

## A novel mouse model of hyperuricemia and gouty nephropathy

Jin Guan<sup>1</sup>, Xiao-Qi Huang<sup>2,3</sup>, Jia-Le Dong<sup>1</sup>, Hui-Min Lu<sup>4</sup>, Yue-Wei Lin<sup>5</sup>, Mei Liu<sup>2,3</sup>, Zhi-Biao Yi<sup>3</sup>, Li-Min Wu<sup>4</sup>, Yong-Ming Huang<sup>5</sup>, Tian Lan<sup>1</sup>

<sup>1</sup>Department of Pharmacology, School of Pharmacy, Guangdong Pharmaceutical University, Guangzhou 510006, China;

<sup>2</sup>Guangzhou University of Chinese Medicine, Math Engineering Academic Chinese Medicine, Guangdong Province of Key Lab New Drug Dev & Res Chinese, Guangzhou, Guangdong 510006, China;

<sup>3</sup>Guangzhou University of Chinese Medicine, Dongguan Math Engineering Academic Chinese Medicine, Dongguan, Guangdong 523808, China;

<sup>4</sup>Foshan Santanyi Biotech Co., Ltd, Foshan, Guangdong 528031, China;

<sup>5</sup>Second Affiliated Hospital of Guangzhou University of Chinese Medicine, Guangzhou, Guangdong 510006, China.

*To the Editor:* Hyperuricemia, the second most common metabolic disease after diabetes mellitus in China, is characterized by the elevated serum uric concentration which is due to the overactive uric acid production or underexcretion.<sup>[1]</sup> Recent study supported the notion that hyperuricemia is implicated in renal tubular injury, subsequent tubulointerstitial fibrosis, and finally gouty nephropathy.<sup>[2]</sup> However, the lack of a suitable and stable mouse model to study hyperuricemia and gout nephropathy delays and limits the development of mechanism research and new drugs. Further understanding of the pathogenesis of hyperuricemia and gouty nephropathy has been largely hampered by a lack of preclinical animal models that reliably capture the characteristic phenotypes of hyperuricemia and gouty nephropathy. In this study, we aimed to develop a novel mouse model for hyperuricemia and gouty nephropathy.

Male C57BL/6J mice (30 g) were divided randomly into three groups the control group (treated with carboxymethyl cellulose sodium [CMC-Na] alone); the 100 mg/kg adenine and 500 mg/kg potassium oxonate group (Ade-100 + OXO-500); and the 150 mg/kg adenine and 300 mg/kg OXO group (Ade-150 + OXO-300). Mice were administered intragastrically with a mixture of adenine and OXO (diluted in 0.5% CMC-Na) daily consistently for 3 weeks [Figure 1A]. The food intake, water consumption and weight gain of mice were measured before administration. Mice were anesthetized with chloral hydrate and the blood samples were collected through the outer canthus for the measurement of serum uric acid and creatinine weekly. On the last day of the experiment, mice were kept in individual metabolic cages to collect 24-h urine for the determination of the urinary uric acid and protein. And then, mice were

sacrificed 3 h after the last treatment. Blood was taken for the analysis of biochemistry indices. Kidneys were harvested for histologic examination and protein analysis.

Mice treated with adenine and OXO exhibited severe kidney injury, as revealed by the elevated level of serum creatinine at the seventh day after the initiation of the study [Figure 1B]. And the elevated serum uric acid at the second and third weeks of the experiment suggested that the hyperuricemia mouse models were successfully established [Figure 1C].

In line with previous studies, adenine and OXO administration significantly decreased the body gain and food intake of mice but increased the water intake of mice. The 24-h urine volumes were also remarkably increased [Supplementary Figure 1, <http://links.lww.com/CM9/A256>]. Strikingly, the activity of serum xanthine oxidase, the key enzyme catalyzing the production of uric acid, was significantly up-regulated by the treatment with Ade-100 + OXO-500 resulting in an obvious decrease in urinary uric acid [Figure 1D and 1E]. In addition, histologic examination of kidney revealed that a significant damage by adenine and OXO treatment [Figure 1F]. Furthermore, not only the dilated kidney tubules and ectatic Bowman spaces, but also the dark needle-like urate crystals, focal tubulointerstitial fibrosis and glycometabolic disorder were more evident in the kidneys of mice treated with Ade-100 + OXO-500, further illustrating the more severe kidney injury [Figure 1G and 1H] in this group of mice. Immunohistochemistry of kidneys from mice administrated with adenine and OXO showed massive accumulation of URAT1 and GLUT9 in injured tubules, meaning the increase of uric acid reabsorption in the proximal renal tubules.<sup>[3]</sup> Western blotting

Jin Guan and Xiao-Qi Huang contributed equally to the work.

**Correspondence to:** Tian Lan, Department of Pharmacology, School of Pharmacy, Guangdong Pharmaceutical University, Guangzhou Higher Education Mega Center, 280 Wai Huan Dong Road, Guangzhou, Guangdong 510006, China  
E-Mail: [lantian012345@163.com](mailto:lantian012345@163.com)

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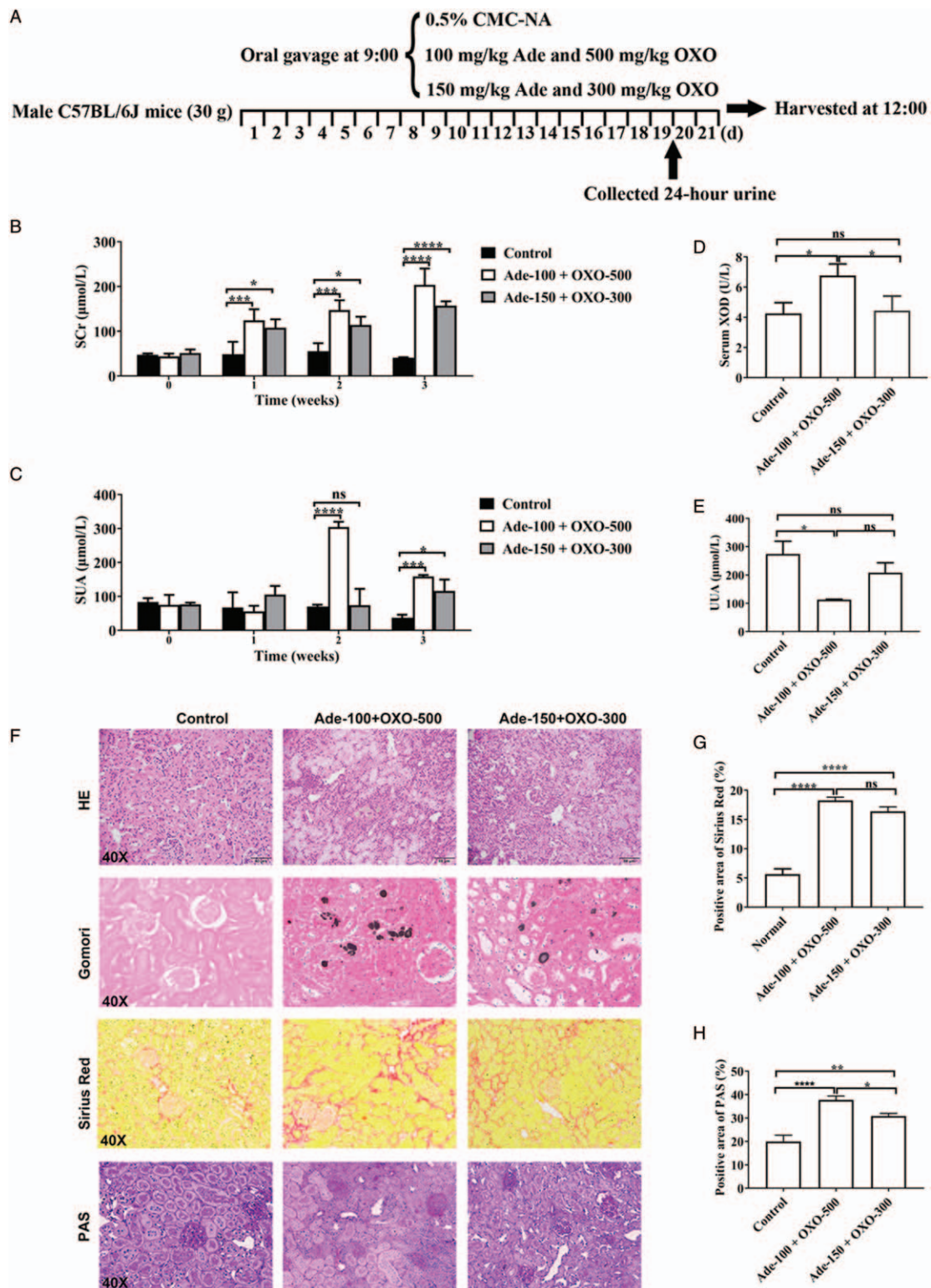


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**Figure 1:** Effects of adenine (Ade) and OXO on renal function and uric acid distribution in mice. (A) Overview of the hyperuricemia model procedure. (B) Serum creatinine (Scr) level. (C) Serum uric acid (SUA) level. (D) Activity of serum XOD. (E) Urinary uric acid (UUA). (F) Representative histology of H&E, Gomori methenamine silver, Sirius red, and PAS staining (original magnification  $\times 40$ ). (G, H) Quantification of positive staining areas was measured by Image J software. Data are presented as mean  $\pm$  standard deviation.  $n = 6$  per group. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , \*\*\*\* $P < 0.0001$ . Similar results from three independent experiments are shown. CMC-Na: Carboxymethyl cellulose sodium; XOD: Xanthine oxidase; H&E: Hemotoxylin and eosin; OXO: Oxonate; PAS: Periodic acid-Schiff; ns: Not significant.

assay also showed a remarkably increased renal expression of URAT1 and GLUT9 in mice treated with adenine and OXO treatment. Importantly, the increase of protein levels of URAT1 and GLUT9 in the kidney was further elevated in

the Ade-100 + OXO-500 group [Supplementary Figure 2, <http://links.lww.com/CM9/A256>]. In addition, increased expression of inflammatory cytokine tumor necrosis factor (TNF)- $\alpha$  has been involved in the immune response during

gout. Interleukin (IL)-1 $\beta$ , which is cleaved to the mature form by active Caspase 1, is also identified as the primary motivating factor of gouty inflammation. We next assessed the effects of adenine and OXO co-stimulation on systemic inflammation in mice by measuring the levels of serum TNF- $\alpha$  and IL-1 $\beta$  in mice. As expected, adenine and OXO treatment remarkably increased the serum concentration of TNF- $\alpha$  and IL-1 $\beta$  in mice [Supplementary Figure 3, <http://links.lww.com/CM9/A256>]. Consistently, mice administered with Ade-100 + OXO-500 had exhibited severe inflammation. Taken together, our results suggest that mice treated with Ade-100 + OXO-500 is more favorable than Ade-150 + OXO-300 to generate disease phenotypes of mouse model of hyperuricemia and gouty nephropathy.

Accumulating evidences indicated that hyperuricemia was an independent risk factor for the new-onset and progression of chronic kidney disease. Methods such as adding yeast cream, gavaging adenine or potassium oxazinate, or intraperitoneally injecting inosine 5-monophosphate and guanosine monophosphate have been used to create mouse model of hyperuricemia with variable degree of reproductivity.<sup>[4]</sup> However, due to the inaccurate dose, high mortality rate and inconvenient operation, the hyperuricemia models in previous research were difficult to reproduced.<sup>[5]</sup> Initially, we started out the experiment by treating mice with 100 mg/kg adenine or 250 mg/kg OXO for 7 days. However, the serum concentration of uric acid in mice was increased provisionally and was less than 200  $\mu$ mol/L. Hence, we attempted to create mouse model of hyperuricemia by injecting mouse with adenine and OXO. Notably, our results showed that administration of adenine and OXO (100 and 500 mg/kg, respectively) increases the intake of purine, inhibits the uricase activity, leading to the elevation of serum uric acid which eventually hyperuricemia and subsequent gouty nephropathy.

In summary, we have successfully established a mouse model of hyperuricemia and gouty nephropathy by administrating adenine and OXO. The validity of our mouse was confirmed by strict parameters of disease phenotypes such as serologic pathology, renal tissue histology examination, the expressions of uric acid trans-

porters, and the inflammatory factors. Our animal model of hyperuricemia and gouty nephropathy can serve as an animal model to evaluate the efficacy of therapeutic agents.

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### Conflicts of interest

None.

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