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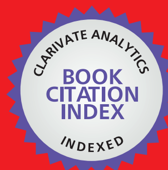

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Chapter 11

Mechanism of Urinary Bladder Carcinogenesis Induced by a Xanthine Oxidoreductase Inhibitor, in Rats

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Additional information is available at the end of the chapter

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1. Introduction

Studies on urinary bladder cancer have underpinned that the urolith is one of the key factors in the carcinogenesis process when rodents are treated with thymine, melamine [1] and uracil [2-5], all of which induce intrarenal crystal deposition/calculus formation. Fukushima et al. [2] and Cohen et al. [6-8] have investigated in detail about factors implicated in the urolith formation as well as the role of calculi in urinary bladder carcinogenesis. The crystal or calculus acts as a subsequent sustained proliferative stimulus ultimately leading to tumors. However, rodent bladder tumors cannot be directly extrapolated for humans. In fact, there is a suggestion of a weak association concerning calculi in humans as a potential risk factor for bladder cancer [9]. A major difference between rodent and human bladders, which potentially affects the carcinogenic hazard from calculi, is related to the horizontal versus vertical status. Rodents, because of their horizontal stature, have a bladder that can retain calculi in the bladder for long periods of time, without completely obstructing the urinary flow.

Xanthine oxidoreductase catalyzes the last two reactions of purine catabolism: the hydroxylation of hypoxanthine to xanthine and of xanthine to uric acid. Xanthine oxidoreductase inhibitors such as allopurinol and febuxostat are used as therapeutic agents against gout and hyperuricemia. They induce decreases in circulating uric acid and concomitant increases in blood xanthine, followed by intrarenal xanthine deposition leading to nephropathy in rodents. The xanthine crystals or calculi mediated by xanthine oxidoreductase inhibitors may repeatedly stimulate the epithelium of the renal pelvis and/or urinary bladder leading to proliferative lesions. Indeed, it has been described that carcinomas of the urinary bladder occurred in the carcinogenicity study of febuxostat [10].

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However, the pathomechanism of urinary bladder carcinogenesis due to xanthine deposits remains undetermined. With the above background, the mechanism of urinary bladder carcinogenesis induced by FYX-051 [11-13], 4-(5-pyridin-4-yl-1H-[1, 2, 4]triazol-3-yl)pyridine-2-carbonitrile, a xanthine oxidoreductase inhibitor, in rats, has been investigated.

Thus far, we have performed studies of FYX-051-induced nephropathy in rats [14-17]. In this chapter, first, general aspects concerning calculi formation and its bladder carcinogenesis are described. Second, the characteristics of FYX-051-induced nephropathy are described with respect to both identification of renal deposits precipitated after FYX-051 administration and marked species difference in nephropathy. Next, our aim was to prevent xanthine deposition after FYX-051 administration in rats by simultaneous treatment with citrate, and have established the experimental model. Using the model, a mechanistic study by simultaneous treatment with citrate for 52 weeks was performed. Based on the results of the 52-week study, finally, the extrapolation of carcinogenesis in rats for humans is discussed.

2. Formation of calculi and its effects on bladder

Solid materials can be generated in the urine by administration of a variety of chemicals, which lead to the formation of calculi [18]. Urinary calculi in rats and/or mice can be produced via variety of mechanisms as described by Cohen et al. [6]. The calculi can arise from the administered chemical itself such as melamine [19, 20], or from one of its metabolites such as diethylene glycol leading to calcium oxalate [21], or from an endogenous substance that results from the administration of the chemicals such as glycine leading to orotic acid [22]. Disorders of intermediate metabolism can also result in similar effects in the kidney and lower urinary tracts, such as the formation of urate calculi following surgical portacaval shunt in rats [23].

A major consequence of a calculus in the bladder lumen is abrasion of the mucosa surface, resulting in erosion and ulceration. These cause an acute inflammatory reaction, but are always accompanied by marked regenerative hyperplasia. Urothelial cell number is markedly increased secondary to not only simple hyperplasia but papillary and nodular hyperplasia, frequently a diffuse papillomatosis. In addition, the rate of proliferation is also increased 10-100 times. This results in 1000-10000 times increase in the number of cell divisions compared with normally quiescent adult urothelium. Although the formation of proliferative lesions produced by these foreign materials are reversible as evidenced by an example of uracil [24], stimulation of the urothelium for a long period leads to papilloma followed by carcinoma.

3. Factors affecting calculus formation

Of particular importance to factors that affect the formation of calculi in the urine are species, strain and sex [7, 25-27]. Rats and mice are the most commonly used species in carcinogen bioassays and in most mechanistic studies with animal models. Of major importance for comparison to humans are the extremely high levels of protein, osmolality,

and concentrations of various salts in the urine of rats and mice. There are marked variations in urinary composition and in response to treatment between rats and mice. Calculi tend to more readily form in rats than in mice [25, 28]. The proliferative response seems to be greater in rats than in mice, partly because of the reaction; primarily papillomatous response in rats, whereas there is a nodular response involving small number of cells in mice [4, 24]. As to the sex difference, male rodents are frequently affected to a greater extent than females. Several factors including potential hormonal effects and total amounts of specific types of urinary proteins have been suggested for this difference [25, 27, 29, 30, 31].

The major factor, however, related to calculus formation is the dose of the chemicals administrated [32, 33]. An adequate amount of the chemical is needed to be administered to generate a sufficiently high concentration in the urine to lead to precipitation and calculus formation. The dose effect is the greatest fundamental factor in extrapolating the results in rodents to potential carcinogenic hazards in humans. Other factors that strictly affect the formation of calculi are physical, chemical and physiological properties. Urinary pH is a particularly fundamental factor [7, 25-27]. Effects of urinary pH have been identified for a variety of chemicals, based on its effects on the solubility of various salts, for example, urate or xanthine being greater at increased pH, while calcium phosphate crystals are formed more readily by the same condition.

4. Nephropathy and identification of renal deposits precipitated after FYX-051 administration

In the long-term toxicity study of FYX-051 in rats, nephropathy with intrarenal deposits was observed. HPLC and LC-MS/MS analyses of intrarenal deposits in rats treated with FYX-051 have proven that the entity was xanthine [14]. The pathomechanism of nephropathy could be speculated as follows. Treatment with FYX-051 induced high blood xanthine levels due to its pharmacological activity, and consequently, renal xanthine deposition occurred mainly during the process of urine-condensation from distal tubules to collecting ducts. When the amount of deposited xanthine exceeds the capacity of the kidney to excrete foreign materials, renal tubules and collecting ducts (particularly remarkable in distal tubules) are occluded by these deposits, leading to secondary interstitial nephritis.

5. Species difference in nephropathy induced by FYX-051

Thirteen-week toxicity study of FYX-051 in rats and dogs demonstrated that the no observed adverse effect level (NOAEL) was estimated to be 0.3 and 10 mg/kg/day, respectively. On the other hand, no changes were seen even at 300 mg/kg/day in the case of 52-week treatment of monkeys (Table 1). It was shown that rats are very susceptible to FYX-051-induced intrarenal xanthine deposition and subsequent nephropathy. The mechanism of marked species difference on nephropathy was investigated. As a result, three factors were found to be involved in the species difference. First, urinary excretion of purine metabolites in terms of body weight as an index of the rate of purine metabolism, which is the key factor

of species difference, was thirty-fold higher in rats than in monkeys (Table 2). As the second factor, urinary xanthine solubility was two- and six-fold higher in dogs and monkeys, respectively, than in rats (Table 3). The fact was mainly mediated by the higher urinary pH of the former two species than the latter, because urinary xanthine solubility is dependent on urinary pH. Third, exposure level of FYX-051 was five-fold higher in rats than in other species (Table 4). The species difference of FYX-051-induced nephropathy seems to be caused by the combined effects of the above three factors.

	NOAEL (mg/kg/day)		
	Rats	Dogs	Monkeys
13-week	0.3	10	> 100
26-week	0.2	NT	NT
52-week	NT	NT	> 300

Changes observed in rats and dogs after repeated oral treatment with FYX-051 were nephropathy. NT: Not tested.

Table 1. No observed adverse effect level (NOAEL) of FYX-051 in repeated toxicity studies using rats, dogs and monkeys

Species	(A) Body weight (kg)	(B) Urinary excretion of purine metabolites (μmol/day)	B/A (μmol/kg/day)	Ratio (to monkeys)
Rats	0.209 ± 0.023	400.7 ± 58.9	1934.7 ± 385.0	31.2
Dogs	9.8 ± 0.7	3929.2 ± 790.3	402.8 ± 74.0	6.5
Monkeys	4.51 ± 0.18	280.4 ± 123.5	62.0 ± 26.0	1.0
Humans	64.1 ± 7.5	3364.3 ± 419.3	52.8 ± 7.0	0.9

Data were cited from the references [15, 16].

Table 2. The daily urinary excretion of total purine metabolites in each species and its ratio per body weight

	Rats	Dogs	Monkeys	Humans
pH	6.7	7.8	9.0	5.5
Urinary xanthinesolubility at 37°C	ca. 150	ca. 350	ca. 950	ca. 130

Data were cited from the references [15, 16].

Table 3. Estimated urinary xanthine solubility in the control urine in each species

	Rats 3 mg/kg	Dogs 10 mg/kg	Monkeys 10 mg/kg	Humans 3 mg/kg
AUC_{0-t} (μg･h/ml)	13.9	8.81	9.89	2.82
Ratio (to monkeys)	4.7	0.9	1.0	1.0

The ratio to monkey's level was calculated based on AUC of FYX-051 at 10 mg/kg estimated from those at 3 mg/kg. Data were cited from the references [15, 16].

Table 4. Exposure levels of FYX-051 in each species

6. Establishment of simultaneous treatment model with citrate for preventing nephropathy

In the carcinogenicity testing in which FYX-051 was orally given to rats for 2 years, transitional cell papilloma and carcinoma were seen in the urinary bladder of male rats, along with the xanthine calculus in the cavity at doses of 1 and 3 mg/kg. Urinary bladder tumors occurred confined to the apex, the site that easily receives physical stimulation. No bladder tumors occurred in animals that died at less than 16 months.

In order to clarify the mechanism of urinary bladder carcinogenesis observed, we tried to prevent the xanthine deposition in the urinary tract by using co-treatment with citrate. Citrate is a urine-alkalizing agent and its treatment with large amount of water causes an elevation of urinary pH and an increase in urine volume, which are attributable to rapid increase in xanthine solubility in urine. Citrate appears to act as an inhibitor of the experimental urolithiasis formation in the urine [34]. Due to its chelating effect, citrate in the urine may be expected to inhibit the formation of calcium-containing precipitates. The experimental conditions suitable for the 52-week simultaneous treatment with FYX-051 and citrate in rats were determined.

Some sodium salts, such as saccharate and ascorbate, at high doses, can lead to the formation of calcium phosphate-containing precipitate [35]. In the present study, citrate was used as a mixture of its tri-sodium salt, tri-potassium salt, and free acid in a ratio of 2:2:1, calculated as anhydrous at molar base, leading to the reduced amount of sodium salt treated.

6.1. Study on the duration of effects by citrate

Duration of effects by citrate was examined as shown in Figure 1a. Male F344 rats received a single oral dosing of FYX-051 alone at a dose of 30 mg/kg (Group 1) or FYX-051 + 2,000 mg/kg citrate (Group 2) orally at a dosing volume of 10 ml/kg. Urine just after urination was collected during 1.5 - 2 h, 3.5 - 4 h, 5.5 - 6 h, and 7.5 - 8 h after oral administration of FYX-051. Insoluble xanthine concentration in each urine sample was determined as the difference of xanthine concentration between whole urine and filtered urine through a 0.45 μm pore size filter.

Simultaneous treatment with citrate remarkably increased urinary citrate concentrations at 3.5 - 4 h (Figure 2a). In both groups, xanthine was dissolved in urine at 1.5 - 2 h after administration, since the insoluble xanthine concentration was nearly zero (Figure 2b). At 3.5 - 4 h, deposition of xanthine was observed in Group 1. Simultaneous treatment with citrate significantly prevented the xanthine deposition. Thereafter, the effects of citrate disappeared; no significant difference was observed between the two groups at 5.5 - 6 h and 7.5 - 8 h after administration. The results indicate a preventative effect of citrate on xanthine deposition in urine but also a lack of its durability.

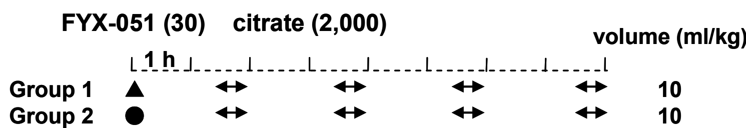
6.2. Seven-day simultaneous treatment study with citrate

Because of the lack of durability of citrate effects, it was suggested that at least daily twice treatments of citrate are needed to prevent xanthine deposition. We carried out a 7-day

simultaneous treatment study by two daily treatments to F344 rats, that is, FYX-051 (6 mg/kg) and citrate (2000 mg/kg) followed by citrate alone treatment under the conditions of selected dosing intervals, the second dose of citrate, and dosing volume. In order to establish the experimental conditions suitable for the 52-week mechanistic study, in which burdens to both animals and technicians should be reduced, the daily first treatment of citrate was done as the mixture with FYX-051, and we tried to reduce the second dose of citrate and/or dosing volume. Experimental designs are shown in Figure 1b.

a Study on the duration of effects by citrate (single dosing)

FYX-051 (30) citrate (2,000) volume (ml/kg)



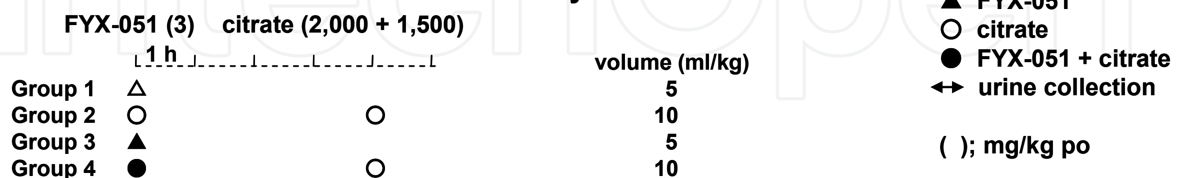
b Seven-day simultaneous treatment study with citrate

FYX-051 (6) citrate (2,000 + 2,000 or 2,000 + 1,500)

	1 h				citrate (2nd)	volume (ml/kg)	No. of rats with granular deposits in the renal pelvis/ no. of rats examined
Exp. 1							
Group 1	△					10	0/5**
Group 2	▲					10	8/8
Group 3	●	○			(2,000)	15	1/8**
Group 4	●	○			(1,500)	15	2/8**
Group 5	●	○			(1,500)	10	3/8*
Group 6	●		○		(2,000)	15	0/7**
Group 7	●		○		(1,500)	15	0/8**
Group 8	●		○		(1,500)	10	0/8**
Group 9	●			○	(2,000)	15	2/7**
Exp. 2							
Group 10	△					10	0/7**
Group 11	▲					10	7/8
Group 12	●			○	(1,500)	15	3/8
Group 13	●			○	(1,500)	10	2/8*

c Four-week simultaneous treatment study with citrate

FYX-051 (3) citrate (2,000 + 1,500)



△ 0.5% CMC-Na
▲ FYX-051
○ citrate
● FYX-051 + citrate
↔ urine collection

( ); mg/kg po

Figure 1. Experimental design (Studies a, b and c) and results (Study b) of the simultaneous treatment study with FYX-051 and citrate for the determination of experimental conditions suitable for the 52-week study. Vehicle of FYX-051 is 0.5% CMC-Na. *: P<0.05, **: P<0.01 vs. FYX-051 alone group (group 2 or group 11, by Fisher's exact probability test). Results of 'Study a' are shown in Figure 2. Results of 'Study c' are shown in Figures 3, 4 and Table 5. Figure is modified from reference [17].

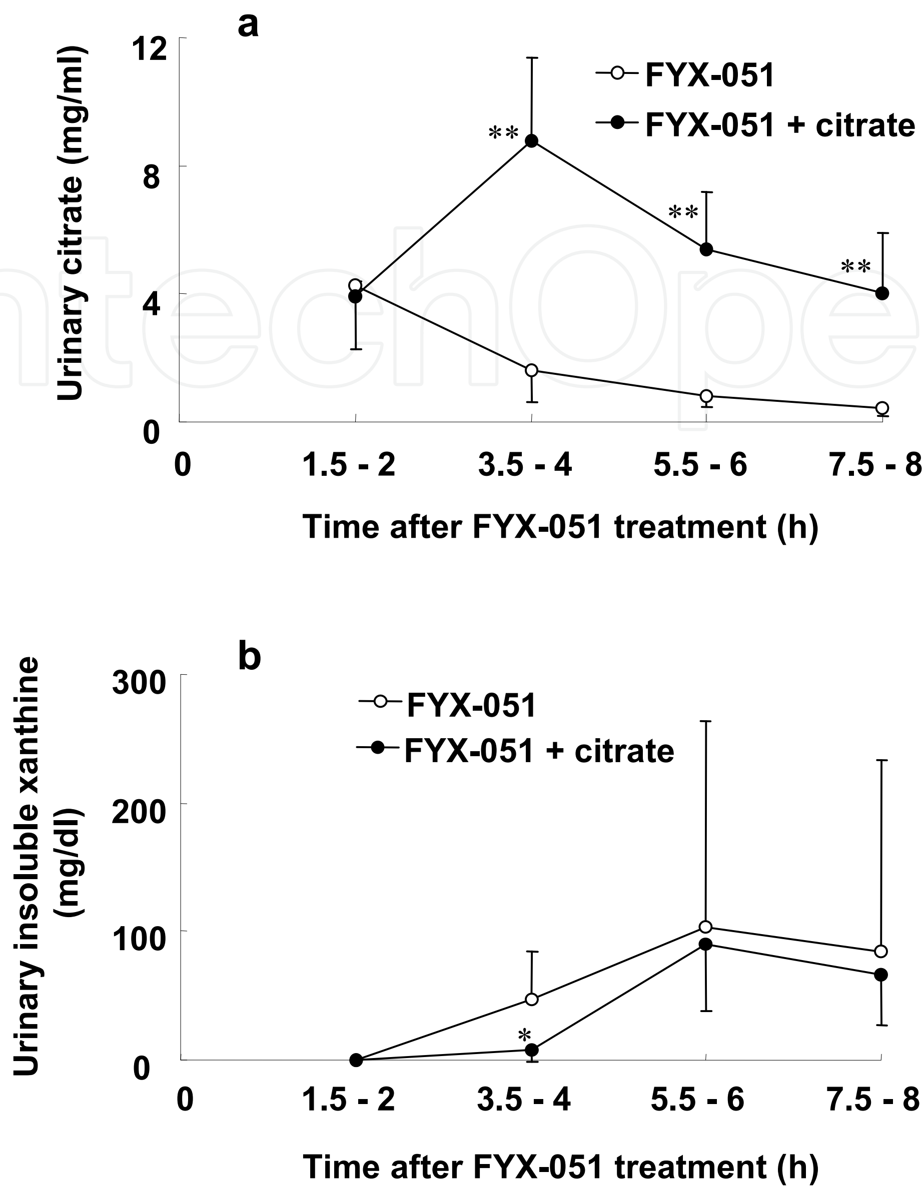


Figure 2. Changes in urinary citrate concentration (a) and insoluble xanthine concentration in urine (b) in rats receiving the single oral treatment of 30 mg/kg FYX-051 (Group 1, open circles) or 30 mg/kg FYX-051 + 2,000 mg/kg citrate (Group 2, closed circles). Each value represents the mean ± SD of 5 animals. *: P<0.05, **: p<0.01, significant difference was observed between the two groups by Student's t test. Figure is from reference [17].

Relative kidney weights and blood urea nitrogen (BUN) level significantly increased in the FYX-051 group (Group 2) compared to the controls (Group 1). Autopsy revealed yellowish-white granular deposits on the cut surface of the kidney or in the urinary bladder in all rats of Group 2. In groups treated with citrate, increased kidney weights and BUN were prevented almost completely (data not shown). Treatment of citrate, 2,000 and 2,000 mg/kg at an interval of 3 h with dosing volume of 15 ml/kg (Group 3) remarkably reduced granular deposits on the cut surface of the kidney and in the urinary bladder, although the deposition still remained in the renal pelvis in 1 out of 8 rats (Figure 1b). When the interval of citrate

treatments was 5 h (Group 9), granular deposits were seen in the renal pelvis in 2 out of 7 rats. In case of 4 h (Group 6), however, no granular deposition in the renal pelvis was observed. Neither a reduction of citrate dose from 2,000 to 1,500 mg/kg (Group 7) nor decreased dosing volume from 15 to 10 ml/kg (Group 8) revealed apparent effects on the incidence of crystal deposition. Similarly, the dose of citrate and dosing volume failed to affect the granular deposition when the dosing interval was 3 h (Groups 4 and 5 vs. Group 3) or 5 h (Groups 12 and 13 vs. Group 9).

The present results indicate that the dosing interval of citrate has a great significance, and is optimal at 4 h, but not at 3 or 5 h, because this treatment completely inhibited intrarenal xanthine deposition. A decrease in dose of citrate for the second treatment or the dosing volume was well tolerated for prevention of granular deposits in the renal pelvis.

7. Effects of simultaneous treatment model with citrate on nephropathy and bladder carcinogenesis in rats

7.1. Four-week simultaneous treatment study with citrate

A 4-week study under the established conditions was carried out as shown in Figure 1c. The male F344 rats were divided into 4 groups of 8 animals each. In the simultaneous treatment group with citrate, first, animals received 3 mg/kg FYX-051 and 2000 mg/kg citrate, and 4 h later 1500 mg/kg citrate alone at a volume of 10 ml/kg, based on a 7-day study.

In the treatment group with FYX-051 alone (Group 3), laboratory investigations revealed chylous urine, granular deposits (xanthine crystals) in urinary sediments, and trends of increased serum creatinine compared with the control group (Group 1) (Table 5). Pathological examinations revealed increased relative kidney weights and gross renal findings such as focal pale changes, focal rough surface, scar, and white patch. Histopathologically, these were characterized by basophilia and dilatation of renal tubules, cell debris in tubular lumen, crystalline materials in the cortex, medulla, and papilla, calculus, fibrosis, and transitional cell hyperplasia (Figure 3a). In addition, calculus and transitional cell hyperplasia were also present in the urinary bladder in one instance each (Figure 4a, Table 5).

Citrate treatment alone (Group 2) and simultaneous treatment with FYX-051 (Group 4) apparently increased urinary pH compared with Groups 1 and 3 (Table 5). In Group 4, no noticeable changes were seen in the kidneys nor were alterations seen in the urinary bladder (Figures 3b, 4b). The serum creatinine level in the Group 4 was equivalent to the Group 1, with attaining a significant decrease compared with the Group 3. Additionally, treatment with citrate exhibited no remarkable effect on the exposure levels of FYX-051. These observations showed that FYX-051-induced nephropathy could be markedly depressed by simultaneous treatment with 3,500 mg/kg of citrate, leading to the disappearance of transitional cell hyperplasia in the urinary bladder.

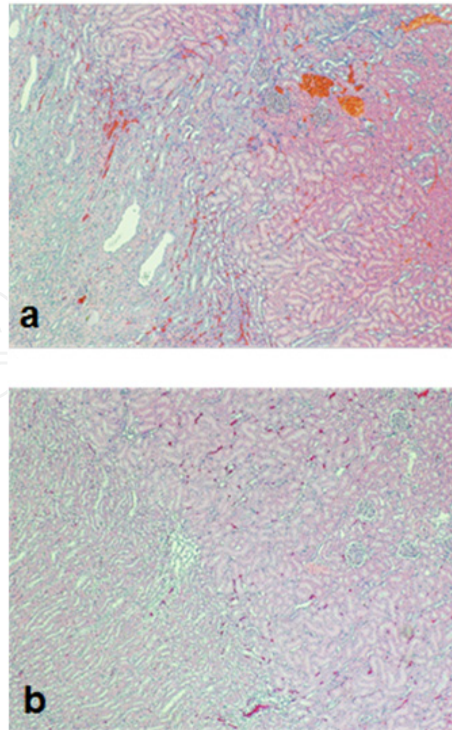


Figure 3. Photomicrographs of the kidney (cortex to outer medulla) from a male F344 rat treated orally with (a) 3 mg/kg FYX-051 (Group 3) or (b) 3 mg/kg FYX-051 + 3,500 mg/kg citrate (Group 4) for 4 weeks. Note (a) mild interstitial nephritis and (b) normal histology; magnification 100×, HE staining.

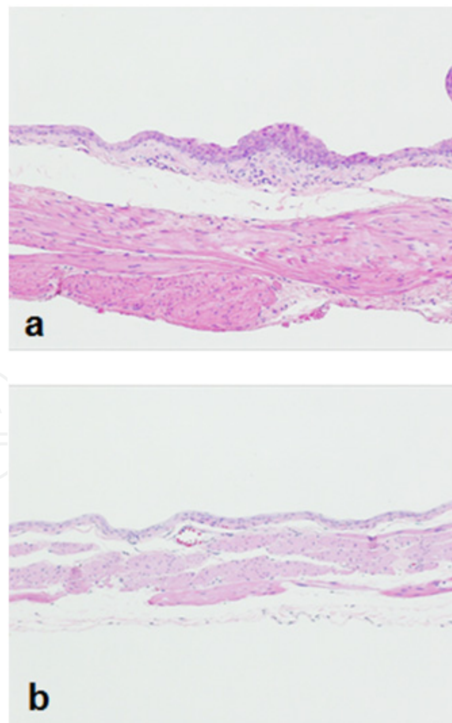


Figure 4. Photomicrographs of the urinary bladder from a male F344 rat treated orally with (a) 3 mg/kg FYX-051 (Group 3) or (b) 3 mg/kg FYX-051 + 3,500 mg/kg citrate (Group 4) for 4 weeks. Note (a) mild epithelial hyperplasia and (b) normal histology; magnification 200×, HE staining. Figures are from reference [17].

Group	1	2	3	4
No. of animals	8	8	8	8
Urinary pH				
8	1		2	
8.5	7	6	6	4
≥9		2		4
Xanthine crystals				
present	0	0	4	0
Serum creatinine (mg/dl)	0.22 ± 0.02	0.22 ± 0.03	0.26 ± 0.05	0.21 ± 0.02*
BUN (mg/dl)	20.4 ± 0.7	23.3 ± 2.3	22.5 ± 4.4	22.1 ± 1.4
Relative kidney weight (%)	0.717 ± 0.028	0.777 ± 0.016††	0.778 ± 0.070†	0.765 ± 0.022
Autopsy				
-Kidney				
pale, focal	0	0	5	0
rough surface, focal	0	0	3	0
scarred	0	0	2	1[c]
white patch	0	0	1	0
-Urinary bladder				
calculus	0	0	6	0
Histopathology				
-Kidney				
calculus	0	0	5	0
cellular infiltration	0	0	4	0
cell debris, tubular lumen	0	0	6	0
crystalline material, cortex	0	0	2	0
crystalline material,medulla	0	0	6	0
crystalline material, papilla	0	0	4	0
dilatation, tubule	0	0	7	0
tubular basophilia [a]	0	0	7	1
foreign body reaction	0	0	4	0
suppurative inflammation	0	0	1	0
pyelitis	0	0	2	0
fibrosis	0	0	5	0
hyperplasia,transitional cell	0	0	5	0
interstitial nephritis [b]	0	0	1	0
-Urinary bladder				
calculus	0	0	1	0
hyperplasia,transitional cell	0	0	1	0
Toxicokinetics				
No. of animals			6	5
$AUC_{0\text{-}24h}$ (μg･h/ml)			5.51 ± 0.75	5.03 ± 0.48

Data were expressed as the mean ± SD. [a] Regeneration [b]Diagnosis [c]No histopathological changes
*Significant difference compared with Group 3 (P<0.05, by Aspin-Welch's t test).
†Significant difference compared with Group 1 (P<0.05, by Aspin-Welch's t test).
††Significant difference compared with Group 1 (P<0.01, by Student's t test). Table is from reference [17].

Table 5. Data of urinalysis, blood chemistry, relative kidney weights, histopathology of urinary organs and toxicokinetics in male rats receiving FYX-051 alone or simultaneous treatment with citrate

7.2. Mechanistic study by simultaneous treatment with citrate for 52 weeks

The male F344 rats were divided into 4 groups of 17 males each. Treatment of FYX-051 and citrate was done in a same manner as 4 week study. As shown in Table 6, in the FYX-051 alone treatment group, the following findings were observed: a significant decrease in urinary osmolality, crystals in sediments, hematuria, significant increases of serum BUN and creatinine, pathological alterations in the kidney such as calculus, atrophy, rough

	Vehicle control (n=17)	3500 mg/kg citrate (n=16)	3 mg/kg FYX-051 (n=17)	3 mg/kg FYX-051 + 3500 mg/kg citrate (n=17)
Urinalysis Crystal in sediments	 0 / 17	 0 / 16	 5 / 17	 0 / 17
Blood biochemistry BUN (mg/dl) Creatinine (mg/dl)	 15.9 ± 1.3 0.27 ± 0.03	 22.6 ± 1.7 0.24 ± 0.02	 42.0 ± 12.8 ** 0.72 ± 0.19 **	 21.7 ± 2.7 ## 0.24 ± 0.02 ##
Kidney weight Absolute (g) Relative (g%)	 2.27 ± 0.14 0.602 ± 0.028	 2.32 ± 0.09 0.732 ± 0.027	 2.00 ± 0.15 ** 0.616 ± 0.049	 2.38 ± 0.14 ## 0.737 ± 0.027 ##
Gross pathology	–	–	Kidney: atrophy (13), calculus (4), rough surface (5), white patch (6) Urinary bladder: calculus (16)	–
Histopathology	–	–	Kidney: interstitial nephritis (17), simple hyperplasia of transitional cell (17) Urinary bladder: simple hyperplasia (4) and papillary hyperplasia (1) of transitional cell	–

–: No remarkable change

*, ** Significant difference was observed in FYX-051 alone group when compared with the vehicle control group (p < 0.05. 0.01, by Student's t-test).

#, ## Significant difference was observed in the simultaneous treatment group with citrate when compare with FYX-051 alone group (p < 0.05, 0.01, by Student's t-test).

Table 6. Data for urinalysis, blood chemistry, kidney weight, gross pathology, and histopathology in rats receiving simultaneous treatment with FYX-051 and citrate for 52 weeks

surface, interstitial nephritis, simple hyperplasia of transitional cells. In the urinary bladder, calculus, simple and papillary hyperplasia of transitional cells was observed in 16, 4 and 1/17 males in the FYX-051 alone group, respectively. Oyasu [36] has postulated that commonly, distinction between papillary hyperplasia and papilloma of transitional cells is difficult. Accordingly, papillary hyperplasia observed in this 52-week mechanistic study could be regarded as precancerous lesions for bladder carcinogenesis. Neither xanthine crystals nor lesions in any of the kidney and urinary bladder samples were observed in the simultaneous treatment group with citrate.

Thus, simple and papillary hyperplasia of transitional cells followed by papilloma and carcinoma in the urinary bladder in rats occurring after the long-term treatment of FYX-051 were secondary changes caused by xanthine crystals being deposited in the kidney, and no other causes could be implicated in these changes. Proposed histogenesis of urinary bladder carcinogenesis obtained in this study is shown in Figure 5.

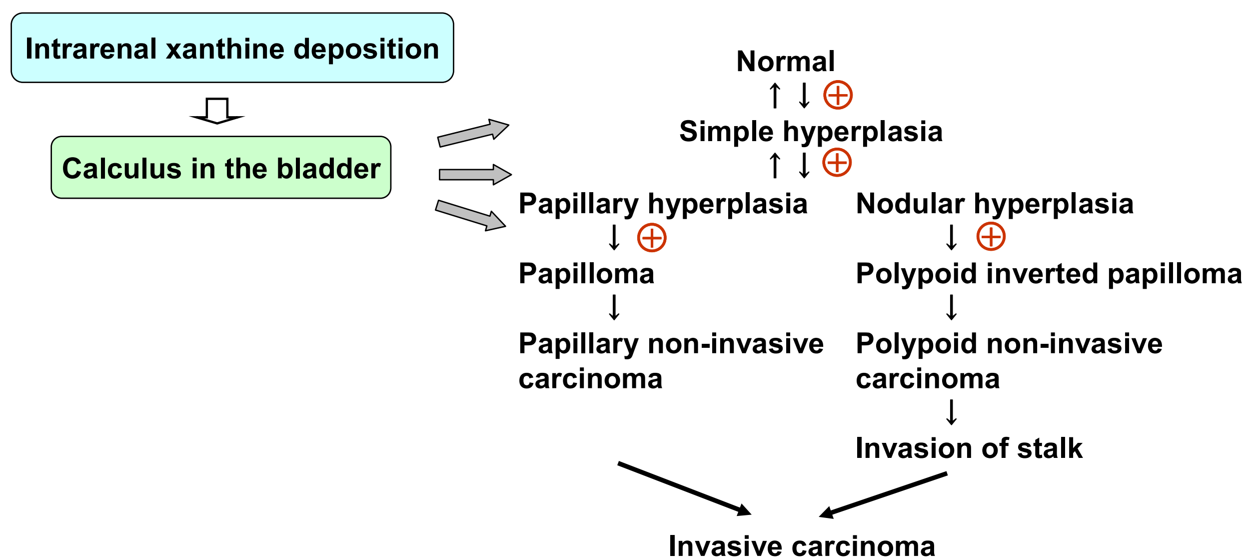


Figure 5. Proposed histogenesis of urinary bladder carcinogenesis induced by xanthine oxidoreductase inhibitors in rats

8. Extrapolation of carcinogenesis in rats for humans

The extrapolation of the results in rats into humans can be considered as follows. A marked species difference between rats and monkeys in FYX-051-induced nephropathy was suggested to be mediated by the combined effects of three factors; the rate of purine metabolism, urinary xanthine solubility and exposure level of FYX-051 as described above. A comparison of these factors relevant to species difference between monkeys and humans demonstrated that urinary xanthine solubility in the former was seven-fold higher than in the latter, without any differences in the remaining two factors. It should be emphasized that in the clinical trial of FYX-051, in which adequate hypouricemic effect was obtained, neither xanthine crystals in urine nor serious side effects have been reported, although attention must be paid in the case of Lesch-Nyhan syndrome in which hypoxanthine-

guanine phosphoribosyltransferase is congenitally absent [37]. Further, small crystals or calculi can be excreted from the bladder in humans in contrast to rodents, as described in the introduction section. The outcomes of genotoxicity studies were negative, revealing that FYX-051 is a non-genotoxic compound (unpublished data). Thus, urinary bladder carcinogenesis, which would be mediated by xanthine deposition in rats after long-term treatment with FYX-051 cannot be extrapolated for humans due to extremely low occurrence of xanthine deposition in humans.

9. Conclusion

It is generally accepted that crystal or calculus in the urinary bladder of rats would ultimately cause carcinoma by acting as a proliferative stimulus. However, no direct evidence of calculus-mediated carcinogenesis was obtained in the treatment of xanthine oxidoreductase inhibitors. In this chapter, the pathomechanism of bladder carcinogenesis in rats induced by a xanthine oxidoreductase inhibitor has been clearly shown by disappearance of precancerous lesions in the case of prevention of xanthine deposition. This work will throw a light on the future studies of urinary bladder carcinogenesis in animals, since it was demonstrated that modifying experimental design that affects calculus formation produced great differences in the results of studies by long-term treatment.

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