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The mechanism of renal stone formation and renal failure induced by administration of melamine and cyanuric acid

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Abstract Renal stone formation and renal failure among Chinese infants administered melamine-containing formula were increasingly reported in 2008. We investigated the mechanism by which melamine and cyanuric acid induce renal stone formation and renal failure. Ten-week-old rats were administered either melamine [2.4, 24, or 240 mg/kg/day], both melamine and cyanuric acid [each at 1.2, 12, or 120 mg/kg/day], or water (controls). Blood and 24-h urine samples and kidney sections were evaluated on days 3, 7, and 14. In rats administered melamine alone or the low-dose melamine/cyanuric acid combination [1.2 mg/kg/day], crystals were not detected. On day 3, crystal formation was observed in the renal distal tubular lumens and collecting ducts of rats administered the intermediate-dose melamine/cyanuric acid [12 mg/kg/day], and the number of crystals increased during the course of the experiment. In rats administered the high-dose melamine/cyanuric acid [120 mg/kg/day], crystals were found in the proximal tubular lumens of the renal cortex on day 3, but acute renal failure resulted in death by day 7. Polarized light optical microphotography and scanning electron microscopy revealed tubular lumens occluded by a layer of axle-shaped crystals. X-ray diffraction findings revealed a nitrogen component but no calcium. The upper regions of occluded tubes were expanded, and the epithelium was thin. Melamine and cyanuric acid in combination, but not by melamine alone induce crystal formation and affected renal functioning. Renal failure due to melamine cyanurate crystals appears to occur via tubular occlusion.

Keywords Melamine · Cyanuric acid · Kidney stone · Tubular obstruction · Renal failure

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Introduction

Recently, cases of renal stones and acute renal failure have been increasingly reported among Chinese children who were fed infant formula contaminated with melamine. According to a 2008 WHO report, 294,000 babies showed symptoms, 51,900 were hospitalized, and 6 died from renal failure [1–3]. The source of melamine contamination was a Chinese milk dealer, who added the substance to make the milk's protein content appear higher than it was. This is because protein content is measured in terms of total nitrogen, an element richly contained in melamine. The effects of the melamine-contaminated milk on kidney function and renal stone formation has been of considerable interest worldwide.

Between 2004 and 2007, melamine-containing pet food was associated with numerous deaths among cats and dogs in North America. Pathological kidney examinations showed that some renal tubules of the affected animals were occluded by melamine cyanurate crystals [4]. Melamine and cyanuric acid, a by-product of melamine production, were mixed into raw materials of pet food imported from China. Triazin ring containing melamine is primarily used to produce melamine resin and in high nitrogen fertilizers. Cyanuric acid resembles melamine structurally, and is used as a water stabilizer in swimming pools and hot tubs to minimize the decomposition of hypochlorous acid by light [5]. Generally, neither

melamine nor cyanuric acid are present in human or animal foods. While the ingestion of either melamine or cyanuric acid alone does not induce renal disorders, the ingestion of a combination of these has been reported to affect renal function [6]. However, the mechanism by which melamine and cyanuric acid induce renal failure and kidney stone formation in combination has not been investigated.

In the present study, we established an animal model for investigating melamine cyanurate renal stone formation, and determined the conditions in which melamine and cyanuric acid induce these effects. We also conducted morphological observations of melamine-cyanurate crystals in the kidney tissue and urine, and identified a mechanism for melamine stone formation and renal failure.

Materials and methods

Experimental protocol

Ten-week-old male Sprague–Dawley rats (Japan SLC, Inc. Shizuoka, Japan) were assigned in equal numbers to one of the three groups: melamine administration (Group-M, $N = 54$), combined melamine/cyanuric acid administration (Group-M + C, $N = 54$), or Control group ($N = 18$). The animals were housed individually in plastic shoebox cages with wood shavings. All of the experimental procedures were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals, and were approved by both the Animal Care and Use Committee and Biological Safety Committee of Nagoya City University.

Control rats were administered 1 mL of water daily. Group-M rats were administered melamine alone, at doses of 2.4, 24, or 240 mg/kg/day (each group, $N = 18$). Group-M + C rats were administered both melamine and cyanuric acid, at doses of 1.2, 12, or 120 mg/kg/day (each group, $N = 18$). All administrations were made via a stomach tube over a maximum period of 2 weeks. Melamine and cyanuric acid (Sigma–Aldrich, Milwaukee, USA) were mixed with water at room temperature. Samplings of blood, 24-h urine, and kidney sections were performed on days 3, 7, and 14 (each end point, $N = 6$).

In vitro observations of melamine and cyanuric acid crystal morphology

Solutions containing melamine (10 mmol/L), cyanuric acid (10 mmol/L), or a mixture of both were observed by light microscopy and polarized light optical microphotography (AX80, Olympus, Tokyo, Japan).

Blood biochemistry and urinary variables

Blood biochemistry and urinary calcium, phosphate, creatinine, and magnesium measurements were conducted by the Mitsubishi Chemical Medience Corporation (Tokyo, Japan). Urinary pH and volumes were measured manually.

Urinary sediments

Bladder urine was aspirated with a 22 G needle during a sacrifice procedure on either day 3 or 7 of administration, and centrifuged at 1,500 rpm for 10 min at room temperature. Crystals were observed using an light microscopy and by polarized light optical microphotography. Ultramicrostructural observations were made using a scanning electron microscope (S-4800, Hitachi, Tokyo, Japan).

Kidney tissue processing

Resected kidney specimens were immediately fixed in 4% paraformaldehyde and embedded in paraffin. Cross sections, which were 4-μm-thick, were stained with HE, Oil Red O (a stain for lipid and plastics), and Von Kossa (a stain for phosphates). For confirmation of positive staining materials as crystals under each staining methods, each sections were observed by polarized light optical microphotography. Crystal formation was assessed quantitatively using the NIH Image 1.61 software (Scion Inc., Bethesda, USA).

Microstructural observations via SEM

For each rat, one kidney was processed for optical microscopy, while the other was processed for SEM. In the latter case, the tissue was cut into 4-μm sections. These paraffin-embedded sections were dewaxed, and washed with a phosphoric acid buffer. They were then re-fixed, first with 2.5% glutaraldehyde, and then with 2% osmium tetroxide. Dehydration was conducted using a 50–100% ethanol series. The samples were embedded in epoxy resin, coated with platinum, and then photographed using an SEM. The elemental spectra of crystals were determined via energy-dispersive X-ray analysis (Horiba EMAX-5770 system, Horiba, Kyoto, Japan).

Statistical analysis

Data are presented as the mean ± SD. Differences between groups were assessed using Mann–Whitney U test; $p < 0.05$ was considered significant.

Results

Morphology of in vitro crystals

Solutions containing melamine (10 mmol/L) or cyanuric acid (10 mmol/L) were colorless and transparent (Fig. 1a, b). Upon mixing melamine and cyanuric acid, a cloudy solution of melamine cyanurate was rapidly formed (Fig. 1c). Microscopy revealed that the crystals in solutions of melamine or cyanuric acid were granular in shape (Fig. 1d, e). The form of crystals in the mixed solution was needle-like (Fig. 1f). All crystals were seen as characteristically birefringent with polarized light optical microphotography (Fig. g–i). The ultramicrostructure of crystals in the mixed solution was observed via SEM (Fig. 1j, k).

Macroscopic Renal Changes Induced by Melamine and Cyanuric Acid

There were no significant differences in the gross appearance of kidneys between Control group and Group-M (Fig. 2a, b). The surfaces of the kidneys were smooth and dark reddish-brown. In the high dose of Group-M + C [120 mg/kg/day], the kidneys were a yellow ocher color and had small irregularities on their surface (Fig. 2c).

There were no significant differences in body weight between Control group and the low dose of Group-M + C [1.2 mg/kg/day]. In Group-M [24 mg/kg/day], body weight was decreased on day 14. In the intermediate dose of Group-M + C [24 mg/kg/day], body weight was decreased on day 7 and 14 (Fig. 2A).

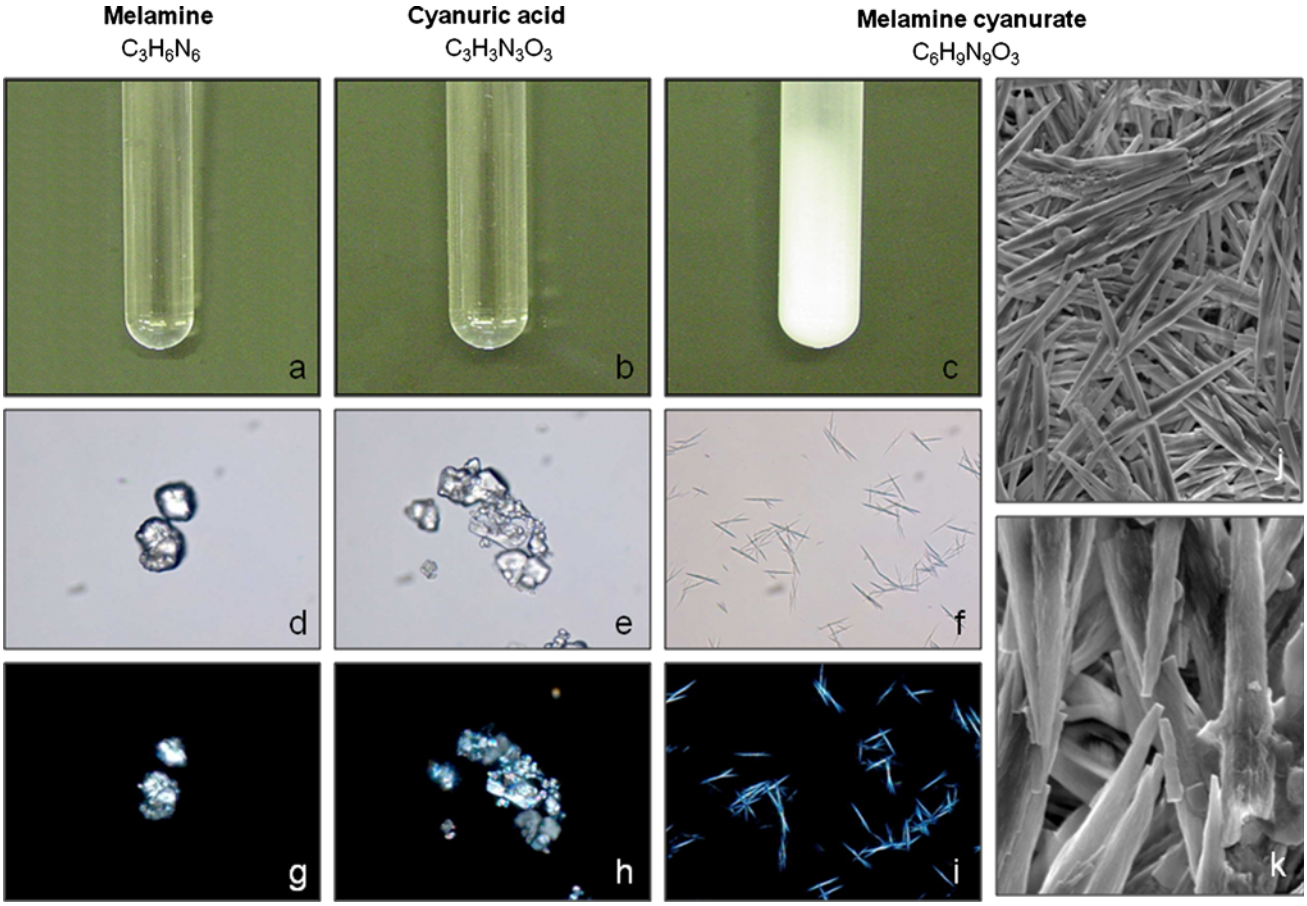

Fig. 1 Macro- and microscopic morphology of in vitro crystals of melamine, cyanuric acid and mixture of both. **a–c** The solution findings in vitro. The appearance of solution of melamine (10 mmol/l) (**a**) and cyanuric acid (10 mmol/l) (**b**) were colorless and transparence. The mixture (melamine cyanurate) (10 mmol/l) (**c**) appeared cloudy. Light microscopic crystal findings of crystals generated in melamine (**d**) and cyanuric acid (**e**) demonstrated granular shapes. The crystal structure of the mixture (**f**) showed needle-like form. The images by polarized light optical microphotography and all crystals had characteristic birefringent (**g–i**). SEM findings of melamine cyanurate crystals were demonstrated in **j** ×7,000 and **k** ×25,000

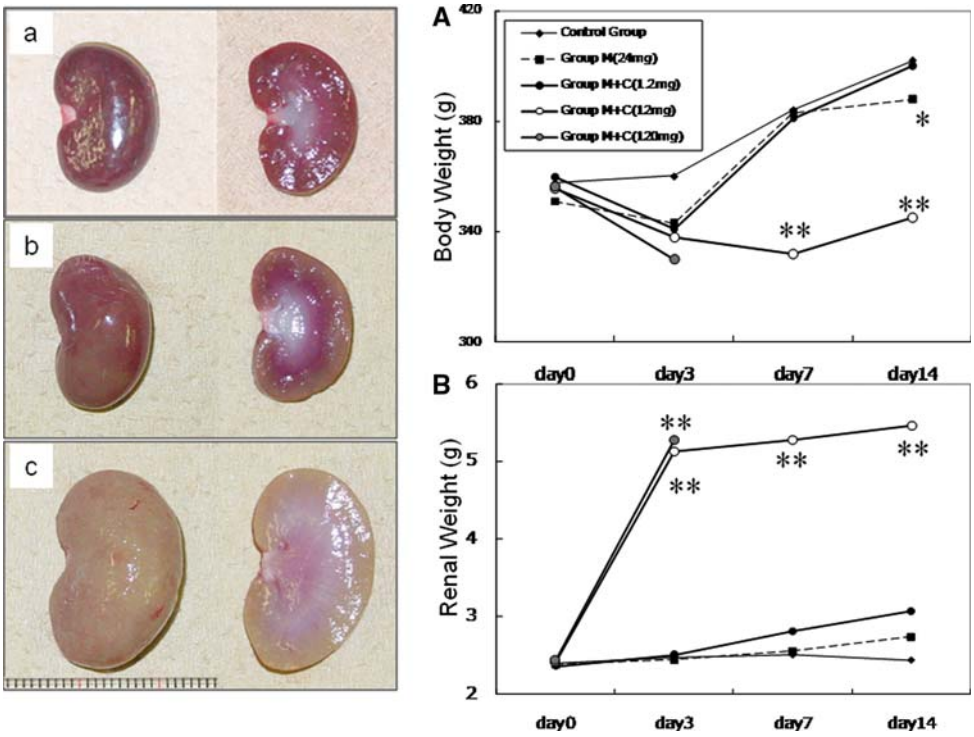

Fig. 2 The gross appearance of extracted kidneys administrated melamine and/or cyanuric acid. **a** Control group at day 7, **b** Group M [24 mg/kg/day] at day 7, **c** the intermediate dose of Group M + C [12 mg/kg/day] at day 7. **a**, **b** The surface of the kidneys were *smooth* and *dark reddish-brown*. In sectioned surfaces, color of the corticomedullary junction and the cortex were same *reddish-brown*. **c** The surface of the kidneys had the small irregularities and had the *yellow ocher color*. But corticomedullary junction seemed to be *white*. The change of body weight and renal weight were indicated as **A** and **B**. The significant differences of the values compared with the each time point of control group were indicated as $*p < 0.05$ and $**p < 0.01$

Kidney appearance and weight were not altered in the low dose of Group-M + C [1.2 mg/kg/day]. Kidney weight was increased on day 3 in higher doses of Group-M + C [12 or 120 mg/kg/day] (Fig. 2B).

Serum and urinary biochemistry

Serum and urine biochemistry data are shown in Table 1. There were no significant differences in serum creatinine levels across Control group, Group-M, the low dose of Group-M + C [1.2 mg/kg/day]. Higher dose of Group-M + C [12 or 120 mg/kg/day] had serum creatinine levels significantly higher than those of Control group.

Administration condition and distribution of crystal formation

Renal crystals could be observed using polarized light optical microphotography. Crystals were not found in any dose of the Group-M, or in the low dose of Group-M + C [1.2 mg/kg/day] (Fig. 3l).

In contrast, crystals were found to have formed by day 3 in the intermediate dose of Group-M + C [12 mg/kg/day] (Fig. 3e). The number of crystals increased across days 3, 7, and 14, with the main regions of formation being the corticomedullary junction and renal papilla (Fig. 3e, i, m). Crystals stained light brown by HE were found in the lumens of renal distal tubules and collecting ducts (Fig. 3f, g, j, k, n, o). The lumens of renal proximal tubules were expanded, and the tubular wall was thin (Fig. 3l, p). There were some crystals on the surface of the renal papilla, of the same form as those found in renal tubular lumens.

Crystals were found on day 3 in the high dose of Group-M + C [120 mg/kg/day] (Fig. 3q). There were no significantly changes in the collecting duct and distal tubules lumen of the high dose of Group-M + C (Fig. 3r, s). These crystals were located mainly in lumens of proximal tubules from the renal cortex (Fig. 3j), and were distributed differently as compared with those found in the intermediate dose of Group-M + C [12 mg/kg/day].

All rats administrated the high dose [120 mg/kg/day] of combined melamine/cyanuric acid died of acute renal failure before day 7.

Crystal characteristics

Crystal characteristics in the proximal tubules of the intermediate dose of Group-M + C were demonstrated on the consecutive kidney sections, which were indicated by HE staining (Fig. 4a), polarized light optical microphotography (Fig. 4b), Oil Red O staining (Fig. 4c), and von Kossa staining (Fig. 4d). The crystals were indicated as light brown materials by H-E staining and red ones with axle-shaped layers by ORO staining. In Von Kossa staining, the crystals could not be stained.

Table 1 Serum and Urinary Biochemistry

	Control Group			Group M [2.4 mg/kg/day]			Group M [24 mg/kg/day]		
	Day 3	Day 7	Day 14	Day 3	Day 7	Day 14	Day 3	Day 7	Day 14
Serum Cr (mg/dl)	0.23 ± 0.27	0.22 ± 0.02	0.24 ± 0.03	0.20 ± 0..2	0.23 ± 0.02	0.24 ± 0.12	0.22 ± 0.01	0.20 ± 0.02	0.20 ± 0.02
Serum Ca (mg/dl)	9.57 ± 0.12	10.20 ± 0.28	10.32 ± 0.26	10.41 ± 0.16	10.26 ± 0.27	10.30 ± 0.30	10.58 ± 0.59	10.55 ± 0.14	10.33 ± 0.19
Serum P (mg/dl)	8.20 ± 0.36	9.35 ± 0.45	9.02 ± 0.25	8.51 ± 0.44	9.31 ± 0.44	9.03 ± 0.92	9.38 ± 1.60	8.10 ± 0.47	7.87 ± 0.71
Urine volume (ml)	22.33 ± 2.08	14.83 ± 6.08	22.50 ± 4.93	14.83 ± 2.38	15.75 ± 5.42	21.38 ± 4.93	19.00 ± 3.10	13.67 ± 3.39	15.80 ± 4.32
Urine pH	8.50 ± 0.00	7.50 ± 0.55	8.17 ± 0.26	8.58 ± 0.20	8.20 ± 0.44	8.06 ± 0.32	8.25 ± 0.61	8.50 ± 0.00	8.40 ± 0.22
Urine Ca (mmol/g/Cr)	0.05 ± 0.03	0.08 ± 0.03	0.06 ± 0.02	0.09 ± 0.04	0.10 ± 0.05	0.10 ± 0.03	0.14 ± 0.08	0.18 ± 0.10	0.08 ± 0.05
Urine P (mmol/g/Cr)	2.16 ± 1.07	6.87 ± 0.74	5.94 ± 0.77	1.48 ± 1.03	0.81 ± 0.71	0.48 ± 0.58	0.65 ± 0.59	0.48 ± 0.51	1.83 ± 0.92
Urine Mg (mmol/g/Cr)	0.05 ± 0.01	0.42 ± 0.50	0.08 ± 0.17	0.17 ± 0.27	0.31 ± 0.63	0.55 ± 0.48	0.16 ± 0.18	0.83 ± 0.58	0.05 ± 0.02

	Group M [240 mg/kg/day]			Group M + C [1.2 mg/kg/day]			Group M + C [12 mg/kg/day]			Group M + C [120 mg/kg/day]
	Day 3	Day 7	Day 14	Day 3	Day 7	Day 14	Day 3	Day 7	Day 14	Day 3
Serum Cr (mg/dl)	0.21 ± 0.02	0.22 ± 0.02	0.22 ± 0.04	0.26 ± 0.05	0.26 ± 0.06	0.23 ± 0.02	0.81 ± 0.57*	1.03 ± 0.89**	0.48 ± 0.27*	1.53 ± 1.29*
Serum Ca (mg/dl)	10.40 ± 0.20	10.60 ± 0.23	10.88 ± 0.28	9.93 ± 0.23	9.83 ± 0.25	9.87 ± 0.19	10.78 ± 1.01	9.73 ± 0.28	9.55 ± 0.19	10.48 ± 0.46
Serum P (mg/dl)	8.91 ± 1.31	10.00 ± 0.42	8.89 ± 0.49	7.78 ± 0.35	7.57 ± 0.45	8.10 ± 0.32	9.45 ± 1.59	11.22 ± 4.12	7.93 ± 0.83	9.83 ± 2.56
Urine volume (ml)	18.20 ± 5.21	22.25 ± 4.49	14.88 ± 4.42	14.17 ± 1.33	18.17 ± 7.39	20.17 ± 10.59	38.17 ± 8.08	38.56 ± 12.30	82.00 ± 33.98	35.20 ± 25.40
Urine pH	8.33 ± 0.33	7.91 ± 0.49	8.16 ± 0.51	8.08 ± 0.20	8.16 ± 0.26	8.00 ± 0.00	8.08 ± 0.74	7.75 ± 0.42	8.00 ± 0.00	7.80 ± 0.20
Urine Ca (mmol/g/Cr)	0.17 ± 0.11	0.15 ± 0.09	0.09 ± 0.05	0.09 ± 0.07	0.05 ± 0.02	0.09 ± 0.05	0.10 ± 0.04	0.10 ± 0.08	0.19 ± 0.12	0.07 ± 0.05
Urine P (mmol/g/Cr)	4.43 ± 2.59	4.90 ± 0.25	1.58 ± 1.15	6.57 ± 12.28	2.38 ± 1.00	2.13 ± 2.36	0.95 ± 1.40	2.89 ± 3.31	1.70 ± 0.79	2.65 ± 2.13
Urine Mg (mmol/g/Cr)	0.37 ± 0.51	0.09 ± 0.15	0.26 ± 0.45	0.26 ± 0.31	0.10 ± 0.15	0.25 ± 0.35	0.99 ± 1.02	1.01 ± 1.15	1.28 ± 1.32	0.59 ± 0.72

Data are presented as the mean ± SD

* $p < 0.05$, ** $p < 0.01$ versus each time point of control group

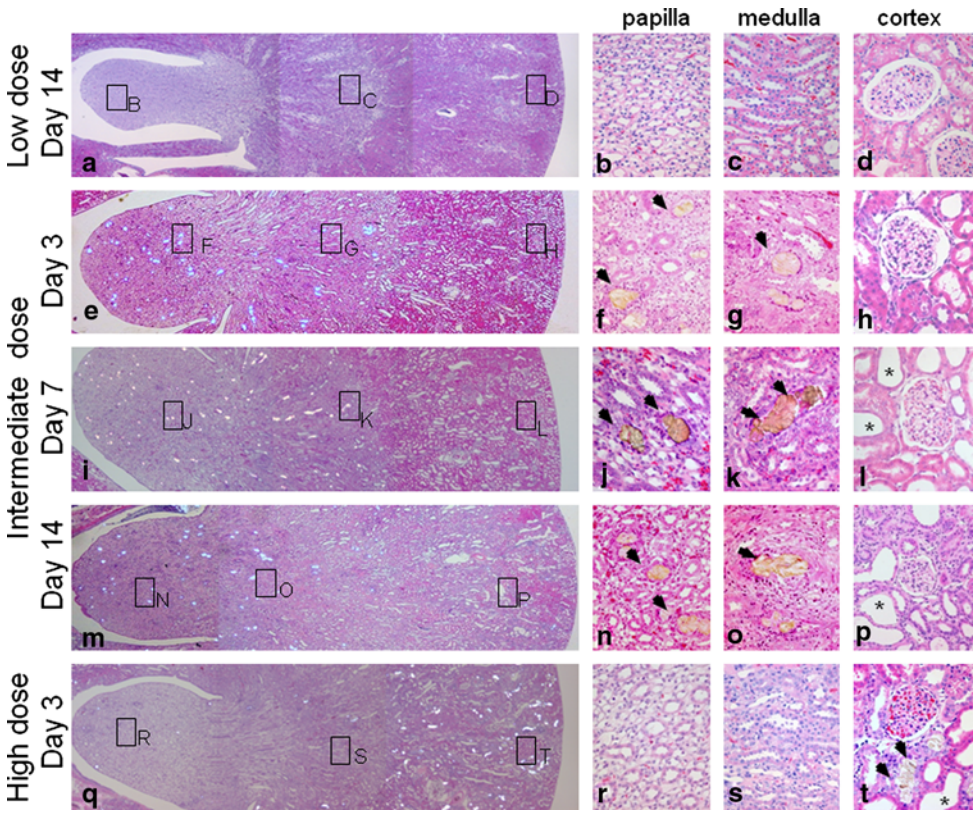

Fig. 3 Crystal distributions in the kidney sections were demonstrated with polarized light optical microphotography (**a**, **e**, **i**, **m**, **q**) and light microscopy. HE staining of the paraffin-embedded axial section in the low dose of Group M + C [1.2 mg/kg/day] at day 14 (**a**–**d**) and intermediate dose of Group M + C [12 mg/kg/day] at day 3 (**e**–**h**), day 7 (**i**–**l**) and day 14 (**m**–**p**), and high dose of Group M + C [120 mg/kg/day] at day 3 (**q**–**t**) (×40, ×400). Crystals were not found in the low dose of Group M + C [1.2 mg/kg/day] (**a**). The crystals of the middle dose of Group M + C [12 mg/kg/day] were generated at renal medulla and renal papilla (**f**, **g**, **j**, **k**, **n**, **o**), and the crystals of the high dose of M + C [120 mg/kg/day] group mainly at renal cortex (**q**). The *black arrowheads* meant the crystals in the renal tubules. The expanded tubular lumens (*) were observed at (**l**, **p**) and (**t**)

Urinary crystals

Urinary crystals were not found in specimens taken from any of the Group-M rats, or from the low dose of Group-M + C. In contrast, light brown crystals were found in the urine of higher dose of Group-M + C. These crystals were 10–20 μm in diameter (Fig. 5a). The crystals were circular and had slightly uneven surfaces. SEM was used to observe the ultramicrostructure of urinary crystals, the surface of which appeared dull and uneven (Fig. 5b).

Crystal characteristics and appearance using SEM with energy dispersive X-ray analysis

The ultramicrostructure of crystals in kidney tissue was observed via SEM. Crystals were seen to be gathered together in a clump (Fig. 6A), a property that appeared uniformly across specimens (Fig. 6B).

Energy dispersive X-ray analysis revealed the crystals as consisting of carbon (C) nitrogen (N) and oxygen (O) without calcium (Fig. 6a). In contrast, the components of peripheral tissue were carbon and oxygen but not nitrogen (Fig. 6b).

Discussion

Melamine does not normally pose a threat to human health because it is primarily used to produce melamine resin for tableware and is present in food only in small amounts [7]. On the basis of previous data [8, 9], the WHO set the tolerable daily intake (TDI) of melamine at 0.2 mg/kg/day. A previous study showed that a high dose of melamine, i.e., more than 150 mg/kg, administered daily to a male rat for 90 days induced urinary bladder stones and indirectly led to the formation of bladder tumors [8–10]. Acute toxicity of melamine in rodents is observed after the administration of an oral lethal dose 50 (LD50) of 3,100 mg/kg in male rats and 3,900 mg/kg in male mice [8]. In the present study, we observed that a high dose of melamine

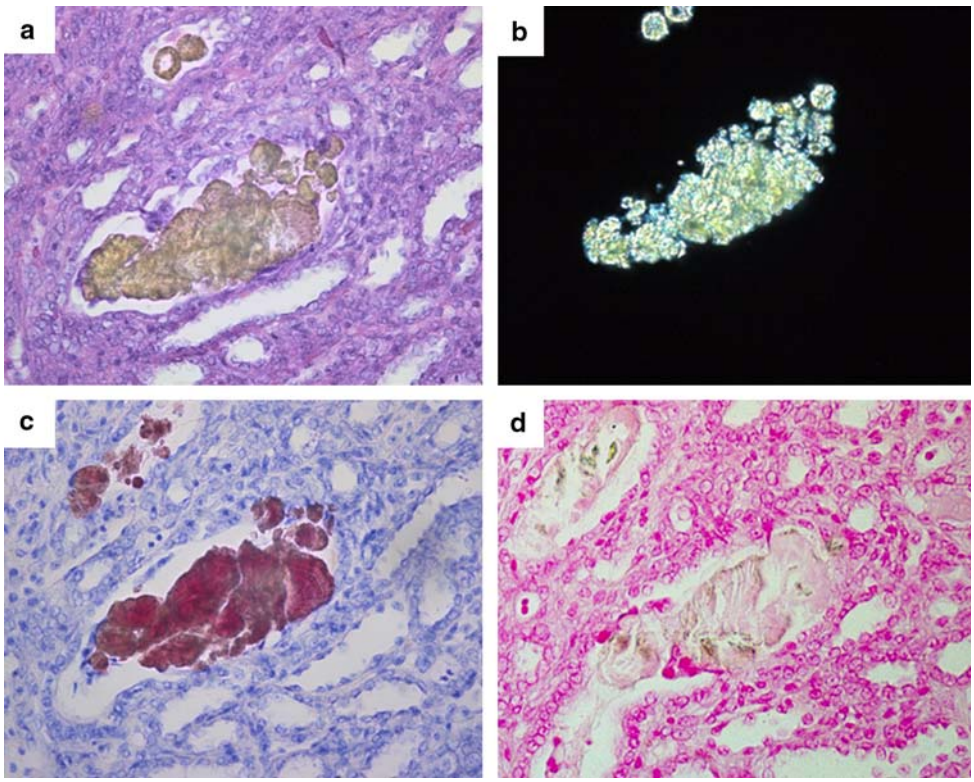

Fig. 4 Crystal characteristics in the distal tubules of intermediate dose of Group M + C were demonstrated on the consecutive kidney sections, which were indicated by H–E staining (**a**), polarized light optical microphotography (**b**), Oil Red O staining (**c**) and von Kossa staining (**d**) (×200). The crystals were indicated as *light brown* materials by H–E staining (**a**) and *red* ones with axle-shaped layers by ORO staining (**c**). In von Kossa staining, the crystals could not be stained (**d**)

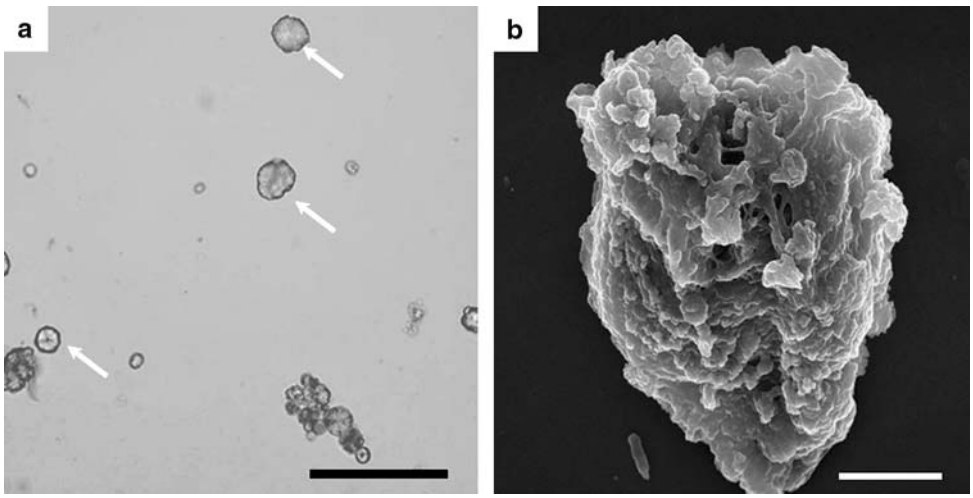

Fig. 5 The morphology of urine crystals was demonstrated by light microscopy **a** ×400 and SEM **b** ×8,000. There were *round-shaped* crystals, the diameter was 5–20 μm (*white head arrow*). Ultramicrostructural findings of urine crystals showed *circular* shape and irregular surface (**b**). The bars mean **a** 50 μm and **b** 2.5 μm

[240 mg/kg/day] administered over 14 days in rats did not influence renal function, kidney structure, or blood biochemistry.

Toxicity of cyanuric acid has also been evaluated in rats and mice. It has been shown that urinary bladder stones and bladder epithelium hyperplasia related to bladder stones were induced after the ingestion of cyanuric acid sodium at semi-chronic levels (either 700 or 2,200 mg/kg) [11].

Information on the toxicity of combined administration of melamine and cyanuric acid is limited, except for the reports on induction of death in animals after the consumption of melamine and cyanuric acid-contaminated pet food in 2004 and 2007. Combined administration of melamine and cyanuric acid has been linked to acute renal failure in cats and dogs. Cats that died after consuming pet food containing melamine and cyanuric acid had crystals of melamine combined with cyanuric acid in the kidneys [12, 13]. In the present study, we observed crystals in the renal tubules of rats that were administered melamine and cyanuric acid combination at a dose of more than 12 mg/kg/day. No effects were observed when melamine was administered individually. Our results are consistent with those of previous animal experimental studies on dogs and cats [14] or fish and pigs [13]. However, none of the

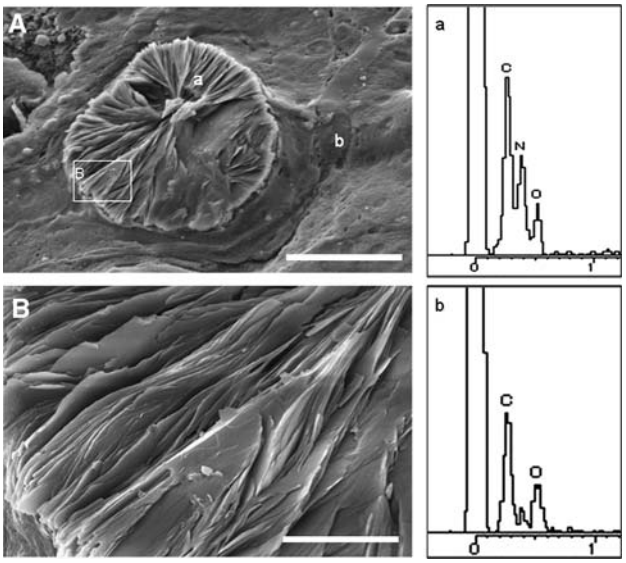

Fig. 6 Ultramicrostructural observation and qualitative analysis of the renal crystals were showed by SEM (**A**, **B**) and energy dispersive X-ray analysis (**a**, **b**). The magnification is **A** ×1,000 and **B** ×7,000. The energy dispersive X-ray analysis indicated the crystal components as carbon (C), nitrogen (N) and oxygen (C) without calcium (**a**) and those of peripheral tissue as carbon and oxygen (**b**). The bars mean **A** 10 μm and **B** 4 μm

previous studies discussed the detailed mechanism of stone formation and renal failure induced by the administration of melamine and cyanuric acid or analyzed the differences in the mechanism of melamine cyanurate stone formation and that of calcium oxalate stone formation.

In the present study, the characteristics of crystals formed in the Group-M + C rats, such as brown color staining with HE), birefringence under polarized light optical microphotography, no staining with von Kossa, and red color staining with Oil Red O, are similar to those of melamine cyanurate crystals from animals that died in 2007 after consuming pet food containing melamine/cyanuric acid. This finding indicates that melamine cyanurate crystals are completely different from the typical calcium-related stones.

For accurate evaluation of the mechanism underlying melamine cyanurate crystal formation, it would be necessary to study the distribution of the generated crystals. It has been previously reported that 90% of both melamine and cyanurate are excreted via urine within 24 h after consumption [15]. Histopathological, toxicological, and clinicopathological evaluation of the animals that were affected during the widespread occurrence of pet food-associated renal failure revealed that melamine cyanurate crystals were localized in the distal tubules and collecting ducts [14]. In the present study, we observed that in Group-M + C rats that were administered intermediate dose of melamine and cyanuric acid, first, melamine cyanurate crystals were observed in the distal tubules of the corticomedullary junction and the collecting duct of the renal papilla, following which renal function was affected, and finally, melamine cyanurate crystals appeared in the urine. Thus, it is likely that the dose used in our study is similar to that ingested by the pets via contaminated pet food. On other hand, high doses of melamine/cyanuric acid led to crystallization mainly in the proximal tubules of the renal cortex. The differences in the distribution of crystals after the administration of intermediate dose (12 mg/kg) and high dose (120 mg/kg) of melamine/cyanuric acid might be due to the difference in the threshold levels of intratubular crystal deposition. That is, intermediate dose of melamine/cyanuric acid could lead to crystal deposition under the influence of intratubular urine concentration caused by water absorption that generally occurs in the region from the distal tubules to collecting ducts, and the melamine cyanurate crystals might cause obstruction of the tubular lumen. On other hand, crystals formed after the administration of high dose of melamine/cyanuric acid could deposit in the proximal tubular lumens before the Henle's loop depending on the urinary concentration caused by the gradient of physiological osmotic pressures. On the basis of these results, we concluded that renal failure after the administration of melamine/cyanuric acid combination seemed to be caused by the obstruction of renal tubules. On other hand, calcium oxalate crystals adhere to the renal tubular epithelial cells and grow by expressing stone-matrix proteins, such as osteopontin or calprotectin [16, 17]. It is likely that at initial stages, the mechanism of stone formation and renal failure induced by melamine and cyanurate acid is completely different from that induced by calcium oxalate.

Taken together, our study demonstrated that there is an increased risk associated with the simultaneous ingestion of melamine and cyanuric acid. However, to comprehensively assess the health risks associated with the consumption of contaminated food, further research is warranted to determine the lowest dose of melamine/cyanuric acid combination that can cause renal failure.

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