Inhibition of Mitochondrial Complex-1 Restores the Downregulation of Aquaporins in Obstructive Nephropathy

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Running title: Rotenone and AQPs in obstructive nephropathy

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Abstract

Obstructive kidney disease is a common complication in the clinic. Downregulation of aquaporins (AQPs) in obstructed kidneys has been thought as a key factor leading to the polyuria and impairment of urine-concentrating capability after the release of kidney obstruction. The present study was to investigate the role of mitochondrial complex-1 in modulating AQPs in obstructive nephropathy. Following 7-day unilateral ureteral obstruction (UUO), AQP1, AQP2, AQP3, and V2 receptor were remarkably reduced as determined by qRT-PCR and/or Western blotting. Notably, inhibition of mitochondrial complex-1 by rotenone markedly reversed the downregulation of AQP1, AQP2, AQP3, and V2. In contrast, AQP4 was not affected by kidney obstruction or rotenone treatment. In a separate study, rotenone also attenuated AQPs’ downregulation after 48h UUO. To study the potential mechanisms in mediating the rotenone effects on AQPs, we examined the regulation of COX-2/mPGES-1/PGE2/EP pathway and found that COX-2, mPGES-1, and renal PGE2 content were all significantly elevated in obstructive kidneys, which was not affected by rotenone treatment. For EP receptors, EP2 and EP4 but not EP1 and EP3 were upregulated in obstructive kidneys. Importantly, rotenone strikingly suppressed EP1 and EP4 but not EP2 and EP3 receptors. However, treatment of EP1 antagonist SC-51322 could not affect AQPs’ reduction in obstructed kidneys. Collectively, these findings suggested an important role of mitochondrial dysfunction in modulating AQPs and V2 receptor in obstructive nephropathy possibly via prostaglandin-independent mechanisms.

Key words: obstructive nephropathy, mitochondrial dysfunction, water channels, COX-2, PGE2
Introduction

Obstructive nephropathy is a common clinical complication presenting with enhanced renal fibrotic and inflammatory responses, progressive loss of renal function, and impaired urine-concentrating capability following the release of renal obstruction(8, 9, 22, 24). One of the recognized reasons leading to the impairment of renal concentration capability in obstructive nephropathy is the global reduction of AQPs including AQP1, AQP2, and AQP3 in the kidney(11, 18, 20, 22, 37). Generally, the downregulation of AQPs were found in both obstructed kidneys and obstruction-released kidneys (11). The impaired urine-concentrating capability could result in severe imbalance of water and electrolytes metabolism. The pathogenic mechanisms leading to the global reduction of renal AQPs and the polyuria has thought to be related to the stimulation of COX-2-derived prostaglandins (PGs), particularly PGE2, a known modulator of renal water metabolism(26, 28). Inhibition of COX-2 attenuated AQPs reduction in obstructed kidneys and improved the polyuria following the obstruction release (4, 25). In addition, Ang II was also reported to be attributable to the downregulation of AQPs possibly via the stimulation of COX-2 in obstructive kidney disease(10, 17, 18). However, more intensive study is necessary for further revealing the pathogenesis of AQPs dysregulation in this pathological process.

Recently, several reports from our and other groups demonstrated that mitochondrial dysfunction contributes to the pathogenesis of CKDs including obstructive kidney disease and 5/6nephrectomy animal model(3, 31). Mitochondria play critical roles in regulating energy production, signaling transduction, cell proliferation and cell cycle(5, 6). Impaired mitochondria elevated oxidative stress,
reduced ATP production, and initiated apoptotic process via the release of mitochondrial DNA, cytochrome C et al (33, 34). By now, the pathogenic role of mitochondrial injury in mediating the pathological response in chronic and acute kidney diseases has been widely recognized (12, 29, 31, 32, 36, 39). However, whether mitochondrial dysfunction play a role in regulating AQP5s in a CKD model of UUO is still unknown.

In the present study, employing a pharmacological strategy, we defined: 1) effect of mitochondrial complex-1 inhibition on the downregulation of AQP5s in obstructive kidney disease; 2) effect of mitochondrial complex-1 inhibition on COX-2/mPGES-1/PGE2/EP signaling pathway.

Methods

Animals

The C57BL/6J mice were originally purchased from Jackson lab, which was propagated at the Nanjing Medical University. In our studies, 3- to 4-month-old male mice were used. All mice were maintained under a 12:12 hour light-dark cycle. This study was approved by the Nanjing Medical University Institutional Animal Care and Use Committee.

UUO mouse model and rotenone treatment

Unilateral ureter obstruction was established as described previously (30, 31). Sham-operated animals served as the controls. Rotenone was incorporated into a chow-based
diet (LabDiet Rodent Chow 5001; Purina) at a level of 500 ppm. The gelled diet was made by melting agar (1.5% by weight) in water (60%), cooling, and adding the drug, ground chow (38.5%). Animals were free access to the jelled diet with or without rotenone and bottle water. In a separate study, a specific EP1 antagonist SC-51322 was administered to the UUO mice at a dose of 10mg/kg by osmotic minipumps for 48h. After two or seven days of UUO with rotenone or SC-51322 treatment, mice were anesthetized with an intraperitoneal injection of a ketamine/xylazine/atropine mixture, and the kidneys and blood were collected for the analysis.

**Immunoblotting**

Kidney tissues were lysed using the protein lysis buffer containing 50 mM Tris, 150 mM NaCl, 10 mM EDTA, 1% Triton X-100, 200 mM sodium fluoride, and 4 mM sodium orthovanadate, as a protease inhibitor (pH 7.5) and protein concentration was determined by Coomassie reagent. Following adding SDS-PAGE buffer, the lysates were denatured in boiling water for 10mins, separated by SDS-polyacrylamide gel electrophoresis, and transferred onto nitrocellulose membranes. The blots were blocked for an hour with 5% milk in Tris-buffered saline (TBS), followed by incubation for 1h with rabbit anti-AQP1 (Cat #: SPC-502D, StressMarq Biosciences), anti-AQP2 (Cat #: SPC-503D, StressMarq Biosciences), anti-AQP3 (Cat #: SPC-504D, StressMarq Biosciences), anti-AQP4 (Cat #: SPC-505D, StressMarq Biosciences) or anti-EP1 (Cat #: 101740, Cayman Chemical) at a dilution of 1:1000. After being washed with TBST, blots were incubated with a goat anti-rabbit IgG peroxidase-conjugated secondary antibody (1:1000 dilution) and visualized with ECL kits (Amersham, Piscataway, NJ USA).
Immunohistochemistry

Kidney samples were fixed with 3% paraformaldehyde and embedded in paraffin. Kidney sections were incubated in 3% H₂O₂ for 10 minutes at room temperature to block endogenous peroxidase activity. Then the slides were boiled in antigen retrieval solution (1 mmol/L Tris-HCl, 0.1 mmol/L EDTA, pH=8.0) for 10 minutes at high power in a microwave oven. The sections were incubated overnight at 4°C with primary antibodies at appropriate dilutions (anti-AQP2, Cat #: SPC-503D, StressMarq Biosciences). After washing with PBS, the secondary antibody was applied and the signals were visualized using an ABC kit (Santa Cruz Biotechnology).

qRT-PCR

Total RNA isolation and reverse transcription were finished as previously described(39). Oligonucleotides were designed using Primer3 software (http://frodo.wi.mit.edu/primer3/) and the sequences are shown in the Table 1. qPCR amplification was performed using the SYBR Green Master Mix (Applied Biosystems, Warrington, UK) and the Prism 7500 Real-Time PCR Detection System (Applied Biosystems, Foster City, CA, USA). Cycling conditions were 95°C for 10min, followed by 40 repeats of 95°C for 15s, and 60°C for 1 min.

Enzyme Immunoassay

The kidney tissue was homogenized in phosphate-buffered saline and then centrifuged for 5 min at 10,000 r.p.m. The supernatant was diluted 1:50 with enzyme immunoassay
buffer. Kidney PGE2 content was determined by enzyme immunoassay according to manufacturer’s instructions (Cayman Chemicals).

**Statistical Analysis**

All values are presented as mean ± SE. Statistical analysis was performed using one-way ANOVA followed by Bonferroni tests or unpaired student’s t test with SPSS 13 statistical software. Differences were considered to be significant when $P<0.05$. 
Results

Kidney obstruction led to Mitochondrial Abnormality.

Following 7 days UUO, we evaluated the mitochondrial status via the examination of mitochondrial DNA copy number (mtDNA), mitochondrial transcription factor (mTFAM), and mitochondria-encoded NADH dehydrogenase 1 (mtNd1) using qRT-PCR. As expected, 7-day ureteral obstruction resulted in striking reduction of mtDNA (Fig. 1A), mTFAM (Fig. 1B), and mtNd1 (Fig. 1C), indicating that mitochondrial abnormality occurs in obstructed kidneys.

Effects of rotenone on AQPs expression in obstructed kidneys.

Following 7-day UUO, the expression of AQP1 was dramatically decreased in the obstructed kidneys as compared to sham controls (Fig. 2A & B). Strikingly, administration of rotenone, a well-established mitochondrial complex-1 inhibitor completely reversed the reduction of AQP1 in obstructed kidneys (Fig. 2A & B), which indicated a mitochondrial role in regulating AQP1 expression in obstructive nephropathy.

AQP2 is mainly expressed on the apical membranes and intracellular vehicles of the renal collecting duct principle cells, and is sensitive to vasopressin (7, 21). As shown by Western blotting data, rotenone prevented the reduction of AQP2 expression (Fig. 3A-C) in the obstructed kidneys, which was further confirmed by immunohistochemistry (Fig. 4). Similarly, vasopressin receptor 2 (V2) also showed a significant reduction in obstructed kidneys and was completely reversed by rotenone administration (Fig. 5). This data suggested that V2 receptor may play a role in mediating AQP2 regulation in this experimental setting at least to some extent.
AQP3 is located in the basolateral cell membrane of principal collecting duct cells and is not regulated by vasopressin. As shown in Fig. 6, UUO led to a robust downregulation of AQP3, and rotenone treatment partially but significantly attenuated AQP3 reduction (Fig. 6), suggesting that other mechanisms besides mitochondria might be also attributable to AQP3 downregulation in obstructive nephropathy.

AQP4 is expressed in the inner medullary collecting ducts and also localized to basolateral membranes in the S3 segment of proximal tubules. In the present model, we didn’t find significant changes of AQP4 in obstructed kidneys with or without rotenone treatment (Fig. 7).

**Effects of rotenone on AQPs in kidneys after 48h obstruction.**

Then we further examined rotenone effects on AQPs’ regulation in kidneys after 48h obstruction. As shown by data in Fig. 8A-C, 48h ureteral obstruction caused significant reduction of AQP1, AQP2, and AQP3, which was markedly attenuated by rotenone treatment as determined by qRT-PCR. By immunohistochemistry, we further confirmed AQP2 regulation at protein level (Fig. 9). These date indicated that rotenone could restore AQPs’ reduction at an early stage of obstructive nephropathy.

**Effects of rotenone on AQPs in non-obstructed kidneys.**

To evaluate the effects of rotenone on the expression of AQPs in non-obstructed kidneys, mice were subjected to the jelly diet with or without rotenone for 7days. Then we checked the expressions of AQP1, AQP2, and AQP3 by qRT-PCR. As shown by the data, rotenone did not affect AQPs’ mRNA expressions (Fig. 10A-C). By Western blotting, we further confirmed the expression of AQP2 at protein level (Fig. 10D-F).
Rotenone had no effect on regulation of COX/PGES/PGE2 cascade in obstructed kidneys.

In order to investigate the potential mechanism in mediating rotenone’s effects on AQPs regulation in obstructed kidneys, we examined COX2/mPGES-1/PGE2 pathway. It has been reported that COX-2 inhibitors prevented downregulation of AQPs in obstructed kidney(25), which suggested that COX-2-derived prostaglandins, particularly PGE2 may play a role in modulating AQPs under this disease condition. Therefore, we speculate that rotenone may affect the AQPs regulation in obstructive nephropathy though COX/PGE2 pathway. By qRT-PCR, we globally examined PGE2 production-related enzymes including COX-1, COX-2, mPGES-1, mPGES-2, cPGES, and 15-PGDH (15-hydroxyprostaglandin dehydrogenase, a key enzyme for the degradation of prostaglandins). Interestingly, only COX-2 and mPGES-1, but not other enzymes were upregulated in obstructed kidneys (Fig. 11A-F). However, rotenone had no effect on the regulation of all these enzymes (Fig. 11A-F). We further confirmed protein expression of COX-2 via Western blotting and found a similar pattern as its mRNA level (Fig. 12A&B). Moreover, the induction of kidney PGE2 content was also not affected by rotenone (Fig. 12C). These data highly indicated that rotenone treatment played no role in regulating COX/PGES/PGE2 cascade in this experimental setting.

Effects of rotenone on PGE2 receptors in obstructed kidneys.

PGE2 functions through four receptors including EP1, EP2, EP3, and EP4. Although COX-/PGES/PGE2 cascade was not altered by rotenone, we can’t rule out a possible
role of rotenone in regulating PGE2 receptors which finally decide PGE2 action.

Interestingly, EP2 and EP4 but not EP1 and EP3 were upregulated in obstructive kidneys as determined by qRT-PCR (Fig 13A-D). Importantly, rotenone strikingly suppressed EP1 and EP4 but not EP2 and EP3 receptors (Fig. 13A-D). EP1 regulation was further confirmed by Western blotting (Fig. 14A&B). These results suggested that suppression of EP1 and/or EP4 receptors possibly played a role in protecting against AQPs downregulation in rotenone-treated obstructed kidneys.

Antagonism of EP1 receptor did not affect AQPs’ reduction in obstructed kidneys.

In consideration of the diuretic role of EP1, we treated the UUO mice with EP1 antagonist SC-51322. Unexpectedly, we did not find its effect on the downregulated AQPs in UUO kidneys as determined by qRT-PCR (Fig. 15A-C). The data indicated that rotenone may affect AQPs’ expression in obstructed kidneys via a EP1-independent mechanism.

Discussion

As a common clinical complication, obstructive nephropathy leads to a remarkable downregulation of AQPs (11, 18, 22, 37). The release of kidney obstruction is followed by polyuria, which could lead to severe disorders of fluid metabolism. In the past years, studies related to the pathogenesis of obstruction-induced downregulation of renal AQPs have been performed. Nørregaard R et al. found that activation of COX-2/prostaglandin E2 signaling might be a mechanism leading to the dysregulation of renal AQPs(26, 27).
Several studies reported that mitochondrial abnormality is an obvious phenomenon in obstructed kidneys(12, 31, 36, 39). In this study, we also confirmed this phenomenon as shown by the significant reduction of mtDNA copy number, mTFAM, and mtNd1. Mitochondrial injury reduces ATP depletion, increases mitochondria-derived reactive oxygen species (ROS) production, and induces the release of proapoptotic factors (cytochrome C, mitochondrial DNA, etc.), which is highly involved in pathogenesis of organ injuries(5, 6, 23). Recently, our group reported a mitochondrial role in mediating renal fibrosis in obstructed kidneys(31), in line with the attenuation of mitochondrial dysfunction and ROS production. In the present study, employing a specific inhibitor of mitochondrial complex-1, we further investigated the role of mitochondria in modulating AQPs in obstructive nephropathy.

In agreement with previous findings (11, 22), kidney obstruction caused robust downregulation of AQP1-3. Interestingly, mitochondrial complex-1 inhibitor rotenone largely prohibited the downregulation of AQP1 and AQP2, and partially attenuated AQP3 reduction after kidney obstruction for 48 hours and 7 days. All these data highly suggested that mitochondrial dysfunction in obstructed kidneys plays a key role in the dysregulation of AQP1 and AQP2. For AQP3, the less response to rotenone treatment suggested a more complex regulatory mechanism. Moreover, reduction of vasopressin receptor V2 in obstructed kidneys was also restored by rotenone, which indicated that dysregulation of V2 receptor may play a role in modulating the downregulation of AQP2 in obstructive nephropathy at least to some extent.

In order to examine the potential mechanism involved in the rotenone effect on the restoration of AQPs in obstructed kidneys, we analyzed COX2/PGE2 signaling pathway
that has been considered to be responsible for the AQPs downregulation in obstructive kidneys. Unexpectedly, although COX-2/mPGES-1/PGE2 cascade was strikingly stimulated in obstructed kidneys, rotenone played no role in regulating this cascade. However, further examination of PGE2 receptors revealed that rotenone suppressed EP1 and EP4 expression, which highly suggested that rotenone may regulate AQPs and V2 receptor via PGE2/EP1 and/or PGE2/EP4 signaling. The biologic action of PGE2 is mediated by G protein-coupled E-prostanoid receptors including EP1, EP2, EP3 and EP4(1). Among the four EP subtypes, the EP1 receptors play a particular role in regulating renal function. Northern blot analysis demonstrates the highest expression of EP1 mRNA in the kidney (35). By in situ hybridization, EP1 expression in the kidney is chiefly localized in the collecting ducts where this EP subtype is considered to be of importance in mediating the diuretic action of PGE2 possibly via targeting V2, AQP2, and epithelial sodium channels (ENaCs) (2, 13, 15). EP4 receptors mainly account for the pro-inflammatory actions of PGE2, and its role in regulating fluid metabolism is not well-defined(38). Although the results suggested a potential of EP receptors in modulating V2 and AQPs in this study, we can’t rule out the direct effect of rotenone on V2 and water channels under this pathological condition. In agreement with this notion, a EP1 antagonist SC-51322 could not affect AQPs’ reduction in obstructed kidneys. In the future, cell-specific deletion of EP1 and other EPs in nephron will be an ideal strategy to define their roles in this experimental setting, which can avoid the impact of their antagonists and/or agonists on cardiovascular system and vasopressin production(14, 16, 19).
In general, application of a mitochondrial complex-1 inhibitor could lead to systemic inhibition of mitochondrial activity. However, a relatively lower dose of mitochondrial complex-1 inhibitor (rotenone) may not result in significant mitochondrial abnormality and organ dysfunction under normal conditions due to the compensatory response of the body (39). In agreement with this notion, rotenone alone at a dose of 500 ppm in diet did not affect AQP5 expression in the kidneys of sham controls. In obstructed kidney, an extensive mitochondrial damage could occur and contribute to a global reduction of AQP5. Under this situation, inhibiting the activity of those abnormal mitochondria could be beneficial in antagonizing pathogenesis, subsequently leading to the attenuation of AQP5 reduction.

In summary, we demonstrated that inhibiting the activity of dysfunctional mitochondria in obstructed kidney by a mitochondrial complex I inhibitor remarkably prevented the downregulation of AQP5 possibly through inhibiting PGE2/EP1 and/or PGE2/EP4 signaling pathways, but not COX-2 itself. Findings from this study not only provided a novel pathogenic mechanism resulting in AQP5 dysregulation and dysfunction, but also suggested potential targets for treating the urine-concentrating defect in obstructive kidney disease.

Acknowledgments

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Program of China (973 Program) (no. 2012CB517602), and the Natural Science Foundation of Jiangsu Province (no. BK2012001).

Contributions

Z.J, Y.Z, and Y.S designed the experiments. Y.Z, Y.S, Z.J, and G.D performed all the experiments and analyzed all the data. Z.J, A.Z, S.H, and X.Y discussed the results and co-wrote the paper. All authors reviewed the manuscript.

Conflict of Interests

There is no conflict of interest to disclose.

References


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**Figure legends**
Fig. 1. Kidney obstruction resulted in mitochondrial abnormality. (A) qRT-PCR analysis of mtDNA copy number. (B) qRT-PCR analysis of mTFAM. (C) qRT-PCR analysis of mtNd1. The presented data are means ± SE. N=5 in each group.

Fig. 2. Protein expression of AQP1 in obstructed kidneys following rotenone treatment. (A) Western blot analysis of AQP1. (B) Densitometric analysis of AQP1. The presented data are means ± SE. N=5 in each group.

Fig. 3. Protein expression of AQP2 in obstructed kidneys following rotenone treatment. (A) Western blot analysis of AQP2. (B) Densitometric analysis of 29kDa AQP2. (C) Densitometric analysis of 35-55kDa AQP2. The presented data are means ± SE. N=5 in each group.

Fig. 4. Immunohistochemistry of AQP2 in obstructed kidneys following rotenone treatment. N=5 in each group.

Fig. 5. mRNA expression of V2 receptor in obstructed kidneys following rotenone treatment. The presented data are means ± SE. N=5 in each group.

Fig. 6. Protein expression of AQP3 in obstructed kidneys following rotenone treatment. (A) Western blot analysis of AQP3. (B) Densitometric analysis of AQP3. The presented data are means ± SE. N=5 in each group.

Fig. 7. Protein expression of AQP4 in obstructed kidneys following rotenone treatment. (A) Western blot analysis of AQP4. (B) Densitometric analysis of AQP4. The presented data are means ± SE. N=5 in each group.
Fig. 8. mRNA expressions of AQPs in kidneys with 48h obstruction following rotenone treatment. (A) mRNA expression of AQP1 determined by qRT-PCR. (B) mRNA expression of AQP2 determined by qRT-PCR. (C) mRNA expression of AQP3 determined by qRT-PCR. The presented data are means ± SE. N=5 in each group.

Fig. 9. Immunohistochemistry analysis of AQP2 in obstructed kidneys (48h) following rotenone treatment.

Fig. 10. Expressions of AQPs in non-obstructed kidneys with 7-day rotenone treatment.

(A) mRNA expression of AQP1 determined by qRT-PCR. (B) mRNA expression of AQP2 determined by qRT-PCR. (C) mRNA expression of AQP3 determined by qRT-PCR. (D) Western blot of AQP2. (E) Densitometry of 35-55 kDa AQP2. (F) Densitometry of 29 kDa AQP2. The presented data are means ± SE. N=5 in each group.

Fig. 11. mRNA expression of PGE2 generation-related enzymes in obstructed kidneys following rotenone treatment. (A) mRNA expression of COX-1 determined by qRT-PCR. (B) mRNA expression of COX-2 determined by qRT-PCR. (C) mRNA expression of mPGES-1 determined by qRT-PCR. (D) mRNA expression of mPGES-2 determined by qRT-PCR. (E) mRNA expression of cPGES determined by qRT-PCR. (F) mRNA expression of 15-PGDH determined by qRT-PCR. The presented data are means ± SE. N=5 in each group.

Fig. 12. Protein expression of COX-2 and kidney PGE2 content in obstructed kidneys following rotenone treatment. (A) Western blot analysis of COX-2. (B) Densitometric
analysis of COX-2. (C) EIA assay of kidney PGE2 content. The presented data are means ± SE. N=5 in each group.

Fig. 13. mRNA expression of PGE2 receptors in obstructed kidneys following rotenone treatment. (A) qRT-PCR analysis of EP1. (B) qRT-PCR analysis of EP2. (C) qRT-PCR analysis of EP3. (D) qRT-PCR analysis of EP4. The presented data are means ± SE. N=5 in each group.

Fig. 14. Protein expression of EP1 in obstructed kidneys following rotenone treatment. (A) Western blot analysis of EP1. (B) Densitometric analysis of EP1. The presented data are means ± SE. N=5 in each group.

Fig. 15. Expressions of AQP5s in obstructed kidneys (48h) with SC-51322 treatment. (A) mRNA expression of AQP1 determined by qRT-PCR. (B) mRNA expression of AQP2 determined by qRT-PCR. (C) mRNA expression of AQP3 determined by qRT-PCR. The presented data are means ± SE. N=5 in each group.
Fig. 1

A

mtDNA/Genome DNA

B

mTFAM/GAPDH

C

mtNd1/GAPDH

P<0.01
Fig. 2

A

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β-actin

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B

![Bar graph showing AQP1/β-actin ratio](image)

- Sham: P<0.01
- UUO: P>0.01
- UUO+Rotenone: P<0.01
Fig. 3

A

Sham UUO UUO+Rotenone

35-55 kDa AQP2

P<0.05

29 kDa AQP2

β-actin

B

P<0.05

29 kDa AQP2

Sham UUO UUO+Rotenone

C

P<0.05

35-55 kDa AQP2

Sham UUO UUO+Rotenone
Fig. 4

Sham  UUO  UUO+Rotenone

Cortex

Medulla
Fig. 5
Fig. 6

A

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B

- AQP3/β-actin
  - Sham: 1.2
  - UUO: 0.2
  - UUO+Rotenone: 0.5

Significance:
- P<0.01
- P<0.01
- P<0.01
Fig. 7

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B

![Bar chart showing AQP4/β-actin ratios in Sham, UUO, and UUO+Rotenone groups.](chart.png)
Fig. 8

A

\[ \text{AQP1/GAPDH} \]

- \( P < 0.01 \)
- \( P < 0.05 \)

B

\[ \text{AQP2/GAPDH} \]

- \( P < 0.01 \)
- \( P < 0.05 \)

C

\[ \text{AQP3/GAPDH} \]

- \( P < 0.01 \)
- \( P < 0.01 \)
Fig. 9

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Fig. 10

(A) AQGP1/GAPDH

(B) AQGP2/GAPDH

(C) AQGP3/GAPDH

(D) Western blot analysis of AQGPs in control (Cont) and Rotenone-treated samples. The bands at 35-55kDa and 29kDa are highlighted.

(E) AQG2 (35-55kDa ratio to cont).

(F) AQG2 (29kDa ratio to cont).
Fig. 11

A. COX-1/GAPDH

B. COX-2/GAPDH

C. mPGES-1/GAPDH

D. mPGES-2/GAPDH

E. cPGES/GAPDH

F. 15-PGDH/GAPDH

Sham UUO UUO+Rotenone

P<0.05
Fig. 12

A

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72 kDa

43 kDa

B

![Graph showing COX-2 and β-actin expression levels with statistical significance (P<0.01)].

C

![Graph showing kidney PGE2 content with statistical significance (P<0.05)].
Fig. 14

A

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42 kDa

43 kDa

B

![Bar chart showing EP1/β-actin levels](chart.png)

- Sham: 1.5
- UUO: 1
- UUO + Rotenone: 0.5

$P < 0.01$
Fig. 15

A

\[ P < 0.05 \]

\[
\begin{array}{ccc}
\text{Sham} & \text{UOO} & \text{UOO+SC51322} \\
\end{array}
\]

\[ \frac{\text{AQPI/GAPDH}}{} \]

B

\[ P < 0.05 \]

\[
\begin{array}{ccc}
\text{Sham} & \text{UOO} & \text{UOO+SC51322} \\
\end{array}
\]

\[ \frac{\text{AQPI/GAPDH}}{} \]

C

\[ P < 0.05 \]

\[
\begin{array}{ccc}
\text{Sham} & \text{UOO} & \text{UOO+SC51322} \\
\end{array}
\]

\[ \frac{\text{AQPI/GAPDH}}{} \]
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