion depends on the chemical forms of uranium, and that the fecal excretion of uranium increases if the concentration of uranium in the serum exceeds the capacity of the kidneys to process the uranium. In other words, the capacity of the kidneys to excrete uranium might be less than the liver, in comparing the excretion rates of uranium in the feces and urine.

Uranium induces acute dysfunction and alterations of tissue in the kidneys [1, 17-18]. Severe damage in the kidneys is induced at least 1 hour after DU administration. This might be due to the fact that UO_2^{2+} acts directly against the epithelial cells of the renal tubes, and thereafter affected by other chemical forms of uranium that changed in the body, because the damages were sever in the pH 7 solution, judging from the results of histological observations of kidneys. In the serum examination, creatinine and BUN increased significantly within 1 hour in the DU (pH 1) group. Creatinine did not increase in the groups receiving only pH 1 solution. BUN increased significantly in the groups administered only the solution. NAG/Cre is a useful marker for assessing not only the renal damage, but also the levels of the chemical toxicity of uranium [17-19]. However, the NAG/Cre levels were variable within 6 hours after DU (pH 1) injection. Namely, NAG/Cre may not always be useful for estimating the renal concentration of uranium early after the intake or in cases when the intake dose is high. Therefore, creatinine might be the most useful marker for accurately diagnosing acute renal damage early after intake following a radiation accident.

GOT and GPT increased significantly at 1 hour after the injection of DU, but did not increase in the pH 1 solution group. Uranyl nitrate induced severe degeneration in the liver [20]. Therefore, the results indicated that the uranium deposited rapidly in the liver and induced acute dysfunction, significantly increasing GOT and GPT. In addition, ALP increased significantly in the groups of DU and only solution. Therefore, ALP may not be useful for diagnosing acute damage in the liver. About 80% of uranium deposits in bone [1]. Various kinds of damage to bone are induced by uranium [18, 21]. ALP was also not a useful indicator for assessing acute damage to bone, or to damage in the liver.

In experiment II, when CBMIDA were infused within 120 min after DU (pH 1) injection, the skin damages observed in experiment I were improved as well as that in the DU-CBMIDA group. The improvement might be due to the neutralization of solution of pH 1 by mixture with the CBMIDA solution, because that solution is pH 6.8, and also because the inhibition of chemical toxic actions of uranium occurs by the chelating effects of CBMIDA.

The uranium retained in the injected site of DU-CBMIDA (pH 1) decreased to 4 % of that at 24 hours in the 4 mg/kg DU (pH 1) group in experiment I. Also, the uranium concentrations in the DU-injected site when treated by CBMIDA within 120 min after DU injection decreased significantly (Fig. 3). This indicated that CBMIDA combines effectively with uranium (UO_2^{2+}) in wounds if treatment is carried out within 120 min after DU contamination. At the same time, CBMIDA treatment within 120 min after the intake demonstrated the profitable effects in accelerating the excretion of uranium in the urine and feces (Fig. 4), and significantly decreasing the concentrations of uranium in the kidneys and femur (Fig. 5). The effects of CBMIDA on the kidneys were supported by the results that creatinine in the serum and NAG/Cre in the urine were at the same level as in the intact group. BUN increased significantly in this study, but it did not increase when DU in the pH 1-10 solutions was administered intraperitoneally in rats, except that of the rats in the pH 7 group [12]. Also, severe damages to the kidneys, which was observed in the groups without CBMIDA treatment in experiment I, were not found. The results of the data on the DU (pH 1) groups that were treated by CBMIDA indicate that if CBMIDA is infused into the wounds at least within 120 min after contamination with DU, the various kinds of damage induced by uranium might decrease.

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On the other hand, even if CBMIDA were administered within 30 min after the intake of DU (pH 7), the effects of CBMIDA were not obtained. In the groups in which CBMIDA was administered after 60 or 120 min post-injection of DU (pH 7), the amount of uranium in the DU-injected site, serum, urine, and bones increased significantly. This may be due to the fact that CBMIDA combines with some of the chemical forms of uranium produced by pH and constituents in the body. Uranium (UO_2^{2+}) below pH 3 changes to various forms at pH 7, but to only $UO_2(CO_3)_3^{4-}$ at pH 10 [6]. CBMIDA was effective for the toxicity of uranium in pH 10 solution [12]. Therefore, CBMIDA can combine with $UO_2(CO_3)_3^{4-}$, suggesting that delayed treatment of CBMIDA may be effective, although further study on this is needed.

5. Conclusion

The present study was carried out to clarify the acute toxicity of uranium within 24 h after subcutaneously injected DU as a simulated wound model (experiment I), and the effects of CBMIDA by local treatment in DU-contaminated wounds (experiment II). To clarify the toxicity by the differences in the chemical forms and doses of uranium, two doses (4 and 16 mg kg-1) of DU dissolved in pH 1 and 7 solution were injected in rats in experiment I. The results indicate that when the uranyl nitrate (UO_2^{2+}) in the pH 1 solution reaches the subcutaneous tissue in wounds, about 60% of the uranium is retained within a few hours after the intake, and the DU-injected site becomes the source from which uranium is released throughout the body. The uranium excretes rapidly in urine in time and dose-dependent after the intake; at the same time uranium deposits in the liver, kidneys and bone, and induces dysfunctions within at least 1 hour after the intake. The data in the DU (pH 7) group are useful for assessing the chemical toxicity of uranium that might change in the body. In experiment II, the effects of CBMIDA when it was infused into the DU-injected site after DU (pH 1) injection were examined. The results demonstrated that CBMIDA can decrease significantly the concentration rates of uranium in the DU-injected site (to 6-17% of that at 24 h post-injection in the groups without CBMIDA treatment) and significantly accelerate the excretion of uranium in the urine and feces, and also decrease the concentration of uranium in the kidney and bone, when CBMIDA is administered as early as possible (within 120 min) after intake.

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