Endothelial dysfunction in human intramyocardial small arteries in atherosclerosis and hypercholesterolemia

ANNE COOPER AND ANTHONY M. HEAGERTY
Department of Medicine, University Hospital of South Manchester, Manchester M23 9LT, United Kingdom

Cooper, Anne, and Anthony M. Heagerty. Endothelial dysfunction in human intramyocardial small arteries in atherosclerosis and hypercholesterolemia. Am. J. Physiol. 275 (Heart Circ. Physiol. 44): H1482–H1488, 1998.—Vascular responses of human intramyocardial small arteries were examined in vitro to assess the influence of atherosclerosis and risk factors for coronary artery disease on endothelium-dependent relaxation. Recipient hearts were obtained from patients with ischemic (n = 14) and nonischemic (n = 13) cardiomyopathy undergoing heart transplantation. Small intramyocardial coronary arteries (mean internal diameter 313 ± 11 μm) were mounted on a wire myograph for measurement of morphology and isometric tension. Vasodilation was examined after preconstriction with U-46619, a thromboxane A₂ analog. Endothelium-dependent relaxation to acetylcholine and bradykinin was impaired in patients with ischemic compared with nonischemic cardiomyopathy (P < 0.01 and P < 0.001, respectively). Endothelium-independent relaxation to sodium nitroprusside was preserved. Incubation with L-arginine (3 mmol/l) did not improve endothelium-dependent relaxation to acetylcholine or bradykinin. With the use of stepwise multivariate analysis, hypercholesterolemia, but no other risk factor for atherosclerosis, was independently associated with impaired endothelium-dependent relaxation to acetylcholine (r = −0.50, P = 0.05) but not to bradykinin. Endothelial dysfunction in intramyocardial small arteries may predispose patients with nonobstructive epicardial atherosclerosis and hypercholesterolemia to myocardial ischemia.

endothelium-derived relaxing factor; microcirculation

THE VASCULAR ENDOTHELIUM plays a crucial role in modulating vasomotor tone in both large conduit and small resistance-sized arteries through the release of vasoactive hormones such as endothelium-derived relaxing factor (EDRF), prostacyclin, endothelium-derived hyperpolarizing factor (EDHF), and endothelin (15).

It is well established that atherosclerosis often potentiates vasoconstrictor responses and impairs endothelium-dependent relaxation (10, 23). This has been demonstrated in human coronary epicardial arteries by in vivo (14, 25) and in vitro (7) studies. However, the coronary microcirculation is the major determinant of coronary blood flow and myocardial perfusion (1). Animal studies in which a model of atherosclerosis induced by a high-cholesterol diet was used suggest that the functional consequences of atherosclerosis extend into the coronary microcirculation despite the absence of overt atherosclerotic lesions there (13, 21). Although the epicardial atherosclerosis induced is similar to that in humans (13), the time for induction is much shorter and levels of serum cholesterol are up to 20 times above normal, levels rarely encountered in human coronary artery disease. Because hypercholesterolemia is known to decrease endothelium-dependent relaxation even before morphological evidence of atherosclerosis appears (2, 23), abnormalities detected in the coronary microcirculation could have been due to hypercholesterolemia rather than proximal atherosclerosis per se.

Only a limited number of studies have examined the vasomotor control of the human coronary microcirculation and have done so indirectly using indexes of coronary blood flow through the whole coronary circulation (6, 25). However, in vitro studies of intramyocardial small arteries have certain advantages over in vivo techniques, because there is considerable heterogeneity in responses of vessels of different size and in vitro studies are not confounded by activation of compensatory control mechanisms. The in vitro myograph permits the examination of small arteries of ~300 μm. Intramyocardial small arteries of this size contribute ~25% of total coronary resistance (1) and, unlike epicardial arteries, do not develop atherosclerosis (3).

Our aims were to examine, in vitro, human intramyocardial small arteries to determine 1) contractile and dilator responses, 2) the influence of proximal epicardial atherosclerosis and coronary risk factors on endothelium-dependent and -independent relaxation, and 3) possible mechanisms for impaired endothelium-dependent relaxation. In particular, we examined endothelium-dependent relaxation to a number of receptor agonists acting on different signal transduction pathways and the effect of incubation with the EDRF precursor L-arginine.

METHODS

Patient demographics. Human recipient hearts were examined from patients undergoing heart transplantation for idiopathic and ischemic cardiomyopathy or heart-lung transplantation for pulmonary hypertension secondary to patent ductus arteriosus. All patients with idiopathic or ischemic cardiomyopathy had end-stage congestive heart failure. Diagnosis was made preoperatively on the basis of coronary angiography demonstrating the absence or presence of coronary artery disease and/or myocardial biopsy demonstrating evidence of cardiomyopathy and later confirmed by histological examination of the explanted heart. An assessment of left ventricular function was made by left ventriculography and echocardiography.

Group A consisted of 13 patients with normal nonatherosclerotic epicardial coronary arteries. Group B consisted of 14 patients with atherosclerotic epicardial coronary arteries and ischemic cardiomyopathy. Intramyocardial small arteries obtained from these patients were used to examine vasomotor responses and the influence of proximal atherosclerosis and coronary risk factors on endothelium-dependent and -independent relaxation. Coronary risk factors identified included age,
Lumen diameter was calculated at would have under a transmural pressure of 100 mmHg. Vasodilator responses were assessed to acetylcholine (10^{-6} mol/l) in the presence or absence of propranolol (10^{-6} mol/l) to produce a stable plateau before and after incubation with 3 mmol/l L-arginine for 45 min to determine whether impairment of endothelium-dependent relaxation resulted from a deficiency of this physiological precursor of EDRF. Time-control experiments were performed to ensure that there was no deterioration in the response to these agents during this incubation period (n = 4).

Statistical analysis. Demographic and morphological data were compared using an unpaired Student’s t-test. Analysis of variance for repeated measures was used to compare dose-response curves in groups A and B. Because there was a significant difference in age and a near-significant difference in serum cholesterol between groups A and B, the effect of proximal atherosclerosis and coronary risk factors on endothelium-dependent relaxation to acetylcholine and bradykinin were examined in these groups. Coronary risk factors were considered as continuous and/or categorical variables. Simple linear regression analysis was used to assess the effects of continuous variables (serum cholesterol and age). The unpaired Student’s t-test was used to examine the effects of categorical coronary risk factors [previous cigarette smoker, family history of ischemic heart disease (IHD) and hypertension] and proximal atherosclerosis. Limited numbers precluded the evaluation of the effect of gender and diabetes on endothelial function. The effects of various coronary risk factors and proximal atherosclerosis on endothelium-dependent relaxation to acetylcholine and bradykinin were then examined by multiple stepwise regression analysis. All data are expressed as means ± SE. Significance was accepted at P < 0.05.

RESULTS

Demographic data. Baseline demographic data for groups A and B are shown in Table 1. The mean small artery internal diameter was 316.7 ± 16 µm in group A and 312.8 ± 17 µm in group B. There was a significant difference in the mean age (P < 0.01) and a near-significant difference in serum cholesterol (P = 0.06). Ten patients in each group were previous cigarette smokers, and patients in group B included five patients with a previous history of hypertension and three patients with diabetes mellitus (2 diet controlled, 1

<table>
<thead>
<tr>
<th>Table 1. Demographic data</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Sex, M/F</td>
</tr>
<tr>
<td>Age, yr</td>
</tr>
<tr>
<td>Cholesterol, mmol/l</td>
</tr>
<tr>
<td>Glucose, mmol/l</td>
</tr>
<tr>
<td>SBP, mmHg</td>
</tr>
<tr>
<td>DBP, mmHg</td>
</tr>
<tr>
<td>LVEF, %</td>
</tr>
<tr>
<td>LVDD, cm</td>
</tr>
<tr>
<td>Heart weight, g</td>
</tr>
<tr>
<td>Vessel diameter, µm</td>
</tr>
</tbody>
</table>

Values represent means ± SE. M/F, male/female; SBP and DBP, systolic and diastolic blood pressure; LVEF, left ventricular ejection fraction; LVDD, left ventricular end-diastolic diameter. *P < 0.01, Student’s t-test between groups A and B.
Table 2. Type of medication and number of patients taking each medication at time of transplantation

<table>
<thead>
<tr>
<th>Type of Medication</th>
<th>Group A</th>
<th>Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACE inhibitors</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td>Diuretics</td>
<td>10</td>
<td>13</td>
</tr>
<tr>
<td>Anticoagulation</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Digoxin</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>Amiodarone</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Nitrate</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Allopurinol</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Ranitidine/cimetidine</td>
<td>2</td>
<td>5</td>
</tr>
</tbody>
</table>

ACE, angiotensin-converting enzyme. The following medications were also taken by no more than 1 patient in either group: persantin, flosequinan, calcium antagonist, quinine, enoxamone, xamoterol, thiamine.

There was no difference in glucose levels at the time of surgery. Medication usage at the time of transplantation in groups A and B is indicated in Table 2. Baseline demographic data for group C are also shown in Table 1.

Morphology studies. Morphological data for groups A and B are shown in Table 3. There is no significant difference in small artery internal diameter, intima thickness, media thickness, media-to-lumen ratio, or cross-sectional area between the two groups.

Small artery studies. U-46619, a thromboxane A2 analog, produced a dose-dependent constriction. U-46619 was the only agonist that produced significant vasoconstriction. There was no overall difference between groups A and B, and no significant difference in the maximum tension induced (3.46 ± 0.46 mN in group A, 2.74 ± 0.24 mN in group B; Fig. 1).

No contractile response was obtained in vessels of either group to norepinephrine in the absence or presence of propranolol. In preconstricted arteries, norepinephrine produced a dose-dependent relaxation (maximum: 35.1 ± 8.9% in group A, 22.1 ± 3.2% in group B; P = not significant). Norepinephrine-induced relaxation was predominantly β-adrenergic because relaxation was attenuated but not abolished by the presence of propranolol (maximum: 19.8 ± 8.6% in group A, 10.5 ± 2.0% in group B; P < 0.05). There was no significant difference in norepinephrine-induced relaxation between the two groups, either in the absence or presence of propranolol.

In general, acetylcholine did not produce a contractile response, although four vessels in group B produced a minimal degree of nonsustained constriction (tension = 0.15 ± 0.04 mN/mm) at the highest concentration of acetylcholine, 10−5 mol/l. Acetylcholine produced a dose-dependent relaxation in preconstricted arteries. Some vessels reconstructed at the higher concentrations of acetylcholine. Endothelium-dependent relaxation was significantly impaired in group B compared with group A in response to acetylcholine (P < 0.01, Fig. 2).

In preconstricted arteries, bradykinin and substance P produced dose-dependent relaxation. Endothelium-dependent relaxation to bradykinin was significantly impaired in group B compared with group A (P < 0.001, Fig. 3). Although substance P produced vasodilation, problems with tachyphylaxis were encountered. There was a trend toward impaired endothelium-dependent relaxation to substance P in group B, but this was not significant (Fig. 4). In contrast, endothelium-independent relaxation to sodium nitroprusside produced identical relaxation responses in small coronary arteries from groups A and B (P = 0.76; Fig. 5). Relaxation to acetylcholine, bradykinin, and substance P was abolished by endothelial denudation, suggesting that relaxation to these agonists is endothelium dependent.

Risk factors. There were no significant relationships between relaxation responses to bradykinin and age, cholesterol, hypertension, smoking, or family history of IHD by univariate analysis. Proximal atherosclerosis

Table 3. Morphology data

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th>Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intimal thickness, µm</td>
<td>1.65 ± 0.27</td>
<td>1.60 ± 0.25</td>
</tr>
<tr>
<td>Media thickness, µm</td>
<td>10.02 ± 0.77</td>
<td>10.72 ± 0.84</td>
</tr>
<tr>
<td>Media-to-lumen ratio, %</td>
<td>3.31 ± 0.30</td>
<td>3.64 ± 0.37</td>
</tr>
<tr>
<td>CSA, µm² × 10⁵</td>
<td>10.22 ± 1.2</td>
<td>10.89 ± 0.81</td>
</tr>
</tbody>
</table>

Values represent means ± SE. CSA, cross-sectional area.
significantly impaired relaxation to bradykinin at $10^{-7}$ mol/l ($P < 0.01$), $10^{-6}$ mol/l ($P = 0.0001$), and $10^{-5}$ mol/l ($P < 0.05$) after adjustment for risk factors by multiple linear regression analysis.

There were no significant relationships between relaxation responses to acetylcholine and age, hypertension, smoking, or a family history of IHD by univariate analysis. The maximum relaxation response to acetylcholine (which was not necessarily at the highest concentration of acetylcholine) and the relaxation response to acetylcholine at $10^{-5}$ mol/l both correlated negatively with serum cholesterol ($r = -0.50$ and $-0.46$, respectively; $P < 0.05$). Both proximal atherosclerosis and serum cholesterol independently predicted the acetylcholine response by multiple linear regression analysis. Cholesterol had more influence at higher concentrations of acetylcholine ($10^{-5}$ mol/l and maximum relaxation, $P < 0.05$), whereas proximal atherosclerosis also influenced acetylcholine relaxation at slightly lower concentrations ($10^{-6}$ mol/l ($P < 0.01$) and $10^{-5}$ mol/l ($P < 0.01$)) and maximum relaxation ($P < 0.05$).

Response to L-arginine. Preincubation of small arteries from group C with 3 mmol/l L-arginine did not improve endothelium-dependent relaxation to acetylcholine (Fig. 6) or bradykinin (Fig. 7). Time-control experiments demonstrated that there was no decline in responses to acetylcholine or bradykinin after the 45-min incubation period (Fig. 8, A and B, respectively).

**DISCUSSION**

This is the first in vitro examination of human intramyocardial small arteries. In contrast to its role in atherosclerotic epicardial arteries, acetylcholine produces vasodilation rather than vasoconstriction of intramyocardial small arteries. Both proximal atherosclerosis and hypercholesterolemia are independently associated with an impairment of endothelium-dependent relaxation in human intramyocardial small arteries. Endothelium-dependent relaxation is impaired to more than one receptor agonist acting on different signal transduction pathways in patients with epicardial atherosclerosis but is selectively impaired to acetylcholine in patients with hypercholesterolemia. A history of hypertension, age, and previous smoking and a family history of IHD are all coronary risk factors that were not associated...
with an impairment of endothelial function in intramyocardial small arteries. Endothelium-independent relaxation is maintained, suggesting normal vascular smooth muscle function. Furthermore, we demonstrated that incubation with L-arginine, a physiological precursor to EDRF, did not restore endothelium-dependent relaxation in patients with ischemic cardiomyopathy, unlike previous studies in hypercholesterolemia, suggesting adequate stores of L-arginine.

Proximal atherosclerosis is associated with impairment of endothelium-dependent relaxation to both acetylcholine and bradykinin, which act on different intracellular signal transduction pathways, suggesting a nonspecific abnormality in endothelial function. This is not accounted for by morphological abnormalities because human intramyocardial small arteries of this size, in contrast to epicardial arteries, do not develop atherosclerotic lesions (3). Morphological data do not demonstrate any differences in media or intima thickness. Impairment of endothelium-dependent relaxation has been demonstrated previously (6) to acetylcholine in vivo in small arteries of patients with proximal atherosclerosis. This observation can now be extended to in vitro studies and to the bradykinin receptor. Although it is uncertain whether endogenous acetylcholine plays a role in control of vasomotor tone, bradykinin does play a role in humans in stimulating the release of EDRF under baseline conditions and during increases in flow (9). As shown by multivariate analysis, hypercholesterolemia is associated with a selective impairment of endothelium-dependent relaxation to acetylcholine, possibly due to disruption of the signal transduction pathway. This selective impairment has been seen in human epicardial arteries and animal models with progression to a generalized impairment only when atherosclerosis has developed (22, 23). This suggests that the mechanisms associated with impairment of endothelium-dependent relaxation in intramyocardial small arteries in the presence of proximal atherosclerosis and hypercholesterolemia are different.

Other differences in the potential mechanism(s) responsible for endothelial dysfunction are evident. EDRF ultimately causes relaxation by activating guanylate cyclase in vascular smooth muscle. Endothelium-independent relaxation to sodium nitroprusside was maintained in ischemic cardiomyopathy, indicating that impaired endothelium-dependent relaxation is not secondary to an abnormal vascular smooth muscle response. Studies have suggested, however, that hypercholesterolemia is associated with a mild impairment in endothelium-independent relaxation in human small arteries from the peripheral circulation (8).

L-Arginine undergoes hydroxylation of its terminal guanidino nitrogen group to form EDRF, a reaction catalyzed by nitric oxide synthase. Endogenous intracellular stores of L-arginine are usually sufficient to saturate the enzyme nitric oxide synthase under normal conditions. Incubation of intramyocardial small arteries from patients with ischemic cardiomyopathy with L-arginine did not improve endothelium-dependent relaxation, suggesting adequate stores of L-arginine. A lack of improvement in this study could be attributed to the chosen concentration of L-arginine or incubation time, but identical parameters have been shown to improve endothelium-dependent relaxation in our laboratory in hypercholesterolemic human peripheral small arteries in the small vessel myograph (8). Therefore, extracellularly added L-arginine must penetrate endothelial cells under these conditions. In
contrast to its role in atherosclerosis, l-arginine has improved endothelium-dependent relaxation in hypercholesterolemia (5, 8, 13).

Because EDRF is degraded by oxygen-derived free radicals, impairment of endothelium-dependent relaxation in atherosclerosis and hypercholesterolemia could be due to an inadequate endothelial intracellular antioxidant defense system and accelerated degradation of EDRF. A number of studies (16, 17) support the concept of accelerated degradation rather than decreased production of EDRF in atherosclerosis. Thus the mechanisms by which hypercholesterolemia and atherosclerosis impair endothelium-dependent relaxation remain unknown, although many potential mechanisms probably contribute.

A limitation to the study was the use of cardiomyopathy patients as “control” patients and the presence of congestive cardiac failure in both groups. However, it is clearly not possible to examine normal human hearts in vitro. No unused donor hearts were available for study. Cardiomyopathy has been shown to impair endothelium-dependent relaxation to acetylcholine in the coronary microcirculation, with a modest impairment of endothelium-independent relaxation (11, 24). These observations suggest endothelial dysfunction, but extra-vascular forces may also have contributed to the impaired coronary blood flow response. Endothelial dysfunction in idiopathic cardiomyopathy may be secondary to heart failure. Recent studies (4, 12, 20) have demonstrated impaired endothelium-dependent relaxation to acetylcholine in congestive cardiac failure in peripheral and coronary arteries. Congestive cardiac failure was present in patients from both groups, and therefore any impairment in the acetylcholine response as a result of heart failure would not necessarily be detected.

In conclusion, proximal atherosclerosis is associated with impairment of endothelium-dependent relaxation via different intracellular signal transduction pathways in human small intramyocardial arteries. It is not secondary to an impaired responsiveness of vascular smooth muscle, because endothelium-independent relaxation was normal, or to a deficiency of l-arginine substrate, because incubation with l-arginine did not improve endothelium-dependent relaxation. Hypercholesterolemia, but no other risk factor, is associated with an impairment of endothelium-dependent relaxation, but this abnormality is confined to the acetylcholine response. Impairment of endothelium-dependent relaxation in intramyocardial small arteries will be associated with a decrease in coronary flow reserve and therefore will play an important role in the pathogenesis of myocardial ischemia, exacerbating the pathophysiological consequences of coronary artery disease.

We thank the cardiac transplantation team and coordinators at Wythenshawe Hospital, Manchester, U.K., for their help and cooperation in providing specimens for this study.

Address for reprint requests: A. Cooper, Dept. of Cardiology, Hope Hospital, Scott Lane, Salford, Manchester M6 BHD, U.K.

Received 20 October 1997; accepted in final form 22 June 1998.

REFERENCES


