Experimental and Molecular Pathology 91 (2011) 590–595

Contents lists available at ScienceDirect

Experimental and Molecular Pathology

journal homepage: www.elsevier.com/locate/yexmp

Angiographic evaluation of the rat carotid balloon injury model

Ludwig D. Orozco [a,*], Huiling Liu [a], Betty B. Chen [a], Jonathan D. Fratkin [b], Eddie Perkins [a,c]

[a] University of Mississippi Medical Center, Department of Neurosurgery, 2500 N State Street Jackson, MS 39216, USA
[b] University of Mississippi Medical Center, Department of Pathology, 2500 N State Street Jackson, MS 39216, USA
[c] University of Mississippi Medical Center, Department of Anatomy, 2500 N State Street Jackson, MS 39216, USA

ARTICLE INFO

Article history:
Received 25 May 2011
Available online 23 July 2011

Keywords:
Angiography
Balloon-injury
Carotid
Method
Rat

ABSTRACT

Rationale: The rat carotid balloon-injury (BI) model is a widely used model of intimal hyperplasia (IH) and vascular remodeling. A variable degree of IH after BI has been previously reported, and we have encountered technical challenges and suboptimal results with the original method.
Objective: To evaluate the original rat carotid artery BI method with the use of micro-angiography. We tested the hypothesis that in order to obtain an optimal arterial response, BI should be limited to the common carotid artery with preservation of blood flow.
Methods and results: The left common carotid artery (CCA) was injured by one of three different methods. Carotid angiograms and pathology were examined 14 days after BI.
A 2 F Fogarty balloon catheter inflated to 2 atm inside the aortic arch would not slide back into the common carotid artery until deflation to 0.5 to 0.7 atm. Four out of five (80%) vessels injured with this method developed excessive inflammation without discernible IH. Six out of nine (66%) arteries that underwent BI limited to the CCA at 2 atm developed the largest angiographic stenosis ($p = 0.003$) and IH (0.20 ± 0.03 mm^2, $p = 0.028$). Ten out of eleven (91%) arteries injured with a variable pressure of 1.5 to 2.2 atm, based on the operator's feedback, developed considerable IH (0.12 ± 0.02 mm^2). All injured carotid arteries with preserved blood flow on angiography developed IH with intact histological boundaries.
Conclusions: Optimal IH with preservation of histological boundaries is achieved by graded BI limited to the CCA that preserves carotid blood flow.

Introduction

Investigation into the response of blood vessels to injury is of pivotal importance in understanding the pathophysiology of vascular disorders. It is acknowledged that blood vessels respond to intraluminal injury by neointima formation, also referred as intimal hyperplasia (IH), and vascular remodeling. This often occurs after balloon angioplasty and is also observed in hypertension, renal and radiation injuries (Schwartz et al., 1990). IH has been viewed as an initial step in the development of atherosclerosis and restenosis following angioplasty and stenting (Indolfi et al., 1995; Koletsky and Snajdar, 1981).

Experimental models in various animal species have been used to study the pathological intimal formation and vessel remodeling as a response of vessel wall damage in a cumulative effort to relate these findings to the human condition. In this context, the rat carotid artery balloon-injury (BI) is one of the most convenient, rapid and thoroughly investigated models for the assessment of the cellular and molecular mechanisms and treatment of IH (Bauters and Isner, 1997; Reidy et al., 1992; Seki et al., 2000).

Although most of the rat studies adopted the standard method described by Clowes and colleagues (1983a, 1983b, 1986), Clowes and Clowes (1985) to produce balloon injury, a variable degree of IH was reported in different studies (Clozel et al., 1993; Daemen et al., 1991; Fingerle et al., 1990; Jawien et al., 1992; Kranzhöfer et al., 1993; Mitsuka et al., 1993; Rakugi et al., 1994). The standard technique produces injury of the carotid artery by passing a 2F Fogarty balloon-catheter, inflated to 2 atm, from the aortic arch to just below the carotid bifurcation, three consecutive times (Clowes and Clowes, 1985; Clowes et al., 1983a, 1983b, 1986; Indolfi et al., 1995; Tulis, 2007). This process denudes the vessel of endothelium and disrupts the elastic lamina and underlying smooth muscle cells (Gabeler et al., 2002). It has been demonstrated that the proliferative response of the injured vessel is related to the degree of injury, specifically the balloon inflation pressure (Indolfi et al., 1995).

The aim of the present study was to evaluate the standard rat carotid artery balloon-injury method with the use of micro-angiography. We found that contrary to the original method, BI cannot be started from the

Abbreviations: Atm, Atmospheres; BI, Balloon-injury; CCA, Common carotid artery; IH, Intimal hyperplasia; I:M, Intima to media; NASCET, North American Symptomatic Carotid Endarterectomy Trial; PACS, Picture archiving and communication system; SMC, Smooth muscle cell.

* Corresponding author at: University of Mississippi Medical Center, Department of Neurosurgery, 2500 N State Street Jackson, MS 39216, USA. Fax: +1 601 984 4733.
E-mail addresses: lorozco-castillo@umc.edu (L.D. Orozco), huliu@umc.edu (H. Liu), bchen@umc.edu (B.B. Chen), jfratkin@umc.edu (J.D. Fratkin), eperkins@umc.edu (E. Perkins).

0014-4800/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.yexmp.2011.06.011

aortic arch. We hypothesize that in order to obtain an optimal arterial response, BI should be limited to the common carotid artery with preservation of blood flow.

Materials and methods

Study design

Twenty-five Sprague–Dawley male rats weighing 340 to 400 g, at 12–14 weeks of age, were obtained from Harlan laboratories (Indianapolis, IN, USA). Animals were assigned into three groups: Aortic arch–common carotid with standard balloon-inflation (group I) (n = 5), common carotid with standard balloon-inflation (group II) (n = 9), and common carotid with variable balloon-inflation (group III) (n = 11).

The animals had free access to rat chow (Harlan laboratories, Indianapolis, IN, USA) and tap water acidified to pH 4.0. They were maintained at constant humidity (60 ± 5%), temperature (24 ± 1 °C) and light cycle (06:00 to 18:00 h). All protocols were approved by the Institutional Animal Care and Use Committee at the University of Mississippi Medical Center and were consistent with the Guide for the care and Use of Laboratory Animals (NIH publication 85-23, revised 1996).

Rat carotid artery balloon injury

Before the study was started, extensive experiments were executed to gain skill in handling the 3 different surgical procedures. All animals were anesthetized with inhalational isoflurane as previously described (Kissin et al., 1983).

A left paramedian neck incision was performed and the skin, sternocleidomastoid and omo/thyrohyoid muscles were retracted with 3–0 vicryl sutures. Then, under the operative microscope, the left common, internal and external carotid arteries were exposed. In order to prevent back-bleeding during arteriotomy and BI, the external carotid artery was ligated with a 5–0 silk suture, 5–6 mm distal to the carotid bifurcation. The proximal common and internal carotid (including the occipital artery) were temporarily clamped with 5–0 silk suture loops. The superior thyroid artery branching off the proximal external carotid artery was coagulated with bipolar electrocautery. Lidocaine (5 mg/kg) was applied to prevent vasospasm. A Fogarty 2F arterial embolectomy catheter (Edwards Lifesciences, Irvine, CA, USA) was introduced into the external carotid artery through an arteriotomy incision and one of the following three BI techniques employed.

Aortic arch–common carotid injury with standard balloon-inflation (group I)

Under fluoroscopic guidance (C-arm, Ziehm Imaging, Nurenberg, Germany) the Fogarty catheter was advanced to the aortic arch and inflated to 2.0 atm (Figs. 1A to D). The balloon would not go back into the common carotid artery until deflation to 0.5–0.7 atm (Figs. 1E to G). Once in the common carotid artery the balloon was inflated back to a pressure of 2 atm. Then the inflated balloon was withdrawn with rotation towards the carotid bifurcation (Fig. 1H). This was repeated three times, going back to the aortic arch each time. Finally, the catheter was withdrawn, and the external carotid ligated.

Common carotid injury with standard balloon-inflation (group II)

Once inside the common carotid artery, the Fogarty catheter was advanced a total of 20 mm, this will keep the catheter above the aortic arch. Occasionally, the catheter meets resistance where the cervical myo-fascial band comes together at the base of the neck (Fig. 1I). This can be overcome with re-orientation of the deflated catheter. Once in an adequate position, the balloon was inflated to a standard pressure of 2.0 atm, and then withdrawn with rotation towards the carotid bifurcation. This was repeated three times, the catheter was withdrawn, and the external carotid ligated.

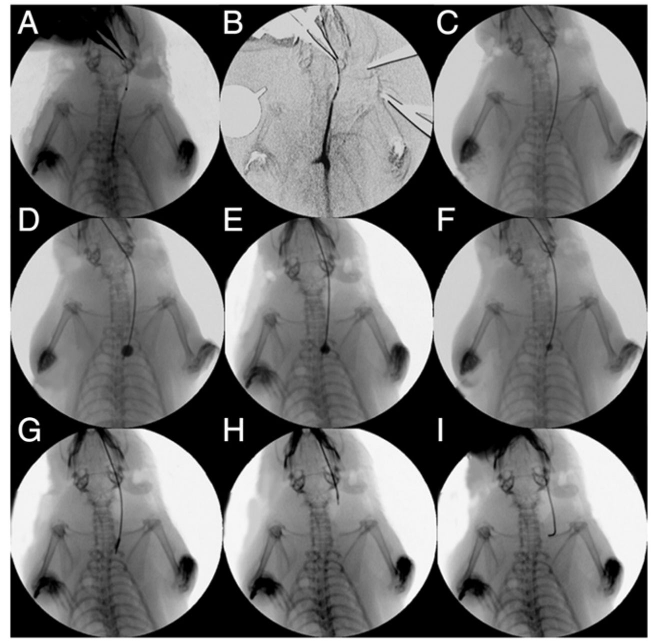

Fig. 1. Antero-posterior (AP) radiographs demonstrate the original balloon-injury technique. Initial left common carotid artery (CCA) angiogram (A) and roadmap (B) show the origin of the CCA at the level of the third rib. A deflated 2F Fogarty balloon catheter is advanced to the aortic arch (C) and inflated to 2 atm (D). The inflated balloon would not pass from the aortic arch into the common carotid artery (E), until deflation to 0.5–0.7 atm (F). Once in the proximal CCA the balloon is inflated to 2 atm (G) and withdrawn by careful traction and rotation toward the carotid bifurcation (H). Occasionally, the catheter will meet resistance where the cervical myo-fascial band comes together at the base of the neck (I). This can be overcome by re-orientation of the balloon catheter.

Common carotid injury with variable balloon-inflation (group III)

This technique was done in similar fashion as the previous one. The only difference is that the balloon was inflated to a variable pressure ranging from 1.5 to 2.2 atm. Balloon pressure was constantly graded, based on the operator's visual and tactile feedback, for the balloon to slide without excessive resistance or vessel distortion/stretching.

Immediately after injury, carotid artery pulsatility and patency were checked, and the degree of balloon inflation validated. The skin was closed in one layer of continuous 2–0 vicryl sutures.

Micro-angiography of injured carotid arteries

Fourteen days after balloon injury, inhalational anesthesia was induced as described above. After a standard laparotomy, the suprarenal aorta was exposed, and hemostatic 5–0 silk suture loops applied above and below the planned arteriotomy. After aortic arteriotomy, an Echelon microcatheter (ev3 Neurovascular, Irvine, CA, USA) with an Agility 10 microwire (Cordis, Miami, FLA, USA) was introduced into the abdominal aorta. Under fluoroscopic guidance, this microcatheter–wire system was navigated into the right brachiocephalic artery first, and to the left common carotid artery second (Figs. 2 to 4). Right and left carotid angiograms were obtained by injecting 0.6–0.8 ml of 50% diluted Omnipaque-300 (General Electric Healthcare, Princeton, NJ, USA). The microcatheter was then removed and the animal euthanized.

Isite picture archiving and communication system (PACS) (Koninklijke Philips, Eindhoven, Netherlands) was used to analyze the carotid angiograms. Under normal circumstances, the common carotid of the