Original Article

Non-invasive evaluation of bilateral renal regional blood flow and tubular dynamics during acute unilateral ureteral obstruction

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Abstract

Background. Global renal haemodynamic responses to acute unilateral ureteral obstruction (AUUO) have been studied extensively in animals, yet little is known about the concurrent changes in haemodynamics and tubular fluid dynamics that occur within the distinct regions of the kidney during AUUO. The advent of electron beam computerized tomography (EBCT) now allows us to evaluate non-invasively intrarenal haemodynamics and tubular fluid dynamics in vivo.

Methods. Using EBCT, we quantified total, cortical and medullary renal blood flow (RBF, C-RBF and M-RBF), and the concurrent intratubular fluid contrast concentration (ITCC) from contrast media dilution curves, prior to, and at 30 and 90 min after the onset of AUUO in five pigs.

Results. At 30 min after AUUO, there was a small 17±7% fall in C-RBF that did not quite reach significance (P = 0.076), whereas RBF, M-RBF, glomerular filtration rate (GFR) and ITCC were preserved. At 90 min, both C-RBF and RBF had fallen by 54±8 and 45±5%, respectively (P < 0.05). GFR also tended to decrease (by 49±8%, P < 0.06), whereas there was preservation of M-RBF. ITCC increased in the proximal and distal tubules, and tended to increase in Henle’s loop. In the contralateral kidney, AUUO did not alter the haemodynamics, but transiently decreased ITCC in all tubular segments.

Conclusion. EBCT allows evaluation of AUUO-induced changes in intrarenal haemodynamics and tubular fluid dynamics. AUUO decreased cortical, but not medullary perfusion of the ipsilateral kidney, and increased the ITCC in most tubular segments, suggesting increased tubular reabsorption that may have helped maintain GFR and tubular fluid flow.

Keywords: obstructive nephropathy; renal function; renal imaging

Introduction

Obstruction of the urinary tract is a relatively common cause of renal dysfunction in both adults and children, and thus has been the subject of intense investigation [1]. It can be caused by numerous congenital or acquired abnormalities. Regardless of the aetiology, once obstruction is present, a cascade of events is initiated that leads to alterations in glomerular haemodynamics and tubular function, and, if the obstruction is not relieved, progressive irreversible renal damage ensues. Consequently, numerous studies have been performed to provide insight into the time course and the mechanisms implicated in the haemodynamic and tubular effects of ureteral obstruction [1–8]. However, the methods used in these studies, while useful, have significant limitations. For instance, some are invasive and cumbersome (clearance techniques require collecting urine from the obstructed kidney, intravascular Dopplers require cannulating the arterial bed, etc.), while others such as plasma disappearance rates are not very accurate, particularly when tubular back-leak may be present [9–11]. Furthermore, there are at present no available techniques that can simultaneously measure intrarenal blood flow distribution, single-kidney glomerular filtration rate (GFR) and tubular function in a simple, accurate and minimally invasive manner [12]. Thus, there is considerable interest in developing techniques that permit us to better assess the changes in intrinsic renal function that occur during obstructive nephropathy. Such techniques have the potential to help (i) identify and evaluate mechanisms by which ureteral obstruction alters renal function; (ii) determine the effectiveness of therapeutic manoeuvres in delaying progression of the
damage; and (iii) determine whether there is potentially recoverable renal function.

The development of electron beam computerized tomography (EBCT) provides a unique opportunity to assess intrarenal haemodynamics and tubular dynamics non-invasively in the single kidney in vivo [13,14], during normal and pathological conditions. EBCT has several advantages. First, it provides fast, reliable and simultaneous measurements of renal blood flow (RBF) in each kidney (which may be especially useful when evaluating unilateral ureteral obstruction). EBCT measurements of RBF exhibit a linear correlation throughout a wide range of flow rates, with reference standards including electromagnetic flow probes [13] and intravascular Doppler measurements [14]. Secondly, EBCT accurately and non-invasively quantifies the regional distribution of the blood flow within the intact kidney. Finally, EBCT allows us to measure concurrent tubular fluid dynamics, an index of tubular fluid reabsorption and tubular fluid dynamics during acute unilateral ureteral obstruction (AUUO) in an animal model whose kidneys resemble human kidneys, namely pigs. Specifically, we determined: (i) AUUO-induced changes in whole kidney RBF and GFR of the obstructed and contralateral kidneys; (ii) whether the changes in whole kidney RBF were accompanied by changes in the distribution of intrarenal (cortical and medullary) blood flow; and (iii) the concomitant changes in intratubular contrast concentration (ITCC; which represents bulk tubular fluid reabsorption since the contrast media behave like inulin).

Materials and methods

The Institutional Animal Care and Use Committee of the Mayo Foundation approved all experiments. Five female domestic pigs (30–35 kg) were maintained on standard diet with free access to water until the night before the experiments when all food was withheld. On the day of the study, each animal was anaesthetized with an intravenous bolus of 10 ml of xylazine/ketamine cocktail (1 g of ketamine and 150 mg of xylazine) followed by a continuous infusion of 4.35 mg/ml ketamine, 0.65 mg/ml xylazine and 5 ml of heparin in normal saline, at a rate of 1 ml/min. The animals were then intubated and mechanically ventilated with oxygen and room air. Under sterile conditions, we inserted a 7F arterial guide into the left carotid artery to monitor mean arterial pressure, and a pigtail catheter was positioned in the right atrium, for contrast media injections. Heart rate was monitored with ECG leads. Both ureters were carefully exposed through bilateral flank incisions. A tie was loosely placed around the left ureter for future obstruction. Following the surgical preparation, the animals were transferred and positioned in the EBCT (Imatron C-150, Imatron Inc., South San Francisco, CA) scanning gantry. After transfer and stabilization, basal scans were performed to obtain the baseline renal haemodynamics, tubular fluid dynamics and renal volume (see below). We allowed 15 min for contrast wash-out and then tightened the tie on the left ureter to induce complete obstruction. Scans were repeated at 30 and 90 min after obstructing the ureter. Following completion of the studies, the pigs were euthanized with 20 ml of sodium pentobarbital given intravenously.

EBCT scanning sequence

Each EBCT study was performed during respiratory suspension at end-expiration as described previously [14]. Using abdominal localization scans, all tomographic levels containing both kidneys (from cranial to caudal pole) were identified and two mid-hilar tomographic levels selected. To study the intrarenal haemodynamics and tubular fluid dynamics, the kidneys were scanned in standard resolution (exposure time of 50 ms/image) multislice flow mode using one target ring, resulting in two 8 mm thick tomographic sections through the hilar regions of the left and right kidneys. Forty consecutive scans (over a 3 min period) were performed over the pre-selected levels immediately after a bolus injection (0.5 ml/kg over 1 s) of the non-ionic, low-osmolar contrast medium iopamidol (Isovue®-370, Squibb Diagnostics, Princeton, NJ) into the right atrial catheter, after which a renal volume study was performed as described previously [14].

Image analysis

All images were reconstructed using a filtered back-projection algorithm on the EBCT workstation and then transferred and displayed on a Sun® workstation for density measurements. Regions of interest were selected by manually tracing the aorta, and the renal cortex and medulla of the obstructed and contralateral kidneys, after which their densities were sampled [13,14]. Time-density curves were generated for each region, describing the change in tissue density consequent to transit of contrast in the vascular and tubular compartments of that region. Renal cortical time-density curves exhibit three sequential peaks with EBCT and are fitted with a mathematical model [14]. These dilution curves maintained a similar pattern in the obstructed kidney, which permitted us to perform the same analysis for the various compartments (Figure 1). The three bell-shaped dilution curves were treated separately to obtain the dynamic characteristics of X-ray contrast medium through the vascular compartment as well as through the proximal and distal nephron segments. Changes of contrast density were also measured in the medullary tip to calculate transit through the loop of Henle. From each segment of the curve, the area enclosed under this segment, and its first moment or moment of inertia, were calculated as: 

$$M(t) = \int_{0}^{t} C(t) \, dt$$

where $C(t)$ is contrast concentration (tissue density) at time $t$, $M(t)$ is the area under the curve, and $C(t)$ is contrast concentration (tissue density) at a time $t$.

Blood volume (ml blood/ml tissue), which is the tissue vascular volume fraction, was calculated as: area under the...
tissue vascular curve/area under the aortic curve (see above) in each region.

Tissue perfusion, which is the blood flow normalized per unit tissue (ml/min/ml of tissue), was calculated as 60 times the blood volume/MTT.

Cortical RBF (C-RBF), medullary RBF (M-RBF) and total RBF were calculated as the product of cortical and medullary perfusion and their corresponding volumes. Total RBF was obtained from the sum of C-RBF and M-RBF.

The intratubular fluid contrast concentration (ITCC) represents the degree of concentration or dilution of intratubular contrast media. Each peak observed following the vascular phase in each regional time-density curve was analysed separately to derive the area under the curve and its first moment. From this, ITCC relative to blood was calculated for each nephron segment as the ratio of its area under the curve to that under the cortical vascular curve. To normalize for alterations in contrast delivery, we corrected for the difference in GFR between the periods.

Normalized GFR (ml/min/ml of tissue) was calculated from the cortical time-density curves as described previously [14]: (maximal slope of the proximal tubular curve x 2 x cortical vascular MTT/area under the aortic curve) x 60. The maximal slope of the ascending arm of the proximal tubular curve represents the rate (per s) of contrast accumulation secondary to glomerular filtration. The duration of transit of the contrast bolus in the cortical vasculature (estimated as twice the cortical MTT) approximates the duration of this process, and division by the area under the aortic curve normalizes the measurement for contrast input. Since the filtration occurs in the cortex, the normalized GFR was multiplied by the corresponding cortical volume to obtain single kidney GFR in units of ml/min.

Statistical analysis

Results are expressed as mean ± SEM. Statistical comparisons between baseline and subsequent experimental periods were performed using two-tailed paired *t*-test. *P* < 0.05 was considered significant.

Results

Mean arterial pressure

The MAP was 93 ± 9 mmHg at baseline and did not change during the course of the experiments (103 ± 10 and 99 ± 8 mmHg at 30 and 90 min, respectively).

Renal and intrarenal haemodynamics

Figure 2 depicts C-RBF, M-RBF and total RBF of both the obstructed and contralateral kidneys during the course of the study, whereas Table 1 shows cortical and medullary perfusion values in each kidney. Obstructing the ureter caused a progressive decrease in C-RBF of the obstructed kidney which started as a trend at 30 min (from 311 ± 24 to 261 ± 33 ml/min; *P* = 0.076), and reached statistical significance at 90 min (143 ± 30 ml/min, *P* < 0.05). This decrement in C-RBF was also reflected by significantly decreased cortical tissue perfusion of 27 and 61% at 30 and 90 min, respectively. On the other hand, neither M-RBF nor medullary tissue perfusion of the obstructed kidney changed at either 30 or 90 min after the onset of obstruction. Total RBF of the obstructed kidney followed a similar pattern to C-RBF but with a lesser magnitude at 30 min: RBF was unchanged from baseline at 30 min (454 ± 46 vs 415 ± 50 ml/min; *P* = NS) but decreased significantly by 90 min (to 250 ± 34 ml/min; *P* < 0.005).

Blood flow to all regions of the contralateral kidney was essentially unaffected by AUUO. C-RBF remained unchanged (327 ± 24, 325 ± 37 and 331 ± 38 ml/min at baseline and at 30 and 90 min, respectively) as did cortical tissue perfusion. M-RBF was also unaltered (149 ± 26, 176 ± 15 and 142 ± 11 ml/min; baseline,
30 and 90 min after the onset of obstruction, respectively), but medullary tissue perfusion transiently increased at 30 min. Despite the increase in medullary perfusion, total RBF remained unchanged (476 ± 45, 501 ± 48 and 473 ± 30 ml/min at baseline and at 30 and 90 min, respectively; P = NS).

### Glomerular filtration rate

As shown in Figure 2, GFR in the obstructed kidney did not change at 30 min (32 ± 5 vs 37 ± 6 ml/min), but showed a strong tendency to decrease at 90 min (to 17 ± 1 ml/min, P = 0.06). GFR remained unchanged in the contralateral kidney (32 ± 4, 46 ± 9 and 32 ± 6 ml/min, at baseline, 30 and 90 min, respectively).

### Tubular fluid dynamics

Figure 3 shows the ITCC profiles in the obstructed and contralateral kidneys at 30 and 90 min after the onset of obstruction. ITCC in the obstructed kidney was not altered at 30 min, but had increased in the proximal and distal tubules, and strongly tended to increase in the loop of Henle at 90 min (albeit not quite reaching statistical significance in the loop: P = 0.06). In contrast, ITCC decreased in all the nephron segments of the contralateral kidney at 30 min. However, this decrease was transient as ITCC returned to baseline in the proximal and distal tubular segments by 90 min, while the loop of Henle had increased ITCC.

### Discussion

In the present study, we used EBCT technology to examine non-invasively the changes in the intrarenal haemodynamics of both the obstructed and contralateral kidneys that occur at 30 and 90 min after the onset of AUUO. Furthermore, we assessed the concomitant changes in single-kidney GFR and we report for the first time the tubular fluid dynamics during AUUO. We found that perfusion to the cortex of the obstructed kidney progressively decreased, whereas M-RBF was preserved throughout the duration of the study. The prominent reduction in C-RBF at 90 min accounted for the large reduction in whole kidney RBF, and was associated with a reduction in GFR (that was of borderline statistical significance). There was also an increase in ITCC, suggesting that tubular fluid reabsorption was enhanced. On the other hand, all the haemodynamic parameters were unchanged in the contralateral kidney throughout the study, whereas there was a transient diffuse decrease in ITCC suggesting a diluting effect in all the nephron segments at 30 min.

Renal haemodynamic responses to AUUO have been evaluated by various studies [1–8]. Most have reported a biphasic haemodynamic response; there is an initial transient increase in RBF followed by a progressive decrease in renal perfusion [1–4]. However, these studies were performed in animals with unipapillary kidneys (i.e. rodents and dogs). Humans have multipapillary kidneys, which may behave somewhat differently. However, there are very few studies that have evaluated haemodynamic changes in response to AUUO in animals that have multipapillary kidneys [5–8]. Thus, using EBCT, we sought to assess these parameters during AUUO in pigs, which have multipapillary kidneys with similar structural and functional characteristics to human kidneys. Unlike the studies in animals with unipapillary kidneys, we did not detect a renal vasodilator phase. This lack of a vasodilator phase is unlikely to be due to the use of contrast media because the non-ionic low osmolar contrast media used are associated with only minimal perturbation of renal haemodynamics and function [15,16] and do not inhibit renal vasodilatory response to vasoactive substances [14,16,17]. In fact, previous studies have also reported the lack of a vasodilatory phase to AUUO in pigs [4–6]. Thus, while we cannot completely rule out the existence of an early, transient and/or labile effects of AUUO.

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**Table 1.** Regional perfusion changes in the obstructed and contralateral kidney at baseline and 30 and 90 min after the onset of acute unilateral ureteral obstruction in pigs

<table>
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<tr>
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<th>Obstructed kidney</th>
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<th>Contralateral kidney</th>
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<tbody>
<tr>
<td></td>
<td>Baseline 30 min</td>
<td>90 min</td>
<td>Baseline 30 min 90 min</td>
<td></td>
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<tr>
<td>Cortical perfusion (ml/min/ml of tissue)</td>
<td>4.93 ± 0.29</td>
<td>3.56 ± 0.29*</td>
<td>1.89 ± 0.31*</td>
<td>4.93 ± 0.18</td>
</tr>
<tr>
<td>Medullary perfusion (ml/min/ml of tissue)</td>
<td>3.45 ± 0.46</td>
<td>3.54 ± 0.32</td>
<td>2.79 ± 0.41*</td>
<td>3.43 ± 0.45</td>
</tr>
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*P < 0.05 vs baseline.
Renal vasoconstriction is commonly observed during AUUO [1–8]. This is usually detected as either a fall in RBF, or more recently by an increase in the renal vascular resistive indices [8]. Fast CT and dynamic contrast-enhanced magnetic resonance imaging techniques are also proving to be useful for evaluating renal haemodynamics in this condition [5,18]. For instance, Singal et al. [5] used dynamic CT to measure the changes in RBF in response to increases in intrapelvic pressure during ureteral obstruction. They found a progressive decline in RBF that was confirmed using radiolabelled microspheres. However, that study was limited to evaluating whole kidney haemodynamics. In the present study, we were not only able to follow the decreases in whole kidney RBF, but in addition we were able to detect the changes in the intrarenal distribution of blood flow. We found differential regional responses to AUUO; it caused cortical vasoconstriction that completely accounted for the fall in whole kidney RBF observed at 90 min. This fall in RBF was similar to that found in separate experiments in which we used electromagnetic flow probes (unpublished data), and also to that previously reported by others [5–7]. Furthermore, the predominant cortical vasoconstriction which we observed is consistent with that seen in previous studies in which intrarenal haemodynamics were determined via ex vivo analysis of renal tissue using autoradiography, microspheres or silicone rubber injection techniques [1,4]. Taken together, the data suggest that AUUO does not elicit the classic triphasic haemodynamic response in pigs, rather there is progressive cortical vasoconstriction. EBCT allowed us to follow the sequential changes in RBF and intrarenal haemodynamics of each kidney in a simple, accurate and non-invasive manner.

In the present study, we calculated real time single-kidney GFR using EBCT by following the filtration of contrast (which behaves like inulin) from the vascular to the tubular compartment via sequential imaging. This feature of EBCT may be particularly useful because there are no simple techniques available to obtain accurate measurements of GFR from obstructed kidneys. Classic clearance techniques require either collecting urine from the obstructed kidney via a nephrostomy, or unclamping the obstruction (both of which result in temporary relief of obstruction, potentially altering the results). Renal scintigraphy compares relative GFR between the kidneys, but does not reliably quantify a bilateral decrease in GFR, cannot be used for longitudinal comparisons and can be skewed by renal depth [19]. Others have calculated GFR of the obstructed kidney either by cannulating the renal veins to obtain measures of renal extraction [6] or by obtaining plasma disappearance curves and then subtracting the inulin clearance rate from the contralateral kidney [9]. These methods are not only cumbersome, but may also be unreliable in pathological conditions [9–11]. They are based on the assumptions that the tracer used (i.e. inulin or EDTA) is neither retained in the kidney nor reabsorbed back into the circulation, when in fact reabsorption may occur during ureteral obstruction via the tubules, the lymphatics or pyelovenous backflow [11]. This reabsorption of the tracer could lead to significant underestimation of GFR if prolonged collection periods are needed. While EBCT may be bound by the same assumptions, the data are obtained in real time, within seconds (from the appearance of contrast in the aorta to its first pass through the kidney). Thus this extremely brief period minimizes the potential for contrast reabsorption (if any). Indeed, we have demonstrated previously the accuracy of GFR measurements by EBCT and its excellent correlation with inulin clearance in a variety of conditions [14]. It the present study, we found that GFR was preserved at 30 min but showed a strong tendency to be decreased at 90 min. This decrease in GFR paralleled that of the RBF, and was consistent, albeit less marked than that seen using the renal extraction method [6]. The smaller fall in GFR in our study may be due to differences in the experimental protocols or perhaps to underestimation of GFR by the renal extraction technique (as discussed above).

There are few methods that can evaluate tubular function in vivo. EBCT and Mag-3 renography have both been used; however, we recently found that EBCT was the more sensitive modality [20]. In the present study, we found that ITCC (which reflects bulk fluid reabsorption along the nephron) was increased in the obstructed kidney at 90 min, suggesting that tubular reabsorption is enhanced at this time point. This is in marked contrast to later stages of obstruction in which both sodium and water reabsorption are impaired [1,2]. The mechanisms involved in the enhanced tubular reabsorption were not evaluated in the present study but may be due to increased humoral factors that act directly upon the tubule, the concurrent changes in intrarenal haemodynamics or perhaps increased intratubular pressures. It is also theoretically possible that the increase in ITCC was caused by leakage of contrast media across disrupted cells and intercellular junctions. However, this is unlikely because the contrast media and fluid would then equilibrate and thus result in lower ITCC indices in the obstructed kidney. In contrast to the obstructed kidney, the contralateral kidney showed a transient decrease in ITCC, suggesting decreased tubular reabsorption at 30 min, with the values returning to normal by 90 min. This is consistent with the compensatory increase in diuresis and natriuresis that commonly occurs in the contralateral kidney and may be due to inhibition of renorenal reflexes.

In summary, the present study shows that it is possible to study non-invasively single-kidney regional haemodynamics, GFR and concomitant tubular dynamics of both kidneys during AUUO. These studies show for the first time that a predominant
cortical hypoperfusion characterizes the progressive decreases in RBF and GFR that occur in the obstructed kidney during AUUO. The cortical hypoperfusion is associated with increased ITCC (tubular reabsorption). This increase in tubular reabsorption tends to preserve tubular fluid flow, which in turn may blunt the increase in intratubular pressure and thus facilitate the maintenance of GFR. On the other hand, all the haemodynamic parameters in the contralateral kidney were unchanged, but there was a transient diluting effect in all of the nephron segments 30 min after the onset of AUUO. Because the integrity of both vascular and tubular function, as well as GFR, can be determined non-invasively by EBCT, it may potentially prove to be helpful in evaluating the pathophysiology and the effectiveness of therapeutic manoeuvres, or perhaps in predicting recoverable renal function in humans.

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Conflict of interest statement. None declared.

References

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