Comparative Medicine
Copyright 2012
by the American Association for Laboratory Animal Science

Vol 62, No 6
December 2012
Pages 480–486

Case Report

Complications of Elastase-Induced Arterial Saccular Aneurysm in Rabbits: Case Reports and Literature Review

Jason S Villano,[*,†] Christine A Boehm,[‡] Elizabeth L Carney,[#] and Timothy K Cooper

Endoluminal infusion and incubation of elastase with or without collagenase into the rabbit common carotid artery is an established model of arterial saccular aneurysm. The model mimics naturally occurring human cerebral aneurysms in many ways, including histologic and morphologic characteristics, hemodynamic pressures, and shear stresses. However, complications have been associated with the model. Here, we report 2 complications: 1) the first known case of iatrogenic laryngeal hemiplegia in a rabbit; and 2) histopathologically confirmed iatrogenic hippocampal and cerebellar infarcts (stroke). Finally, we present and review data from current literature on the morbidity and mortality associated with this model.

Abbreviations: CCA, common carotid artery; RLN, recurrent laryngeal nerve.

Elastase-induced aneurysm in the rabbit common carotid artery (CCA) is an established model of arterial saccular aneurysm[2,6] that provides an alternative to large animal (for example, swine and canine) models of saccular aneurysm. The degradation of the elastic lamina with elastase leads to a cascade of changes in extracellular matrix proteins and progressive inflammatory infiltration as seen in naturally occurring cerebral and aortic aneurysms.[15] These changes lead to thin-walled aneurysms[9] that are morphometrically[28] and histologically[1] similar to naturally occurring human cerebral aneurysms. In addition, the created aneurysms have hemodynamic pressures and shear stresses similar to those of human cerebral aneurysms,[24] because the rabbit's carotid artery is also comparable in size to human middle cerebral arteries.[1] Rabbits also have comparable thrombotic and thrombolytic profiles to those of humans.[16]

Despite the advantages, this rabbit model has limitations. These include the inability to control the size of aneurysms (potentially corrected by adjusting the ligation site);[10] sacrifice of the arterial access for each angiogram;[4] absence of clear atherosclerotic changes, thus limiting data extrapolation to the "true human degenerative aneurysm";[27] and small size of rabbits, thus limiting the evaluation of large devices or techniques requiring multiple catheters.[13] In addition, rare spontaneous ruptures of elastase-induced aneurysms have been reported;[1,6,17,19] these ruptures were postulated to be due to residual collagen in the vessel wall, given that collagen is important to maintain the wall's tensile strength.[33] Therefore, the natural history of human intracranial aneurysm is not precisely produced in this rabbit model. Because alterations in either the quantity[14,26,30] or quality[32] of collagen may be related to the development of cerebral aneurysms in humans, the traditional technique for creating these aneurysms in rabbits was modified to include the infusion and incubation of collagenase into the CCA.

Creating the model involves careful dissection of the ventral neck region to isolate the CCA and infuse it with elastase with or without collagenase. Complications arising from this technique have been reported. Here, we present 2 clinical cases based on our experience: the first known case of iatrogenic laryngeal hemiplegia in a rabbit and a histopathologically confirmed case of iatrogenic rabbit stroke. We also review the literature regarding complications associated with creating this model.

Materials and Methods

Aneurysm creation. Male New Zealand white rabbits (n = 19; age, 8 mo; Robinson Services, Mocksville, NC) were used in an IACUC-approved protocol to create saccular aneurysm models at an AAALAC-accredited research facility. Vendor surveillance reports indicated that the rabbits were from colonies seronegative for *Encephalitozoon cuniculi*, cilia-associated respiratory bacillus, and *Treponema paraluiscuniculi* and were negative for *Salmonella* spp., *Klebsiella* spp., *Citrobacter* spp., helminth endoparasites, and arthropod ectoparasites. The rabbits were housed in a vivarium that was not SPF for *Bordetella bronchiseptica*, but *Pasteurella multocida* was a controlled pathogen. Rabbits were singly housed in stainless-steel cages in animal rooms with constant environmental conditions (61 to 72 °F [16.1 to 22.2 °C]; relative humidity, 30% to 70%; 12:12-h light:dark cycle) and were fed with a fixed-formula rabbit diet (2031 Teklad Global High Fiber Rabbit Diet, Harlan

Received: 30 Apr 2012. Revision requested: 25 May 2012. Accepted: 01 Jun 2012.
Department of Comparative Medicine, Penn State Hershey Medical Center, Hershey, Pennsylvania.
Current affiliations: [†]Animal Resources Center, University of Texas Medical Branch, Galveston, Texas; [‡]Laboratory Animal Resource Center, Indiana University, Indianapolis, Indiana; [#]York Veterinary Housecalls, York, Pennsylvania.
[*]Corresponding author. Email: jason.villano@utmb.edu

Laboratories, Madison, WI) and supplemented with alfalfa hay once daily.

The rabbits were premedicated with meloxicam (0.3 mg/kg SC), buprenorphine (0.005 mg/kg SC), and acepromazine (0.5 mg/kg SC), followed by ketamine (20 mg/kg IM) and medetomidine (0.25 mg/kg IM) for anesthesia induction. The rabbits then were intubated by using 3.0-mm endotracheal tubes and maintained with 1% to 2% isoflurane delivered in 100% oxygen. The surgical procedure involved fluoroscopy (OEC 9800 Plus, GE Medical Systems, Salt Lake City, UT)-guided advancement of a 3-French balloon catheter (Fogarty, Baxter Healthcare, Irvine, CA) through a vascular sheath into the right CCA and aneurysm creation as described previously.[33] Briefly, porcine elastase (100 U) and type I collagenase (1.5 mg; both from Sigma–Aldrich, St Louis, MO) were infused and incubated for 20 and 15 min, respectively, within the lumen of the right CCA to induce saccular aneurysm. Postoperative care included buprenorphine (0.005 mg/kg SC) twice daily for at least 2 d and enrofloxacin (5 mg/kg SC) once daily for 5 d.

Necropsy, histopathology, and postmortem diagnostic procedures. Two rabbits were euthanized (150 mg/kg IV or IC; Euthasol, Vedco, St Joseph, MO) because of study complications. Tissues from major organs (including the right CCA) were harvested, fixed in 10% buffered formalin, embedded in paraffin, sectioned at 4 to 6 µm, and processed and stained with hematoxylin and eosin. Gram and Wright–Giemsa staining of selected tissues also was performed. Frozen kidney sections of one rabbit were submitted (IDEXX RADIL, Columbia, MO) for *E. cuniculi* PCR assay. Tympanic bullae of the same rabbit were sampled (BBL Liquid Stuart Medium Swabs, Becton Dickinson, Franklin Lakes, NJ) for bacterial culture. Culture media used were 5% sheep blood, Columbia, MacConkey, and chocolate agar (Becton Dickinson, St Joseph, MO). Samples also were cultured by using 5% sheep blood agar incubated in CO_2 to rule out *P. multocida*. Gram staining of the resultant colonies was performed, and Gram-negative organisms were identified (API-20NE Test Kit, bioMerieux, Hazelwood, MO).

Literature review. We performed a computerized search of Medline–PubMed for reports on complications associated with creating this model. We used aneurysm, rabbit, and elastase as keywords; collagenase was not used as a keyword because the addition of this enzyme was only a protocol modification, and elastase is the primary enzyme used for aneurysm induction. The search was not limited by language, country of origin, or date, to be as inclusive as possible and reduce the number of missed reports. Abstracts of or full-paper references were reviewed and screened regarding their relevance to our topic. We did not include complications unrelated to aneurysm creation (for example, anesthesia), those that are considered to be consequences of aneurysm (for example, aneurysm rupture), and those arising from and related to experimental treatment procedures (for example, embolization and stent deployment).

Results

Case report 1. One day after uneventful surgery, a rabbit presented with reduced appetite and vocalization with a roaring-like sound during and after handling. Examination revealed normothermia, and inspiratory stridor exacerbated by manipulation. Top differential diagnoses included laryngeal paralysis due to damage to the recurrent laryngeal nerve (RLN) and tracheitis–laryngitis due to intubation. At 4 d after surgery and in the absence of any clinical sign of improvement, carprofen (3 mg/kg SC) and dexamethasone (1 mg/kg SC) were initiated to reduce inflammation. Dexamethasone and subcutaneous fluid therapy were continued for another 2 d. The rabbit failed to respond to therapeutic intervention, and its condition deteriorated; it was anesthetized with ketamine (20 mg/kg IM) and xylazine (5 mg/kg IM) and euthanized.

Necropsy revealed no significant gross abnormalities other than clotted blood within the pericardial sac, consistent with intracardiac administration of the euthanasia agent. Histopathology showed multiple, related findings, including unilateral focally extensive atrophy, degeneration, and necrosis of the myocytes of the cricoarytenoideus dorsalis and ipsilateral cricoarytenoideus lateralis muscle (Figure 1 A). Some cells were regenerative with myotube formation, and there was nascent fibroplasia within the endomysium. The ipsilateral cricothyroideus and thyroarytenoideus muscles were unaffected. Sections of the ipsilateral RLN contained numerous swollen granular hypereosinophilic axons (spheroids; Figure 1 B). The trachea had heterophils transmigrating from the submucosa to the surface epithelium, which showed focal areas of squamous metaplasia and ulceration (Figure 1 C). Gram and Wright–Giemsa staining revealed low numbers of Gram-negative bacilli associated with the tracheitis and laryngitis (Figure 1 D). Other histopathology findings included moderate suppurative bronchopneumonia and atrial myocarditis associated with morphologically identical bacteria.

Case report 2. Another rabbit had nystagmus (with the rapid phase to the left), head tilt to the right, and an alternating strabismus immediately after recovery from anesthesia for aneurysm creation. The rabbit showed muscle weakness in the hindlimbs and lack of anal and tail tone. Differential diagnoses included stroke, transient ischemic attack, vertebral fracture, and vestibular syndrome. To rule out vertebral fracture, the rabbit was sedated for radiography; no fractures were present. One day after surgery, the rabbit was dorsally recumbent, tachypneic, and hyperthermic (42.8 °C). The nystagmus had decreased in the left eye but was still present in the right eye. Anal, tail, and hindlimb muscle tone was normal. Because no further improvement occurred, the rabbit was euthanized.

Necropsy revealed no gross abnormalities other than focal hemorrhages of the subcutaneous tissue and fascia of the underlying muscles of the ventral neck and the left thoracic wall and axillary region, consistent with the surgical manipulation. The right eye had mild focal conjunctival petechial hemorrhage on the dorsal aspect of the sclera. Histopathology demonstrated unilateral diffuse pallor of the hippocampus neuropil, with neurons that contained pyknotic, karyolytic, and karyorrhectic nuclei and hypereosinophilic cytoplasm (necrosis) (Figure 2). The cerebellum had focally extensive pallor with loss and necrosis of Purkinje cells. One section of the right CCA showed a blood-filled space within the tunica adventitia, indicating a dissecting aneurysm; a few sections of the same artery showed constriction and an empty lumen (Figure 3 A). The artery also had blood-filled dilatation, transmural hemorrhage, and severe necrotizing and heterophilic inflammation (Figure 3 B).

Because of the neurologic deficits, we ruled out *E. cuniculi* and *P. multocida.* Cultures of tympanic bullae grew negligible numbers of *Pseudomonas aeruginosa* and *B. bronchiseptica* on one side; and negligible numbers of 4 different Gram-positive organisms,

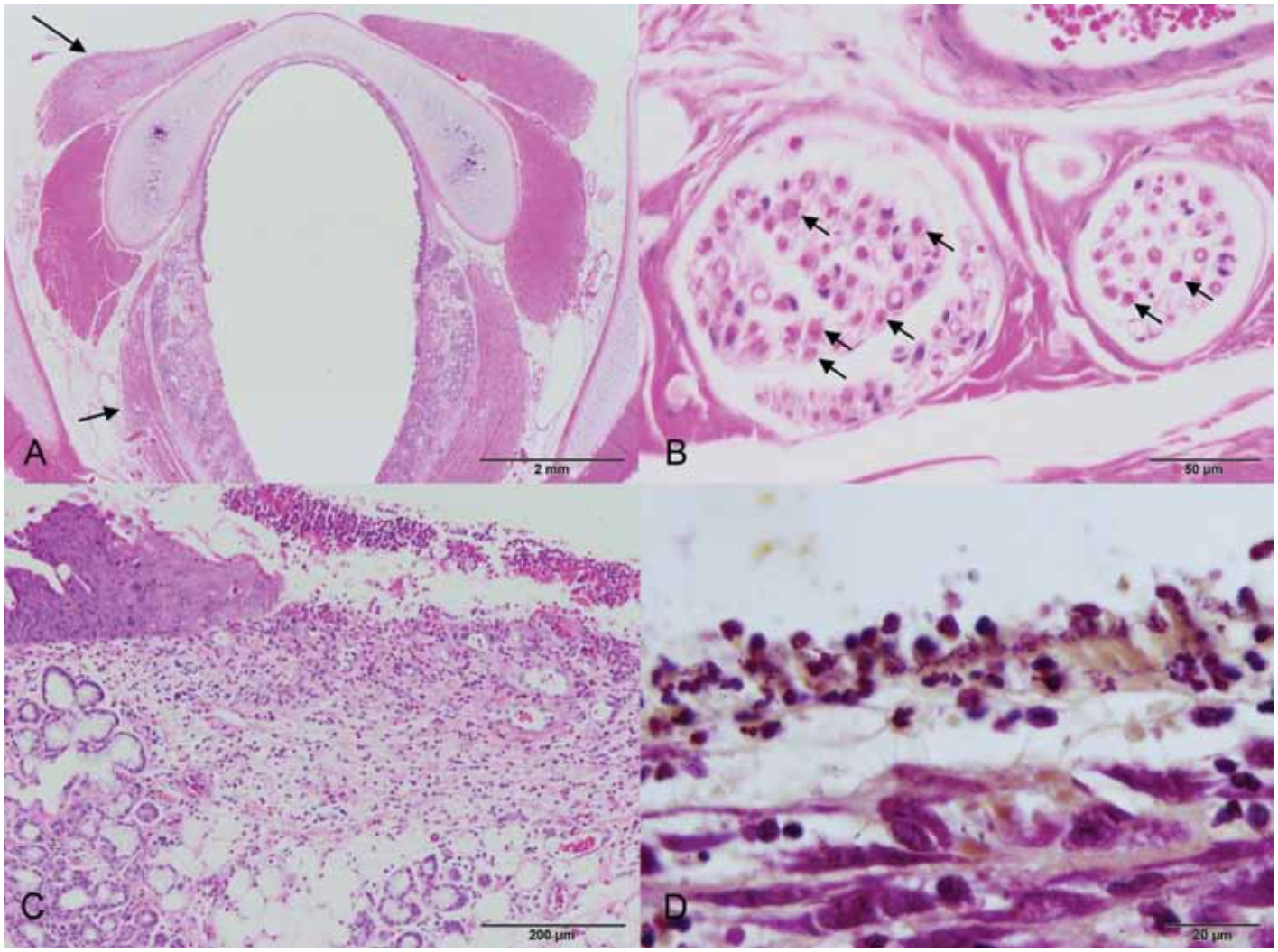

Figure 1. Transverse section of larynx through cricoid and thyroid cartilages. (A) Unilateral atrophy and degeneration of the cricoarytenoideus dorsalis (long arrow) and cricoarytenoideus lateralis (short arrow). Hematoxylin and eosin stain; bar, 2 mm. (B) Axonal spheroids (arrows) within the ipsilateral recurrent laryngeal nerve. Hematoxylin and eosin stain; bar, 50 µm. (C) Ulcerative and heterophilic laryngitis with squamous metaplasia. Hematoxylin and eosin stain; bar, 200 µm. (D) Tracheitis and laryngitis with associated Gram-negative short bacilli. Gram stain; bar, 20 µm.

moderate amounts of *P. aeruginosa*, and rare *P. multocida* on the contralateral side. Kidney sections were PCR-negative for *E. cuniculi*. In light of these results and the experimental history, it was unlikely that these pathogens caused the neurologic deficits.

Literature review. Our literature search resulted to 109 articles as of February 2012. All articles except one (Chinese) were published in English. The paper in Chinese was not included because its English abstract described a different aneurysm creation technique. The summary of reported complications is shown in Figure 4. One group of authors reported neurologic deficits but stopped short of referring to the deficits as manifestations of stroke.[8] One concentration-escalation study reported thrombosis but did not indicate whether thrombosis led to neurologic deficits.[27] Although not a complication, we included reported failure to induce aneurysm, because the presence of aberrant arteries similar to those associated with hemorrhagic tracheal necrosis was implicated.[2,25]

Discussion

Histopathology for our first case revealed unilateral degeneration of the cricoarytenoideus dorsalis and lateralis muscles and ipsilateral degeneration of the RLN. This lesion resulted in the inability to abduct the vocal fold, leading to a narrowed airway and a roaring noise (inspiratory stridor), the rabbit's clinical sign. The focally extensive inflammation and ulceration of the laryngeal mucosa with squamous metaplasia were consistent with local chronic irritation, probably related to inspiratory dyspnea and turbulent flow. The tracheitis and laryngitis may have permitted a local overgrowth of normal upper respiratory tract flora, including the Gram-negative short rods that eventually colonized the lungs to cause bronchopneumonia. Such bacteria also were associated with the heterophilic atrial myocarditis, indicating that bacteremia had occurred. The bacteria were morphologically consistent with *B. bronchiseptica* and *P. multocida*, both primary pathogens of rabbits, among other Gram-negative rods.

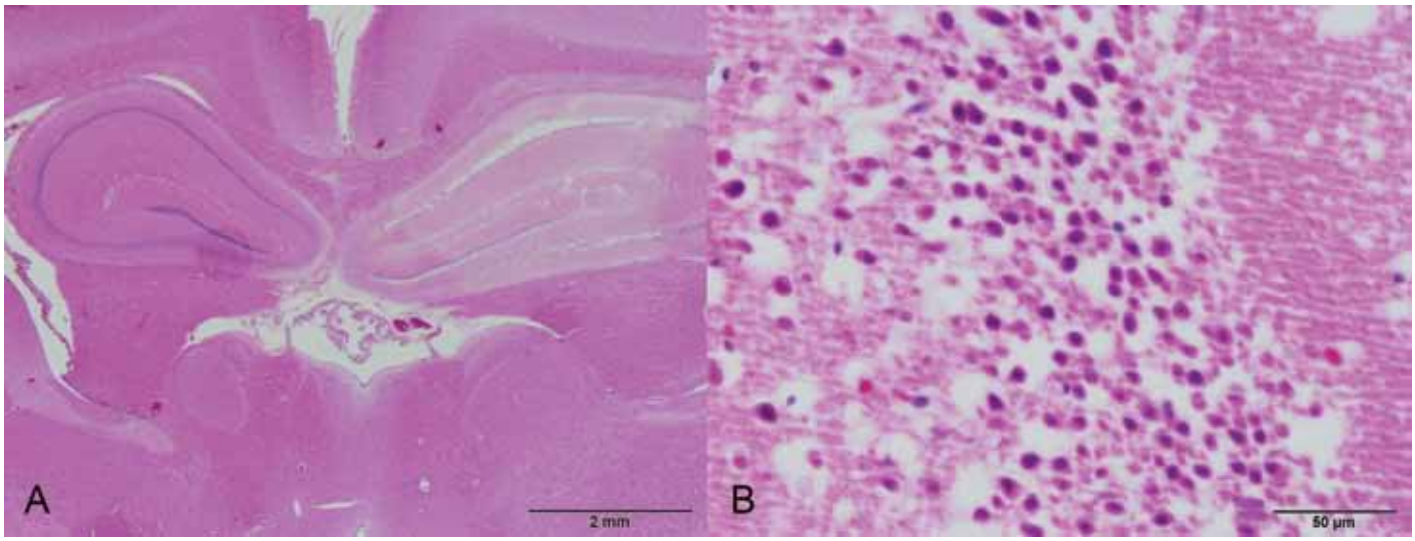

Figure 2. (A) Unilateral pallor and cell necrosis in the hippocampus. Hematoxylin and eosin stain; bar, 2 mm. (B) Diffuse neuronal necrosis in the hippocampus. Neurons that have pyknotic, karyolytic, and karyorrhectic nuclei and hypereosinophilic cytoplasm are indicative of necrosis. Hematoxylin and eosin stain; bar, 50 µm.

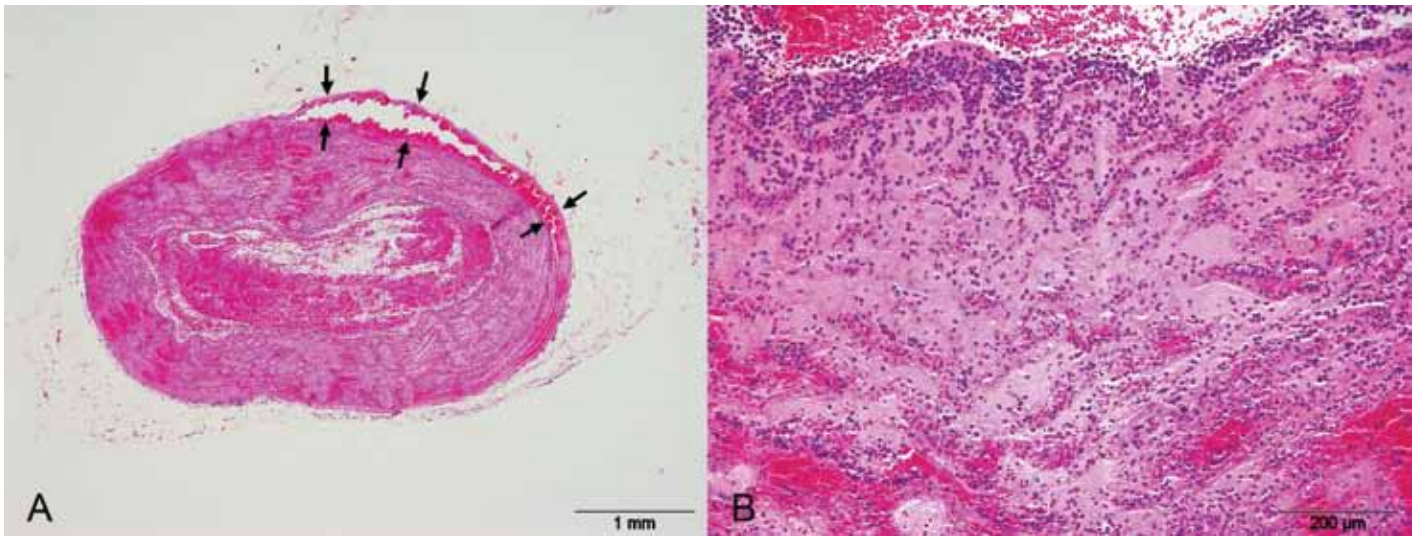

Figure 3. Wall of the right common carotid artery. (A) Dissection surrounded by arrows. Hematoxylin and eosin stain; bar, 1 mm. (B) Heterophilic inflammation, necrosis, and hemorrhage. Hematoxylin and eosin stain; bar, 200 µm.

The case is similar to left recurrent laryngeal hemiplegia (roaring) in horses after degeneration of the left RLN. Laryngeal hemiplegia is rarely reported as a spontaneous idiopathic finding in other species. Although our rabbit case may have resulted from RLN trauma during experimental manipulation, given that the RLN is located dorsal to the right CCA, ischemic damage as a result of thromboembolism is a more likely explanation. Both the cricoarytenoideus dorsalis and lateralis receive the same ventral laryngeal branch of the caudal laryngeal artery.[5] Although the unaffected ipsilateral thyroarytenoideus is supplied by the same branch, this is augmented by a collateral from the dorsal laryngeal branch; the blood supply to the unaffected ipsilateral cricothyroideus is entirely separate. In the microsurgical construction model of CCA bifurcation aneurysm, postoperative vocal cord paralysis and hemorrhagic aspiration pneumonia occurred even in experienced hands, possibly due to laryngeal dysfunction caused by either superior laryngeal nerve injury or muscle dysfunction due to the extensive neck dissection.[16]

Literature review revealed that other respiratory complications have been reported to occur in elastase-induced aneurysm rabbit models primarily because of washing of elastase (that is, elastase washout) into aberrant arteries. Such arteries include the right tracheoesophageal branch, which usually has its source via the bronchial branch from the right supreme intercostal artery, which originates from the subclavian artery.[5] Occasionally, the right bronchial branch originates from the proximal right CCA instead, and its tracheoesophageal branch ascends to the groove between the trachea and esophagus.[3] This condition caused elastase washout and hemorrhagic tracheal necrosis in one rabbit.[25] Another variant is the tracheobronchial artery that originates from the proximal CCA at a near 90° angle to the trachea in a rope-ladder fashion[29] and has caused similar tracheal necrosis with

Complication	Incidence	Comments	References
Neurologic deficits	15 of 38	Nystagmus, dyspnea, head tilt, level of consciousness varying from clouded to comatose, loss of balance, rolling and pedaling; 4 rabbits had thrombosis, and 4 rabbits fully recovered	8
Stroke	5 of 25	All died and was the leading cause of death (5 of 13 deaths)	11, 18
	1 of 19	Clinical signs included nystagmus, head tilt, and strabismus	Current study
Clot formation along parent vessel walls	6 of 30	4 of 6 rabbits with clot formation had neurologic deficits	8
Thrombosis	11 of 34	Concentration-escalation study; did not indicate whether thrombosis led to neurologic deficits	27
Hemorrhagic tracheal necrosis caused by washout of elastase into aberrant arteries	9 of 24	CCA had tracheobronchial artery; manifested as pulmonary edema; all affected rabbits died and was the leading cause of death (9 of 13 deaths)	29
	1 of 20	CCA had tracheoesophageal branch	25
	"High percentage" of 15 rabbits	Aberrant arteries to the trachea; affected rabbits died within 24 h	20
Laryngeal hemiplegia	1 of 19	Rabbit was roaring; with subsequent bronchopneumonia; most possibly caused by thromboembolism	Current study
Death	14 of 38	Without neurologic deficits; 3 died within 24 h; 11 died or were euthanized because of neurologic deficits	8
	59 of 700	Both acute and chronic aneurysm creation; 43 other rabbits died from embolization procedures	21
	6 of 34	Concentration-escalation study	27
	27 of 700	For both acute and chronic creation; theorized to be due to stroke	21
Aneurysm induction failure due to washout of elastase into aberrant arteries	1 of 20	CCA had tracheoesophageal branch	25
	3 of 20	Abnormally deep origin of superior thyroid artery	25
	1 of 9	CCA had tracheobronchial artery	2

Figure 4. Summary of reported complications of elastase-induced saccular aneurysms in rabbits.

subsequent acute pulmonary edema and death. The same branch carried away elastase and caused failure of the right CCA stump to dilate in a rabbit.[2] Such failures have also been reported for rabbits with abnormally deep origin of the superior thyroid artery.[25]

The surgeon creating the model should be familiar with the anatomy of the rabbit ventral neck and its vascular peculiarities[12] and, to avoid potential respiratory complications, should prevent inadvertent damage or elastase washout to structures adjacent to the CCA. One article[5] provides a particularly good review of the normal and anomalous vascular anatomy of the rabbit cervical viscera. In addition, other possible refinement methods in aneurysm creation include modified techniques that minimize the neck dissection needed, such as the use of temporary arcuate aneurysm clips.[31] Finally, performing digital subtraction angiography or contrast-enhanced magnetic resonance angiography and monitoring for washout of contrast material can be performed to determine correct placement of the vascular sheath and complete CCA occlusion. For example, when washout occurs, the sheath can be adjusted proximally to avoid hemorrhagic tracheal necrosis.[20,29]

Stroke is a major complication and was the suspected major cause of rabbit mortality in this model.[11,21] In our second case, extensive acute neuronal necrosis was present unilaterally and diffusely in the hippocampus and focally in the cerebellum. These neuronal populations are exquisitely hypoxia-sensitive.[7] Four plausible iatrogenic etiopathologies of stroke in this model are presented in Figure 5. Of these 4, thrombosis has been documented by digital subtraction angiography and was significantly correlated with neurologic deficits such as nystagmus, head tilt, and coma.[8] Swept emboli may reach the brain via the right vertebral

Cause	Mechanism	Means to minimize risk
Occlusion of the CCA with inflated balloon catheter	Although rabbits have a circle of Willis, which typically mitigates unilateral occlusion of the artery, biologic variability in the adequacy of the compensatory flow likely occurs in some animals.[21]	Minimize occlusion time or perform intermittent occlusions (for example, 2 consecutive incubation sessions: 1. 5 min 2. 10 min (after angiographic evaluation of the results of the first occlusion)[23]
Occlusion of multiple arteries due to balloon placement[a]	1. Total occlusion of the subclavian artery and subsequent blockage of the right vertebral artery;[8] or 2. Partial occlusion of the left CCA when the balloon is located low in the brachiocephalic trunk, occluding the subclavian artery, with the distal part of the balloon reaching the origin of the left CCA. This situation leads to simultaneous occlusion of the 3 arteries (right and left CCA and right vertebral artery) that supply blood to the brain.[8]	1. Position balloon precisely and correctly 2. Avoid using long balloons 3. Be cognizant of possible anatomic variations in the rabbit vasculature.[12]
Thrombosis or thromboembolism	Extensive maneuvering of the catheters inside the vessels, chafing the inflated balloon against arterial wall leading to endothelial damage and exposure of the subendothelium, and frequent manipulation of the blood flow during aneurysm creation may trigger coagulation cascade activation and, when the balloon is deflated and flow is restored, swept emboli may reach the brain via the right vertebral artery.[8]	1. Administer heparin[b] 2. Minimize injury to vessel wall by precise, slow movements and refined techniques 3. Minimize endovascular procedure time
Cerebral arterial air embolism	Introduction of air during angiography (for example, at the time of injection of angiographic contrast, possibly by the formation of cavitation bubbles under pressure)[22]	1. Use an 8- or 9-French guide catheter to protect the port of entry of the balloon and microcatheter into the introducer sheath and use 2 rotating hemostatic valves, stopcocks, and check valve to help create a closed environment.[8] 2. Allow contrast media (and elastase) to stand prior to injection and flush catheters with saline slowly.[22]

[a]Not significantly correlated with neurologic deficits.[8]
[b]Heparin (100 U/kg) is administered routinely through the vascular sheath. It is not a potent antithrombotic agent, and its activity against platelet deposition and thrombus formation has been assessed as moderate.[34]

Figure 5. Plausible iatrogenic etiopathologies of stroke in elastase-induced aneurysm model in rabbits.

artery once the balloon is deflated,[8] whereas occlusive thrombus of the CCA can disrupt perfusion to the head and the neck, leading to ischemia or infarction. However, thrombosis does not always cause neurologic deficits, and if deficits are present, rabbits may still fully recover.[8] We deem that these etiopathologies can be affected by lengthy endovascular procedures, as was determined for thrombosis,[8] and can therefore be related to surgeon experience, among other factors.[21] In fact, when the length of the endovascular procedures was limited to less than 25 min, no complications were observed.[23] In our case, the additional time (15 min) needed to occlude the CCA for collagenase incubation may have increased the risk of stroke for the rabbit.

Based on literature, complications related to elastase-induced aneurysm model in rabbits were primarily due to neurologic deficits or stroke, followed by hemorrhagic tracheal necrosis. Figure 4 summarizes the reported complications arising from model creation only and does not include other procedures that may carry further risk. For example, embolization procedures were associated with 43 of 102 deaths in one study.[21] The longest time rabbits were kept after surgery was 5 y, during which 11 of the 12 rabbits that survived had fully patent aneurysms, whereas 13 died, including 5 from stroke.[11,18] The greatest number of rabbits used for a single study was 700, spanning 4 y, in which suspected stroke-associated mortality reached 8% for aneurysm creation.

In summary, we here present 2 case reports and a literature review of the complications of creating elastase- and collagenase-induced aneurysm in the rabbit CCA, a commonly used model of saccular aneurysms. Surgeons should be cognizant of these complications, the most common of which is stroke, and incorporate measures to prevent them to increase survival rate. In addition, structures in the ventral neck region, including the RLN and laryngeal muscles, can be damaged inadvertently and lead to laryngeal hemiplegia. Furthermore, aberrant arteries should be avoided during elastase–collagenase infusion and incubation to avoid hemorrhagic tracheal necrosis and failure of aneurysm induction.

Acknowledgment

We thank Dr Ronald Wilson, Dr Xuwen Peng, Joy Ellwanger, Sherry Cooper, Weifang Lin, and Ellen Mullady.

References

1. **Abruzzo T, Shengelaia GG, Dawson RC, Owens DS, Cawley CM, Gravanis MB.** 1998. Histologic and morphologic comparison of

experimental aneurysms with human intracranial aneurysms. Am J Neuroradiol **19:**1309–1314.

2. **Altes TA, Cloft HJ, Short JG, DeGast A, Do HM, Helm GA, Kallmes DF.** 2000. 1999 ARRS Executive Council Award. Creation of saccular aneurysms in the rabbit: a model suitable for testing endovascular devices. American Roentgen Ray Society. Am J Roentgenol **174:**349–354.
3. **Blanding JD, Ogilvie RW, Hoffmann CL, Knisely WH.** 1964. The gross morphology of the arterial supply to the trachea, primary bronchi, and oesophagus of the rabbit. Anat Rec **148:**611–614.
4. **Bouzeghrane F, Naggara O, Kallmes DF, Berenstein A, Raymond J; International Consortium of Neuroendovascular Centres.** 2010. In vivo experimental intracranial aneurysm models: a systematic review. Am J Neuroradiol **31:**418–423.
5. **Bugge J.** 1967. Arterial supply of the cervical viscera in the rabbit. Acta Anat (Basel) **68:**216–227.
6. **Cawley CM, Dawson RC, Shengelaia G, Bonner G, Barrow DL, Colohan AR.** 1996. Arterial saccular aneurysm model in the rabbit. Am J Neuroradiol **17:**1761–1766.
7. **Cervós-Navarro J, Diemer NH.** 1991. Selective vulnerability in brain hypoxia. Crit Rev Neurobiol 6**:**149–182.
8. **Cesar L, Miskolczi L, Lieber BB, Sadasivan C, Gounis MJ, Wakhloo AK.** 2009. Neurological deficits associated with the elastase-induced aneurysm model in rabbits. Neurol Res **31:**414–419.
9. **Cloft HJ, Altes TA, Marx WF, Raible RJ, Hudson SB, Helm GA, Mandell JW, Jensen ME, Dion JE, Kallmes DF.** 1999. Endovascular creation of an in vivo bifurcation aneurysm model in rabbits. Radiology **213:**223–228.
10. **Ding YH, Dai D, Danielson MA, Kadirvel R, Lewis DA, Cloft HJ, Kallmes DF.** 2007. Control of aneurysm volume by adjusting the position of ligation during creation of elastase-induced aneurysms: a prospective study. Am J Neuroradiol **28:**857–859.
11. **Ding YH, Dai D, Kadirvel R, Lewis DA, Kallmes DF.** 2010. Five-year follow-up in elastase-induced aneurysms in rabbits. Am J Neuroradiol **31:**1236–1239.
12. **Ding YH, Dai D, Layton KF, Lewis DA, Danielson MA, Kadirvel R, Cloft HJ, Kallmes DF.** 2006. Vascular anatomic variation in rabbits. J Vasc Interv Radiol **17:**1031–1035.
13. **Ding YH, Danielson MA, Kadirvel R, Dai D, Lewis DA, Cloft HJ, Kallmes DF.** 2006. Modified technique to create morphologically reproducible elastase-induced aneurysms in rabbits. Neuroradiology **48:**528–532.
14. **Gaetani P, Tartara F, Grazioli V, Tancioni F, Infuso L, Rodriguez y Baena R.** 1998. Collagen cross-linkage, elastolytic, and collagenolytic activities in cerebral aneurysms: a preliminary investigation. Life Sci **63:**285–292.
15. **Halpern VJ, Nackman GB, Gandhi RH, Irizarry E, Scholes JV, Ramey WG, Tilson MD.** 1994. The elastase infusion model of experimental aortic aneurysms: synchrony of induction of endogenous proteinases with matrix destruction and inflammatory cell response. J Vasc Surg **20:**51–60.
16. **Heilman CB, Kwan ES, Wu JK.** 1992. Aneurysm recurrence following endovascular balloon occlusion. J Neurosurg **77:**260–264.
17. **Hoh BL, Rabinov JD, Pryor JC, Ogilvy CS.** 2004. A modified technique for using elastase to create saccular aneurysms in animals that histologically and hemodynamically resemble aneurysms in human. Acta Neurochir (Wien) **146:**705–711.
18. **Kadirvel R, Ding YH, Dai D, Lewis DA, Kallmes DF.** 2011. Gene expression changes: 5 years after creation of elastase-induced aneurysms. J Vasc Interv Radiol **22:**1447–1451.
19. **Kallmes DF, Fujiwara NH, Berr SS, Helm GA, Cloft HJ.** 2002. Elastase-induced saccular aneurysms in rabbits: a dose-escalation study. Am J Neuroradiol **23:**295–298.
20. **Krings T, Möller-Hartmann W, Hans FJ, Thiex R, Brunn A, Scherer K, Meetz A, Dreeskamp H, Stein KP, Gilsbach JM, Thron A.** 2003. A refined method for creating saccular aneurysms in the rabbit. Neuroradiology **45:**423–429.
21. **Lewis DA, Ding YH, Dai D, Kadirvel R, Danielson MA, Cloft HJ, Kallmes DF.** 2009. Morbidity and mortality associated with creation of elastase-induced saccular aneurysms in a rabbit model. Am J Neuroradiol **30:**91–94.
22. **Markus H, Loh A, Israel D, Buckenham T, Clifton A, Brown MM.** 1993. Microscopic air embolism during cerebral angiography and strategies for its avoidance. Lancet **341:**784–787.
23. **Miskolczi L, Gounis MJ, Onizuka M, Cesar L, Lieber BB, Wakhloo AK, Anaya CA.** 2004. Elastase-induced saccular aneurysms in rabbits: instructions for the rest of us, p 352. Proceedings of the 42nd Annual Meeting of the American Society of Neuroradiology, Seattle, WA. Oak Brook (IL): American Society of Neuroradiology.
24. **Miskolczi L, Guterman LR, Flaherty JD, Szikora I, Hopkins LN.** 1997. Rapid saccular aneurysm induction by elastase application in vitro. Neurosurgery **41:**220–228.
25. **Möller-Hartmann W, Krings T, Stein KP, Dreeskamp A, Meetz A, Thiex R, Hans FJ, Gilsbach JM, Thron A.** 2003. Aberrant origin of the superior thyroid artery and the tracheoesophageal branch from the common carotid artery: a source of failure in elastase-induced aneurysms in rabbits. Am J Roentgenol **181:**739–741.
26. **Ostergaard JR, Oxlund H.** 1987. Collagen type III deficiency in patients with rupture of intracranial saccular aneurysms. J Neurosurg **67:**690–696.
27. **Reinald N, Fournier B, Naveau A, Couty L, Lemitre M, Seguier S, Coulomb B, Gogly B, Lafont A, Durand E.** 2010. Fusiform aneurysm model in rabbit carotid artery. J Vasc Res **47:**61–68.
28. **Short JG, Fujiwara NH, Marx WF, Helm GA, Cloft HJ, Kallmes DF.** 2001. Elastase-induced saccular aneurysms in rabbits: comparison of geometric features with those of human aneurysms. Am J Neuroradiol **22:**1833–1837.
29. **Thiex R, Hans FJ, Krings T, Möller-Hartmann W, Brunn A, Scherer K, Gilsbach JM, Thron A.** 2004. Haemorrhagic tracheal necrosis as a lethal complication of an aneurysm model in rabbits via endoluminal incubation with elastase. Acta Neurochir (Wien) **146:**285–289, discussion 289.
30. **van den Berg JS, Limburg M, Pals G, Arwert F, Westerveld A.** 2001. Type III collagen deficiency in a family with intracranial aneurysms. Cerebrovasc Dis **11:**92–94.
31. **Wang K, Huang Q, Hong B, Xu Y, Zhao W, Chen J, Zhao R, Liu J.** 2009. Neck injury is critical to elastase-induced aneurysm model. Am J Neuroradiol **30:**1685–1687.
32. **Whittaker P, Schwab ME, Canham PB.** 1988. The molecular organization of collagen in saccular aneurysms assessed by polarized light microscopy. Connect Tissue Res **17:**43–54.
33. **Yang XJ, Li L, Wu ZX.** 2007. A novel arterial pouch model of saccular aneurysm by concomitant elastase and collagenase digestion. J Zhejiang Univ Sci B **8:**697–703.
34. **Zaman AG, Osende JI, Chesebro JH, Fuster V, Padurean A, Gallo R, Worthley SG, Helft G, Rodriguez OX, Fallon JT, Badimon JJ.** 2000. In vivo dynamic real-time monitoring and quantification of platelet-thrombus formation: use of a local isotope detector. Arterioscler Thromb Vasc Biol **20:**860–865.