Urinary oxygen tension: a clinical window on the health of the renal medulla?

Roger G. Evans, Julian A. Smith, Christopher Wright, Bruce S. Gardiner, David W. Smith & Andrew D. Cochrane

Department of Physiology, Department of Surgery (Monash Medical Centre) and Faculty of Medicine, Nursing and Health Sciences, Monash University, Melbourne, Australia.

School of Computer Science and Software Engineering, The University of Western Australia, Perth, Australia.

Running title: Urinary oxygen tension

Author for correspondence:
Dr Roger Evans
Department of Physiology
PO Box 13F, Monash University, Victoria 3800, Australia
Tel: 61 3 9905 1466
Fax: 61 3 9905 2566
Email: Roger.Evans@monash.edu
Abstract

We describe the determinants of urinary oxygen tension (PO$_2$) and the potential for use of urinary PO$_2$ as a ‘physiological biomarker’ of the risk of acute kidney injury (AKI) in hospital settings. We also identify knowledge-gaps required for clinical translation of bedside monitoring of urinary PO$_2$. Hypoxia in the renal medulla is a hallmark of AKI of diverse aetiology. Urine in the collecting ducts would be expected to equilibrate with the tissue PO$_2$ of the inner medulla. Accordingly, the PO$_2$ of urine in the renal pelvis changes in response to stimuli that would be expected to alter oxygenation of the renal medulla. Oxygen exchange across the walls of the ureter and bladder will confound measurement of the PO$_2$ of bladder urine. Nevertheless, the PO$_2$ of bladder urine also changes in response to stimuli that would be expected to alter renal medullary oxygenation. If confounding influences can be understood, urinary bladder PO$_2$ may provide prognostically useful information, including for prediction of AKI after cardiopulmonary bypass surgery. To translate bedside monitoring of urinary PO$_2$ into the clinical setting, we require (i) a more detailed knowledge of the relationship between renal medullary oxygenation and the PO$_2$ of pelvic urine under physiological and pathophysiological conditions, (ii) a quantitative understanding of the impact of oxygen transport across the ureteric epithelium on urinary PO$_2$ measured from the bladder, and (iii) a simple, robust medical device that can be introduced into the bladder via a standard catheter, to provide reliable and continuous measurement of urinary PO$_2$.

Word count: 249

Keywords: Acute kidney injury, biomarker, cardiopulmonary bypass surgery, hypoxia, intensive care.
Introduction

Many hospital settings are associated with a significant risk of the development of acute kidney injury (AKI) (49). Patients at particular risk are those with heart disease, who undergo major surgical procedures, those administered radiocontrast or other nephrotoxic agents, or those who develop sepsis (49). The presence of pre-existing renal dysfunction, as evidenced by elevated serum creatinine, greatly increases the risk of hospital acquired AKI, particularly in the setting of cardiac surgery (38). Development of even relatively mild AKI in a hospital setting is associated with considerable increased risk of mortality (24).

Plasma and urinary biomarkers are the source of much new hope for early diagnosis of hospital acquired AKI. Baseline serum creatinine can help identify those patients at risk of hospital-acquired AKI (38). Serum creatinine can also be used to follow the progression of AKI. However, increases in serum creatinine lag many hours behind the development of AKI. Multiple plasma and urinary biomarkers, including neutrophil gelatinase-associated lipocalin (NGAL), have shown some efficacy in early prediction of AKI (17), but such molecules are markers of renal damage. As with assessment of changes in serum creatinine, diagnosis lags hours behind the initiating insult. An ideal biomarker would provide information about the physiological status of the kidney in individual patients, so that conditions likely to promote development of AKI can be avoided.

In the first part of this article, we review the evidence that continuous measurement of urinary oxygen tension (PO$_2$) might provide a means to monitor kidney health in hospital settings. Our hypothesis is based on the evidence that medullary hypoxia is a critical initiating event in the development of multiple forms of AKI, and that urinary PO$_2$ provides an index of medullary oxygenation. Because the delay between medullary hypoxia and measurement of urinary hypoxia should be only minutes, this biomarker has the potential to aid in the management of patients at risk of AKI, and so to possibly prevent development of AKI. In
the second part of the article we outline the significant challenges that must be overcome in order to translate this concept into clinical practice. These include the need to (i) account for the various factors that might confound interpretation of bladder urine PO$_2$ as an index of oxygenation of the renal medulla, (ii) develop an approved medical device for continuous measurement of bladder urine PO$_2$ in a hospital setting, and (iii) formulate computational tools to help clinicians use this new information to better manage their patients.

Evidence for the clinical utility of urinary PO$_2$

**Medullary hypoxia in the pathogenesis of acute kidney injury**

Despite the fact that the kidneys receive a quarter of the cardiac output, they are susceptible to hypoxia (6, 9). Multiple factors render the renal medulla particularly susceptible to development of hypoxia. These include the fact that medullary blood flow, per unit tissue, is much less than cortical blood flow (11). Furthermore, diffusive oxygen shunting between counter-current vessels in both the cortex (from arteries and veins) (13, 14, 39) and medulla (from descending and ascending vasa recta) (7), reduces oxygen delivery to medullary tissue. The outer medulla contains the thick ascending limbs of Henle’s loop which reabsorb much of the filtered sodium, and so require a large amount of oxygen. Yet this region has a comparatively meager oxygen supply (7). Consequently, in human AKI tubular damage is most often seen in the distal segments of nephrons in the outer medulla (thick ascending limbs and collecting ducts) (20).

Evidence that prevention of kidney ischemia and hypoxia should reduce the risk of AKI comes from studies of AKI after cardiopulmonary bypass (CPB) surgery. Many of the risk factors for AKI after CPB surgery are associated with reduced renal perfusion (and thus oxygen delivery) and/or increased renal oxygen consumption. Pre-operative risk factors likely to promote renal hypoxia include pre-existing renal dysfunction (elevated pre-operative serum creatinine), diabetes, peripheral vascular disease, and cardiogenic shock. Intraoperative
risk factors likely to promote renal hypoxia include the duration of CPB, hypotension, low pump flow and hemodilution (43). Moreover, whole body oxygen delivery, which could be modified by careful management of pump flow and the level of hemodilution, has been shown to have a considerable impact on the risk of AKI in this setting (8, 43).

There is also evidence from experimental studies to support the notion that tissue hypoxia is a final common pathway in multiple forms of AKI (1, 18, 30, 31). Tissue hypoxia has been consistently observed in experimental AKI and initiates multiple pathways leading to tissue damage and dysfunction (11, 18). In renal ischemia-reperfusion injury, depletion of cellular ATP initiates signalling cascades leading to apoptosis and necrosis. Hypoxia activates pro-inflammatory pathways, including those driven by the transforming growth factor β and Smad pathways. Multiple downstream pathways, including oxidative stress, then exacerbate tissue hypoxia. Multiple factors also lead to reduced efficiency of oxygen use and so increased oxygen consumption, including oxidative stress and loss of Na,K-ATPase polarity and tight junctions between tubular cells. Cellular protective mechanisms such as hypoxia inducible factor driven gene expression appear to protect the kidney when hypoxia is mild and/or brief, but fail when hypoxia is severe and/or protracted (18, 37).

It has been argued that tissue hypoxia may initiate a vicious cycle in AKI, leading to progression of kidney disease and worsened tissue hypoxia (11, 19, 46). But despite the availability of methods to monitor kidney oxygenation by blood oxygen dependent magnetic resonance imaging (BOLD MRI), it is not currently feasible to continuously monitor renal oxygenation in a clinical setting. Might continuous measurement of urinary PO₂ provide an adequate proxy measure of medullary oxygenation? It has long been assumed that urinary PO₂ reflects medullary PO₂. This makes sense from an anatomical perspective, as the collecting ducts exiting at the renal papilla run parallel, and in close association with, the vasa
recta (Fig. 1). To assess the potential utility of urinary PO₂ as a marker of medullary oxygenation, and as a biomarker for risk of AKI, we review the relevant literature below.

The oxygen tension of pelvic urine

Observations of the oxygen content within the kidney and urinary tract go back to the works of Claude Bernard and Eduard Pflüger in the 19th century (cited in (45)). But Rennie and colleagues were the first to study urinary oxygenation in a truly systematic manner (44, 45). Their studies in anesthetized dogs confirmed that pelvic urinary PO₂ is consistently lower than renal venous PO₂. The relatively low PO₂ of pelvic urine led Aukland and Krog to propose that this reflected a relatively low oxygen tension of the renal medulla, which they later confirmed in anesthetized dogs using silver electrodes (4). Aukland and Krog (3) recognized the importance of the anatomical arrangement of the medullary tubular and vascular elements, which has more recently been defined in great detail through the work of Pannabecker and Dantzler and their colleagues (41). The intimate association of collecting ducts with (particularly ascending) vasa recta in the inner medulla indicates that urinary oxygen content is likely in equilibrium with inner medullary interstitial oxygen (Fig. 1). Aukland and Krog (2, 3) were able to interpret the abilities of vasoactive agents to alter urinary PO₂ through their effects on medullary oxygen supply (i.e. medullary blood flow) and oxygen demand (i.e. thick ascending limb sodium transport), even though they had no direct measure of either of these critical variables.

In 1964, Landes et al demonstrated, in humans, the ability of diuresis to increase pelvic urinary PO₂, and of infusion of hypertonic saline to reduce pelvic urinary PO₂ (29). They suggested that these effects were mediated by altered medullary blood flow. It seems likely that altered medullary metabolic activity also contributed to these effects. Leonhardt and Landes showed that the response of human pelvic urinary PO₂ to inspiration of 100% oxygen, is altered in a number of disease states, including chronic pyelonephritis, hydronephrosis,
essential hypertension, arteriolar nephrosclerosis and renal artery stenosis (29, 33). They interpreted their findings in terms of medullary perfusion, arguing that, in the absence of changes in medullary metabolic activity, the degree to which urinary PO$_2$ increases upon breathing 100% oxygen, and the rate at which it increases, is an indirect measure of medullary perfusion. They also showed reduced pelvic urinary PO$_2$ in response to vasopressin administration.

The notion that urinary (and so by implication medullary) PO$_2$ depends on oxygen utilization for sodium reabsorption in the loop of Henle was put on a firmer footing by the studies of Washington and colleagues, published in 1966 (52). They observed, in anesthetized dogs, reduced pelvic urinary PO$_2$ in response to treatments that inhibit proximal tubular sodium reabsorption (e.g. hypertonic mannitol or sodium induced diuresis) which were reversed by the loop diuretic, ethacrynic acid. These observations accorded with those of Leonhardt and colleagues, who were able to show in humans that pelvic urinary PO$_2$ is relatively low under antidiuretic conditions, but increases during diuresis (32). These considerations probably also explain why pelvic urinary PO$_2$ is relatively normal (or at least not reduced) in chronic experimental (35, 53) and clinical (5, 33, 35) renal artery stenosis, and chronic kidney disease (33), conditions likely associated with both reduced medullary oxygen delivery and oxygen consumption.

Taken together, the studies reviewed above provide strong evidence that the oxygen tension of pelvic urine provides a useful index of medullary oxygenation. But measurement of the PO$_2$ of pelvic urine in the clinical setting would be rather invasive. Might it not be possible to obtain useful information from the measurement of PO$_2$ of urine in the bladder via a catheter deployed as part of standard surgical practice?
Oxygen tension of bladder urine

Multiple factors might confound measurement of urinary PO\(_2\) in the bladder. Firstly, some materials that might be used for construction of bladder catheters, such as polyethylene (45) and silicone rubber (16) are permeable to oxygen. Polyvinyl chloride is impermeable to oxygen (3). Rennie and colleagues also found that urine could consume oxygen. In the fasted state, urinary oxygen consumption was found to be negligible. But the presence of reducing agents in the urine, after a human subject ate two oranges, was found to greatly increase urinary oxygen consumption.

Rennie and colleagues argued that oxygen exchange along the ureter would invalidate the measurement of PO\(_2\) in urine collected from a bladder catheter in humans. (44, 45). However, their observations were based on experiments in only two anesthetized dogs. Leonhardt and Landes found, in normal human subjects, that urinary PO\(_2\) fell, from ~48 mmHg to ~33 mmHg along the course of the ureter from the renal pelvis to the bladder (29, 32). Nevertheless, they were able to observe consistent changes in the PO\(_2\) of freshly voided urine in response to changes in hydration. Furthermore, they were able to show low PO\(_2\) (10-15 mmHg) in urine from the bladder of patients with circulatory shock of multiple causes or azotemia due to nephrosclerosis. Moreover, the PO\(_2\) of the urine from the bladder of these patients could be markedly increased by fluid loading, presumably due to the combined effects of increased medullary blood flow and inhibition of tubular sodium reabsorption.

More recently, Kitashiro showed an excellent correlation (\(r = 0.94\)) between the PO\(_2\) of urine in the bladder versus that in the ureter (mid-way between the kidney and bladder), in dogs subjected to treatments that alter cardiac output, even though mean PO\(_2\) of urine in the bladder was consistently (12 ± 3 mmHg) less than the PO\(_2\) of urine in the ureter (25).

Collectively, these data show that oxygen transport between urine and the epithelium of the ureter and bladder does alter urinary PO\(_2\). Nevertheless, changes in the PO\(_2\) of bladder urine
do appear to reflect those in pelvic urine. If our hypothesis is correct, urinary oxygen tension should have prognostic value in patients at risk of AKI.

**Prognostic value of urinary oxygen tension**

Kainuma studied the effects of acute changes in renal blood flow, induced by constriction of the abdominal aorta to reduce renal perfusion pressure, on the PO$_2$ of pelvic urine in anesthetized dogs (22). They found urinary PO$_2$ to fall as renal blood flow fell. Kitashiro and colleagues demonstrated good agreement between bladder urine PO$_2$ and renal cortical blood flow ($r = 0.92$) when cardiac output was manipulated in anesthetized dogs (25). Similarly, Weems and colleagues observed reduced PO$_2$ of pelvic urine during acute renal artery constriction in anesthetized dogs (53). From what we now know of the factors that influence medullary oxygenation (11), it seems likely that these observations reflect a strong correlation between medullary oxygenation (not measured in these studies) and renal blood flow under the conditions of these experiments. Other indirect evidence that changes in human urinary PO$_2$ reflect changes in medullary oxygenation include the observations in humans of increased bladder urine PO$_2$ after furosemide administration (15), although others have found the PO$_2$ of pelvic urine to fall after furosemide treatment (47). Although loop diuretics reduce medullary metabolic activity, they can also induce renal vasoconstriction in humans (50). Thus, their effects on medullary oxygenation, and so presumably urinary PO$_2$, may vary depending on the relative changes in medullary oxygen supply versus demand.

There is good evidence that measurement of urinary PO$_2$ can provide prognostic information regarding kidney health in patients undergoing CPB surgery. Kainuma and colleagues measured the PO$_2$ of bladder urine in 96 patients undergoing CPB surgery (23). They found that urinary PO$_2$ progressively decreased after the start of CPB and partially recovered at weaning from CPB. Critically, post-operative serum creatinine concentration was greater in those patients whose urinary PO$_2$ decreased after CPB, providing good evidence that it has
prognostic value. Indeed, post-operative recovery of urinary PO$_2$ ($r = -0.57$) predicted post-operative renal dysfunction (as defined by peak post-operative serum creatinine) better than did pre-operative serum creatinine ($r = 0.42$) or blood urea-nitrogen ($r = 0.43$), duration of CPB ($r = 0.40$), or post-operative cardiac index ($r = -0.33$) or urine flow ($r = -0.22$). Farahani et al also observed reduced PO$_2$ of bladder urine during CPB (12).

The PO$_2$ of bladder urine has also been shown to fall during non-cardiac surgical procedures associated with compromised renal function, including laparoscopic surgery requiring carbon dioxide pneumo-peritoneum (26), but remain unchanged during surgical procedures associated with well-maintained renal function (27, 28). Blood transfusion in anesthetized patients rendered anemic by blood loss also resulted in increased PO$_2$ of bladder urine (48). These observations are consistent with the ability of hemodilution to induce medullary hypoxia in anesthetized rats (21). Morelli and colleagues demonstrated, in stable critically ill patients, the ability of the dopamine agonist fenoldopam to increase urinary PO$_2$ measured via a sensor inserted into the bladder, in association with a brisk diuresis and natriuresis (36). They attributed the effects of fenoldopam to increased medullary perfusion, but did not directly measure any indices of renal hemodynamics or renal metabolic activity.

Collectively, the experimental and clinical observations described above demonstrate the potential for urinary PO$_2$ to provide a novel biomarker for risk of AKI. But there are numerous challenges that need to be overcome before this approach to be employed in a wide-spread manner.

**Challenges for clinical translation of urinary PO$_2$**

**Critical unknowns**

In multiple studies, performed from the early 1960s to late 1990s, in both anesthetized dogs and conscious and anesthetized humans, urinary PO$_2$ was found to be markedly altered by
maneuvers that were presumed to alter either blood flow or metabolic activity in the medulla (3, 15, 29, 33, 52) or total renal blood flow (22, 25, 53) The critical term here is presumed, because at that time there were no (or limited) methods available to measure medullary perfusion, and measuring kidney tissue PO₂ was technically challenging. Consequently, the only direct evidence that urinary PO₂ reflects medullary tissue PO₂ comes from observations in the mid-1960s in three patients (34). In a total of 9 paired measurements (3 per patient), there was excellent agreement between pelvic urinary PO₂ and medullary tissue PO₂ (Fig. 1). As compelling as these preliminary data are, they hardly constitute the level of evidence required for translation of urinary PO₂ to the clinical setting as a diagnostic or prognostic tool. Thus, there remains a need for experiments in anesthetized animals to fully characterize the relationship between medullary tissue PO₂ and urinary PO₂.

We also require a better understanding of the impact, on urinary PO₂, of oxygen transport across the epithelium of the ureter and bladder. Indeed, it should be possible to use the results of studies in experimental animals, which quantify the impact of such oxygen transport, as the basis of a computational model to predict pelvic urine (and thus medullary) PO₂ from the PO₂ of urine bladder. Such models could also incorporate data from additional sources, such as urine production rate, body temperature, arterial blood gas status, and cardiac output, to allow estimation of the PO₂ of pelvic urine under a range of conditions.

**Methods for measurement of urinary PO₂**

Consideration should be given to the best approach for measurement of the PO₂ of bladder urine in humans. Polarographic electrodes have been used extensively (3, 22, 25, 29, 33, 36, 52, 53). This is unlikely to be a feasible approach for bedside monitoring, both because of the fragile nature of these electrodes and because it is not possible to provide complete electrical isolation of the patient. Urine oximetry could also potentially be performed using a standard blood gas analyser (48), although this would require considerable care during collection of
samples and hardly constitutes a method for continuous measurement of urinary PO$_2$. MRI can be used to measure urinary PO$_2$ in a non-invasive manner (51). However, MRI would hardly be a feasible approach for monitoring patients in the operating theatre or intensive care unit. Our favoured approach would be the use of fiber optic probes that could be inserted into a standard bladder catheter without blocking urine flow. These probes, such as those that operate on fluorescence lifetime oximetry (http://www.oxford-optronix.com/sensor12/sensor-for-Oxygen-Monitors.html), have to our knowledge not yet been used for measurement of urinary PO$_2$ in animals or humans and are certainly not currently approved for human use. Nevertheless, they have the advantage that they are relatively sturdy, are pre-calibrated by the manufacturer, and can be operated with complete electrical isolation from the patient. They have been applied to in vivo measurement of the PO$_2$ of a range of tissues (including the kidney (10)) and body fluids (e.g. oviductal fluid (42)).

**How could this new information be used?**

Clinical trials of the utility of measurement of urinary oxygen tension should initially focus on the potential of this biomarker to predict development of AKI. However, if it can be shown to be prognostically useful, we also see the potential for urinary PO$_2$ to provide feedback to guide the on-going management of renal health in individual patients, including in the operating theatre and intensive care unit. Given the complexity of kidney oxygenation, the real challenge in implementing this would be to provide some level of predictive certainty for clinicians when they make decisions about the management of their patients. For example, during CPB surgery it would not be a trivial matter to predict how changes in pump flow or hematocrit would translate to changes in total and regional blood flow and glomerular filtration rate, and thus renal tissue oxygen delivery and consumption. One way in which this could be achieved is through the use of a computational model of kidney oxygenation to aid clinical decision-making. Clearly, such a model would require extensive validation in the clinical setting before it could be fully implemented.
Conclusions

Urinary PO$_2$ was recently identified as one of a panel of potential ‘physiological’ biomarkers of AKI (40). Our review of the literature indicates to us that this potential merits further investigation in studies in both experimental animals and humans. We suspect that this approach might be particularly useful in patients undergoing CPB surgery, and in the intensive care unit, where the risk of development of AKI is high and the placement of a bladder catheter is relatively routine.

Acknowledgements

The authors’ work is supported by grants from the National Health and Medical Research Council of Australia (606601 & 1024575).

Disclosures

None
References


Urinary oxygen tension


Fig. 1 Evidence that urinary oxygen tension (PO2) provides an index of medullary tissue PO2. Panels A-C, reproduced from Pannabecker and Dantzler (41) (with permission from the American Physiological Society), show the intimate relationship between vasa recta (particularly ascending vasa recta) and the collecting ducts. Panels A and B show three dimensional reconstructions of a cluster of collecting ducts in the rat inner medulla. Scale bars = 500 µm. Panel A shows descending vasa recta in green, the descending thin limbs of the loop of Henle in red or grey, and collecting ducts in blue. Panel B shows the same cluster of collecting ducts (in blue) but ascending vasa recta are shown in red and ascending thin limbs are shown in green. Panel C shows a transverse section from near the base of the inner medulla, showing ascending vasa recta (red) positioned around collecting ducts (blue). Descending vasa recta are shown in green. Scale bar = 30 µm. Panel D shows the results of 9 paired measurements in three human subjects, of urinary PO2 and mean medullary PO2. The line of identity is also shown (dashed). The figure was re-drawn from Leonhardt et al (34).