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Sodium bicarbonate protects uranium-induced acute nephrotoxicity through uranium-decorporation by urinary alkalinization in rats

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Running title: Reduction of Nephrotoxiciy by Uranium Decorporation in Rats

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ABSTRACT

To evaluate the effectiveness of sodium bicarbonate (SB) in removing uranium and protecting animals from uranium toxicity, we intramuscularly administered 1 mg/kg of uranyl nitrate to 8-wk-old male SD rats, and 20 min after administration of uranyl nitrate, the animals were given a single oral administration of SB at 0.1, 0.3 or 1 g/kg. The SB treatment at a dose of 0.3 g/kg or more raised the pH of the rats' urine until 4 h after treatment, and it significantly reduced the uranium amounts in the kidneys at 1 day after treatment. In another experiment, rats were intramuscularly administered 1 mg/kg of uranyl nitrate, and 20 min later, the animals were treated with sodium bicarbonate (0.1 or 1 g/kg). The rats were autopsied at 1, 3 and 7 days after uranium treatment. High-dose SB resulted in a significant increase in urinary uranium excretion in the first 24 h and a reduction of uranium deposition in the kidneys and femurs, and it also significantly suppressed uranium-induced renal toxicity, as shown by both histopathology and clinical chemistry at 3 days after uranium treatment. Low-dose SB did not show such marked effects. Our findings demonstrated that the uranium decorporation effect of sodium bicarbonate was observed at the dosage showing urine alkalinization in rats and that decorporation effect of sodium bicarbonate might be beneficial if it is administered immediately after incorporation of soluble uranium.

Key words: uranium, decorporation effect, sodium bicarbonate, urine alkalinization, rat

INTRODUCTION

Uranium, an actinide element that has been present since Earth's formation, is used as fuel for nuclear power plants due to its reactivity. In the case of internal exposure, the major target organs of uranium are kidney and bone. Accidental intake of uranium induces acute renal toxicity in humans and animals through accumulation in the kidneys[1, 2]. In parenteral ingestion of soluble-form uranium, the uranium rapidly enters the systemic circulation as uranyl ions, and it is also immediately deposited in the kidneys[1].Uranium accumulates mainly in the renal proximal tubules of the outer stripe of the outer medulla, and a high accumulation of uranium causes tubular damage such as renal tubular necrosis[1−3].

Uranium is also one of the bone-seeking elements, and it is deposited on the bone surface, where it remains for a long period[1]. As one of the alpha-particle-emitting radionuclides, uranium is thus thought to possibly increase the risk of a stochastic effect, such as bone malignancies[1, 4]. Following an accidental intake of toxic levels of uranium, decontamination therapy should therefore be performed to prevent uranium toxicity including acute renal toxicity and the risk of bone cancer development.

In decontamination therapy, uranium excretion-enhancing drugs such as chelating agents can be used to induce the excretion of as much uranium as possible in the early period. Sodium bicarbonate has been proposed as a representative agent for uranium decontamination[5−7]. The treatment regimen described in a 2010 National Council on Radiation Protection & Measurements (NCRP) report is a slow intravenous infusion of sodium bicarbonate solution, or an oral administration of sodium bicarbonate tablets until the urine reaches a pH of 8.0 to 9.0[5]. Although increasing the blood level of bicarbonate ions and alkalinizing the urine were thought to effective for amelioration of acute

uranium contamination in affected humans[8], the effectiveness of uranium decontamination by sodium bicarbonate has not been supported by controlled studies with laboratory animals under realistic conditions[9]. Indeed, two research groups reported that treatment with sodium bicarbonate produced almost no decontamination effects in uranium-contaminated rats[10, 11]. The dosage of sodium bicarbonate in these studies was approx. 0.1 g/kg, which is almost equal to the clinical human dose, and the urine pH of the treated animals was not monitored in these studies. In addition, sodium bicarbonate showed urinary alkalinization at a dose level higher than the clinical human dose. Chiu et. al. reported that cortical uptake of gentamicin was inhibited by urinary alkalinization due to a 1-h infusion of a solution of sodium bicarbonate (0.3 mol/L) at a dose of 6.3 mL/h (calculated as approx. 0.5 g/kg) preceding the administration of gentamicin, and by a continuous infusion of 0.15 or 0.30 mol/L sodium bicarbonate for 3 h (calculated as approx. 0.75 or 1.5 g/kg) after the administration of gentamicin[12].

Hattori et al. reported that sodium bicarbonate at a dose of 1 g/kg showed a significant urine alkalinization effects in rats[13]. We thus hypothesized that the lack of a uranium decorporation effect in the laboratory animals in the Henge-Napoli et al.[10] and Fukuda et al.[11] studies described above may have been due to the dosage of sodium bicarbonate, which produced insufficient urine alkalinization. In the present study, to determine whether sodium bicarbonate at a dose producing urine alkalinization has uranium decontamination effects, we used a uranium-contaminated rat model to examine the effectiveness of sodium bicarbonate for removing uranium and protecting against uranium-induced acute renal toxicity.

MATERIALS AND METHODS

Compound

Uranium nitrate was purchased from Wako Pure Chemical Industries. (Osaka, Japan). The uranium was dissolved in distilled water, and the administration volume was 1 mL/kg body weight. Sodium bicarbonate (SB) was purchased from Nippon Chemiphar (Tokyo). The SB was dissolved in distilled water, and the administration volume was 10 mL/kg body weight.

Experiment I: Effect of sodium bicarbonate on the pH of the urine in uranium-contaminated rats

We used 8-wk-old male Crl:CD (SD) rats (n=24; Charles River Laboratories Japan, Kanagawa, Japan). The animals were randomly assigned into four groups (six animals per group). All animals were administered 1 mg/kg of uranyl nitrate intramuscularly into the right femoral muscle at 10:40 a.m. Twenty minutes after the uranium injection, the animals in the four groups were given a single oral administration of SB at the dose levels of 0, 0.1, 0.3 and 1 g/kg, respectively. The dosages used were based on the report by Hattori et al[8]. For the uranium control group, distilled water (DW) only was administered in the same manner as that used for SB.

The urine of each animal was collected just before and at 1, 2, 4, 9 and 23 h after the uranium injection. Each animal was placed in a plastic animal cage, and the naturally excreted urine was collected. The pH of the urine was measured with a pH meter (model D-51, Horiba, Kyoto, Japan). At 24 h after the uranium injection, the animals were euthanized under ketamine/xylazine anesthesia, the left kidney was removed, and the concentration of uranium in the kidney was measured with an inductively coupled plasma-mass spectrometer (ICP-MS, SII SPQ9700-II, SII Nanotechnology, Chiba,

Japan) after separating the uranium from matrix components using a closed-vessel microwave digestion system (Discover SP-D, CEM Corp., Matthews, NC, USA). Based on the concentration of uranium in the kidney, the uranium amount per left kidney was calculated and used for the evaluation.

Experiment II: Effect of urinary alkalinization on the removal of uranium and uranium-induced renal toxicity

For this experiment, we used 8-wk-old male Crj:CD rats (n=60; Charles River Laboratories Japan Inc., Kanagawa, Japan). Fifty-four animals were randomly assigned into three groups. All animals were administered 1 mg/kg of uranyl nitrate intramuscularly into the right femoral muscle at 10:40 a.m. At 20 min after the uranium injection, the animals in the three groups were given a single oral administration of SB at the dose levels of 0 (uranium control), 0.1 and 1 g/kg, respectively. Six animals in each group were autopsied at 1, 3 and 7 days after the uranium injection, respectively. Two dose groups of SB were selected based on the results of Experiment I. The high dose was expected to show significant urine alkalinization, and the low dose was expected to show no significant urine alkalinization. Before autopsy, 24-h urine collection was performed using metabolic cages. From this 24-h urine, 0–5-h urinary samples and 5–24-h urinary samples were collected. The urinary volume was measured, and the urine samples were subjected to a uranium amount analysis and urinary biochemistry. The animals were euthanized under ketamine/xylazine anesthesia, and blood samples obtained from each animal were used for plasma biochemical analyses.

Plasma urea nitrogen (UN), plasma creatinine (CRE), urinary total protein (uTP) and urinary glucose (uGLU) were analyzed using an automatic biochemistry analyzer

(CA 400, Furuno, Nishinomiya, Japan). Beta-2-microblobulin (β2-MG) in urine was determined by a commercial enzyme-linked immunosorbent assay (ELISA) kit (LSI Medience Corp., Tokyo, Japan). We evaluated the total urinary excretions of uTP, uGLU, and β2-MG in the 24-h urine samples.

In addition, each rat's right kidney was removed, and a part of the kidney was fixed in 10% neutral buffered formalin. Paraffin sections cut at 5 μm were stained with hematoxylin and eosin and subjected to histopathological examinations. The left kidney and the left femur were weighed and stored in a freezer until analyses. The concentrations of uranium in the left kidney, left femur and urine were measured by the method described above. Based on the concentrations of uranium in the left kidney, left femur and urine, the uranium amount per left kidney or left femur and 24-h urinary uranium excretion were calculated and used for the evaluation.

The six remaining rats were assigned into the normal control group. These animals were administered DW intramuscularly into the right femoral muscle, and 20 min after administration of the DW, the animals were given a single oral administration of DW. The rats' 24-h urine was collected and used for determination of the normal level of urinary β2-MG excretion. One day after the DW administration, the animals were euthanized under ketamine/xylazine anesthesia, blood samples were obtained, and the separated plasma was used for plasma biochemical analyses.

All numeral data are presented as means±standard deviation (SD). The statistical analysis was performed using Dunnett's test after an analysis of variance (ANOVA). All animal experiments were carried out with permission and under regulation of the Institutional Committee for Animal Safety and Welfare at the National Institute of Radiological Sciences.

RESULTS

In Experiment I, the urinary pH of the uranium-treated rats fell immediately after the uranium injection and returned to near neutral more than 4 h post injection (Fig. 1). Sodium bicarbonate significantly suppressed the decrease in urinary pH of the uranium-treated rats at a dosage of 0.3 g/kg or more. The highest dose of SB raised the urinary pH from 2 h until more than 4 h after the treatment, and the urine alkalinization effect was maintained until 9 h after treatment. The pH of the urine of the middle-dose group was significantly higher than that of the uranium control group from 2 until 4 h after treatment. Although the lowest dose of SB did not alkalinize the urinary pH, the acidity of the urea in the uranium-treated rats was significantly improved in this group at 4 h after treatment. The SB treatment dose-dependently reduced the uranium amounts in the kidney (Fig. 2).

In Experiment II, one animal in the uranium control group died on day 7 due to acute renal failure induced by uranium. This animal was excluded from the evaluation. The urine volume increased in the uranium control group at 3 and 7 days after uranium treatment. The high-dose SB treatment significantly improved this polyuria. However, the urine volume of the SB low-dose group increased in a manner similar to that in the uranium control group (Fig. 3). A significant increase in urinary uranium excretion was noted in the first 24-h urine of the SB high-dose group (Fig. 4). This increase was due to the increase in the uranium excretion in 0–5-h urine of the SB high-dose group, and the uranium excretion in the 5–24-h urine of the SB high-dose group was similar to that of the uranium control group (Fig. 4). The urinary uranium excretion at 3 or 7 days after the uranium treatment decreased in the SB high-dose group (Fig. 4). The uranium

amounts in the kidney and in the femur were significantly decreased in this group on day 1 and remained at a low level throughout the experiment period. The uranium amounts in the kidney and in the femur of the SB low-dose group were lower than those in the uranium control group on day 1. However, the uranium amounts in the kidney and in the femur were similar to those in the uranium control group on days 3 and 7 (Fig. 5).

The clinical chemistry results showed that the high dose of SB had remarkable protective effects against uranium-induced acute renal toxicity. Plasma UN and CRE were increased in the uranium control group after day 3, whereas the levels of these clinical markers in the SB high-dose group remained almost normal (Fig. 6). The levels of uTP, uGLU and β2-MG, which indicate uranium-induced renal tubular damage, were increased in the uranium control group on day 3, and then began to recover (Fig. 7). These markers were significantly lower in the SB high-dose group than in the uranium control group. The low-dose SB group did not show marked improvement of these renal markers; only a mild but significant suppression of β2-MG on day 3 was noted.

Histopathologically, the uranyl nitrate treatment induced acute tubular necrosis. On day 1, mild degeneration and single-cell necrosis of the tubular epithelium in the outer stripe of the outer medulla were sporadically seen in the uranium control group (Fig. 8a). On day 3, severe tubular necrosis was observed mainly in the outer stripe of the outer medulla in the uranium control group (Fig. 8b). In these lesions, in addition to the necrosis and/or degeneration of proximal tubular epithelial cells (which were usually detached from the basement membrane), basophilic epithelial cells were also observed in the affected tubules. On day 7, although marked regeneration of damaged renal tubules was observed, tubular dilatation with casts consisting of cellular debris was observed in the uranium control group (Fig. 8c). The cellular casts were commonly seen

in the outer stripe of the outer medulla, and proteinaceous casts and mild congestion were observed in the inner stripe of the outer medulla of the rats in the uranium control group (Fig. 8c).

Glomerular abnormalities were not seen in the uranium control group. In the SB high-dose group, the kidney was histopathologically normal (Fig. 8d) on day 1, and only mild degeneration and necrosis of the tubular epithelium were observed (Fig. 8e) on day3. On day 7, mild and focally regenerated tubules were seen as basophilic tubules in the SB high-dose group (Fig. 8f). The renal lesions in the SB low-dose group observed on days 1, 3 and 7 were comparable to those in the uranium control group at each time point (data not shown).

DISCUSSION

The results of the present study demonstrated that sodium bicarbonate had a decorporating effect for uranium contamination at the dosage showing urine alkalinization. From the results of Experiment I, we found that oral treatment with sodium bicarbonate decreased renal uranium deposition at the dosage showing urinary alkalinization, and we suspect that the degree of the reduction of renal uranium deposition was related to the dose level of sodium bicarbonate. The results of Experiment II clearly demonstrated that sodium bicarbonate protected the rats against uranium-induced renal toxicity at the dosage showing urinary alkalinization.

In light of the results of Experiments I and II, we speculate that 0.1 g/kg of sodium bicarbonate — which slightly improved the urinary pH of uranium-contaminated rats but did not alkalinize their urine pH — might have a weak or limited decorporating effect, resulting in the apparent renal-protection effects not being seen in the rats

administered 0.1 g/kg of sodium bicarbonate. In contrast, the urine alkalinization caused by 1 g/kg of sodium bicarbonate immediately after the uranium challenge enhanced urinary uranium excretion and showed subsequent renal protective effects. These results indicate that urinary alkalinization immediately after uranium ingestion is important for uranium decorporation.

Uranyl tricarbonate is a dominant species at about pH 8.0 or more[14], and it is considered to be stable[14]. Sodium bicarbonate may increase the uranyl tricarbonate levels in blood and urine by increasing the blood level of bicarbonate ions, and the increased stable uranyl ion complex may lead to a decrease in both the interaction between uranyl ion and renal tubular cells and the deposition of uranium in the tubular epithelial cells of the kidney.

Ethane-1-hydroxy-1,1-bisphosphonate (EHBP), a bisphosphonate used for the treatment of Paget's disease and the prevention of osteoporosis, has been reported to chelate uranium and show a uranium decorporation effect in rats[15], and it is listed as one of the possible agents for uranium decontamination therapy in humans[5]. In a comparison of the degree of effectiveness of sodium bicarbonate and that of EHBP, the effectiveness of sodium bicarbonate was thought to be larger than that of the decorporating effect of EHBP in uranium-contaminated animals[15]. In that report, EHBP was administered 5 or 30 min after an intramuscular injection of uranyl nitrate in rats, and the deposition in the kidney was decreased on the first day by a factor of approx. 5 or 2, respectively[15]. In our experiment, the uranium deposition in the kidney was decreased by a factor of approx. 5 with 1 g/kg of sodium bicarbonate treatment 30 min after the uranyl nitrate treatment.

In conclusion, our present findings clearly demonstrate that the urine

alkalinization agent sodium bicarbonate had a significant decorporation effect in the uranium-contaminated rat model. Regarding optimization of the decontamination treatment, further studies using sodium bicarbonate in rats could be conducted to examine parameters such as a delay between exposure and treatment of 30 min or more. Treatments for simultaneous contaminations with other nuclides and uranium could also be examined. In addition, urine alkalinization medicine may be useful as a decorporation agent for uranium-decontamination therapy.

Conflict of interest statement:

There are no conflicts of interest to be reported.

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LEGENDS FOR FIGURES

Fig. 1. Changes in urinary pH of rats treated with uranyl nitrate alone or in combination with sodium bicarbonate (SB). *p<0.05 vs. uranium control group at each examination time point (Dunnett's test); **p<0.01 vs. uranium control group at each examination time point (Dunnett's test).

Fig. 2. Uranium amount of the left kidney of rats treated with uranyl nitrate alone or in combination with SB. **p<0.01 vs. uranium control group (Dunnett's test).

Fig. 3. Changes in the urine volume of rats treated with uranyl nitrate alone or in combination with SB. *p<0.05 vs. uranium control group at each examination time point; **p<0.01 vs. uranium control group at each examination time point. #p<0.05 vs. uranium control group before administration (Dunnett's test); ##p<0.01 vs. uranium control group before administration (Dunnett's test).

Fig. 4. Urinary uranium excretions in 24-h urine of rats treated with uranyl nitrate alone or in combination with SB. The bar with a grid pattern represents the collected urine during 0–5 h post administration of uranyl nitrate. **p<0.01 vs. uranium control group at each examination time point (Dunnett's test).

Fig. 5. Uranium amounts in the left kidney (a) and left femur (b) of rats treated with uranyl nitrate alone or in combination with SB. *p<0.05 vs. uranium control group at each examination time point (Dunnett's test); **p<0.01 vs. uranium

control group at each examination time point (Dunnett's test).

Fig. 6. Blood biochemical analyses. Concentrations of urea nitrogen (a) and creatinine (b) in the plasma of rats treated with uranyl nitrate alone or in combination with SB. *p<0.05 vs. uranium control group at each examination time point; **p<0.01 vs. uranium control group at each examination time point. ##p<0.01 vs. normal control group (Dunnett's test).

Fig. 7. Urinary biochemical analyses. Total urinary excretions of total protein (a), glucose (b) and β2-microglobulin (c) in 24-h urine of rats treated with uranyl nitrate alone or in combination with SB. *p<0.05 vs. uranium control group at each examination time point; **p<0.01 vs. uranium control group at each examination time point. #p<0.05 vs. uranium control group before administration (a, b) or normal control (c) (Dunnett's test); ##p<0.01 vs. uranium control group before administration (a, b) or normal control (c) (Dunnett's test).

Fig. 8. Light micrographs of the kidneys from the uranium control group (a–c) and the group that received uranium combined with high-dose SB (d–f). The outer stripe of the outer medulla of the kidney from rats treated with uranyl nitrate on day 1 (a, d), day 3 (b, e) and day 7 (c, f).

Fig.1

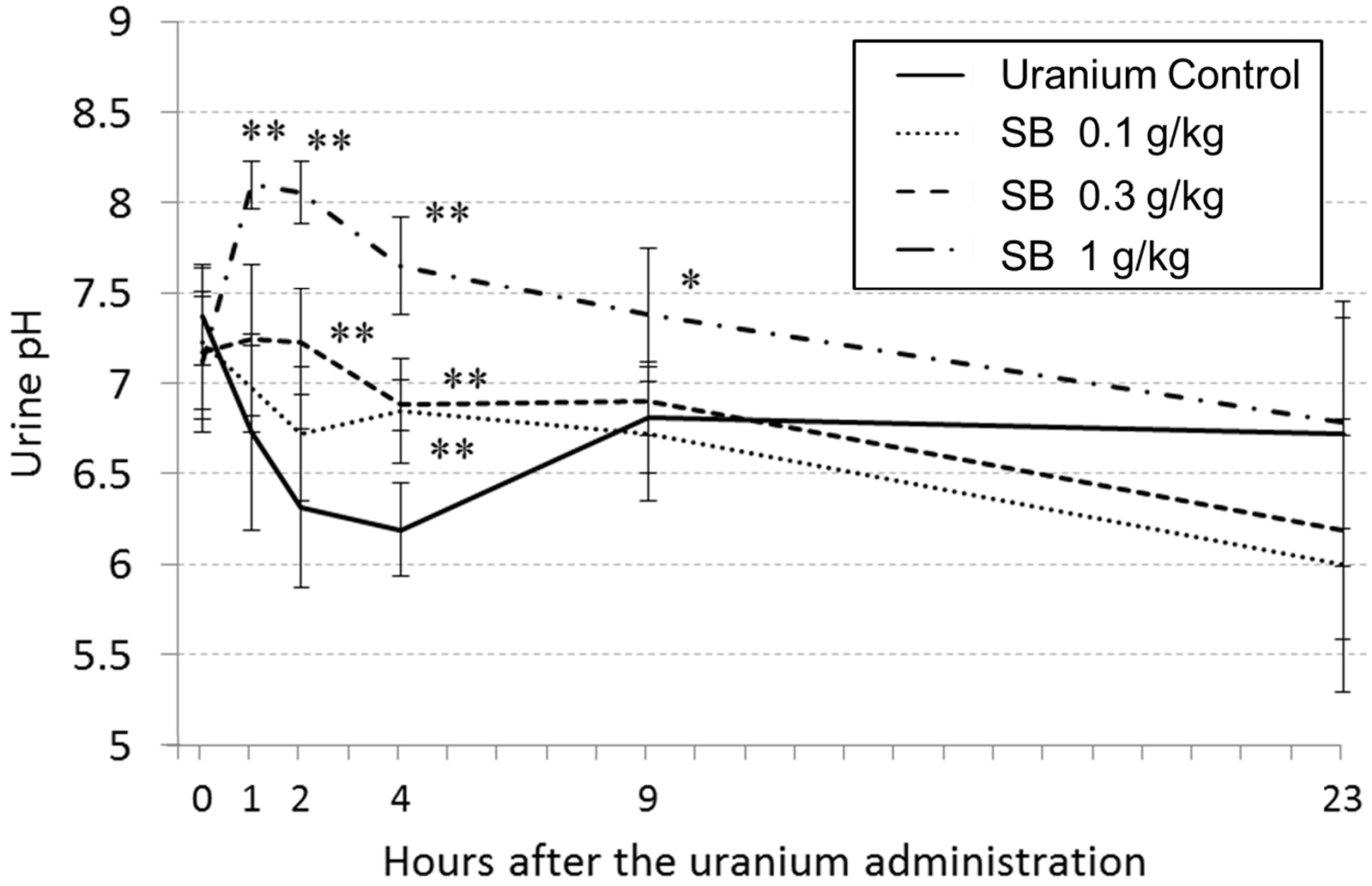
Uranium Control
SB 0.1 g/kg
SB 0.3 g/kg
SB 1 g/kg
Urine pH
9
8.5
8
7.5
7
6.5
6
5.5
5
0
1
2
4
9
23
Hours after the uranium administration
**
**
**
**
**
*

Fig.2

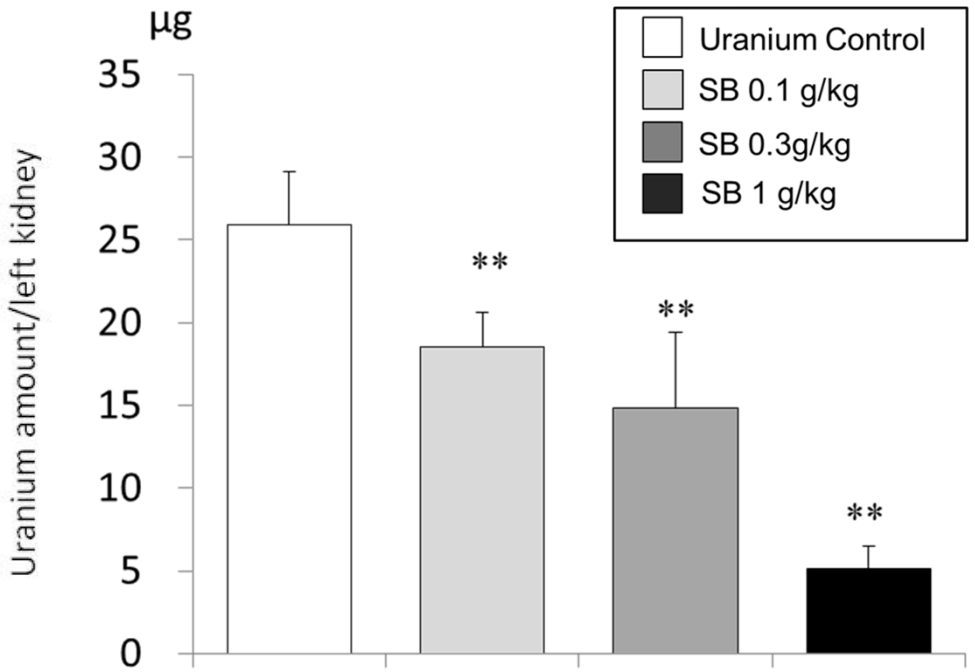
μg
Uranium amount/left kidney
35
30
25
20
15
10
5
0
Uranium Control
SB 0.1 g/kg
SB 0.3g/kg
SB 1 g/kg
**
**
**

Fig.3

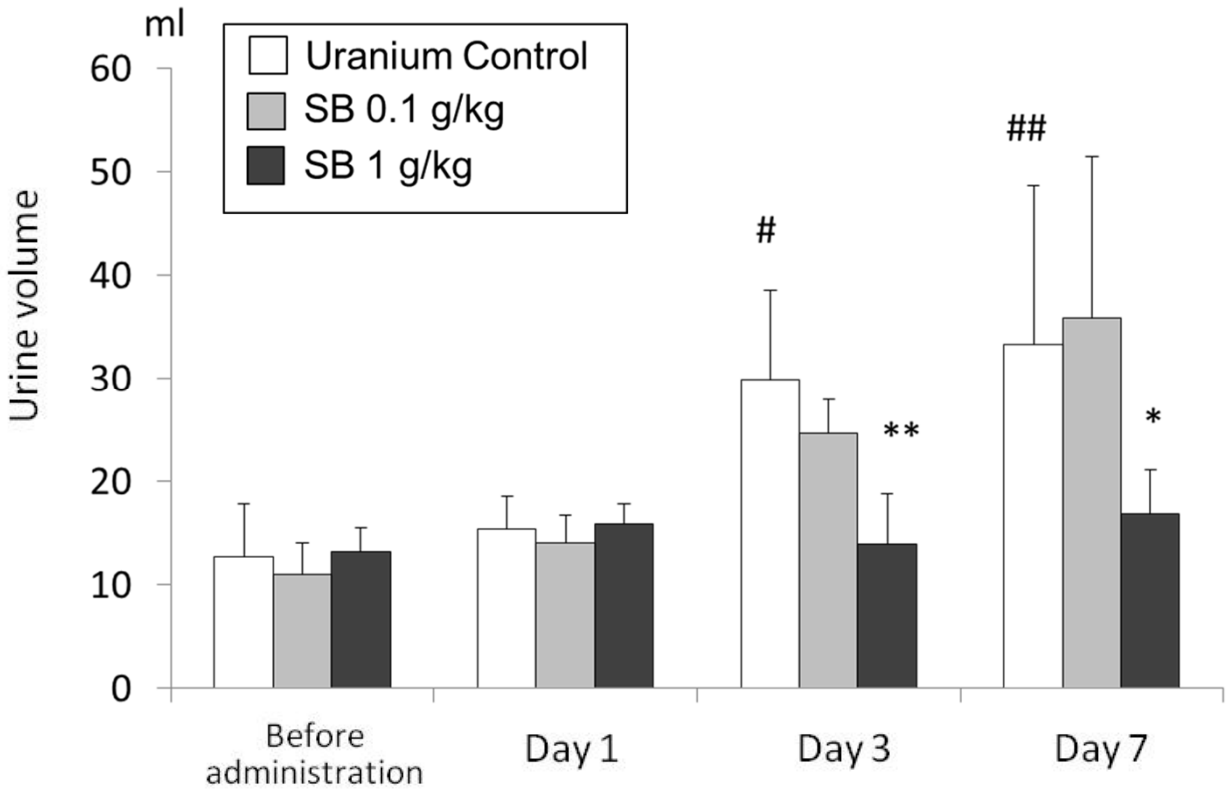
ml
Uranium Control
SB 0.1 g/kg
SB 1 g/kg
Urine volume
60
50
40
30
20
10
0
#
##
**
*
Before administration
Day 1
Day 3
Day 7

Fig.4

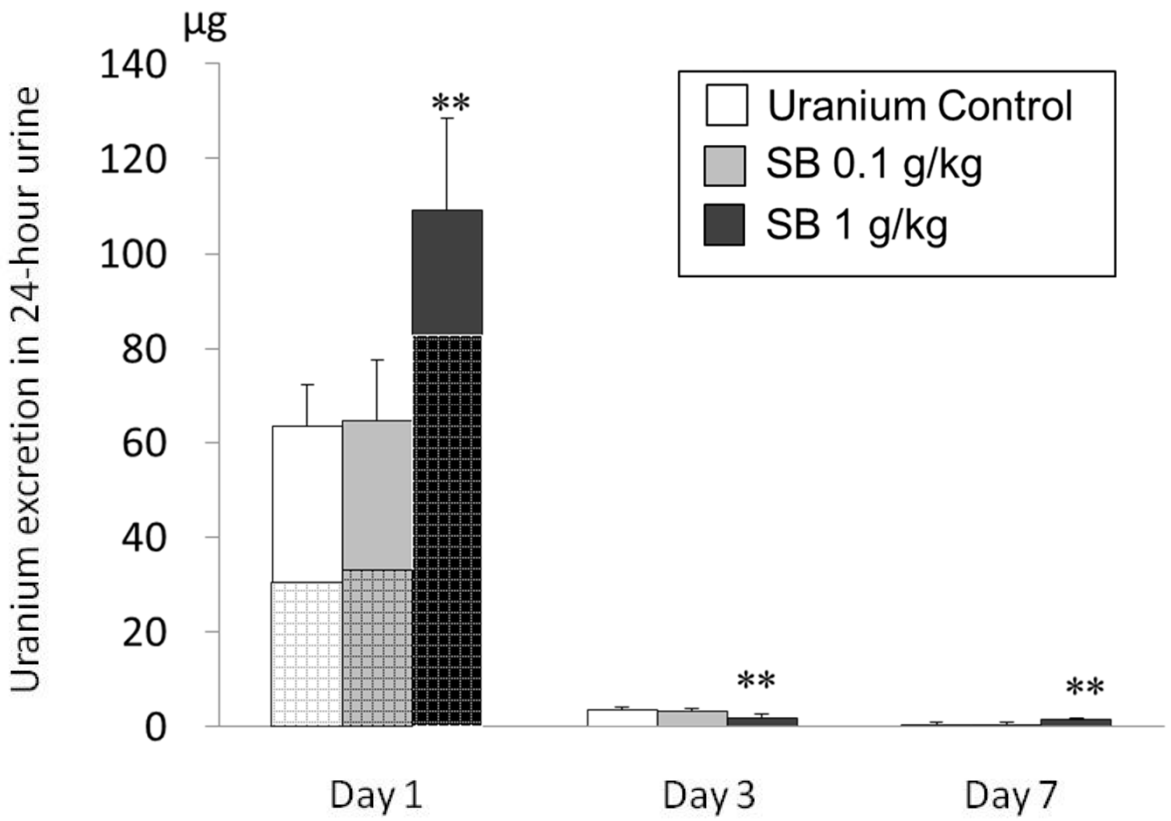
µg
140
120
100
80
60
40
20
0
Uranium excretion in 24-hour urine
**
**
**
Uranium Control
SB 0.1 g/kg
SB 1 g/kg
Day 1
Day 3
Day 7

Fig.5

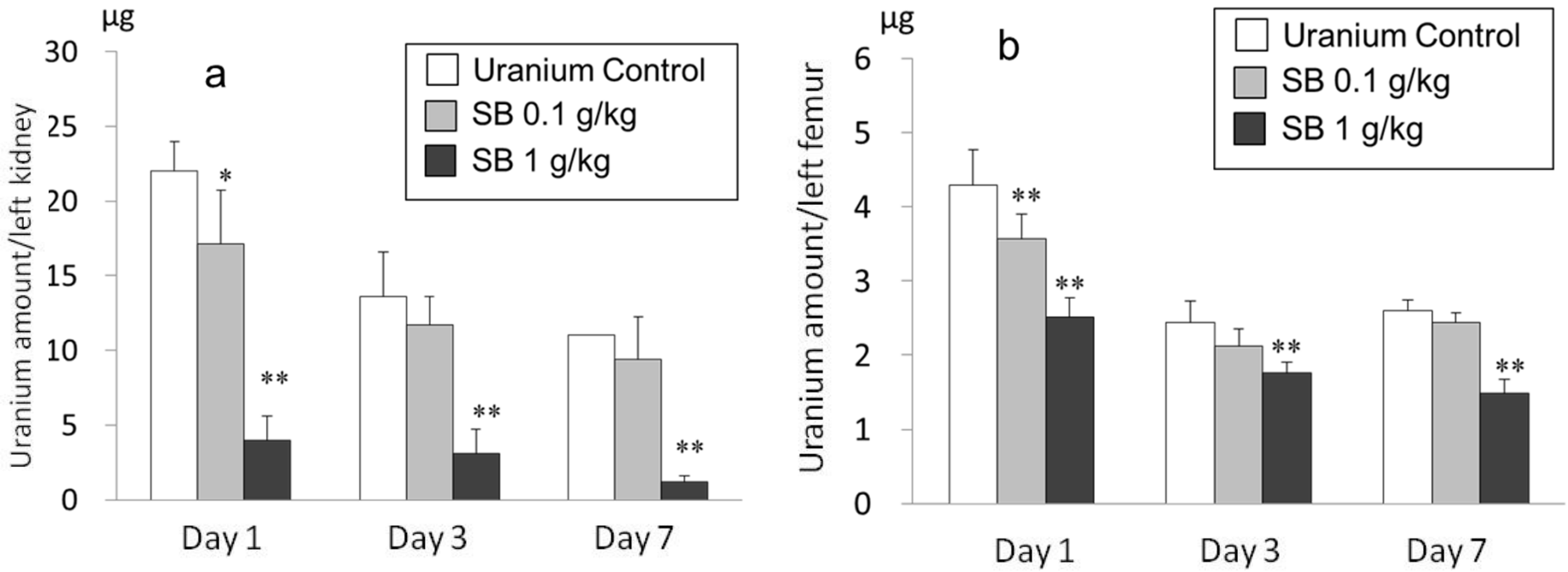
a
μg
Uranium amount/left kidney
30
25
20
15
10
5
0
Uranium Control
SB 0.1 g/kg
SB 1 g/kg
*
**
**
**
Day 1
Day 3
Day 7
b
μg
Uranium amount/left femur
6
5
4
3
2
1
0
Uranium Control
SB 0.1 g/kg
SB 1 g/kg
**
**
**
**
Day 1
Day 3
Day 7

Fig.6

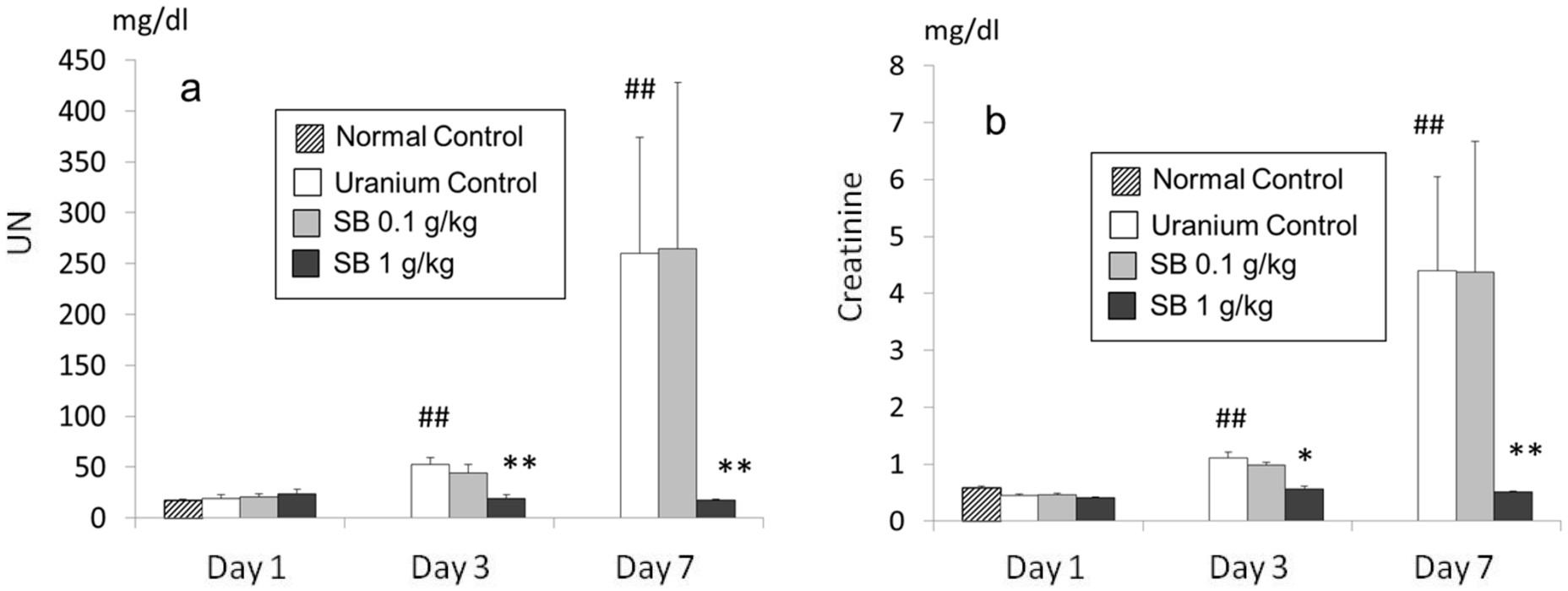
mg/dl
a
UN
450
400
350
300
250
200
150
100
50
0
Normal Control
Uranium Control
SB 0.1 g/kg
SB 1 g/kg
##
##
**
**
Day 1
Day 3
Day 7
mg/dl
b
Creatinine
8
7
6
5
4
3
2
1
0
Normal Control
Uranium Control
SB 0.1 g/kg
SB 1 g/kg
##
##
*
**
Day 1
Day 3
Day 7

Fig.7

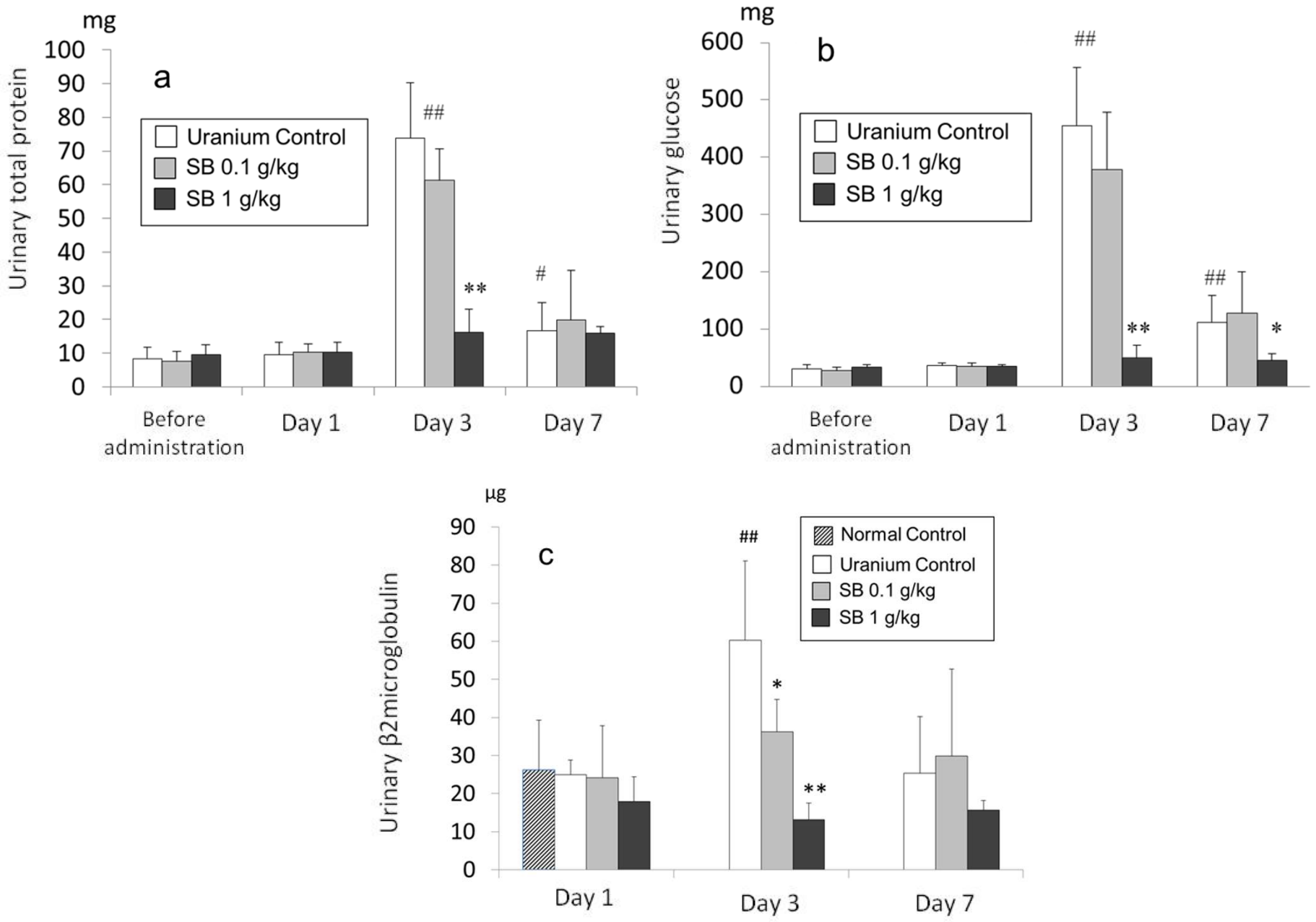
mg
Urinary total protein
a
Uranium Control
SB 0.1 g/kg
SB 1 g/kg
##
**
#
Before administration
Day 1
Day 3
Day 7
mg
Urinary glucose
b
Uranium Control
SB 0.1 g/kg
SB 1 g/kg
##
**
##
*
Before administration
Day 1
Day 3
Day 7
μg
Urinary β2microglobulin
c
Normal Control
Uranium Control
SB 0.1 g/kg
SB 1 g/kg
##
*
**
Day 1
Day 3
Day 7

Fig.8