Potential renoprotective effects of chronic remote ischemic conditioning in a rodent model of sleep apnea

[1,2]**Benjamin G. Madigan (BA)**, [1,2]Katherine Harbeck (BS), [1,2]Raina Gerritts (BS), [1,2]Kalie A. Savage (BS), [1]Sarah C. Clayton (PhD), Kiefer W. Kious (MS), [3]Kelsey S. Schwartz (BS), [3]James A. Lang (PhD), [1]Noah J. Marcus (PhD)

[1]Department of Physiology and Pharmacology, Des Moines University Medicine and Health Sciences, Des Moines, IA. [2]College of Osteopathic Medicine, Des Moines University Medicine and Health Sciences, Des Moines, IA, [3]Department of Kinesiology, Iowa State University, Ames IA.

Introduction

Obstructive sleep apnea (OSA) is a highly prevalent chronic disease characterized by repetitive hypoxic episodes during periods of sleep. There is an association between OSA and chronic kidney disease (CKD), which may be partly attributed to reactive oxygen species (ROS) forming in the kidneys during or after hypoxic episodes[1]. Remote ischemic conditioning (RIC) is the application of intermittent ischemia to an extremity with the intent of protecting a distant organ from subsequent ischemic insults. RIC has been shown to mitigate extreme increases in reactive oxygen species[2], which provides renal protection during ischemic insults[3]. For this reason, RIC may have the potential to affect the pathogenesis of CKD in patients with OSA. In this study, we investigated the effects of RIC in a rodent model for sleep apnea (chronic intermittent hypoxia, CIH).

Hypotheses

- RIC will mitigate differences in the expression of molecular markers for oxidative stress, inflammation, fibrosis, and vascular function between AIR and CIH rats.
- RIC will reduce baseline reductions in renal cortical PO_2 between AIR and CIH rats.
- RIC will attenuate hypoxia-evoked reductions in renal perfusion and PO_2 between AIR and CIH rats.

Experimental Methods

Figure 1. Experimental Protocol

Chronic Intermittent Hypoxia

- RIC-CIH animals were exposed to chronic intermittent hypoxia (60 sec. FiO_2 10%, 120 sec. FiO_2 21% 8h/day) for 14 days. RIC-AIR animals were exposed to air-air cycling.

Remote Ischemic Conditioning

- For the final 7 days, both groups of animals received 3 treatments of remote ischemic conditioning (5 min. hind leg ischemia, 5 min. no ischemia) every 48 hours under isoflurane anesthesia (1.5% in air).

Measurement of Renal Perfusion and PO_2

- Following CIH, renal perfusion and PO_2 (Oxford Optronics) were measured under isoflurane anesthesia (1.5% in air) during 10 episodes of acute intermittent hypoxia (AIH) challenges (30 sec. FiO_2 10%, $FiCO_2$ 3%, 60 sec. FiO_2 21%). Renal perfusion was also measured using laser speckle contrast imaging (Moor FLPI-2, Moor instruments).

Measurement of Gene Expression

- RNA was extracted from kidney cortex tissue (ZYMO research). It was converted to cDNA and quantified using SYBR green real time qPCR (Thermo Fisher Scientific).

Figure 2. Evaluation of Gene Expression

Physiologic Experiment Data Analysis

- Baseline cortical PO_2 and renal perfusion data was collected pre-exposure (average of 300 sec.) and at each AIH baseline (average of 10 sec. at peak response). Recovery was assessed 5 minutes after 10 AIH exposures (average of 10 sec.). Percent change from baseline was calculated and averaged for each group. Statistical comparisons were made using unpaired t-tests.

Molecular Experiment Data Analysis

- Gene expression was assessed by determining RT-qPCR cycle threshold (Ct) values in duplicate for each animal. The difference between a given gene's Ct values and β-actin (housekeeping gene) Ct values were calculated (ΔCt) for each animal. Fold change was calculated by finding the difference between an animal's ΔCt and the ΔCt of an arbitrarily selected RIC-AIR animal (ΔΔCt), then calculating $2^{-\Delta\Delta Ct}$.

Results

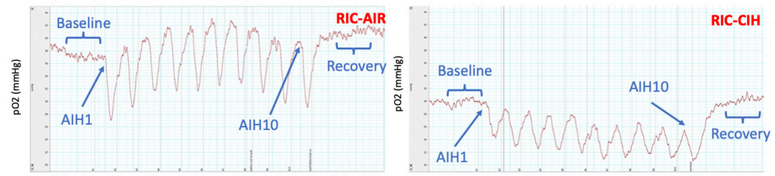


Figure 3. Differences in cortical PO_2 between RIC-AIR and RIC-CIH rats during hypoxic challenges. With subsequent exposure to 10% FiO_2, RIC-AIR rats (left) exhibited a relatively stable cortical PO_2 between hypoxic challenges; cortical PO_2 for RIC-CIH rats (right) trended downward. AIH1 and AIH10 points were compared to baseline to assess the effects of successive hypoxic challenges.

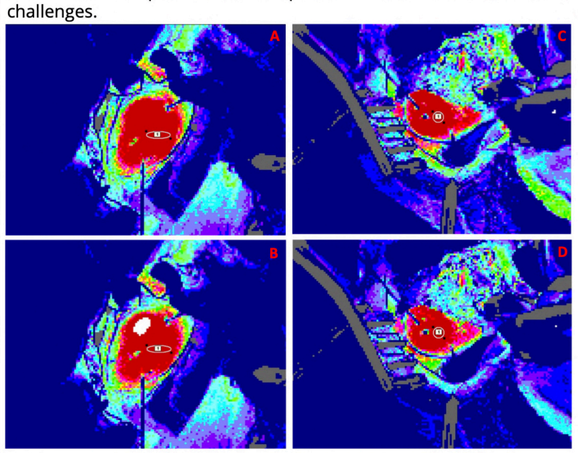


Figure 4. Laser Speckle Contrast Imaging for RIC-AIR and RIC-CIH rats at baseline and AIH10. A slight decrease in perfusion was observed between RIC-AIR baseline (A) and RIC-AIR AIH10 (B). Similarly, but with greater magnitude, a decrease in perfusion was observed between RIC-CIH baseline (C) and RIC-CIH AIH10 (D). The white marking in figure B is an artifact that did not affect the data.

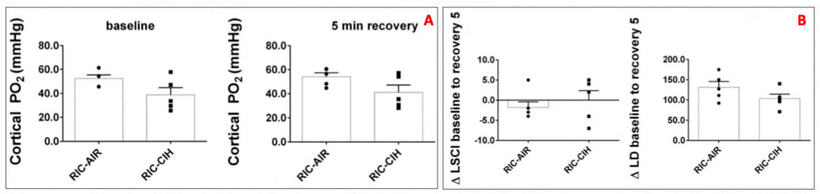


Figure 5. Cortical PO_2 and perfusion at baseline and 5 minutes recovery for RIC-AIR and RIC-CIH rats. No difference was noticed between baseline PO_2 and recovery PO_2 between RIC-AIR and RIC-CIH (A). Laser Speckle Contrast Imaging and Laser Doppler Flow displayed no differences in perfusion between baseline and recovery (B).

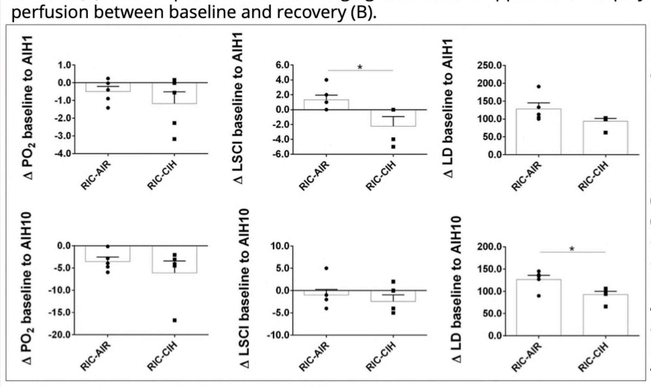
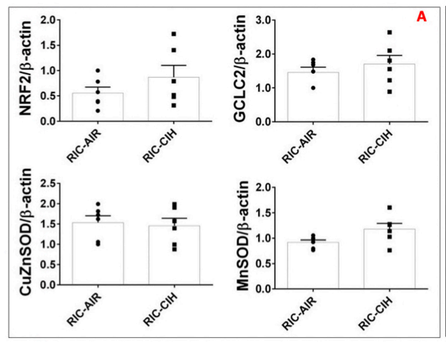
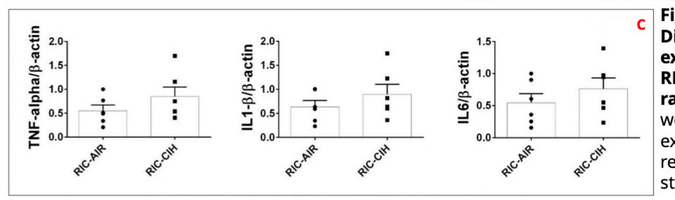


Figure 6. Differences in cortical PO_2 and perfusion between baseline values for RIC-AIR and RIC-CIH rats and their respective AIH1 (ΔAIH1) and AIH10 (ΔAIH1) values. No differences were observed in cortical PO_2 for ΔAIH1 or ΔAIH10. Laser Speckle Contrast Imaging displayed a 4% lower RIC-CIH perfusion than RIC-AIR for ΔAIH1, which was corroborated by a similar trend in Laser Doppler Flow. Laser Doppler Flow displayed a 34% lower perfusion for RIC-CIH at ΔAIH10 than RIC-AIR, which was supported by a similar finding in Laser Speckle Contrast Imaging.

Results





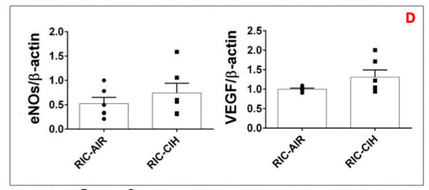


Figure 7. Differences in gene expression between RIC-AIR and RIC-CIH rats. No differences were noticed in the expression of genes related to oxidative stress (A), fibrosis (B), inflammation (C), or vascular function (D). Past studies between AIR and CIH rats displayed differences in IL6, IL1-β, NRF2, CuZn-SOD, MnSOD, KLF15, CTGF, and Collagen III. RIC treatments applied to both CIH and AIR groups appeared to attenuate these differences, indicating that RIC may provide a salutary effect on the renal cortex.

Conclusions

- RIC mitigated differences between CIH and AIR groups in molecular markers for inflammation, fibrosis, vascular function, and oxidative stress.
- RIC reduced baseline and hypoxic-evoked reductions in renal cortical PO_2 between AIR and CIH rats.
- RIC did not attenuate hypoxia-evoked reductions in renal perfusion between AIR and CIH rats.
- RIC may confer a salutary effect on renal physiology with exposure to CIH

Limitations

- The effects of isoflurane anesthesia on RIC are unknown.
- The data from RIC-AIR and RIC-CIH was not compared to groups that did not receive RIC treatments, which makes it difficult to ascertain the direct impact of RIC on animals exposed to CIH.

References

1. Lin, C. H., Lurie, R. C., & Lyons, O. D. (2020). Sleep Apnea and Chronic Kidney Disease: A State-of-the-Art Review. Chest, 157(3), 673–685.
2. Heusch, G. (2015). Molecular Basis of Cardioprotection. Circulation Research, 116(4), 674-699.
3. Giannopoulos, G., Vrachatis, D. A., Panagopoulou, V., Vavuranakis, M., Cleman, M. W., & Deftereos, S. (2017). Remote Ischemic Conditioning and Renal Protection. Journal of Cardiovascular Pharmacology and Therapeutics, 22(4), 321-329.

Support

- Supported by the Mentored Student Research Program (MSRP) at Des Moines University
- Iowa Osteopathic Education Research Fund (IOER)