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ORIGINAL ARTICLE

Polydatin Lowers Serum Uric Acid Levels by Increasing its Excretion and Suppressing Production

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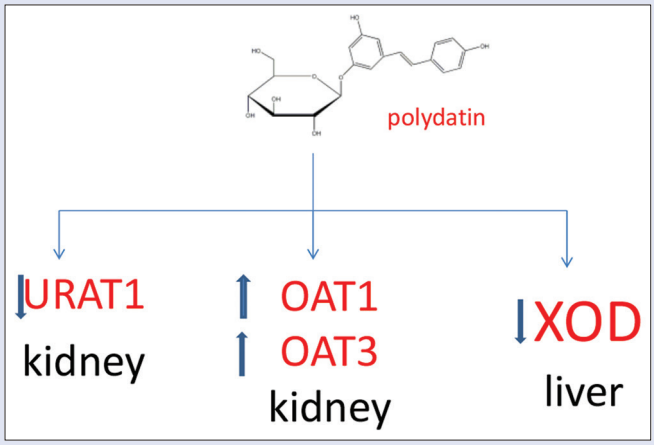
ABSTRACT

Background: Polydatin has significant uricosuric effects. This study was conducted to examine the xanthine oxidase inhibition and to detect protein levels of urate transporter 1 (URAT1), organic anion transporters 1 (OAT1), and organic anion transporters 3 (OAT3) in the hyperuricemic mice to understand the role of antihyperuricemic mechanism of polydatin. **Materials and Methods:** Hyperuricemia mice model was established with uricase inhibitor (potassium oxonate), and uric acids in serum were observed. Kidney tissues were used to detect gene contents of URAT1, OAT1, and OAT3 by real-time-PCR (polymerase chain reaction) and to detect pathological features. The activity of xanthine oxidase (XOD) in the liver tissues of mice was detected. **Results:** The polydatin experimental groups and the positive control group (benzbromarone) showed significantly inhibited levels of serum uric acid when compared with the model group. Polydatin significantly inhibited the increasing tendency of the mRNA and the protein levels of OAT1 and OAT3, and decreased the tendency of the mRNA and the protein level of URAT1. Polydatin significantly inhibited the level of XOD in the liver tissues of mice in a concentration-dependent manner. Polydatin showed a protective effect on the pathological injury of the kidney. **Conclusion:** Polydatin has an antihyperuricemic effect in oxonate-induced hyperuricemic mice. The effect was related to the downregulation of renal URAT1, upregulation of renal OAT1 and OAT3, and inhibition of XOD in the hyperuricemic mice.

Key words: Hyperuricemia, organic anion transporter, polydatin, urate transporter, uric acid, xanthine oxidase

SUMMARY

- Our data provided further evidence that polydatin can decrease the expression of urate transporter 1 (URAT1) , increase OAT1 and OAT3 levels and inhibit XOD activity, and protect the kidney from renal damage in the hyperuricemic mice.



Abbreviations used: URAT1: urate transporter 1; OAT1: organic anion transporters 1; OAT3: organic anion transporters 3; XOD: xanthine oxidase; PCR: C-reactive protein; NSAIDs: nonsteroidal anti-inflammatory drugs; CMC-Na: Carboxymethylcellulose sodium.

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INTRODUCTION

Uric acid, a weak organic acid with a pKa of 5.75, is the end product of purine metabolism. As urate exists as an organic anion, membrane transporters are essential for it to permeate the plasma membrane in the body. Purine bases and their nucleotides are converted into hypoxanthine, then into xanthine, and into uric acid by the xanthine oxidase in the liver.[1] Invertebrates, uric acid is oxidized to water-soluble allantoin by the urate oxidase in live and later into urea. The non-functioning uricase gene in humans led to humans having higher uric acid levels than other mammals.[2] Urate homeostasis depends on the balance between production and excretion. It is estimated that uric acid is mainly excreted by the kidneys (70%) and intestines (30%).[3] In the human kidney, urate is freely filtered through the glomerulus. However, the largest part (greater than 90%) is usually reabsorbed in the kidney, which is mediated via membrane transport proteins and returns to the bloodstream. Thus, the balance of the renal reabsorption and secretion of urate contributes to high-serum urate levels (about 300 mol/L) in human beings.[4] High-plasma uric acid is a prerequisite for gout and renal calculi. In the last 20 years, epidemiological studies showed that hyperuricemia is an independent risk factor for metabolic syndrome and cardiovascular diseases,

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particularly in hypertensive and diabetic individuals.[5-8] There is strong epidemiological evidence that the prevalence of gout and hyperuricemia is increasing worldwide.[9]

Previous studies have shown that approximately 90% of hyperuricemia is caused by renal urate under excretion.[10] Because the kidney plays a major role in maintaining plasma urate levels through its excretion, the reabsorption and secretion of uric acid are controlled by transporters in renal apical and basolateral membranes. The transporters carry out bidirectional transport of their endogenous and exogenous substrates across the tubular epithelial cells, thus regulating the homeostasis of these substrates. Recent molecular studies indicate that the proximal tubule is the major site of renal urate transport. Since the identification of the urate/anion exchanger urate transporter 1 (URAT1) in 2002,[11] several organic anion transport proteins (OATs) such as organic anion transporters OAT1 to OAT4 have been identified successively.[12] Most hyperuricemic patients (up to 90%) are urate under-excretors and URAT1 is responsible for most of the uric acid reabsorption. Benzbromarone, a uricosuric agent used clinically for the treatment of hyperuricemia and gout, and other uricosuric substances such as probenecid, fenylbutazone, sulfinpyrazone, nonsteroidal anti-inflammatory drugs (NSAIDs), and diuretic drugs, is the URAT1 inhibitor.[13] Urate secretion in renal proximal tubules is performed mainly by the OAT1 and/or OAT3 and the urate channel at the basolateral membrane. Therefore, these renal OATs may be one of the important targets for the effective agents to prevent and treat hyperuricemia.[14]

Polygonum cuspidatum Sieb. and Zucc. (Polygonaceae), also known as Huzhang in Chinese, is a traditional Chinese medicine, used for the treatment of various inflammatory diseases, such as hepatitis, skin burns, tumors, and diarrhea. It is officially listed in the Chinese Pharmacopoeia. It has been traditionally used in folk medicine as a crude drug for joint pain induced by gout.[15] As one of its main ingredients, polydatin (also named resveratroside) is a kind of glycoside, which exists widely in the plants like grape, earthnut, giant knotweed, black-false hellebore, sickle senna, etc. In human body, polydatin is hydrolyzed in the intestine by glycosidase to resveratrol to display its pharmacological action, such as liver protection, antiinflammation, antioxidative, antitumor, and antipathogenic microbe, cardiomyocyte protection, vascular smooth muscle dilation, antiplatelet aggregation, antithrombotic effect, and atherosclerosis prevention.[16]

Our previous studies have demonstrated that polydatin has significant uricosuric effects on the hyperuricemic mice.[17] This study aimed to understand the antihyperuricemic mechanism of polydatin. First, an animal model of hyperuricemia was established to verify the uric acid-lowering effect of polydatin. To discover the definite mechanisms of polydatin, we detected gene contents of URAT1, OAT1, and OAT3 and to detect pathological features of kidney tissues. Furthermore, we also determined the activity of XOD in liver tissues. Therefore, polydatin owns promising application prospects and important research value in reducing uric acid.

MATERIALS AND METHODS

Methods

Experimental animals

ICR mice (SPF grade, male, 10-week-old, weighing 18–20 g) were supplied by Shanghai Lake Science Experimental Animal Center, of which the laboratory animal license No. was SCXK (shanghai) 2007-0005. Before the experiment, all mice were adaptively raised for 1 week, with free drinking and standard feeding during the experiment. The experiments were performed in accordance with the guidelines of the Animal Care and Use approved by the Second Military Medical University Experimental Animal Ethics Committee. The committee's reference number: 20170106025.

Materials

Polydatin (Batch number was 130123) was provided by Shanghai Yuanye Biotechnology Co., Ltd. (Shanghai) .Potassium oxonate (approximately 2 mg) (Shanghai) was weighted and dissolved with 200 mL 0.8% carboxymethylcellulose sodium (CMC-Na) solution to prepare 10 g/L potassium oxonate injection. Trizol was provided by Invitrogen Company. Fluorescent quantitative detection kit, DNA Marker and M-MLV reverse transcription kit were purchased from Dalian Takara Biotechnology Co. Ltd. Tubes for quantitative detection PCR and sterile centrifuge tubes were provided by Axygen Company. Primer synthesis and DNA sequencing were conducted in Shanghai Invitrogen Company. All other chemicals used in this study were of analytical grade, made in China.

Establishment of hyperuricemia mice model and routine detection[18]

Sixty male ICR mice were randomly divided into six groups with 10 mice in each group, that is polydatin experimental groups (5, 10, and 20 mg/kg), a positive control group (benzbromarone, 16.7 mg/kg), a model group, and a negative control group. Polydatin and benzbromarone were prepared with 0.8% CMC-Na solution to get the corresponding concentration.

The mice in the normal control group and model group were administered by gastric infusion with 0.8% CMC-Na solution, once daily for continuous 7 days, and the final administration was given one hour after the molding. Blood samples were drawn at 2 hr when all the experimental groups and model group were given intraperitoneal injections of 250 mg/kg potassium oxonate, while the normal control group was given 0.8% CMC-Na solution. One hour after the administration, blood samples were drawn from the ophthalmic venous plexus in mice, then 100 μL serum was collected by centrifugation (4000 × *g*, 5 min), and the blood uric acid levels were detected by TOSHIBA7060 model automatic biochemistry analyzer. At the end of the observation period, all animals were sacrificed by cervical dislocation and vital organs (liver, kidney) were removed from all animals for gross and histopathological examination. Mice livers were extracted and stored at −80°C until XOD detection. Kidney tissues of mice were collected, one part was stored at −80°C and used for total RNA and protein extraction to detect the mRNA as well as protein contents, and another part was fixed in 4% paraformaldehyde for pathological detection.

Content detection of URAT1, OAT1, and OAT3 mRNA in kidney tissues of mice

The relative mRNA contents of URAT1, OAT1, and OAT3 genes in kidney tissues of mice were detected by real time-PCR. Kidney tissues (50 mg) stored at −80°C were removed with a scalpel quickly, transferred to a 1.5 mL aseptic centrifugetube and washed in 1 mL of 4°C ice-cold dPBS (1 mol/L, pH 7.4) twice, removed to a new 1.5 mL centrifugetube, added with 1 mL Trizol, and homogenized adequately, and total RNA was extracted by phenol-chloroform. RNA (1 μg) was reversely transcribed into cDNA with random primer, and 1 μL reverse transcription product was detected by PCR analysis while the reference gene was beta actin (NM_007393.3). Primer sequences: beta actin-forward primer: 5'-CCTAAGGCCAACCGTGAAAAGATG-3' and beta actin-reverse primer: 5'-GTCCCGGCCAGCCAGGTCCAG-3', and the size of the PCR product was 219 bp. OAT1 (NM_008766.3) primers: OAT1-forward

primer: 5'- CTTTCCCGCACAATGGCACAGAGG-3' and OAT1-reverse primer: 5'- GTCCGCCAGGTAGCCAAACATCAT-3', and the size of the PCR product was 209 bp; OAT3 (NM_031194.5) primers: OAT3-forward primer: 5'- CCAAACGCCAGTCTTCCCAATGA-3' and OAT3-reverse primer: 5'- TGCGGCCAAACCTGTCTGA-3', and the size of the PCR product was 211 bp; URAT1 (NM_009203.3) primers: URAT1-forward primer: 5'- TTCATGCCCACCTTCCCCCTC TAC-3' and URAT1-reverse primer 5'- CATCCTCCAGCTGCGCACACCATA -3', and the size of the PCR product was 207 bp. The RCR system of 20 μL contained Takara SYBR Premix Ex Tap 10 μL, 0.2 μL 0.2 μmol/L of each primer, cDNA 2 μL and complemented dH_2O, while the reactive conditions were 40 cycles of denaturation at 95°C for 5 s, annealing at 60°C for 20 s and extension at 72°C for 20 s. The relative contents of mRNA were calculated by ΔCt method, and the expression level of the target gene to that of the reference gene was expressed as 2ΔCt = 2 Ctm − Ctn.

Content detection of URAT1, OAT1, and OAT3 in kidney tissues of mice by western blotting

Kidney (100 mg) tissues stored at −80°C were removed with a scalpel quickly, transferred to a 1.5 mL aseptic centrifugetube, washed in 1 mL of 4°C ice-cold dPBS twice, removed to a new 1.5 mL centrifugetube, added 500 μL lysis buffer T-TER, and homogenized adequately; then the lysate was transferred to a new 1.5 mL centrifugetube, centrifuged at 12,000 × *g*, 4°C for 10 min, and the supernatant was collected. The total protein level was detected by the method of BCA. Total protein (10 μg) was analyzed by SDS-PAG electrophoresis using 11% separation gel at a voltage 120 V for 100 min, and transferred to PVDF membranes using the wet transfer at 400 mA electric current for 90 min. The membranes were blocked in TBST containing 5% nonfat milk at room temperature for 2 hr, incubated overnight at 4°C with the primary antibody (diluted with TBST, the diluent rates of the antibodies against URAT1, OAT1, OAT3, and beta actin C were 1:500, 1:300, 1:400, and 1:800, respectively), washed in 4°C ice-cold TBST three times and incubated with 1:3,000 dilution of the goat antimouse secondary for 2 hr. The membrane was washed three times again and ECL solution was added, and the optical signal of HRP was determined by a scanner. The software of densitometry Totallab10.1 was used to determine the gray value of the objective band, to analyze the relative content of URAT1, OAT1, and OAT3.

Activity detection of XOD in liver tissues of mice

The activity of XOD in liver tissues of mice was detected by Xanthine Oxidase Assay Kit (ab102522). Liver tissues (200 mg) stored at − 80°C were obtained, added 0.2 mL lysis buffer, homogenized adequately, and centrifuged at 16,000 × *g* for 10 min, and the supernatant was collected, 50 μL of which was taken to determine the activity of XOD according to the instructions of the kit. The calculation formula was as following:

$$\text{XO Activity} = \frac{(\text{B x Diluation Factor})}{(T_2 - T_1)xV} = \text{nmol / min / ml/ = mU / ml}$$

An enzyme-labelling measuring instrument (Enzyme labeling analyzer SK201, Shenzhen Shengxinkang Technology Co., Ltd) was used to measure the absorbance value in 570 nm quickly: the first reaction time and reading were recorded as T1 and A1, respectively; the sample was retested after being placed in 25°C for 2 min, and the second reaction time and reading were recorded as T2 and A2. The variation value of absorbance was ΔA = A2 − A1. It is essential to read A1 and A2 in the reaction linear range and we will choose A1 and A2 in the reaction linear range. Where B is the amount of H_2O_2 (nmoL) generated by XO from a standard curve, T1 is the time of the first reading (A1) (in min), T2 is the time of the second reading (A2) (in min), and V is the pretreated sample volume (mL) added into the reaction well.

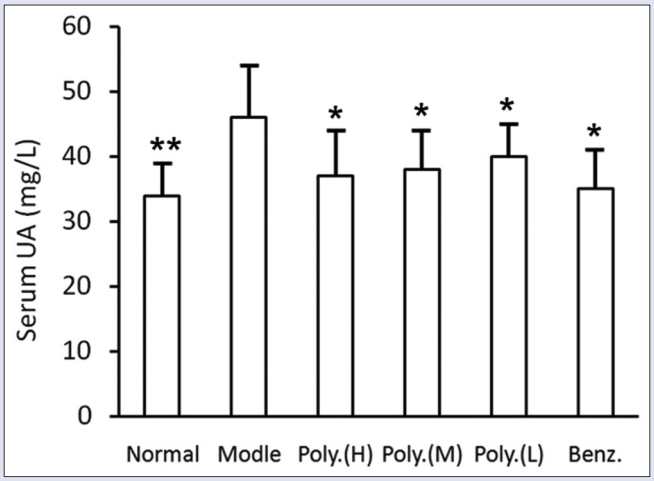

Figure 1: Effects of polydatin on serum uric acid levels. Mice were administrated with 0.8% CMC-Na (normal or model groups) or polydatin (5, 10, or 20 mg/kg) orbenzbromarone (Benz, 16.7 mg/kg) once daily for continuous 7 days, and received 0.8% CMC-Na (normal) or 250 mg/kg potassium oxonate (model, poly-H, poly-M, ploy-L and benz) by intraperitoneal injections one hour before the last administration of the drugs. Blood samples were collected 2 hr later and the blood uric acid levels were detected. Results are expressed as mean ± SEM. * $P < 0.05$ and ** $P < 0.01$ vs. the model group

Pathological detection in kidney tissues of mice

The tissues were fixed, cut into sections and stained with HE staining according to the routine procedure.

Data analysis

The statistical analysis of the experimental data was performed using statistic software SPSS 10.0 (Version 21; New York, NY, USA). The analysis of variance test was applied in comparing the intergroup difference of measurement data, while Ridit analysis was used for ranked data. A statistical significance was considered when $P < 0.05$.

RESULTS

Effects of polydatin on serum uric acid level in hyperuricemic mice

According to the results in Figure 1, the polydatin experimental groups (5, 10, and 20 mg/kg) and the positive control group (benzbromarone, 26 mg/kg) showed significantly inhibited levels of serum uric acid when compared with the model group. Variance analysis indicated that the level of serum uric acid was remarkably elevated in the model group when compared with the normal control group, and the statistically significant differences between groups ($P < 0.01$) suggested that the model was successfully established. The level of serum uric acid was lower in the benzbromarone control group compared with the model group, and there were no statistical differences between benzbromarone control group and the normal control group ($P < 0.05$), suggesting that benzbromarone decreased the level of serum uric acid to the normal one. The levels of serum uric acid were higher in the polydatin experimental groups compared with the normal control group, but lower than in the model group, and there were no statistical differences among the

three dosage groups ($P > 0.05$) suggesting that polydatin significantly decreased the level of serum uric acid, however, the efficacy of polydatin had no obvious relation with the doses within a range of 5-20 mg/kg.

Assay analysis of the mRNA levels

The relative contents of URAT1, OAT1, and OAT3 were calculated according to the value of Ct with beta actin as the reference gene. The data in Figure 2 show that the relative level of URAT1 is increased in the model group compared with the normal control group ($P < 0.01$), and polydatin significantly inhibits the increasing tendency of URAT1 levels. There were statistically significant differences between the high dosage group and the model group ($P < 0.01$), suggesting that the inhibitory action of polydatin on URAT1 was concentration-dependent. The data in Figure 2 also show the relative levels of OAT1 and OAT3 are significantly decreased in the model group compared with the normal control group ($P < 0.01$), and polydatin significantly inhibits the decreasing tendency of OAT1 and OAT3 levels. There were statistical differences between the high, middle dosage group, and the model group ($P < 0.05$), suggesting that the inhibitory action of polydatin on OAT1 and OAT3 was obviously concentration-dependent. Similar results were observed in the benzbromarone group.

Assay detection of proteins

The data of protein detection in Figure 3 show that the protein levels of OAT1 and OAT3 were significantly increased in the hyperuricemic mice, whereas the protein level of URAT1 was decreased remarkably, and there were statistical differences among groups ($P < 0.05$). The data also show that the inhibitory action of polydatin on the protein level of OAT1, URAT1, and OAT3 were concentration-dependent, and there were statistical differences between the high, middle dosage groups, and the model group ($P < 0.05$). Similar results were observed in the benzbromarone group, indicating the high reliability of the experimental system.

Content detection of XOD in liver tissues of mice

According to the results in Figure 4, the levels of XOD in the normal group and model group are 2.41 ± 0.40 U/L and 11.15 ± 2.09 U/L, respectively, and there is a statistic significance between the two groups ($P < 0.05$). The levels of XOD in the high-, middle-, and low-dose groups were 7.89 ± 1.20, 7.89 ± 0.79, and 10.41 ± 0.79 U/L, respectively, of which the level in the high-dosage group was significantly lower than that of the model group ($P < 0.05$). The level of XOD in the positive control group (benzbromarone) was 5.18 ± 1.04 U/L, significantly lower than that of the model group ($P < 0.05$). The results indicated the model had high reliability, and polydatin significantly inhibited the level of XOD in the liver tissues of mice in a concentration-dependent manner.

Pathological detection

The results of HE staining in Figure 5 suggest that there were kidney tissue pathological changes in hyperuricemic mice, and polydatin and benzbromarone showed a protective effect on the pathological injury of the kidney. The Figure also shows in comparison with the normal structure of the kidney, no cloudy swelling and normal size of glomeruli in the normal control group, there were glomerular disturbance, severe hemorrhage, and inflammatory cells infiltration of glomerular periphery in the model group. It also indicates that glomeruli were arranged nearly uniformly with a few of inflammatory cells around them in the low dosage group. In the middle dosage group, the glomeruli were

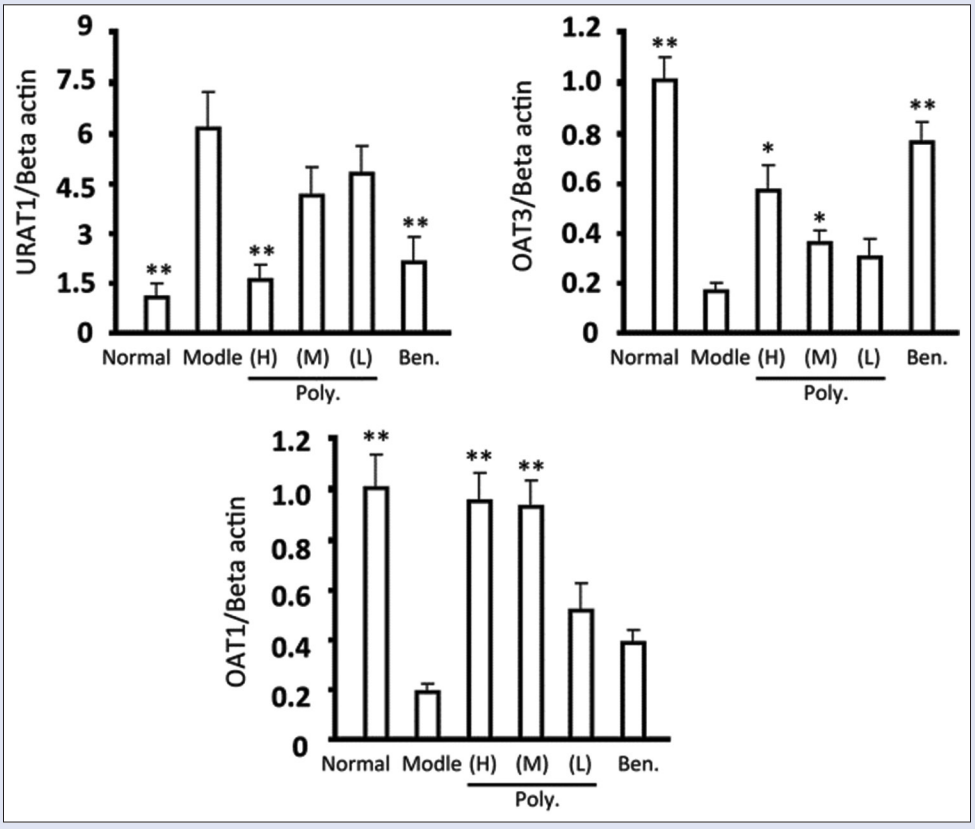

Figure 2: Effects of polydatin on renal URAT1, OAT3 and OAT1 mRNA levels. The mRNA levels in the kidney tissues from the treated mice were detected by quantitative PCR. Results are expressed as mean ± SEM. * $P < 0.05$ and ** $P < 0.01$ vs. the model group

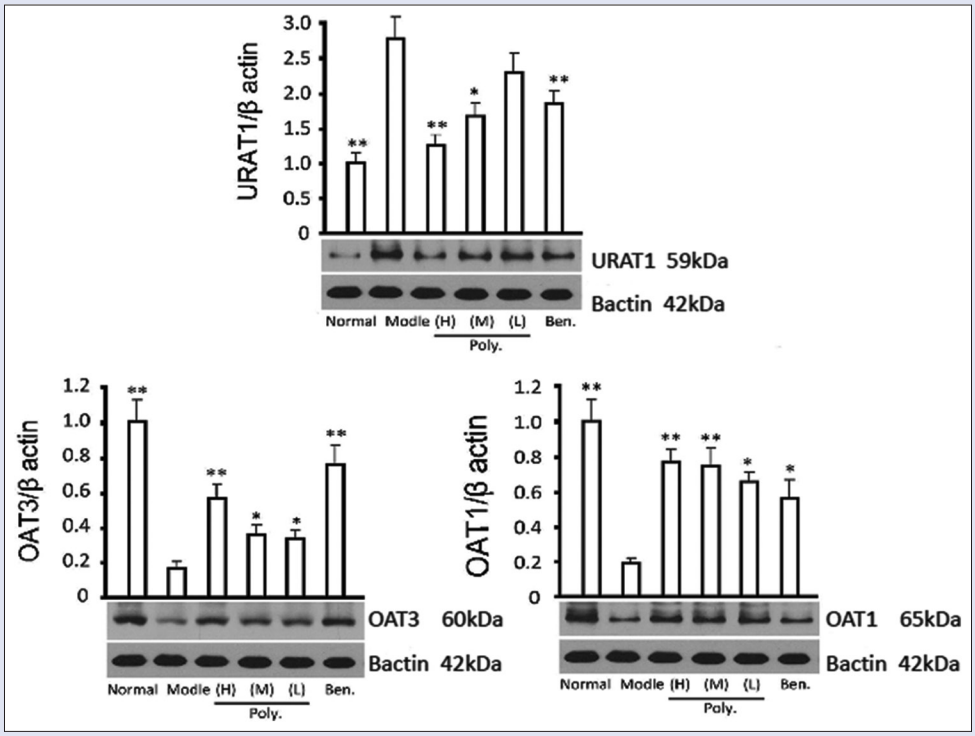

Figure 3: Effects of polydatin on renal URAT1, OAT3 and OAT1 protein levels. The protein levels in the kidney tissues from the treated mice were detected by western blotting. Results are expressed as mean ± SEM. * $P < 0.05$ and ** $P < 0.01$ vs. the model group

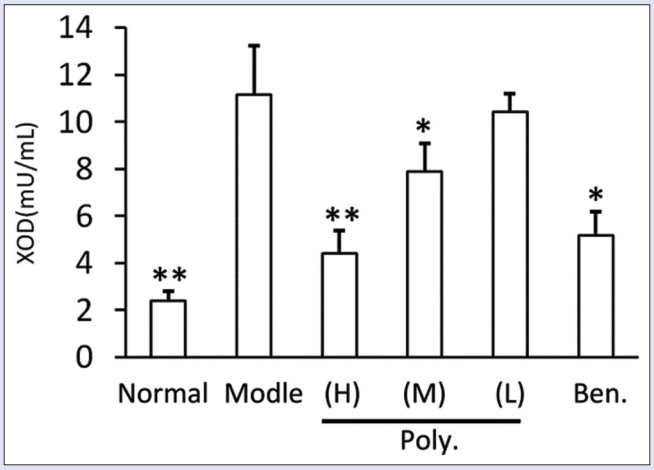

Figure 4: Effects of polydatin on liver XOD activity. The XOD activities in the liver tissues from the treated mice were detected with Xanthine Oxidase Assay Kit. Results are expressed as means ± SD. * $P < 0.05$ and ** $P < 0.01$ vs. the model group

arranged relatively uniformly, without obvious hemorrhage but a few of inflammatory cells around them. The glomeruli were arranged highly uniformly with a relatively complete structure where there was a little hemorrhage in the high dosage group. The Figure also indicated that glomeruli were arranged relatively uniformly with a little hemorrhage in the benzbromarone group.

DISCUSSION

In this study, we examined the antihyperuricemic effect of polydatin in the oxonate-induced hyperuricemic mice. The effect was related to the regulation of renal urate transporter 1 (mURAT1), organic anion transporter 1 (mOAT1) and organic anion transporter 3 (mOAT3) in the hyperuricemic mice. Polydatin was found to downregulate mRNA and protein levels of mURAT1, as well as up-regulate mOAT1 and mOAT3 in the kidney of the hyperuricemic mice. Moreover, the inhibition of XOD in liver tissues of mice as well as protective effects of renal function also contributed to the antihyperuricemic effect of polydatin. These findings suggested that polydatin might inhibit uric acid formation, enhance uric acid excretion and decrease of uric acid reabsorption, thus in turn reduce serum uric acid levels in the hyperuricemic mice.

Uric acid exit from blood is mainly controlled by the kidney.[10] In the kidney, uric acid and urate as waste products are initially filtered and additionally secreted from the blood to the urine. However, most of the filtered urate (90%) is usually reabsorbed and returns to the blood.[1] The kidney plays a dominant role in the determination of the plasma urate level. Therefore, it is important to understand the mechanism of renal urate handling because a decrease in urate excretion has been demonstrated in the majority of hyperuricemic patients.[10] URAT1 transports urate reabsorption in exchange for Cl- or other organic anions from the kidney tubule lumen to blood and maintains systemic circulation urate homeostasis. URAT1 is inhibited completely by the classical uricosuric agent benzbromarone.[19] URAT1 is the sole transporter that mediates urate reabsorption on the apical side of the proximal tubule is established, based on the fact that loss of URAT1 function causes renal hypouricemia.[11] Our study revealed that polydatin reduced serum urate levels by downregulating mURAT1 expression to inhibit urate reabsorption in the kidney of the hyperuricemic mice. The polydatin and benzbromarone groups showed the same kind of effects. The organic anion transporters OAT1 and OAT3 in the basolateral membranes of renal proximal tubules mediate renal urate secretion by operating as organic ion/dicarboxylate exchangers, which are recognized as the first step in renal secretion.[20] Our data suggest that polydatin

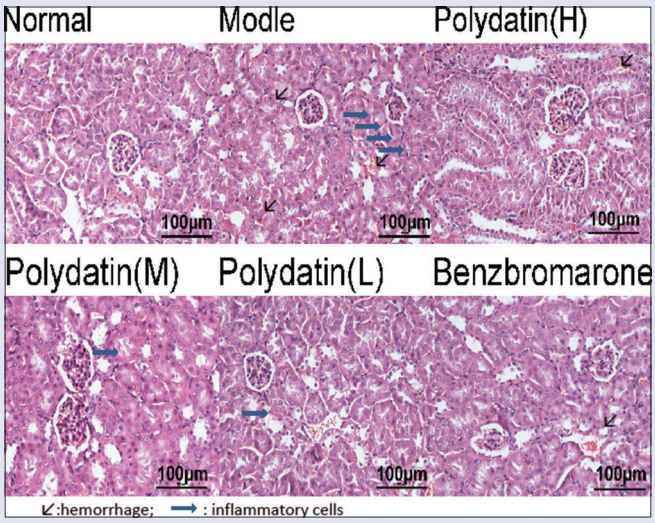

Figure 5: Effects of polydatin on pathological changes in kidney tissues. The kidney tissues from the treated mice were fixed, cut into sections and stained with HE according to the routine procedure and the representative images are shown

significantly inhibited the decreasing tendency of OAT1 and OAT3 levels in the hyperuricemic mice. A study has been shown that down-regulation of renal rOAT1 and rOAT3 was previously observed in the hyperuricemicrats induced by oxonic acid,[21] indicating that OAT1 and OAT3 may play an important role in the pathogenesis of hyperuricemia. Our data suggested that polydatin exerted antihyperuricemic actions by simultaneously up-regulating renal mOAT1 and mOAT3 expression to elevate urate secretion. These results suggested that polydatin exhibited antihyperuricemic effects through the regulation of different renal urate transport-related proteins to enhance renal urate excretion in the hyperuricemic mice. In comparison with the positive control drug, antihyperuricemic efficacies of polydatin seemed to be similar to those of benzbromarone, or even more potent at higher doses in this study.

XOD has been implicated as a key oxidative enzyme that catalyzes purine catabolism. The highest activity of XOD is detected in liver, intestine, and endothelium. XOD catalyzes oxidation of hypoxanthine to xanthine and of xanthine to uric acid, and reactive oxygen species (ROS) are generated as a by-product. XOD plays a pivotal role in regulating the production of uric acid. The results from experimental studies imply that polydatin at the low, middle and high doses significantly inhibited the activity of XOD in liver tissues of mice in a concentration-dependent way. Beside controlling purine catabolic pathway as a key oxidative enzyme, XOD plays a crucial in the oxidative stress. Inflammatory cytokines may up-regulate the synthesis of XOD, and in turn, XOD has a putative role in inflammatory signal transduction. Uric acid also acts as a protective factor for antioxidants and can be responsible for 2/3 of total plasma antioxidant capacity. An alternative hypothesis to explain the association between hyperuricemia and increased vascular risk has been proposed: hyperuricemia is recognized as a surrogate marker for high levels of damaging oxidative stress associated with increased xanthine oxidase activity, rather than being directly responsible for vascular injury and the subsequent increase in risk.[22] Dijana *et al.*[23] have demonstrated that an up-regulated XOD may be implicated in hemodialysis-induced oxidative injury. In this study, we observed that polydatin has a protective effect on the pathological injury of the kidney as benzbromarone in the hyperuricemic mice. A series of studies have recently demonstrated that polydatin has been suggested to have the properties of antioxidative, antiinflammatory and nephroprotective effects.[24] Chen *et al.*[16] have reported that polydatin has inhibitory activities on the xanthine oxidase to repress the level of serum uric acid *in vivo* and *in vitro*. This demonstrated that the nephroprotective activities of polydatin were not only due to the effects on remarkably attenuating the oxidative stress induced by uric acid but also on markedly suppressing the oxidative stress-related inflammatory cascade. Hyperuricemia is one of well-described risk factors for kidney function disorders. Our results showed that there were kidney tissue pathological changes in the hyperuricemic mice, consistent with a previous study that polydatin ameliorates renal injury. Our results should give strong pathophysiological support to the positive correlation between XOD activity and renal damage. In fact, uric acid reflects upregulated XOD activity, and is associated with a pro-inflammatory state in human subjects and particularly with an increase in inflammatory markers.[25] The previous studies showed that hyperuricemia was frequently noted in patients either with cardiovascular disease or at a high risk of cardiovascular diseases such as hypertension, coronary heart disease, peripheral vascular disease, heart failure, metabolic syndrome, and stroke.[26] Findings from this study support the potential use of serum urate as an independent biomarker for poor all-cause and coronary heart disease mortality in patients with recent acute myocardial infarction through increased oxidative stress and inflammation.[27] Therefore, we propose that polydatin has nephron protective effects by inhibiting XOD activity for uric acid reduction and suppressing the inflammatory response.

The current pathological classification of hyperuricemia is based on the understanding of its mechanism by which hyperuricemia results from either overproduction of urate, or under excretion by abnormal renal urate transport activity, or the combination of the two. Our study provides two facts: polydatin not only increases renal urate excretion of uric acid through the regulation of different renal urate transport-related proteins; but also reduces uric acid production by inhibiting the levels of XOD enzymes in the liver. Existing urate-lowering agents in clinical practice usually have single mechanism-uricosuricagents such as benzbromarone and probenecid enhance the excretion of uric acid and xanthine oxidase inhibitors such as allopurinolattenuate the production of urate. Because polydatin has the antihyperuricemic effect with a potentially dual mechanism-combination of the underproduction of urate and increasing renal urate excretion, polydatin is expected to become a more effective and safer uric acid lowering agent.

CONCLUSION

This study has clarified that polydatin has an antihyperuricemic effect in oxonate-induced hyperuricemic mice. The effect was related to the down-regulation of renal URAT1, up-regulated renal OAT1 and OAT3 and inhibition XOD in the hyperuricemic mice. This study supports the notion that polydatin is a choice therapeutic strategy for preventing and treating hyperuricemia. Nonetheless, because of the complexity of hyperuricemia pathology, new related action targets of polydatin in the treatment of hyperuricemia should be studied in detail.

Author contributions statement

S. Wu, G. Wu, MM and H. Wu, MM were principal investigators of this study and had full access to all of the study data and take responsibility for their integrity and the accuracy of the analysis, and were major contributors in writing the manuscript. H. Jiang participated in the study design, collected data, and drafted the manuscript. All authors read and approved the manuscript.

Ethics approval

The experiments were performed in accordance with the guidelines of the Animal Care and Use approved by the Second Military Medical

University Experimental Animal Ethics Committee. The committee's reference number: 20170106025.

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Conflicts of interest

There are no conflicts of interest.

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