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Changes in Intrarenal Oxygenation as Evaluated by BOLD MRI in a Rat Kidney Model for Radiocontrast Nephropathy

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Abstract

The pathogenesis of radiocontrast nephropathy is poorly understood. In an animal model, inhibition of the synthesis of nitric oxide and prostaglandins appears to predispose rats to severe renal injury following the administration of radiocontrast. Here we have investigated whether administration of radiocontrast, as well as changes in renal medullary oxygenation following pharmacologic inhibition of nitric oxide and prostaglandin synthesis, might be evaluated by blood oxygenation level-dependent (BOLD) MRI. Nineteen anesthetized (Inactin 100 mg/kg) rats were studied. BOLD MRI measurements were performed following administration of L-NAME (N-nitro-L-arginine methyl ester, 10 mg/kg), Indomethacin (10 mg/kg), and a radio-contrast agent (sodium iohalamate 60%, 6 mL/kg). Marked sequential changes in medullary R_2^* , presumably reflecting decline in medullary pO_2 , were noted after each of the pharmacological interventions employed. These results, obtained by noninvasive MRI, are consistent with prior direct recordings of pO_2 and doppler flow in the rat renal medulla after administration of L-NAME, Indomethacin and iohalamate. Medullary oxygenation in rats was reduced by inhibition of the synthesis of prostaglandins and nitric oxide, as well as by intravenous injection of radiocontrast agents. BOLD MRI can noninvasively evaluate changes in medullary oxygenation in rats that appear to predispose acute renal failure.

Index terms

renal medulla; oxygenation; radiocontrast; acute renal failure; MRI

Transient or irreversible renal failure induced by intravenous radiographic contrast agents (radiocontrast media induced nephropathy (RCIN)), is said to be the third most common cause of hospital acquired renal failure (1), accounting for more than 30% of all such patients with this iatrogenic disorder (2). Patients with pre-existing renal insufficiency, diabetes or congestive heart failure that are exposed to a large volume of high-osmolality radiocontrast (3) are at increased risk of developing RCIN. Renal hemodynamic changes are thought to play a key role in RCIN. The exact pathophysiological mechanisms are poorly understood, though disturbed endothelial dysfunction has been surmised (4). Nitric oxide and prostaglandins are important vasoactive mediators released by the vascular endothelium. Both play a role in the homeostasis of oxygen supply to the renal medulla, a region normally

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operating on the verge of hypoxia. Inhibition of these vasoconstrictors predisposed rats to severe renal injury following radiocontrast administration in studies employing invasive doppler flow probe measurements of regional blood flow (5).

The effects of radiocontrast agents on renal hemodynamics has been studied extensively in rat kidneys (6–12). The laboratory rat has also been used in a variety of experiments designed to study the mechanism of the other forms of acute renal failure (13–15) and their therapy (12,16–19). Such preventive strategies are usually designed to improve or maintain medullary oxygenation. Until now, a limitation in the effective testing of these hypotheses has been the absence of a suitable non-invasive, reproducible method to assess regional oxygenation of rat kidneys. BOLD MRI promises to be such a technique.

We have previously demonstrated that BOLD MRI can evaluate intra-renal oxygenation in a non-invasive way in humans (20,21). The technique is ideally suited to estimate sequential changes in medullary oxygenation owing to the relatively low tissue pO_2 localized in this region (22). Our studies in humans have focused both on maneuvers that improve medullary oxygenation and circumstances that might block such improvement (23). In order to apply this technique to animal models, we have developed a BOLD MRI method to image rat kidneys (24). Here we investigated whether BOLD MRI would be sensitive both to reductions in renal medullary oxygenation that follow pharmacologic inhibition of the synthesis of nitric oxide and prostaglandins, and the administration of a radiocontrast agent (5).

METHODS

Animal Preparation

Nineteen anesthetized (inactin 100 mg/kg) Sprague-Dawley rats weighing 44.8 ± 9 g (mean $\pm$ s.e.) were studied. All studies were approved by the institutional animal care and use committee. A femoral vein was cannulated for the administration of a nitric oxide synthase inhibitor (L-NAME), an inhibitor of prostaglandin synthesis (Indomethacin, 10 mg/kg), and a radiocontrast agent (Angio-Conray 60%, 6mL/kg). Four sets of experiments were performed in which these substances were administered in different order:

n = 3, Baseline, Indomethacin, L-NAME

n = 6, Baseline, L-NAME, Indomethacin

n = 7, Baseline, L-NAME, Indomethacin, radiocontrast

n = 3, radiocontrast alone

Imaging

All studies were performed on a 1.5 T whole-body MR scanner (Vision; Siemens Medical Systems, Erlangen, Germany) using a multiple gradient recalled echo (mGRE) sequence (TR/TE/FA= 60 msec/10–56 msec/30°) to acquire 16 T_2^* -weighted images (24). Other relevant parameters were FOV = 128 mm, Matrix size = 256×256 . The result was an effective pixel size of 0.5×0.5 mm, slice thickness = 3 mm. An R_2^* ($1/T_2^*$) map was reconstructed online, based on the signal of the sixteen T_2^* -weighted images (24). A flexible wrist coil was used to receive the signal. Rats were placed on their right side to reduce the susceptibility artifacts on the kidneys caused by bowel gas. We obtained baseline BOLD MRI data and followed the R_2^* changes every three minutes after administration of the pharmacological agents. We acquired images for 15 minutes following the injection of each of the pharmacological agents.

The R_2^* was measured using regions of interest (ROI, consisting of at least six pixels) in the renal medulla and cortex. When evaluating the time series, the same ROI locations were utilized for each image in the series. Statistical significance of the differences in the pre- vs. post-pharmacologic maneuver was assessed based on a two-tailed paired Student's t-test.

RESULTS

Figure 1a shows a set of R_2^* maps of the right kidney before and after administration of L-NAME followed by Indomethacin and iohalamate. Figure 1b plots the corresponding R_2^* values in the cortex and medulla. Table 1 summarizes R_2^* and ΔR_2^* for cortex and medulla, measured at 15 minutes after administration of each of the pharmacological agents. Note the increase in R_2^* values following administration of L-NAME and Indomethacin, signifying reductions in the oxygenation status of the medulla. After iohalamate was injected intravenously, there was an immediate but short lived fall in medullary R_2^* (consistent with brief medullary hyperemia) and an increase in pO_2 . This was followed consistently by a rapid return to baseline hypoxic levels. This hyperemic response following iohalamate was seen whether or not the animals had been pretreated with Indomethacin and L-NAME, but the final levels of medullary R_2^* , measured 15 minutes after injection, were much lower in the latter case (Table 1).

DISCUSSION

The marked hypoxia of renal medulla, as compared with the renal cortex and most other body tissues, has important implications for human disease (22). In particular, it has been postulated that anoxic injury to renal medullary structures initiates the phenomenon of acute renal failure (25). If so, physiological or pharmacological maneuvers that adversely influence medullary oxygenation should confer susceptibility to acute renal failure. Consistent with this hypothesis, it has been shown in rats that inhibition of the synthesis of nitric oxide and prostaglandins predisposes renal failure from intravascular injection of iohalamate characterized by extensive necrosis of medullary, thick, ascending limbs (5). This model of radiocontrast nephropathy does not require preliminary uninephrectomy or chronic salt depletion (13,26).

As seen in Fig. 1 and Table 1, the BOLD MRI technique readily detects the reduction in medullary oxygenation that follows the administration of various pharmacologic agents. Owing to the relatively small number of animals used for Indomethacin and Indomethacin + LNAME, the changes in R_2^* did not reach statistical significance. However, the changes after L-NAME and Indomethacin appear comparable, and their effects are additive, regardless of the order of administration. By comparison, the effect of these agents on cortical R_2^* was much less pronounced. These changes in medullary R_2^* values following administration of L-NAME and Indomethacin are consistent with the changes in regional blood flow that have been measured in rats (5). The observed medullary changes in R_2^* are also consistent with previous invasive pO_2 measurements following nitric-oxide inhibition by L-NMMA (26) and Indomethacin (27). BOLD MRI, however, may be less sensitive to changes in blood flow in the renal cortex than in the medulla. This is due to the relatively high baseline pO_2 of the cortex and to the fact that oxygen utilization in the renal cortex tends to be proportional to blood flow.

The reason for the transient decrease in medullary R_2^* values immediately following radiocontrast administration is not quite clear. Agmon et al. (5) report an increase in medullary blood flow (consistent with our observation of transient improvement in

medullary pO_2) following iohalamate without pretreatment. Conversely, Heyman et al. (27) noticed a drop in pO_2 following iohalamate. Agmon et al. (5) also found a small drop in blood flow following iohalamate administered after pretreatment with L-NAME and Indomethacin. In the experiments of Heyman (27) and Agmon (5), however, the right kidney had been exposed through a long abdominal incision, then de-encapsulated and fixed in position, whereas the present data with BOLD MRI was obtained from intact rats not subjected to surgical stress. It seems possible that the transient increase in medullary pO_2 observed directly after intravenous injection of iohalamate may have been a consequence of sudden expansion of the blood volume and, perhaps, osmotic diuresis.

The marked and sustained falls in medullary pO_2 seen after Indomethacin or L-NAME have important clinical implications. Non-steroidal anti-inflammatory drugs that inhibit prostaglandin synthesis are known to predispose acute renal failure (28), and the chronic use of similar agents has been implicated in the papillary necrosis of analgesic nephropathy (29). A theoretical concern in the clinical use of inhibitors of nitric oxide synthase for septic shock and hepatorenal syndrome is the possible deleterious effect of such therapy on the oxygenation of the renal medulla. It seems possible that patients with disorders of renal synthesis of prostaglandin or nitric oxide may be unusually susceptible to ischemic renal injury in clinical situations that predispose acute renal failure. Finally, it would be prudent to ensure that drugs inhibiting prostaglandin synthesis are interdicted for patients scheduled to receive radiocontrast agents in diagnostic tests.

Further studies are warranted to correlate the observed BOLD MRI changes with both conventional renal function measurements and histologic changes in the medulla. Future studies are also necessary to elucidate the transient decrease in medullary R_2^* following radio-contrast administration. It may also be possible to correlate the BOLD MRI responses to direct and simultaneous measurements of tissue pO_2 and regional blood flow using novel optical probe technology (30).

PRACTICAL APPLICATIONS

The BOLD MRI technique facilitates studies that elucidate pathophysiological mechanisms responsible for acute renal failure, both in humans and in animal models. The present data and our earlier studies in humans permit the conjecture that some day the application of the technique might help to “identify with greater sensitivity those patients at high risk for ischemic or nephrotoxic renal injury” (31).

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Figure 1.

a: The R_2^* map obtained at different time points in one representative animal. The top row shows the baseline images. The next three rows are images obtained after L-NAME, Indomethacin, and iohalamate, respectively. The five images in these rows were obtained at three-minute intervals for 15 minutes after administration of the agent. The brighter inner concentric region is the medulla, and the relatively darker outer region is the cortex. Higher R_2^* values imply relatively lower blood pO_2 , hence lower tissue pO_2 . **b:** Plot describing the temporal changes in R_2^* for cortex and medulla following the administration of L-NAME, Indomethacin, and radiocontrast. The vertical lines indicate the time of administration of each of the agents. Error bars represent standard deviation within the ROI of this one representative animal.

Table 1

R_2^* of Medulla and Cortex Following Various Pharmacologic Agents

	R_2^* in Medulla (Hz)		R_2^* in Cortex (Hz)		ΔR_2^* (Hz)	
	Baseline	Post	Baseline	Post	Medulla	Cortex
Indomethacin, $n=3$	21.6 $\pm$ 3.6	29.5 $\pm$ 6.1	18.4 $\pm$ 2.1	19.3 $\pm$ 2.3	7.9 $\pm$ 6.6	0.8 $\pm$ 0.5
Indomethacin + L-NAME, $n=3$	—	40.2 $\pm$ 5.3	—	24.9 $\pm$ 0.7	18.6 $\pm$ 2.5	6.4 $\pm$ 2.6
L-NAME, $n=13$	22.8 $\pm$ 1.0	32.2 $\pm$ 1.6 ^a	18.3 $\pm$ 0.6	21.6 $\pm$ 1.1 ^a	9.3 $\pm$ 1.3	3.8 $\pm$ 0.9
L-NAME + indomethacin, $n=13$	—	39.9 $\pm$ 2.8 ^b	—	24.0 $\pm$ 1.5	15.5 $\pm$ 2.2	6.2 $\pm$ 1.0
L-NAME + indomethacin + Contrast, $n=7$	—	37.4 $\pm$ 4.1	—	20.5 $\pm$ 1.4	15.3 $\pm$ 3.3	2.1 $\pm$ 1.2
Contrast, $n=3$	26.4 $\pm$ 5.6	24.0 $\pm$ 2.9	21.0 $\pm$ 1.7	17.3 $\pm$ 2.8	-2.4 $\pm$ 1.7	-3.7 $\pm$ 1.2

ΔR_2^* , $R_2^*(\text{post}) - R_2^*(\text{baseline})$; $R_2^*(\text{post})$ measured at 15 minutes after administration of the respective pharmacologic agent and $R_2^*(\text{baseline})$ is the measurement made before any agent is administered. All values shown are mean $\pm$ s.e.

^a $P < 0.05$ for two-tailed Student t-test when compared with baseline.

^b $P < 0.05$ for two-tailed Student t-test when compared with post-L-NAME.