Loss of Microvessel Density Correlates with the Onset of Atrophy in 2 Kidney 1 Clip Hypertension.

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BACKGROUND

Renal artery stenosis (RAS) affecting the elderly population is a major cause of renovascular hypertension and leads to chronic renal injury. Currently, there is no optimal management for RAS. Understanding the mechanisms leading to the inflammation, oxidative stress and microvessel density loss can help to correlate the reversibility of stenotic lesions with renal histopathologic alterations. In present study, we assessed the % atrophy, microvessel density loss and development of hypoxia in stenotic kidney (STK) as function of time.

METHODS

C57BL/6J male mice were used for the present study. RAS was induced on the right renal artery of animals by putting a polytetrafluoroethylene cuff and sham surgery was performed with manipulation of the right renal artery without cuff placement. MRI images were captured using an Avance DRX 700WB spectrometer with a vertical 16.4 T wide bore magnet connected with the imaging tools following RAS or sham surgery at day 3, 7, 14 and 28 (N=35 RAS, N=13 Sham). BOLD CMR was performed with respiratory gated conventional 2-D multi-gradient Echo (MGE). R^2 * values to assess regional hypoxia using Blood oxygen level dependent MRI (BOLD MRI) was obtained with MATLAB. The perfusion and volume on captured images were calculated using ParaVision version 5.1 and Analyze software respectively. The animals were harvested at day 3, 7, 14 and 28 following surgery and kidney tissues were collected for histology. Atrophy was assessed by analyzing % atrophic tubules over the entire cortical area using H&E stain. CD31 staining was performed using CD31 antibody (1:500, Santa Cruz Biotechnology, Dallas, Texas, USA) at day 3, 7, 14 and 28 (N=32 RAS and N=13 sham) following RAS or sham surgery. Olympus Bx50 microscope (Olympus Optical Co. Ltd., Buffalo Grove, IL, USA and NIS elements BR 4.13.00 64-bit image analysis system (Nikon Instruments INC., Melville, NY, USA) was used for CD31 count analysis. The data analysis was performed using GraphPad Prism 6.

RESULTS

STK did not show any significant histopathological changes at day 3 but showed higher % atrophy at day 7 (47±14.7%), 14 (85±4.6%, p=0.0007) and 28 (72.1±12.5%, p=0.007) compared to their respected shams. We observed significantly reduced microvessel count at day 3 (129±8.6, p=0.03) which continued to show significant reduction at day 7 (90±8.0 p=0.007), 14 (72±4.0, p=0.0003) and 28 (70±9.5, p=0.0002) compared to their respective shams. We observed that 57% STK showed high R2* value indicating hypoxia following RAS at day 3 and did show significance when compared with the sham (p=0.0003). Similarly, significantly higher hypoxia in cortex of STK was observed at day 7 (p<0.0001), 14 (p<0.0001) and 28 (p=0.0002) compared to their respective sham.

The % atrophy correlated negatively and strongly with MVD count (r=-0.8585). The % reduction in the renal blood flow correlated strongly and negatively with the MVD count (r=-0.8425) and right kidney weight (r=-7197). The % reduction in the renal blood flow correlated strongly and positively with % atrophy and R2* value (r=0.6683).

CONCLUSIONS

Microvessel density is reduced as early as 3 days following RAS surgery and progressively decreases with time. MR-BOLD shows evidence of cortical hypoxia as early as 3 days. Development of renal atrophy at later time points is associated with reduction of microvessel density and persistence of cortical hypoxia. These studies provide the basis for future studies to correlate reversibility of stenotic lesions with renal histopathologic alterations.

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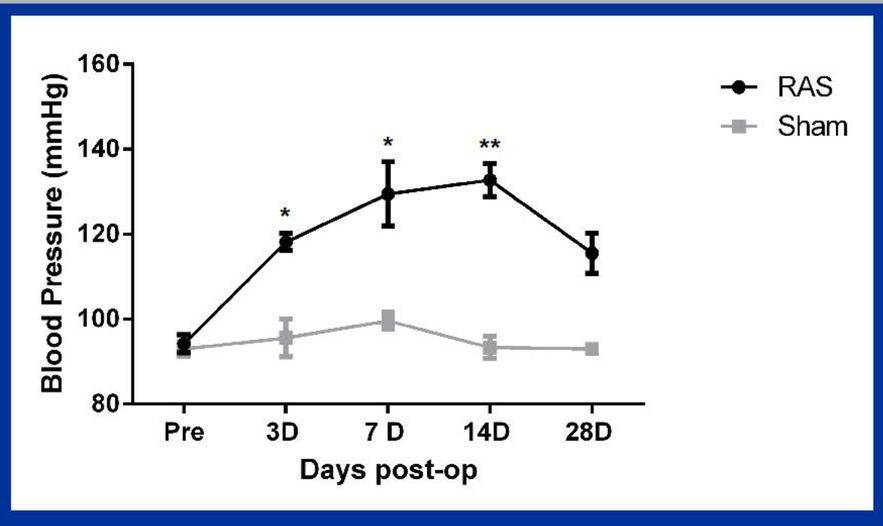

Figure 1. Showing the mean maximum systolic blood pressure pre and post surgery. *P ≤ 0.05, **P ≤ 0.001 for RAS in comparison to their respective shams.

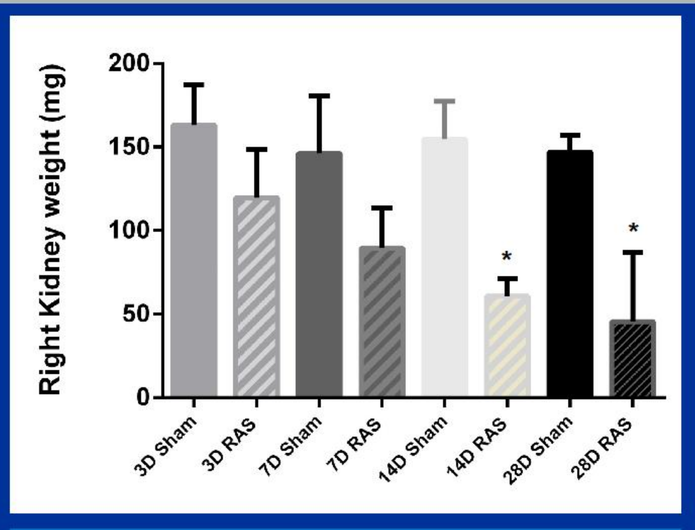

Figure 2. Showing mean Right kidney weight following surgery at different time points. *P ≤ 0.01 for RAS in comparison to their respective shams.

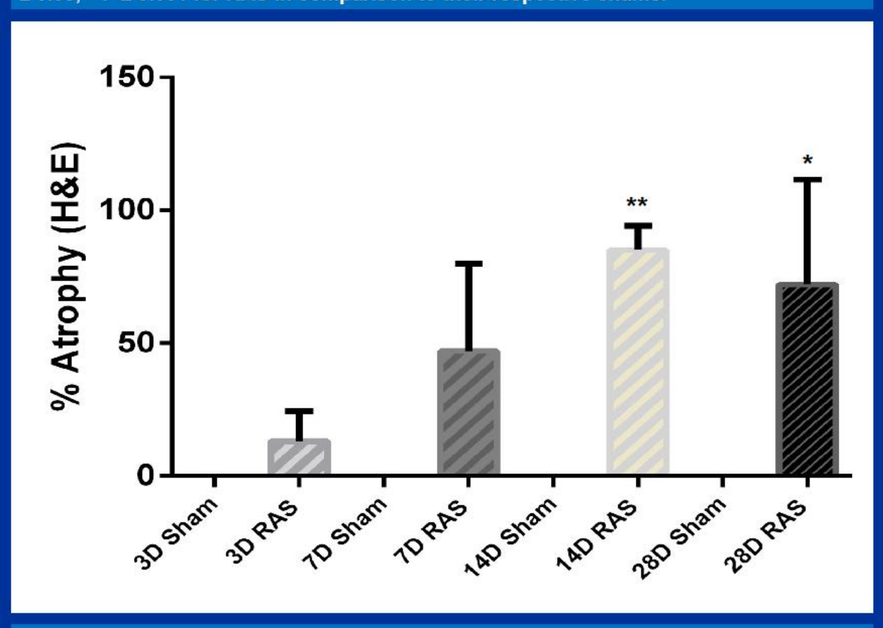

Figure 3. Showing mean % atrophy following surgery at different time points. *P ≤ 0.01, **P ≤ 0.001, for RAS in comparison to their respective shams.

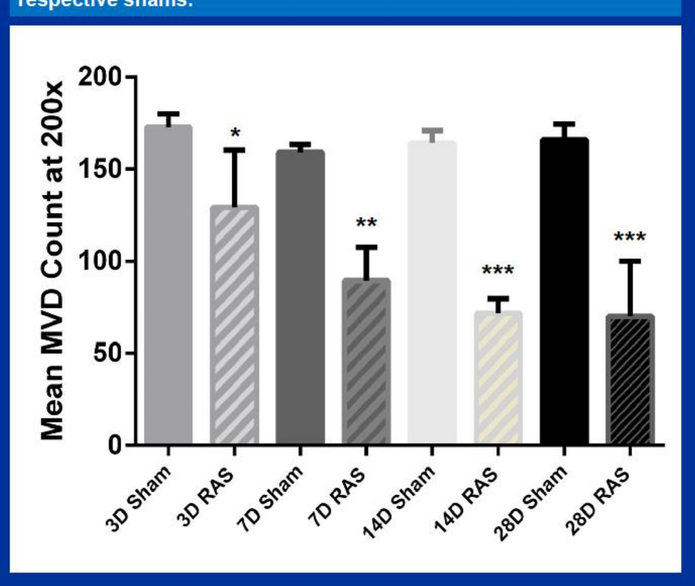

Figure 4. Showing the mean microvessel density count following surgery at different time points. *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001 for RAS in comparison to their respective shams.

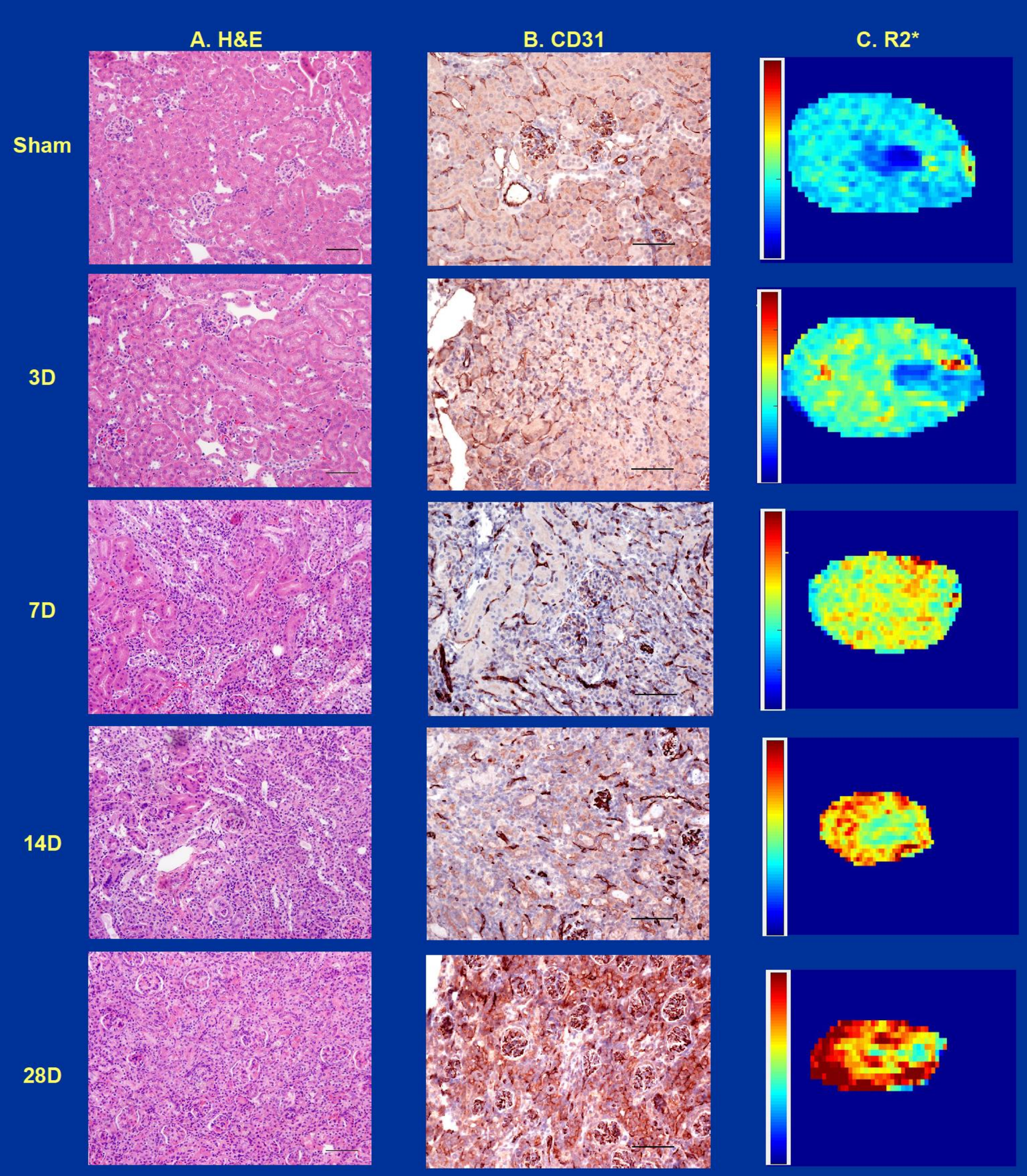

Figure 5. A. Stenotic kidney shows progressive atrophy following RAS surgery. Representative images showing H&E staining at 200X magnification following 3, 7, 14 and 28 days after RAS surgery compared to sham. B. The microvessel density reduces progressively following RAS surgery. Representative images showing Anti-CD31 staining at 200X magnification following 3, 7, 14 and 28 days after RAS surgery compared to sham. C. Hypoxia increases progressively following RAS surgery. Representative images showing the R2* values indicating right cortical hypoxia following RAS surgery compared to sham.

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