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Original Article

Establishment of rat models for screening slow-acting drugs of hyperuricemia

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Abstract: Hyperuricemia, caused by dietary, environmental and genetic factors, has been recognized as a high risk factor for gout, cerebrovascular and cardiovascular diseases. Most hyperuricemic animal models were carried out on rats with short-term treatment (<1-15 days), which were not suitable for screening of agents with long-term effect and mechanisms of action, especially for traditional Chinese medicines. In this study, several combinations of two types of model drugs were adopted to establish a stable model of hyperuricemia in rats during 15-45 days. Results showed that either co-administration of adenine plus oteracil potassium or administration of toteracil potassium alone was able to increase serum uric acid levels in a time dependent manner. Allopurinol significantly decreased elevated serum uric acid levels in all models, while benzbromarone only took effect in the model using oteracil potassium alone. In addition, prominent macroscopic and microscopic abnormity of kidney were observed in adenine-treated groups and appreciably attenuated by allopurinol, which suggested that morphologic indexes or enzyme parameters regarding renal injury may have the potential to be an indicator of drug efficacy. The test platform we established based on different approaches may play an important role in screening for the majority slow-acting antihyperuricemia agents acting by different mechanisms.

Keywords: Hyperuricemia, animal model, rat, drug screening, kidney injury

Introduction

Hyperuricemia, defined as a serum urate concentration higher or equal to 6.8 mg/dL (0.408 mmol/L), is strongly associated with the risk of gout [1]. During the last few years, a growing body of evidence demonstrated that high serum uric acid levels with or without gout are both associated with kidney diseases [2] and cardiovascular diseases (*e.g.* hypertension, coronary heart diseases and peripheral vascular diseases) [3], although it was unclear whether serum uric acid is an independent risk factor.

Despite the current in-depth knowledge of the pathophysiological role of hyperuricemia in human diseases and the availability of valid therapeutic options, the management of hyperuricemia still largely relies on the pharmaceutical approaches [4]. However, the target serum urate goals are not always achieved by using fist-line agents, including allopurinol and febuxostat, due to multiple factors. And varying degrees of side effects such as gastrointestinal reactions and liver injury have been frequently reported during medication application [5]. In addition, the key point about the management of hyperuricemia is lowering serum urate below saturation concentrations in long-term, but some patients will develop drug resistance in the medication process.

Some traditional Chinese medicine (TCM) and many natural plants-origin products (*e.g.* the water extract of *Leonurus artemisia*) have been proved efficient in down-regulating uric acid levels [6, 7]. Data from a recent meta-analysis of eleven randomized controlled clinical trials showed that TCM had a similar effect compared with western medicine for treatment of hyperuricemia, but with fewer side effects [8]. Besides, the moderate time-effect courses of TCM may be more appropriate for serum urate maintaining in the management of hyperurice-

Table 1. Treatment Groups (n=13)

Group	Treatment	
	Modeling	Intervention
Control	1% Na-CMC in sterile water	Distilled water
I-M	100.0 mg/kg·bw adenine and 1.50 g/kg·bw oteracil potassium in 1% Na-CMC	Distilled water
I-A		27.0 mg/kg·bw allopurinol in distilled water
I-B		4.5 mg/kg·bw benzbromarone in distilled water
II-M	50.0 mg/kg·bw adenine and 1.50 g/kg·bw oteracil potassium in 1% Na-CMC	Distilled water
II-A		27.0 mg/kg·bw allopurinol in distilled water
II-B		4.5 mg/kg·bw benzbromarone in distilled water
III-M	1.50 g/kg·bw oteracil potassium in 1% Na-CMC	Distilled water
III-A		27.0 mg/kg·bw allopurinol in distilled water
III-B		4.5 mg/kg·bw benzbromarone in distilled water

Notes: I, model I; II, model II; III, model III; M, model control; A, allopurinol; B, benzbromarone; Na-CMC, sodium carboxymethyl cellulose.

mia. Animal models play an important role in pilot screening of antihyperuricemic agents and preliminary exploration of the mechanisms of action. It should be noted that most animal models established before were with short time treatment for both modeling and intervention [9-11], which was less suitable for the screening for slow-acting agents such as TCM.

In this study, in an effort to explore the efficacy evaluation profiles of natural products with long-course treatment, different doses and combinations of several hypericemia-inducing drugs were adopted to establish an ideal model for hyperuricemia in rats. Analysis of kidney injury and the efficacy of positive control drugs were also discussed in this paper. This study was set up to establish the test platform in which the efficacy of slow-acting agents, e.g. several traditional Chinese herbs will be thoroughly evaluated.

Materials and methods

Reagents

Oteracil potassium (CAS No. 2207-75-2) was purchased from Jinan Chenghui Shuangda Chemical Co., Ltd. (Jinan, Shanghai, China). Adenine (CAS No. 73-24-5) was purchased from Amresco L.L.C. (Solon, OH, USA). Allopurinol (CAS No. 315-30-0) was purchased from Tokyo Kakoki Co., Ltd. (Kamiina, Nagano, Japan). Benzbromarone (CAS No. 3562-84-3) and Sodium carboxymethyl cellulose (Na-CMC, CAS No. 9004-32-4) were purchased from Sigma-Aldrich Inc. (St. Louis, MO, USA). All chemicals used were of analytical grade.

Test animals

SPF-grade male Sprague-Dawley rats (6-week of age) were procured from Dashuo Laboratory Animal Reproduction Center (Chengdu, Sichuan, China) (Certificate No. SCXK2013-24). The rats were kept at the Animal Laboratory Center of West China School of Public Health (Chengdu, Sichuan, China) (Certificate No. SCXK2013-11). Five rats were housed in each cage and were given free access to standard commercial rodent feed and drinking water. The animals were acclimatized for 7 days before treatment. Standard ethical guidelines of the Ethical Committee for Research on Laboratory Animals of Sichuan University were followed.

Experimental approach

To eliminate the interference from the extreme values, rats of which serum uric acid (UA) were out of range of 95% baseline (P2.5-P97.5) at day-1 were excluded. The rats were randomized to nine treatment groups (n=13 in each group) according to serum UA, *i.e.* a control group, 3 model groups (model I, II, III) and 6 positive groups (allopurinol and benzbromarone intervention groups in each model). Model drugs, dose levels, vehicles employed and other relevant information are summarized in **Table 1**. The animal doses of allopurinol and benzbromarone were extrapolated from human doses by a conversion based on body surface area [12]. The model drugs were administrated at 9:00 am and the positive control drugs were administrated at 3:00 pm in all groups for con-

Table 2. Final body and relative organ weights for male rats treated with modeling and intervention drugs for 45 days (n=13)

Group	Body weight (g)	Liver weight (g)	Liver weight/ Body weight	Kidney weight (g)	Kidney weight/ Body weight
Control	458.0 ± 21.8	13.58 ± 0.88	2.97 ± 0.20	2.74 ± 0.28	0.60 ± 0.05
I-M	434.7 ± 36.4	12.71 ± 1.69	2.92 ± 0.21	4.70 ± 0.49▲▲	1.08 ± 0.04▲▲
I-A	446.9 ± 25.0	12.69 ± 1.67	2.83 ± 0.27	3.85 ± 0.46*	0.86 ± 0.08**
I-B	436.1 ± 26.2	12.33 ± 1.05	2.83 ± 0.18	4.85 ± 0.56	1.11 ± 0.11
II-M	448.1 ± 19.4	13.37 ± 1.27	2.98 ± 0.23	3.37 ± 0.25▲▲	0.75 ± 0.06▲▲
II-A	458.1 ± 22.7	13.78 ± 1.37	3.00 ± 0.27	3.29 ± 0.55	0.72 ± 0.10
II-B	447.4 ± 25.2	13.90 ± 1.34	3.11 ± 0.22	3.60 ± 0.39	0.81 ± 0.09
III-M	457.8 ± 31.8	12.81 ± 2.53	2.79 ± 0.47	2.85 ± 0.42	0.62 ± 0.07
III-A	463.5 ± 26.1	12.80 ± 1.47	2.76 ± 0.28	2.93 ±0.39	0.63 ± 0.07
III-B	465.1 ± 32.7	13.38 ± 2.46	2.87 ± 0.48	2.99 ± 0.43	0.64 ± 0.09

Notes: I, model I; II, model II; III, model III; M, model control; A, allopurinol; B, benzbromarone. ▲▲Significantly different from the control value at the levels of $P<0.01$. *,**Significantly different from the corresponding model value at the levels of $P<0.05$, $P<0.01$, respectively.

tinuous 45 days (days 1-45). All treatments were via oral gavage in a volume of 10 mL/kg·bw. Body weight was measured and recorded before treatment on the first day of dosing and twice per week thereafter and prior to necropsy.

Blood collection and measurement

The rats were fasted eight hours prior to blood collection with free access to water. Peripheral blood was collected on day -1 (as baseline), 15, 30 and 45. On day -1, 15 and 30, blood was obtained by nicking a lateral tail vein with a surgical blade after animals were warmed briefly under a heat lamp. At the end of the experiment, all animals were sacrificed and the abdominal aorta blood samples were collected. After centrifugation at 1300 g for 10 min, the serum was isolated and stored at -80°C for following detection. The serum creatinine (Cr), urea nitrogen (UN) and uric acid were all determined by an autoanalyzer (Beckman Coulter AU480, USA). For six treatment groups, the percentage of decrease in uric acid in positive groups compared with corresponding model groups was calculated using the following equation on day 15, 30 and 45:

$$\text{percentage of decrease in uric acid (\%)} = \frac{(\text{UA in model groups} - \text{UA in positive groups})}{\text{UA in model groups}} \times 100\%$$

Necropsy

After the blood samples were collected, the livers and kidneys were excised and weighed. Relative organ weights [(organ/body weight) × 100%] were calculated for all organs.

Histopathology and image analysis

Livers and kidneys were fixed in 10% buffered formalin, embedded in paraffin and stained with hematoxylin and eosin (H&E). Light microscopy studies were done under a photomicroscope (Olympus BX50F4, Japan).

The extent of the damage was evaluated by the Image-Pro Plus System 5.0 (Media Cybernetics Inc., Silver Spring, MD, USA). The images of whole tissue sections from all animals were captured under × 200 optical magnification. Successive fields of each slide (ten to fifteen images) were then analyzed with the software above. For each image, positive staining was measured as a percentage of the area of total image. The calculation was performed as follows: (a) define the area to be analyzed, (b) set the colors that identified the positive, and (c) automatically measure by computer. All histological determinations were done by a blinded observer.

Statistical analyses

Results were expressed as mean values with standard deviations (mean ± S.D.). Statistical analysis was performed using Statistical Product and Service Solutions (SPSS, Chicago, IL, USA) Version 11.0, and the level of significance was set at alpha = 0.05. For all data one-way analysis of variance (ANOVA) was used after

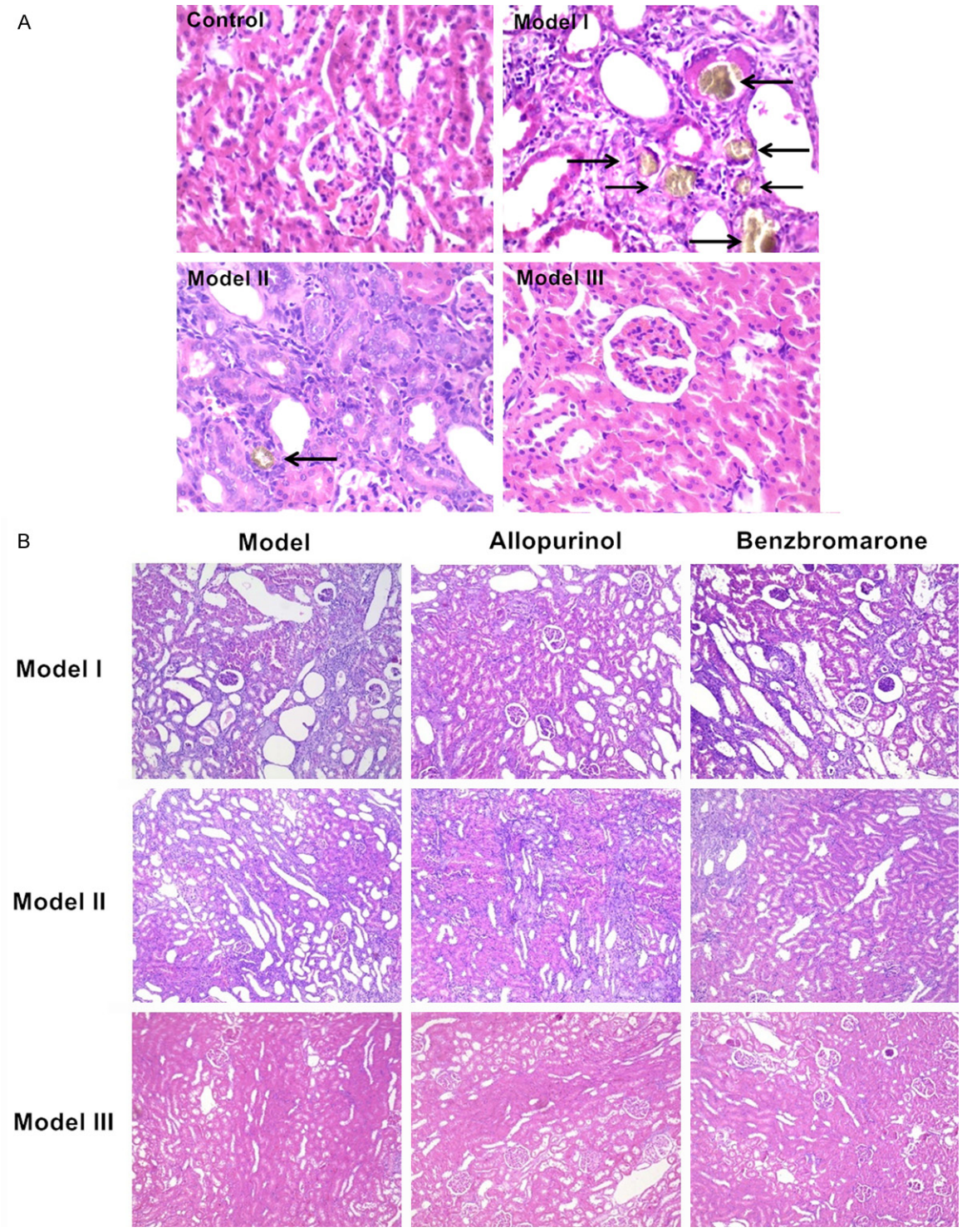

Figure 1. Hematoxylin & Eosin stains of kidney sections. A: Images from control and 3 model groups (*i.e.* model I, model II and model III) under × 400 magnification. Tubulointerstitium appeared normal in control and model III rats, while prominent injuries including severe inflammatory cell infiltration, vascular congestion, interstitial fibrosis and crystalline-type deposit were shown in model I and II. Kidney stones were indicated by arrows. B: Images from all groups under × 200 magnification. In model I and II, the injuries were appreciably attenuated by allopurinol, whereas benzbromarone had no such effects.

Table 3. Injury severity of kidney sections evaluated by the Image-Pro Plus System on H&E stains (n=13)

Group	Injury area (%)
Control	NO
I-M	74.32 ± 13.11
I-A	32.82 ± 09.28**
I-B	71.14 ± 12.53
II-M	58.72 ± 09.83
II-A	27.13 ± 12.24**
II-B	60.40 ± 07.12
III-M	NO
III-A	NO
III-B	NO

Notes: I, model I; II, model II; III, model III; M, model control; A, allopurinol; B, benzbromarone; NO, not observed. Injury area expressed as the percentage of the entire field of each slide. **Significantly different from the corresponding model value at the levels of $P<0.01$.

checking for homogeneity of variance, or the Kruskal-Wallis rank sum test was performed. Comparison between groups was undertaken with a Dunnett's test while $P \leq 0.05$ in ANOVA.

Results

General behavior, body weight and organ weight

No rat died during the experiment. Later in the 45 consecutive days, yellow and clutter in furs, apathetic and burnout in spirit were observed in animals in adenine feeding groups (model I and II), while neither of the symptoms were observed in model III and control group. Animals in model I and II gained less weight than control animals, but the differences were modest and did not reach statistical significance ($P>0.05$, **Table 2**).

Significant increase ($P<0.05$) of absolute and relative kidney weights was observed in rats treated with adenine (model I and II) compared to those of controls, and the increase was more profound at 100.0 mg/kg·bw. There was no significant difference ($P>0.05$) in absolute and relative organ weights in rats treated with oteracil potassium alone (model III) compared with those of controls. In model I, allopurinol decreased both absolute and relative kidney weights as compared to those of the corresponding model group ($P<0.05$), but benzbromarone had no such effects. Slight but non-significant ($P>0.05$) reduction in the absolute and relative kidney weights was observed in allopurinol intervention group of model II (**Table 2**).

Necropsy and histopathologic findings

At the time of autopsy, marked renal swelling, white discoloration and granular change were observed in the gross morphology of kidney in model I and II, whereas none of those abnormal changes were found in control and model III. On H&E stains of kidney section slides (**Figure 1A**), both tubulointerstitium and glomeruli of rats in control and model III appeared normal, while rats in model I and II had considerable renal damage. Kidney slices of model I and II showed different amounts of crystals in tubular lumina and interstitium. Furthermore, scattered dilation and obstruction of tubular lumina, formation of foreign body granuloma, subsequent degeneration and necrosis of tubular epithelia were found. There were no macroscopic and microscopic findings of livers in rats from all groups considered to be related to treatment.

Quantified analysis was performed as previously described on H&E stains of kidney sections (**Table 3** and **Figure 1B**). There were no renal damages in control and model III groups. Renal injury was extensive in two adenine treated model groups (model I and II), and injury area on average was 74.32% at dose of 100.0 mg/kg·bw larger than 58.72% at 50.0 mg/kg·bw ($P<0.05$). In model I and II, the injuries were appreciably attenuated in allopurinol intervention groups ($P<0.05$), whereas similar effects were not observed regarding benzbromarone.

Serum uric acid, creatinine and urea nitrogen levels

Measurements of serum UA, Cr and UN were performed on day 0, 15 d, 30 d and 45 d (**Figure 2**; **Tables 4** and **5**). The two dose of adenine (model I and II) induced a statistically significant increase in serum UA, Cr and UN, as early as day 15, with further increases at day 30 and 45 ($P<0.05$). Oteracil potassium alone (model III) also induced a statistically significant increase in UA ($P<0.05$) in a time-dependent manner. In contrast to co-treatment of adenine plus oteracil potassium, the increase of Cr and UN levels in the model group used oteracil potas-

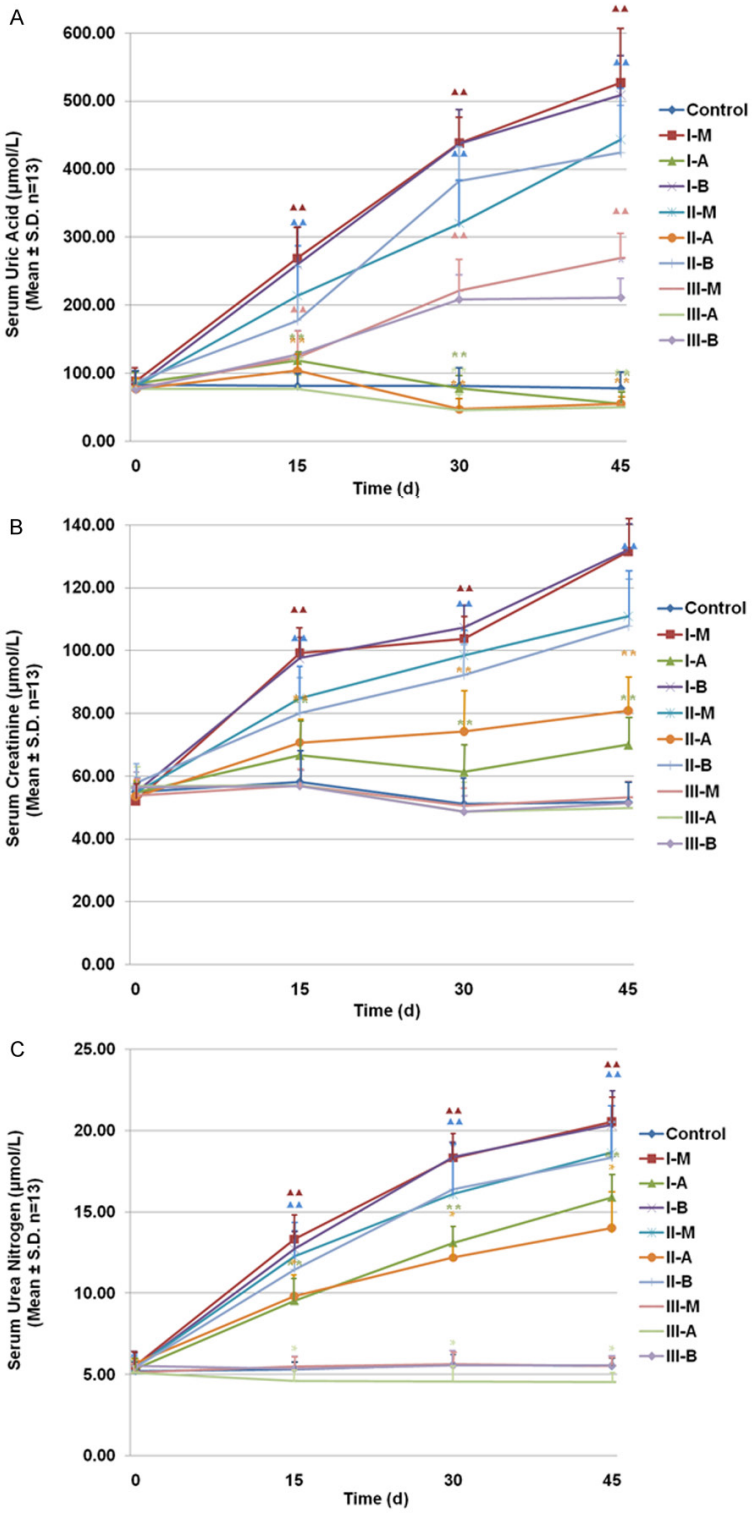

Figure 2. Time courses of responses for different treatments in hyperuricemic rats. The response of (A) serum uric acid levels, (B) serum creatinine levels, and (C) serum urea nitrogen levels for 45 days. Notes: I, model I; II, model II; III, model III; M, model control; (A) allopurinol; (B) benzbromarone. ▲▲Significantly different from the control value at the levels of $P<0.01$. *,**Significantly different from the corresponding model value at the levels of $P<0.05$, $P<0.01$, respectively.

sium alone was not observed at any time point.

For all 3 models, the level of UA decreased after treatment with allopurinol compared with corresponding model groups at any time point from day 15 to day 45 ($P<0.05$, **Figure 2A** and **Table 4**). Meanwhile, the percentage of decrease in UA was gradually increased over time. Allopurinol also induced decreases in serum Cr and UN levels in model I and II ($P<0.05$, **Figure 2B**, **2C** and **Table 5**), whereas only the decrease of UN levels was showed in model III ($P<0.05$). There was no significant difference in treatment with benzbromarone in attenuating the increase in UA levels in model I and II ($P>0.05$), and the UA levels was not attenuated until day 45 in model III (**Figure 2A** and **Table 4**). There was likewise no significant difference in treatment with benzbromarone in both UN and Cr responses among the three model groups ($P>0.05$, **Figure 2B**, **2C** and **Table 5**).

Discussion

Uric acid in most mammalian species is converted to allantoin by uricase, which is lacking in the higher apes and human [13]. Hence, inhibitors of the uricase (*e.g.* oxonic acid) [14], and precursors of uric acid (*e.g.* adenine) [15] have generally been adopted as modelling agents for animal hyperuricemia, while transgenic rat [16] and fructose [17] have also been employed. Previous studies on animal hyperuricemia models showed that daily ingestion of uricase inhibitors and precursors of uric acid may lead to morphological alter in renal structure as-

Table 4. Serum UA and percentage of decrease in UA of rats treated with modeling and intervention drugs during 45 days (n=13)

Group	UA (μmol/L)				Percentage of decrease in UA (%)		
	Day 0	Day 15	Day 30	Day 45	Day 15	Day 30	Day 45
Control	82.86 ± 15.70	80.89 ± 13.80	80.83 ± 20.03	77.54 ± 17.97	-	-	-
I-M	87.98 ± 13.51	268.92 ± 36.06▲▲	438.32 ± 34.00▲▲	527.16 ± 66.18▲▲			
I-A	84.12 ± 12.53	118.52 ± 9.76**	77.45 ± 15.64**	54.84 ± 13.16**	55.93	82.33	89.60
I-B	80.68 ± 15.47	259.59 ± 44.84	437.02 ± 42.28	508.67 ± 48.84	3.47	0.30	3.51
II-M	80.92 ± 12.35	213.79 ± 59.88▲▲	320.00 ± 55.01▲▲	443.55 ± 64.21▲▲	-	-	-
II-A	76.74 ± 8.41	103.66 ± 17.33**	46.99 ± 11.91**	55.27 ± 6.80**	51.51	85.32	87.54
II-B	87.59 ± 11.08	177.47 ± 64.42	382.18 ± 41.81	423.97 ± 59.13	16.99	-19.43	4.41
III-M	78.07 ± 10.83	123.05 ± 27.90▲▲	221.28 ± 37.15▲▲	269.35 ± 29.84▲▲	-	-	-
III-A	76.61 ± 09.41	76.32 ± 13.80**	45.21 ± 16.38**	49.68 ± 15.87**	37.98	79.57	81.56
III-B	75.69 ± 10.06	127.41 ± 29.56	208.28 ± 28.88	211.15 ± 24.58*	-3.54	5.87	21.61

Notes. I, model I; II, model II; III, model III; M, model control; A, allopurinol; B, benzbromarone; UA, uric acid. Percentage of decrease in serum uric acid was calculated by the formula listed in the text. ▲▲Significantly different from the control value at the levels of $P<0.01$. *,**Significantly different from the corresponding model value at the levels of $P<0.05$, $P<0.01$, respectively.

Table 5. Serum Cr and UN of rats treated with modeling and intervention drugs during 45 days (n=13)

Group	Cr (μmol/L)				UN (μmol/L)			
	Day 0	Day 15	Day 30	Day 45	Day 0	Day 15	Day 30	Day 45
Control	55.02 ± 7.05	58.13 ± 9.08	51.19 ± 7.23	51.77 ± 5.04	5.23 ± 0.37	5.35 ± 0.37	5.61 ± 0.50	5.53 ± 0.40
I-M	52.03 ± 4.17	99.19 ± 7.00▲▲	103.77 ± 5.89▲▲	131.51 ± 9.39▲▲	5.56 ± 0.74	13.33 ± 1.31▲▲	18.33 ± 1.40▲▲	20.56 ± 1.38▲▲
I-A	54.74 ± 2.34	66.68 ± 9.71**	61.35 ± 7.52**	70.00 ± 7.77**	5.35 ± 0.56	9.56 ± 1.29**	13.12 ± 1.05**	15.92 ± 1.39**
I-B	54.75 ± 2.18	97.57 ± 6.20	107.38 ± 6.21	132.06 ± 7.08	5.48 ± 0.60	12.75 ± 1.02	18.37 ± 0.91	20.37 ± 2.01
II-M	54.78 ± 3.07	84.82 ± 8.94▲▲	98.43 ± 7.50▲▲	110.97 ± 13.08▲▲	5.45 ± 0.41	12.26 ± 1.97▲▲	16.10 ± 2.77▲▲	18.66 ± 2.84▲▲
II-A	53.50 ± 3.75	70.68 ± 6.41*	74.22 ± 10.77**	80.85 ± 9.78**	5.70 ± 0.31	9.82 ± 1.23*	12.20 ± 1.86*	14.03 ± 2.22*
II-B	57.73 ± 4.70	80.14 ± 10.13	92.19 ± 8.49	107.85 ± 13.25	5.48 ± 0.44	11.49 ± 2.21	16.42 ± 2.51	18.36 ± 2.05
III-M	53.76 ± 4.27	57.06 ± 4.89	50.58 ± 4.40	53.29 ± 4.30	5.16 ± 0.41	5.51 ± 0.51	5.66 ± 0.58	5.57 ± 0.42
III-A	56.83 ± 7.67	52.83 ± 5.55	48.61 ± 7.62	49.70 ± 4.02	5.11 ± 0.62	4.59 ± 0.60*	4.55 ± 0.60*	4.53 ± 0.55*
III-B	56.42 ± 3.85	56.89 ± 4.28	48.61 ± 4.30	51.37 ± 4.55	5.54 ± 0.79	5.36 ± 0.73	5.56 ± 0.78	5.57 ± 0.48

Notes. I, model I; II, model II; III, model III; M, model control; A, allopurinol; B, benzbromarone; Cr, creatinine; UN, urea nitrogen. ▲▲Significantly different from the control value at the levels of P<0.01. *,**Significantly different from the corresponding model value at the levels of P<0.05, P<0.01, respectively.

sociated with the molding mechanisms of hyperuricemia. In animal models induced by uricase inhibitors, serum UA was increased by a competitive-inhibition of the UA transformation. Therefore, kidney injury in this model was caused by the separation and deposition of urate crystals [18], which was similar to the renal injury of primary hyperuricemia implicated in pathological states such as gout and inherited purine disorders. On the contrary, the crystal kidney stones deposited in tubular lumina in adenine induced models were oxidized adenine (*i.e.* 2,8-dihydroxyadenine) [19], followed by the increase of UA level as a result from kidney dysfunction. The renal injury in adenine-induced animals was analogous to those in secondary hyperuricemia.

Some researchers have suggested that renal diseases possibly implicated the progression hyperuricemia [20]. Ma *et al.* [21] and Wang *et al.* [22] have used renal histopathological changes to reflect drug efficacy in fructose-induced or oxonate-induced hyperuricemia respectively. Therefore, we tested the hypothesis that the attenuation degree of renal injury may be an indicator of drug efficacy in two types of hyperuricemia models. In this study, rats co-treated with adenine at 50 mg/kg·bw or 100 mg/kg·bw plus oteracil potassium developed crystal-related kidney inflammation and injury. Allopurinol could ameliorate elevated UA levels and renal injuries in both two co-treated models, while those improvements were not observed in benzbromarone intervention groups.

We were also able to induce hyperuricemia by oteracil potassium alone, but the kidneys were devoid of crystals and were normal by light microscopy. The failure of observation of renal injury was probably attributed to that light microscopy was not sensitive enough to detect the small injuries and animals were not treated for long enough. Elevated UA levels were also attenuated by allopurinol in this model. Notably, benzbromarone was found to significantly decrease the UA levels at day 45 in this model, in contrast with its null effect in other two co-treated models.

It can be deduced that the variation of drug efficacy of allopurinol and benzbromarone observed in two types of hyperuricemia models was associated with their mechanisms of action. Adenine and benzbromarone are representative agents of xanthine oxidase inhibitors and uricosurics, two main categories for antihyperuricemia drugs, respectively. Allopurinol is a nonspecific competitive xanthine oxidase inhibitor [5]. Some researchers have suggested that allopurinol is able to slow the progression of renal diseases and improve kidney function in hyperuricemic patients [23]. These effects were validated in our study in the models co-treated of adenine plus oteracil potassium. The precise mechanism is currently unknown, but probably related to alleviation in endothelial dysfunction [24]. By contrast, benzbromarone takes effect by increasing renal urate excretion mediated by selective inhibition of organic anion transporters [5], and this might be the reason why benzbromarone did not work on animals with kidney dysfunction.

Moderate degree of hepatic damages induced by adenine was reported by Al Za'abi *et al.* [25] and Song *et al.* [26]. In the former study that Wistar rats were treated with adenine at the dose of 0.25% (w/w) for 35 days, liver tissue showed infiltration of inflammatory cells under light microscopy, and significant elevations in some plasma enzymes were also observed [25]. In the latter study that used adenine at the dose of 10 mg/kg·bw for 14 days in Wistar rats, Song *et al*. [26] also reported significant increases in some enzymes. Dark grain material and increase of lysosome were observed under ultra microstructure. However, in contrast with the observation reported by the two studies listed above, no liver abnormities showed up by histological analyses in our study.

Currently, the courses of TCM therapy of hyperuricemia usually last for 20-30 days [27, 28], and the patients with comorbidities such as renal impairment need to receive treatment for even longer periods (56-90 days) [29, 30]. Hence, we adopted the administration time of 15-45 days, which was appropriate for screening the majority slow-acting drugs relative to human medication period. Our study provided evidence that either co-administration of adenine plus oteracil potassium or using oteracil potassium only was available to establish a stable hyperuricemia model in rats during 15-45 days. The former model was similar to secondary hyperuricemia in human, and it was able to screen xanthine oxidase inhibitors but uricosurics. The latter model was similar to primary hyperuricemia, which was suitable for screening both classes of antihyperuricemic agents. Considering that severe renal injury may affect enzyme activity in drug action, treating with adenine at the dose of 50.0 mg/kg·bw was recommended rather than that at 100.0 mg/kg·bw.

In this study, treatment with adenine plus oteracil potassium or oteracil potassium alone was both able to establish stable hyperuricemia animal models during 15-45 days, providing a valuable platform for efficacy screening of slow-acting antihyperuricemia agents, especially for TCM. The kidney injury induced by adenine mitigated by allopurinol may play an important role in screening for agents as xanthine oxidase inhibitors. In addition, these findings supported the need for additional studies to clarify the mechanistic pathways of crystalline deposition induced by adenine for better understanding of the profile and efficacy of antihyperuricemic agents. It is promising that animal models established in this study will serve as a valuable research tool in this area of effective agents screening.

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Disclosure of conflict of interest

None.

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