Effect of NOS Inhibition by L-NAME on Intra-Renal BOLD MRI: Dose Response in Rat and Human Kidneys

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INTRODUCTION

Previous BOLD MRI with NOS inhibition with rat kidneys have been performed using 10 mg/kg of L-NAME (N^G-nitro-L-arginine methyl ester) administered as a single bolus. However, when translating to human use, the proposed methods in the literature suggest a maximum dose to-date of 4 mg/kg administered as an infusion over 30 mins. Based on our preliminary experience the effect of another NOS inhibitor L-NMMA (N^G-monomethyl-L-arginine, 4.25 mg/kg) [Proc ISMRM 2004:565] and L-NAME (2 mg/kg) resulted in a change in R_2* much smaller compared to what was seen in rat kidneys with bolus administrations. Also, when using infusion it is important to know the temporal response. In this study, we have evaluated the effect of dose of L-NAME in rat and human kidneys, and obtained the temporal response during and after the 30 min infusion period.

METHODS

Rat Studies: Studies were performed in anesthetized (Inactin 100 mg/kg) Sprague-Dawley rats (n=6; wt: 339.2±10.3gm; age=12 weeks, Harlan Laboratoy, WI). Blood pressure was monitored *via* femoral artery using an invasive monitor DBP1001 (Kent Scientific Co., Torrington, CT, USA) and Powerlab data acquisition system (ADInstruments, Colorado Springs, CO). After obtaining a set of baseline, L-NAME (Sigma-Aldrich, St. Louis, MO, USA) solution (2, 4, 10, mg/kg) was infused using PHD 2000 (Harvard Apparatus, Holiston, MA, USA) *via* femoral vein for 30 minutes. MRI studies were performed on a short bore Signa Twin speed 3.0T (GE Healthcare, Milwaukee, WI) using a multiple gradient echo sequence (TR/TE/Flip angle/FOV/BW/matrix/Thk/NXE = 70ms/4.4-57.7ms /30^o /42 kHz /256x256 /2mm /10) to acquire 16 T_2* weighted images. A quadrature extremity coil was used for signal reception. The signal intensity *vs.* time data was fit to a single exponential function to obtain R_2* using the FUNCTOOL. Regions of interest of at least 4 pixels were placed on renal medulla to obtain values for the mean and standard deviation of R_2*. R_2* and MAP were obtained every 3 minutes for one hour.

Human Studies: To-date, four healthy male subjects (31.3±6.3 years old) participated in this study and were scanned on two different days with different doses of L-NAME. Each volunteer gave informed consent to a protocol approved by our Institutional Review Board. The subjects came to the study after abstaining from food and water for about 12 hours. The arterial blood pressure was monitored every 3 minutes using MRI compatible patient monitoring system (Magnitude, Invivo, Orlando, FL, USA). After obtaining baseline BOLD MRI data, L-NAME (Clinalfa, Bad Soden, Germany); 2mg/kg or 4mg/kg was infused intravenously by infusion pump (Medfusion 2010, Duluth, GA, USA) over 30 minutes. L-arginine (Clinalfa, Bad Soden, Germany) 200mg/kg was infused for 15 minutes afterward to reverse the effects of L-NAME. Post-L-NAME images were continually acquired from 5 axial slices during and following the infusion of L-NAME in 3 minutes interval. The experiments were conducted on a GE Signa Vh*i* 3.0T whole body scanner (GE Medical Systems, Milwaukee, WI) using a multiple gradient echo (mGRE) sequence (TR/TE/Flip angle/BW=60/6.4-40.8ms/30/62.5 kHz) with selective water excitation pulse to acquire 16 T_2* weighted images within a single breath-hold of about 12 s. A FOV 36x27 cm with 256 by 256 matrix size applied. A standard four-coil torso array was used for signal reception. ROIs covering at least 10 pixels were drawn on the anatomic template from each of the 5 slices acquired and on both kidneys. The statistical significance was assessed using the two-tailed paired Student's t-test.

RESULTS

Figure 1 shows representative pre- and post-L-NAME R_2* map with different L-NAME infusion dose. Note the relatively brighter medulla in the post-L-NAME map as compared to pre-L-NAME map for each dose, signifying a reduction in medullary oxygenation. The brightness in medulla increased in the post-L-NAME with increasing dose. The window and level settings for pre- and post-L-NAME R_2* maps are exactly the same.

Figure 2 shows representative medullary R_2* *vs.* time for each infusion dose. The data were normalized to baseline. The error bars indicate the standard deviation in each measurement. Note all the 3 doses of L-NAME produced a dose-dependent increase in R_2*. Also the time to the maximum response was progressively shorter for higher dose.

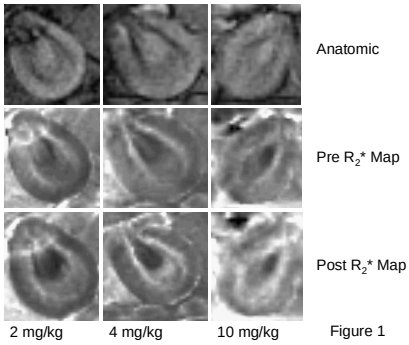
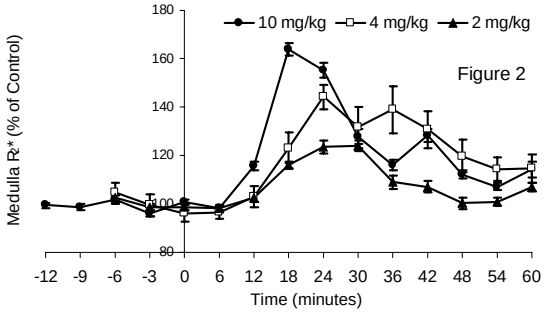


Figure 1



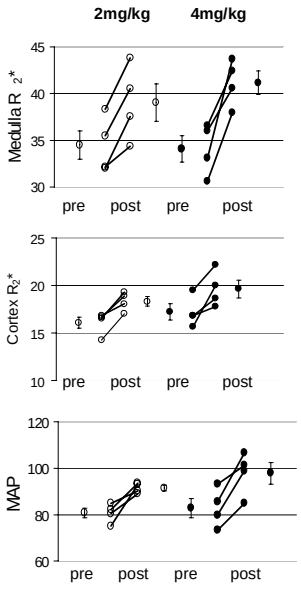


Figure 3

Figure 3 illustrates the individual changes of R_2* in medulla and cortex as well as MAP post-L-NAME in four human subjects. Owing to varying temporal profile during the 30 min infusion period, the maximum response after L-NAME administration was used as post-L-NAME data. Mean pre- and post-L-NAME data is the average over all subjects.

Table is averaged peak response over baseline (%) in R_2* in renal medulla and MAP measurement for 2 rats of each dose and 4 human subject for each dose.

DISCUSSION AND CONCLUSION

The L-NAME infusion caused a dose-dependent increase in R_2* followed by a prompt return towards baseline in rat kidneys. However, at the same time while MAP shows similar increase post-L-NAME, it does not recover over the one hour observation period. This apparent disconnection is an intriguing observation. It is probably related to paradoxical increase in NO bioavailability in hypoxic tissue during L-NAME administration [Microcirculation 1999; 6: 199]. In humans, there is similarly a dose dependent increase in R_2* post L-NAME. However, we have not performed measurements beyond 4 mg/kg owing to lack of evidence in the literature for use of higher doses for systemic administration.

Table. Summary of Peak Response over Baseline (%)

	In Rats (n=2) for each			In Human (n=4)	
	2mg/kg	4mg/kg	10mg/kg	2mg/kg	4mg/kg
R2*	15.57	40.95	79.87	13.17	21.14
MAP	18.96	30.35	55.97	13.62	18.37

ACKNOWLEDGEMENTS

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