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Rat Carotid Artery Balloon Injury Model

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i. Summary

Numerous and diverse experimental animal models have been used over the years to examine reactions to various forms of blood vessel disease and/or injury across species and in multiple vascular beds in a cumulative effort to relate these findings to the human condition. In this context, the rat carotid artery balloon injury model is highly characterized and commonly used for investigating gross morphological, cellular, biochemical, and molecular components of the response to experimentally-induced arterial injury. The mechanical damage caused by the balloon catheter completely removes the intimal endothelial lining and creates a distending mural injury in the operated vessel. This elicits a reproducible remodeling response characterized by vascular smooth muscle cell (SMC) mitogenesis and migration (via phenotypic switching), SMC apoptosis, partial vascular endothelial cell regeneration, enhanced matrix synthesis, and establishment of an invasive neointima in time-dependent fashion. This multi-factorial process allows for investigation of these many important pathophysiological processes and can serve as a valuable “proof-of-concept” tool to verify and substantiate *in vitro* results; however, inherent anatomical and adaptive constraints of this *in vivo* model ration comparison to the diseased human system (see **Note 1**). In this chapter, brief overview of the materials needed and the methodologies commonly employed for successful routine performance of this important experimental animal model will be provided. Individual sub-sections will cover animal care and handling, pre- and post-operative procedures, and the surgery proper. Protocols for histopathology and morphometry and procedures for data management and interpretation pertinent to the rat carotid artery balloon injury model will be discussed in Chapter ___ of this series. **Notes** will conclude with important caveats, limitations, and considerations for practical use of this technique.

Keywords

adventitia; balloon injury; common carotid artery; vascular endothelial cell; extracellular matrix; media; neointima; rat; remodeling; vascular smooth muscle cell

1. Introduction

Investigation into the response of blood vessels to injury is of pivotal importance in understanding the pathophysiology of vascular disorders. Injury-based experiments are generally designed for examination of gross, cellular, biochemical, and/or molecular mechanisms that contribute to the vascular injury response, a phenomena particularly important in both basic science and clinical medicine. Injury-based approaches consist of mechanical or other artificial intervention(s) to elicit a primary or secondary injury. Responses to the injury,

then, provide adaptive measures for study. This should not be confused with approaches used to study the pathophysiology of vascular disease which often employ genetic, dietary, and/or environmental induction.

The rat carotid artery balloon injury method was originally described in several seminal articles by Clowes and colleagues (1-4) and has since been employed and thoroughly characterized in a plethora of basic and clinical science research endeavors. Briefly and from a methodological perspective, this approach consists of isolating a segment of carotid artery vasculature in an anesthetized laboratory rat, creating an arteriotomy incision in the external carotid branch through which the balloon catheter is inserted, advancement of the catheter through the common carotid artery, repeated inflation and withdrawal of the catheter to induce endothelial cell loss and mural distension, and removal of the catheter with closure of the arteriotomy and resumption of blood flow through the common carotid artery and internal carotid artery branch. As detailed in Chapter __ of this series, histological protocols germane to this model can then be employed that allow acute measures of cellular and molecular changes and longer-term qualification of neointima development and vessel wall restructuring along with morphometric analyses and quantification.

In this chapter, comprehensive methods are provided along with special considerations for practical use of this technique as suggested by the author; however, individual experiences and preferences may dictate alternate more suitable practices on a case-by-case basis. This chapter is not intended for use as a scientific review of the model nor of the mechanisms contributing to the vascular injury response. Excellent summaries of neointima formation and vascular remodeling following injury including the rat carotid artery balloon injury model are recommended for interested readers (5-8).

2. Materials

All of the materials needed to perform this experimental method are listed below in appropriate subheadings as in Section 3, **Methods**. Where suitable, a short description of their use if included, and mention is made of special requirements for particular materials which are cross-referenced in Section 4, **Notes**. For specific items, name-brands are included of reagents and/or supplies preferred by the author; however, choice of suitable alternate materials is up to the discretion of the investigator(s).

2.1. Animals

A variety of rat strains has been utilized for this method; however, perhaps the most highly used and characterized strain is Sprague-Dawley (Hsd, Harlan). Male rats are preferred due to the potential impact of hormone levels on various cellular function(s) that has been identified in females. A wide range of animal weights and ages has been used in this approach, yet most citations report body weights between 350 and 500 grams. Nonetheless, it is important to use fully grown animals as vessel caliber will directly impact the severity of the injury from use of a standard-sized (2 French) inflated balloon catheter. Retired breeders may be used as long as they are of appropriate age/weight. Unless otherwise desired, animals can be kept on standard rodent chow and water *ad libitum* peri-operatively.

2.2. Pre-operative Procedures

Solutions:

Betadine solution (The Purdue Frederick Company), or other topical anti-septic/bactericide/virucide agent

Absolute alcohol (to cleanse surgical area on animal skin)

70% alcohol (to cleanse surgery platform)

Ophthalmic ointment/lubricant (Phoenix Pharmaceutical, Inc., see **Note 2**)

Anesthetic of choice (see **Note 3**)

Pre-warmed phosphate buffered saline (PBS), Lactated Ringers (LR) solution, normal saline solution, or alternate choice for supplemental fluids (see **Note 4**)

Supplies:

Glass bead sterilizer (Germinator 500, Roboz Surgical) or other suitable instrument sterilizer

Animal hair clippers (A-5 Clipper, size 40 blade, Oster)

Scissors, medium (for removing fine hairs)

Needles (26 g. for anesthetic if parenteral administration; 18-20 g. for supplemental fluids)

Syringes (1 ml for anesthetic if parenteral administration; 3-5 ml for supplemental fluids)

Gauze

Cotton-tipped applicators (for use in topically applying anti-septic/bactericide/virucide agent and ophthalmic ointment/lubricant)

Tape (cut to appropriate lengths (~3") and placed nearby for easy access)

Rodent operating table or other surgery platform

Surgical blanket, sterile

Rodent limb tie-downs or restrainers (see **Note 5**)

Animal weighing scale (capable of weights up to ~600 grams)

Sterile water (used to fill balloon catheter ahead of time)

Drinking water (to moisten tongue, mouth) (see **Note 2**)

2.3. Surgery Proper

Solutions:

Lidocaine hydrochloride (see **Note 6**)

Ophthalmic ointment/lubricant (see **Note 2**)

Supplemental fluids (see **Note 4**)

Anesthetic, supplemental (see **Note 3**)

Supplies:

Needles and syringes (see above)

Gauze, sterile

Cotton-tipped applicators, sterile

Tape

Fogarty balloon embolectomy catheters, 2 French (#120602F, Edwards Lifesciences Germany)

Trocar guiding needle (#305185, Becton Dickinson) or suitable alternative (see **Note 7**)

Two-way stopcocks with luer-lock end (used for attachment of syringe to balloon catheter)

Inflation device for balloon catheter (Encore 26 Advantage, Boston Scientific; see **Note 8**)

Suture: 4-0 black braided silk (Roboz), 6-0 Prolene blue monofilament (Ethicon; see **Note 9**)

Ample lighting (see **Note 10**)

Heating source (heat lamps, heating blanket; see **Note 11**)

Rodent operating table or other surgery platform

Rodent limb tie-downs or restrainers

Surgical blanket, sterile

Micro-caliper (#RS-6460, Roboz) or small metric ruler (to measure vessel lengths)

Surgical tools: (see **Note 12**)

Large scissors (to cut skin; #RS-6812SC, Roboz; see **Note 13**)

Medium scissors (#RS-5910, #RS-5912SC, Roboz)

Small micro-scissors (McPherson-Vannas, #RS-5600, Roboz)

Small curved forceps (Micro-dissecting tweezers, Pattern 7S, #RS-4935, Roboz)

Small arterial clamps (#RS-5424, #RS-5426, #RS-5433, Roboz)

Retractors (large for skin, small for tissues; see **Note 14**)

Skin staples (7-9 mm) or skin suture

Skin stapler

Skin staple remover (see **Note 15**)

2.4.-2.5. Post-operative Procedures, Animal Recovery

Solutions:

Topical anti-septic/bactericide/virucide agent

Supplemental fluids (see **Note 4**)

Ophthalmic ointment/lubricant (see **Note 2**)

Water for drinking (see **Note 2**)

Surgical instrument cleaner, detergent

70% alcohol

Analgesic of choice (see **Note 16**)

Supplies:

Gauze

Sterile blanket

Surgical drapes, towels (see **Note 17**)

Animal cage with cover and water supply

Rodent chow

3. Methods

3.1. Animals

All animal care and experimental procedures must adhere strictly to the recommendations of the Guide for Care and Use of Laboratory Animals (DHEW (NIH) 85-23 (revised 1985) and the Public Health Service Policy on Humane Care and Use of Laboratory Animals (revised 1986), as well to the guidelines of the local institutional animal care and use committee.

Continual monitoring of the state of anesthesia and well-being of the animal by the investigator (s) is imperative throughout this surgery, and guidelines for early euthanasia should be strictly followed (detailed in Section 3.4, *Post-operative Procedures*).

3.2. Pre-operative Procedures

Anesthesia—Choice of appropriate anesthetic and route(s) of delivery are decisions for the investigator; however, the major consideration is that a surgical plane of anesthesia be maintained for the duration of the surgery. This can range from approximately 30 minutes for an experienced surgeon to over 90 minutes for a novice or in the case of unexpected problems. The level of anesthesia should be continually monitored via toe or tail pinch, inspection of breathing rate and pattern, and inspection of heart rate throughout the surgery to ensure that the rat is adequately sedated. If the animal appears to be coming out of anesthesia during the surgery, immediately provide supplemental anesthesia (following institutional guidelines or approximately 10% original dose) and pay particular attention to the level of sedation for the remainder of the surgery. Supplemental oxygen is not normally needed for this surgery; however, if the investigator deems it necessary oxygen or supplemental air can be provided to the rat via nasal cannula without interference to the surgeon.

Setup—It is imperative that the investigator have everything prepared ahead of time and that the surgical area correctly be setup in order to avoid complications or emergencies during the surgery proper. Be aware that the more expediently the surgery is completed (in scientifically and medically sound fashion of course), the better chance for rapid recovery by the animal and the higher chance for success. Preparation and sterilization of the surgical area, preparation of all solutions and reagents, cleaning and sterilization of all necessary surgical tools, and correct setup of the surgical area with convenient placement of all materials and supplies that will be needed throughout the surgery should take place prior to sedating the animal. Also, try to have available any item that might be needed in case of emergency (see **Note 18**).

A major task to complete prior to surgery is preparation of the balloon catheter and trocar (if one is to be used). This can take 10 or 15 minutes to complete, so plan accordingly. Remove the new balloon embolectomy catheter from its package and remove packaging components and the luer-lock cap and discard. Fill a 1 ml syringe (without needle) completely with sterile water and remove air bubbles. Carefully fill a two-way stopcock with sterile water to create a water-filled system and similarly remove air bubbles. Attach the syringe to the stopcock without introducing air bubbles (see **Note 19**). In similar fashion, fill the luer-lock portion of the balloon catheter with sterile water (ejected from the syringe through the stopcock, again removing air bubbles) and remove any trapped air in the catheter opening. Maintaining a closed system of water, firmly attach the stop-cock to the luer-lock end of the balloon catheter. Depress the syringe slightly to check for leaks and to ensure that balloon inflation occurs. A suggested capacity of the inflated balloon for these surgeries is 0.02 ml; however, be sure to note that the maximum inflation capacity (as stated in Indications for Use in the product insert) is 0.2 ml. For these studies, be sure to make a note regarding the degree of balloon inflation using 0.02 ml water and the appropriate markings on the syringe. Upon inspection, do not be surprised if a small bubble appears in the balloon itself. This is impossible to remove from the catheter and will soon dissipate. If using a manual barometer and inflation device, fill the device with water

(through the luer lock tubing) while removing air bubbles according to manufacturer's directions. Similarly remove air from the luer-lock end of the balloon catheter and attach the catheter end of the catheter to the luer lock of the inflation device. Manually inflate the device to an appropriate pressure (~2.0 atm) and watch the gauge closely. Be careful because a little pressure from this device can inflate the balloon a large degree. Also be cautious that there can exist a delay in the pressure induced by the device (by rotating the handle) and the distension of the balloon. This can lead to over-pressurizing the balloon to higher than desired levels. A photo of an arterial balloon catheter attached to a 1.0 ml syringe via a stopcock along with an 18 g. thin-walled guiding trocar and an automated balloon inflation device is shown in Figure 1.

A surgical mask, surgical gloves, and/or protective goggles are recommended to be worn by the surgeon during the surgery proper. This will minimize the influence of human exposure on the animal as well as protect the animal from potential infection. This will also serve to protect the surgeon from animal dander and exposure. A glass bead sterilizer or other sterilizing apparatus (mini-autoclave) is recommended and should be pre-heated, and all surgical tools should be sterilized and placed conveniently on the surgery platform (see **Note 20**). All other reagents and treatment(s), solutions, gauze, cotton-tipped applicators, tape, suture, etc. should also be setup ahead of time. Cut appropriate lengths of suture and tape (see **Note 9**), and make sure gauze and ample cotton swabs are within easy reach. Cleanse and sterilize the operating surface on surgery area/platform/table with 70% alcohol. Lay out all items to be used for surgery as well as anything else that you might potentially need in case of emergency or other unexpected occurrence (see **Note 18**). A photo of a typical pre-operative surgical setup for a left-handed surgeon performing a rat carotid artery balloon injury study is shown in Figure 2.

Once the animal is sedated to a surgical level of anesthesia (verified by toe or tail pinch, breathing and heart rate patterns) and holding the animal supine in one hand (making sure to stabilize the head), shave the surgery site with animal hair clippers. This area to be shaved is the ventral neck region from the chin down to just above the sternum. Be careful not to press too deeply while shaving, as any pressure here will compress the thorax and impede the animals ability to breathe. Use both side-to-side and up-and-down motions with the clippers and try to remove all hair in this region (see **Note 21**). Following use of the clippers, remove lingering fine hair with small scissors. Again, do not press too deeply. Empty all hair into the trash and place the animal supine on the surgery table in a heated environment (see **Note 11**) with head towards the surgeon. Harness the arms and legs and gently retract the head (see **Note 5**). Place a piece of gauze on the lower groin (covering the penis) to soak up urine flow that will occur. Swab the cervical area with a topical anti-septic/bactericide/virucide agent followed by 70% alcohol. An ophthalmic solution should be placed on the open eyes with a cotton swab in order to prevent drying. The tongue and mouth of the animal can also be moistened with drinking water to avoid drying. At this time provide 3-6 ml supplemental fluids.

3.3. Surgery Proper

Given the important details of this protocol and the many procedures involved, this section is presented in numerical steps to simplify instruction and to make it easy to follow. At this point the animal is sedated and lying supine on the operating platform with head towards the surgeon and appendages retracted, with neck area shaved and cleansed and all surgical items conveniently located nearby within easy access.

1. Using sharp-blunt serrated-edge scissors (see **Note 13**) and starting immediately below the chin of the animal, make a straight incision in a direction towards the tail all the way to the top of the sternum just above the ribcage. Make the incision as straight as possible and not too deep (this is for purposes of cutting the skin only). Keep the scissor tips up! (see **Note 22**).

2. Using medium hemostats and/or dull forceps, blunt dissect underlying glandular tissue from skin. Keep the tips of your instruments parallel to the tissues during this process so not to puncture the skin or the underlying tissue. With blunt dissection, go in with tips closed, gently separate the tips, and remove the instrument with the tips wide open. In this fashion, gently separate the skin from underlying tissue circumferentially around the entire incision wound. This will aid in suturing the underlying tissues independently from the skin and in layers following surgery. Do not be afraid if small vessels break and if there is some minor bleeding. Swab these areas with a cotton tipped applicator to stop bleeding. Keep area moist with warm sterile PBS or other fluid. Following this step the skin should be completely separated from underlying tissues all the way around the incision. Use of a medium to large skin retractor at this stage to keep the skin out of the way is recommended (see **Note 14**).
3. Using medium scissors, cut through the fascia overlying the glandular tissue (again, keep tips up) to expose underlying glands. Again, make this incision as straight as possible, as this will be sutured following surgery. Gently separate glands to expose underlying muscular layer via blunt dissection.
4. Carefully separate muscular tissues with dissection using sharp 7S forceps (be careful not to dig too deep, as these forceps are very sharp and can easily penetrate tissues). For purposes of this protocol, procedures are indicated for surgery to be performed on the left common carotid artery (see **Note 23**). Blunt dissect along the longitudinal left aspect of the central and adjacent muscular tissues (sternocleidomastoid, omohyoid, thyrohyoid, sternohyoid) and remember to avoid pressure on these muscles as below them lay the thorax and the animals' ability to breath! Gently separate the central muscle from parallel neck muscles and the diagonal thin muscular band (omohyoid) lying directly over the carotid vasculature (see **Note 24**). At this point retraction of skin and muscular tissues is highly recommended and virtually essential for visualization of the underlying carotid artery vasculature. During all of these procedures, keep the tips of the instruments up and keep all tissues moist. Following thorough dissection and retraction of tissues, at this stage the surgeon will be able to view the left common carotid artery, the vagus nerve (a thin white sheath lying adjacent to the carotid artery), and adjacent nerves and vessels (see **Note 25**).
5. Continue blunt dissection alongside the left carotid artery distally towards the head to expose the carotid artery bifurcation into the internal and external branches. Use of lidocaine HCl at this stage on the exposed carotid vasculature is recommended to keep it moist (see **Note 6**). As the surgeon looks at it, upon exposure the artery lying on top (ventral aspect) distal to the bifurcation will be the external carotid artery branch, while the internal carotid artery branch digs dorsally and is not usually apparent unless the carotid vasculature is moved aside. To ensure identification of the internal carotid artery and location of the bifurcation, gently move the distal portion of the common carotid artery to the right and the internal branch should appear directly below it. Make a note of where the common carotid bifurcation exists for each animal (see **Note 26**). At this point the surgeon might also be able to visualize the hyoid cartilage directly under the chin. Avoid damage to the hyoid cartilage and to all nerves that are present, especially the vagus nerve and nerve plexus. These nerves control many aspects of physiologic function, and perturbation can result in severe cardiovascular depression. As before, use retraction to keep the tissues separated. Keep in mind to moisten all exposed tissues throughout the surgery and to use liberal amounts of lidocaine on exposed vasculature.
6. Expose the left common carotid artery the entire length of the skin incision down to the sternum. In an adult rat, this should be ~20 mm in length proximally from the bifurcation (some variation exists between animals). The entire length of the adult

common carotid artery is estimated to be 35-40 mm. The surgeon will be able to visualize vasodilation with each arterial bolus (heartbeat) if the vasculature is tented up with forceps (be careful not to over-manipulate the vessel). Add lidocaine on top of the exposed common carotid artery and let it incubate for several minutes (see **Note 6**). At this stage lidocaine will serve to dilate the blood vessel and its branches thus allowing easier vascular access. Swab away excess lidocaine before the next step.

7. Before placement of sutures around specific sections of the carotid vasculature can take place (see Fig. 3) (9), the surgeon must make sure that these vessel sections are completely separated from all adjacent tissues. If this is not the case, then during the retraction and/or clamping procedures complete cessation of blood flow will not occur due to the presence of additional tissue in the clamp or retractor. This will result in unexpected bleeding, severely hamper progress of the surgery, and cause undue stress on the animal. At each site for suture placement (Fig. 3), carefully blunt dissect away all adjacent tissues from the vessel so that at least 2-3 mm of the vessel is free from extraneous tissue.
8. This step involves placement of sutures around certain sections of the carotid artery vasculature to be used for retraction and/or clamping and hemostasis (see **Note 27**). For exact anatomical location of these sutures, please refer to Figures 3 and 4. When wrapping sutures around the vessels, use the small forceps (7S micro-dissecting tweezers). At the most proximal site on the left common carotid artery (as close to the sternum as possible) and having separated the vascular fascia and vagus nerve from the artery, loop a single ~3" suture (size 4-0, black braided silk) around the artery (see **Note 9**; #1 in Figs. 3, 4). Next, immediately distal to the bifurcation of the common carotid artery, loop and loosely tie one ~3" 4-0 silk suture around the external carotid artery branch (the branch that lies on the surface in the prone animal; #2 in Figs. 3, 4). Do not tie this off but keep it loosely looped for later (this will be the suture that will be pulled tight immediately after removal of the balloon catheter from the arteriotomy incision). Next, loop and loosely tie one 5-6" 4-0 black silk suture on the external artery branch as distally and far away from the bifurcation as possible (#3 in Figs. 3, 4). When loosely tying this suture, keep one end of the wrapped suture as long as possible as this will be used to retract the vessel in a direction towards the head. In this effort, try to obtain as long a section on the external carotid artery branch between the two sutures as possible (see **Note 28**). A longer isolated section of the external carotid artery will significantly improve chances for successful balloon intervention. At this point three individual pieces of 4-0 silk suture have been loosely placed around the carotid vasculature: one as proximal on the common carotid as possible, one on the external carotid branch immediately distal to the bifurcation and loosely looped, and one as distal as possible on the external branch and loosely looped with one long end. No sutures are tied yet and blood flow is still patent throughout the entire vasculature at this step.
9. As the surgeon is dissecting around the bifurcation and along the external carotid artery branch, several small arteries appear (in most cases) that if not ligated could be a source for retrograde blood loss during balloon intervention. If arterial branching off the external carotid artery is apparent, the ascending pharyngeal, occipital and/or superior thyroid arteries should be completely tied off using short lengths of 6-0 Prolene blue monofilament suture (see **Note 29**; see Figs. 3, 4).
10. The surgeon now must decide whether to access the internal carotid artery branch which, as the surgeon is looking at the animal, lies immediately below the carotid bifurcation and the external carotid artery branch and quickly moves into deep tissue in a dorsal direction (#4 in Fig. 3). The reason to access the internal artery is that it can be a significant source for retrograde blood loss if its flow is not adequately

controlled. Access to this branch can be difficult as there is not a lot of space with which to work and the available length of the internal branch is usually minimal. The surgeon must determine if it is in the best interest for the animal and for the research study itself to try to access this vessel to provide another degree of localized hemostasis. The author does not routinely access the internal carotid artery during the surgical technique; moreover, the small amount of bleeding that occurs from internal carotid artery retrograde blood flow is minimal in his hands. This choice for the surgeon must be taken seriously, especially for a novice who might take more time to successfully introduce the catheter into the arteriotomy (and hence, allow more time for blood loss via internal carotid artery retrograde flow). It is recommended that the surgeon attempt this surgery both with and without accessing the internal carotid artery and choose whichever approach works best in their hands. If the surgeon chooses to isolate the internal carotid artery branch and control its flow, then the exact site of the common carotid artery bifurcation needs to be determined and a note made of it as mentioned before. At this site, the middle suture (or the more proximal suture on the external carotid branch) should be easily accessible, and the surgeon can use this to gently retract the overlying vessels to the right in order to visualize the underlying internal carotid artery. Using the small forceps (7S), gently blunt dissect adjacent tissues from the internal carotid branch immediately adjacent to the bifurcation, exposing as much of the internal carotid as possible. Loop a section of 4-0 silk suture around the artery and keep it un-tied. Control of the internal carotid artery blood flow can then be achieved with either arterial micro-clamps or with retraction of the suture (see **Note 14**; see Fig. 3). If using an arterial clamp, do not place it too close to the common carotid bifurcation, as this will physically impede advancement of the balloon catheter and the clamp will have to be removed and placed at a more distal site on the internal carotid artery. Similarly, if using suture to retract the internal carotid branch, be careful not to alter the geometry of the carotid vasculature and create an angle that might obstruct insertion and advancement of the catheter through the vessel lumen.

11. Now, the internal carotid artery may or may not be retracted and/or clamped (#4 in Fig. 3), and it is time to tie the other sutures already in place (one on the common carotid, two on the external carotid) and to clamp the common carotid artery in advance of the arteriotomy incision and balloon catheterization. First, using a double-knot tie the most distal suture on the external branch, retract it towards the head, and adhere it to the operating surface with tape (#3 in Fig. 3). Be careful here not to pull too tightly, and constantly watch the carotid artery during retraction to ensure that the vasculature is not being stressed too much. Avoid undue pressure on the vasculature and try to maintain normal vessel geometry (avoid angles). Gently retracting the carotid artery during this step lifts up the external carotid artery segment for easier access.
12. Gently retract the proximal suture on the common carotid artery and place an arterial clamp on the vessel in order to stop common carotid artery blood flow (see **Notes 30, 31**; #1 in Fig. 3). Be careful here and try not to traumatize the vessel. Try to place the clamp exactly perpendicular to the vessel and avoid catching adjacent tissues in the clamp. Lidocaine can be applied to the vasculature and incubated for several minutes. At this point the external carotid artery has been retracted distally, the common carotid artery blood flow has been stopped via clamping, most or all (if internal carotid artery is involved) retrograde blood flow is controlled via ligation of miscellaneous small arteries, and suture is loosely looped around the external branch just distal to the bifurcation of the common carotid artery. The common and external carotid arteries should now be lying straight with easy access to the portion on the external carotid

artery between the two sutures. This is the critical site for performing an arteriotomy incision and introducing the balloon catheter.

13. The balloon catheter should have been calibrated well ahead of time (see **Section 3.2.**) and should be located within easy reach of the surgeon. With a cotton swab in one hand and small micro-scissors in the dominant hand, very delicately snip a portion of the external carotid branch perpendicular to its axis and as distally as possible (towards suture nearest the head; see Figs. 3, 4). This will allow sufficient length of external carotid artery branch if there presents a problem with this initial arteriotomy incision (see **Note 28**). The cut should be straight with a length $\sim 1/4$ to $1/3$ the circumference of the vessel. Do not make the cut too deep or cut completely through the vessel, as this will make things more difficult for introduction of the catheter (see **Notes 32, 33**). Immediately following the arteriotomy gently swab it to stop bleeding (there should be minimal bleeding if adequate hemostasis was achieved). It may help to gently retract the distal external carotid artery ligature to make the arterial section taught prior to making the incision.
14. Several investigators recommend flushing the lumen of the common carotid artery (after adequate hemostasis is ensured) with 1 ml heparin solution (50 IU/ml) or with PBS via the arteriotomy incision prior to inserting the balloon catheter as well as immediately following the injury and removal of the catheter (10). This practice is not performed nor recommended by the author as heparin has been shown to inhibit vascular SMC proliferation, an essential component of the neointimal response to injury (3,11). Additionally, other investigators recommend removing the adventitia from the carotid artery prior to performing balloon injury (12). This is not encouraged as the adventitia is a valuable source for sensory nerves, immune elements including macrophages and mast cells, and fibroblasts that have been implicated in the response to balloon injury in rats (13,14).
15. Following a successful arteriotomy incision on the external carotid artery branch and holding the balloon catheter alone or inserted through a trocar guiding needle (see **Note 7**), gently insert the un-inflated balloon into the arteriotomy hole and advance it all the way to the arterial clamp on the common carotid artery using 7S forceps held sideways to guide the catheter. Be careful not to damage the balloon with the forcep tips and hold the catheter at the same plane as the blood vessel during this process. Remove the clamp on the common carotid to allow passage of the un-inflated balloon catheter all the way down the common carotid to the aortic arch (~ 35 -40 mm total length). Try to feel the resistance when the balloon tip reaches the back wall of the aortic arch. Make sure to watch the markings on the catheter sheath when this happens, which generally occurs 10-15 mm between the first black band and the arteriotomy incision (see Fig. 5). Also, watch for excess bleeding out of the arteriotomy incision around the catheter, which can occur with an un-inflated balloon.
16. Now, slowly inflate the catheter to a pre-determined volume (suggested 0.02 ml) or pressure (~ 2.0 atm) manually (via syringe) or with the use of an inflation device and barometer. If performing this step manually, remember to lock the stopcock between the syringe and the catheter to maintain appropriate pressure in the balloon. Keep an eye on the catheter and its markings here because if the catheter is inserted too far into the aortic arch, aortic flow will take the inflated balloon and pull it down the thoracic aorta! With the inflated balloon in the common carotid artery, get a feel for the degree of resistance as it is slowly withdrawn. Gently remove the catheter using rotation (see **Note 34**) all the way almost up to the arteriotomy incision (you will be able to see the inflated balloon inside the carotid artery as it approaches the arteriotomy site). Be very careful here and do not withdraw the catheter too close to the arteriotomy hole. If the inflated balloon gets too close to this hole, it will slip out and an open

arterial bleed will ensue. This, of course, will cause significant blood loss if the common carotid is not immediately clamped or retracted (see **Note 31**). If this does happen, stop blood flow immediately with a clamp or suture retraction on the common carotid artery and re-group.

17. Deflate the balloon and re-insert the catheter all the way to the aortic arch again. Inflate the balloon at desired volume or pressure and withdraw the catheter with rotation. Perform this procedure a total of three times with rotation to ensure complete and reproducible removal of the endothelial lining and distension of the vessel wall (see **Note 35**).
18. Now for the removal of the catheter and closure of the arteriotomy site. Hold a 7S forcep in each hand and grab the ends of the loosely tied proximal suture on the external carotid artery (#2 in Figs. 3, 4). These will be pulled tight to tie the suture and to close off the arteriotomy following removal of the catheter. With the inflated balloon near the arteriotomy hole on the external carotid and the ends of the suture held in the forcep tips, quickly deflate the balloon, remove the catheter from the vessel, and tie the suture to close the arteriotomy hole (see **Note 36**). Place the catheter aside and tie this suture again. Quickly clean up pooled blood and inspect the surgical site for arterial bleeding (see **Note 37**). If the internal carotid artery is clamped or retracted, release it to restore blood flow. Again, check for any leaks. Release the retracted distal suture on the external carotid artery but cutting the suture (keep the knot tied), and remove the suture still looped on the common carotid artery. Make sure the common carotid artery is patent and pulsatile (tent it up) with luminal blood flow. Check for any other bleeding or unusual happenings (lack of pulsatility, no blood flow, apparent thrombosis, etc.). Add topical lidocaine and check for any leaks.
19. Swab away the lidocaine and any pooled blood and remove dried blood from the muscles, skin, and all body areas. Make sure all tissues are kept moist.
20. Remove all clamps and excess suture if still present. Place overlying tissues on top of the carotid vasculature in layers.
21. Close glandular tissue using 6.0 blue monofilament sutures and a running (continuous) suture (a.k.a., “mattress stitch”). Be sure to double-knot and tuck both ends.
22. Tent up the skin for the folds to meet and close the skin using either skin sutures or standard rodent wound staples and skin stapler. If using skin staples keep them closely approximated from end to end (see **Note 15**). Following skin closure, inspect the wound and check for any openings and correct if needed.
23. Swab an anti-septic/bactericide/virucide agent on all sides of wound (see **Note 38**) to reduce likelihood of infection.
24. Inject 3-5ml supplemental fluids to amend for fluid loss, swab ophthalmic ointment over eyes, moisten mouth and tongue (see **Note 2**), and provide an appropriate analgesic for the animal according to institutional guidelines (see **Note 16**). Continue with post-operative animal care described below.

3.4. Post-operative Procedures

1. Once adequate steps are taken to ensure animal comfort (see **Notes 2, 4**), return the animal to his cage and place the animal prone on suitable bedding. Avoid using corncob bedding as this can get lodged in the wound area and be a source of discomfort or infection. It is recommended to use sterile gauze pads directly below the wound to maintain a degree of cleanliness during recovery. Tuck the hind legs under the body

for support, prop-up the upper thorax of the animal using rolled-up surgical blankets or towels, and tuck the front legs under the neck/head (see **Note 17**). This aids breathing during recovery. Remember to keep the animal prone throughout recovery until ambulatory. It has been recommended to the author that placement of a heating blanket (set at a low to medium temperature) or a microwaveable heating pad inside the animal cage during recovery aids the animal during recovery (see **Note 11**). Also, during this time it is important to monitor breathing rate and rhythm. If these become labored or slow, supplemental air or oxygen via a nasal cannula should be used.

2. Monitor the animal routinely during recovery until sternally recumbent and ambulatory. Make sure to keep the eyes, mouth, and tongue moist. Inspect the wound to make sure there is not bleeding or that it does not become dehiscent and open for infection.
3. Once animal is ambulatory, provide sufficient water to drink and food and return to animal care facility. A symptom commonly demonstrated by balloon-injured rats is partial ptosis of the left eye due to nerve manipulation associated with the surgery and ensuing malfunction of the eyelid elevator muscles.
4. Carefully remove the trocar from the balloon catheter sheath and thoroughly clean. This can be sterilized as well. Heparin is suggested to be used on the trocar to prevent formation of blood clots within the trocar lumen.
5. Thoroughly but carefully clean the balloon catheter, especially making sure to remove all clotted blood that might be around the balloon-catheter junction.
6. It is very important to check the degree of balloon inflation after every surgery to make sure that the balloon inflated the appropriate extent during the operation. With any given ballooning procedure, the balloon can become distended or otherwise lopsided or even perforated, so it is imperative to make sure the balloon is still able to inflate to the expected volume or pressure. Return the balloon tip to an aliquot of sterilized water for storage (see **Note 39**).
7. Collect all instruments in a stainless steel surgical pan, add surgical instrument cleaner or detergent, thoroughly clean each instrument (see **Note 40**), and dry.
8. Clean and sterilize the entire surgery area, and swab the area with 70% alcohol.
9. Specific to the research design, plan next series of experiments accordingly to obtain relevant vascular tissues for use in histology, expression analyses, or other endpoints as described in Chapter ___ of this book series. Several examples of photomicrographs from rat uninjured or balloon-injured carotid arteries are presented in Figures 6 and 7.

3.5. Animal Recovery

It is mentioned here for the investigator to be especially aware of the common signs of animal morbidity during the recovery period which can serve as indications for early euthanasia. General guidelines include immobility, huddled posture, inability to eat and/or drink, ruffled fur, self-mutilation, vocalization, wound dehiscence, hypothermia, and/or greater than 20% weight loss.

4. Notes

Note 1

A primary caveat of the rat carotid artery balloon injury model is that this intervention uses a normal eutrophic blood vessel lacking pre-existing atherogenic or vasoproliferative pathology.

This is in sharp contrast to clinical balloon angioplasty procedures performed on diseased vasculature in humans. Although the response of healthy vessels to balloon intervention involves many of the same cellular and molecular signals that are involved during the response in diseased vessels, the investigator must be aware that these are independent processes and should not be confused. Vascular SMCs are virtually completely responsible for the adaptive response to injury in the rat, whereas in diseased human vasculature a mixed population of vascular SMCs and endothelial cells, macrophages, and T-cells interact in response to the inimical stimulus. Anatomical constraints of this model include lower percentage of medial wall elastin, a condensed subintimal layer, and lack of an existing vasa vasorum (15). Studies using this model often report success in minimizing the extent of arterial remodeling using varied treatments that are not replicated in human studies, thus suggesting that this model may be a “ubiquitous responder” to wide-ranging experimental regimens. However, despite these inherent limitations this experimental approach remains a valuable tool with which to study many of the diverse mechanisms involved in the injury response.

Note 2

In this method, several steps are taken by the investigator to ensure that appropriate animal care is provided and that the animal's welfare is considered seriously. In this regard, provision of ophthalmic solution for the eyes and water for the mouth and tongue is performed before surgery as well as during and following surgery to prevent drying of these tissues and discomfort to the animal.

Note 3

An appropriate anesthetic should be chosen according to the specifics of the research project and in accordance with institutional guidelines for the care and use of animals. The anesthetic of choice should be placed adjacent to the surgical area along with a syringe and needle with an appropriate supplemental dose (10% original dose) already withdrawn in the syringe and ready to inject if needed.

Note 4

Provision of supplemental fluids is essential during the surgery as the animals become dehydrated under anesthesia. Generally and unless significant blood loss occurs, 8-10 ml fluid per animal is provided via subcutaneous injection (using an 18-20 g. needle and a 3-5 ml syringe). If substantial blood loss occurs during surgery, then additional fluids should be provided as well as supplemental nutrients (Lactated Ringers solution is suggested). Supplemental fluids can be given immediately prior to performing the surgery as well as through the duration of the surgery and during the recovery period.

Note 5

One can also simply use long pieces of adhesive tape to hold down the rat appendages. It is suggested for the surgeon to loosely tie-down both arms and legs as well as to prepare a loop of suture (size 0, chromic gut) attached to a piece of tape and to use this to gently retract and hold down the head of the animal by looping around teeth. It is not recommended to restrain the tail of the animal as the rat uses the tail to regulate body temperature as well as the fact that the tail often flinches if the level of sedation becomes too light. The surgeon should regularly monitor tail movement as an indication that supplemental anesthesia might be needed. Keeping the tail unrestrained also allows easy access to the tail vasculature if the surgeon desires intravenous or intra-arterial intervention.

Note 6

The author uses topical warm lidocaine HCl (0.8 g to 40 ml PBS) to provide mild anesthesia to the exposed tissues, to decrease incidence of muscular spasm of the carotid vasculature, and to provide a moderate degree of vasodilation to simplify insertion of the catheter through the arteriotomy incision.

Note 7

Insertion of the balloon catheter into the arteriotomy incision on the external carotid artery is simplified if the surgeon uses an introducing guiding needle as a trocar. Prior to surgery the balloon tip must be advanced through the trocar and the catheter with trocar should be readily available during surgery. The trocar allows precise insertion of the balloon tip into the arteriotomy hole without interference caused by the bulbous head of the balloon. The author recommends using a trocar suitable for 2 French catheters, namely 18 g. thin-walled or ultra-thin walled needles. Caution must be practiced, however, when using a trocar such as a thin-walled needle. Immediately upon insertion of the needle bevel into the arteriotomy, carefully lower the luer-lock end of the needle in order to slightly raise the bevel tip. Advance the trocar bevel slightly more into the lumen and proceed with insertion of the balloon catheter through the trocar. Once the balloon catheter is inserted and advanced to the clamp on the common carotid artery, gently slide the trocar down the catheter sheath towards the syringe and out of the way. If during balloon advancement the balloon tip does not enter the artery lumen but instead moves alongside the vessel (and therefore out of the arteriotomy), withdraw it slightly and gently advance and rotate the trocar bevel in the direction that the balloon tip is moving. This will help guide the balloon into the artery lumen and keep it from moving into the arteriotomy at an angle. One other note regarding use of a trocar necessitates mention here. The tip of the trocar needle is extremely sharp and will easily penetrate the backside of the vessel ("backwall") if the trocar is inserted at too sharp of an angle. If this happens, the catheter will still be able to be advanced through the trocar; however, the balloon will not be inserted into the artery lumen and will usually be located underneath or adjacent to the common carotid artery. Remove the balloon catheter and the trocar and re-attempt insertion of the trocar into the arteriotomy hole and into the lumen. Once a "backwall" has happened, effort must be made to keep the catheter from going in that same direction upon repeated attempts.

Note 8

A regulated manual (or automatic) inflation pump can be used to achieve consistent and reproducible inflation of the balloon at a given pressure (atmosphere). This is a good measure to use for calibration of the balloon and for verification of the extent of inflation. The surgeon must be aware, however, of the limitations and practical difficulties of using an inflation device during surgery. It is recommended that if an inflation device is to be used during surgery, that a separate operator performs the inflation of the balloon while the surgeon performs manipulation of the catheter inside the vessel along with withdrawal of the inflated balloon to induce injury.

Note 9

Prior to placing suture sections around various sites on the carotid artery vasculature, it expedites the process for the surgeon to have cut several lengths of suture (4-0 black braided silk, 6-0 Prolene blue monofilament) ahead of time (during the setup) and to have these readily available. Approximately 3" sections of both silk and blue monofilament sutures as well as 5-6" lengths of silk suture will be needed.

Note 10

It is highly recommended that the surgeon use several independent sources of light or multi-directional fiber-optic lighting during the surgery in order to reduce incidence of shadows and to enhance visualization of the carotid vasculature.

Note 11

A temperature-regulated (via anal probe) heating pad is the best choice to use during the surgery to avoid hyperthermia for the animal. If heating lamps are used, make sure to precisely control the temperature of the animal to avoid hyperthermia. It can be difficult to adequately control the temperature of the animal when using heat lamps.

Note 12

A broad range of surgical tools can be used with complete confidence for a successful surgery; therefore, specific item numbers or manufacturing information is included for a majority of these items yet these are simply recommendations made by the author. The most important point concerning the surgical instruments is that the surgeon be comfortable and at ease with their use.

Note 13

For scissors to be used to cut the skin, the author recommends using large straight, sharp-blunt scissors with one serrated edge on the sharp side. When cutting through the skin, use the sharp serrated side of the scissors to penetrate the skin, and remember to keep the tip up during the skin incision to avoid penetrating underlying tissue.

Note 14

Pertinent to all retraction procedures performed in this method, the surgeon can choose between using a surgical retractor (commercially available) and using sutures to keep skin and/or tissues out of the way. The use of surgical retractors is straight-forward. If the operator wishes to use suture, it is recommended to use 6-0 suture with suture needle attached. Once the needle has been passed through the tissue to be retracted, gently tie the suture thread, retract the suture away from the animal and clamp the suture thread taut with a hemostat or tape it down to the operating table out of the way of the surgeon.

Note 15

A skin staple remover is highly recommended to be available to the surgeon during post-operative closing procedures if the surgeon chooses to use skin staples to close the skin (instead of skin suture). A common occurrence during this procedure is the placement of unapposed skin staples that creates gaps between the skin folds and openings of the underlying tissues. This can lead to dehiscence and infection in the animal. Use of a manual skin staple remover makes removal of incorrectly placed staples simple and straight-forward.

Note 16

The author recommends use of an appropriate analgesic post-operatively if this does not interfere with the investigators research study and if it abides by the local institutional animal care and use guidelines. Buprenorphine is the analgesic of choice of the author for use in rats (0.1-0.5 mg/kg SC immediately following surgery and then every 12 hours as needed, or according to institutional policy).

Note 17

The author uses rolled-up surgical drapes or towels propped under the thorax of the animal during recovery to keep animal upright (~30° angle) in an effort to ease breathing until consciousness is attained.

Note 18

The primary example of an emergency that can occur in this protocol is unexpected rupture of the carotid artery or accidental removal (slippage) of the balloon catheter from the artery. Items to keep readily available for such an occurrence include arterial clips or clamps of various sizes, large and small retractors, extra sections of tape and suture, and additional gauze and plenty of cotton tipped applicators. Also, extra surgical tools (in case one drops and breaks) and extra balloon catheters along with trocar(s) should be available. The more one is prepared, the better chance of dealing with an unexpected situation successfully and the better prognosis for the animal in case of emergency.

Note 19

When removing air bubbles from the stopcock and catheter end, the author recommends using a broken wooden stick from the cotton swabs to “sweep out” any lodged air bubbles. Otherwise, shaking these items will inadvertently remove the water and the surgeon will have to start over again. It is essential to create a closed system here with water filling the syringe and stopcock. The closed system will also include the lumen of the balloon catheter which will similarly be filled with water.

Note 20

For sterilizing surgical tools, the author recommends using a glass bead sterilizer. This apparatus should be situated nearby and needs to be pre-heated for 30 minutes. The tips or other operating part of the surgical tool needs to be immersed in the glass beads for 10-15 seconds (longer times can eventually damage the instrument), after which the tool should be placed on a sterile surgical blanket at an appropriate and convenient location in the surgical area. Keep in mind not to touch anything with the sterilized portion of each surgical tool after sterilization. In the case of surgical forceps (especially Pattern 7S), never lay them down on their tips! Always lay forceps with tips up to maintain sterility and to keep from damaging the tips.

Note 21

When using the clippers to remove hair, be careful as the neck area is very pliable and will depress with minimal pressure thus causing difficulty in breathing for the animal. At the sternum, it is easy for the clippers to nick the skin and to cause minor cuts that could bleed and cause discomfort to the animal. It might be better to use scissors for hair removal near the chest area.

Note 22

Do not be tempted to use a scalpel to perform the neck incision. Even with the use of brand new and sharp scalpels one must exert sufficient pressure on the blade in order to make the cut (this tissue is very pliable). This will depress the trachea and impede the ability of the animal to breathe and should not be attempted.

Note 23

These methods are for left carotid artery injury; however, injury can be performed on the right carotid artery just as well. The left carotid artery presents a longer section for intervention than

does the right carotid and is the choice for this author and many other investigators. If one chooses the right carotid artery for injury, remember that the right carotid artery branches off the innominate (brachiocephalic) artery after bifurcation from the aortic root, hence shortening the available section for injury. The innominate artery is not suitable for inclusion in the injury protocol due to larger caliber compared to the right common carotid.

Note 24

If the surgeon is using instruments in his or her right hand and performing this surgery on the left carotid artery, then caution must be practiced to ensure that the surgical instruments in use are not impinging upon the trachea and causing difficulty in breathing for the animal. This inadvertent act is easy to do when focusing on blunt dissection and the carotid vasculature. Be aware of the breathing rate and rhythm of the animal and the location of the surgical instrument (s) during this and all steps of the surgery.

Note 25

As one moves proximally along the common carotid artery towards the sternum, the vagus nerve travels alongside the artery in parallel fashion. However, in many animals just before the carotid artery moves under the rib cage the vagus nerve moves across the artery in a medial direction where it continues to travel parallel to the artery. This creates inconvenience for the surgeon who must avoid contact with and manipulation of the vagus nerve. Additionally, oftentimes a nerve plexus exists overlying the carotid vasculature which should not be manipulated or cut. This is especially imperative at the carotid bifurcation, a site for blood pressure control. If necessary, these vital nerve components should be carefully moved aside and out of the way of the carotid artery. Do not attempt to cut these essential nerves.

Note 26

Considerable variation can exist in the exact anatomical location of the common carotid artery bifurcation. In most animals the branch point for the internal and external carotid arteries occurs on the distal common carotid at a site that provides easy access to the external branch for surgical intervention. However, in some animals the bifurcation occurs more distally towards the head, thus making a shorter segment on the external branch for vascular access. Make a note for each animal as to the exact location on the common carotid artery the bifurcation is located (i.e., 5 mm from hyoid cartilage, 15 mm from sternum, etc.) using a ruler or micro-caliper.

Note 27

Hemostasis in the main carotid artery should be induced and all retrograde blood flows controlled before insertion of the catheter into the lumen of the carotid artery. If not, the surgery can still proceed but there will be bleeding once the arteriotomy is made. This can lead to dramatic blood loss and difficulty in visualization of the surgical area. It is highly recommended by the author that temporary cessation of blood flow (via retraction and/or clamping) takes place as an essential component of this method. If blood flow is halted for a few moments before making the arteriotomy incision and entry of the catheter, then once the arteriotomy hole is made it will be clearly seen and insertion of the catheter will be greatly simplified. Once the catheter is inserted into the arteriotomy and advanced a bit, then the retractors and/or clamps can be released. This will restore blood flow only partially though because the catheter will now be inside the vessel lumen which will halt most blood flow, even in an un-inflated state (some leaking is still expected with an un-inflated balloon).

Note 28

In this step, all adjacent tissues must have been separated from the external carotid artery as distally up the vasculature as possible towards the head. This will create a workable length of the external carotid branch thus providing a longer section with which to make the arteriotomy hole and to insert the balloon catheter. If a problem exists with the first arteriotomy incision (which is made as distally as possible), then that site will be closed and the surgeon will move proximally if there exists a sufficient length of external carotid artery branch remaining with which to work. However, caution must be practiced if the arteriotomy hole is made too close to the most distal suture knot (#3 in Figs. 3, 4). In that case, the suture knot itself could impede insertion of the balloon tip, and the surgeon may have to make a second arteriotomy at a more proximal site.

Note 29

Up to this point no vascular intervention or blood flow alteration has occurred; yet once these small vessels are ligated then the surgeon must work as quickly but in as scientifically and medically sound fashion as possible. This is a critical time during the surgery, and prolonged time under blood flow cessation may cause undesired side-effects. From the time when the first vessel is ligated through when the catheter is removed and the arteriotomy incision is closed is the most critical and significant portion of the entire method.

Note 30

As mentioned before, retraction and stoppage of blood flow can take place either through the use of an arterial clamp or with a suture looped around the vessel and retracted. For the common carotid artery, the author recommends using an arterial clamp to ensure that all blood flow is stopped through this vessel. If suture is used here, it must be significantly retracted to a large degree in order to stop blood flow and the normal geometry of the carotid artery is dramatically perturbed; therefore, the author recommends against using suture to halt common carotid artery blood flow in this step.

Note 31

It is recommended to keep the suture in place here even though an arterial clamp has been placed on the common carotid artery to stop blood flow. The reason being is that this clamp will be removed during the balloon injury, and if there occurs a major arterial leak from any number of reasons (accidental removal of the balloon tip during the catheter withdrawal procedure being a primary example), then this suture is already looped around the common carotid artery and can easily and quickly be retracted to stop common carotid artery blood flow. However, if during advancement of the balloon catheter the balloon tip gets “caught” or “hung-up” on the suture that is looped around the common carotid artery and therefore cannot be advanced, then either gently loosen that suture or remove it all together. If removed, be aware that if a carotid artery leak should occur then hemostasis will be more difficult without that suture already in place.

Note 32

If the arteriotomy incision is made too deep or if the entire vessel is cut through, don't panic! Since this initial arteriotomy site was far up the external carotid artery on the most distal portion of that vessel (hopefully), ample length of external carotid artery should still exist to try this again. But first, if the vessel is cut deeply but is still intact, try to perform insertion of the balloon catheter and the balloon injury as normal but be especially prudent of the amount of pressure applied to the vessel by the catheter. If too much pressure is applied, then this will surely cause the arteriotomy incision to expand and the vessel to break. If the vessel breaks (either from the initial arteriotomy attempt or from too much pressure from the catheter), the

surgeon has several options. If the catheter was already inserted into the artery, then the balloon injury itself could still take place if the external artery tissue is held fast by forceps or hemostats. This is often too difficult to perform and can lead to tears and more breaks in the artery. Alternatively and the preferred choice of the author, the surgeon can attach the broken proximal portion of the external artery to the broken distal portion via sutures and pull these two sections together. Joining the two broken portions of the external artery will then provide additional vessel length with which to attempt another arteriotomy incision, this time proximal to the initial cut and to the re-attached vessel, and will also importantly restore normal vascular geometry. Remember, the entire external carotid artery will be ligated after injury, so this does not cause further complication to the animal or to the scientific integrity of the experiment.

Note 33

Another alternative for the surgeon if the vessel breaks is to gently grab the lip of the proximal section with fine forceps (7S), and then to try to insert the catheter into the vessel by holding or tenting it up. This is similar to putting on a sock and using only two fingers on one hand! Generally, once the catheter is inserted into the common carotid, it will advance without further complication.

Note 34

The author recommends manual withdrawal of the balloon catheter with rotation as this is simpler to perform than when using forceps. Rotation of the balloon catheter is essential in order to ensure concentric injury and to maintain consistency in the injury along a cross-section of the vessel. This is especially important if the balloon does not inflate perfectly round but instead becomes lop-sided inside the blood vessel. An alternative to full rotation of the balloon catheter is to partially rotate the catheter using a side-to-side motion.

Note 35

The degree of balloon inflation will directly determine the extent of vascular injury and ensuing cellular and molecular mechanisms responsible for neointima development and medial wall remodeling (16); therefore, maintaining consistency and reproducibility of balloon inflation within and between animals is essential.

Note 36

For removal of the deflated balloon catheter and closure of the arteriotomy site, it is recommended that the surgeon use an assistant. If the surgeon performs this technique alone, then successful removal of the catheter can be achieved without significant blood loss by holding the catheter (near the syringe) in his or her mouth and gently moving their head back to remove the catheter from the vessel. The surgeon should try to maintain their head at the same level as that of the animal. Once the catheter is removed, quickly pull back on the forceps in each hand to tie the suture and close the arteriotomy hole. This approach was devised by the author and is used routinely in his laboratory with much success.

Note 37

Visualization of an arterial bleed is made easier by adding lidocaine to the vasculature. This will serve to dilate the artery and will enhance blood flow thus augmenting existing leaks in the vessels. Generally speaking, if leaks are present they are usually located around the arteriotomy incision and are due to inadequate closure of that suture. If this is the case, tie another suture around the arteriotomy at that site (#2 in Figs. 3, 4) to stop the bleeding.

Note 38

Do not to apply the topical anti-septic/bactericide/virucide agent directly on top of the wound incision but instead spread it all around the periphery of the wound using cotton tipped applicators.

Note 39

Following surgery and cleaning of the balloon catheter, always store the balloon tip in sterilized water or other suitable liquid medium. If not, the balloon will quickly desiccate and will not be able to be used again. With proper care, a single balloon can be used repeatedly for these animal surgeries as long as the balloon remains intact and not perforated, inflates the appropriate degree, and is completely circular or concentric during inflation. An estimate by the author for use of a single balloon catheter is approximately 15 surgeries without complications.

Note 40

When cleaning the surgical tools, remember to open all scissors and hemostats and to clean both the inside and outside edges of the blades. Open arterial clamps and clean their insides as well. Be very careful with the instruments during cleaning, as this is a time when most accidents occur that can ruin expensive tools!

5. Conclusions

The rat carotid artery balloon injury model described in these methods presents a practical and highly useful *in vivo* animal model that mimics many biophysical, cellular, chemical, and molecular mechanisms found in humans with injured and/or diseased vasculature; however, knowledge of the caveats and limitations of this model is imperative for successful completion of such studies, scientifically valid interpretation of results, and legitimate comparisons to the human condition. Described herein are peri-operative protocols for the rat balloon injury model that have been used over many years with great success; however, modification of these methods may be required for individual needs and/or based on individual experiences. Histological and morphometric methods pertinent to the rat carotid artery balloon injury model and other animal vascular injury models are described in Chapter __ of this series and should be consulted prior to designing such research studies.

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7. References

1. Clowes AW, Reidy MA, Clowes MM. Mechanisms of stenosis after arterial injury. *Lab. Invest* 1983;49:208–215. [PubMed: 6876748]
2. Clowes AW, Reidy MA, Clowes MM. Kinetics of cellular proliferation after arterial injury. I. Smooth muscle growth in the absence of endothelium. *Lab. Invest* 1983;49:327–333. [PubMed: 6887785]
3. Clowes AW, Clowes MM. Kinetics of cellular proliferation after arterial injury. II. Inhibition of smooth muscle growth by heparin. *Lab. Invest* 1985;52:611–616. [PubMed: 4040189]
4. Clowes AW, Clowes MM, Reidy MA. Kinetics of cellular proliferation after arterial injury. III. Endothelial and smooth muscle growth in chronically denuded vessels. *Lab. Invest* 1986;54:295–303. [PubMed: 3512908]
5. Clowes AW, Reidy MA. Prevention of stenosis after vascular reconstruction: Pharmacologic control of intimal hyperplasia – A review. *J. Vasc. Surg* 1991;13:885–891. [PubMed: 2038110]
6. Majesky MW. Neointima formation after acute vascular injury. *Texas Heart J* 1994;21:78–85.

7. Schwartz SM, deBlois D, O'Brien ERM. The intima: soil for atherosclerosis and restenosis. *Circ. Res* 1995;77:445–465. [PubMed: 7641318]
8. Zubilewicz T, Wronski J, Bourriez A, Terlecki P, Guinault A-M, Muscatelli-Groux B, Michalak J, Melliere D, Becquemin JP, Allaire E. Injury in vascular surgery – the intimal hyperplastic response. *Med. Sci. Monit* 2001;7:316–324. [PubMed: 11257743]
9. Sapru HN, Krieger AJ. Carotid and aortic chemoreceptor function in the rat. *J. Appl. Physiol. (Respirat. Environ. Exercise Physiol.)* 1997;42:344–348.
10. Gabeler EEE, van Hillegersberg R, Statius van Eps RG, Sluiter W, Gussenhoven EJ, Mulder P, van Urk H. A comparison of balloon injury models of endovascular lesions in rat arteries. *BMC Cardiovasc. Disord* 2002;2:16–28. [PubMed: 12350231]
11. Majesky MW, Schwartz SM, Clowes MM, Clowes AW. Heparin regulates smooth muscle S phase entry in the injured rat carotid artery. *Circ. Res* 1987;61:296–300. [PubMed: 3621492]
12. West JL, Hubbell JA. Separation of the arterial wall from blood contact using hydrogel barriers reduces intimal thickening after balloon injury in the rat: the roles of medial and luminal factors in arterial healing. *Proc. Natl. Acad. Sci. USA* 1996;93:13188–13193. [PubMed: 8917566]
13. Wallner K, Sharifi BG, Shah PK, Noguchi S, DeLeon H, Wilcox JN. Adventitial remodeling after angioplasty is associated with expression of tenascin mRNA by adventitial myofibroblasts. *J. Am. Coll. Cardiol* 2001;37:655–661. [PubMed: 11216993]
14. Li G, Chen S-J, Oparil S, Chen Y-F, Thompson JA. Direct in vivo evidence demonstrating neointimal migration of adventitial fibroblasts after balloon injury of rat carotid arteries. *Circulation* 2000;101:1362–1365. [PubMed: 10736277]
15. Sims FH. A comparison of structural features of the walls of coronary arteries from 10 different species. *Pathology* 1989;21:115–124. [PubMed: 2682490]
16. Indolfi C, Esposito G, Di Lorenzo E, Rapacciuolo A, Feliciello A, Porcellini A, Avvedimento VE, Condorelli M, Chiariello M. Smooth muscle cell proliferation is proportional to the degree of balloon injury in a rat model of angioplasty. *Circulation* 1995;92:1230–1235. [PubMed: 7648670]

Figure 1. Photograph of an arterial balloon embolectomy catheter attached to a water-filled 1.0 ml syringe via a stopcock. Also shown is an 18 g. thin-walled needle that can be used as a trocar and a standardized inflation device for the balloon catheter.

Figure 2.

Typical pre-operative surgical setup for rat carotid artery balloon injury studies. Suggested sites for convenient placement of all items needed as well as those potentially needed for successful completion of the surgery are included and depicted for a left-handed surgeon. It is imperative that comprehensive pre-operative procedures be performed in this method including preparation and calibration of the balloon catheter, cutting appropriate lengths of suture and tape, sterilizing all necessary surgical tools, preparation of all solutions and reagents, and proper well-situated location of these items for the surgery proper.

Figure 3.

Simplified diagram of the rat carotid artery vascular anatomy along with sites for placement of arterial clamp(s), suture, and ligations (9). Proximal and distal anatomical locations are indicated, as well as numerical steps identified in the protocol. The arteriotomy site for insertion of the balloon catheter is on the external carotid artery branch between the bifurcation of the common carotid artery and the site of distal ligation and retraction. Abbreviations: LCC: left common carotid artery; EC: external carotid artery; IC: internal carotid artery.

Figure 4.

Photograph of the surgical site on the ventral aspect of the neck of a rat undergoing carotid artery balloon injury. At this point in the procedure, skin and underlying muscles have been retracted and sutures have been carefully placed around the most accessible proximal site on the left common carotid artery (#1) and looped but not tied around the external carotid artery branch immediately distal to the bifurcation (#2) and as distally as possible (#3). The suture at (#2) will be tied immediately upon removal of the balloon catheter following surgery, while the suture at (#3) will be tied and used to retract the carotid vasculature prior to performing the arteriotomy. These numbers correspond to those included in Figure 3. The internal carotid artery branch was not accessed in this animal and therefore is not indicated. Also shown are sites for the arteriotomy on the external carotid branch (arrowhead) and the un-ligated superior thyroid artery (*) which will be tied off prior to surgery.

Figure 5. Photograph of the rat left carotid artery vasculature during balloon injury. Illustrated is the distal end of a balloon catheter inserted into an arteriotomy incision on the external carotid artery, with a section of suture wrapped around the catheter and an arrowhead indicating the first black band from the balloon tip on the catheter. The suture will be tied following injury and removal of the balloon catheter.

Figure 6. Cross-sectional photomicrographs of rat uninjured (**A**) or balloon-injured (**B**) carotid arteries perfusion-fixed and treated with Verhoeff's elastin stain and Van Gieson counterstain. Injured arteries demonstrate robust and concentric neointima with luminal stenosis 2 weeks post-injury. Magnification for photos is 100X.

Figure 7. Detailed photomicrographs of rat uninjured (A) or balloon-injured (B) carotid artery sections demonstrating a matrix-rich neointima. Specifics of histological protocols and morphometric analyses germane to the rat carotid artery balloon injury model are discussed in Chapter __ of this book series. Magnification for photos is 400X.