Renal blood flow and vascular resistance responses to angiotensin II in irreversible and reversible unilateral ureteral obstruction rats; the role of angiotensin II type 1 and 2 receptors

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1. Background

Unilateral ureteral obstruction (UUO) is an important model of progressive renal disease characterized by renal hemodynamic impairment (1). Functional and hemodynamic impairments (1,2) are associated with elevated pressure in the renal glomeruli, tubules, and ureter that can lead to histopathological (1) and morphological changes in the kidney with UUO (3). UUO decreases the renal blood flow (RBF) and increases the renal vascular resistance (RVR) (4) progressively in ipsilateral kidney until the UUO is removed, while UUO removal (RUUO) can prevent the complications of acute UUO (5). Renin-
angiotensin system (RAS) is one of the main systems that is activated in UUO condition, and local RAS activation leads to the excessive elevation of intrarenal angiotensin II (Ang II) content (6) and induces inflammation and fibrosis in kidney suffering from UUO (7). Moreover, UUO induces podocyte injury in renal bowman's capsule and then plasma angiotensigenen is filtered within the kidney and converted to Ang II (8). This mechanism is a new pathway for local renal Ang II generation (9). Ang II alters the hemodynamic parameters in the ipsilateral kidney after UUO and can induce injury via constriction of afferent and efferent kidney arterioles (10,11). Ang II as the main bioactive product of the RAS (12), acts via Ang II receptors subtypes 1 (AT₁R) and subtypes 2 (AT₂R) (13). The most effects of Ang II are done via AT₁R in adult mammals (14). Therefore, the main classical Ang II functions such as activation of fibroblasts, inflammation, and oxidative stress are mediated by AT₁R (14,15). Studies have shown that AT₁R antagonists have a renoprotection effect and attenuate the interstitial fibrosis and apoptosis in the kidney with UUO (16,17). Ang II via AT₁R induces diuresis, natriuresis, vasodilatation, and antiproliferative responses (15). Moreover, it is reported that AT₁R antagonist (PD123319) increases the renal interstitial collagen accumulation after UUO (18). Also, it has been seen that interstitial fibrosis increases in the AT₁R knockout UUO mice (19). Renal hemodynamic responses to Ang II may act differently during UUO and after removal UUO (RUUO).

2. Objectives
The main purpose of this study was to evaluate the role of AT₁R and AT₂R antagonists on renal vascular responses to graded Ang II infusion after both UUO and RUUO in male rats.

3. Materials and Methods

3.1. Animals
Male Wistar rats (215 ± 10 g) obtained from Water and Electrolyte Research Center Animal House, Isfahan, Iran. The animals were housed at 23–25°C with a 12-hour light/dark cycle and allowed 1 week to acclimatize to this situation. The rats were fed with rat chow and had free access to tap water.

3.2. Induction of UUO and RUUO models
Rats were anesthesized with chloral hydrate (450 mg/kg, I.P., Sigma St. Louis USA) (20) and surgery was performed through an incision on left quadrant of the abdomen and the left ureter was exposed and ligated by 4-0 nylon suture to induce UUO induction (groups 4-9). A similar procedure was applied in sham groups except for UUO induction (group 1-3). But in groups 7-9 (RUUO), UUO was removed under anesthesia after three days and allowed to recover for 24 hours. In summary, the following groups were designed (n=7-10 in each group).

Groups 1-3 (named sham): sham-operated groups that treated with vehicle (group 1), losartan (group 2) and PD123319 (group 3), and then they were subjected to receive graded Ang II infusion (21).

Groups 4-6 (named UUO): UUO groups that treated with vehicle (group 4), losartan (group 5) and PD123319 (group 6), and then they were subjected to receive graded Ang II infusion.

Groups 7-9 (named RUUO): RUUO groups that treated with vehicle (group 7), losartan (group 8) and PD123319 (group 9), and then they were subjected to receive graded Ang II infusion.

3.3. Surgical preparation
Rats were anesthetized with 1.7 g/kg bodyweight urethane (Sigma St. Louis USA). The trachea was exposed and the air ventilation tube inserted to facilitate breathing. The left jugular vein was isolated, ligated distally, and cannulated with polyethylene tubing (PE 9658, Microtube Extrusions, North Rocks NSW, Australia) for measuring mean arterial pressure (MAP) and renal perfusion pressure (RPP), respectively. The left kidney was exposed, placed and fixed in special kidney cup. Renal artery was separated from the renal veins then an ultrasound flow probe interfaced with a compatible flow meter (T108; Transonic Systems) was placed around the renal artery for RBF measuring. An adjustable clamp was placed around the abdominal aorta (above renal arteries) to maintain RPP in base levels during Ang II infusion. MAP, RPP, and RBF were monitored continuously during the experiment.

3.4. Experimental protocol

3.4.1. Baseline measurement and antagonist response
The animals were allowed to stabilize for 30-45 minutes as equilibrium time for baseline measurement. The baseline data for the MAP, RPP and RBF were obtained over the last 5 minutes of equilibrium time. Based on groups specified the animals were subjected to received either vehicle (saline), AT₁R antagonist; losartan (Merck & Co. Inc., Rathway, NJ, USA), or AT₂R antagonist;
Renal blood flow and angiotensin II

PD123319 (Sigma, St. Louis, MO, USA). Losartan and PD123319 dissolved in 0.9% w/v saline were administered as bolus doses of 5 mg kg⁻¹ and 1 mg kg⁻¹ followed by continuous infusions of 5 mg kg⁻¹ h⁻¹ and 1 mg kg⁻¹ h⁻¹ respectively using a microsyringe infusion pump (New Era Pump System Inc., Farmingdale, NY, USA) during the experiment. The dose of losartan and PD123319 were selected based on previous studies (22, 23), while Macari et al (24) reported that PD123319 had a high affinity for AT₁R at doses less than 1000 μg/kg/min. Thirty minutes post vehicle or antagonists infusion were considered as antagonist’s effect time for the measurement. MAP, RPP, and RBF were determined over the last 5 minutes period of antagonists’ effect time. MAP/RBF also was calculated as RVR.

3.4.2. Response to graded Ang II infusion
Ang II was administrated intravenously 30 minutes after antagonist or vehicle started. Graded Ang II infusion (30, 100, 300, and 1000 ng kg⁻¹ min⁻¹) was commenced using microsyringe pump. Each dose of Ang II was infused for a 15-minute period while RPP was maintained at pre-Ang II infusion levels by manipulation of the aortic clamp. MAP, RPP, and RBF responses to graded Ang II infusion were determined over the final 5 minutes of each infusion. At the end of the study, the animals were sacrificed humanely via an overdose of anesthetic, and the wet weight of left kidney was determined.

3.5. Ethical issues
The research was approved by Ethics Committee of Isfahan University of Medical. Prior to the experiment, the protocols were confirmed to be in accordance with the guidelines of Animal Ethics Committee of Isfahan University of Medical Sciences (code# IR.MUI.REC.1395.3.353).

3.6. Statistical analysis
The data were expressed as mean ± SEM and analysis were performed using SPSS version 20 software. The baseline data and the effect of vehicle/antagonist were analyzed by the one-way analysis of variance (ANOVA). Inter and between groups comparisons were followed by least significant difference (LSD) test. A repeated measure ANOVA was applied to compare the effect of each treatment response to Ang II. Significant differences were considered with values of $P \leq 0.05$.

4. Results
4.1. Baseline measurements
No significant differences were detected in terms of MAP, RBF, RPP, and RVR between the groups in each set of animal models (sham, UUO, and RUUO) in control or equilibrium period before infusion of vehicle or antagonists (Figure 1: A-L).

4.2. Effect of vehicle or antagonists
The results showed that vehicle (saline) infusion had no significant effect on the MAP, RPP, RBF, and RVR between the groups in each set of animal models (sham, UUO, and RUUO) 30 min post vehicle infusion (Figure 1: A-L). However, in sham-operated, UUO and RUUO models, AT₁R antagonist, losartan compared to vehicle or PD123319 decreased MAP, $P \leq 0.004$, Figure 1: A, E and I), RPP, $P \leq 0.002$, Figure 1: F and J), and RVR, $P \leq 0.0001$, Figure 1: L) significantly, but in RUUO compared to sham-operated model, RBF was increased significantly ($P < 0.05$) by losartan, and such observation was not detected in UUO model.

4.3. Response to graded Ang II infusion
The intravenous graded Ang II infusion increased the percentage changes of MAP and RVR and decreased RBF significantly in vehicle or PD123319 treated rats in sham, UUO and RUUO models ($P < 0.05$), but no significant difference was observed between antagonists treated groups in each model. However, MAP ($P$ dose $\leq 0.0001$, Figure 2: A, E and I), RBF ($P$ dose $\leq 0.0001$, Figure 2: C, G and K) and RVR ($P$ dose $\leq 0.0001$, Figure 2: D, H and L) were attenuated in losartan-treated groups when compared with the other groups in each models. For example; Ang II with the dose of 1000 ng kg⁻¹ min⁻¹ increased RBF percentage changes to 11.05% ± 6.3, -20.4% ± 8.4, 13.01 ± 5.04 respectively in sham, UUO and RUUO rats treated with losartan.

5. Discussion
The main findings indicated that RBF response to graded Ang II infusion in sham-losartan treated rats was significantly different from UUO and RUUO animal treated with losartan. Evidences have demonstrated that after UUO, local RAS strongly activates and leads to a prominent elevation of Ang II in ipsilateral kidney (25,26). Ang II via AT₁R also contributes to kidney injury (15). The previous report has shown that RVR increased and RBF decreased progressively in ipsilateral kidney suffering from UUO (27). In line with these results, in our study, 3-day UUO was associated with a significant reduction in RBF and a significant increase in RVR in the ipsilateral kidney (Figure 1). It is shown that losartan directly binds to AT₁R (28) and reduces the vasoconstriction response in kidney (27,29).
Furthermore, losartan improves the renal architecture, and it has renoprotective effects against tubulointerstitial fibrosis, renal ischemia and hypoxia, and oxidative stress (29,30). Our study showed that losartan decreased RVR, and also increased RBF in UUO and RUUO rats significantly (Figure 1). In concordant with our result, it has been shown that continuous injection of losartan can block the AT$_1$R mediated effects in the ipsilateral kidney in pigs with UUO (27). This study also supports the idea that Ang II is the main vasoconstrictor mediator that involved in hemodynamic disorder resulting from UUO (27). MAP, RVR and RBF responses to graded Ang II infusion in the losartan-treated group were significantly different from PD123319 or vehicle-treated rats in sham, UUO and RUUO models (Figure 2).

Studies have confirmed a significant rise in local Ang II content, angiotensin converting enzyme, renin, and AT$_1$R expression in the ipsilateral kidney with prolonged UUO (29,31,32). Furthermore, Hammad et al (33) confirmed that the proportion and/or affinity of AT$_1$R / was increased in UO kidney. Therefore, based on these studies, it can be concluded that AT$_1$R overexpression, and its functional effects, possibly alter the responses. Also, it has been found that after UUO, mechanical strain can up-regulate the AT$_1$R expression in podocytes; leads to podocyte injury, and then increases the local Ang II production (34). Also, it has been proved that Ang II stimulates releasing and activation of the endothelin-1 (35), while endothelin-1 has a crosstalk with Ang II in UUO (36) which mediates some of its vasoconstriction effects (37). Ang II also stimulates the release of noradrenaline from renal sympathetic nerve terminals dose dependently (38), and noradrenaline exacerbates the renal vasoconstriction (31). Together, it is suggested that Ang II may cause vasoconstriction through other mechanisms not directly related to AT$_1$R. To consider the renal vascular response in RUUO model, Ito et al (39) have demonstrated that, in 3-day UUO model, RBF returned to normal at 14 days after RUUO. Moreover, even short-term UUO is followed by progressive kidney
Figure 2. The percentage changes of the MAP, RPP, RBF and RVR in vehicle or losartan or PD123319 administration rats’ responses to graded Ang II infusion in sham or UUO or RUUO models. Data are shown as mean ± SEM of percentage changes from baseline. The $P$ values were derived from repeated measure ANOVA. *; Represents significant difference from the vehicle or PD123319 ($P$ dose < 0.05); n = 8–10 per group. UUO; Unilateral ureteral obstruction, RUUO: UUO removal, MAP: Mean arterial pressure, RPP: Renal perfusion pressure, RBF: Renal blood flow, RVR: Renal vascular resistance.

Figure 3. Effects of the vehicle (left panels) or losartan (midst panels) or PD123319 (right panels) on RBF and RVR percentage changes responses to graded Ang II infusion in sham, UUO, and RUUO rats. Data are presented as mean ± SEM. UUO: Unilateral ureteral obstruction, RUUO; UUO removal, RBF: Renal blood flow, RVR: Renal vascular resistance. $P$ values were derived from repeated measure ANOVA. *; Represents significant difference from the vehicle or PD123319 ($P < 0.05$).
injury and its harmful side effect remains after RUUO.
Our study also indicated that RBF and RVR responses to PD123319 infusion in UUO model were different with other PD123319 administrated groups (Figure 1). It is well-known that AT_{1}R mRNA expression and its antifibrotic effect were decreased in the kidney with UUO (40, 41), while AT_{2}R function and its vascular effect are against AT_{1}R (42). Moreover, PD123319 can interact with AT1/Mas receptors in the absence of AT_{2}R (43, 44). Perhaps PD123319 acts via another mechanism due to decreased AT_{2}R functional response in this model. PD123319 didn’t alter the hemodynamic parameter in response to Ang II significantly (Figure 3).
The possible reason could be related to overexpression of AT_{2}R, because it is documented that the renal AT_{2}R affinity for binding to Ang II is similar to AT_{1}R (45) but Ang II binding sites for AT_{1}R is one-fifth less than AT_{2}R (45).

6. Conclusions
It is concluded that both AT_{1}R and AT_{2}R are affected by 3-day UUO, but the regulatory role of AT_{1}R on renal hemodynamic parameters was greater than AT_{2}R. Also, paradoxical renal hemodynamic (RBF, RVR) responses to graded Ang II infusion in UUO or RUUO rats treated with losartan compared to sham-losartan rats were observed. This finding suggests that Ang II can cause vasoconstriction independent to direct AT_{1}R stimulation. Moreover, possibly the AT_{2}R expression is increased by UUO. However, the inhibition of AT_{1}R may be considered as therapeutic interventions on renal hemodynamic parameters in UUO or RUUO conditions.

Authors’ contribution
JH and MM conducted the experimental procedures and helped to prepare the first draft of the manuscript. MN; designed, supervised and analyzed the research and completed the manuscript. All authors read and signed the manuscript.

Conflicts of interest
The authors declare no conflict of interest.

Ethical considerations
Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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Renal blood flow and angiotensin II


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