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Intra-Renal Oxygenation in Rat Kidneys During Water-loading: Effects of COX Inhibition & NO Donation

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Abstract

Purpose—To evaluate intra-renal oxygenation by blood oxygenation level dependent magnetic resonance imaging (BOLD MRI) in rat kidneys during water-loading and to investigate if the nitric oxide donating moiety in naproxen could compensate the effect of cyclooxygenase inhibition of naproxen.

Materials and Methods—Nineteen male Sprague Dawley rats were divided into three groups and dosed with vehicle, naproxen or naproxenod by gavage for two weeks. On the day of the experiment, hypotonic saline with glucose was infused intravenously to induce water diuresis. BOLD MRI data to monitor renal oxygenation and timed urine samples for estimation of prostaglandins and urine flow were obtained.

Results—The data in this study is consistent with previous experience in humans in that pre-treatment with naproxen abolished the improvement in medullary oxygenation during water-loading. In addition, the inhibition of prostaglandins by naproxenod reached similar levels as naproxen but maintained the improvement in oxygenation in renal medulla during water-loading.

Conclusion—This suggests that naproxenod may have less nephrotoxicity and that the nitric oxide donating moiety partially compensates for the hemodynamic effects of prostaglandin inhibition by naproxen.

Keywords

kidney; oxygenation; BOLD; cyclooxygenase inhibition; nitric oxide donation

INTRODUCTION

The role of renal medullary hypoxia is well accepted in the context of acute (1) and chronic kidney disease settings (2,3). Evaluating factors that influence renal oxygenation status is hence important both for understanding pathophysiology and in developing preventive and/or interventional strategies.

Non-steroidal anti-inflammatory drugs (NSAIDs) have been widely used to treat pain and inflammation. NSAIDs are very effective for pain management (4,5). However, it is also

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known that chronic consumption of NSAIDs has broad range of adverse nephrotoxic effects on the kidneys (6,7). Unfortunately, the use of NSAIDs is on the rise, particularly in the elderly population owing to chronic conditions such as arthritis (8).

NSAIDs primarily inhibit the enzyme cyclooxygenase (COX) (9), which is responsible for the production of prostaglandins. PGs regulate vascular tone, control tubular function and renin release (10). Among the many effects of PGs, especially PGE₂, is to increase blood flow in the kidney (11). On the other hand, PGs are also involved in developing inflammation, pain, and fever (12). Hence, these symptoms can be relieved by inhibiting COX.

A recent attempt to mitigate the ill effects of NSAIDs has been the design of the Cyclooxygenase-Inhibiting Nitric Oxide Donators (CINOD), which have demonstrated preliminary benefits to cardiovascular and renal functions (13–15). One such drug is naproxcinod (16,17) in which the NSAID part is represented by naproxen. During Phase II and III clinical trials, the drug has been shown to be equally effective compared to equimolar doses of naproxen in pain management while more protective to the GI tract (18). Also demonstrated a reduced pressor effect of naproxcinod when compared to naproxen (13).

Acute effects of NSAIDs on intrarenal oxygenation have been evaluated using blood oxygenation level dependent (BOLD) MRI in humans (19,20). Ibuprofen (19) and naproxen (20) have been shown to abolish the improvement of renal medullary oxygenation during water-loading. However, the effects of water-loading in rats have not been extensively studied even though effects of prostaglandin inhibition on renal BOLD MRI have been previously evaluated (21). Since naproxcinod is not yet approved for human use, we have for the first time implemented a water-loading paradigm to evaluate its effects on intra-renal oxygenation in rat kidneys using BOLD MRI.

In this preliminary study, we wanted to demonstrate feasibility of performing waterload studies in rats using BOLD MRI and test the hypothesis that the intra renal oxygenation level will show improvement during water diuresis in animals pre-treated with naproxcinod compared to those with naproxen.

MATERIALS AND METHODS

The study protocol was approved by the Institutional Animal Care and Use Committee. The rats were purchased from Charles Rivers (Chicago, IL, US) and housed at the institutional animal care facility that is approved by the American Association for the Accreditation of Laboratory Animal Care. The rats were housed for at least three days before the experiments. The rats had free access to food and water throughout the study. Nineteen male Sprague Dawley rats (weight 318.6 ± 6.5 grams) were randomly divided into three groups. Group 1 of six rats were fed with vehicle (carboxymethylcellulose/DMSO, Sigma Chemical Co., St. Louis, MO, USA), group 2 of six rats were fed with equimolar naproxen (10mg/kg, Sigma Chemical Co., St. Louis, MO, USA), and group 3 of seven rats were fed with naproxcinod (14.5 mg/kg, NicOx SA, Sophia Antipolis, France). Each group had been dosed by gavage for two weeks prior to scanning. There was no significant weight or age difference among groups.

On the day of the experiment, rats were anesthetized with Ketamine (60–100 mg/kg ip, Abbott Laboratories, North Chicago, IL, USA) and thiobutabarbital (Inactin, 100 mg/kg ip, Sigma Chemical Co., St. Louis, MO, USA). A catheter (PE-50) was placed in femoral vein to induce water diuresis by infusion of hypotonic saline (0.25% NaCl, 0.5% glucose) (22,23) using an infusion pump (Genie Plus, Kent Scientific, Litchfield, CT, USA). The urinary

bladder was catheterized through a suprapubic incision for urine collection. Urine was sampled every 10 minutes for volume and PGE₂ estimation after three continuous scans using multiple gradient recalled echo sequence (mGRE). The urine volume was calculated gravimetrically. The baseline and 90 min urine samples were sent for analysis of PGE₂ content to Cayman Chemical (Ann Arbor, Michigan, USA).

MRI acquisitions were performed on a short bore Signa Twin speed 3.0T (GE Healthcare) using a multiple gradient echo sequence (TR/TE/Flip angle/BW/matrix/Thk = 70ms/4.4–57.7ms/30°/42 kHz/256×256/2mm) to acquire 16 T₂* weighted images similar to previous reports (24). FOV is 10 × 6 cm. In order to minimize artifacts produced by respiration motion, multiple acquisitions were performed for averaging. Image acquisitions were performed during both positive and negative lobes of the read out gradient. Only auto shimming was used. A quadrature extremity coil was used for signal reception. Animals were placed on their right side inside the coil to minimize bulk susceptibility artifacts from bowel gas. The signal intensity vs. time data was fit to a single exponential function to generate R₂* map using the FUNCTOOL (GE Healthcare, Waukesha, WI, USA). After obtaining three sets of baseline images, hypotonic saline containing glucose was infused at 1.5ml/100g body weight/hour *via i.v.* for 2 hours to induce the water-diuresis in each rat. R₂* maps were obtained every 3 minutes for 2 hours. Regions of interest (ROIs) were placed on renal medulla and cortex area in anatomic image and the ROIs were automatically copied to R₂* map in FUNCTOOL. The mean and standard deviation of R₂* were recorded. An increase in R₂* implying a decrease in oxygenation and vice versa.

The statistical significance of the differences between pre- and post-diuresis (90 mins) measurements for PGE₂, urine flow and medullary R₂* (MR₂*) and cortical R₂* (CR₂*) was evaluated by two-tailed paired Student's t-test. Since R₂* measurements in each rat was made in a longitudinal fashion and multiple observations within each animal were correlated, a linear mixed-effects model with appropriate variance-covariance structure (first-order auto-regressive moving average) was used to assess the difference between the groups, treating group and time as fixed effects and individual rat as random effects. The most appropriate variance-covariance structure was determined by AIC (Akaike's Information Criterion). We also used restricted maximum likelihood approach to obtain parameter estimates given the small sample size. From these, a linear slope of change over time was estimated for MR₂* and CR₂* for each group. *p* < 0.05 was considered for statistical significance. SAS 9.1 (Cary, NC, USA) was used to perform the statistical analyses.

RESULTS

Figure 1 shows representative images of rat kidney acquired in this study. Shown are an anatomic image (one of the T₂* weighted images) and calculated R₂* map.

Figure 2 summarizes the temporal response during water diuresis in MR₂* and CR₂* measurements acquired in all rats pretreated with either naproxen, naproxinod or vehicle. The MR₂* decreased with time in control group suggesting an improvement in oxygenation as shown in Figure 2(A). A similar improvement of oxygenation in renal medulla was completely abolished in the naproxen group indicated by no change in MR₂* during water diuresis, which is consistent with previous findings in humans (20). In the naproxinod group, the MR₂* response to water-loading appears similar to control group, even though the urinary PGE₂ (Figure 3(B)) is substantially reduced in both naproxen and naproxinod groups. Figure 2(B) shows the temporal response to water-loading in cortical R₂* in the three groups of rats. As seen, the CR₂* decreases only slightly with time in all three groups.

Figure 3 summarizes urine flow, PGE₂ and R₂* measurements. The control group produced the largest increase in urine flow during water diuresis (A). At baseline, the naproxen and naproxinod groups had lower PGE₂ level than the control group, consistent with COX inhibition (B). The post water diuresis PGE₂ in the control group showed that it reduced considerably but did not reach statistical significance due to the large variations among individuals. The PGE₂ level increased greatly in naproxinod group and also lacked statistical significance due to the large variations among individuals. There was no significant difference in baseline MR₂* and CR₂* among the three groups (C, D), even though the mean values were noticeably different. The post MR₂* at 90' shows a significant reduction only in control group, and not in naproxinod and naproxen group.

The use of mixed effects model analysis inherently assumes of normality and heteroscedasticity of the data. Based on the descriptive statistics (mean, standard deviation, etc.) over time by group for CR₂* and MR₂* (data not shown), MR₂* measures from rat#4 in the naproxen and rat#1 in the naproxinod groups were excluded from the mixed effects model estimation as they were outliers and severely digressed from the normality and homogeneous assumptions. Table 1 summarizes the mixed effects model estimates for MR₂* and CR₂*. It shows that water-loading in control and naproxinod groups resulted in significantly decreased R₂* over time both in cortex and medulla while measures in naproxen group remained constant over time. MR₂* tended to decrease faster than CR₂* in control and naproxinod groups. A first-order autoregressive moving average variance-covariance structure was specified for all mixed effects models.

DISCUSSION

Measurements of oxygen consumption in the kidneys have been pursued for more than half a century. Early measurements depended on the whole organ determinations by evaluating arterio-venous difference in oxygen saturation (25). These studies demonstrated very small difference in arterio-venous oxygen saturation across the kidney and consequently also resulted in minimal change following water-loading. However, later measurements involving invasive microelectrodes illustrated a significant gradient in tissue oxygenation within the kidney (26). In fact, it was suggested that the kidney should be considered to be made up of two organs, the cortex and the medulla (27) based on their significant hemodynamic differences. While the cortex is supplied with blood flow far in excess of its metabolic needs, the medulla receives very little supply. Further, active transport of sodium chloride in the medullary thick ascending limbs is associated with high energy demand. Together, the medullary oxygenation is commonly described as being at hypoxic levels (1).

Measurement of tissue oxygenation using microelectrodes is a labor intensive and extremely fragile technique and hence limited to a handful of laboratories around the world. Availability of alternate and more robust technologies could allow for more widespread use of these measurements. BOLD MRI uses blood as a reporter of tissue oxygenation (28). It has been shown to be very useful in monitoring intrarenal oxygenation (29–33). A major advantage of the non-invasive BOLD MRI is the ability to translate findings in animal models to humans. This is the first time, by our knowledge, that oxygenation changes during water-loading have been investigated in a small animal model by any method. Our findings with BOLD MRI are consistent with previous experience in humans (6,19,20). MR₂* was significantly lower during water-loading in the control group, suggesting an improved renal medullary oxygenation, while R₂* showed minimal change in naproxen group (Figure 3 & Table 1). These results are consistent with the previously reported human data (20). The novel finding in this study is that the naproxinod group shows a response to water-loading similar to the control group. This finding supports our hypothesis that the NO donor added to naproxen could effectively compensate for the hemodynamic effects of COX inhibition.

This may indicate that naproxcinod has similar efficacy in anti-inflammatory effect and pain relief as traditional NSAIDs as shown by previous reports (18), but may reduce the nephrotoxicity associated with chronic NSAID use.

The magnitude of change in MR_2^* reported in this study (~10%) is much smaller than our previous studies in healthy young humans (27.1% in (20) and 29.6% in (19)). This may be related to the differences in the water-loading maneuver. In humans, water was orally ingested at 20 ml/kg within a short duration of time (~15 mins). In this study, hypotonic saline (15 ml/kg) was infused *via i.v.* over a period of 2 hours. This is also reflected in the urine flow changes during water-loading. In the present study, in control group the flow increased by a factor of 10 (from 0.01 to 0.1 ml/min) compared to young human subjects where the flow increased by a factor of ~25 (from 0.44 to 10.9 ml/min).

The urine flow rate increased in all groups during water-loading (90 min) compared to baseline (Figure 3(A)). Both naproxcinod and naproxen groups had less of an increase in urine flow during water-loading compared to the control group, but only the naproxen group reached statistical significance.

The urinary PGE_2 level showed substantial reduction in both naproxen and naproxcinod groups in baseline compared to the control group (Figure 3(B)). However, the inter-animal variability in PGE_2 estimation was relatively high both in the control and naproxcinod groups resulting in no statistical significance (Figure 3(B)). The previous study in humans showed an increase in PGE_2 following a water diuresis in healthy young volunteers (19). We fail to see a similar effect in the control group in this study. It may be explained by the fact that the volume of urine samples in rats is much less compared to humans, and urinary PGE_2 excretion varies directly with urine flow rate (34). Also, we only used females in the human study because in males the prostate also contributes to the urinary PGE_2 . We used male rats in this study.

The trend of R_2^* response to water-loading in naproxcinod group is similar with the control group as shown in Figure 2. However none of the individual post water-loading measurements reached statistical significance when compared to the baseline. This is the reason we used all the data points during the water-loading to look for statistical significance. A linear mixed-effects model with appropriate variance-covariance structure did show statistical difference between baseline and post water-loading measurements in the control and naproxcinod groups.

In conclusion, the preliminary results presented here suggest that renal oxygenation changes during water-loading can be investigated in rodent models. The results in control and naproxen group are consistent with previous human data. The novel finding is that the NO-donating moiety of naproxcinoid could mitigate the renal hemodynamic consequences of COX inhibition. Further studies with larger number of animals, and probably other confirmatory studies may be needed to fully validate these observations. If and when naproxcinod is approved for human use, these findings could be translated in humans.

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Figure 1.

Example of anatomic image and corresponding R_2^* map from one representative rat in the control group. The image in the left is the anatomic image. The R_2^* map displayed as gray scale (in the middle) and color (on the right). Arrow points to the renal medulla and cortex. The renal medulla in the R_2^* map is relatively brighter compared to the cortex signifying a lower oxygenation there.

Figure 2.

Temporal change in medullary (A) and cortical (B) R_2^* . Summary plots show R_2^* response in renal medulla to water-load in three groups of rats (control n=6; naproxen n=6; naproxinod n=7). Considering the variation of the baseline measurement across the groups, data was normalized and expressed as a percentage of the baseline value. Time zero is the baseline before induction of water diuresis. Each point is the average of three measurements in 10 minutes interval. Error bars represent the standard error over the different animals.

Figure 3.

Summary of urine flow (A), PGE₂ (B) and R₂* (C, D) measurements. Data expressed as mean±SE and post represents 90 min time point. In order to illustrate the small changes observed in the naproxen group, the y axis on (B) was plotted using a logarithmic scale. Error bars represent the standard error in the measurements from different animals. * implies $p < 0.05$ compared to baseline by two tailed Student's t-test.

Table 1

Linear mixed effects model for MR_2^* (s^{-1}) and CR_2^* (s^{-1}) vs. time (up to 90 minutes)

MR_2^* in	Slope estimate	SE	P value
Control	<i>-0.044</i>	0.011	<i><.0001</i>
Naproxen	-0.001	0.012	0.924
Naproxcinod	<i>-0.027</i>	0.011	<i>0.015</i>
CR_2^* in	Slope estimate	SE	P value
Control	<i>-0.013</i>	0.006	<i>0.030</i>
Naproxen	-0.001	0.006	0.833
Naproxcinod	<i>-0.014</i>	0.005	<i>0.008</i>

SE: standard error