Laser-sealed arteriotomy: a reliable aneurysm model

MATTHEW R. QUIGLEY, M.D., KENNETH HEIFERMAN, M.D., HAU C. KWAAN, M.D., DANKO VIDOVICH, M.D., PETER NORA, AND LEONARD J. CERULLO, M.D.

Division of Neurosurgery, Department of Surgery, and Division of Hematology/Oncology, Department of Medicine, Northwestern University Medical School and Veterans Administration Lakeside Medical Center, Chicago, Illinois

Laser-assisted vascular anastomosis (LAVA) is associated with a significant aneurysm problem when it is applied to small arteries. The etiology of this phenomenon was investigated by creating arteriotomies of different lengths and orientation in the rat carotid artery and sealing them with the milliwatt CO2 laser. It was found that increasing the arteriotomy length from 0.5 to 1.0 mm significantly raised aneurysm occurrence (4/17 vs. 25/28, chi-square: p < 0.001) regardless of orientation. Systemic hypertension (systolic blood pressure > 170 mm Hg) also significantly affected the aneurysm rate among the 0.5-mm arteriotomy group, raising aneurysm occurrence from 23.5% (4/17) to 100% (14/14) (p < 0.001). Assuming that the stay-sutures used for LAVA's act as rigid supports, the rate of aneurysm occurrence must be related to the distance between sutures. This phenomenon has been exploited to create a reliable aneurysm model.

KEY WORDS • aneurysm • hypertension • laser • arteriotomy • rat

Several studies from our laboratory have outlined the vessel histopathology following laser-assisted vascular anastomosis (LAVA) in the rat femoral artery model. The salient histopathological observations from these studies include long-term medial degeneration, the loss of elastic lamina, and the presence of significant intimal proliferation. Recently, we reported a large series of animals followed for at least 1 week after LAVA in which a 30% aneurysm rate was documented. The present investigation was undertaken to determine the histopathological process contributing to this problem, and to develop a reliable aneurysm model.

Materials and Methods

Sprague-Dawley rats, each weighing 250 to 350 gm, were operated on according to the standards of care outlined by our University's Animal Research Committee.

Laser-Sealed Arteriotomy

After induction of anesthesia with intraperitoneal pentobarbital (50 mg/kg), the common carotid arteries were exposed bilaterally. On the right side, an approximator clamp was placed on the vessel and a 0.5-mm or 1.0-mm arteriotomy was made with an iridectomy knife either parallel (longitudinal) or perpendicular (axial) to the long axis of the vessel. The arteries were then irrigated free of blood with heparinized saline (200 IU/ml). Two stay-sutures of 10-0 nylon were placed at either end of the arterial incisions, and traction was placed on each to evert the vessel edges. Laser energy (70 mW, 150-μM spot size) was applied for 0.1 seconds to the vessel edges in multiple bursts, and copious irrigation was employed between applications. The endpoint of laser application was a visible tissue change that we have shown accompanies "welding." The opposite side of the vessel underwent clamping only. The laser-sealed arteriotomy (LSA) wounds were closed in two layers with 3-0 silk sutures, and no antibiotics or systemic anticoagulant agents were administered. Two weeks later, the animals underwent reexploration of their neck wounds, at which time the presence of aneurysms (≥ 50% dilatation of the parent vessel) was noted. The rats were sacrificed with an intracardiac perfusion of Zenker's solution at physiological pressure. Vessels were harvested, embedded in paraffin, cut in 5-μM sections, and stained with hematoxylin and eosin or Movat's solutions.

Terumo, Inc., Chapel Hill, North Carolina.

* Laser, Model 7600, manufactured by Bioquantum, Houston, Texas.
Laser-sealed arteriotomy aneurysm model

Hypertension and Laser-Sealed Arteriotomy

Another group of animals was rendered hypertensive by the method of Hashimoto, et al.\textsuperscript{4} In brief, the rats were subjected to unilateral nephrectomy and were given a 1% saline solution as drinking water. Twice weekly they received subcutaneous injections of deoxycorticosterone acetate at a dose of 2.5 mg/100 gm body weight prepared as a 1.5-gm suspension in sesame seed oil. Blood pressure was determined weekly in the unanesthetized animals by the tail-cuff auto-pickup method, and hypertension was diagnosed when the systolic blood pressure reached 170 mm Hg. The rats were then subjected to a 0.5-mm axial or longitudinal arteriotomy and a laser-sealing procedure as well as contralateral carotid clamping as described above.

The animals underwent reexploration 2 weeks after surgery and were sacrificed with an intracardiac infusion of buffered saline. Harvested vessels were reserved for a subsequent experiment.

Laser Application Only

After unilateral carotid artery exposure, another series of rats underwent arterial clamping. This was followed by evacuation of blood from the occluded segment through a No. 30 needle hole and the removal of blood with heparinized saline. Then, 150 0.1-second applications of laser power (70 mW, 150-μM spot size — the total laser use for an average LAVA) were made evenly in a circumferential manner at a site away from the needle hole. The needle hole was then closed with one 10-0 nylon stitch and the clamp was removed. The wounds were reexplored at 2, 4, and 8 weeks after the procedure, the animals were sacrificed, and specimens were prepared as described above for the LSA animals.

Statistical Analysis

Statistical comparisons employed the chi-square distribution and Fisher’s exact probability techniques.

Results

All groups of animals subjected to arteriotomies exhibited aneurysms. No aneurysms were noted among the vessels that were only clamped, in either the normotensive or hypertensive groups. Among the animals with laser exposure but no arteriotomy, arterial thrombosis was evident in one animal each in the 4- and 8-week groups, but no aneurysms were seen in any vessel examined at 2 weeks (nine rats), at 4 weeks (eight rats), or at 8 weeks (eight rats). The difference in aneurysm occurrence associated with arteriotomy lengths of 0.5 mm and 1.0 mm was statistically significant (p < 0.001) as shown in Table 1. Significant differences (p < 0.001) were also noted when comparing normotensive and hypertensive animals that received a 0.5-mm arteriotomy (Table 1). All differences were also significantly related to orientation (longitudinal vs. axial) of the arteriotomy. The gross appearance of all aneurysms was of the saccular or berry type (Fig. 1).

Histological Findings

The vessel pathology seen in all aneurysm cases was identical to that described previously.\textsuperscript{14,16} The elastic laminae terminated at the aneurysmal neck. The sac itself was composed of myofibroblasts and myointimal cells embedded in a delicate elastin network (Fig. 2). The aneurysmal dome was often buttressed with reactive connective tissue, and the thinnest portion of the aneurysm was found near the neck.

The non-aneurysmal vessels showed gaps of approximately 150 μM in the elastic lamina at the arteriotomy site. Medial architecture was not normal, again consisting of myointimal and myofibroblast cells. Arteries that were subjected to only laser application demonstrated small (50-μ) segments of medial coagulation necrosis with very occasional loss of elastic lamina continuity. This area was eventually repopulated with myofibroblasts, as seen in specimens harvested at 8 weeks. The

\textbf{TABLE 1

Effect of laser-sealed arteriotomy length and hypertension on aneurysm formation

<table>
<thead>
<tr>
<th>Variable</th>
<th>Axial</th>
<th>Longitudinal</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>arteriotomy length</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5 mm</td>
<td>3/11 (27.2)</td>
<td>1/6 (16.7)</td>
<td>4/17 (23.5)</td>
</tr>
<tr>
<td>1.0 mm</td>
<td>19/22 (86.3)</td>
<td>6/6 (100)</td>
<td>25/28 (89.3)</td>
</tr>
<tr>
<td>significance</td>
<td>x² = 9.01, p &lt; 0.05*</td>
<td>x² = 17.2, p &lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>0.5-mm arteriotomy normotensive</td>
<td>3/11 (27.2)</td>
<td>1/6 (16.7)</td>
<td>4/17 (23.5)</td>
</tr>
<tr>
<td>hypertensive†</td>
<td>8/8 (100)</td>
<td>6/6 (100)</td>
<td>14/14 (100)</td>
</tr>
<tr>
<td>significance</td>
<td>p &lt; 0.01*</td>
<td>p &lt; 0.05*</td>
<td>x² = 15.4, p &lt; 0.001</td>
</tr>
</tbody>
</table>

* Fisher’s exact test.
† Systolic blood pressure 170 mm Hg or more.
control vessels (those with clamping only) showed only spotty medial necrosis with no loss of elastic lamina integrity.

Discussion

Our previous study of 150 LAVA procedures revealed: 1) an 18.6% aneurysm occurrence rate overall in the rat femoral artery model; 2) a 30% rate in vessels followed for at least 1 week; and 3) that aneurysm formation occurred within 1 week after the procedure, beyond which time the aneurysm occurrence rate remained static. Stabilization of the aneurysm rate beyond 1 week is explained by a previous study which showed that by 2 weeks the bursting strength of the LAVA site approximated that of suture controls, but was significantly weaker before that time. The current study suggests that the aneurysm occurrence rate in the LAVA model is only 30% because the formation rate is a function of length of arteriotomy welded; aneurysm rates approached 100% with 1.0-mm defects. The mechanism is presumably related to the extent of elastic lamina damage. In the rat femoral artery LAVA model, the stay-sutures acted as rigid supporting structures and therefore shortened the "functional" length of the arteriotomy spanned by the laser weld to approximately 0.5 mm. In an end-to-side LAVA model using rat carotid arteries with four stay-sutures placed more than 1 mm apart, a 100% aneurysm rate was observed. The critical determinant in the rate of aneurysm formation in LAVA procedures appears to be the distance between stay-sutures. Other investigators employing experimental LAVA techniques have also noted aneurysm occurrence rates between 10% and 70%. Of the three, only the last type produces intracranial aneurysms in a clinically relevant anatomical distribution which are histologically similar to the human situation. The difficulty with this third model is that it is limited to rats, in which the vessels are very small and not amenable to manipulation. Our model of LSA produces a reliable aneurysm rate with histologically faithful material in an artery large enough to manipulate and which is easily accessible surgically.

Acknowledgments

We thank Clara Lastre for technical assistance and Bonnie Osborn for preparing the manuscript.

References

Laser-sealed arteriotomy aneurysm model


Manuscript received October 22, 1986.
This research was sponsored in part by a generous gift in memory of Mrs. June Taylor.
Address reprint requests to: Hau C. Kwaan, M.D., 333 East Huron Street, Chicago, Illinois 60611.