Morphometry of Air-Drying-Induced Arteriosclerosis in Rat Carotid Artery

Effect of Air-Flow Rate

Elizabeth van Pelt-Verkuil, Wilfrid van Pelt, and Dick Jense

Arteriosclerosis in the Wistar rat carotid artery was induced by air drying of the endothelium, a procedure that caused media necrosis. We describe a number of technical modifications that facilitate the procedure and minimize damage to the vessel wall (i.e., media necrosis). A morphometric study of vessel wall changes induced by endothelial denudation at various air-flow rates showed that necrosis of the inner medial layer and lesion size and position were constant at all flow rates used (≥ 28 ml/min). The extent of necrosis of the outer medial layers, the endothelial repair, and the shape of the lesions varied with the air-flow rate used. Since at an air-flow rate of 28 ml/min medial necrosis was minimal and myointimal lesion development was as extensive as at higher flow rates, we consider this air-flow rate to be optimal for this model of experimental arteriosclerosis. We hypothesize that the shape of the myointimal lesion is determined by the timespan of endothelial denudation and by the availability of smooth muscle cells in necrotic and normal media. Furthermore, the proliferation of the smooth muscle cells and their migration into the intima is probably dominant over the repopulation of the media by these cells.

(Arteriosclerosis 3:441-451, September/October 1983)

The formation of arteriosclerotic, fibromuscular-elastic intimal thickening following experimental endothelial denudation has been well documented in a number of animal models.1-4 In rat arteries mechanical injury,5 air-drying,6 and balloon catheterization7,8 have been shown to remove the endothelium and to cause myointimal thickening. Although these experiments were aimed at specifically causing endothelial injury, the underlying media was often affected as well.6-10 Fishman et al.6 and Clowes et al.11 used the air-drying technique in the rat and claimed that injury, at least at the time of denudation, was confined to the endothelium. This apparent selectivity led us to choose the air-drying model for a study of the effect of experimental conditions on lesion development.

In our study however, we found that this injury was not as selective as in other studies;6 media necrosis (disappearance of smooth muscle cells) occurred in certain areas of the vessel wall. Furthermore, a different intimal thickening pattern was observed. Preliminary studies indicated that the velocity of infused air was important. To find the optimal conditions, we performed the air-drying method by lowering the air-flow rate in steps of approximately 5 ml/min down to the lower limit of lesion induction. The air-drying technique of Fishman et al.6 was modified, thereby facilitating surgery, avoiding mechanical injury at air inflow and outflow holes, and minimizing media necrosis.

In this paper we shall present a univariate quantitative analysis of the changes in vessel wall morphology that we found when applying diminished air-flow rates to denude the artery. Special attention will be paid to parameters describing the resulting myointimal lesions, endothelial repair, and media necrosis. To describe the interrelations between parameters that related to the vessel wall response to endothelial injury both within and between air-flow rate groups, we performed a multivariate analysis. This analysis, which was based on the data presented here, will be published in a separate paper.
Methods

Surgical Procedure

Male Wistar rats (Centraal Proefdierenbedrijf TNO, Zeist, The Netherlands) weighing 350–400 g were used. Air-drying injury was induced using a modification of Fishman's method and applying different air-flow rates. The rats were anesthetized with Nembutal from Abbott Laboratories (0.1 ml/100 g body weight) and the right or left carotid artery was chosen at random for surgery. Only small areas of the carotid artery were exposed at the distal and proximal ends, giving enough space to place aneurysmal clips (20 g/cm²; Aesculap FD 561 R, Aesculap Werke AG; Tuttingen; West Germany) to arrest the blood flow. We preferred this procedure to ligation, which cause deep mechanical injury, a type of injury that reportedly induced a strong atherosclerotic response in the rat. Clips with pressures of 40 g/cm² or 120 g/cm² caused intimal damage, as shown by endothelial denudation, platelet adherence, fibrinlike immunoreactivity at the subendothelium, and strong Evans blue permeation (Figure 1). In contrast, the clips with a pressure of 20 g/cm² did not cause morphological evidence of injury to endothelial cells or to the underlying media. Since some Evans blue permeated into the media (Figure 1A), minor endothelial injury must have occurred.

Figure 1. Evans blue permeation reveals vessel wall damage on carotid artery by aneurysmal clips of varying types and pressures (A = 20 g/cm²; B = 40 g/cm²; C = 120 g/cm²). P = proximal end of artery; D = distal end of artery. Bar indicates 5 mm.

The same was observed in control experiments using an air-flow rate of 0 ml/min (saline only). These vessels showed no signs of myointimal lesion development at Day 14. The 20 g/cm² clips, inducing the smallest injury to the vessel wall, were selected for our experiments. At the proximal end, a 30-gauge hypodermic needle was inserted. Since local lesion development occurred at the entrance region when the needle was pushed accidentally into the opposite part of the carotid artery, we did not pass the needle through the vessel to make the outflow hole as described elsewhere. Instead, a small hole (0.15 mm) was made at the distal end at the bifurcation (dorsally and ventrally) with a 00-insect needle. Before air-drying we washed away the blood with saline; thereafter, sterile, medical air was allowed to flow through the artery for 4 minutes. Atmospheric pressure (as determined at the cylinder outlet) was maximally 0.4 atm/cm². Flow rates of 58 ml/min (n = 14), 45 ml/min (n = 15), 40 ml/min (n = 4), 36 ml/min (n = 4), 32 ml/min (n = 4), 28 ml/min (n = 15), 24 ml/min (n = 4), 16 ml/min (n = 4), 12 ml/min (n = 4) and 0 ml/min (n = 4) were used. The contralateral carotid artery was used as a control in all animals. We removed the clips after refilling the lumen with saline (the distal one first). Bleeding from the puncture sites was stopped for 10 minutes, by applying light pressure with tampons soaked in saline. The total operation time was about 30 minutes. The skin wound was closed and the animals were housed separately.

Endothelial Denudation

The completeness of the endothelial denudation was confirmed in separate animals at 1, 3, 5, 7, and 24 hours after surgery. We used perfusion silver staining and Evans blue permeation of whole vessels, longitudinal sections, and cross-sections (at 125 μm intervals) along the control artery. The latter were evaluated using the criteria for endothelial denudation described under Morphometry.

Tissue Processing

One-half hour before sacrifice (14 days after surgery), animals were anesthetized with Nembutal. To assess the vessel wall permeability, we injected Evans blue dye (6 mg/100 g body weight). The rats were heparinized (100 U/100 g body weight iv) and perfused via the thoracic aorta with phosphate-buffered saline (PBS) for 2 minutes and then with 100 ml 1.25% 1-ethyl-3,3-(diaminopropylcarbodiimide) in PBS (pH 7.4) for 1 hour at 4°C at a pressure of 130 cm water. This fixative is a bifunctional reagent which crosslinks macromolecules without major effects on the tertiary structure. Therefore, the antigenic properties are better preserved. This allowed the demonstration of various antigens in serial sections. Additionally, tissue hardening as caused by formalin or glutaraldehyde fixation was avoided, thereby facilitating serial sectioning. The carotids were left in situ at 4°C for 3 to 4 hours, after which 20 mm of both the control and the experimental arteries (including the distal bifurcation) were excised and postfixed at their physiological length for 48 hours at 4°C. The entire dissected carotids were embedded in paraplast and 5 μm-thick cross-sections were made at 125 μm intervals. To allow various staining procedures, five successive sections were mounted for each 125 μm step. For immunohistochemical purposes, other carotid arteries (n = 10; flow rate 29 ml/min) were cryostat-sectioned using the same sectioning schedule.
Histological Procedures

Connective tissue elements were stained by a combination of Weigert's stain (elastin), Hanssen's iron hematoxylin (nuclei) and van Gieson's picrofuchsin (collagen). Smooth muscle myosin and platelets adhering to the denuded vessel wall were stained with Levanol-Fast Cyanine SRN.

Immunohistochemical Procedures

Antigens were demonstrated on cryostat sections by means of indirect techniques. Primary antibodies were diluted in 12.5% nonimmune serum derived from the animal in which the secondary antibody had been raised. As controls, diluted (1:20) nonimmune sera of rabbit, swine, and goat were applied. The incubation time of the primary antibody step was 30 minutes. Secondary antibodies: goat-antirabbit IgG, peroxidase conjugated (Nordic, Tilburg, The Netherlands); swine-antirabbit IgG, FITC conjugated (DAKO Immunoglobulins Limited, Copenhagen, Denmark) were diluted (1:40) in PBS and incubated for 30 minutes as well. Peroxidase and FITC were visualized with diaminobenzidine and incident light fluorescence respectively. Between each successive step, sections were thoroughly washed with PBS.

We used rabbit antihuman F VIII R:Ag (Centraal Laboratorium Bloed-transfusiedienst, Amsterdam, The Netherlands) which was free of antifibronectin and antifibrinogen antibodies at a 1:320 dilution. This antiserum yielded a single precipitation line against human cryoprecipitate in double immunodiffusion and immunoelectrophoresis. The peroxidase conjugated antibody was used in the second step. Smooth muscle cell (SMC) myosin (rabbit-antichicken gizzard myosin kindly provided by Professor Dr. Ute Gröschel-Stewart, TH Darmstadt, West Germany) was demonstrated using a 1:800 dilution of the primary antibody, followed by the FITC conjugated antibody in the second step.

Platelet antigens were detected using a 1:50 diluted primary rabbit antiserum against platelets made in our laboratory accordingly to published methods. The antiserum was absorbed with rat plasma using a solid phase absorbent. It contained no antibodies against F VIII R:Ag. Secondary antibodies were FITC conjugated.

Morphometry

The changes in vessel wall morphology were determined quantitatively, with respect to the position and size of a number of phenomena: 1) myointimal lesion development, 2) endothelial repair, (further characterized by the area of platelet adherence and Evans blue permeation), and 3) media damage. There were a total of 24 parameters (Figures 2, 3; Table 1).

Position

The position of each phenomenon along the denuded artery (entrance-outflow distance) was determined from the sections and expressed in millimeters as the distance from the entrance point of air (Figure 2). We corrected for the discrepancies between the entrance-outflow (EO) distance as measured in situ and after calculation from the sections.

![Figure 2. Parameters indicating the position of the phenomena along the denuded artery segment as determined from cross-sections. E = entrance of air; O = outflow of air.](http://atvb.ahajournals.org/Downloaded from http://atvb.ahajournals.org/ by guest on June 28, 2017)
Figure 3. Schematic presentation of the phenomena describing endothelial repair at a luminal surface view of an opened artery. Data is from the 45 ml group. The still fully denuded part of the artery is represented by LDe, whereas in the case of incomplete endothelial repair, platelet adherence was present (dotted area). The zone of macroscopically detectable permeation of Evans blue is shaded; BEB = beginning of Evans blue permeation; EEB = end of Evans blue permeation; BPL = beginning of platelet adherence to subendothelium; EPL = end of platelet adherence to subendothelium; PRe = proximal end-point of reendothelialized area; DRe = distal end-point of reendothelialized area; EO-distance = distance between air entrance (E) and air outflow (O) holes.

Table 1. Mean Values (± SEM) of All Parameters Describing Vessel Wall Morphology at Day 14 after Endothelial Denudation (as Used for Statistical Analysis)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Flow rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>28 ml/min (A)</td>
</tr>
<tr>
<td>Myointimal arteriosclerotic lesion</td>
<td></td>
</tr>
<tr>
<td>BL begin (mm)</td>
<td>1.8 ± 0.5</td>
</tr>
<tr>
<td>EL end (mm)</td>
<td>9.4 ± 0.6</td>
</tr>
<tr>
<td>LL length (mm)</td>
<td>7.6 ± 0.8</td>
</tr>
<tr>
<td>AL absolute length (mm)**</td>
<td>6.6 ± 0.6</td>
</tr>
<tr>
<td>LI size**</td>
<td>14.0 ± 2.7</td>
</tr>
<tr>
<td>MLT maximal thickness (μm)</td>
<td>59.5 ± 10.6</td>
</tr>
<tr>
<td>LF intima covering (%)</td>
<td>15.4 ± 2.5</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Endothelial repair</td>
<td></td>
</tr>
<tr>
<td>PRe proximal end (mm)</td>
<td>5.6 ± 0.3$</td>
</tr>
<tr>
<td>DRe distal begin (mm)</td>
<td>6.4 ± 0.2*</td>
</tr>
<tr>
<td>LDe length of still fully denuded intima</td>
<td>0.7 ± 0.4‡</td>
</tr>
<tr>
<td>Platelet adherence</td>
<td></td>
</tr>
<tr>
<td>BPL begin (mm)</td>
<td>5.4 ± 0.4</td>
</tr>
<tr>
<td>EPL end (mm)</td>
<td>7.6 ± 0.2*</td>
</tr>
<tr>
<td>LP length (mm)</td>
<td>2.2 ± 0.5</td>
</tr>
<tr>
<td>Evans blue permeation</td>
<td></td>
</tr>
<tr>
<td>BEB begin (mm)</td>
<td>3.8 ± 0.4</td>
</tr>
<tr>
<td>EEB end (mm)</td>
<td>7.5 ± 0.4†</td>
</tr>
<tr>
<td>LEB length (mm)</td>
<td>3.6 ± 0.5</td>
</tr>
<tr>
<td>Media damage</td>
<td></td>
</tr>
<tr>
<td>Necrosis of inner medial layer</td>
<td></td>
</tr>
<tr>
<td>BSI begin (mm)</td>
<td>1.9 ± 0.2</td>
</tr>
<tr>
<td>ESI end (mm)</td>
<td>9.0 ± 0.7</td>
</tr>
<tr>
<td>LSi length (mm)</td>
<td>7.1 ± 0.7</td>
</tr>
<tr>
<td>SIF fraction (%)</td>
<td>40.0 ± 4.3</td>
</tr>
<tr>
<td>Necrosis of outer medial layer</td>
<td></td>
</tr>
<tr>
<td>BSo begin (mm)</td>
<td>5.8 ± 0.4$</td>
</tr>
<tr>
<td>ESo end (mm)</td>
<td>8.1 ± 0.4</td>
</tr>
<tr>
<td>LSSo length (mm)</td>
<td>2.3 ± 0.6*</td>
</tr>
<tr>
<td>SOf fraction (%)</td>
<td>9.3 ± 2.4†</td>
</tr>
</tbody>
</table>

In all three groups, EO distance was 14.7 mm. Univariate statistics using three air-flow rate groups (A = 28 ml; B = 45 ml; C = 58 ml/min) performed with ANOVA. When Kruskall-Wallis' test had to be applied, this is indicated with (K).

A ≠ (B = C); significant at p < 0.05.
A ≠ B = C; A # C; significant at p < 0.05.
A = (B = C); significant at p < 0.05.
(A = B) ≠ C; significant at p < 0.05.
(A = C) ≠ B; significant at p < 0.05.

**This length measure excludes vessel intersections between BL and EL without a lesion.
***Compare legend to Figure 8; square number (in arbitrary units).
MORPHOMETRY OF RAT ARTERIOSCLEROSIS van Pelt-Verkuil et al. 445

Size

The distance between the beginning and the end point of a phenomenon was used as the measure of size (length).

Instead of an intima/media ratio, which was strongly influenced by media necrosis, the lesion size (LI) was described by the integral of the lesion thickness along the artery (cf legend for Figure 7), its maximal thickness (MLT) and the fraction of the intima covered by the lesion (LF). The latter parameter was quantitated as follows: at 0.5 mm intervals the fraction of the intimal circumference in cross section covered by the lesion was scored as 0, 0.33, 0.66, and 1.0 (for 0%, 33%, 66%, and 100% respectively of the intima covered). The quotient of \( \Sigma \) (cross-sectional scores)/(number of scored cross sections along EO distance) \( \times 100\% \) was used as the parameter (LF).

The fraction of the media with necrosis (disappearance of SMCs) in inner (SiF) and outer (SoF) medial layers was determined in the same way as described for the fraction of the intima covered by the lesion.

Specifications of Parameters

Endothelial cells and proliferated SMCs were discriminated on the basis of morphological and immunohistochemical characteristics. Platelet adherence, which could be easily demonstrated using Levanol-Fast cyanine 5RN, was found only in still denuded areas (Figure 3) and was recorded when groups of four or more platelets were distinguished at the intima. Media necrosis was indicated by the absence of SMCs with or without extracellular matrix. Two medial zones were defined: the inner medial layer bordered by the elastica interna and the second elastin lamella, and the outer medial layers up to the adventitia.

Statistical Methods

One way analysis of variance (ANOVA) was chosen as a model to test the overall hypothesis of the equality of parameter means of all individual variables, for Group A (28 ml/min), Group B (45 ml/min) and Group C (58 ml/min). To determine the position of individual sample means, we applied an a posteriori simultaneous-testing procedure after ANOVA, using the least significant difference as a criterion.20 We used these procedures after inspection of sample distribution characteristics and ascertaining normality with Fisher’s test on the third and fourth moments.21 The equality of variances was tested and established using Levene’s test.22 Variables not fulfilling these criteria were tested with the nonparametric Kruskall-Wallis test,23 followed by an a posteriori simultaneous testing.

Results

General Observations

The pattern of vessel wall response at 14 days after endothelial denudation was qualitatively similar for all air-flow rates greater than 16 ml/min. This pattern is described in the following paragraphs. At lower air-flow rates (< 24 ml/min), endothelial denudation was incomplete; myointimal lesions were no longer observed below flow rates of 12 ml/min.

Myointimal, Arteriosclerotic Lesion Development

We observed this development in 57 of 60 animals at air-flow rates greater than 16 ml/min. It varied from massive intimal thickening with large amounts of collagen and elastin formation to smaller numbers of intimal SMCs with sparse matrix deposition. Luminal pseudoendothelial SMCs apparently contained more myosin than those deeper in the lesion.
**Endothelial Repair**

Endothelial repair progressed from the distal and proximal ends, and newly proliferated endothelium was present at minimally 60% of the intima. Complete reendothelialization occurred in 16% of the vessels. New endothelium (containing F VIII R:Ag; Figure 5) had a much higher cellular density (Figure 6 C) than that of control arteries (Figure 6 D).

Adhering platelets were always seen on the intimal surface of still denuded parts of the arteries, but never in reendothelialized areas. Although platelets could not be morphologically detected under lesions, or under new endothelium, platelet immunoreactivity was found at those locations (Figure 4). White thrombi, mostly 0.1–0.2 mm in diameter and never longer than 2 mm, were occasionally found in still denuded areas. As van Aken and Emeis concluded, these were young unorganized thrombi (3 to 12 hours old). In areas of white thrombi, leukocytes often adhered to the lesion or to the subendothelium.

**Figure 5.** The localization of F VIII R:Ag in a reendothelialized, nonlesion area on Day 14 after air drying (flow 28 ml/min). The antigen is present in the endothelial cells and in the inner medial layer (cryostat section, no counterstain). Bar indicates 20 μm.

**Figure 6.** Different types of medial necrosis in air-dried arteries (A, B, C) as compared with control (D) on Day 14 after air drying (flow 28 ml/min; connective tissue staining). A. Disappearance of smooth muscle cells (SMC) in the inner medial layer (I) underneath a nonlesion platelet-covered intima. B. Disappearance of SMCs underneath a lesion-covered intima (L = lesion). C. Disappearance of SMCs from the entire media (arrowheads). The adventitia is on the left and the lumen is on the right. D. Control. Bar indicates 20 μm.
Vessel Wall Permeability

Plasma proteins were found permeated into large areas of the media. Fibrin, albumin, and IgG were demonstrated immunocytochemically and inventoried in a similar way as media necrosis (unpublished observations). F VIII R:Ag and platelet antigens were also found in the media (Figure 4 inset, Figure 5).

Maximal lesion thickness (μ)

Figure 7. The pattern of intimal thickening along the length of the denuded artery (mean ± SEM) for the flow groups of 28, 45, and 58 ml/min). These curves were generated as follows: the thickest point of the myointimal lesion was measured every 0.5 mm. These values were plotted on graph paper (Y axis) against the distance between air entrance and outflow (EO distance) (X axis) for each individual rat. These graphs were read by means of a digitizer tablet yielding a continuous series of Y values (intimal thickening). For a comparison of each individual thickening pattern, these graphs were transformed into new ones in the following way: the X axis was divided into 50 equidistant points, by which 50 corresponding Y values were found. These 50 Y values were considered as homologous for each individual. They were used to calculate the mean lesion thickness at 50 equidistant points along the X axis for the three air-flow rate groups which are shown in Figures 7 and 8 (± SEM). The data were also used to calculate a measure for lesion size (LI) by means of an integral of lesion length and the Y values. Thus the LI parameter is the surface of the mean curve along the EO distance representing the thickest points of the lesion.

Medial Damage

Necrotic areas (medial damage) were found in all arteries investigated. In these areas SMCs had disappeared either with or without their extracellular matrix (Figure 6). Media necrosis mostly occurred in the more proximal portion of the vessel and occupied a larger area in the inner medial layer. Even at air-
**Figure 8.** The best fit of the maximal lesion thickness for the flow series along the damaged artery, as described by a 10th degree polynomial. Areas of significant differences (50 Y values tested) between the flow groups are indicated for (28, 45) ↔ 58 (p = 0.05; t-test; double-dotted area) and (58, 45) ↔ 28 (p < 0.05; ANOVA; dotted area).

**Figure 9.** A visualization of the relevant intergroup differences of the flow series: mean myointimal lesion shape, the denuded area (deE), and the zone of necrosis of the outer medial layers (SM₀). For the calculation of lesion shape, see Figure 8. E = entrance point of air; O = outflow of air; reE = endothelial repair; reS = repopulation direction of SMCs in outer medial layers. The thickness of the intima and media are shown on the scale.
flow rates at or under the lower limits of lesion induction (12–16 ml/min) SMC disappearance was observed. The highest extent of necrosis was always found in still denuded parts of the vessel, and sometimes it extended to the entire vessel circumference. Medial thickness was maximally reduced by 40% in comparison with the contralateral control vessel. In contrast, other vessel wall areas with little cell death showed edema. The remaining medial SMCs were always less immunoreactive toward the antmyosin antiserum and they required longer staining times with Levanol.

Quantitative and Qualitative Differences in the Flow Series

We noted differences in the vessel wall response at 14 days after endothelial denudation at various air-flow rates, (Table 1; Figures 7, 8, 9). To describe these differences quantitatively, we used the three groups with the largest sample sizes: 28 ml/min (n = 15), 45 ml/min (n = 15), and 58 ml/min (n = 14).

Myointimal, Arteriosclerotic Lesion Development

This development was not significantly influenced by reducing the air-flow rate to 28 ml/min with respect to its position (BL, EL) on the one hand, and length (AL, LL), maximal lesion thickness (MLT), and the integral of the intima thickening pattern as size measures (LI) on the other hand (Table 1). The pattern of intimal thickening (lesion shape) along the vessel axis was clearly different for the three flow groups (Figures 7, 8). In the 58 ml/min group this pattern showed two peaks at about one-fourth and three-fourths of the EO-distance (Figure 8). At lower flow rates, the dip in MLT values diminished (p = 0.05), together with a higher MLT maximum, at one-fourth of the EO-distance, and a fall of the maximum at three-fourths in the 28 ml/min group (p < 0.05). The lesion mass moved into a more proximal position at lower air-flow rates, but never achieved its maximal thickness in the middle of the damaged, still denuded area.

We had the impression that the amount and composition of extracellular matrix differed. In the highest air-flow rate group (58 ml/min), a number of lesions with diminished elastin and collagen formation were present, especially in the middle section of the still denuded area (cf Figure 6 B).

Endothelial Repair

Endothelial repair, and thereby platelet adherence and Evans blue permeation, differed significantly between the flow groups. The mean speed of endothelial repair was 0.38 mm/day and 0.43 mm/day respectively for proximal (PRe) and distal (DRe) outgrowth of the endothelium in the 45 ml/min and 58 ml/min groups. Endothelial repair occurred significantly faster in the lowest air-flow rate group, as judged from a smaller length of the denuded area (LDe), and a more proximal position of distal endothelial repair (DRe; 0.59 mm/day) (Table 1; cf Figure 9). Due to differences in the speed of endothelial repair along the vessel circumference, the area with platelet adherence to still denuded parts was greater than that of complete denudation in all groups (cf Figure 3). The length of Evans blue permeation was significantly greater than that of endothelial denudation or platelet adherence in all three groups (p = 0.05).

Media Damage

The disappearance of SMC from the inner medial layer did not differ significantly between the air-flow rate groups. A relatively large, posteriorly situated zone of the inner media was devoid of SMC (Table 1; BSi, ESi). There was a nonsignificant trend toward less SMC disappearance in the 28 ml and 45 ml groups, compared to the 58 ml group (SIf; LSi).

The necrosis of the outer medial layers was significantly smaller in the lowest air-flow rate group (28 ml), as represented by all parameters. Most extensive medial damage was found at 58 ml/min (Table 1 and Figure 9). The beginning of the necrotic area was more distal (BSo), and both the length (LSo) and the fraction (SoF) of the media involved were smaller at 28 ml/min. A small zone with an extensive outer media necrosis was still present at the middle of the denuded artery (Figure 9, SMo). The position of the most intense media necrosis at 58 ml/min roughly coincided with the lowered values of maximal lesion thickness in the middle region.

Discussion

Response to Injury of the Rat Carotid Artery

General Remarks

The aim of the present study was to induce in the vessel wall a reproducible endothelial injury that would lead to arteriosclerotic lesion development. This response pattern could then be used in experiments aimed at experimentally influencing the development of arteriosclerosis.

In our study, the air-drying procedure in the rat carotid artery endothelium also caused media necrosis. The results showed that the degree of media necrosis could be minimized under well defined surgical conditions at a low air-flow (28 ml/min), but that some medial damage is unavoidable in Wistar rats.

Media Necrosis

SMC disappearance was found, in contrast to the findings of other authors using this animal model.6,11 The SMC disappearance was not accompanied by higher densities of SMCs elsewhere in the vessel.
wall, either before or during myointimal lesion development. Since disappearance of SMCs from the outer medial layers occurred as early as 1 day after surgery (unpublished observations) and could be diminished in the outer medial layers by lowering the air-flow rate, we assumed this death to be dependent on the air-flow rate.

The disappearance of SMCs from the inner medial layer was also observed as early as 1 day after surgery. The extent of this disappearance was only slightly diminished at lower air-flow rates (Si-parameter), and local areas without SMCs were then observed at air-flow rates less than 24 ml/min. Therefore, the disappearance of SMCs from the inner medial layer seems to be much less related to air-flow rates than is true with the outer media. Factors in the air-drying procedure other than the flow rate might have more effects on the inner media.

Myointimal Arteriosclerotic Lesion

Following endothelial denudation, the vessel wall responded with the formation of a myointimal lesion. Although the different air-flow rates used to induce denudation led to varying extents of media necrosis, the size and the position of the myointimal lesion was constant. However, the lesion shape (the course of the MLT values along the denuded artery) was considerably influenced by the rate of the air flow.

To compare the induced intimal lesions with published data, we determined the maximal intima/media ratios in those single arteries without media necrosis. These values were of the same magnitude as published data, we determined the maximal intima/media ratios in those single arteries without media necrosis. The minor lesion development in the distal part of the 28 ml group might have been caused by a faster endothelial repair than in the outer media. Factors in the air-drying procedure other than the flow rate might have more effects on the inner media.

Effect of Medial Necrosis on Myointimal Lesion Development

Lesion Shape

Differences in myointimal lesion shape between the three air-flow rate groups in response to endothelial denudation (Figure 9), are probably related to the rate of endothelial repair and the extent of medial damage.

Endothelial denudation caused myointimal thickening at all air-flow rates. The minor lesion development in the distal part of the 28 ml group might have been caused by a faster endothelial repair than in the other groups (0.59 mm/day), the latter showing a similar growth rate (0.42 mm/day) as already described. Distal medial SMCs might not have been exposed to the blood long enough to induce large scale intimal proliferation at 28 ml/min. These vessels were 100% permeable to Evans blue at 1 day after air drying, but they sometimes showed focal clumps of endothelial cells in the most distal 1.2 mm. These cells may be responsible for the faster endothelial repair, which also might have been facilitated by lesser overall damage.

If local shortage of SMCs is important for the local thickening pattern, the dip in MLT values in the middle region of the 58 ml group might have been caused by a shortage of SMCs, as is most extensive at that location. A comparable phenomenon has been described for rabbit aorta after deep mechanical injury when crater-like lesions develop over areas with extensive media necrosis. Since the outer media necrosis of the 45 ml group was less severe with respect to the percentage of the media involved (Table 1; SoF), more SMCs might have migrated into the intima, leading to a smaller dip. Relatively small lesion thickness in the region of the 28 ml group might also have been caused by a local shortage of SMCs. The area with the outer media necrosis was smaller (LSO), but the percentage of the media involved (SoF) was the same when compared with the 45 ml group. An optimal proliferative response seems to have taken place in the proximal part of the 28 ml carotids, as the highest MLT values were found there and there was no necrosis of the outer medial layers.

Lesion Size and Position

Medial SMC necrosis in the inner and outer medial layers was greater on the first day after surgery, and repopulation of the media took place gradually thereafter (unpublished observations). This medial repair might have interfered with the extent of migration and proliferation of the medial SMCs in the intima. Surprisingly, however, no great differences in lesion size and position were found between the air-flow rate groups at Day 14 (Table 1). This may indicate a certain dominancy of myointimal lesion development over repopulation of the media by SMCs. Additionally, more damage may provide a greater stimulus for SMC proliferation. It is unclear why the phenomenon of medial necrosis has not yet been described for the air-drying model. Medial SMCs of Sprague Dawley rats, which were used for many studies, may show a different response to the air-drying injury than Wistar rats, but we found some medial damage in Sprague Dawley rats (unpublished observations).

It would be preferable to deal with a selective endothelial injury model. With the exception of the small defined injury of the Reidy and Schwartz method, rat models producing complete endothelial denudation suffer from more or less media damage. At least the degree of media damage has become reproducible in the catheterization technique and in the present method. We have chosen the air-drying model (flow rate 28 ml/min) for future research. Under these conditions, there is the least medial damage and an optimal proliferative response seems to have taken place. Furthermore, the lesions are most homogeneous and the variation coefficients of all other parameters, smallest.
References


30. van Pelt-Verkuil E, Karnovsky MJ. Failure of certain antiplatelet drugs to affect myointimal thickening following arterial endothelial injury in the rat. Lab Invest 1977;35:452–464


Index Terms: rat • carotid artery • endothelial denudation • myointimal lesion • media necrosis • morphometry
Morphometry of air-drying-induced arteriosclerosis in rat carotid artery. Effect of air-flow rate.
E van Pelt-Verkuil, W van Pelt and D Jense

doi: 10.1161/01.ATV.3.5.441

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1983 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/3/5/441

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/