Correlation of Caveolin-1 With Vascular Intimal Thickness for Different Locations of Catheter Tips in a Dog Model

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Original Paper

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Keywords. hemodialysis, tunneled cuffed catheter, intimal thickness, caveolin-1

Introduction. Many studies have reported increased intimal thickness around the catheter tip after catheterization. Caveolin-1 is a protein in the endothelial cell that acts as a shear sensor causing vascular remodeling. This study aimed to elucidate the suitability of different catheter locations and determine the role of caveolin-1 in canine models.

Materials and Methods. Tunneled silicone 14.5-F catheters were inserted into the left jugular vein and right femoral vein in 8 dogs. The dogs were separated into 2 groups by catheter location and were followed up for 28 days. All dogs underwent extracorporeal circulation 3 times a week. After animal sacrifice, histological and immunohistochemical assays were performed to measure specific cell populations.

Results. There were higher catheter dysfunction rates and lower blood flow rates in the right femoral vein group compared to the left jugular vein group. There was intimal hyperplasia around the catheter tip in both groups with no significant difference between the two groups. There were caveolin-1 expression in the intimal layer of venous wall around the catheter tip location sites in both groups.

Conclusions. These findings indicate that different catheter tip locations may influence catheter function and specific targeting of caveonlin-1 could be a strategy of possible future novel therapies for catheter-related vein stenosis.

IJKD 2018;12:232-9
www.ijkd.org

INTRODUCTION

Although the number of arteriovenous fistulas in hemodialysis patients has been increasing, the use of tunneled hemodialysis catheters is still widespread.[1] There is a subset of hemodialysis patients that require long-term catheter use due to limited access sites, poor vasculature resources, or refusing access creation.[2] However, there may be complications related to prolonged catheter use.[3]

When a catheter becomes dysfunctional, it is usually because the catheter tip becomes surrounded by a fibrin sheath or due to thrombosis.[4] There can also be increased intimal thickness around the catheter tip, which may cause vein stenosis.[5] Many studies have found a high fluid shear change during hemodialysis at the catheter tip.[6,7] However, it is unclear whether this reflects an interaction between endothelial cells and the catheter tip. Caveolin-1 is a protein in the endothelial cell that acts as a shear sensor that may transmit mechanical change into biochemical signals and final vascular remodeling.[8] An important question is whether caveolin-1 plays a role in structural changes at the catheter tip, limiting catheter effectiveness.

Additionally, the optimal location for a long-term tunneled central venous hemodialysis catheter tip is controversial.[9] The National Kidney Foundation recommends that tunneled hemodialysis catheters are placed with the tip in the mid-right atrium to obtain maximize flow rates, as suggested by previous studies.[10,11] However, the National Kidney Foundation guidelines emphasized that further research should be done to investigate the ideal catheter tip position.[10] The Food and Drug Administration analyzed many reports before the common use of fluoroscopy, and recommended that catheter tip should be located in the superior vena cava due to the concern of potential cardiac-related complications and arterial thrombosis if the catheter tip was located in the right atrium.[11,12]

The correlation of tip location and the caveolin-1 signaling that elicits vascular remodeling around the catheter tip has not been studied. However, it is known that endothelial cells drive most vascular tissue responses to altered shear stress.[13] To elucidate the suitability of different catheter locations and determine the role of caveolin-1, we used catheters in canine models. Our results was aimed to provide significant insight into the biologic responses of endothelium to local shear stress and the role ofcaveolin-1 near catheter tip sites.

MATERIALS AND METHODS

Experimental Design

A total of 8 dogs (initial weight, 25 kg) were used in the study. Animal handling and experiment conformed to standards set forth in The Guide for the Care and Use of Laboratory Animals.[13] The study was approved by the institutional animal use committee of the 2nd Hospital of Tianjin Medical University, China. The dogs were randomly divided into 2 groups (4 in each group) and a catheter was inserted in the right femoral vein or in the left internal jugular vein. Then animals were sacrificed after a catheterization period of 28 days.

Surgical Procedure

The dogs were anesthetized with xylazine hydrochloride injection (1 mg/kg, intramuscularly), at least 30 minutes prior to the surgical procedure. All operations were done under aseptic surgical procedures. The dogs' hair was shaved from the ventral and left lateral sides of the neck to at least the lateral midline on both sides or from left abdomen to left leg. The dogs were then transferred into the operating room, the surgical site was cleaned by applying povidone-iodine solution and then 70% isopropyl alcohol solution, and draped for aseptic surgery.

The left internal jugular vein or right femoral vein was punctured with guidance. Access was achieved with a 21-gauge needle that was exchanged for a 3 coaxial dilator over a 0.018-inch guide wire (Covidien, Mansfield, MA, USA). A 16-F peel away introducer sheath was advanced into the access vein over a stiff 0.35-inch guidewire. A 14.5-F 19-cm Palindrome catheter was used (Covidien). After catheter placement, an X-ray was taken to assess the location of the tip of the catheter. When the tip of the catheter was between the fifth and sixth ribs or between the third and fourth lumbar vertebra, we determined that the catheter tips were in the proximal end of the superior vena cava or in the inferior vena cava, based on our knowledge of canine anatomy.

Extracorporeal Circulation

The dogs underwent extracorporeal circulation, under aseptic conditions, 3 shifts per week, in order to mimic the hemodynamic shear stress changes that occur during hemodialysis. In brief, dogs were anesthetized, and subsequently, after priming with saline, 2 dialysis tubes were separately connected to the catheter adapters, and a loaded heparin dose of 3000 IU was given intravenously. The dogs underwent extracorporeal circulation for 30 minutes, using a blood pump with a blood flow rate of 250 mL/min. For prevention of thrombosis, a heparin lock was used at the end of procedure.

Catheter Performance Evaluation

Catheter dysfunction was considered when Qb was less than 100 mL/min. If this occurred, the catheters were instilled with 5000 IU/mL of urokinase per port to restore the flow. Tunnel infection of catheters was diagnosed by signs of infection near the cuff area, such as erythema, tenderness, and induration in the tissues overlying the catheters more than 2 cm from the exit site.[12]

Specimen Processing

The dogs were sacrificed after a catheterization period of 28 days. The tissues of vessels around the catheter tip both in the superior vena cava

and inferior vena cava were fixed in formalin for at least 1 day. Transverse slices of the associated venous wall of approximately 0.5 cm thickness were put into cassettes and embedded in paraffin.

Histochemistry and Immunohistochemistry

Standard hematoxylin-eosin staining was used for histological examination. Verhoeff-van Gieson staining was used to identify elastic laminas. For immunohistochemical staining, after deparaffinization and hydration, sections were incubated with the blocking agent, 3% hydrogen peroxide, for 10 minutes and then blocked with rabbit serum for 30 minutes. The sections were then incubated with primary antibody (anti-alpha smooth muscle actin antibody, abcam, ab5694, 1: 100 and anti-Caveolin-1 Cell Singal 1:200) overnight at 4°C. The next day, sections were incubated with the biotinylated goat anti-polyvalent antibody for 30 minutes and with streptavidin peroxidase for 30 minutes at 37°C. The sections were then treated with the 3′-diaminobenzidine color developing solution and hematoxylin. Following dehydration, sections were mounted and dried.

Image Analysis

Images of the stained sections were analyzed using a microscope (Nikon Eclipse 90i) fitted with a camera head (DS-5M) and a controller (DS Camera Control Unit DS-L1), which transmitted the image to a computer (IBM). Digital Managing System (NIS-Elements F.3.0) was used for image capture and analysis.

To calculate the thickness and area of the intimal and medial layers, the image analysis software was calibrated to provide measurements in μm. For the hematoxylin-eosin stained sections, each segment was analyzed by drawing 6 equally spaced lines perpendicular to the venous wall, and measuring the thickness of the intima and media at that location. The data was then averaged to calculate the thickness of the laminate for each segment.

Statistical Analysis

Data are expressed as the mean ± standard deviation. Comparisons were made using a 2-tailed Student *t* test. The difference was considered significant at *P* values less than .05. Statistical analysis was performed using the SPSS software (Statistical Package for the Social Sciences, version 20.0, IBM Corp, New York, NY, USA).

RESULTS

Catheter Performance

At the beginning of the study, we assumed that the catheter function might be different in both groups. After the period of 28 days, we analyzed catheter performance to investigate the difference of catheter tip location. There was more catheter dysfunction in the femoral vein group than in internal jugular vein group ($P < .05$), but the catheter infection rate was comparable between the two groups (Table).

Intimal Thickness of Venous Wall

After the 28th day of catheterization, there was intimal hyperplasia in both groups near the location of catheter tips (Figure 1). Meanwhile, when the intima-media ratio of the thickness was calculated, we found no significant differences in the catheter location sites for the left interjugular vein group and the right femoral vein group (Figure 2).

Radiographic Examination of Catheter Tip Location

To determine the catheter tip location, we did radiographic examinations for the two groups of dogs. The catheter tip was located in the superior vena cava for the left jugular vein group and in the inferior vena cava in the right femoral vein group (Figure 3).

Cellular Phenotypes of Venous Wall

After the 28th day of catheterization, there were

Comparison of Catheter Function Between the Study Groups*

Parameters	Left internal jugular vein catheter group	Right Femoral vein catheter group	*P*
Blood flow, mL/min	236 ± 21	197 ± 13	.001
Episode of catheter dysfunction	1 (12.5)	5 (62.5)	.04
Episode of catheter infection	3 (37.5)	4 (50)	.61

*Values are mean ± standard deviation or frequency (percentage).

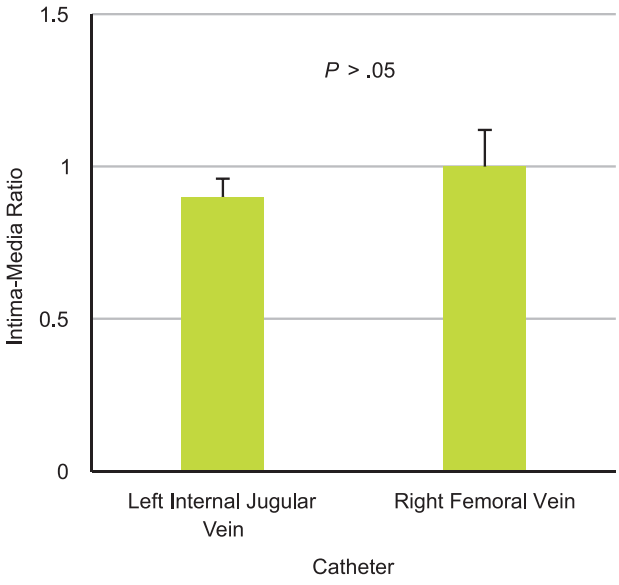

Figure 1. Comparison of intimal-medial ratio of thickness between the two groups of dogs with right femoral vein or in the left internal jugular vein catheters.

fibrin sheaths in both groups. Furthermore, there were fibrin sheaths in the venous cavity with less prominent cellularity and more prominent collagen content (Figure 4) in both groups, while intimal layer was located above the internal elastin layer (Figure 5). Additionally, smooth muscle cells were detected under the intimal layer on day 28 at both vascular walls around the different catheter tips, as indicated by their positive staining with the anti-actin antibody (Figure 6). Meanwhile, there was caveolin-1 expression in the intimal layer of venous wall around the catheter tip location sites in both groups (Figure 7).

DISCUSSION

In our study, we compared two different locations of catheter tips to gain insight into the effect of tip location on catheter function. To our knowledge, this is the first report using extracorporeal circulation to mimic the hemodynamic change with catheters in the vascular wall. We found catheter function was better when catheter tip was positioned in the superior vena cava than when positioned in the inferior vena cava, similar with our previous studies.[14,15] This may be due to the different direction of blood flow in the two locations, which also is complicated by gravity due to the anatomical difference between the inter jugular vein and femoral vein. Additionally, the ongoing movement of the dog with the catheter within the femoral vein caused endothelial damage and can trigger the development of mural thrombi that encroach on the lumen of the catheters until there is dysfunction.

Although the intimal hyperplasia has been well studied for arteriovenous fistula stenosis in terms of fluid shear stress.[16,17] Little is known about how these events happen with a tunneled cuffed catheter tip and associated vascular wall remodeling. Due to the complexity of the integrated response elicited by venous biomechanical cues, exposure to the tip of the catheter and blood flow change during circulation may be an important stimulus for the vascular remodeling of a venous wall around the catheter tip.[5,7]

Caveolae are noncoated micropatches in the plasma membrane of different shapes (flat,

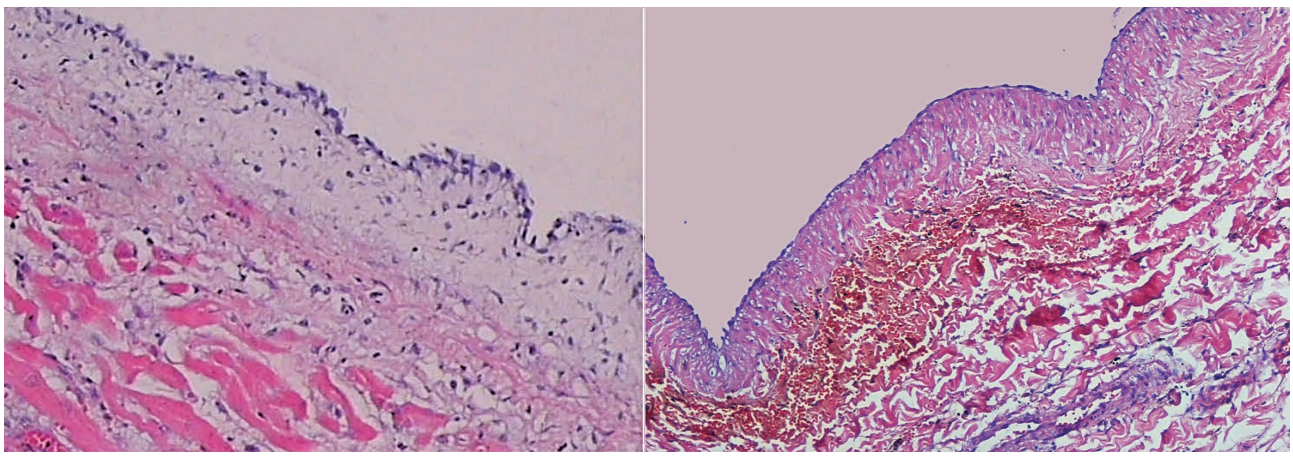

Figure 2. Intimal thickness in both groups of dogs with right femoral vein or in the left internal jugular vein catheters. Left, Intimal hyperplasia of vascular wall near catheter tip in the left internal jugular group. Right, Intimal hyperplasia of vascular wall near catheter tip in the right femoral vein group (hematoxylin-eosin, × 100).

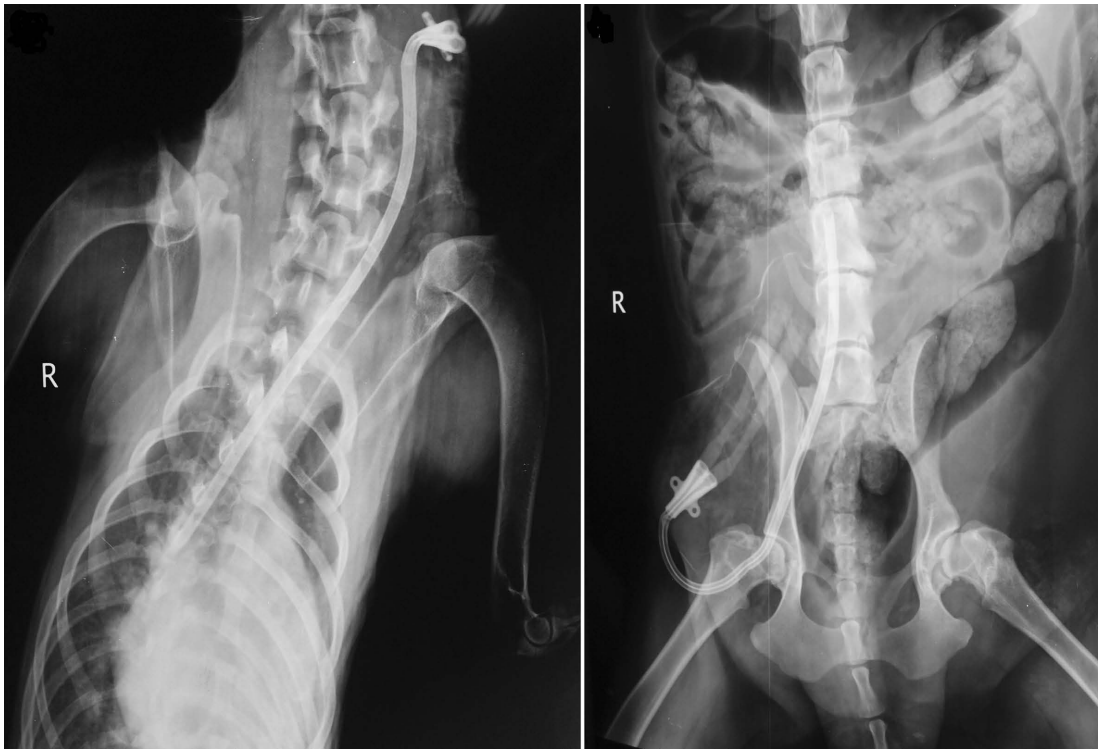

Figure 3. Left, Catheter inserted into the left internal jugular vein of the dog. Right, Catheter inserted into the right femoral vein of the dog.

invaginated, and tubular).[18] Many cell types including endothelial cells, fibroblasts, smooth muscle cells, and adipocytes have caveolae. Caveolin, a 21- to 24-kDa membrane protein, is a principal component of caveolae, and also binds directly to cholesterol and interacts with signaling molecules including G protein a-subunits, Ras, Src family kinases, and the endothelial form of nitric

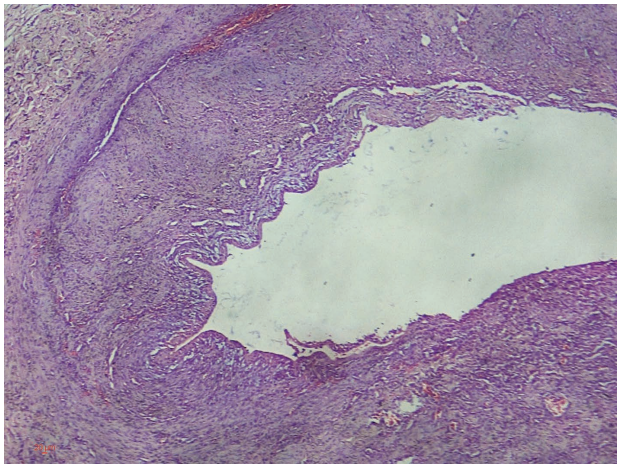

Figure 4. Fibrin sheath in the vascular wall near the catheter tip. Sheaths in the venous cavity on day 28 showed less prominent cellularity and more prominent collagen content. (hematoxylin-eosin, × 40).

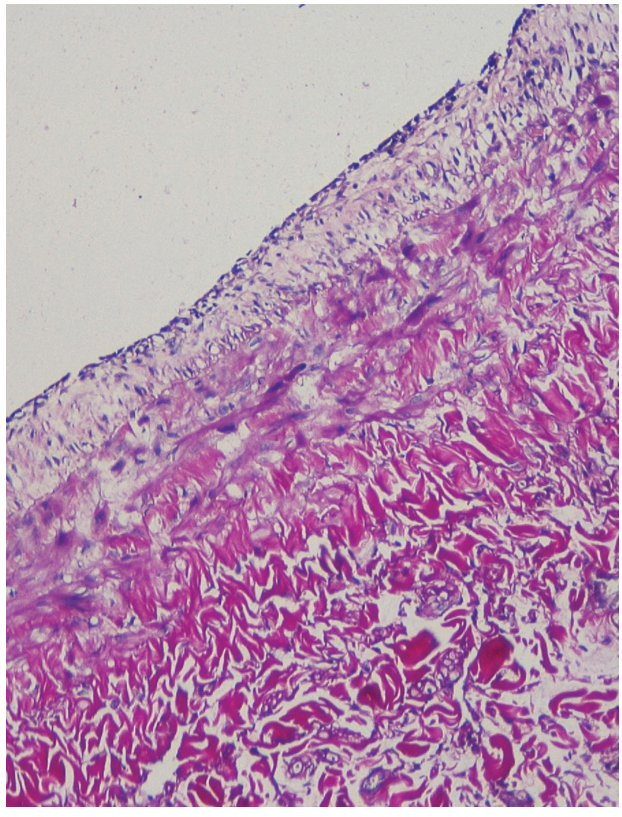

Figure 5. Collagen distribution in the laminar membrane was detected between intimal and media layers in the vascular wall near the catheter tip (Verhoeff-van Gieson's elastin [lamina], ×100).

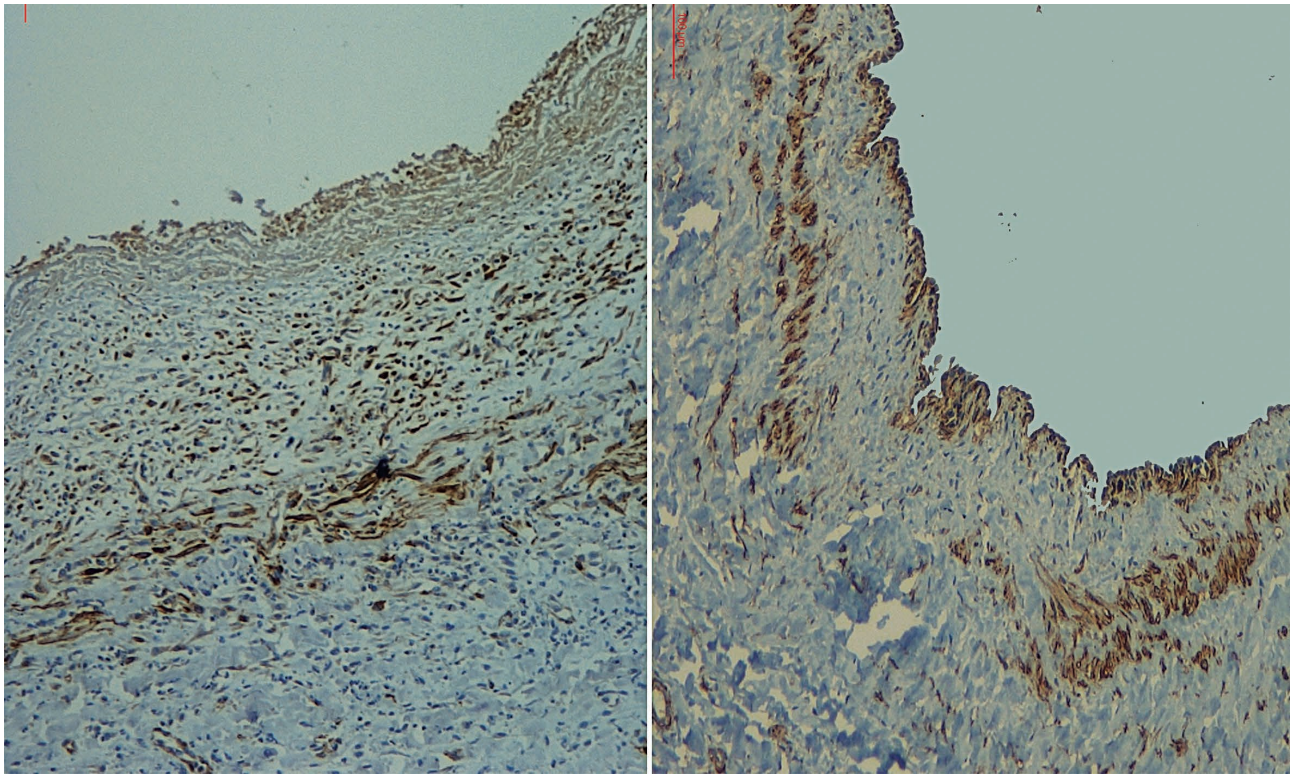

Figure 6. Cells staining positive for smooth muscle actin were present under intimal layer near the catheter tip. Left, The vascular wall in the left inter jugular group. Right, The vascular wall in the right femoral vein group (hematoxylin-eosin, × 100).

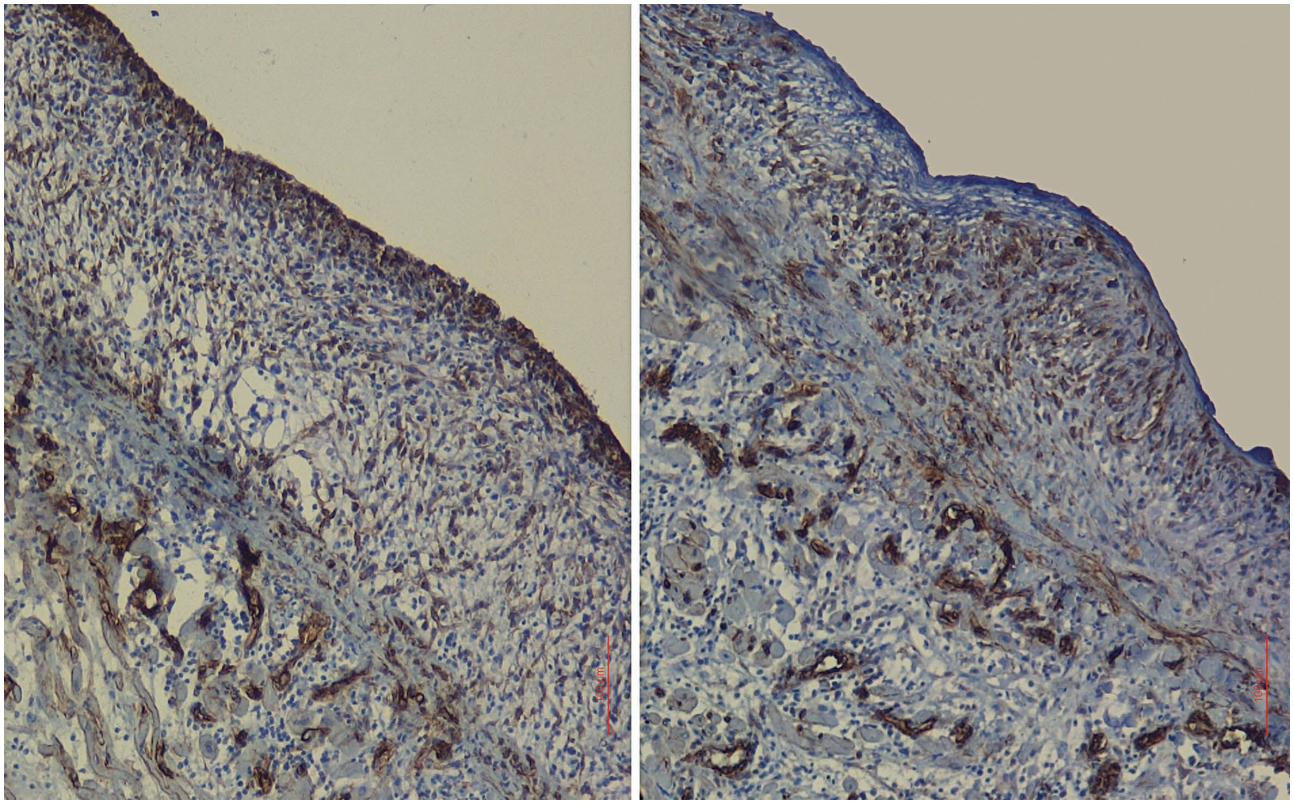

Figure 7. Caveolin-1 expression under intimal layer near the catheter tip. Left, Caveolin-1 expression in the vascular wall in the left jugular vein group. Right, Caveolin-1 expression in the vascular wall in the right femoral vein group (hematoxylin-eosin, × 100).

oxide synthase.[19,20] Three caveolin family members (caveolin-1, caveolin-2, and caveolin -3), including 2 isoforms (caveolin-1a and caveolin-1b), of the caveolin gene family in mammalian cells have been identified. Among them, caveolin-1 is abundantly expressed in endothelial cells. It has been supposed to be a sensor of shear stress which endothlial cell is facing and trigger subsequent signal pathways of vascular remodeling.[21]

The catheter tip can alter blood flow with high shear stress and cause different flow patterns,[7] which may activate caveolin-1 in the proximal endothelium cells. In our study, we found that intimal hyperplasia around the catheter tip. Caveolin-1 was expressed in both groups near the catheter tip, which suggests a potential involvement of caveolin-1 in intimal hyperplasis by sensing the change of shear stress. Other reports also concluded that the accumulation of neointima cells results from migration and proliferation of adventitial myofibroblasts, characterized by positive staining of smooth muscle actin-α.[22] In our study, we found that focal areas of contact between the catheter tip and the vein lead to endothelial denudation and the development of intimal hyperplasia. Migrating (from the media) smooth muscle cells were observed under the venous intima, indicating that smooth muscle cells may be involved in the vascular remodeling that results from the increased shear stress from the catheter tip.

Our study has some limitations. First, the number of dogs in each group was small. Compared with other studies,[16] focusing on catheter-related complications, rodent models can more easily utilize a large number of animals in a single study, which is more difficult to do with canine models. However, our data were sufficient to suggest vascular remodeling around the catheter tip. Second, we did not measure the actual wall shear stress inside the venous wall, limiting applicability to human studies. However, through the use of extracorporeal circulation, we simulated the hemodynamic change that occurs during hemodialysis and the sequence of vascular remodeling should be the same in terms of the dynamics of circulation and the ongoing response of the vascular wall.[23]

CONCLUSIONS

We developed a novel canine model of venous intimal hyperplasia to test different locations of a catheter tip. This model will facilitate investigation of early cellular and molecular adaptations of venous vessels in response to a shear stress of the local biomechanical environment generated by the catheter tip. The use of this model in the context should help in the future identification and functional characterization of pathways implicated in venous stenosis after catheterization. These results underscore our current lack of understanding about possible phenotype switching within the venous neointima and suggest that targeting of specific caveonlin-1 could be applied as a future novel therapy for hemodialysis vascular access dysfunction.

FUNDING SUPPORT

This research received grants from the General Program of Tianjin Medical University (No 2016KYZM11), Project of Chinese Society of Blood Purification Administration (No CHABP201605), and the National Natural Science Foundation of China (No 81600591).

ACKNOWLEDGMENTS

We thank all the doctors in our center. Lihua Wang and Lan Jia contributed equally to this work and should be taken as co-first authors.

CONFLICT OF INTEREST

None declared.

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Received January 2018
Accepted March 2018