Optimization of a local type rabbit model for arteriovenous fistula, focused on study neointimal hyperplasia

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ABSTRACT

Background: Hemodialysis vascular access dysfunction is a significant cause of morbidity and mortality in hemodialysis patients. Over the past decades, numerous animal models have been developed. However, there needs to be more animal model protocol with rabbits to investigate neointimal hyperplasia in the arteriovenous fistula. This study aimed to explore an alternative rabbit model using a wild-type local model for investigating neointimal hyperplasia in the arteriovenous fistula.

Methods: One local-type male rabbit performed arteriovenous fistula, in which the right carotid communis artery was anastomosed end-to-side to the right internal jugular vein. When examining vascular tissue, measurements were taken of the thicknesses of the tunica intima and tunica media, as well as the areas of the tunica intima and tunica media, vascular diameters, and vascular lumen areas.

Result: We successfully induced neointimal hyperplasia in local-type rabbits, indicated by increased tunica intima thickness after 28 days of the arteriovenous fistula procedure.

Conclusion: Optimization of the Rabbit model using a wild-type local model for investigating neointimal hyperplasia in arteriovenous fistula may be susceptible and considered as it is accessible and cost-effective besides producing the same outcome as a laboratory rabbit.

Keywords: Local rabbit, optimization animal model, neointimal hyperplasia, arteriovenous fistula.


INTRODUCTION

Nearly 4 million people worldwide live on kidney replacement therapy (KRT), and hemodialysis (HD) remains the most typical form of KRT, accounting for approximately 69% of all KRT and 89% of all dialysis.1 Arteriovenous fistula is the most preferred vascular access for the initial choice of chronic hemodialysis access.2 Arteriovenous fistula (AVF) shows superior long-term patency and lower infection rates than arteriovenous grafts (AVG) or central venous catheters.3 Functional and durable vascular access is required to deliver successful, consistent, and sustainable dialysis therapy.4 Hemodialysis vascular access dysfunction is a significant cause of morbidity and mortality in hemodialysis patients.5 The most common etiology of vascular access dysfunction is venous stenosis at the vein-artery anastomosis in arteriovenous fistula and the vein-graft anastomosis in arteriovenous grafts (AVG).6 Although considered a preferred technique, among successfully created AVF, the primary patency rates range only 50–70% and 30–40% at 1 and 2 years, respectively.6 AVF maturation failure, resulting from early neointimal hyperplasia development and poor vascular remodeling, is the most common problem in AVF dysfunction.6

There needs to be a better understanding of the biological mechanisms that lead to AVF maturation failure and AVG stenosis. Consequently, few effective therapies treat vascular access dysfunction in AVFs and AVGs. That has been studied in experimental and clinical studies to prevent vascular access dysfunction in AVF and AVG.3 As a result, animal models have become increasingly important as a research tool.

Over the past decades, numerous animal models have been developed to unravel the vascular pathology of AV access failure and to design new therapeutic strategies to improve the durability of these vascular conduits.8 Different species have been researched for reporting neointimal hyperplasia, such as rats,9–12 mice,13–18...
dogs,\textsuperscript{19} pigs,\textsuperscript{20–22} sheep,\textsuperscript{23,24} and primates.\textsuperscript{25,26} However, animal model protocol with rabbits is lacking to investigate neointimal hyperplasia in the arteriovenous fistula.\textsuperscript{27}

Although humans and mice or rats share a high level of genetic similarity, there are significant differences in their metabolism that restrict the translational potential of murine research.\textsuperscript{28} On the other hand, rabbits provide a greater translational capability for the development of new drugs and diagnostic methods, as they offer a bridge between basic research in rodents and more translational research in larger animals.\textsuperscript{28,29} Amongst various strains, New Zealand white strains of rabbits are commonly being used for research activities.\textsuperscript{27} These strains are less aggressive in nature and have fewer health problems as compared with other breeds.\textsuperscript{29} However, some researchers may need help obtaining this rabbit type. In this study, we aimed to explore an alternative rabbit model using a wild-type local model for investigating neointimal hyperplasia in the arteriovenous fistula.

\textbf{METHODS}

\textbf{Animal Preparation}

One wild-type male rabbit that weighed around 2000-2500 gram (Figure 1) and aged between 8-12 weeks were performed AVF surgery. Our standard protocols for pre-experiment care were implemented. They were approved by an animal use protocol by the Institutional of Animal Care and Use Committee, Faculty of Veterinary of Universitas Syiah Kuala (Ref.144/KEPH/VI/2022).

\textbf{Surgical Procedure}

The researcher, who is a thoracic cardiac and vascular surgeon as well as a veterinarian, performed all of the anastomoses. The animals were anesthetized with 50 mg/kg of intramuscular ketamine and 5 mg/kg of intramuscular xylazine. The incision areas were shaved to provide better visibility during surgery and were disinfected with povidone-iodine. The right carotid communis artery was accessed through a vertical incision in the neck (Figure 2). A dose of 100 IU/kg of intravenous heparinization was administered. The right carotid communis artery was then anastomosed end-to-side to the right internal jugular vein. The anastomosis was completed by suturing one by one with a 7-0 polypropylene suture, and the tissues were closed in an anatomical layer (Figure 3). The left internal jugular vein was unintervened and indicated as the study’s control group.

\textbf{Tissue harvesting}

Throughout the study, the experimental animals remained alive. There were no instances of wound site infection or neurological problems observed at the end of the postoperative 28th day. After 28 days, the rabbits were sacrificed to evaluate the development of neointimal hyperplasia. The right and left non-anastomosed segments were removed and sent to the histology laboratory for examination. Both the anastomosed and non-anastomosed components were fixed with a 10% buffered neutral formaldehyde solution and embedded in paraffin. 5 \textmu m serial sections were obtained using a rotary microtome (Figure 4), and the sections were stained with Hematoxylin Eosin (Figure 5) and Masson’s trichomes (Figure 6). The prepared slides were then examined under a light microscope. During the examination of the vascular tissue, measurements were taken of the thicknesses of the tunica intima and tunica media, as well as the areas of the tunica intima and tunica media, vascular diameters, and vascular lumen areas. These parameters were assessed for both the anastomosed fistula and non-anastomosed segments, and the non-anastomosed component was collected from the left internal jugular vein.

\textbf{Histomorphometric analysis}

All the specimens underwent a histomorphometric analysis to measure intima-media area per unit length (IMA/L). We divided the intima-media area by the circumference of the lumen to correct the degree of dilatation. This histological report was done in

\begin{figure}[h]
\centering
\includegraphics[width=0.8\textwidth]{figure1.png}
\caption{A wild-type male local rabbit that weighed around 2000-2500 grams was prepared for AVF Surgery.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=0.8\textwidth]{figure2.png}
\caption{A macroscopic view of the right neck before anastomose. The red arrow showed the arterial segment of the right carotid artery. The green arrow showed the venous part of the right internal jugular vein.}
\end{figure}
the Histology Laboratory, Faculty of Veterinary of Universitas Syiah Kuala, Banda Aceh.

RESULT
We successfully induced neointimal hyperplasia in wild-type local rabbits, indicated by increased tunica intima thickness after 28 days of the arteriovenous fistula procedure. The results of the histological measurements of anastomosed and non-anastomosed fistula of the experimental and control groups are presented in (Figure 6), using Masson’s trichrome staining. Figure 6A showed a microscopic view from non-anastomosed vein harvesting tissue after Masson’s trichrome staining. The width of the tunica intima of the left internal jugular vein is 1.17 μm (black dash). A microscopic view from anastomosed AVF vein harvesting tissue after Masson’s trichrome staining showed in Figure 6B. It shows that the width of the tunica intima of the left internal jugular vein is 22.14 μm (black dash).

DISCUSSION
Vascular access-related complications constitute a significant cause of morbidity for patients on chronic hemodialysis. The pathogenesis of venous neointimal hyperplasia in arteriovenous (AV) graft stenosis and late AV fistula stenosis has been well described and is commonly divided into upstream and downstream events. Upstream events are the initial insults responsible for endothelial injury, leading to a cascade of mediators (downstream events) that regulate oxidative stress, endothelial dysfunction, and inflammation. Downstream events represent the response to endothelial (vascular) injury from the upstream events, resulting in the migration of smooth muscle cells from the media to the intima, eventually forming neointimal hyperplasia.

Laboratory animal models play an essential role in the study of human diseases. Using appropriate animals is critical for basic research and the development of therapeutics and diagnostic tools. Animal models closely mimicking human pathology are of utmost importance to unravel the pathophysiology of hemodialysis access failure.

Although humans and mice or rats share a high level of genetic similarity, there are significant differences in their metabolism that restrict the translational potential of murine research. On the other hand, rabbits provide a greater translational capability for the development of new drugs and diagnostic methods, as they offer a bridge between basic research in rodents and more translational research in larger animals.

Over the years, three primary rabbit breeding lines have emerged as popular choices for cardiovascular research: New Zealand White, Japanese White, and Watanabe heritable hyperlipidemic. While transgenic rabbits have also been...
used in research, their utilization is not as widespread as rodents due to the higher costs and greater difficulty of genetic manipulation.\textsuperscript{34} Despite their importance, compared with the mouse, the use of rabbit models still needs to be improved.

No study investigated neointimal hyperplasia in arteriovenous fistula using wild-type rabbits to date. However, our study has a similar result to a study using laboratory rabbits. One study by Mehrad et al. generated neointimal hyperplasia in a New Zealand white rabbit. Histopathological results showed progressive, smooth muscle cell proliferation in the intimal layer after four weeks.\textsuperscript{27} Compared to their wild ancestors, laboratory rabbits are significantly larger in size, which means they require more space to be housed properly.\textsuperscript{28} On the contrary, wild-type rabbits in our study may be susceptible to effectively reducing research costs and still showed regular chow diet and behavior throughout the course like laboratory rabbits.

A significant advantage of rabbits other than rats or mice is that the vessel of a rabbit is large enough for human instruments. Several vessels are targeted for cardiovascular research with rabbit animal models; carotid artery, aorta, iliac artery, and femoral artery.\textsuperscript{28} In this study, we experimented with anastomosis end-to-side in the right femoral artery-right femoral vein, and the abdominals artery-abdominal vein, which turned out the rabbit died in the following days.

The surgical procedure used to induce vascular damage in rabbits is similar to that in rats and mice. However, the focus is more on angioplasty techniques that closely resemble those used in humans.\textsuperscript{34} This makes arteriovenous fistula models particularly useful in rabbits as they can simulate realistic clinical scenarios for testing new therapeutic and diagnostic approaches, emphasizing the importance of translatability in research.

**CONCLUSION**

Rabbit model using a wild-type model for investigating neointimal hyperplasia in arteriovenous fistula may be susceptible and considered as it is accessible and also cost-effective besides producing the same outcome as laboratory rabbit.

**ETHICAL CLEARANCE**

Ethical Clearance already approved by an animal use protocol by the Institutional of Animal Care and Use Committee, Faculty of Veterinary of Universitas Syiah Kuala,
CONFLICT OF INTEREST
No conflict of interest.

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AUTHOR CONTRIBUTION
All authors have contributed equally from the conceptual framework, data acquisition, and data analysis until the study results are reported through publication.

REFERENCE