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*† 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† that are morphometrically28 and histologically5 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;1 absence of clear athereosclerotic changes, thus limiting data extrapolation to the “true human degenerative aneurysm”;7 and small size of rabbits, thus limiting the evaluation of large devices or techniques requiring multiple catheters.11 In addition, rare spontaneous ruptures of elastase-induced aneurysms have been reported.16,17,19 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 quantity14,26,30 or quality32 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
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 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 of a 3-French balloon catheter (Fogarty, Baxter Healthcare, Irvine, CA) through a vascular sheath into the right CCA and aneurysm created as described previously. 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 CO₂ 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 lateralisis muscle (Figure 1 A). Some cells were regenerative with myotube formation, and there was nascent fibroplasia within the endomyxium. The ipsilateral cricothyroides 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,
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.

**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. One concentration-escalation study reported thrombosis but did not indicate whether thrombosis led to neurologic deficits. 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.

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.
Complications of aneurysm creation in rabbits

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. Occasionally, the right bronchial branch originates from the proximal right common carotid artery instead, and its tracheoesophageal branch ascends to the groove between the trachea and esophagus. This condition caused elastase washout and hemorrhagic tracheal necrosis in one rabbit. Another variant is the tracheobronchial artery that originates from the proximal common carotid artery at a near 90° angle to the trachea in a rope-ladder fashion and has caused similar tracheal necrosis with

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 common carotid artery, 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. 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 common carotid artery 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.

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.
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 necro-
sis.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 document-
ed by digital subtraction angiography and was significantly cor-
related with neurologic deficits such as nystagmus, head tilt, and
coma.8 Swept emboli may reach the brain via the right vertebral

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

The surgeon creating the model should be familiar with the
anatomy of the rabbit ventral neck and its vascular peculiarities12
and, to avoid potential respiratory complications, should prevent
inadvertent damage or elastase washout to structures adjacent
to the CCA. One article5 provides a particularly good review of
the normal and anomalous vascular anatomy of the rabbit cer-
vical 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.25 Finally, performing digital subtraction angiog-
raphy or contrast-enhanced magnetic resonance angiography and
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 rab-
bbits with abnormally deep origin of the superior thyroid artery.25

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<table>
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<tr>
<th>Cause</th>
<th>Mechanism</th>
<th>Means to minimize risk</th>
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<tr>
<td>Occlusion of the CCA with inflated balloon catheter</td>
<td>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.</td>
<td>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))</td>
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<tr>
<td>Occlusion of multiple arteries due to balloon placement*</td>
<td>1. Total occlusion of the subclavian artery and subsequent blockage of the right vertebral artery; 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.</td>
<td>1. Position balloon precisely and correctly 2. Avoid using long balloons 3. Be cognizant of possible anatomic variations in the rabbit vasculature.</td>
</tr>
<tr>
<td>Thrombosis or thromboembolism</td>
<td>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.</td>
<td>1. Administer heparin* 2. Minimize injury to vessel wall by precise, slow movements and refined techniques 3. Minimize endovascular procedure time</td>
</tr>
<tr>
<td>Cerebral arterial air embolism</td>
<td>Introduction of air during angiography (for example, at the time of injection of angiographic contrast, possibly by the formation of cavitation bubbles under pressure)</td>
<td>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. 2. Allow contrast media (and elastase) to stand prior to injection and flush catheters with saline slowly.</td>
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*Not significantly correlated with neurologic deficits. *
*Bivin (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. 

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

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**References**


