



## EFFECTS OF RECEPTORS FOR ADVANCED GLYCATION END PRODUCTS INHIBITION ON ARTERIOVENOUS FISTULA MATURATION

Samantha Vranes<sup>1</sup>, Yuxia He<sup>2</sup>, Jason Chieh Sheng Tey<sup>2</sup>, Yan-Ting Shiu, PhD<sup>2</sup>

<sup>1</sup>Department of Biomedical Engineering, <sup>2</sup>Division of Nephrology and Hypertension

The arteriovenous fistula (AVF) is the most common and preferred vascular access type for hemodialysis (HD) patients with end-stage kidney disease. This is due to the lower risk of infection and mortality, and yet higher ability to ensure long-term functional vascular access than other vascular access times, rendering the AVF as the most cost-effective among all vascular access types<sup>1,2,3,4</sup>. However, up to 60% of AVFs fail to mature and achieve the high blood flow rate required for HD cannulation due to neointimal lesion formation and impaired lumen expansion<sup>2</sup>. The interaction between advanced glycation end products (AGE) and the receptor for advanced glycation end products (RAGE) triggers a cascade of increased oxidative stress and endothelial dysfunction<sup>5,6</sup>, which may be linked to neointimal lesion formation and impaired lumen expansion, leading to AVF maturation failure. This study investigates the effect of inhibiting RAGE on neointimal lesion development in AVF maturation failure. This project hypothesizes that inhibition of RAGE via genetic knockout (KO) or a pharmaceutical inhibitor, FPS-ZM1, leads to decreased neointimal lesion development and increased vein dilation, thereby improving AVF maturation.

Carotid-external jugular AVFs were surgically created in 8 to 12 weeks old C57BL/6 wild-type (WT) mice, genetically engineered RAGE KO mice on C57BL/6 background, and WT mice treated with the pharmaceutical RAGE inhibitor FPS-ZM1. N=3 for each group in my study. The pharmaceutical inhibitor, FPS-ZM1 (catalog no. 553030; Sigma-Aldrich), was administered by intraperitoneal injection at a dose of 1.0 mg/kg daily, starting from three days prior to AVF creation and continuing until euthanasia. Likewise, 100  $\mu$ l of PBS was administered to the WT mice for control by intraperitoneal injection. AVF veins were harvested one-week post-creation, processed through a tissue processor, embedded into paraffin tissue blocks, sectioned to 5  $\mu$ m thick vein sections with a microtome, and mounted on slides. Histology samples were stained with Verhoeff-van gieson (VVG) stain (catalog no. HT25A-1KT; Sigma-Aldrich) to visualize the internal elastic lamina (IEL)-enclosed area and open lumen area. Image analysis and statistical analysis were used to quantify the IEL-enclosed area, open lumen area, and neointimal lesion area to assess for vein dilation and stenosis.

Results show that, in the AVF veins, RAGE KO mice had smaller IEL area (236,097 $\pm$ 62,279  $\mu$ m<sup>2</sup> WT vs. 131,987 $\pm$ 41,374  $\mu$ m<sup>2</sup> RAGE KO,  $p < 0.05$ ) and neointimal lesion area (202,475 $\pm$ 62,480  $\mu$ m<sup>2</sup> WT vs 99,949 $\pm$ 48,813  $\mu$ m<sup>2</sup> RAGE KO,  $p < 0.05$ ) than the WT mice. There was no significant difference in the open lumen area in the AVF vein between the RAGE KO mice and WT mice comparison. Between the FPS-ZM1-treated WT mice and non-treated WT mice, there was no significant difference in the IEL-enclosed area, open lumen area, or

neointimal lesion area in the AVF vein. These results increase our understanding of the effect of inhibiting RAGE for hemodialysis AVF remodeling and maturation.

Histological analysis of the AVF vein showed a reduction in neointimal lesion area in the genetic RAGE KO mice, as expected. However, the other results were not consistent with the hypothesis. There were no statistically significant differences in the neointimal lesion area of the AVF vein between the non-treated WT mice and FPS-ZM1-treated mice. Thus, no conclusion can be drawn about AVF maturation through the pharmaceutical RAGE inhibitor, FPS-ZM1. This study provides evidence that inhibiting RAGE leads to decreased neointimal lesion formation in AVF remodeling. Further work would need to be done to conclude if the chosen pharmaceutical RAGE inhibitor, FPS-ZM1, is effective for studies regarding vascular access in end-stage kidney disease. This work will serve as preliminary data for future work in the Shiu lab regarding blockade of the AGE-RAGE axis for improved vascular access.

## References

1. J. Karim, L. Kumbar. "Kidney Replacement Therapy". *Divisions of Nephrology & Hypertension and General Internal Medicine Chronic Kidney Disease (CKD) Clinical Practice Recommendations for Primary Care Physicians and Healthcare Providers a Collaborative Approach*. Henry Ford Health Systems. ed. 6, pp. 48-50, 2011.
2. P. Roy-Chaudhury, V. Sukhatme, A. Cheung. "Hemodialysis vascular access dysfunction: A cellular and molecular viewpoint". *J Am Soc Nephrol*. vol 17 pp. 1112-1127, 2006.
3. M.A. Siddique, S. Ashraff, D. Santos, et al. "An overview of AVF maturation and endothelial dysfunction in an advanced renal failure". *Ren Replace Ther*. vol 3, 2017. <https://doi.org/10.1186/s41100-017-0123-x>.
4. T. Vachharajani. "Atlas of Dialysis Vascular Access". *Wake Forest University School of Medicine*, 2010.
5. D. Aronson. "Potential role of advanced glycosylation end products in promoting restenosis in diabetes and renal failure". *Medical Hypothesis*. vol 53 pp. 207-301, 2002.
6. A. E.M. Stingham, Z. Massey, H. Vlassara, G. Striker, A. Boullier. "Uremic toxicity of advanced glycation end products in CKD". *J Am Soc Nephrol*. vol 27 pp. 354-370, 2016.