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Assessment of Vascular Geometry for Bilateral Carotid Artery Ligation to Induce Early Basilar Terminus Aneurysmal Remodeling in Rats

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Abstract

Bilateral common carotid artery (CCA) ligation in rabbits is a model for basilar terminus (BT) aneurysm formation. We asked if this model could be replicated in rats. Fourteen female Sprague Dawley rats underwent bilateral CCA ligation (n=8) or sham surgery (n=6). After 7 days, 5 ligated and 3 sham rats were euthanized for histological evaluation of BT aneurysm formation, while the remaining rats were imaged with magnetic resonance angiography, euthanized, and subjected to corrosion casting of the Circle of Willis (CoW). 3D micro computed tomography images of CoW casts were used for flow simulations at the rat BT, and electron micrographs of the casts were analyzed for aneurysmal and morphological changes. Results from these analyses were compared to rabbit model data (n=10 ligated and n=6 sham). Bilateral CCA ligation did not produce aneurysmal damage at the rat BT. While the surgical manipulation increased rat basilar artery flow, fluid dynamics simulations showed that the initial hemodynamic stress at the rat BT was significantly less than in rabbits. Rats also exhibited fewer morphological and pathological changes (minor changes only occurred in the posterior CoW) than rabbits, which had drastic changes throughout the CoW. A comparison of CoW anatomies demonstrated a greater number of

branching arteries at the BT, larger CoW arteries in relation to basilar artery, and a steeper BT bifurcation angle in the rat. These differences could account for the lower hemodynamic stress at the BT and in the cerebrovasculature of the rat. In conclusion, bilateral CCA ligation in rats does not recapitulate the rabbit model of early flow-induced BT aneurysm. We suspect that the different CoW morphology of the rat lessens hemodynamic insults, thereby diminishing flow-induced aneurysmal remodeling.

Graphical abstract

Bilateral carotid ligation in rabbits is a model of flow-induced basilar terminus intracranial aneurysm. In rats the same manipulation did not recapitulate the model because of geometrical differences in the cerebrovasculature between the two species. (BT = basilar terminus, WSS = wall shear stress)

Keywords

intracranial aneurysm; computational fluid dynamics; cerebral blood flow; animal models; Circle of Willis; vascular remodeling; carotid artery ligation; hemodynamics; micro computed tomography; vascular corrosion casting

Introduction

Hemodynamic insult is considered a major factor in the genesis of intracranial aneurysms (IAs). (1) In past experimental studies, we were able to induce IAs in rabbits using a purely hemodynamic manipulation. Bilateral common carotid artery (CCA) ligation drastically increased flow in the basilar artery (BA) and led to aneurysm initiation at the basilar terminus (BT) in rabbits as early as two days after ligation.(2) The caliber of the rabbit vessels allowed us to acquire 3D cerebral vascular geometry by rotational digital subtraction angiography. By combining reconstructed 3D geometry with flow velocity measurements from ultrasound, we performed computational fluid dynamics (CFD) analyses to obtain the inciting hemodynamics causing aneurysm development. By co-mapping the hemodynamics with histology of the nascent aneurysms, we found that aneurysmal damage was localized to

areas of elevated wall shear stress (WSS) and positive WSS gradient (WSSG) in flow acceleration zones at the BT.(3) In addition to BT aneurysm initiation, this bilateral CCA ligation model has also been used in multiple studies to evaluate morphological and pathobiological changes in the rabbit circle of Willis (CoW) following drastic flow redistribution caused by carotid occlusion.(4–6)

Although this rabbit model enabled us to describe the detailed hemodynamics responsible for IA initiation, it suffers from several limitations including the restricted availability of antibodies and biochemical assays, limited genetic information and minimal genetically engineered strains, and high cost. An attractive alternative to the rabbit for studying the complex pathobiology of flow-induced IA initiation is the rat if the model could be scaled down, since the rat is smaller and better described biologically with a wider array of tools available for *in vivo* and *ex vivo* analyses. However, only a small number of studies have used flow manipulation alone to induce IAs in rats (7–10), and none of them directly investigated the hemodynamics associated with aneurysm formation.

The objective of this study was to explore the feasibility of recapitulating the rabbit IA initiation model in the rat and obtaining relevant BT hemodynamics using CFD. We also aimed to perform a detailed assessment of the cerebrovasculature of the rat and the rabbit in order to understand how the anatomical differences between the two species might impact the scalability of the aneurysmal remodeling model. To this end we performed bilateral carotid ligation in rats and investigated the subsequent pathological and hemodynamic consequences. To perform CFD analysis at the rat BT, we used magnetic resonance angiography (MRA) to find inlet flow velocity in the BA and micro-scale high resolution X-ray computed tomography (micro-CT) of vascular corrosion casts to obtain high resolution 3D geometry.(11) We then compared the characteristics and magnitudes of the aneurysmal damage and hemodynamic insult at the BT with those from the original rabbit model.(3) We also used scanning electron microscopy (SEM) of the rat corrosion casts to assess morphological and pathological effects of carotid ligation throughout the CoW. These images were compared to electron micrographs of rabbit casts prepared in the same manner in a previous study.(5)

Materials and Methods

Bilateral common carotid artery ligation

All animal procedures were approved by the Institutional Animal Care and Use Committee of the State University of New York at Buffalo. Figure 1A provides an overview of the rat experimental procedures. A total of 14 adult, female Sprague Dawley rats (4–6 months old) underwent either bilateral CCA ligation (n=8) or sham surgery (n=6). The histology group (n=5 ligated and n=3 sham) was used to evaluate the aneurysmal damage at the BT after ligation. The CFD group of rats (n=3 ligated and n=3 sham) was subjected to MRA to obtain BA velocity. Subsequently, vascular corrosion casting was performed to facilitate micro-CT imaging of the 3D CoW geometry and morphological/pathological analysis by SEM. For all surgical procedures, anesthesia was induced with 4% isoflurane gas and maintained with 1%. In the ligated group, both CCAs were ligated at two locations and then severed between the ligatures with a cautery. (12) In sham animals, the carotid arteries were exposed, but not

ligated or severed. Rats were maintained for 7 days, 2 days longer after ligation than rabbits, to allow extra time for development of observable aneurysmal damage.

Raw rabbit data was obtained from 16 adult, female New Zealand White rabbits that had been used in past studies,(3, 5) and was reanalyzed. Ten rabbits had been subjected to bilateral carotid ligation and 6 to a sham operation, as described in detail previously.(3) Figure 1B details the experimental procedures used to produce the rabbit cohorts that were compared to rat data in the present study. The Histology and CFD group (n=7 ligated and n=3 sham) was used to assess BT aneurysm formation in histological sections and was also imaged by rotational angiography for 3D geometry and Doppler ultrasonography for inlet flow velocity (inputs for CFD simulations).(3) The morphology group (n=3 ligated and n=3 sham) was subjected solely to corrosion casting of the CoW for analysis of geometrical and pathological changes.(5)

Histology and quantitation of aneurysmal damage

Seven days after surgery, 3 sham and 3 ligated rats were euthanized by intraperitoneal injection of 100 mg/kg sodium pentobarbital and were perfused with 1 U/mL heparin in 0.9% saline solution. These rats were pressure fixed with 10% phosphate-buffered formalin for 20 minutes at 150 mmHg, after which their brains were excised and placed in 10% phosphate-buffered formalin for at least 24 hours. For each specimen, the basilar bifurcation was excised, embedded in paraffin, sectioned longitudinally at 4 μ m, and stained with Van Gieson's stain. Rat histology was compared with histological sections of 7 ligated rabbits euthanized at 5 days and 3 sham rabbits. Details of the histological preparation of rabbit tissues are described elsewhere.(3, 13)

To assess aneurysmal damage on histology, 3 characteristics were measured as the summed lengths of vessel segments containing each individual characteristic.(13) The 3 aneurysmal characteristics were: *internal elastic lamina (IEL) loss* – mural segments where IEL was absent; *medial thinning* – segments containing >30% loss of media thickness compared to distal, undamaged, neighboring regions; and *bulging* – segments containing outward pouching of the vessel wall. To quantify the degree of aneurysmal damage in the histological sections, we used a previously-described aneurysm development score (ADS).(13) The ADS was defined as the sum of the lengths of vessel wall segments where all 3 aneurysmal characteristics were simultaneously present, multiplied by the percentage of media thinning (total segment length of media thinning divided by total length of lumen multiplied by 100) and normalized to BA diameter to account for differences in arterial size between animals and species.

In vivo magnetic resonance imaging

To obtain flow information for CFD in rats, 3 ligated and 3 sham rats were imaged with magnetic resonance imaging (MRI) immediately prior to euthanasia 7 days after surgery. Regrettably, we did not measure the BA flow in rats immediately after ligation, which would have allowed CFD simulations capturing the initial insult of hemodynamics. This decision was made due to concerns about the added trauma that would be placed on the rats, namely moving the animal to the imaging facility and additional anesthesia during imaging.

For MRI, rats were anesthetized and imaged in a 4.7 T/33 cm horizontal bore scanner, incorporating AVANCE™ digital electronics (ParaVision Version 4.0 Operating System, Bruker Biospin, Billerica, MA, USA). High-resolution MRA was carried out using a flow-compensated, gradient echo (GEFC) time-of-flight imaging protocol with the following parameters: echo time (TE)/repetition time (TR) = 3/15 ms, flip angle = 90°, field of view (FOV) = 2.82×1.76 cm, matrix size = 256×160, 42 slices, slice separation = 0.22 mm, averages (NEX) = 18. Blood flow velocities were acquired by imaging a slice containing the BA using a GEFC scan with bipolar flow-encoding gradients with the following parameters: TE/TR = 5/15 ms, FA = 30, FOV = 2.82×1.76 cm, matrix = 160×160 zero-filled to 256×160, slice thickness = 1 mm, NEX = 36, Venc = 50 cm/s. Average velocity was calculated as (maximum velocity)/2 with the Hagen-Poiseuille flow model. Seven day flow data was compared to previously published values from our rabbit experiments also measured 7 days after ligation.(14)

Vascular corrosion casting

The 3D geometry obtained from the MRI described above had poor spatial resolution. Therefore, we obtained 3D vessel geometries by creating vascular corrosion casts of the CoW. The MRI-imaged rats were euthanized by intraperitoneal injection of 100 mg/kg sodium pentobarbital, and corrosion casting was immediately performed, as described by Jamous et al.(15) In brief, laparotomy and thoracotomy were performed after euthanasia, and a plastic cannula was inserted into the left ventricle of the heart and secured at the ascending aorta with a ligature. The right atrium was punctured for drainage and rats were perfused with 1 U/mL heparin in 0.9% saline solution, followed by manual injection of Batson's No. 17 corrosion casting polymer mixture (Polysciences, Inc., Warrington, PA, USA). Whole specimens were kept at 4°C for 24 hours during resin polymerization, after which brains containing vascular endocasts were excised and placed in 20% potassium hydroxide for 24 to 72 hours to dissolve tissue. After the brain tissue had dissolved, the endocasts were isolated and rinsed with distilled water and pruned of small perforating vessels.

Postmortem micro-CT imaging

To obtain 3D geometry, rat endocasts were imaged with a custom-build micro-CT system. (16) The system consisted of a high resolution micro angiographic detector developed in our lab and a micro-focus Ultrabright Oxford x-ray tube with XYZ rotary stage for sample mounting. The detector had a 1024×1024 matrix with an effective pixel size of 43 microns. (17) We scanned each endocast in increments of 1° over 360°, with a tube voltage of 40 kVp and tube current of 1mA. Projection images were acquired and calibrated using a self-aligning algorithm.(18) The data was reconstructed using a Feldkamp algorithm.(19) Each volume consisted of 512×512×512 30µm isotropic voxels.

CFD simulation

To determine the hemodynamic environment at the rat BT, 3D geometries were reconstructed from micro-CT imaging of the endocasts and CFD simulations were performed as previously described using velocity obtained from MRA.(20) The 3D geometries were segmented using Vascular Modeling Toolkit (Orobix, Bergamo, Italy), then

cropped and refined to include just the BT and connecting vessels. The segmented models were converted to volumetric meshes using ICEM-CFD (ANSYS, Inc., Canonsburg, PA, USA) and the flow governing Navier–Stokes equations were solved in the commercial software Star-CD (CD-Adapco, Melville, NY, USA) under steady state conditions. The inlet boundary condition at the BA was defined as the average velocity obtained from MRA, and the outlet boundary conditions were defined as having equal WSS at all branches, assuming the principle of optimal work.(21, 22)

Computational fluid dynamics simulations were performed for the 3 sham and three 7-day ligated rats that underwent MRI prior to euthanasia (see Figure 1A). We estimated the initial hemodynamic insult at the rat BT by performing CFD analyses using sham geometry and 7-day ligated BA flow rate (flow rate has been shown to increase immediately after ligation and stay elevated even to 6 months(14)). We used the sham geometries because compensatory vascular remodeling during the 7 days after ligation changes the vessel geometry, and thus the hemodynamic stresses measured with 7-day geometries would likely not reflect the initial hemodynamic insult caused by ligation. Post-processing was performed in Tecplot 360 (Tecplot, Inc., Bellevue, WA, USA) to obtain average and maximum WSS and WSSG at the BT bifurcation zone. This zone was defined as the vascular surface contained within a $200\text{ }\mu\text{m}^3$ cube centered at the bifurcation apex (a measurement empirically determined to inscribe the BT without including other bifurcations or vessel walls opposite to the BT).

The sham and 7-day ligated rat data were compared against previously collected raw CFD data from 3 sham and seven 5-day ligated rabbits with imaging immediately after ligation. (3) For these rabbits, we had performed 3D digital subtraction angiography with a Toshiba Infinix, VX-i medical suite (Toshiba Medical System Corporation, Tustin, CA, USA) to obtain posterior CoW geometry and transcranial Doppler ultrasonography (Spencer Technologies, Northborough, MA, USA) to obtain inlet flow velocities. Details on rabbit CFD simulation are reported elsewhere.(3, 13) For these rabbits, new analysis was performed on raw CFD data in Tecplot with a BT bifurcation zone demarked by a $600\mu\text{m}^3$ cube (scaled based on BA diameter to match the rat CFD analysis).

SEM imaging of vascular endocasts

For SEM imaging, the 6 rat vascular endocasts were electrically coupled to a stage with graphite, scatter-coated with molecular carbon under vacuum, and imaged at 5kV, with a Hitachi SU-70 SEM (Hitachi High Technologies America, Inc., Roslyn Heights, NY, USA). Mosaic images capturing the entire CoW at 50 $\times$ were created using Photoshop software (Adobe Systems, Inc., San Jose, CA, USA) as described previously.(5) For comparison with rabbits, we used 6 CoW endocasts that were created in the same manner from 3 sham and three 5-day ligated rabbits. These endocasts were also imaged in the same manner.(5)

Quantitation of CoW morphology

We first compared native morphology of the rat and rabbit CoW using casts of sham-operated animals. On mosaic SEM images, we measured vessel diameter on the BA, P1 segment of the posterior cerebral artery (PCA), superior cerebellar artery (SCA), P2 segment

of the PCA, posterior communicating artery (PCom), internal carotid artery (ICA) distal to the PCom junction, middle cerebral artery (MCA), anterior cerebral artery (ACA), and olfactory artery (OfA – only in rats) or ophthalmic artery (OpA – only in rabbits). We normalized these measurements by the average BA vessel diameter of each species. We also measured bifurcation angles of the BT, SCA/PCA bifurcation, PCA/PCom junction, ICA junction, MCA/ACA bifurcation, and OfA/OpA branch in both rats and rabbits.

To quantify morphological effects of ligation in both species, we analyzed vascular morphology between ligated and sham animals using mosaic SEM images. We quantified vessel diameter and length in sham and ligated animals. We also quantified tortuosity using a previously-established tortuosity index (TI), where $TI = (L - C) / C$ (L = total length of vessel, C = straight line chord distance).

Quantitation of flow-induced aneurysmal damage on mosaic SEM images

To quantify aneurysmal damage on CoW casts, we analyzed 10 arterial regions on mosaic SEM images (Figure 2): the BA, BT, SCA origin, PCA P1, PCom origin, PCom, distal ICA, ACA/MCA bifurcation, and the OfA (rats) or OpA (rabbits) origin. To quantify vascular damage, each region was graded with a 5-point damage score where: 0 = no damage, 1 = endothelial cell (EC) irregularities, 2 = IEL fenestrations, 3 = smooth muscle cell (SMC) imprints, 4 = small bulges, and 5 = large bulges. A given region was scored based on the most severe type of damage that was present. Damage was defined as follows: (5) *EC irregularities* – loss of spindle-shaped, flow-aligned EC morphology, typically interpreted as a sign of EC dysfunction; (15) *IEL fenestrations* – well demarked shallow indentations in the vessel lumen due to localized matrix degradation; (23) *SMC imprints* – circumferential striations running orthogonal to the direction of endothelial orientation that indicate regions of massive IEL loss, which permits contact between the intima and media leaving luminal SMC imprints; (24) and bulges (small or large) – pre-aneurysmal arterial protrusions with low dome height.

Statistical analysis

All values are expressed as mean $\pm$ standard error. Statistical analyses were performed using Microsoft Excel software (Microsoft Corporation, Redmond, WA, USA). A Student's *t*-test was used to test for statistical significance for comparisons between experimental groups and species. For all statistical tests, differences were considered significant at $p < 0.05$.

Results

Bilateral carotid ligation did not produce aneurysmal damage at the rat BT

Histological analysis was performed on 5 ligated rats and 3 sham rats to assess IA initiation at the rat BT after bilateral carotid ligation. Sections of BTs stained with Van Gieson's stain revealed no significant difference in IEL loss, bulging, and media thinning between the sham and 7-day ligated group (Figure 3A, B, and C). Consequently, there was no significant difference in the ADS, an aggregate metric of aneurysmal damage, between these groups. Analysis performed on histological sections of rabbit BTs stained with Van Gieson's stain from a group of sham ($n=3$) and 5-day ligated ($n=7$) rabbits (3) demonstrated that BTs of

ligated rabbits had clear damage even at 5 days (Figure 3D, E and F). The ligated rabbits had significantly higher ADS than sham, $p=0.03$. Compared to 5-day rabbits, 7-day ligated rats had significantly less damage at the BT, despite having longer time to develop. Ligated rabbit BTs had significantly greater IEL loss (1.07 ± 0.31 rabbit vs. 0 rat, $p=0.013$), bulge formation (0.83 ± 0.2 rabbit vs. 0 rat, $p=0.0060$) and media thinning (0.79 ± 0.16 rabbit vs. 0.048 ± 0.02 rat, $p=0.0033$). Five-day ligated rabbits also had greater ADS (8.1 ± 3.1) than 7-day ligated rats (0, $p=0.03$) (Figure 3C and F).

Bilateral carotid ligation increased flow through the BA in rats

To confirm that bilateral carotid ligation increased blood flow through the BA in rats, we imaged the 3 ligated and 3 sham rats with MRA 7 days after surgery. Flow measurement during MRA indicated that BA flow velocity in 7-day ligated rats (19.3 ± 1.7 cm/s) was significantly greater than in sham rats (8.2 ± 1.3 cm/s, $p=0.0014$). As shown in figure 4A, this translated into a 4.25 fold increase in flow rate between sham (0.24 ± 0.033 ml/min) and the 7-day ligated rats (1.02 ± 0.077 ml/min, $p=0.0007$). Comparatively, this increase in flow rate was greater than the increase previously measured in rabbits ($n=5$) after 7 days of ligation (which showed a 2.8 fold increase in flow rate) in a study from our group (Figure 4B).(14)

Flow insult at the BT was less in rats than in rabbits

The lack of aneurysmal damage at the rat BT compared to rabbit could be explained if the rat BT experienced lower local hemodynamic insult. To determine hemodynamic forces at the BT in rats and rabbits we performed CFD simulations. To estimate the hemodynamic insult immediately after ligation at the rat BT, we performed CFD analyses using sham vascular geometry (equivalent to rat geometry immediately after ligation but before substantial remodeling) and 7-day ligated rat BA flow rate (equivalent to the flow rate immediately after ligation, as the rate has been shown to increase sharply after ligation and remain elevated (14)). Our CFD analysis (Figure 5A, B, and C) demonstrated that 7-day ligated rats showed an increase in maximum BT WSS (46.3 ± 11.3 Pa) compared to sham (9.33 ± 0.88 Pa), however the increase was not significant ($p=0.078$), nor was there a significant change in average WSS or maximum WSSG. Only the average WSSG was increased significantly in the ligated rats (134.1 ± 11.6 kPa/m) compared to sham (16.7 ± 4.1 kPa/m, $p=0.005$).

In rabbits, ligation produced a significant increase in WSS and WSSG at the BT (Figure 5D–F). The maximum and average WSS (247.0 ± 22.1 Pa and 159.7 ± 14.3 Pa, respectively), and the maximum WSSG (1031 ± 138 kPa/m) were significantly higher in ligated rabbits compared to sham rabbits (all $p<0.0007$) (Figure 5D, E, and F). Comparing the insult between species, we found that rabbits had significantly higher maximum and average WSS at the BT vs. rats ($p=0.0005$ and $p<0.0001$, respectively) (Figure 5B and E). The maximum and average WSSG, however, were not different between the 2 species.

Differences in native CoW geometry exist between rats and rabbits

To explain the disparity between the BT hemodynamics of the rat and the rabbit we investigated CoW geometry, which directly influences flow distribution and forces in the cerebral vasculature. Figure 6 shows representative mosaic images of CoW casts in each

experimental group. Initial inspection of the sham casts (Figure 6A and C) demonstrates intrinsic differences in the rat and rabbit CoW, for example the lack of an OpA coming off the ICA and the presence of the OfA branching from the ACA in the rat. Across our specimens, we also observed much more anatomic variation in the rat geometries than we observed in the rabbits, and even in the total number of rabbits we have imaged in our previous studies.(3, 5, 12, 13, 25) Some rat CoW did not have any P1 segment of the PCA before to the SCA origin, with 4 vessels separating simultaneously at the BT (see Figure 6A), while others had significant PCA P1 segments before the SCA origin and only two vessels separating at the BT. Some BTs were even asymmetric, with both left and right P1 segments of the PCA originating on the same side of the BT bifurcation. We suspect that anatomical variations in rats, namely multiple branching arteries at the basilar bifurcation, could dissipate hemodynamic forces at the BT.

In addition to making qualitative observations of cast geometry, we quantified differences in CoW geometry between sham rats and rabbits on SEM images. The cross sectional area of the vessel (and by extension diameter) is directly proportional to the flow velocity, which is positively related to the level of hemodynamic forces on the artery wall. We compared arterial diameters between rats and rabbits by normalizing diameter measurements in each cast by the average BA diameter of each specimen's respective species. As shown in Figure 7A, rats had greater relative artery diameters in their cerebral vasculature than rabbits. Aside from the PCA P1, the rat CoW had relatively uniform vessel diameters throughout.

Bifurcation angle is a geometrical parameter that can also affect hemodynamic forces by dictating the amount of impinging flow at a vessel branch point or split. Measuring bifurcation angles on mosaic SEM images, we found that rats tended to have smaller angles at major bifurcations in the CoW (Figure 7B). Most importantly, rats had a significantly smaller BT bifurcation angle ($92\pm10^\circ$) compared to rabbits ($142\pm9^\circ$, $p=0.0047$), which could lead to less flow impingement and lower levels of WSS at the rat BT.

Bilateral carotid ligation caused less morphological changes in the rat CoW

To investigate morphological changes in the cerebral vasculature following carotid ligation, we analyzed casts of the CoW using SEM. Comparing sham and ligated casts (Figure 6) we found that bilateral carotid ligation caused gross morphological changes to the CoW in both rats and rabbits. However, it was clear that there were greater changes in rabbits than in rats; rabbit arteries increased in diameter and became more tortuous. Quantitatively, we found significant increase in the diameters of only the BA and PCA P1 vessels in rats (Figure 8A), whereas ligation caused an almost global increase in vessel diameter in rabbits, with every measured location significantly larger in the ligated animals except the distal ICA (Figure 8D). The increase in lengths of CoW vessels was also greater in rabbits than in rats. In rats only the PCA P1 was significantly longer in ligated animals (Figure 8B), while in ligated rabbits the PCA P1, PCom, and ACA all had greater lengths compared to sham (Figure 8E). Furthermore, rats demonstrated increased tortuosity in the PCA P1 (Figure 8C), while rabbits had increased tortuosity in the PCA P1 and, most notably, the PCom (Figure 8F).

Bilateral carotid ligation caused less aneurysmal changes in the rat CoW

In addition to geometrical changes, we used SEM images of the vascular corrosion casts to investigate aneurysmal damage throughout the CoW following carotid ligation. We measured 5 types of vascular damage, (EC irregularities, IEL fenestrations, SMC imprints, small bulges, and large bulges) at 10 arterial regions in the CoW (Figure 2) and used a 5-point Damage Score to quantify the maximum type of damage in each region. In ligated rats, most of the arterial regions showed no induction of aneurysmal damage, equating to a damage score of less than 1 (EC irregularities) (Figure 9A–E). However, the P1 segment of the PCA had significantly more damage, with increased SMC imprints and some scattered small bulges. Because of these changes, this region received a damage score of 3.33 ± 0.42 , a significantly greater score than sham (0, $p=0.00052$) (Figure 9F–J). However, aside from this region, no major vascular damage was noted in the CoW following bilateral carotid ligation. Rabbits presented much more damage, even after only 5 days of bilateral carotid ligation. Notably, the ligated rabbits presented SMC imprints at the BT and along the PCA and PCom (Figure 9G and H). Ligated rabbits also presented significant bulges and SMC imprints at the OA origin (Figure 9I).

Discussion

This study indicates that bilateral carotid ligation in rats does not recapitulate the early hemodynamic induction of IAs at the BT that has been observed in rabbits. In our previous studies using the rabbit bilateral CCA ligation model, this manipulation drastically increased flow in the BA and led to pathological changes mirroring aneurysm genesis at the BT as early as 2 days.(2, 12) The observed aneurysmal remodeling was characterized by IEL loss, medial thinning and bulging, and was localized to the peri-apical regions of the BT. By mapping hemodynamics onto histology of the lesions, we further demonstrated that the aneurysmal damage was specifically localized to areas of high WSS and positive WSSG.(3)

In addition to IA induction at the BT, bilateral CCA ligation in the rabbit has also been shown to elicit morphological and pathobiological changes throughout the CoW due to flow redistribution subsequent to CCA ligation.(4–6) In one of our recent studies we reported widespread vascular remodeling including extensive arterial expansion, aneurysmal bulging, and severe vascular tortuosity throughout the rabbit CoW at 5 days and 6 months after bilateral CCA ligation.(5) In a follow-up study, we further investigated the effect of additional aneurysm risk factors (hypertension and estrogen deficiency) on the development of these vascular changes in the rabbit and demonstrated more widespread aneurysmal changes and more severe tortuosity at 6 months compared to CCA ligation alone.(6) Furthermore, Esegolu et al.(4) explored the role of the sympathetic nervous system on BA remodeling by using the rabbit bilateral CCA ligation model and observed flattening of the inner elastic membrane and dilation of the BA at 2 months. Noting the similarities between these aneurysmal changes and BT aneurysm formation (IEL damage and bulging), we suspect that CCA ligation also produces aneurysm-initiating hemodynamics (high WSS and positive WSSG) at many locations in the rabbit CoW in addition to BT.

However, despite performing the same surgical manipulation (bilateral CCA ligation), in rats we did not see aneurysmal changes at the BT or any other regions of the CoW, except for the

PCA (which demonstrated moderate remodeling). A plausible explanation for the drastic difference in aneurysmal response to the same surgical manipulation between the two species is that lesser hemodynamic insult was produced at the rat BT. Our estimation of the initial hemodynamic insult at the rat BT after bilateral CCA ligation indicates that ligation did increase WSS at the BT in rats, but the increase was not statistically significant and was much less than in rabbits. Metaxa et al.(3) demonstrated that IA damage at the rabbit BT primarily occurs when WSS and WSSG exceed a certain level. Thus, we suspect that bilateral CCA ligation in rats fails to produce local hemodynamic forces that exceed the level necessary to elicit aneurysm initiation at the BT and at other regions throughout the CoW in the rat.

Arterial geometry, which largely dictates hemodynamics through the vasculature, may account for the difference in hemodynamic insult between rats and rabbits. Our morphological analysis of sham rat and sham rabbit CoW casts demonstrates three intrinsic differences between the species. First, rat CoW arteries are more similar in diameter to the rat BA (with the exception of the PCA P1, see Figure 7A), in contrast to the rabbit CoW arteries, which are smaller than the rabbit BA. Based on the flow rate equation ($Q=VA$) and the law of conservation of mass, flow velocity (V) increases as the cross-sectional area of the vessel (A) decreases in order to keep flow rate (Q) constant. Therefore, velocity in rabbit CoW arteries may increase more than it does in rat CoW arteries following BA flow increase, especially in the posterior circulation where significant damage was observed. Since WSS is a function of the flow velocity, we suspect that WSS is also greater throughout the rabbit CoW compared to rats. Second, we observed differences in the rat CoW configuration that could reduce hemodynamic stresses at the BT. Four vessels often diverge simultaneously at the rat BT, instead of the two that typically split in rabbits. This likely disperses hemodynamic forces at the rat BT, since splitting the flow 4-fold causes lower WSS and WSSG than if it were split in half. Third, the rat BT has a smaller bifurcation angle than the rabbit BT. Acute angles produce less flow impingement impact and thus less deceleration and acceleration of flow, resulting in lower WSS and WSSG.(26) Indeed, in humans it has been observed that IAs occur more frequently at bifurcations that experience higher hemodynamic shear stress and stronger flow acceleration,(26) and that IAs occur at a significantly greater incidence at abrupt bifurcations and at bifurcations with narrow branching arteries (27). We suspect that the three anatomical differences between the rat and the rabbit cause the rat CoW to have more dispersed hemodynamic forces, which could explain why the rat does not develop appreciable aneurysmal remodeling following BA flow increase subsequent to bilateral CCA ligation. These anatomical differences could also explain why geometrical and pathological changes in rats only happen in the most posterior regions of the rat CoW, namely the BA and PCA, where flow insult is greatest.

Our study demonstrates the importance of considering vascular geometry in animal models of IA that incorporate hemodynamic modifications. Issues concerning variability in CoW geometry could be ameliorated by using other small mammals or different strains of rats that have more consistent cerebral vascular geometries.(7, 28) Alternatively, previous flow-only rat IA models that did not incorporate CFD could be redesigned to accommodate hemodynamic analysis.(7–10) In this case, the use of more powerful magnetic fields, e.g. 9.4 T or even 21.1 T MRI, could significantly improve sensitivity and resolution compared to

those used in this study.(29) This might allow direct histology-hemodynamic mapping as we have done in our previous rabbit model.(3)

Conclusions

Bilateral common carotid artery ligation in rats does not recapitulate the rabbit model of early flow-induced BT aneurysm. This can be explained by calculations of inciting hemodynamic forces at the BT after ligation, which were found to be much lower in rats than in rabbits. Anatomical examination reveals a greater number of branching arteries at the BT and relatively larger CoW arteries in the rat, which could account for the lower WSS and diminished flow insult at the rat BT. The rat also has a steeper bifurcation angle at the BT than the rabbit, which could also reduce WSS due to weaker flow impingement on the bifurcation apex. Our study demonstrates that the variability of vascular geometry should be taken into consideration when creating animal models of flow-induced IA initiation.

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Conflicts of Interest

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A Rat Experimental Procedures

B Rabbit Experimental Procedures

Figure 1.

Experimental design of the study. Flow charts outlining the specimen numbers and procedures for the rats (A) and rabbits (B) used to produce the data in this study. (BT = basilar terminus, CCA = common carotid artery, CFD= computational fluid dynamics, DSA = digital subtraction angiography, micro-CT = micro computed tomography, MRI = magnetic resonance imaging, SEM= scanning electron microscopy, TCD = transcranial Doppler)

Figure 2.

Comparative anatomy of the rat and rabbit Circle of Willis (CoW). This illustration highlights the regions of interest in both the rat and rabbit CoW. The regions of interest include: basilar artery (BA), basilar terminus (BT), superior cerebellar artery (SCA) origin, P1 segment of the posterior cerebral artery (PCA), posterior communicating artery (PCom) origin, PCom, distal internal carotid artery (ICA), middle cerebral artery (MCA), anterior cerebral artery (ACA), olfactory artery (OfA – rat only), and ophthalmic artery (OpA – rabbit only).

Figure 3.

Histology of aneurysmal damage at the rat and rabbit basilar terminus (BT). A and B). Staining with Van Gieson's stain failed to show substantial internal elastic lamina (IEL) damage, bulge formation, or media thinning at the rat BT in the 7-day ligated or sham animals. C). The aneurysm development score (ADS) for each group of rats was 0. D and E). In contrast, the 5-day ligated rabbit BT showed substantial IEL loss, bulge formation, and medial thinning compared to sham. F). this translated into a large difference in ADS between ligated and sham rabbits. Bars represent mean $\pm$ standard error. (*) indicates statistical significance (Student's *t*-test, $p < 0.05$). (BA = basilar artery)

Figure 4.

Flow rate measurements in rats and rabbits. A). Flow velocity was measured by magnetic resonance angiography in the basilar artery (BA) of rats. The BA flow rate was 4.3-fold higher in ligated rate (1.02 ± 0.077 ml/min) than in sham rats (0.24 ± 0.033 ml/min, $p=0.0007$). B). For comparison, BA flow measurements by transcranial Doppler ultrasonography in ligated and sham rabbits at 7 days from a previous study were used.(14) In these rabbits, bilateral carotid ligation produced a 2.8 fold increase in BA flow rate. Bars represent mean $\pm$ standard error. (*) indicates statistical significance (Student's t -test, $p < 0.05$).

Figure 5.

Computational fluid dynamics analysis in rats and rabbits to characterize basilar terminus (BT) hemodynamics. A). Flow analysis of the rat BT showed an overall increase in wall shear stress (WSS), and wall shear stress gradient (WSSG). B). Ligated rats demonstrated increased maximum BT WSS (46.3 ± 11.3 Pa) vs sham (9.33 ± 0.88 Pa), but the difference was not statistically significant ($p=0.078$). C). Maximum WSSG was the only hemodynamic parameter significantly increased in ligated rats (134.1 ± 11.6 kPa/m) vs. sham (16.7 ± 4.1 kPa/m, $p=0.005$). D). In rabbits, ligation increased WSS and WSSG at the BT. E). In ligated rabbits, the maximum and average WSS (247.0 ± 22.1 Pa and 159.7 ± 14.3 Pa, respectively) was significantly greater than in sham, $p<0.0007$. F). Furthermore, the maximum WSSG in ligated rabbits (1031 ± 138 kPa/m) was significantly higher than in sham, $p<0.0007$. Bars represent mean $\pm$ standard error. (*) indicates statistical significance (Student's *t*-test, $p < 0.05$).

Figure 6.

Representative mosaics of scanning electron microscopy images of the rat and rabbit Circle of Willis (CoW) with and without ligation. A and B). Sham and ligated rat casts. C and D). Sham and ligated rabbit casts. Clear differences in the CoW response to bilateral carotid ligation between rats and rabbits are evident. Specifically, an increase in vessel caliber and tortuosity present in the rabbits is not evident in the rats. (Gaps where casts may have been broken or cracked during preparation/handling, e.g. distal ICA segment in C, were excluded from analysis)

Figure 7.

Morphological differences between sham rat and sham rabbit Circle of Willis (CoW). A) Artery diameter measurements normalized by each species' average basilar artery (BA) diameter. Rats had greater artery diameters in their cerebral vasculature than rabbits, and in general tended to have CoW vessels that were similar in diameter to the BA. B). Rats had smaller angles at most bifurcations in the CoW compared to rabbits. Notably, rats had a significantly smaller basilar terminus (BT) bifurcation angle ($92 \pm 10^\circ$) compared to rabbits ($142 \pm 9^\circ$, $p=0.0047$). Bars represent mean $\pm$ standard error. (*) represents statistical significance (Student's t -test, $p < 0.05$). (ACA = anterior cerebral artery, ICA = internal carotid artery, MCA = middle cerebral artery, OfA = olfactory artery, OpA = ophthalmic artery, PCA = posterior cerebral artery, PCom = posterior communicating artery, SCA = superior cerebellar artery)

Figure 8.

Changes in vessel diameter, length, and tortuosity in rats and rabbits following bilateral carotid ligation. A and B). In ligated rats only the diameter of the basilar artery and posterior cerebral artery (PCA) P1 and the length of the PCA P1 increased compared to sham. C). Ligated rats also demonstrated increased tortuosity on the PCA P1. D and E). In ligated rabbits the diameter of almost all Circle of Willis arteries and the length of PCA P1, posterior communication artery (PCom) an anterior cerebral artery were significantly increased compared to sham. D). The ligated rabbits had more vessels with increased tortuosity than rats, with significant increases in both the PCA P1, PCom. Bars represent mean $\pm$ standard error. (*) represents statistical significance (Student's *t*-test, $p < 0.05$). (ACA = anterior cerebral artery, BA = basilar artery, ICA = internal carotid artery, MCA = middle cerebral artery, OfA = olfactory artery, OpA = ophthalmic artery, SCA = superior cerebellar artery)

Figure 9.

Microscopic structure analysis of aneurysmal damage in the Circle of Willis (CoW) on rat and rabbit casts. A-D). Most of the arterial regions of ligated rats showed little or no aneurysmal damage, with the exception of the posterior cerebral artery (PCA) P1. B2). Arrows indicate smooth muscle cell (SMC) striations and some small bulges present on the rat PCA P1 7 days after ligation. E). The PCA P1 had a significant increase in damage score (3.33 ± 0.42) vs sham (0 , $p=0.00052$). All other arterial regions of the rat CoW had no significant increase in damage score. F-I). In contrast, many regions of the rabbit CoW showed damage. Arrows indicate SMC striations at the BT (F2) and posterior

communicating artery (PCom) (H2), internal elastic lamina (IEL) fenestrations on the PCA P1, and large aneurysmal bulges at the ophthalmic artery (OpA) origin (I2). J). In the ligated rabbits the BT, PCA P1, PCom, and OpA origin all had significantly increased damage score compared to sham, mainly due to the presence of SMC striations. Bars represent mean $\pm$ standard error. (*) indicates statistical significance (Student's *t*-test, $p < 0.05$). (ACA = anterior cerebral artery, BA = basilar artery, ICA = internal carotid artery, MCA = middle cerebral artery, OfA = olfactory artery, SCA = superior cerebellar artery)