Effects of Age on Endothelium-Dependent Vasodilation of Resistance Coronary Artery by Acetylcholine in Humans

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Background. It has been suggested that endothelium-related vasomotion is important in the control of coronary circulation. Our goal was to determine if endothelium-dependent dilation of the coronary vasculature was altered with aging in 18 patients with atypical chest pain (age, 23-70 years) who had angiographically normal coronary arteries and no coronary risk factors.

Methods and Results. We infused an endothelium-dependent vasodilator acetylcholine (1, 3, 10, and 30 \( \mu \)g/min) and an endothelium-independent vasodilator papaverine (10 mg) into the left coronary artery. The large coronary diameter was assessed by arteriography, and the increase in coronary blood flow was measured using the intracoronary Doppler catheter technique. Acetylcholine increased coronary blood flow in a dose-dependent manner with no changes in arterial pressure and heart rate. The maximum increase in coronary blood flow evoked by acetylcholine varied widely among patients (increase in coronary blood flow ranged from 200% to 560%) and was correlated significantly with aging \((r=-.86, P<.001)\), whereas the peak coronary blood flow response to papaverine was affected slightly by aging \((r=-.44, P=.07)\). The percent increase in blood flow response to acetylcholine to the response to papaverine correlated with aging \((r=-.87, P<.001)\). The slope of the coronary blood flow response to acetylcholine also correlated significantly with aging. The large epicardial coronary artery response to the low doses of acetylcholine \((\leq 10 \mu g/\min)\) correlated inversely with aging.

Conclusions. The results of this study suggest that endothelium-dependent dilation of coronary arteries evoked by acetylcholine may be decreased with aging in humans. (Circulation 1993;88:77-81)

Key Words: age • vasodilation • arteries • endothelium • circulation

Age is a risk factor for cardiovascular disease.\(^1\) Aging is associated with morphological and functional changes in the coronary vasculature. It has been shown that aging decreases capillary density and increases collagen content in the arteriolar wall.\(^2\) In addition, aging decreases coronary flow reserve, predominantly in subendocardial myocardium in animals,\(^3\)\(^,\)\(^4\) indicating that myocardial perfusion decreases with aging. In the resistance artery of rats, endothelium-dependent dilation decreases with age.\(^5\),\(^6\)

Recent clinical studies\(^7\)\(^,\)\(^8\) have demonstrated that dilation of large epicardial coronary artery evoked with intracoronary administration of acetylcholine decreases or disappears with aging in patients with angiographically normal coronary arteries, suggesting that aging impairs endothelium-dependent dilation of large epicardial coronary arteries. However, no study has examined the effects of aging on endothelium-dependent vasodilation of resistance coronary artery in humans. It is well recognized that endothelium plays an important role in the control of coronary blood flow by regulating coronary vascular resistance.\(^5\),\(^10\) Our goal was to determine if endothelium-dependent vasodilation of resistance coronary artery evoked by acetylcholine is altered by aging in humans.

Methods

Study Patients

Eighteen patients who had atypical chest pain, normal exercise test results, and angiographically normal coronary arteries were studied. Patients who had arterial hypertension or evidence of left ventricular hyper trophy as assessed by ECG and echocardiography and were receiving an antihypertensive or a cholesterol-lowering drug were excluded. Patients who had hypercholesterolemia (total cholesterol, >220 mg/dL), diabetes mellitus, cardiomyopathy, valvular heart disease, and left or right bundle branch block on the ECG were excluded. Patients with variant angina who had angiographically documented coronary spasm also were excluded.

The research proposal of this study was approved by the institutional review committee for clinical research.
Written informed consent was obtained from each patient after the study protocol was explained.

**Quantitative Coronary Arteriography**

Coronary cineangiograms were recorded on 35-mm cinefilm (60 frames per second) using a Siemens cineangiographic system. A view was selected that allowed the best visualization of the left anterior descending coronary artery (the study artery).

We determined changes in the luminal diameter at the proximal segments of the left anterior descending coronary artery, a segment 2 to 3 mm distal to the tip of the Doppler catheter, as we described previously. An end-diastolic frame was selected on a cineprojector, and the arterial segments under study were scanned with a videocamera. The images were digitized and analyzed with a videodensitometric analysis system (Kontron Instruments, Germany). The diameter of the segment of interest was measured, and the averaged value from triplicate measurements was used for later analysis. A Judkins catheter was used for calibrating the arterial diameter in millimeters. The arterial diameter measurements were done blindly without knowledge of clinical characteristics of the patients.

**Measurements of Coronary Blood Flow Velocity and Estimation of Coronary Blood Flow**

An 8F angioplasty guiding catheter was introduced into the left main coronary artery with a femoral approach. A 3F Doppler flow-velocity catheter (model DC-201, Millar Instruments, Houston, Tex) was introduced into the left anterior descending coronary artery. The Doppler catheter then was connected to a DC-101 Velocimeter (Millar Instruments) to obtain mean and phasic velocity signals. The increases in coronary blood flow evoked by acetylcholine were estimated from the product of the mean coronary blood flow velocity and the cross-sectional area of the proximal arterial segment at the tip of the Doppler catheter, and increases were expressed as percent increases from the baseline level. The increases in coronary blood flow in response to papaverine were assessed from the product of mean blood flow velocity and the baseline coronary cross-sectional area, assuming that the cross-sectional area changed little as a result of administration of papaverine. We did not measure the arterial cross-sectional area after papaverine administration because peak flow velocity after papaverine usually occurred at 20 to 40 s after the drug injection, so it was difficult to record simultaneously peak flow velocity and arterial cross-sectional area in such a brief period of time. We previously examined the effects of the administration of 10 mg papaverine i.c. on the coronary artery diameter in a group of 28 patients and found that the drug dilated the proximal coronary artery modestly (by ±8%) and that age did not influence the degree of proximal coronary vasodilation caused by papaverine (authors' unpublished observation).

**Study Protocol**

Cardiac catheterization was performed with subjects in the fasting state before medication administration of 5 mg diazepam p.o. Twelve of 17 patients were not receiving antianginal drugs, and five patients were receiving calcium channel blockers and nitrates. These drugs were discontinued at least 24 h before the study.

After completion of the diagnostic catheterization, the following interventions were performed: 1) a bolus injection of papaverine (10 mg/5 mL) through the guiding catheter; 2) infusion of saline (0.5 mL/min for 2 minutes) through the Doppler catheter; and 3) infusions of acetylcholine (0.5 mL/min) at the doses of 1, 3, 10, and 30 μg/min (for 2 minutes at each dose) through the Doppler catheter. We confirmed that acetylcholine at the dosage of 30 μg/min caused the maximum increase in coronary blood flow, because infusion of acetylcholine at the dose of 60 μg/min did not further increase coronary blood flow velocity in all patients. It has been demonstrated that papaverine (10 mg) injected into the left main coronary artery evokes the maximal increase in coronary blood flow velocity with no changes in arterial pressure and heart rate.

After completion of the study with one drug, we waited for at least 5 minutes before beginning infusion of the next drug. Coronary arteriography was performed before and 2 minutes after each dose of acetylcholine. Mean and phasic coronary blood flow velocities, arterial blood pressure, and heart rate were monitored continuously and recorded on a recorder (Nihon-Kohden polygraph system, Tokyo). Values during a steady-state condition were used for later analysis.

**Drugs**

Acetylcholine chloride (Dai-ichi Pharmaceutical Co., Tokyo) and papaverine (Dai-Nippon Pharmaceutical Co., Tokyo) were used.

**Statistical Analysis**

Data are expressed as mean±SD. The relationship between various hemodynamic variables such as coronary blood flow and age was assessed by a linear regression analysis. To characterize the blood flow response to acetylcholine, the slope of the percent increase in coronary blood flow in response to acetylcholine (y axis, percent increase in blood flow; x axis, log[doses (μg/min) of acetylcholine]) was determined using a linear regression analysis by plotting the blood flow response against four doses of acetylcholine in each patient. Correlation coefficient ranged from .75 to .99 (mean±SD, .89±.11).

When serial changes in arterial pressure, heart rate, arterial diameter, and coronary blood flow were compared, ANOVA for repeated measures followed by Bonferroni’s multiple comparison test was used. Student’s t test was used for comparison of paired or unpaired data. P<.05 was considered significant.

**Results**

Table 1 summarizes the baseline diameter of the proximal left anterior descending coronary artery and the vasomotor responses of the vessel, mean arterial pressure, and heart rate to acetylcholine. During infusion of either acetylcholine or papaverine, no significant changes in mean arterial pressure or heart rate were noted. There was a significant inverse correlation between age and the baseline vessel diameter (y=3.96 −0.017x, r=−.86, P<.001; y axis, diameter [mm]; x axis, age [years]).
Intracoronary infusion of acetylcholine at the dosages of 1, 3, and 10 µg/min induced variable responses of the epicardial coronary artery in patients with an increase or decrease in the diameter, whereas acetylcholine at the high dosage (30 µg/min) decreased the diameter in all patients. There was a significant correlation between ages and the percent changes in the diameter evoked with acetylcholine at the dosages of 1, 3, and 10 µg/min: \( y = 16.6 - 0.25x \), \( r = -.7, P < .001 \) for 1 µg/min; \( y = 15.6 - 0.26x, r = -.7, P < .001 \) for 3 µg/min; and \( y = 14.9 - 0.41x, r = -.8, P < .001 \) for 10 µg/min; where \( y \) is the percent change in the diameter, and \( x \) is age (years). There was no correlation between ages and the diameter responses to acetylcholine at the dosage of 30 µg/min (\( P = -.137 \)).

Acetylcholine increased estimated coronary blood flow in a dose-dependent manner (increase: 46±45%, 132±82%, 269±118%, and 363±111% at dosages of 1, 3, 10, and 30 µg/min, respectively). The maximum blood flow response occurred in two patients at dosages of 10 µg/min and in 16 patients at the dosage of 30 µg/min. Peak coronary blood flow response to acetylcholine and coronary blood flow response to papaverine in each patient are shown in Table 2. As shown in Fig 1A, the peak increase in coronary blood flow with acetylcholine varied widely among patients (from 220% to 560%) and correlated significantly \( (r = -.86, P < .001) \) with age, whereas there was a trend of decrease in the coronary blood flow response to papaverine with aging \( (r = -.44, P = .07) \). We compared the percentage of the peak coronary blood flow response to acetylcholine with that to papaverine in each patient to assess the effect of aging on endothelium-dependent control of coronary blood flow. The percentage of the response decreased significantly with age (Fig 1B). The slope of the blood flow relation to acetylcholine (\( y \) axis, percent increase in coronary blood flow; \( x \) axis, log[doses of acetylcholine]) also correlated significantly with age \( (r = -.78, P < .001) \) (Fig 2).

### Discussion

The major new finding of this study is that the coronary blood flow response to acetylcholine (an endothelium-dependent vasodilator) decreased significantly with aging, whereas the blood flow response to papaverine (an endo-

<table>
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<tr>
<th>Age (years)</th>
<th>Sex</th>
<th>ACH</th>
<th>PAP</th>
<th>ACH/PAP</th>
<th>Slope of acetylcholine dose-flow relation (%/loggg/min)</th>
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<td>540</td>
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CBF, coronary blood flow; ACH, acetylcholine; PAP, papaverine.

It has been suggested that hypercholesterolemia, hypertension and left ventricular hypertrophy, and mild atherosclerotic lesions are associated with impaired endothelium-dependent vasodilation in epicardial coronary arteries in humans. Therefore, we studied the relatively normal patients who had atypical chest pain and angiographically normal coronary arteries and excluded those who had hypercholesterolemia, hypertension or left ventricular hypertrophy, and diabetes mellitus. Such patient selection allowed us to determine the specific effect of aging on endothelium-dependent dilation of the coronary vasculature.

**Endothelial Dysfunction in Large Epicardial Coronary Arteries**

Our finding of diminished vasodilation of the large epicardial coronary arteries in response to acetylcholine correlated with aging is consistent with results of previous studies. Vita et al showed the inverse correlation between age and the slope of diameter response to intracoronary infusion of acetylcholine in patients with angiographically normal coronary arteries. However, most patients in the study by Vita et al had various risk factors for coronary artery disease. Our results show that vasomotor responses of the epicardial coronary artery to the low dosages (1, 3, and 10 µg/min) correlated inversely with aging but that the response to the high dose (30 µg/min) of acetylcholine did not. The endothelium-dependent vasodilating response of the epicardial coronary arteries to acetylcholine is a composite of receptor-mediated and flow-related stimulation. Therefore, the diminished vasodilation in
the epicardial coronary arteries in response to acetylcholine correlated with age in the results of previous studies,\(^7,8\) as well as the present study might be caused by an effect of aging in the epicardial vessels or by secondary effects due to less flow-mediated vasodilation of the epicardial vessels.\(^21,22\) Lack of correlation between age and response to the high dose of acetylcholine may be due in part to a predominantly direct vasoconstricting effect by the high dose of acetylcholine resulting in blunted endothelium-dependent vasodilation.

An interesting finding of this study is that there was a significant inverse correlation between age and the baseline diameter of the epicardial vessels. This finding may suggest the presence of certain structural changes in coronary arterial wall (early atherosclerosis) as shown by pathological and echocardiographic studies,\(^23,24\) because atherosclerosis is an age-related phenomenon. Age-related structural changes such as intimal thickening may be a cause of the diminished vasodilation of the epicardial vessels in response to acetylcholine demonstrated in this study.

**Endothelial Dysfunction in Resistance Coronary Arteries**

As previously demonstrated in humans with the intracoronary Doppler catheter technique used in the present study,\(^16,25\) intracoronary administration of acetylcholine progressively increased coronary blood flow in our patients. It is assumed that acetylcholine-induced increase in coronary blood flow resulted from endothelium-dependent vasodilation of resistance artery.\(^20,26-28\)

The most important finding of this study is that the endothelium-dependent increase in coronary blood flow evoked by acetylcholine—not only the maximum blood flow response but also the slope of the flow response to acetylcholine—was significantly and inversely correlated with aging. The percentage of the coronary blood flow response to acetylcholine compared with the response to papaverine (ie, coronary flow reserve attributable to endothelium-dependent vasodilation) also was inversely correlated with aging. These findings strongly suggest that endothelium-dependent dilation of resistance coronary artery is impaired with aging in humans. Our results are consistent with previous findings in animals that endothelium-dependent vasodilation in cerebral and peripheral resistance arteries decreased with age.\(^5,6\) This study appears to be the first demonstration that endothelium-dependent dilation of resistance coronary artery is impaired with increasing age in humans.

We consider it unlikely that the attenuated coronary blood flow response to acetylcholine with age resulted from flow-limiting vasoconstriction of the epicardial coronary vessels because the degree of epicardial coronary vasoconstriction by acetylcholine at the high dosage of 30 \(\mu\)g/min was small (15.7±3.6%). It has been reported that
vasoconstriction of large coronary artery by 12±5% (diameter) in response to ergonovine does not alter the coronary blood flow response to intracoronary injection of 10 mg papaverine.29 We did not measure changes in arterial cross-sectional area of the large epicardial coronary vessels after papaverine (see “Methods”), which results in underestimation of the coronary blood flow response to papaverine. It is unlikely that this limitation is critical because vasodilation of the large epicardial vessels in response to papaverine does not correlate with aging (authors’ unpublished observation).

We did not explore mechanisms of the attenuated coronary blood flow response to acetylcholine in this study, but they may relate to age-related decrease in release of endothelium-dependent relaxing factors, inactivation of endothelium-dependent relaxing factors, or concomitant release of constricting factors by microvascular endothelial cells. Kuo et al30 recently demonstrated that impaired endothelium-dependent dilation of coronary microvessels in atherosclerotic animals was restored by administration of L-arginine (a precursor of endothelium-dependent relaxing factors31,32), suggesting the important role of endothelium-dependent relaxing factors in control of coronary blood flow in atherosclerosis. Future studies are needed to determine the role of age-related endothelial dysfunction in the coronary microcirculation.

Conclusions

Endothelium-derived vasoactive substances such as nitric oxide play an important part in myocardial perfusion.9,10,20 This study indicates that endothelium-dependent vasodilation of the coronary vasculature evoked by acetylcholine may be impaired with increasing age in humans, which may contribute to altered myocardial perfusion and the manifestation of coronary artery disease in patients with advanced age. Age-related endothelial dysfunction may contribute to the development of coronary artery disease in humans.

References

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