

Aquaporin-2 expression in the kidney and urine is elevated in rats with monocrotaline-induced pulmonary heart disease

Shao Ouyang¹, Wei Chen² , Gaofeng Zeng¹ and Changcheng Lei¹

Abstract

Objective: Little is known about how renal aquaporin-2 (AQP2) expression is affected by right heart failure caused by pulmonary heart disease (PHD). Therefore, we examined the expression of AQP2 in a rat model of PHD induced by monocrotaline (MCT).

Methods: After 4 weeks of treatment, urine and blood samples were collected from sham-treated and MCT-treated rats. Plasma arginine vasopressin (AVP) levels were measured by radioimmunoassay, and kidney *Aqp2* mRNA expression was detected by reverse transcription (RT)-PCR. Kidney AQP2 protein expression was quantified by immunohistochemistry and western blotting assays. The concentration of urine AQP2 was determined by indirect enzyme-linked immunosorbent assay.

Results: We successfully established an animal model of MCT-induced PHD in rats. MCT-treated rats had significantly higher mRNA and protein levels of AQP2 in their kidney tissue. Following MCT treatment, rats also had markedly increased concentrations of both urine AQP2 and plasma AVP.

Conclusions: AQP2 expression was significantly increased in the kidney tissues and urine of rats with PHD induced by MCT. Our findings suggest that the evaluation of AQP2 expression contributes to an early diagnosis of PHD, and may also be an important reference to improve PHD therapeutics.

Keywords

Aquaporin-2, kidney, plasma, pulmonary heart disease, monocrotaline, pulmonary hypertension

Date received: 7 May 2019; accepted: 21 November 2019

¹Department of Cardiovascular Medicine, The Second Affiliated Hospital, University of South China, Hengyang, Hunan, China

²Department of Respiratory Medicine, The Second Affiliated Hospital, University of South China, Hengyang, Hunan, China

Corresponding author:

Wei Chen, Department of Respiratory Medicine, The Second Affiliated Hospital, University of South China, Hengyang 421001, Hunan, China.
Email: chenwei454945725@163.com



Introduction

Pulmonary heart disease (PHD) typically refers to changes in the structure and/or function of the right ventricle associated with abnormal respiratory function.^{1–3} PHD is caused by pulmonary hypertension induced by chronic lesions in the lungs, thorax, or pulmonary artery. Pulmonary hypertension can cause right ventricular hypertrophy and right ventricle remodeling, leading to right heart failure and abnormal retention of renal water and sodium.^{2,4}

Chronic PHD, with an average prevalence of 0.47%, is a common disease in China, accounting for 38.5% to 46% of patients hospitalized for heart disease.^{5,6} PHD has a high mortality and poor prognosis, and seriously affects patients' quality of life.^{7,8} Early diagnosis and appropriate treatment play important roles in delaying the progression of PHD, increasing the quality of life and reducing the rate of death.

Aquaporin-2 (AQP2) is a member of the AQP family that plays an important role in regulating and adjusting the balance of water and other solutes in the body.⁹ AQP2 is also a key protein that controls the permeability of the kidney collecting duct to water.^{10–12} AQP2 levels are regulated by the neurohypophysial hormone arginine vasopressin (AVP), whose elevation in serum is associated with the enhanced expression of AQP2 in the renal collecting duct epithelium.^{9,13} Under AVP stimulation, AQP2 in intracellular vesicles can be redistributed to the plasma membrane of renal collecting duct principal cells; excessive AQP2 insertion into the plasma membrane results in diseases such as diabetes insipidus.¹¹ Therefore, AVP and AQP2 are critical in regulating the renal water balance, but there is no report on the changes in renal AQP2 expression induced by right heart failure as a result of PHD.

As a pyrrolizidine alkaloid, monocrotaline (MCT) can lead to a pulmonary

vascular syndrome of rats that is characterized by pulmonary hypertension and PHD.¹² The administration of MCT to rats is an extensively studied animal model of right heart failure and right ventricular hypertrophy, which induces relatively rapid pulmonary hypertension.^{13,14}

In this study, we explored the mechanisms underlying kidney water–sodium retention that cause pulmonary hypertension and PHD in rats treated with MCT by investigating the expression of kidney and urine AQP2 and AVP excretion.

Materials and methods

Animal model of pulmonary hypertension

Male Wistar rats (8 weeks old, body weight 200–350 g) were purchased from the Department of Laboratory Animals of the University of South China. Animals were assigned to two groups (40/group): an MCT-induced pulmonary hypertension group and an age-matched sham-treated control group. The pulmonary hypertension group was induced with a single intraperitoneal (i.p.) injection of MCT (50 mg/kg; 1 mL/100 g body weight). Rats in the control group were injected with an equal volume of phosphate-buffered saline (PBS). MCT was purchased from Sigma-Aldrich (St Louis, MO, USA) and freshly dissolved in 1 M HCl and diluted with PBS (pH 7.0) and 0.1 M NaOH, as previously described.¹⁵ Rats were housed two per cage (one control and one MCT-treated). They were permitted free access to food and water and kept on a 12-hour:12-hour light:dark cycle for 4 weeks after injection. All rats were euthanized by the inhalation of an overdose of CO₂, followed by immediate removal of the heart and kidney, as previously reported.¹⁶ All procedures involving animal experiments were approved by the Department of Laboratory Animals at the University of

South China (Protocol number: SYKK (Xiang) 2015-0001).

Collection and pretreatment of specimens

At 29 days after injection, rats were placed into metabolic cages to allow for adaptation for 24 hours. A volume of 5 to 10 ml of fresh urine was collected and stored at -70°C . Rats were anesthetized by i.p. injection of MCT (500 mg, Sigma-Aldrich) at 45 mg/kg body weight. The lateral jugular vein and the left common carotid artery were connected to one end of a pressure sensor (Shenzhen MINDRAY Bio-Medical Electronics Co., Ltd., Shenzhen, China). A PE50 catheter (inner diameter, 0.58 mm; outer diameter, 0.96 mm) was filled with 0.3% heparin saline and inserted through the right jugular vein into the right heart. The chamber was inserted into the right ventricle, and the pressure transducer was connected with the physiological recorder (PM-9000; Shenzhen MINDRAY Bio-Medical Electronics Co., Ltd.). The depth and angles of the chamber were adjusted according to the changes of pressure waves displayed by the monitors. Parameters including central venous pressure (CVP), right ventricular systolic pressure (RVSP), ascending slope of pulmonary artery pressure, right ventricular systole (RVdp/dt), and mean arterial blood pressure (MAP) were recorded.

Rats were sacrificed after 1 mL blood was collected in tubes with 0.13 mmol/L EDTA. Plasma was collected from the supernatant after the centrifugation of whole blood at $3000 \times g$ for 10 minutes, then stored at -70°C prior to the determination of plasma AVP levels. Serum creatinine (Scr), blood urea nitrogen (BUN), and serum uric acid were detected from serum. After obtaining hemodynamic data, rat heart and lung tissues were removed and washed with cold PBS; filter paper was used to remove residual liquid. A formalin

solution (methanol formaldehyde [37%] in solution) was used to fix tissues for hematoxylin and eosin staining. The left ventricle (LV) and right ventricle (RV) plus the septum (S) were removed, then their weights measured and used to calculate the right ventricular hypertrophy index as follows: $\text{RV}/(\text{LV} + \text{S}) \times 100\%$. Kidneys were removed and rinsed in PBS ($\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, pH 7.4), then the medullae were separated and stored in liquid nitrogen in a cryopreservation tube.

Radioimmunoassay

The concentration of plasma AVP was determined by radioimmunoassay with a radioimmunoassay kit (DSL Biological Products, Webster, TX, USA) according to the manufacturer's instructions. Radiolabeling was detected by an FM-2000 γ immune counter (Xi'an Kaipu Electrical, Xi'an, China).

Enzyme-linked immunosorbent assay

The concentration of AQP2 in rat urine was measured by an enzyme-linked immunosorbent assay (ELISA) with antibodies incubated for 90 minutes.¹⁷ The primary antibody was a rabbit anti-AQP2 polyclonal antibody (2 mg/L; New England BioLabs, Ipswich, MA, USA), while the secondary antibody was a horseradish peroxidase-conjugated goat anti-rabbit IgG antibody (1:1000 dilution; New England BioLabs). Optical density values at a wavelength of 450 nm were acquired by an ELx-800 ELISA Detector microplate reader (Dio-Tek Instruments Inc., Winooski, VT, USA).

Immunohistochemistry

After fixation with 10% formalin, tissues close to the renal medulla were embedded in paraffin using conventional histological methods. Tissues were then sliced into 4- μm sections and placed on glass slides. After dewaxing with xylenes and rehydrating with an alcohol gradient, the sections

underwent antigen retrieval by incubating with citric acid solution and heating in a water bath overnight. The activity of endogenous peroxidase was blocked by incubation overnight with 3% hydrogen peroxide at room temperature. After blocking with nonspecific serum, the sections were incubated with a rabbit anti-rat AQP2 antibody (Calbiochem, San Diego, CA, USA) or goat serum (negative control) in a humidified box at 4°C overnight. After washing with PBS, sections were incubated with a biotin-labeled secondary antibody (1:1000 dilution; Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) at room temperature for 20 minutes. Brownish-yellow puncta in the cytoplasm and cell membranes of the kidney specimens were considered to be positive staining, and were detected with Image-Pro Plus V6.0 image analysis software (Media Cybernetics Inc., Silver Spring, MD, USA). The percentage of positively-stained cells was calculated from the staining patterns of five randomly selected visual fields.

Reverse transcription (RT)-PCR

Total RNA was extracted using the TRIzol RNA extraction kit (Takara Biotech Co. Ltd., Dalian, China) according to the manufacturer's instructions. The RNA quality was verified by measuring the A260/A280 ratio, then 2 µg of total RNA per sample was used to synthesize cDNA using M-MuLV reverse transcriptase (MBI Fermentas, Burlington, Canada). cDNA samples (2 µL each of 1200 ng/mL) were amplified with Taq polymerase (Bio-Asia Diagnostics Co., Ltd., Shanghai, China) using the following primers: 18S rRNA (internal control gene), forward: 5'-CGACGGACCCATTCGAACGTCT-3' and reverse: 5'-GCTATTGGAGCTGGAATTACCG-3'; and *Aqp2* (target gene), forward: 5'-CATGTCTCCTTCCTTCGAGC-3' and reverse: 5'-TTGTGGAGAGCATTGACAGC-3'. PCR amplification conditions

were as follows: initial incubation at 95°C for 5 minutes, followed by 30 cycles of 94°C for 30 seconds, 60°C for 30 seconds, and 72°C for 60 seconds, then 72°C for 7 minutes. PCR products (131 bp for *Aqp2*, 312 bp for 18S rRNA) were separated on 2% agarose gels stained with ethidium bromide.

Western blotting

Protein samples were obtained from the homogenates of kidney tissues processed with a Polytron high-speed homogenizer. These samples were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes (Millipore, Billerica, MA, USA). After blocking with 5% skimmed milk at 37°C for 2 hours, the membrane was incubated with a rabbit anti-rat/mouse AQP2 primary antibody (Calbiochem, San Diego, CA, USA) or a rabbit anti-rat/mouse β -actin antibody (Calbiochem; used as an internal control) at 4°C overnight. Then, the membrane was washed with Tris-buffered saline supplemented with Tween-20 (TBS-T) and further incubated with a biotin-labeled goat anti-rabbit secondary antibody (1:1000 dilution; Santa Cruz Biotechnology Inc.) at 37°C for 40 minutes. Finally, the membrane was washed again with TBS-T, then proteins of interest were visualized using an enhanced chemiluminescence kit (EMD Millipore). Band intensities were quantified by densitometry with ImageJ software (version 1.49; National Institutes of Health, Bethesda, MA, USA).

Statistical analysis

SPSS statistical software (version 17.0; IBM, Armonk, NY, USA) was used for statistical analyses. Quantitative data are expressed as the mean \pm standard deviation. Differences between two groups were analyzed and compared by the independent-sample *t*-test. A *P*

value less than 0.05 was considered to be statistically significant.

Results

Successful establishment of an animal model of MCT-induced PHD in rats

At the end of the experiment, all rats were alive in both the control and the MCT groups. However, rats treated with MCT (218 ± 17 g) were significantly lower in weight than rats in the control group (301 ± 13 g). All rats in the MCT group presented with clinical signs of overt heart failure, characterized by lethargy, labored breathing, pleural effusion, vein and liver engorgement, ascites, and cachexia. MCT-treated rats showed a significant increase in CVP, RVSP, RVdp/dt, RV/LV+S, Scr, and BUN, and a significant decrease in MAP compared with sham rats (all $P < 0.01$); there was no significant difference in serum uric acid levels between the two groups (Table 1). Additionally, myocardial fibers in MCT-treated rats were disordered, with some that were bifurcated with large, dense nuclei, and evidence of vacuolar degeneration and myocardial fibrosis (Figure 1). This indicated that we

had successfully established a rat model of MCT-induced PHD.

AQP2 expression was elevated in the kidney tissues and urine of MCT-treated rats

To determine whether renal function in MCT-treated rats was associated with changes in AQP2 expression, we next used RT-PCR and western blotting to evaluate the levels of *Aqp2* mRNA and AQP2 protein in the two groups. In contrast to the control group (0.75 ± 0.08), the semiquantitative RT-PCR results revealed significantly higher *Aqp2* expression in the kidneys of MCT-treated rats (1.18 ± 0.04 , $t = 10.165$, $P < 0.01$, Figure 2a). AQP2 protein expression was normalized against β -actin by quantifying the intensities of the bands obtained by western blotting. As shown in Figure 2b, the relative expression of AQP2 in the kidneys of rats treated with MCT (0.91 ± 0.06) was significantly higher than in control rats (0.57 ± 0.02 , $t = 10.452$, $P < 0.01$). Given the increased levels of *Aqp2* mRNA and protein, we then quantified AQP2 expression by immunohistochemistry. As anticipated, most AQP2 expression was distributed in the cells

Table 1. Comparison of hemodynamic index in MCT-treated and control rats (n = 40 per group).

Treatment group	Control	MCT	P-value
Body weight (g)	301 ± 13	218 ± 17	0.004
CVP (mmHg)	4.46 ± 0.59	12.77 ± 2.01	0.007
RVSP (mmHg)	4.94 ± 0.13	14.12 ± 1.98	0.006
RVdp/dt	972.33 ± 132.41	1420.51 ± 108.21	0.004
MAP	132.11 ± 7.65	79.54 ± 9.31	0.005
RV/LV+S	0.26 ± 0.02	0.78 ± 0.13	0.003
Scr ($\mu\text{mol/L}$)	42.67 ± 2.04	56.13 ± 4.11	0.008
BUN (mmol/L)	5.61 ± 0.21	7.56 ± 0.67	0.007
SUA ($\mu\text{mol/L}$)	86.98 ± 8.45	92.12 ± 11.91	0.008

MCT, monocrotaline; CVP, central venous pressure; RVSP, right ventricular systolic pressure; RVdp/dt, ascending slope of pulmonary artery pressure and right ventricular systole; MAP, mean arterial blood; Scr, serum creatinine, BUN, urea nitrogen; SUA, serum uric acid.

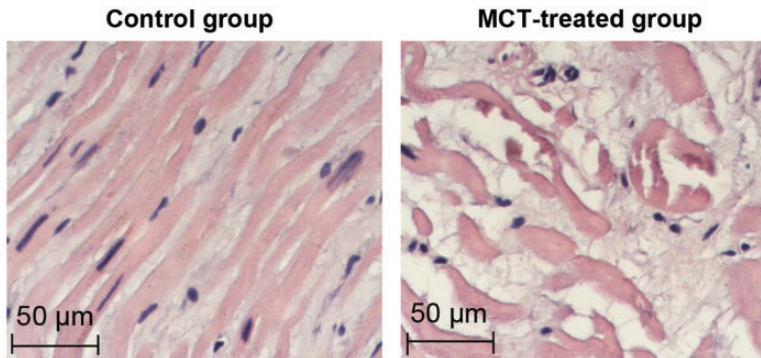


Figure 1. Myocardial pathologic phenotype in the right ventricle tissues of control rats and MCT-treated rats.

Representative images of right ventricle tissues subjected to hematoxylin and eosin staining. Myocardial fibers in MCT-treated rats are disordered, with some bifurcated. The nuclei appear large and dense, and vacuolar degeneration and myocardial fibrosis are evident. Scale bar, 50 μm .

around the collecting ducts of renal tissue near the medullae of the kidney in both groups. However, AQP2 staining was clearly stronger in the kidneys of MCT-treated rats than control rats (Figure 2c). Additionally, the positively-stained area (0.80 ± 0.08) of the kidney sections from MCT-treated rats was significantly wider than that of the control group (0.50 ± 0.05 , $t=10.157$, $P<0.01$; Figure 2c). Compared with the control group, rats in the MCT-treated group also had significantly higher concentrations of plasma AVP and urine AQP2 ($P<0.01$, Table 2).

Discussion

Recent research suggests that PHD caused by right heart failure and sodium water retention has a disability rate and mortality rate no less than those caused by left heart failure. However, because of the lack of evidence and related guidelines, the study of right heart failure has not yet attracted much attention in academic settings. Moreover, although the roles of AQP2 in regulating water permeability in renal disorders and congestive heart failure have been well elucidated, little is known about

how renal AQP2 expression is affected by right heart failure caused by PHD.

In this study, we successfully established a rat model of MCT-induced PHD and compared the expression levels of AQP2 in the kidney and urine from untreated rats with those of MCT-treated rats. Four weeks after MCT exposure, rats had significantly enhanced expression levels of AQP2 in the kidney, and elevated urinary secretion of soluble AQP2 and AVP. Our study therefore highlights the potential of AQP2 as a biomarker for the diagnosis of PHD and for the prediction of drug response in patients with PHD.

MCT pyrrole is mainly retained in the pulmonary arteries, and its immediate effect on arteries in other organs, such as the kidney, is considered minimal.^{18,19} In our model, MCT-treated rats presented with clinical signs of overt heart failure, while RV remodeling is characterized by myocardial hypertrophy, which usually indicates an increased right ventricular weight and right heart failure²⁰ (Table 1). Multiple studies have employed this model to investigate potential therapeutics. For example, Langleben et al.²¹ examined the effects of methylprednisolone on MCT-

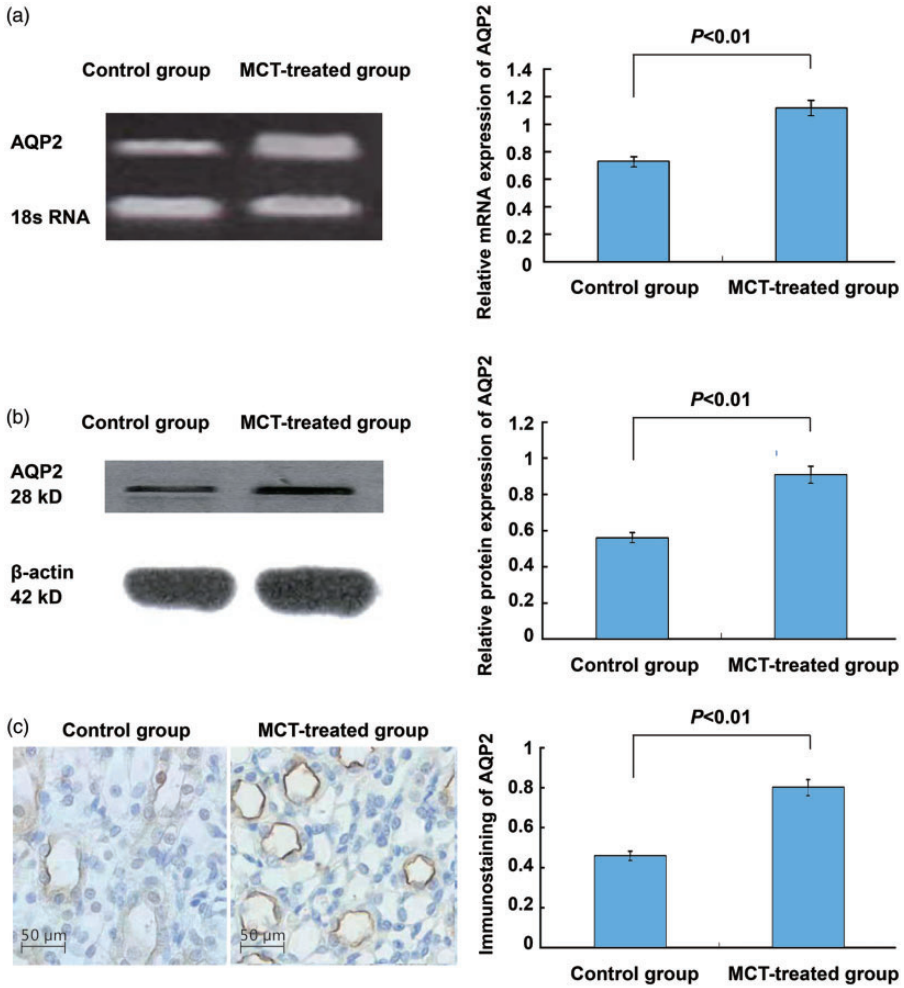


Figure 2. Renal tissues from MCT-treated rats have higher levels of aquaporin-2 expression than those from control rats. (a) Representative gel electrophoresis images showing relative mRNA expression of *Aqp2* and 18S RNA from kidney tissues near the renal medulla. The right panel is the summarized bar graph showing normalized values of *Aqp2* mRNA levels in the control group and MCT-treated group (n=20 per group). (b) Representative images of western blotting bands for AQP2 and β -actin protein in rat kidney tissues. The right panel is the summarized bar graph showing normalized values of AQP2 protein levels in the control group and MCT-treated group (n=20 per group). β -actin was used as a loading control. (c) Representative images of AQP2 by immunohistochemical staining. The right panel is the summarized bar graph showing relative sizes of areas with positive AQP2 staining in the control group and MCT-treated group (n = 5 fields per group). Scale bar, 50 μ m; magnification, $\times 400$.

induced pulmonary hypertension and PHD in rats. These studies suggest that our PHD model will be useful to investigate whether urine AQP2 can be considered a biomarker

for the diagnosis and prediction of therapeutic effects.

AQP2 is an important regulator of the renal collecting duct along with AVP,

Table 2. Comparison of plasma AVP and urine AQP2 in MCT-treated and control rats (n=40 per group).

Treatment group	Control	MCT	P-value
AVP (ng/L)	52.78 ± 11.59	77.66 ± 9.781	0.008
AQP2 (ng/mL)	8.95 ± 2.91	17.54 ± 3.80	0.007

MCT, monocrotaline; AQP2, Aquaporin-2; AVP, arginine vasopressin.

which is associated with heart failure and hypertension. To alter the water permeability of collecting duct main cells, we determined whether the MCT-induced PHD model affected the expression of AQP2. *Aqp2* mRNA and protein levels in kidney tissues surrounding the renal medulla were increased in MCT rats relative to controls, while AQP2 urine concentrations clearly increased after MCT treatment. This indicated that the PHD model resulted in the upregulated expression of AQP2 in renal tissues and in increased urine excretion of AQP2.

Possible reasons for this is that rats with MCT-induced PHD developed the disease from pulmonary hypertension followed by right ventricular remodeling and right heart failure, which eventually led to sodium retention and decreased cardiac output, including insufficient perfusion of the kidney tissue. This progression would increase renin-angiotensin-aldosterone system (RAAS) activity and angiotensin II production, causing the posterior pituitary lobe to release AVP which stimulates the hypothalamic supraoptic nucleus; finally, the synthesis and secretion of AVP would increase in the paraventricular nucleus and be stored in the neurohypophysis.

AVP would then be secreted into the blood where it affects kidney function, including exocytosis-induced activation of the V2 receptor on the peritubular membrane of the synaptic main cell. Additionally, the distribution density of AQP2 on the lumen membrane would increase, and the water channels open to increase permeability, which is a short-term adjustment of the “shuttle mechanism”. AVP levels in the serum would continue to rise for hours,

resulting in intracellular *AQP2* gene activation in the kidney and increased protein expression in the collecting duct. The absolute amount of AQP2 would therefore increase, enhancing its water transport capacity. This phenomenon is a long-term adjustment, resulting in increased urinary output, cardiac filling pressure, and blood flow.

Mechanical disorders can activate the sympathetic nervous system and the RAAS system. These may further upregulate the expression of AQP2 in kidney tissue, thus increasing the retention of water and sodium.²² Consistent with our findings, Xu et al.²³ also observed that both AQP2 protein and mRNA were significantly increased in the kidneys of rats with chronic heart failure induced by a left coronary artery ligation. Taken together, these studies emphasize the potential of urine AQP2 and plasma AVP as biomarkers to evaluate therapeutic efficacy in the treatment of PHD.²⁴

In conclusion, AQP2 expression was significantly increased in the kidney tissues and urine of rats with PHD induced by MCT. Our investigation suggests that AQP2 can objectively reflect the progression of the development of PHD. The evaluation of AQP2 expression could contribute to the early diagnosis of PHD, and may also be an important reference to improve therapeutics.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

Ethics


The study protocol was approved by the Ethics Committees of the Second Affiliated Hospital,

University of South China, Hengyang. All procedures involved in animal experiments were approved by the Department of Laboratory Animals in the University of South China (Protocol number: SYKK (Xiang) 2015-0001).

Funding

This work was supported in part by the Scientific Research Project of the Health and Family Planning Commission of the Hunan province of China (B20180057), the Science and Health Joint Project of the Hunan Provincial Natural Science Foundation of China (2018JJ6069), and the Youth Academic Leadership Project of the Second Affiliated Hospital of the University of South China.

ORCID iD

Wei Chen  <https://orcid.org/0000-0002-7300-9554>

References

- Forfia PR, Vaidya A and Wieggers SE. Pulmonary heart disease: the heart-lung interaction and its impact on patient phenotypes. *Pulm Circ* 2013; 3: 5–19.
- Zangiabadi A, De Pasquale CG and Sajkov D. Pulmonary hypertension and right heart dysfunction in chronic lung disease. *Biomed Res Int* 2014; 2014: 739674.
- Roth TS and Aboulhosn JA. Pulmonary hypertension and congenital heart disease. *Cardiol Clin* 2016; 34: 391–400.
- Calcaianu G, Canuet M, Schuller A, et al. Pulmonary arterial hypertension-specific drug therapy in COPD patients with severe pulmonary hypertension and mild-to-moderate airflow limitation. *Respiration* 2016; 91: 9–17.
- Chen WW, Gao RL, Liu LS, et al. China cardiovascular diseases report 2015: a summary. *J Geriatr Cardiol* 2017; 14: 1–10.
- Wang R, Yan Z, Liang Y, et al. Prevalence and patterns of chronic disease pairs and multimorbidity among older Chinese adults living in a rural area. *PLoS One* 2015; 10: e0138521.
- Krishnan U and Rosenzweig EB. Pulmonary arterial hypertension associated with congenital heart disease. *Clin Chest Med* 2013; 34: 707–717.
- Li JS, Wang HF, Li SY, et al. Shenmai injection for chronic pulmonary heart disease: a systematic review and meta-analysis. *J Altern Complement Med* 2011; 17: 579–587.
- Knepper MA and Inoue T. Regulation of aquaporin-2 water channel trafficking by vasopressin. *Curr Opin Cell Biol* 1997; 9: 560–564.
- Kwon TH, Frokiaer J and Nielsen S. Regulation of aquaporin-2 in the kidney: a molecular mechanism of body-water homeostasis. *Kidney Res Clin Pract* 2013; 32: 96–102.
- Vukicevic T, Schulz M, Faust D, et al. The trafficking of the water channel aquaporin-2 in renal principal cells—a potential target for pharmacological intervention in cardiovascular diseases. *Front Pharmacol* 2016; 7: 23.
- Wilson DW, Segall HJ, Pan LC, et al. Mechanisms and pathology of monocrotaline pulmonary toxicity. *Crit Rev Toxicol* 1992; 22: 307–325.
- Rosenberg HC and Rabinovitch M. Endothelial injury and vascular reactivity in monocrotaline pulmonary hypertension. *Am J Physiol* 1988; 255: H1484–H1491.
- Schultze AE and Roth RA. Chronic pulmonary hypertension—the monocrotaline model and involvement of the hemostatic system. *J Toxicol Environ Health B Crit Rev* 1998; 1: 271–346.
- Umar S, de Visser YP, Steendijk P, et al. Allogenic stem cell therapy improves right ventricular function by improving lung pathology in rats with pulmonary hypertension. *Am J Physiol Heart Circ Physiol* 2009; 297: H1606–H1616.
- Redout EM, van der Toorn A, Zuidwijk MJ, et al. Antioxidant treatment attenuates pulmonary arterial hypertension-induced heart failure. *Am J Physiol Heart Circ Physiol* 2010; 298: H1038–H1047.
- Zhao W, Xu AG, Wu J, et al. Effects of imidapril treatment on aquaporin-2 expression in the kidneys and excretion in the urine

- of hypertensive rats. *Exp Ther Med* 2013; 5: 1327–1331.
18. van Suylen RJ, Smits JF and Daemen MJ. Pulmonary artery remodeling differs in hypoxia- and monocrotaline-induced pulmonary hypertension. *Am J Respir Crit Care Med* 1998; 157: 1423–1428.
 19. Shimoda LA and Laurie SS. Vascular remodeling in pulmonary hypertension. *J Mol Med (Berl)* 2013; 91: 297–309.
 20. Langleben D and Reid LM. Effect of methylprednisolone on monocrotaline-induced pulmonary vascular disease and right ventricular hypertrophy. *Lab Invest* 1985; 52: 298–303.
 21. Wu F, Hao Y, Yang J, et al. Protective effects of alopentine on monocrotaline-induced pulmonary hypertension in rats. *Biomed Pharmacother* 2017; 89: 632–641.
 22. Sakamoto Y, Kameshima S, Kakuda C, et al. Visceral adipose tissue-derived serine protease inhibitor prevents the development of monocrotaline-induced pulmonary arterial hypertension in rats. *Pflugers Arch* 2017; 469: 1425–1432.
 23. Nielsen S, Kwon TH, Christensen BM, et al. Physiology and pathophysiology of renal aquaporins. *J Am Soc Nephrol* 1999; 10: 647–663.
 24. Imamura T and Kinugawa K. Urine aquaporin-2: a promising marker of response to the arginine vasopressin type-2 antagonist, tolvaptan in patients with congestive heart failure. *Int J Mol Sci* 2016; 17: pii: E10.