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Down Stream Vascular Changes after Flow Diverting Device Deployment in a Rabbit Model

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Abstract

Background—Flow diverters (FDs) are increasingly used in the treatment of intracranial aneurysms, and carry the risk for thromboembolic complications, even in patients treated with dual antiplatelet therapy. The effect of FDs on the downstream vascular is unknown. The aim of the study was to investigate vascular wall pulse wave velocity and contractility changes following FD treatment in a rabbit model.

Methods—FDs (Pipeline Embolic Device, Medtronic Inc.) were implanted in the aorta of normal rabbits and sham-operated aorta were used as controls (n=6 per group). Pulse wave imaging with ultra-fast ultrasound at 1600 frames per second (Vantage, Verasonics, Inc.) was performed in the vessel wall distal to FD prior to device implantation and at 8-week follow-up to measure the pulse wave velocity (PWV). Force contraction vascular reactivity studies were conducted in the aortic rings using organ bath.

Results—The difference in mean PWV in the follow-up compared to pre-implantation was significantly higher in the distal vessels compared to sham controls (1.18 m/s [SD=0.54] vs. 0.37m/s [SD=1.09], p=0.03). Conversely, the aortic segments distal to the FD exhibited a 55%

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PKP and DD were involved in tissue harvest, vascular contractility studies, and drafting of the manuscript.

YHD was involved FD deployment and sacrifice

MWU was involved in pulse wave velocity measurement and analysis.

LM and SV were involved in conception and design of vascular contractility studies.

DFK, JC and RK contributed to the conception and design of the study and to revision of the article critically for important intellectual content.

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increase in vascular contractility compared to proximal segments ($p=0.002$). We observed a significant positive correlation between mean PWV and mean vascular contractility.

Conclusion—Implantation of FD was associated with increased PWV and vascular contractility, suggesting that FD implantation causes changes to the vascular wall. Further studies are needed to understand the clinical implication of changes in vascular PWV and contractility.

Keywords

Intracranial aneurysms; Flow diverter complications; distal intraparenchymal hemorrhage; Pulse wave velocity; vascular contractility; rabbit aneurysm model

INTRODUCTION

Endovascular treatment of aneurysms has emerged as one of the primary treatments for intracranial aneurysms.^{1, 2} Endovascular coiling has been primarily used for the treatment of aneurysms in recent years.³ Flow diverters (FDs) are increasingly used, owing to superior occlusion rates compared to coils.⁴ Despite the superior therapeutic efficacy, FD treatments are associated with an overall complication rate of approximately 17%. Complications related to FDs include delayed intraparenchymal hemorrhage, subarachnoid hemorrhage, and ischemic stroke following FD deployment, even in patients treated with dual antiplatelet therapy.⁵ This poses a serious concern for the use of FDs and demands an exploration into weighing the potential risks and benefits of FDs. The pathophysiological mechanism behind these delayed vascular complications is unclear. In this study, we aimed to investigate changes to the vascular wall induced by FD deployment, by measuring downstream vascular pulse wave velocity (PWV) and vascular contractility before and after FD implantation in the rabbit aorta.

METHODS

In vivo studies

The animal research protocol was approved by our Institutional Animal Care and Use Committee. FDs (Pipeline Embolic Devices, Medtronic, Inc.) were implanted in the abdominal aorta of six rabbits ($n=6$), and another group of six rabbits were used as sham-operated controls ($n=6$). Pulse wave imaging was performed ultrafast ultrasound at 1600 frames per second (Vantage, Verasonics, Inc.), on both the anterior wall and posterior wall of the aorta, 5mm distal to the FD device, prior to device implantation and at 8 weeks follow-up to measure the pulse wave propagation.

Flow diverter deployment

Two days before deployment, rabbits were premedicated with aspirin (10 mg/kg orally) and clopidogrel (10 mg/kg orally).⁶ Anesthesia was induced by intramuscular injection of ketamine and xylazine and maintained by 2.5-3% Isoflurane inhalation. Surgical cut-down of the right femoral artery was performed, followed by the insertion of a 5-Fr sheath. A 5-Fr guide catheter (Envoy; Cordis) was placed into the abdominal aorta and digital subtraction angiography (DSA) was performed. A microcatheter (Marksman, Covidien) was placed over

a micro guidewire (Transcend; Boston Scientific) into abdominal aorta through the guide catheter. The micro guidewire was removed, and the FD was advanced into the distal aspect of the microcatheter. The device was deployed below the origin of renal artery, covering at least one lumbar artery. The micro catheter was removed and DSA was performed through a guide catheter. Then the guide catheter and sheath were removed, the femoral artery was ligated, and the animals were allowed to recover. Antiplatelet therapy was continued until the animals were euthanized. For control animals, the same procedure was undertaken except no flow diverter was deployed.

Pulse wave velocity measurement

Pulse wave imaging (PWI) is a method that measures the motion of the arterial wall due to the pressure pulse to quantify the local PWV. This method has been utilized in the aortas of mice with and without aneurysms, and in human aortas and carotid arteries.^{7,8} Utilizing pulse wave imaging has the advantage of assessing the PWV over a short segment (1-9 cm) compared to the longer segment used clinically in cfPWV (carotid to femoral pulse wave velocity).

For the PWV measurement, a programmable ultrasound scanner was used (Vantage, Verasonics, Inc., Kirkland, WA) with a linear array transducer (L11-4v, Verasonics, Inc., Kirkland, WA). Once the aorta distal to the flow diverter was located in the abdomen of the rabbit using real-time B-mode ultrasound, we used an ultrafast imaging sequence to measure the PWV. A compound plane wave imaging approach with three angles (-6° , 0° , $+6^\circ$) performed the imaging with an effective frame rate of 1600 Hz for 0.5 s.⁹ The particle wall velocity was estimated from the acquired in-phase/quadrature (IQ) using an autocorrelation algorithm for analysis. A temporal derivative was computed on the particle velocity to obtain the wall particle acceleration. Measurements were repeated 5-10 times for each rabbit at each occasion of imaging. In some cases, depending on the animal's heart rate, we had multiple measurements of the PWV that could be made from a single acquisition.

The aorta walls were identified manually, and a spatial vector was created for all tracked locations. A window was centered about the manually identified wall for extraction of the data for PWV analysis. To estimate the PWV, we tracked the peak of the acceleration curve and a time-of-flight algorithm was used with a linear regression of tracked temporal peaks versus lateral location along the length of the ultrasound transducer.¹⁰ The PWV was measured on both the anterior wall and posterior wall of the aorta in the B-mode image, 5mm distal to the FD device. All the measurements were averaged, and the results were pooled from both walls for analysis.

Tissue harvest

Eight weeks after FD deployment, the animals were euthanized. The proximal and distal segments of the implanted device in the aorta and the corresponding segments in the control group were harvested and placed in oxygenated physiological salt solution (PSS) or formalin for histological analysis.

***In vitro* vasoreactivity assay**

The aortic segments were cleaned of the surrounding fat and connective tissue and cut into rings 2 to 3 mm long. Vessels were then placed in an organ chamber containing 4 mL of warm and oxygenated PSS. Rings were connected to force transducers and isometric force was recorded continuously. The vascular rings were stretched to determine the optimal point of their length – tension. The output from the transducers was amplified by signal conditioners and transmitted to the nearby computer for analog/digital conversion. For each aorta, 2 proximal and 2 distal rings were studied. The contractile response to a depolarizing concentration of potassium chloride (KCl=80mmol/L) provided a measure of maximal contractile responsiveness in each ring. All of the rings were then constricted with 1 μ M phenylephrine. When maximal response produced by this agonist remained stable, the rings were washed with PSS and allowed to become stable under resting tension.¹¹

Histological analysis

Histological processing was performed as we have previously described,¹² and arterial segments were subsequently embedded in paraffin, serially sectioned (4 μ m) and mounted on glass slides. Then, the slides were stained with hematoxylin and eosin, Masson's trichrome, and Verhoeff-Van Gieson (VVG) stains for assessing changes in cellularity, collagen and elastin, respectively. A trained, experienced (more than 16 years) pathologist evaluated all the slides blinded to the study group.

Statistical analysis

Descriptive statistics are reported at the sample level. The outcome variable for contractility measurements was the average amplitude in relation to baseline and peak amplitudes. The effect of treatment and location on contractility was modeled using a linear mixed effects model, with adjusted denominator degrees of freedom using the Kenwood-Roger method. Analysis was performed using SAS (Cary, NC; version 9.3). Due to small sample size, nonparametric data was assumed for PWV data, and within groups pre- and post-intervention measurements were compared using the Wilcoxon signed-rank test. Between-group PWV results for the FD-treated and sham-operative group were analyzed via the Mann-Whitney test. P-values less than 0.05 were considered significant. PWV data were analyzed using GraphPad Prism (La Jolla, CA) version 7.05.

RESULTS

Of the initial 12 animals, 10 animals (83%, n=5 in each group) were used for assessment of vasoreactivity of blood vessels. Two animals of the initial 12 (17%, one Test and one control) were excluded from the vasoreactivity study due to technical malfunction of the equipment on the day of study. Data from 11 of 12 animals (92%) were useable for the measurement of pulse wave velocity of aorta distal to the FD. One control rabbit died due to complications.

Pulse wave velocity

In 2/6 cases (33%), the data was not adequate for PWV measurement in the posterior wall for the FD-treated group. In most animals (6/6 [100%] for the anterior wall; 3/4 [75%] for

the posterior wall), the PWV was found to increase eight weeks after FD implantation. Accordingly, 8 weeks after FD placement, there was a significant increase in PWV in the FD group (n=6) compared to baseline measurements at the anterior wall (1.18 m/s [SD=0.54], p=0.03), but there was no significant change in the control group (n=5) at 8 weeks post-implantation (0.37 m/s [SD=1.09], p=0.31). Conversely, when PWV was measured at the posterior wall, there was no significant difference between baseline and 8-week measurements for both the control (n=4) and the FD-treated group (n=4) (FD: 0.31 m/s [SD=0.58], p=0.39; control: -0.10 m/s [SD=1.34], p=0.99). We noted a significant increase in the PWV measured in the anterior wall of the aorta distal to the FD (n=6) in comparison to control (n=5) (1.18 m/s [SD=0.54] vs. 0.37 m/s [SD=1.09], p=0.03). PWV measured in the posterior wall of the aorta was not significantly increased in comparison to control (0.31 m/s [SD=0.58] vs. -0.10 m/s [SD=1.34], p=0.73). Figure 1 shows the PWV results from the anterior wall (Fig 1A), and the posterior wall (Fig 1B) for FD-implanted animals and control animals. Data is displayed for both pre-implantation and post-implantation. In some animals, the PWV was observed to increase and decrease after the implantation.

Figure 1D provides a summary showing the trends of the mean values for the anterior wall, posterior wall, and pooled (Total) results before and after implantation of FD. The PWV generally increased about 1 m/s in the rabbits with flow diverters, which represents a substantial increase (~20-50%) in the PWV. Figure 2 shows a visual representation of PWV measurement before and after FD implantation.

Vascular reactivity

The distal region was associated with contractility (p=0.02). Compared to proximal locations, distal locations showed a 55% increase in amplitude, which showed to be statistically significant (1.24 [95% CI: 0.46-2.03] vs. 0.68 [95% CI: -1.0-2.36], p=0.002). Distal locations did not show statistically significant increased contractility compared to controls (0.68 [95% CI: -1.0-2.36], p=0.39). The change in contractility between proximal locations and control regions were not significant (-0.57 [95% CI: -2.25-1.12], p=0.47). A summary of the results from the vascular reactivity experiments is shown in Figure 3.

Histological analysis.

H&E stains showed normal vasculature of aorta in both control and test group. Neither inflammatory cell infiltrate nor neointimal hyperplasia was observed in any of the tissue section. Similarly, our VVG and Masson's trichrome stains showed that the elastin architecture and collagen content were not different between the two groups (Figure 4).

DISCUSSION

In this study, we observed significant increases in both pulse wave velocity and vascular contractility in the distal aortic segment after FD deployment in the rabbit aorta. The findings described here suggest the occurrence of ongoing pathophysiological changes to the vasculature after FD deployment. It is possible that increased PWV and vascular contractility may contribute to complications and adverse events associated with the use of

FDs, and thus future studies are needed to elucidate the potential clinical impacts of these changes.

Several studies have shown significant morbidity and mortality after treatment with FD.¹³⁻¹⁵ In a recent meta-analysis of 1,654 aneurysms, Brinjikji et al. found an aneurysm occlusion rate 75% of after 6 months with procedure-related morbidity and mortality of 5% and 3%, respectively. The complications were comprised of 5% postoperative hemorrhage and 3% intraparenchymal hemorrhage. The rate of ischemic stroke was 6%, with significantly lower perforator infarction in the anterior circulation than the posterior circulation.¹⁵ These findings highlight the critical need to understand the pathogenesis of the complications and adoption of definitive measures to prevent these complications. Although the cause of thrombotic complications associated with FD treatment is unclear, current literature postulates catheter-related thromboembolism as a potential contributing factor, documented by MR imaging in the immediate postoperative period.¹⁶ Another dreaded complications following FD deployment is delayed distal intraparenchymal hemorrhage (IPH).¹⁵⁻¹⁷ The hemodynamic hypothesis suggests one possible cause of IPH, which involves a reduction in the “windkessel effect,” or a decrease in blood vessel elasticity that induces an increase in distal pulse pressure and consequently an increased PWV.¹⁶

We observed increased contractility in the abdominal aorta distal to the FD device in comparison to the proximal segment of the device. We believe that contractility changes may be due to the hemodynamic changes in the structure of the vessel wall as a result of slightly altered flow in the FD-implanted vessel wall. Previous studies have shown that shear stress increases the vascular smooth muscle contractility through the glycocalyx mediated mechanism.¹⁸ The presence of an aneurysm might modify the physiological hemodynamic patterns in parent artery, which may in turn affect the cellular function in the surrounding vasculature. Furthermore, FD treatment in the internal carotid artery has shown to alter the hemodynamics by increasing flow velocities and wall shear stress while lowering resistance in the ipsilateral middle cerebral artery.¹⁹ Further studies are needed to substantiate the mechanism for the changes in contractility of the distal vessel wall and its potential pathophysiological impact.

Moreover, we observed an increase in PWV in the anterior wall of aorta distal to the aneurysm. The reason for differences in PWV in the anterior and posterior walls of the distal vessel remains unclear. The probe’s ability to measure variable boundary conditions of the different walls and conspicuity of the walls in the B-mode imaging is one possible explanation for this difference. Previous studies have shown that increases in PWV are directly related to increases in the stiffness of the vessel wall.⁷ The arterial stiffness can be caused due to a number of reasons. Endothelial denudation and injury were observed during device implantation. We did not find any significant structural differences in the distal vessel wall after FD implantation compared to controls by histological analysis. Because increased arterial stiffness is a known risk factor for thrombogenicity of the vessel and possibly subsequent IPH,²⁰ further studies focusing on biomolecular pathways are warranted to delineate the mechanism and impact of arterial stiffness following FD treatment.

Limitations

There are several methodological limitations to our study. We used healthy aorta for the deployment of FDs instead of pathological aneurysms. The biomechanical properties of aorta are very different from the intracranial aneurysms. In addition, the structural components and properties of elastic artery are employed in the study as opposed to intracranial aneurysms, which are predominantly muscular arteries. The uptake of blood into an aneurysm can lead to reduced sheer stress in the distal vessel wall, contributing to additional stiffness and increase in pulse wave velocity.²¹ This finding cannot be quantified in a healthy aorta without an aneurysm. In the pulse wave velocity measurement of the vessel wall distal to the FD, we were not able to obtain consistent measurements of the posterior wall of the distal vessel. In this study, we focused only on the functional changes of the vessel wall after FD deployment and did not measure the molecular signals that could contribute to the changes of the vessel wall. Additionally, pulse wave velocity is known to correlate with blood pressure. We unfortunately did not measure the blood pressure before and after implantation in this current study.

Conclusion

Our study found changes in the PWV after implantation of the FD, which was associated with vascular contractility. These results could explain vascular wall changes downstream following FD implantation. Future studies with more animals will be needed to make more definite conclusions, along with molecular analysis of the aortas for animals with and without implanted flow diverters.

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Figure 1:

Comparison of pulse wave velocity in the vessel wall before and after flow diverter implantation. (A) Increase in pulse wave velocity in the anterior wall of the vessel compared to the control. (B) Increase in pulse wave velocity in the posterior wall compared to control. (C) Pooled PWV changes in both the anterior and posterior wall. (D) Difference in the pulse wave velocity in the follow-up compared to preimplantation is significantly ($p=0.03$) higher in the distal vessels compared to controls. Red: Flow diverter group; Blue: Control; AW: Anterior Wall; PW: Posterior Wall.

Figure 2:

Visual representation of pulse wave velocity measurement before and after flow diverter implantation. (A) Wall particle velocity measurement before and (B) after flow diverter deployment. (C) Wall particle acceleration measurements before and (D) after deployment of device. The pulse wave velocity was estimated 2.79 m/s before implantation and 5.03 m/s after implantation.

Figure 3:

Vascular contractility results from organ bath experiments of vessel walls distal to and proximal to flow diverter placement. (A) Vascular contractility results shown as average contractility measurement, and (B) results shown as percent contractility as compared to control. (C) Pictorial representation of the difference in amplitude between the vessel wall distal and proximal to flow diverter compared to control animals. There was a significant increase in contractility of the vessel wall distal to the flow diverter compared to the proximal vessel wall ($p=0.002$). The vascular contractility between distal vessel wall and control ($p=0.39$) and proximal vessel wall and control ($p=0.47$) were not statistically significant. Distal: aorta distal to flow diverter; Proximal: aorta proximal to flow diverter.

Figure 4:

Histological analysis of control and flow diverter-implanted aortas. Panels A, B, and C show the cross section of an aorta in a control rabbit; panels D, E, and F show the cross section of an aorta 5mm distal to the flow diverter. (A) and (D) are stained with hematoxylin and eosin showing normal architecture and cellular composition of aortic wall with distinct 3 layers including tunica intima, media and adventitia.; (B) and (E) are stained with Mason Trichrome showing collagen is primarily located in adventitia in both groups; (C) and (F) are stained with Verhoeff-Van Gieson showing concentric continuous elastic fibers with no breaks, fragmentation in both groups. The thickness of the collagen, elastin fibers and tunica media were found to be similar in control and flow diverter-implanted rabbits.