BIOMEDICAL AND ENVIRONMENTAL SCIENCES **18**, 211-219 (2005)

Induction of Bladder Lesion by Terephthalic Acid and Its Mechanism[1]

GUI-DONG DAI, LUN-BIAO CUI, LING SONG, REN-ZHEN ZHAO, JIAN-FENG CHENG, MEI-XIA LIU, JIAN-WEI ZHOU, HANG XIAO, AND XIN-RU WANG[*]

Institute of Toxicology, Nanjing Medical University, Nanjing 210029, Jiangsu, China

Objective To provide more information for rational evaluation of potential risks of terephthalic acid (TPA), we studied the effects of TPA on rats' bladders in 90 days after TPA exposure. **Methods** Sprague Dawley rats were subdivided into five groups, ingesting 0 %, 0.04 %, 0.2 %, 1 %, and 5 % TPA respectively for a sub-chronic feeding study lasting for 90 days. Urine, serum and samples of brain, liver, lung, kidney, bladder, *etc.* were collected and analyzed. **Results** TPA ingesting decreased the value of urinary pH, and increased the contents of Ca^{2+}, Zn^{2+}, Mg^{2+}, Na^{+}, K^{+} in urine. The volume of 24 h urine was significantly increased in male rats in the 1 % and 5 % TPA groups. Urinary white sediment was found in both sexes, and its formation in male rats seemed more susceptible than that in female rats. Alpha 2u-globulin (AUG) in serum and urine of male rats was markedly increased in a dose-dependent manner. Fifteen cases of hyperplasia (simple or atypical) were determined in the 5 % TPA ingesting group, 14/52 in male rats and 1/23 in female rats. Among them 3 male rats had no stone or calculus. Those with either bladder stones or hyperplasia were accompanied with urinary white sediments. **Conclusion** White sediment accompanied with elevated urine AUG is the basis of TPA induced urolith formation, and is also associated with TPA induced bladder epithelial cell proliferation. It can act as an early biomarker for the potential toxic effect of TPA.

Key words: Terephthalic acid; Bladder; Uroliths; Hyperplasia; Sediment; Alpha 2μ-globulin

INTRODUCTION

Terephthalic acid (TPA), one of the most commonly produced chemicals in the world, has been extensively used for the synthesis of certain crystalline polyester resins, films, and fibers. It is estimated that more than 2 million tons of TPA are produced in P. R. China every year[1]. TPA is apparently a non-genotoxic chemical compound, its LD_{50}>1 500 mg/kg[2] (SD rats, ig), and therefore, most occupational workers have almost no special protection.

Laboratory experiments demonstrated that rats exposed to 3%-5% terephthalic acid in the diet for two weeks had formation of bladder calculus, followed with bladder hyperplasia, and finally developed transitional epithelial carcinomas in two years after chronic feeding studies[3-4]. Heck *et al.*[3,5] suggested that bladder cancer was induced by a long-term irritation of bladder stone, and the concentration of TPA to attain super saturation in urine was necessary to form the stone. Thus the dose of TPA intakes was approximately 2.0 g/day, which was obviously impossible for human to absorb such a large quantity.

Although uroliths could induce bladder epithelial hyperplasia[6], epidemiological data are not sufficient to support its actions on carcinogensis[7], and also more problems exist in animal models to explain the paradoxical results. First of all, the frequency of bladder stone formation in male rats was higher than that in female rats[4,8], but the incidence rate of bladder cancer had no obvious difference between sexes[3]. Obviously, ascribing TPA induced bladder carcinomas with no stone formation to the missed stone detection or excretion was somewhat subjective. Secondly, it was generally considered that carcinogens could induce cancer both in high dose administration for a short period of time and in low dose for a long term exposure, suggesting that carcinogen has no obvious threshold dose. On the contrary, the occurrence of TPA induced uroliths had a threshold. These results could not be fully explained by the theory suggested by Heck *et al.*[3-4].

The mechanism of bladder lesion by TPA exposure is pivotal for evaluating its potential risks on human. To provide more rational information for risk assessment, we investigated the toxic effects of TPA

[1]This work was supported by National Natural Science Foundation of China (No. 30170798), the Preliminary Study of Important Project in the National Basic Research, MOST (No. 200150) and the Greatest Project in the National Basic Research (No. 2002 CB512908).

[*]Corresponding should be addressed to Xin-Ru WANG, Tel: 86-025-8686-2939. Fax: 86-025-8652-7613. E-mail: xrwang@njmu.edu.cn

Biographical note of the first author: Gui-Dong DAI, male, born in 1968, Ph. D., majoring in toxicology and pharmacology. E-mail: daiguidong@163.com

0895-3988/2005
CN 11-2816/Q

on rat bladder 90 days after TPA exposure.

MATERIALS AND METHODS

Materials and Animals

Chemicals Terephthalic acid (TPA) in white powder with its purity ⩾99.99%，was obtained from Yi Zheng Chemical Fiber Co. (Jiangsu, China). Rat alpha 2μ-globulin immunoassay kit (R&D), mouse anti-proliferating cell nuclear antigen (PCNA) antibody were from Santa Cruze, U.S.A, and mouse SP kit was from Beijing Zhongshan Biotech Co., Ltd. All other chemicals used were of the highest purity.

Animals Male and female Sprague Dawley (SD) rats (90±10 g) were purchased from Shanghai B & K Laboratory Animal Co. Ltd (Shanghai, China). SPF，No: 152, weighting 80 g-100 g was also obtained.

Methods

Ninety-day subchronic TPA exposure studies SD rats were quarantined for 7 days before experiments. Room temperature and relative humidity were controlled at 22℃±3℃ and 60%±10%, respectively. Fluorescent lighting was provided in a 12 h light/dark cycle. The animals were divided into 5%, 1%, 0.2%, 0.04%, and control groups. The treatment schedule was continued for 90 days.

Sample collection and storage All rats were sacrificed on the 90th day. Blood was taken into glass tubes and clot ted for 2 h at room temperature before it was centrifuged for 20 min at 4℃ 2000×g, serum was removed and stored at -20℃. Ten to 100 μL fresh urine was collected between 0700 and 0900 before the day when rats were sacrificed. Urine pH was immediately detected with MI 129 ISFET pH meter (Switzerland), then stored at -20℃. Twenty-four h urine was collected using metabolic cage and its volume was measured after particulates were removed by centrifugation. The brain, heart, liver, spleen, lung, kidney, and bladder were weighed, and fixed in 10 % formaldehyde solution.

Urine analysis Urine Ca^{2+}, Mg^{2+}, Zn^{2+}, Na^{+}, and K^{+} were analyzed with AA-6501F atomic absorption flame emission spectrophotometer (Japan) and calibrated with creatinine analyzed with the Lisa-500 automatic analyzer (France).

Alpha 2μ-globulin immunoassay Before assay, thawed male serum was centrifuged for 20 min at 4℃ 2000×g and diluted 1000-fold. Male urine was centrifuged for 20 min at 4℃ 2000×g and diluted 100 000-fold. Assay was performed according to the manufacturer's instructions and the optical density of each well was determined within 30 min, using the EL808–Ultra micro plate reader (USA).

Histopathology Four μm thick sections of formalin-fixed and paraffin embedded samples of bladder, liver and kidney *etc.* was stained with hematoxylin and eosin (H & E) for histopathological assessment.

Immunohistochemistry Four μm thick sections of formalin-fixed, paraffin embedded bladders were cut, spread on APES coated slides, deparaffinized in xylene and hydrated using a grade series of ethanol. Endogenous peroxdase activity was quenched by applying 3% hydrogen peroxide for 20 min. Antigen retrieval was performed routinely by microwave heating. Sections were immersed in citrate buffer (pH 6.0) in a glass container and heated to boiling temperature repeatedly and then cooled down to room temperature. Non-specific binding was blocked by incubating sections with non-immune serum at room temperature for 20 min, then the sections were incubated with mouse anti-proliferating cell nuclear antigen (PCNA) antibody diluted 1:100 overnight at 4℃, and sequentially rinsed in PBS before incubated for 35 min with goat anti-mouse biotinylated conjugate. Binding of the primary antibody was detected using streptavidin-peroxidase with diaminobenzidine (DAB) as the chromogen. Slides were rinsed with water, lightly counterstained with hematoxylin, dehydrated in grade ethanol, cleared with xylene and overslipped.

Von Kossa staining Seven μm thick sections of bladder samples were deparaffinized and hydrated, stained in 2% silver nitrate for 60 min, and then changed to 5% sodium hyposulfite for 2 min, counterstained with 1% neutral red.

Statistical Analysis

Student *t*-test was used to calculate the significance of difference between control and experimental values. *P* value less than 0.05 was considered statistically significant.

RESULTS

General Condition

TPA had no obvious effects on body weight and weight gain of rats 90 days after TPA exposure. The relative weight of spleen and kidney in 1% female groups, liver in 1% male groups was increased and that of brain in 0.02 % male groups was slightly decreased (Tables 1 and 2).

TABLE 1

Changes in Relative Organ Weights of Female SD Rats 90 Days After TPA Subchronic Exposure (g/100 g wt, $\bar{x} \pm s$)

Group	*n*	Brain	Thymus	Heart	Lung	Liver	Spleen	Kidney	Adrenal Gland	Ovary	Uterus	Bladder
Control	12	0.608 ± 0.102	0.135 ± 0.045	0.380 ± 0.029	0.541 ± 0.096	3.160 ± 0.295	0.205 ± 0.026	0.776 ± 0.065	0.031 ± 0.009	0.035 ± 0.008	0.187 ± 0.061	0.040 ± 0.008
TPA 5%	23	0.570 ± 0.108	0.153 ± 0.036	0.362 ± 0.039	0.689 ± 0.423	3.125 ± 0.359	0.208 ± 0.050	0.780 ± 0.073	0.030 ± 0.005	0.034 ± 0.010	0.217 ± 0.090	0.051 ± 0.019
1%	12	0.588 ± 0.104	0.138 ± 0.036	0.394 ± 0.023	0.587 ± 0.089	3.295 ± 0.376	0.235 ± 0.023**	0.848 ± 0.037**	0.030 ± 0.006	0.039 ± 0.010	0.223 ± 0.078	0.043 ± 0.010
0.2%	12	0.554 ± 0.151	0.147 ± 0.030	0.367 ± 0.038	0.533 ± 0.064	3.128 ± 0.354	0.193 ± 0.027	0.728 ± 0.139	0.027 ± 0.005	0.033 ± 0.006	0.164 ± 0.043	0.037 ± 0.006
0.04%	12	0.588 ± 0.147	0.140 ± 0.025	0.372 ± 0.040	0.595 ± 0.109	3.329 ± 0.427	0.202 ± 0.036	0.751 ± 0.135	0.040 ± 0.035	0.036 ± 0.008	0.167 ± 0.038	0.041 ± 0.008

Note. Compared with control, $^{**}P<0.01$.

TABLE 2

Changes in Relative Organ Weights of Male SD Rats 90 days After TPA Subchronic Exposure (g/100 g wt, $\bar{x} \pm s$)

Groups	*n*	Brain	Thymus	Heart	Lung	Liver	Spleen	Kidney	Adrenal Gland	Testis	Prostate	Epididymides	Bladder
Control	12	0.507 ± 0.047	0.094 ± 0.033	0.361 ± 0.027	0.481 ± 0.098	2.998 ± 0.431	0.185 ± 0.031	0.719 ± 0.130	0.014 ± 0.003	0.887 ± 0.120	0.1623 ± 0.08	0.169 ± 0.018	0.037 ± 0.006
TPA 5%	52	0.474 ± 0.113	0.080 ± 0.024	0.347 ± 0.038	0.561 ± 0.462	2.940 ± 0.295	0.167 ± 0.030	0.778 ± 0.115	0.015 ± 0.004	0.887 ± 0.143	0.169 ± 0.064	0.185 ± 0.083	0.067 ± 0.105
1%	12	0.487 ± 0.099	0.090 ± 0.024	0.376 ± 0.030	0.576 ± 0.189	3.383 ± 0.363*	0.178 ± 0.021	0.781 ± 0.034	0.017 ± 0.005	0.899 ± 0.114	0.180 ± 0.061	0.175 ± 0.036	0.039 ± 0.008
0.2%	11	0.433 ± 0.078*	0.098 ± 0.027	0.362 ± 0.051	0.506 ± 0.068	3.218 ± 0.118	0.173 ± 0.017	0.748 ± 0.077	0.012 ± 0.004	0.886 ± 0.078	0.173 ± 0.078	0.178 ± 0.026	0.036 ± 0.007
0.4%	12	0.463 ± 0.079	0.083 ± 0.032	0.354 ± 0.028	0.477 ± 0.086	3.270 ± 0.193	0.169 ± 0.029	0.776 ± 0.044	0.013 ± 0.004	0.845 ± 0.113	0.173 ± 0.055	0.168 ± 0.018	0.040 ± 0.010

Note. Compared with control, $^{*}P<0.05$.

TABLE 3

Changes in Urinaryion of SD Rats 90 days After TPA Subchronic Exposure ($\bar{x} \pm s$)

Ion (n=9)	Unit	Male					Female				
		Control (n=12)	0.04% (n=12)	0.2% (n=10)	1% (n=12)	5% (n=9)	Control (n=12)	0.04% (n=12)	0.2% (n=10)	1% (n=12)	5% (n=9)
Ca^{2+}	g/molCr	0.39±0.31	0.45±0.48	0.52±0.36	8.23±7.60**	124.56±15.72**	8.61±6.46	9.69±6.15	9.93±3.98	25.92±23.22	44.12±25.78**
Mg^{2+}	g/molCr	16.20±7.62	30.37±11.06	41.65±11.61**	43.38±21.20**	119.65±55.69**	25.64±16.66	44.45±14.48	47.26±15.02	49.94±15.68	86.85±68.57**
Zn^{2+}	g/molCr	0.10±0.11	0.16±0.07	0.10±0.06	0.18±0.12	0.74±0.72**	0.05±0.02	0.08±0.06	0.11±0.07	0.18±0.26	0.22±0.17*
K^{+}	g/mmolCr	0.11±0.04	0.40±0.05	0.29±0.13	0.40±0.28**	0.31±0.26	0.26±0.14	0.27±0.14	0.21±0.07	0.58±0.30*	0.33±0.28
Na^{+}	g/mmolCr	0.26±0.16	0.48±0.14	0.34±0.18	0.39±0.32	0.91±1.28*	0.50±0.25	0.50±0.22	0.23±0.11	0.60±0.40	0.75±0.14**

Note. Compared with control: *P<0.05, **P<0.01.

TABLE 4

Changes in Urine and Bladder Samples of SD Rats 90 Days After TPA Subchronic Exposure ($\bar{x} \pm s$)

Groups		Male							Female						
		n	Bladder Stone	Urine White Sediment			Bladder Hyperplasia		n	Bladder Stone	Urine White Sediment			Bladder Hyperplasia	
				+	++	+++	Simple	Atypical			+	++	+++	Simple	Atypical
Control		12	0	0	0	0	0	0	12	0	0	0	0	0	0
TPA	5%	52	21	13	9	8	9	5	23	1	8	4	3	1	0
	1%	12	0	6	4	2	0	0	12	0	4	1	0	0	0
	0.2%	11	0	5	0	0	0	0	12	0	2	0	0	0	0
	0.04%	12	0	5	0	0	0	0	12	0	0	0	0	0	0

Note. +: minor, ++: moderate, +++: large.

Urine Analysis

Urine volume, ion and pH Except the rats having a large amount of stones in bladder with urodialysis, the volume of 24 h urine collected from 5% TPA treatment male rats was significantly increased (Table 5). High doses of ingested TPA acidified the urine of both sexes with decreased urine pH (Table 5). After having calibrated with creatinine, the concentration of urinary Ca^{2+}, Mg^{2+}, Zn^{2+}, K^{+}, Na^{+} was increased in a dose-dependent manner, especially in groups of 5% TPA (Table 3).

Observation of Sediment

Urinary white sediment was found in most TPA ingesting rats (Fig. 1). There was a large amount of TPA-sediment in 5% TPA exposure groups, and its incidence in male rats was more susceptible than that in female rats (Table 4).

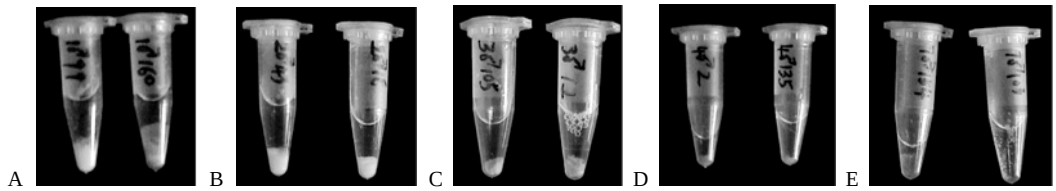

FIG. 1. Observation of white sediments in urine samples collected from SD Rats 90 days after TPA subchronic. A) 5% TPA ingested groups of rats with a large amount of white sediment; B) 1% TPA with a moderate amount of white sediment; C) 0.2% TPA with a moderate to minor amount of white sediment; D) 0.04% TPA with a suspected amount of white sediment; E) groups of control without white sediment.

AUG Levels in Serum and Urine

AUG was the major urinary protein synthesized and secreted by liver, and regulated by multi-hormone and exogenous chemicals. TPA ingestion markedly increased the levels of serum AUG and elevated its urine excretion in rats (Table 6).

Uroliths and Calculus Detection

Among SD rats 90 days after TPA subchronic exposure, 21 uroliths (one in female, 20 in male) were detected. The bladder of one male rat was notably enlarged, and the stone inside was about 3.171 g (Fig. 2). Micro-calculi were also detected in bladders of TPA exposed rats with *Von Kossa* staining (Fig. 3).

TABLE 5
Changes of 24 h Urine Volume and Urinary pH in SD Rats 90 days after TPA Subchronic Exposure ($\bar{x} \pm s$, n=11)

Groups		24 h Urine Volume (mL)		Urine pH	
		Male	Female	Male	Female
Control		14.06 ± 3.39	14.68 ± 3.84	6.53 ± 0.47	6.56 ± 0.06
TPA	5%	22.55 ± 2.72**	17.04 ± 3.48	5.66 ± 0.18**	5.77 ± 0.22**
	1%	17.36 ± 3.26*	15.48 ± 3.69	5.92 ± 0.38	6.08 ± 0.77*
	0.2%	15.24 ± 3.37	15.44 ± 3.25	6.39 ± 0.45	6.27 ± 0.54
	0.04%	11.26 ± 2.83	13.25 ± 5.93	6.05 ± 0.25	6.20 ± 0.56**

Note. Compared with control: *P<0.05, **P<0.01.

TABLE 6
Levels of Urinary and Serum AUG in SD Rats 90 Days After TPA Subchronic Exposure ($\bar{x} \pm s$)

Groups		Urine		Serum	
		n	AUG (g/molCr)	n	AUG (μg/mL)
Control		5	137.6±51.1	5	10.8 ± 1.75
TPA	5%	6	207.2 ± 35.4*	6	13.6 ± 1.5**
	1%	5	230.8 ± 21.9**	5	14.0 ± 0.9**
	0.2%	6	219.6 ± 28.9**	6	12.1 ± 4.1
	0.04%	5	155.2 ± 13.2	5	11.7 ± 2.1

Note. Compared with control: *P<0.05, **P<0.01.

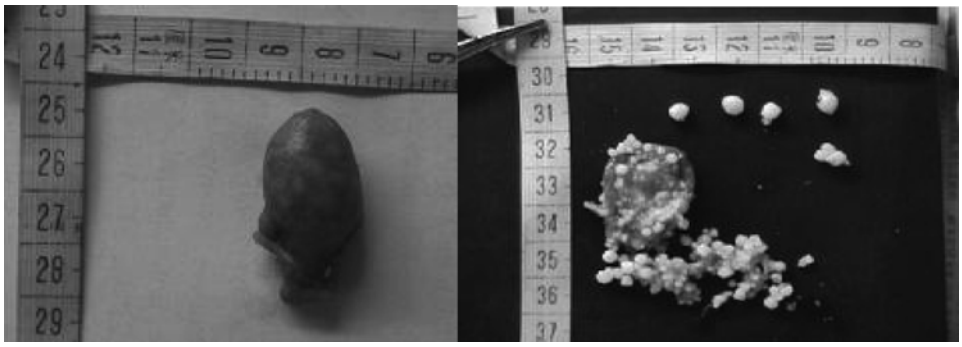

FIG. 2. A large amount of stones in bladder of a male rat 90 days after subchronic exposure to TPA.

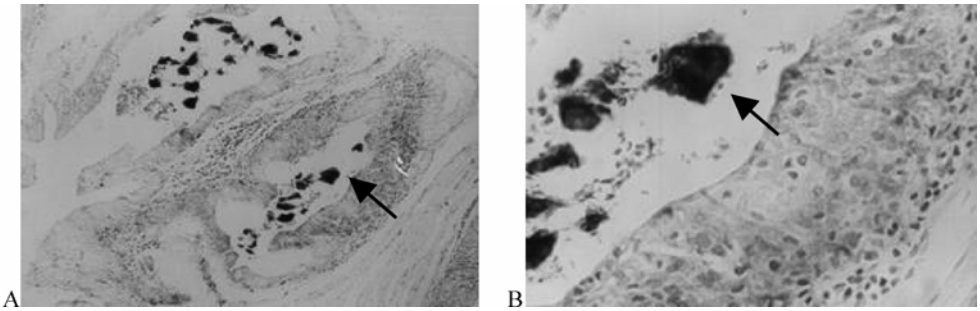

FIG. 3. Calculus embedded in surface epithelium of a male rat 90 days after exposure to 5% TPA (*Von Kossa* staining). A) Black calculus granules in hyperplasic bladder lumen 10×; B) Magnificent of A) 40×.

Histopathology and Immuno-histopathology

Stained with H&E (Fig. 4), bladder hyperplasia was diagnosed only in 5% TPA ingesting rats, and their PCNA positive expression rate was 86.7% (13/15) (Fig. 5). The cases who had either bladder stone or hyperplasia were accompanied with urinary sediment. Among the 15 cases diagnosed as bladder hyperplasia, three cases had no stone or calculus, but the volume of white sediment was more than moderate (++), suggesting that the sediment itself could lead to urothelial cell proliferation.

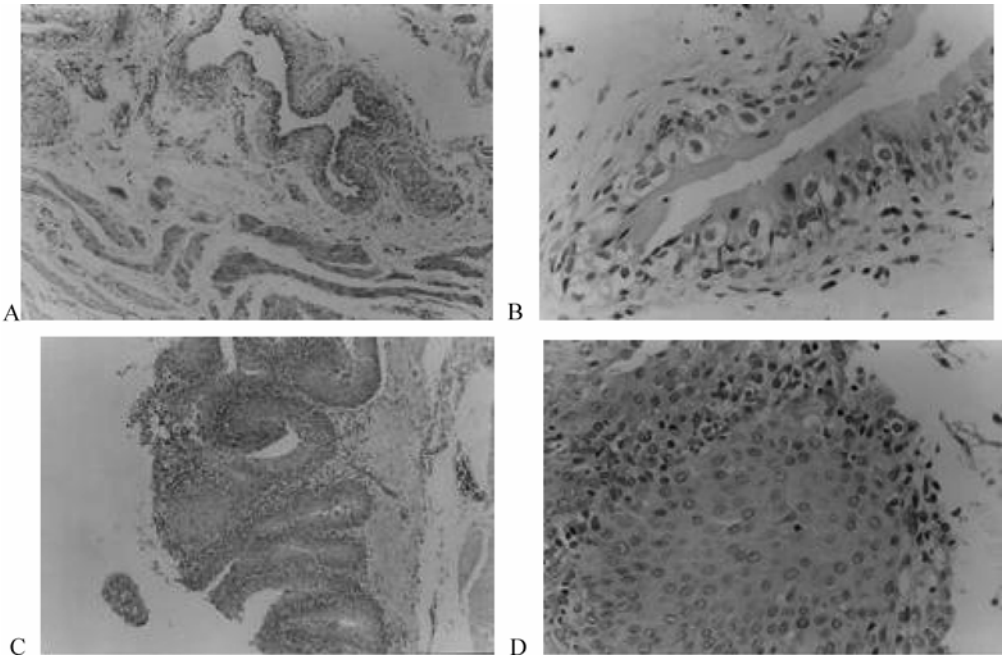

FIG. 4. Bladders of rats 90 days after TPA subchronic exposure (H&E). A) Normal bladder epithelium (10×); B) Magnificence of A) (40×); C) Bladder hyperplasia (10×); D) Magnificence of C) (40×).

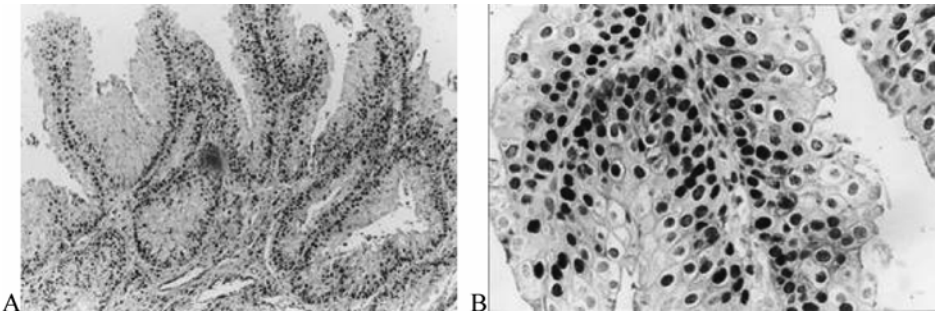

FIG. 5. PCNA positive expression on bladders of SD rats 90 days after TPA sub chronic exposure. A) Bladder hyperplasia with brown nuclear expression 10×; B) Magnificence of A) 40×.

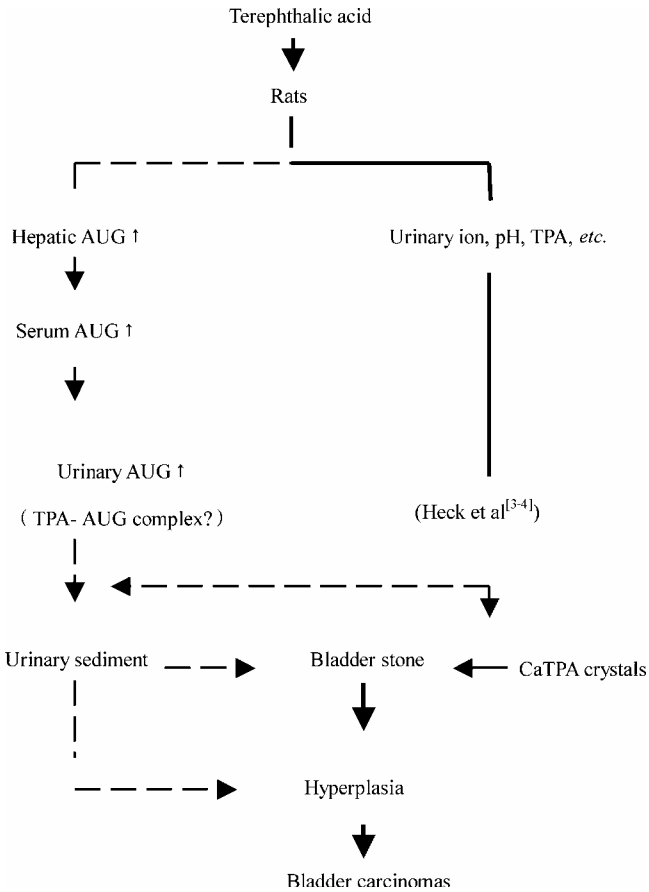

FIG. 6. Mechanism of TPA induced bladder lesions.

Heck *et al.*[3-4] reported that when urinary TPA reached its super saturation, CaTPA crystal started to precipitate and form nidi of bladder stones. Bladder epithelial cells proliferated and ultimately became carcinomas after a long-term urolith irritation. We proposed that TPA administration could increase hepatic AUG synthesis following serum AUG elevation and urinary AUG excretion. TPA induced AUG-based sedimentation and bladder stone formation were affected by urinary pH, ions and TPA concentration. Sediment and uroliths irritated bladder epithelium, then induced bladder hyperplasia and carcinomas.

DISCUSSION

TPA was studied in detail before the 1990s. It was demonstrated that absorbed TPA was mainly distributed in serum, liver and kidney, of which about 60%-70% was actively secreted and reabsorbed in proximal kidney, and excreted in urine in original form[9]. TPA did not accumulate *in vivo*, while no

metabolites were detected with ^{14}C labeled TPA[10]. The genotoxicity of TPA has been evaluated in standard Ames[2-3], bone marrow micronucleus and chromosome aberration test, but no positive results were obtained[2]. Thus, TPA apparently belonged to non-genotoxic compounds. Rats receiving 5% dietary TPA over a 2-week period resulted in bladder calculi, while bladder transitional cell carcinomas occurred two years after chronic exposure[3-4]. It was considered that the changes of urinary composition were responsible for these incidents[11].

Compared with the previous reports, we obtained similar results in the changes of urine ingredient of rats by TPA treatment (Table 3). We also detected the bladder stone and/or hyperplasia in 5% rats after 90 days of TPA subchronic exposure and their formation and incidence rate were higher in male rats than that in females (Table 4). However, we observed a kind of white sediments in TPA administrated rats (Fig. 1) and the occurrence was strongly correlated with the dose of TPA. Also sex difference existed at the same time. Diagnosed bladder cell proliferation was not always accompanied with uroliths or calculi, however it was accompanied with specific white urinary sediment.

AUG is a kind of special proteins biosynthesized and secreted by male rat liver, but its function is still unclear. Molecular weights of AUG are of 18.6 kD (hepatic type) and 15.5 kD (kidney type) respectively[12]. Its active secretion consisted of 35%-40% total protein in male rats urine, and about 60% were reabsorbed by proximal kidney[12]. Previous researches showed that AUG could affect bladder epithelial cell proliferation and development of transitional cell carcinomas[13]. Cohen *et al.*[13-14] suggested that the mechanism underlying saccharin induced bladder carcinomas was due to its action on the synthesis and secretion of hepatic AUG in male rats. Saccharin could also bind to AUG, which could change its structure, decrease its degradation, elevate its urinary level, and finally form AUG-based precipitates under appropriate conditions such as urinary pH, Ca^{2+}, Mg^{2+}, and large amount of chemicals. The sediment affecting urothelial cell proliferation was thus considered to ultimately lead to bladder tumors[13-14].

In this study, we found that both serum and urinary AUG levels increased in a dose-dependent manner in TPA treated rats. White sediment induced by TPA (Fig. 1) was much related to bladder hyperplasia and stone formation, suggesting that TPA-induced urinary sediments were formed with the similar mechanism involving in saccharin exposure. It was clear that the formation of this kind of sediment was earlier than that of bladder stone, indicating that it might be the basis for TPA-calculus formation. Because of the synthesis and secretion of AUG with sex and age specialty[13-18], TPA-induced stone had its characteristics, such as higher frequency in male rats than that in female rats, and more susceptible for weanling rats than adults rats. For the cases of bladder hyperplasia without stone, Heck *et al.*[3-4] ascribed them to the lost or passed stones during processing of tissues for histopathologic examination. However we considered that except for uroliths, the sediment itself, could lead to bladder cell proliferation, and ultimately bladder cancer. We summarized the sequence of events secondary to TPA administration in Fig. 6, and suggested that TPA-induced urinary sediment could be an early biomarker for its potential toxic effects.

ACKNOWLEDGMENT

We thank Dr. Hebrow C. CHANG and Mrs. Jun HE for preparing the manuscript.

REFERENCES

1. Gong, N. and Wang, X. R. (1997). The toxicity and early biological effects of terephthalic acid. *Forei. Med. Sci (Hygiene Section*) **24** (4), 223-226.
2. Gong, N., Xu, X., K., and Wang, X., R. (1999). Study on the acute toxicity and genotoxicty of terephthalic acid. *Chin. Pub. Health* **18** (4), 203-204.
3. Heck, H. D. and Tyl, R. W. (1985). The induction of bladder stones by terephthalic acid, dimethyl terephthalate, and melamine (2,4,6-triamino-s-triazine) and its relevance to risk assessment. *Regul. Toxicol. Pharmacol.* **5** (3), 294-313.
4. Chin, T. Y., Tyl, R. W., Popp, J. A., and Heck, H. D. (1981). Chemical urolithiasis. 1. Characteristics of bladder stone induction by terephthalic acid and dimethyl terephthalate in weanling Fischer-344 rats. *Toxicol. Appl. Pharmacol.* **58** (2), 307-321.
5. Heck, H. D. (1981). Chemical urolithiasis 2. Thermodynamic aspects of bladder stone induction by terephthalic acid and dimethyl terephthalate in weanling Fischer-344 rats. *Fundam. Appl. Toxicol.* **1** (4), 299-308.
6. Clayson, D. B., Fishbein, L., and Cohen, S. M. (1995). Effects of stones and other physical factors on the induction of rodent bladder cancer. *Food Chem. Toxicol.* **33** (9), 771-784.
7. Burin, G. J., Gibb, H. J., and Hill, R. N. (1995). Human bladder cancer: evidence for a potential irritation-induced mechanism. *Food Chem. Toxicol.* **33** (9), 785-795.
8. Cohen, S. M., Masui, T., Garland, E. M., and Arnold, L. L. (1997). Effects of diet on urinary bladder carcinogenesis and cancer prevention. *J. Nutr.* **127** (5 Suppl), 826-829.
9. Moffitt, A. E. Jr, Clary, J. J., Lewis, T. R., Blanck, M. D, and Perone, V. B. (1975). Absorption, distribution and excretion of terephthalic acid and dimethyl terephthalate. *Am. Ind. Hyg. Assoc. J.* **36** (8), 633-641.
10. Hoshi, A. and Kuretani, K. (1967). Metabolism of terephthalic acid. Absorption of terephthalic acid from gastriointestinal tract and detection of its metabolites. *Chem. Pharm. Bull.* **15** (12), 1979-1984.
11. Cohen, S. M. (1995). Cell proliferation in the bladder and implications for cancer risk assessment. *Toxicology* **102** (1-2), 149-159.

12. Wang, Y., Shia, M. A., Christensen, T. G., and Borkan, S. C. (2000). Hepatic alpha 2u-globulin localizes to the cytosol of rat proximal tubule cells. *Kidney. Int.* **57** (3), 1015-1026.
13. Cohen, S. M., Cano, M., Earl, R. A., Carson, S. D, and Garland, E. M. (1991). A proposed role for silicates and protein in the proliferative effects of saccharin on the male rat urothelium. *Carcinogenesis* **12** (9), 1551-1555.
14. Cohen, S. M., Arnold, L. L., Cano, M., Ito, M., Garland, E. M., and Shaw, R. A. (2000). Calcium phosphate-containing precipitate and the carcinogenicity of sodium salts in rats. *Carcinogenesis* **21** (4), 783-792.
15. Caldwell, D. J., Eldridge, S. R., Lington, A. W., and McKee, R. H. (1999). Retrospective evaluation of alpha 2μ-globulin accumulation in male rat kidneys following high doses of diisononyl phthalate. *Toxicol. Sci.* **51** (1), 153-160.
16. Williams, T. M. and Borghoff, S. J. (2001). Characterization of tert-butyl alcohol binding to alpha 2μ-globulin in F-344 rats. *Toxicol. Sci.* **62** (2), 228-235.
17. Borghoff, S. J., Prescott, J. S., Janszen, D. B., Wong, B. A, and Everitt, J. I. (2001). Alpha 2μ-Globulin nephropathy, renal cell proliferation, and dosimetry of inhaled tert-butyl alcohol in male and female F-344 rats. *Toxicol. Sci.* **61** (1), 176-186.
18. Blumbach, K., Pahler, A., Deger, H. M., and Dekant, W. (2000). Biotransformation and male rat-specific renal toxicity of diethyl ethyl- and dimethyl methylphosphonate. *Toxicol. Sci.* **53** (1), 24-32.

(*Received December* 16, 2003 *Accepted June* 24, 2004)