Flow Pattern and Structural Changes at Carotid Bifurcation in Hypertensive Cynomolgus Monkeys

Michael Hennerici, Karl-Friedrich Bürrig, and Michael Daffertshofer

Blood flow pattern and morphological changes within the extracranial carotid system were studied in M. fascicularis before, immediately after, and at various intervals (4 hours, 4 days, 10 days, and 4 weeks) after the introduction of systemic hypertension by surgical coarctation of the thoracic aorta. Intra-arterial hemodynamics were assessed by means of both continuous-wave Doppler signal spectrum analysis and multigate pulsed-wave Doppler flow velocity profile processing. Diminished peak frequencies throughout the carotid system and enlargement of the lumen diameter in the common carotid artery and the carotid bulb were major findings and were suspected to be due to cerebral autoregulation at high levels of intracranial vascular resistance. In the internal carotid artery of pure muscular type, the lumen diameter was diminished so the carotid bulb became an area of structural and hemodynamic transition. As a consequence, flow irregularities already observed in this region before surgery considerably increased during hypertension. In addition, endothelial disarray and leucocytic adherence and activation were associated with these hemodynamic alterations within the carotid bulb. Their mutual role in early atherogenesis is discussed. (Hypertension 1989;13:315–321)

Atherosclerosis of the carotid bifurcation represents a major source for stroke, and may develop from early childhood without corresponding neurological signs or symptoms for decades. Insufficiency of the cerebral circulation in the presence of hemodynamically significant obstructive lesions or cerebral embolism arising from plaques with irregular surfaces have been associated with cerebrovascular episodes. Thus, progression of initially neurologically asymptomatic small lesions to symptomatic arterial obstructions has long been suggested to represent the natural history of atherosclerosis in man. This concept had to be modified due to the results of recent prospective studies that investigated the spontaneous course of atherosclerosis in humans by means of noninvasive ultrasound methods.1–5 These indicated variable stable periods alternating with progressing and relapsing episodes. The results of these studies are in accordance with progression and regression observed in primates exposed to atherogenic dietary manipulations.4,5 They are further supported by the results of recent therapeutic trials such as coronary atherosclerosis regression in patients treated for hypercholesterolemia.6,7

The initial pathomechanisms involved in plaque formation as well as their subsequent developments are, however, still incompletely understood. Although experimental and anatomic studies have shown that the carotid bulb and the proximal part of the internal carotid artery are predilected sites for atherosclerosis and susceptible territories to atherogenic metabolic conditions,8–10 the alternative sequence of morphological changes induced by abnormal hemodynamics has not been established. Because morphological and hemodynamic aspects are intermingled in human atherosclerosis and cannot be studied in vitro experiments,11–13 we have compared the hemodynamic changes subsequent to acute hypertension with morphological alterations at the same place. Flow velocity patterns were recorded by means of a high-resolution, multigate pulsed-wave Doppler system before and after systemic supra-aortic hypertension due to surgical coarctation of the thoracic aorta. Flow velocity profiles from the common, the proximal internal carotid artery, and the carotid bifurcation with the bulb in particular have been assessed because flow separation and recirculation have been hypothesized to represent important mechanisms for the development of atherosclerotic plaques.1,12,14
Materials and Methods

Surgical and Anatomic Procedures

Hypertension was produced in six adult (5-6 years old) male cynomolgus monkeys (M. fascicularis) by surgical coarctation of the aorta. In addition, one animal served as a control. One small segment (about 5 mm) of the midthoracic aorta was stenosed by a Dacron suture until a mean pressure difference of approximately 60 mm Hg between the proximal and distal segments was obtained. On the day of sacrifice, at 4 hours (n=2), 4 days (n=1), 10 days (n=1), and 4 weeks (n=2) after surgery, respectively, the monkeys were anesthetized with a halothane (N₂O-O₂) mixture, brachial and femoral intra-arterial blood pressures were measured, and then the monkeys were killed.

The carotid arteries were perfused at a pressure of 100 mm Hg via the cannulated left heart chamber. Initial perfusion was done with 0.9% NaCl for 1 minute, followed by 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.3, 320 mosm/l glucose added) for 10 minutes. After perfusion the carotid arteries were dissected, pinned to cork plates, and immersed in fresh fixative overnight. One carotid bifurcation was used for routine histology and transmission electron microscopy and the other was washed in buffer, postfixed in 1% buffered osmium tetroxide, dehydrated in graded acetone, critical-point-dried coated with gold, and examined in a Philips SEM 515 (Philips Electronic Instrs., Inc., Eindhoven, The Netherlands) scanning electron microscope.

Ultrasound Methods

The ultrasound examination procedures used have been described in detail before. A 64-gate system operates at 5.2 MHz emission frequency and a pulsed repetition frequency of 16 kHz with 1.2 msec emission duration. Sample interval was 0.6 mm and the lower cut off frequency was 250 Hz. The sample volume of the multigate system as measured in vitro is 1.75 mm³ at a 50 mm distance and 1.35 mm³ at 20 mm distance. Both velocity profiles across the lumen diameter and the instantaneous velocity traces of each individual gate are displayed online. As both velocity profiles and single gate traces recorded during several consecutive cardiac cycles showed only minimal variations on a beat-to-beat basis, 4-10 cycles were usually plotted on a XY recorder (Philips Electronic Instrs., Inc.), for further data analysis. Only a limited number of velocity profiles of each cardiac cycle out of a maximum of 256 available are presented for the sake of clarity. The absolute vessel diameters were assessed with the multigate system from the A-mode according to vessel wall displacement. This A-mode differs from the usual A-mode implemented in ultrasound systems because of the rejection of stationary signals (upper cut off frequency 5 Hz).

In addition, a 4 MHz continuous-wave Doppler system was used. The quadrature (two-direction sensitive) signals were further analyzed with a fast Fourier transform (FFT) analyzer (Echospec, Diagnostic Electronics Corporation, Lexington, Massachusetts). Technical characteristics are frequency range from -7 kHz to 28 kHz, 40 dB dynamic range, 0.15 V peak-to-peak resolution, sampling rate of approximately 4 msec, frequency resolution 280 Hz/horizontal frequency line (126 frequency components were displayed). The ultrasound examination procedure was adjusted from human investigations. Adaptation of both instruments, originally designed for human application, on the considerably smaller caliper of the carotid system in monkeys was restricted to signal processing.

Flow velocity measurements within both extracranial carotid arteries were obtained from each animal before surgery, immediately after coarctation, and before killing in similar anesthetic conditions. Pulsed-wave, multigate velocity profiles and traces as well as continuous-wave Doppler FFT spectra were recorded from the common carotid artery, the carotid bifurcation, and the proximal internal carotid artery as identified by the probe position with regard to the characteristic differences of both audiosignal and Doppler waveform analysis and the anatomic situation. Recordings with the best amplification available, indicating a probe-to-vessel angle of about 60°, were used for further data analysis exclusively; insonation of the carotid artery bulb at steeper angles could not be safely achieved without additional imaging because of the low signal amplitude and the small size of the carotid system in these animals.

Variables evaluated with the multigate, pulsed-wave Doppler system are the systolic arterial diameter, the systolic peak frequency as determined on top of the velocity pattern, the ratio between the number of systolic and diastolic velocity profiles as revealed by electrocardiograph recordings, and an index of backflow phenomena defined by the number of profiles with flow segments oriented toward the probe throughout five subsequent cardiac cycles. The absolute systolic diameters are dependent on the angle of interrogation and hence multiplied by 0.87 (= sine 60°). Variables evaluated from the FFT continuous-wave Doppler signals are the systolic peak frequency, the mean frequency, and the systolic window.

Reproducibility of all variables mentioned is excellent at a given position provided that conditions of examination are stable. Furthermore, even after change of the probes of both systems, a reasonable correlation can be demonstrated for similar parameters (Figure 1).

Results

Hemodynamics

Typical flow velocity profiles recorded from the multigate, pulsed-wave Doppler system before surgery, after coarctation of the aorta, and before the
monkeys were killed are demonstrated in Figure 2. In both the distal common carotid and the proximal internal carotid arteries, peak flow velocities were similar and the velocity profiles were symmetric with only a few irregularities, mainly in the center of the blood stream during systole, but without significant backflow phenomena. In the carotid bulb, however, peak flow velocity was slower and the backflow components somewhat more frequent (Figure 3, Tables 1 and 2). After surgery, a reduction of the peak frequency throughout the carotid system occurred, and the mean frequency tended to decline simultaneously.

This was associated with a remarkable dilatation of the lumen within the common carotid artery but contrasted with constriction of the internal carotid artery (Figure 2, Table 1). Both changes resulted in a significant decrease of the systolic/diastolic ratio, which mirrored the higher flow pulsatility and increasing wall shear stress produced by the increased blood pressure. In the carotid bulb both peak and mean frequencies also tended to decrease, whereas the arterial diameter slightly increased, which was similar to the alterations within the common carotid artery. The systolic/diastolic ratio was also reduced in both the internal and common carotid arteries, but in addition local flow separations were observed from a significant increase of the backflow index in the bulb. This corresponds with a reduced systolic window in the continuous-wave Doppler spectra (Tables 1 and 2).

The parameters mentioned above tended to normalize throughout the postoperative period when recorded again after 4 hours, 4 days, 10 days, and 4 weeks. However, the initial values were never reached, and the systolic/diastolic ratio in particular remained statistically at significantly different levels.

**Morphology**

The morphological findings, which will be presented in detail elsewhere, are summarized as follows: The internal carotid artery in *M. fascicularis* is of pure muscular type, whereas the common carotid artery and the carotid bulb have an elastic structure that ends with a rather abrupt change at the proximal internal carotid artery (Figure 2). In humans, however, the carotid bulb represents an area of a more continuous transition from one morphological type to the other. The media thickness is slightly reduced in the bulb and quite similar to the wall thickness of the internal carotid artery.

Under the scanning electron microscope, the endothelial cells of the common carotid artery and the internal carotid artery upstream from the bulb displayed regular patterns. The endothelial nuclei were arranged in parallel. In contrast, the endothe-

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**Table 1. Flow Parameters Before Surgery and After Surgery With Pulsed-Wave Doppler**

<table>
<thead>
<tr>
<th></th>
<th>Common carotid artery</th>
<th>Carotid bulb</th>
<th>Internal carotid artery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Peak frequency (Hz)</td>
<td>Artery diameter (mm)</td>
<td>Systolic/diastolic ratio</td>
</tr>
<tr>
<td>Before</td>
<td>2,219±520*</td>
<td>4.85±0.99</td>
<td>1.13±0.34†</td>
</tr>
<tr>
<td>After</td>
<td>1,696±397</td>
<td>5.31±1.17</td>
<td>0.52±0.15†</td>
</tr>
<tr>
<td>Before</td>
<td>1,937±869</td>
<td>5.44±1.44</td>
<td>1.23±0.35†</td>
</tr>
<tr>
<td>After</td>
<td>1,339±347</td>
<td>5.9±1.75</td>
<td>0.56±0.18</td>
</tr>
<tr>
<td>Before</td>
<td>2,312±836*</td>
<td>5.55±0.67</td>
<td>1.13±0.36†</td>
</tr>
<tr>
<td>After</td>
<td>1,523±424</td>
<td>5.29±1.45</td>
<td>0.58±0.21</td>
</tr>
</tbody>
</table>

Values are mean±SD. *n=*six monkeys.

* *p<0.05, †p<0.01, and ‡p<0.01 by Student’s *t* test.
profiles are recorded at discrete time intervals for a
and the CCA
patterns from the ICA
(upper row)
(lower row).

Arrows.

Anterior and posterior
walls with regard to the probe position are indicated by

Toluidine blue, x400). Multigate pulsed-wave Doppler velocity
musculoelastic layers are noted
(upper left,
Toluidine blue, x80) shows the
Elastica-von Gieson stain, x80) shows the
internal carotid artery (ICA), the media is entirely com-
upper row.
and
In the

Middle,
the light micro-
iments that the higher number of flow irregularities
are present in other places of the bifurcation. In addition, leukocyte activation was evident from the
spreading of these adherent cells (Figure 3).

Discussion
The data presented indicate combined hemody-
namic and morphological effects on the extracranial
system of M. fascicularis subsequent to the intro-
duction of severe supra-aortic hypertension. In the
common carotid artery and the carotid bulb
(which are of similar morphological structures in
our model), a striking dilatation occurs while the
internal carotid artery shows a lumen narrowing. As
with humans, this vessel is of pure muscular type
and branches with an abrupt morphological change
at the outlet of the bulb. These effects reflect the
increase of intracranial vascular resistance subse-
quently to aortic coarctation and result in a reduction
of peak flow velocities throughout the extracranial
carotid system. Quantitative estimation of this is
difficult because of the influence of surgical and
anesthetic procedures on the cardiovascular system.

In addition, a marked increase of backflow phenom-
ena and a slight reduction of the systolic FFT spec-
trum window occurs in the carotid bulb. These
marked low flow areas migrate between the entry of
the common (diastole) and the outlet of the internal
(systole) carotid artery during each cardiac cycle.

We interpret these alterations as secondary flow
phenomena, the nature of which was recently
reviewed by Schmid-Schönbein and Wurzinger.8
These authors showed that true turbulence charac-
terized by stochastic motion of fluid elements does
not occur in the vascular tree, not even at sites of
atherosclerotic lesions like the carotid bifurcation.
In contrast, they considered flow separation, back-
flow phenomena, and other forms of nonlaminar
flows to represent characteristic features of the
carotid bulb. In addition, flow irregularities may be
further pronounced as a consequence of reduced
aortic elastic properties after surgical coarctation,
which results in a higher pulsatility throughout the
vascular tree. This is reflected by the diminished
systolic-diastolic pulsatility index observed in our
experiments. This interpretation fits with the hemo-
dynamic theory already described by Naumann and
Schmid-Schönbein,17 who showed in vitro exper-
iments that the higher number of flow irregularities
corresponded with higher pulsatility conditions.

The area of major velocity pattern alterations in
this model is the carotid bulb and its outlet into the
intracranial vascular tree. It is in this segment of
the extracranial carotid system that the endothelial
disarray is prominent morphologically, and leuco-
cyte adherence can be observed. The observations
presented suggest that hypertension as a single
risk factor can initiate atherosclerotic plaques
mediated through a separation of low flow areas in

![FIGURE 2. Correlation of morphology and hemodynamics in the carotid bifurcation. Middle, the light micro-
ograph (left, Elastica-von Gieson stain, ×80) shows the
transition zone of the elastic artery (EA) to the muscular
type (MA). The total view of the carotid bifurcation is
given by the scanning electron micrograph (right, ×11). A
more detailed morphology of the media is demonstrated by
semithin sections in the upper and lower row. In the
internal carotid artery (ICA), the media is entirely com-
posed of smooth muscle cells (upper left, Toluidine blue,
×600) whereas in the common carotid artery (CCA)
musculoelastic layers are noted (lower left, Toluidine
blue, ×400). Multigate pulsed-wave Doppler velocity
patterns from the ICA (upper row) and the CCA (lower
row), before (A), immediately after surgery (B), and
before killing (C) are given on the right. Several velocity
profiles are recorded at discrete time intervals for a
different number of cardiac cycles. Anterior and posterior
walls with regard to the probe position are indicated by
arrows.](http://hyper.ahajournals.org/doi/abs/10.1161/HYPERTENSIONAHA.117.128692)
FIGURE 3. Hemodynamic and morphological changes after introduction of hypertension in the carotid bulb. Continuous-wave Doppler spectra before (panel A) and after (panel B) surgery, the latter indicating severe backflow components. Multigate pulsed-wave Doppler profiles with flow irregularities in both investigations obtained before surgery (panel C) and after coarctation (panel D), the latter demonstrating a marked dilatation. Scanning electron micrographs of the carotid bulb in the control monkey showed a slight disarray of the endothelial nuclei, indicating flow irregularities (panel E). Leucocytic adherence was prominent after onset of hypertension (panel F) 4 weeks after aortic coarctation (panel E and F) (×300).

certain vascular segments. These hemodynamic changes induce morphological changes that are probably accompanied by a cascade of biochemical reactions and other mechanisms responsible for subsequent stages of atherogenesis.\textsuperscript{8,18} This interpretation is supported from recent contributions by Davis et al\textsuperscript{19} and Langille and Adamson\textsuperscript{20} who found in in vitro studies very regular endothelial cell patterns with ellipsoidal-shaped cells under laminar flow conditions but disarrangement of the monolayers exposed to shear stress in turbulent flow conditions.

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References


KEY WORDS • atherosclerosis • Doppler ultrasound • carotid artery • hemodynamics
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