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Vascular Architecture of the Liver in SHR and WKY Rats

Charalette Mathwig (Chemistry and Geology)

Ken Willaert (Biological Sciences)

Faculty Mentor: Dr. Michael Bentley, Dept. Biological Sciences

Abstract

The liver is a highly complex vascular organ containing an intricate network of arteries, arterioles, portal veins, veins, venules, and capillaries. This complex network could change in situations related to vascular disease such as hypertension. We examined the vasculature in rat livers by scanning electron microscopy. The tissue was prepared by perfusing either Mercox resin or polyurethane resin into the vasculature. Once the resin polymerized, each liver was placed in concentrated potassium hydroxide to corrode the tissue from the vascular casts. The casts were critical point dried, sputter coated with gold-palladium, and viewed by scanning electron microscopy. Examination of the vascular architecture showed the circulatory patterns in the various lobes of the liver. The capillaries from the arterial and portal venous supply were continuous with the venous drainage. The information gathered in this study may aid ongoing research in hypertension studies and future studies involving liver regeneration.

Introduction

As well documented studies confirm, chronic high blood pressure can lead to an enlarged heart, kidney failure, neurological damage, and retinal damage¹. Individuals with high blood pressure have an increased stiffness and resistance in their peripheral arteries throughout the tissues of the body¹. This increased resistance causes the heart muscle to work harder to pump the blood through these blood vessels or vasculature. As a result of the increased workload, there is an enlargement of the heart and thickening of the arterial walls². The vascular effects of hypertension are well known in many organs¹⁻⁴. However, little information exists on the effects of high blood pressure on the liver and its vascular tissue. A recent study suggested that hypertension may lead to proliferation of capillary beds and decreased diameter of major arterioles within organs².

This project focused on the structural characteristics of vascular tissue in the liver attributable to high blood pressure. To do this we used a scanning electron microscope (SEM) to examine the vasculature in livers of spontaneous hypertensive rats (SHR) and normotensive Wistar-Kyoto (WKY) rats. The hypothesis was that the micro vascular architecture in the hypertensive rats will be abnormal compared to the Wistar-Kyoto rats. It was predicted through this project that we would find decreased diameter in SHR samples. We also expected to find increased vascular growth within liver tissue. The mechanism behind vasculature adaptation will lead to a better understanding of the effects due to hypertension.

Methodology

Livers from six SHR and six WKY rats were used for electron microscopy scanning. First, the rat was anesthetized with inactin at 100mg/kg body weight. A laparotomy was performed to expose both the descending aorta and hepatic portal vein. A catheter was placed into either the aorta or hepatic portal vein, and the rat was injected with a 0.9%

(w/v) sodium chloride and 0.02% (w/v) papaverine solution to dilate the vasculature. Following the injection, polyurethane resin, a plastic derivative, was infused throughout the hepatic vasculature via the descending aorta or hepatic portal vein to provide a framework of the blood vessels. At the conclusion of the infusion, the rats were euthanized by performing acute pneumothorax in accordance with standard protocol³. Once the plastic resin polymerized, the liver was removed. The liver was placed into 5% (w/v) potassium hydroxide solution to digest the tissue around the plastic cast⁴. The remaining structure was dehydrated using a critical point dryer. After the sample was dried, and in order to have viewed it with the scanning electron microscope, the sample was coated with palladium using a sputter coater. The sample was analyzed under the scanning electron microscope. Pictures were taken and evaluated for structural differences³.

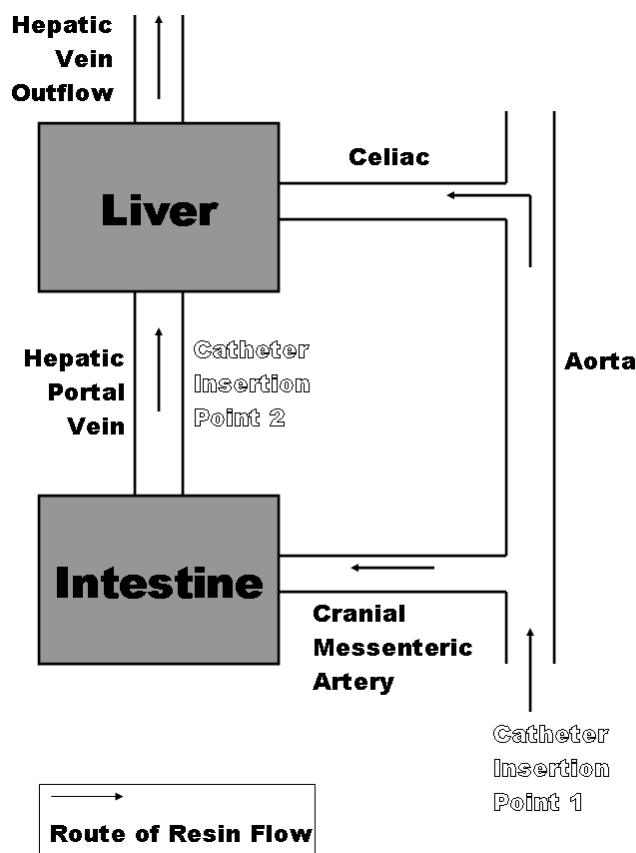


Figure 1. Schematic of the two routes of infusion. Plastic infusion was performed by entering either through the descending aorta or through the hepatic portal vein. Infusion through the hepatic portal vein provided the most efficient method to achieve liver casts.

Results

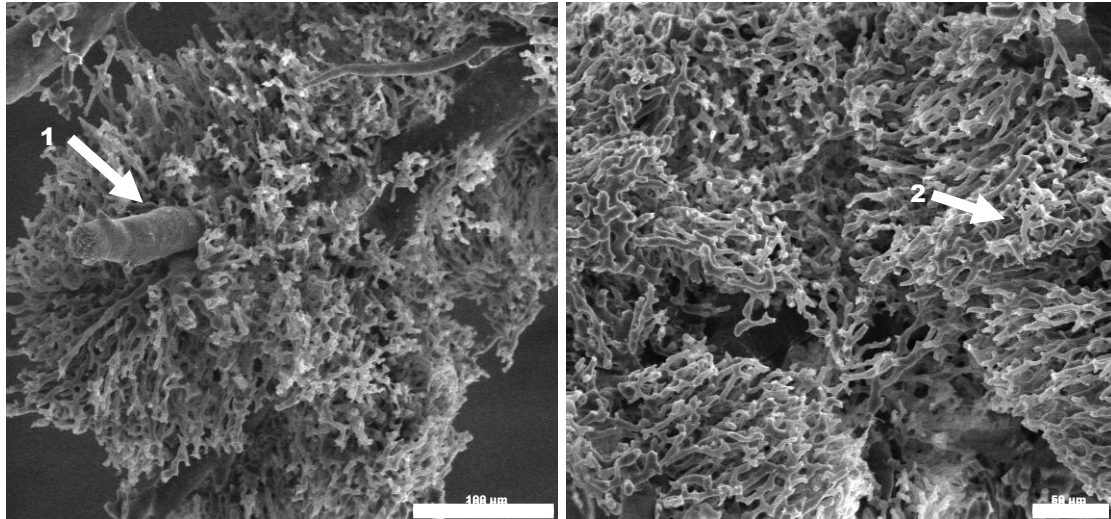


Figure 2. Two panel views of liver vasculature from arterial infusion depicting blunted sinusoidal capillary ends. Arrow 1 points to a branched section of the hepatic portal vein, and arrow 2 points to incomplete sinusoidal capillaries.

SHR

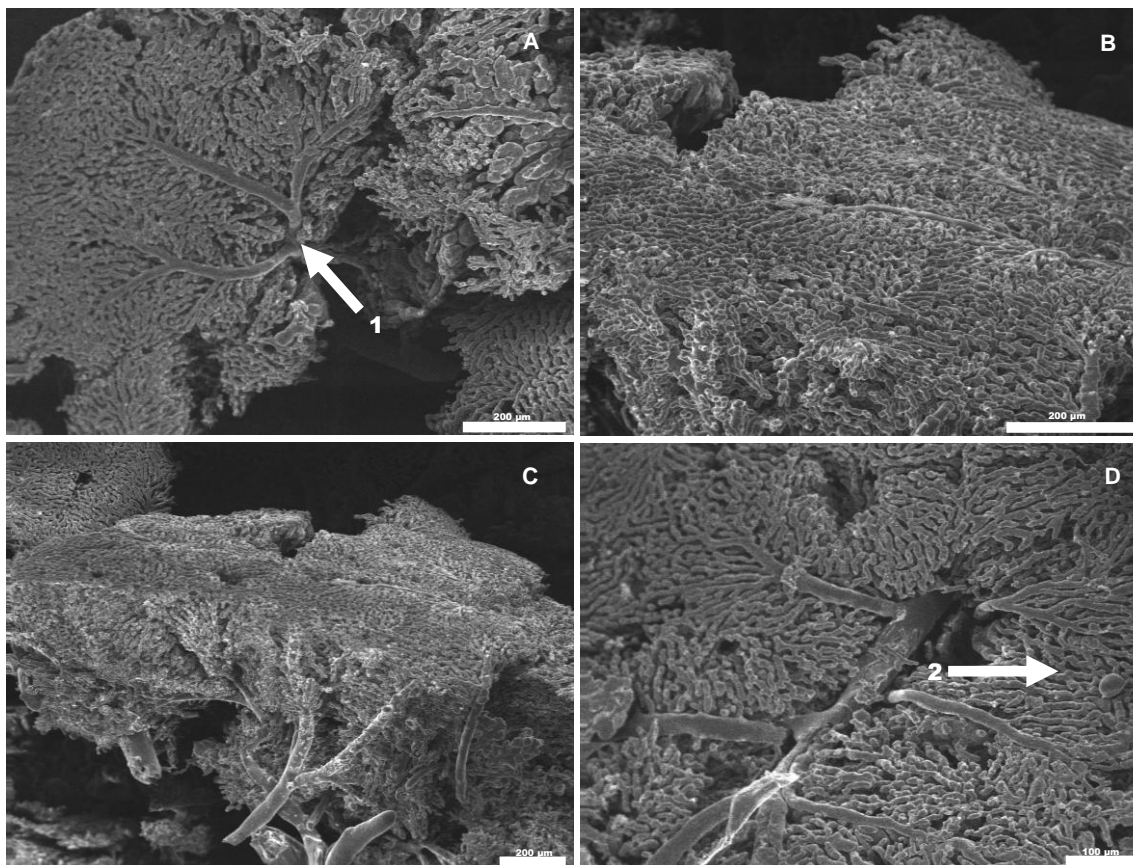


Figure 3. Four panel views of SHR liver vasculature obtained from hepatic portal vein infusion. Panel A: Superficial view of liver vasculature. Panel B: Close-up of the liver vasculature. Panel C: Cross-sectional view of liver showing the deep branching of the hepatic portal vein and extensive corresponding sinusoidal capillaries. Panel D: Deep view of liver vasculature. Arrow 1 points to a branched section of the hepatic portal vein, and arrow 2 points to sinusoidal capillaries.

WKY

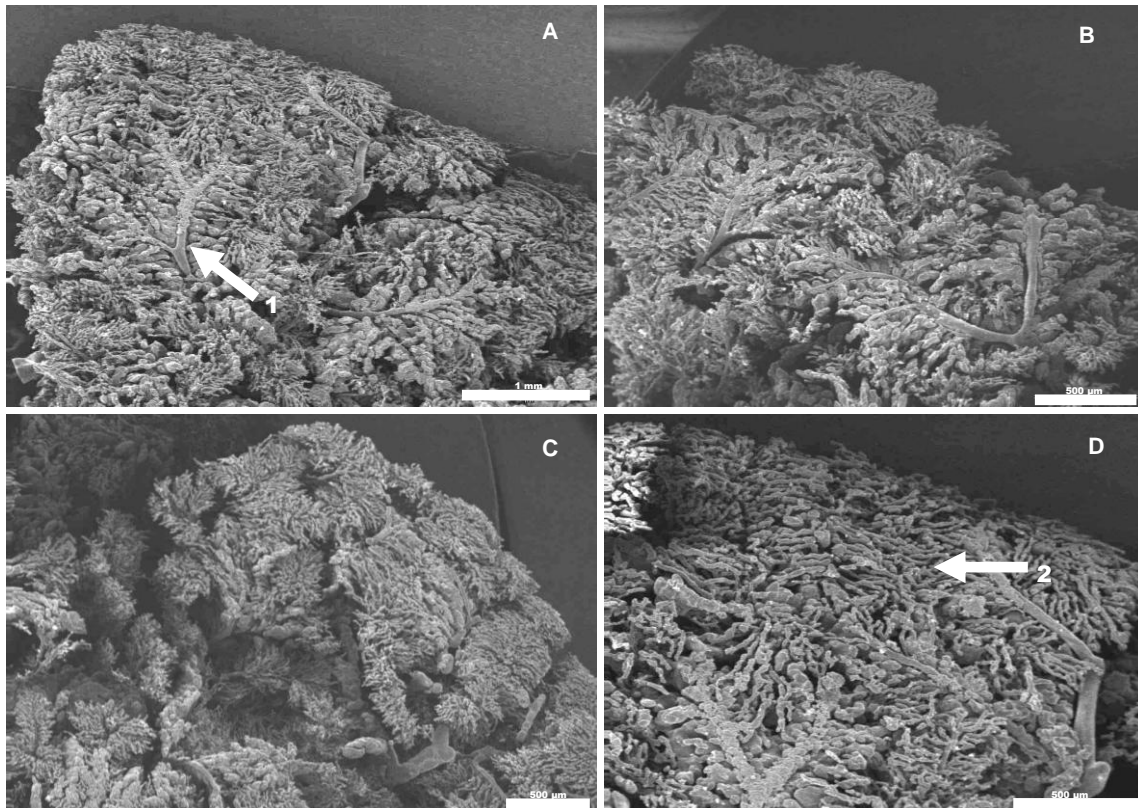


Figure 4. Four panel views of WKY liver vasculature obtained from hepatic portal vein infusion. Panel A: Superficial view of liver vasculature. Panel B: Close-up view of liver vasculature. Panel C: Cross-sectional view of liver. Panel D: Deep view of liver vasculature. Arrow 1 points to a branched section of the hepatic portal vein, and arrow 2 points to sinusoidal capillaries.

Conclusion

Two observations were made throughout this study concerning the method of infusion.

- 1) Arterial infusion gave different results between SHR and WKY vasculature. For instance, SHR vasculature did not fill as readily as that of WKY. Additionally, the microvasculature was not obtainable for either the SHR or WKY even though the diffusion was better within the WKY.
- 2) Hepatic portal vein infusion noticeably filled the vasculature more completely. With that, however, there were still some parts of the vasculature that did not fill.

The more complete infusion with the hepatic portal route could be because the arterial route being more constricted than that of the hepatic portal vein. Additionally, the pathway to the liver via the arterial route seemed to be directed toward and followed the intestinal vasculature before reaching the liver itself, thus giving a poorer infusion. By using the hepatic portal vein the branching pattern of the portal vein down to the sinusoidal level was observed in both the SHR and WKY. In the end, there was no noticeable structural difference seen in SHR and WKY vasculature.

It is important to note the differences in quality of vascular casts between the two infusion routes for both SHR and WKY specimens. These findings could be the start of further investigations encompassing both vasculature research and corrosion casting methods.

Future Research

By understanding the hepatic vascular architecture and the method of infusion, the information gathered in this study may aid ongoing research in hypertension studies and future studies involving liver regeneration.

Acknowledgements

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Charalette Mathwig Biography

Charalette spent her childhood days on a farm in Arlington Minnesota. She moved to LeSueur at the age of twelve. After graduating from LeSueur Henderson High School she was employed at Belle Pharmaceuticals Inc. in Belle Plaine, Minnesota. She worked there from June 1995 to September 2005. Duties that she performed were that of Quality Control, Laboratory Analyst, and Document Control. In June 2005 she took a job at Shopko Pharmacy in Mankato, Minnesota working as a pharmacy technician. She decided that pharmacy was the career she wanted to pursue.

Charalette enrolled at Minnesota State University, Mankato in the fall of 2004 and is currently a Pre-Pharmacy student. She is working on achieving her Bachelor of Science in chemistry and a minor in biology. In January of 2006 she started her research at Minnesota State University, Mankato in the Biology Department under the mentor of Dr. Michael Bentley. She started out working on vascular techniques and other laboratory skills along with her partner Ken Willaert. Charalette is also a member of the Pre-Pharmacy Club since September of 2005 and is currently the treasurer of the club. She has received an Alliss scholarship and has been on the dean's list at MSU.

Her goals for the future are to be accepted into a pharmacy program and to continue conducting research.

Kenneth R. Willaert

Biography

Ken was born in Mankato, MN on January 28, 1985. He was born to Robert and Jeanne Willaert as the third oldest in a family of seven children. For the first few years of his life, Ken grew up in St. Peter, MN, just north of Mankato, where his family owned and operated a farm. His family soon moved to Mankato after struggling through financial hardship.

Ken received most of his education while in Mankato. Upon graduation from Mankato East High School, Ken was accepted to and graduated from Minnesota State University, Mankato. He graduated May 2007, *Summa Cum Laude*, with a Bachelor of Science in biology and a minor in chemistry.

Originating in high school and extending into his college years, Ken participated in a number of activities and organizations. Ken extended his passion for swimming, founded from his younger years, to his first three years at MSU. Ken has also been involved in various student organizations at MSU such as Chemistry, Biology, and Pre-Med. Clubs. Ken served as a student representative for his college in the Student Government, in the Dean Search Committee, and in the Student Advisory Board. During his last few years at MSU, Ken was involved in undergraduate research mentored by Dr. Michael Bentley. He and his partners looked at the vascular architecture within various organs of murine animals.

With his education, Ken plans to pursue a career in the medical field. He wishes to one day settle down in Mankato, where he looks forward to raising a family and involving himself in such a wonderful community.

Michael Bentley

Biography

Michael Bentley has been a professor in the Department of Biological Sciences at Minnesota State University, Mankato (MSU) since 1989. He received a B.S. degree in Biology from Central Michigan University in 1968. From the University of Minnesota, he received an M.S. degree in Cell Biology in 1973 and a Ph.D. degree in Veterinary Anatomy in 1983. Following his Ph.D. degree and prior to his faculty appointment at MSU, he received post-doctoral training and conducted research in nephrology and hypertension at Mayo Clinic. At MSU, he is an advisor for pre-professional students who are majoring in Human Biology and he teaches a variety of courses related to biomedical sciences. His research has mainly involved the application of imaging technology to study vascular alterations in kidneys and other organs during experimental disease conditions.