Diffusion-weighted MRI and cognitive evaluation of the effects of induced hypertension in middle aged cyp1a1-ren2 transgenic rats

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ABSTRACT

Hypertension (HTN) is associated with an increased risk of cardiovascular disease (CVD) and cognitive decline in aging humans, with the onset occurring around middle-age. While prior research has suggested an association between CVD and cognitive decline in the elderly, it is also critical to investigate how this dynamic may evolve from middle to older age and the related changes in brain region-specific anatomy and function prior to and after onset of HTN. We have carried out diffusion-weighted MRI (dMRI), a powerful non-invasive translational tool that can be utilized to analyze microstructural changes to the brain, and cognitive evaluation before and after induced HTN in transgenic rats. In this study, Cyp1a1-Ren2 xenobiotic-inducible transgenic rats (Fischer 344) (n=71) were used to model the gradual rate and age-of-onset of HTN observed in humans. 15-month-old male rats were assigned to either control (n=32) or treatment (n=39) groups and given a 6-week battery of behavior tests. The battery included the hippocampus-dependent spatial version of the Morris watermaze. Following the pre-treatment tests, the treatment group received a diet with 0.15% Indole-3-Carbinol (I3C) while control rats received the same chow without I3C. A post-treatment behavioral battery was given to assess the effect of HTN on cognition. Gradual onset of HTN was confirmed through systolic and diastolic blood pressure changes. Shortly after both pre- and post-treatment behavioral tests, body weights were measured, and neurological MRI was carried out. Multi-shell (b = 0, 1000, 2000 and 3000 s/mm^2), multi-direction (64) dMRI, was carried out with a resolution of 300x300x1000 µm. Fiber orientation distribution functions (FODs) were calculated via constrained spherical deconvolution (CSD) and used to register images to a study-specific population template. A Fischer 344 T2-weighted reference image and corresponding labeled atlas (116 regions of the brain) were registered to a population template, and values of apparent diffusion coefficients (ADC) and regional volumes were compared between groups and HTN status. The I3C-treated group showed a significant increase in diastolic and systolic blood pressure, as well as significant cardiac and renal end organ damage as observed previously (Willeman et al. 2019, Physiol. Report, 7:2051). Analysis of the hippocampus region-specific Morris watermaze data indicate no significant changes in cognitive performance with renal-induced HTN. Noninvasive imaging techniques also found no statistically significant differences between groups in hippocampal volume or mean hippocampal ADC. Overall, no significant correlation was found between the rats' spatial learning performance on watermaze of the Morris watermaze task (pre- and post- treatment) and hippocampal volume and hippocampal mean ADC.

METHODS

Experimental Procedure

Cyp1a1-Ren2 xenobiotic-inducible transgenic male rats at ages 13-15 months (n=71) were first assessed on a pre-treatment behavior battery then assigned to either treated (n= 39) or control (n=32) groups. The battery included the spatial and cued versions of the Morris Water Maze task, as illustrated in **Figure 1**. After a 4-week feeding period of 0.15% Indole-3-Carbinol (I3C) for treated and the same chow without I3C for control rats, groups were retested on the behavior tasks (post-treatment). Indirect systolic and diastolic blood pressure measurements by the tail-cuff method were taken three times a week along with the weights of the rats throughout the experiment. Due to observed weight loss from I3C treatment, half of the control group (n=32) were designated to a diet-restricted group (n=16) and given a reduced amount of chow in order to match the degree of weight loss in the treated group.

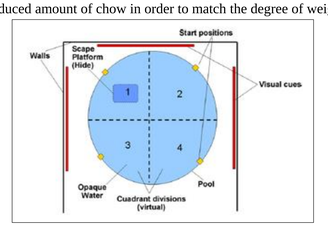


Figure 1: Morris watermaze
Schematic image of watermaze external cue placement, internal platform location and rat drop locations.

After each session of behavioral tests (pre- and post-treatment), a round of MR imaging sequences commenced. Prior to processing, all images were quality assessed; necessary subjects were removed from experimental subsets for reasons such as tumors, death, or irreparable imaging artifacts. 3-dimensional, high-resolution, 150-micron isotropic T2-weighted RARE anatomical images were used for volumetric analyses **(Figure 2-A)**. For each subject image at both imaging time points, the labeled atlas was warped to the subject's native spatial domain. Referring to the unique voxel values of each region in the atlas, a volume of each labeled region could be computed as the sum of voxels multiplied by the voxel resolution (150-micron isotropic). Multi-shell multi-direction EPI DTI sequences (b = 0, 1000, 2000 and 3000 s/mm^2) were processed for use in diffusion analyses, where all images were masked and corrected to reduce imaging artifacts. Diffusion tensor generation was performed on each subject image, from which, a metric map of ADC was created (**Figure 2-B**). Each individual ADC map was registered to a population template and underwent transformational processing to ensure all images were registered to the same spatial domain. Once this was performed, using a corresponding labeled atlas in the same spatial domain, statistics could be gathered for each of the 116 regions of the brain for each subject at both imaging time points.

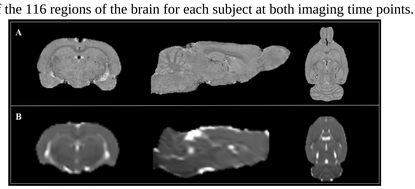


Figure 2: Representative subject images.
A) A representative 3D high-resolution T2 RARE anatomical image, **B)** representative ADC metric map image.

RESULTS

Behavioral Testing Results

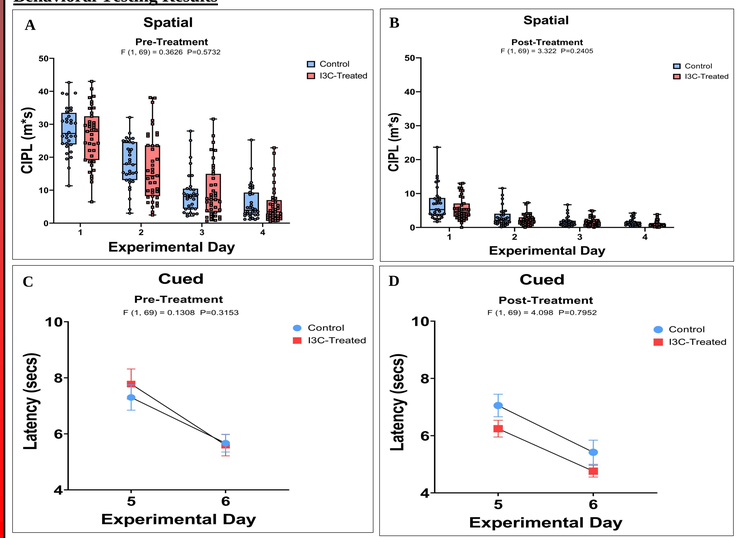


Figure 3: Spatial and Cued Morris Watermaze Performance

No significant differences were found between performance of control and I3C-treated animals on the spatial watermaze, pre- (**A**) and post-treatment (**B**) (respectively, two-way repeated measures ANOVA: treatment: F(1, 69)= 0.36338, p=0.3626 and F(1, 69)=3.322, p=0.2405). Error bars indicate SEM.

Similarly, no significant differences were found between performance of control and I3C-treated animals on the cued watermaze, pre- (**C**) and post-treatment (**D**) (respectively, two-way repeated measure ANOVA: treatment: F(1, 69)=0.1308, p=0.3153 and F(1, 69)=4.098, p=0.7952). Error bars indicate SEM.

These results suggest that induced hypertension did not significantly impair spatial learning and memory or visual perception.

Image Processing Results

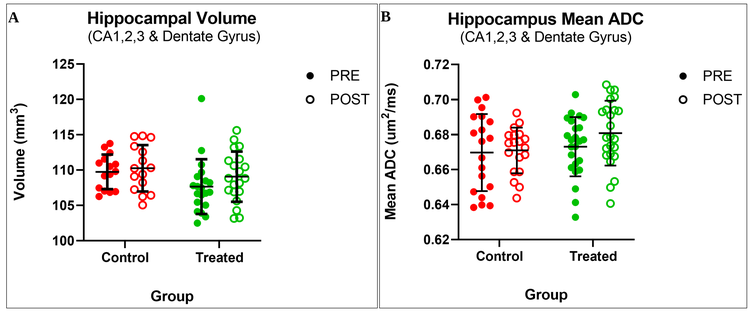


Figure 4: Volumetric and Diffusion Analysis Results for Hippocampus.

No statistically significant differences were found in hippocampal volume between timepoints of control (t=0.56) or treated (t=1.54) groups using a multiple paired, parametric t-test and Holm Šídák multiple comparisons correction **((A).**

Additionally, no significant differences were found in mean hippocampal ADC between timepoints of control (t=0.084) or treated (t=1.313) groups when performing the same statistical analysis **(B).**

Combined Analysis (Behavioral & Imaging)

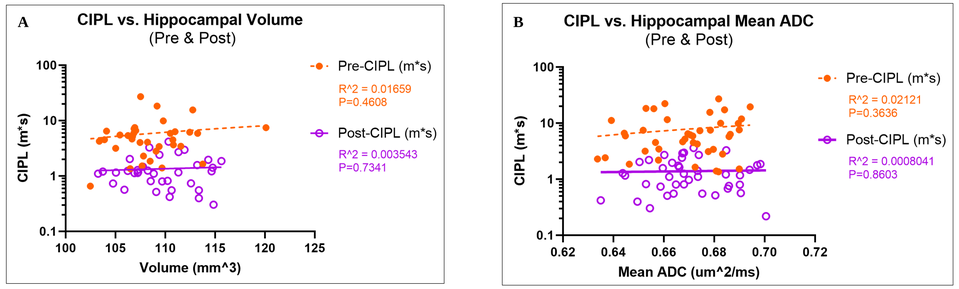
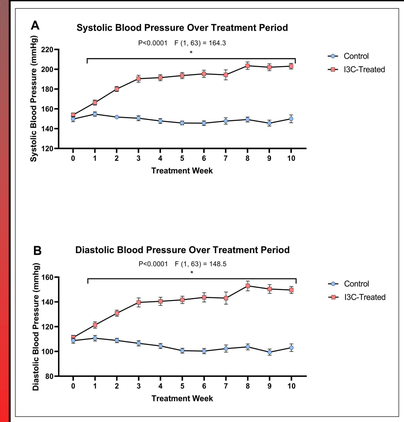


Figure 5. Combined Analysis of CIPL Spatial Water Maze Results and Imaging Results.
(A) A linear regression analysis was performed on average CIPL (last 12 trials) results from the Morris Water Maze task and hippocampal volume. No significant correlation was found between the two variables for either pre-treatment or post-treatment timepoints.
(B) A second linear regression analysis was performed on the same CIPL pathlength data verses mean ADC of the hippocampus. No significant correlation was found between the two variables for either pre- or post-treatment timepoints.



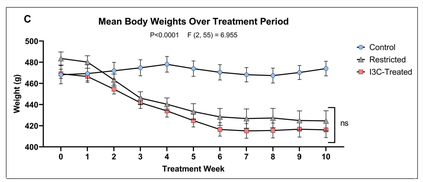


Figure 6: Blood Pressure and Body Weight Measurements

Dietary treatment starts on treatment week 1, and treatment week 5 is the start of the post treatment behavior battery. **A)** By treatment week 2, systolic blood pressure measurements are significantly different between treated and control animals (two-way ANOVA with repeated measures: treatment: F (1, 63) = 164.3 P<0.0001). **B)** Diastolic blood pressure measurements on treatment week 2, are significantly different between treated and control animals (two-way ANOVA with repeated measures: treatment: F (1, 63) = 148.5 P<0.0001).
C) Half of the control animals (n=32) were diet restricted (n=16) to match the treated animals' weights. Treated and restricted groups showed a significant loss of body weight in comparison to the unrestricted group (two-way ANOVA with repeated measures: treatment: F (2, 55) = 6.955 P<0.0001). Error bars indicate SEM **Note:** Asterisk indicates P<0.05 at the treatment week.

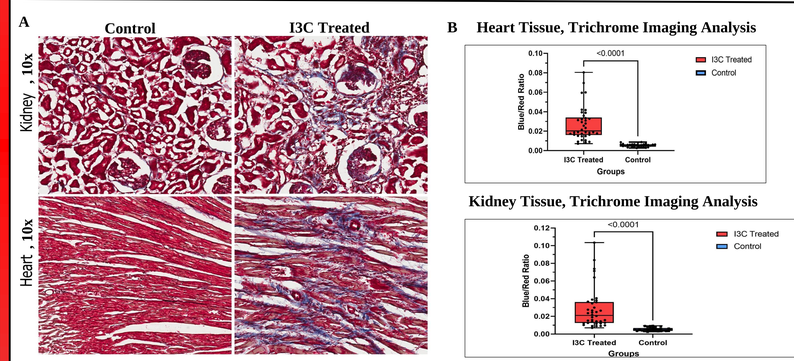


Figure 7: **Cardiovascular and Renal Effects of I3C-induced hypertension.**

A) Images of end organ damage using Masson's trichrome staining to indicate collagen fibrosis. **B)** Heart and kidney tissue analysis indicate significantly greater end organ damage in treated compared to control animals. Blue (fibrotic collagen deposits) versus red (cytoplasmic control) p< 0.000001. Error bars indicate SEM.

DISCUSSION

- Significant increase in diastolic and systolic blood pressure was observed in I3C-treated animals, demonstrating successful model replication with Willeman et al., 2019.
- No correlation was found between induced hypertension and spatial and cued versions of the Morris watermaze.
- The findings of a cognitive deficit in spatial memory that was observed in Willeman et al. (2019) was not observed in this study, likely due to our larger sample size.
- Imaging analysis found no statistically significant differences between I3C-treated and control groups in hippocampal volume or mean ADC.
- Overall, no correlation was found between spatial learning performance (pre- and post-treatment) and hippocampal volume or mean hippocampal apparent diffusion coefficient.

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