Cellular and Molecular Biology of Atherosclerosis

With 37 Figures

Antonio M. Gotto, Jr (Ed.)

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Cover illustrations: Ch. 1, Fig. 2. Segment of a coronary artery which is entirely normal. (Reproduced by permission from Arnett et al. 1979.)
Ch. 7, Fig. 1. Increased leukocyte adherence to the aortic endothelial surface after MM-LDL injection.

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Preface

Atherosclerotic cardiovascular disease remains the major cause of death and disability in Western society. The field of atherosclerosis research has grown tremendously over the last forty years, shedding a great deal of light on the contributing factors and natural history of the disorder and enabling strategies for its treatment and prevention. Some of the greatest strides in this field in recent years have derived from advances in molecular biology techniques. These strides were chosen for emphasis in the most recent Princess Lilian symposium, whose proceedings this volume represents.

Historically, the Princess Lilian meetings have been small ones aimed at bringing together investigators from diverse specialties to discuss a particular subject. The most recent meeting was no exception and included clinicians, clinical investigators, and researchers in basic science.

The symposium began with an extensive review of coronary morphopathological findings in patients who died of coronary heart disease. Any rational hypothesis of atherogenesis must take into account clinical findings, and any attempt to bridge the gap between experimental laboratory findings and studies in man is highly desirable.

Three chapters focus on endothelial injury: one on the nitric oxide pathway in physiology and pathology, a second on the activation of endothelial cells, and a third on the monocyte and endothelial injury. Still another chapter examines growth factors, in particular the fibroblast growth factor in atherogenesis.

Much interest has recently focused on the possible role of oxidized and modified lipoproteins in atherogenesis. Clinical trials are being planned to test whether the administration of antioxidants will protect against atherosclerosis. The roles of modified lipoproteins are reviewed in this volume, as is the possible contribution of lipoprotein [a], an independent predictor of coronary disease. The roles of apolipoproteins are also discussed, from the molecular biology of apolipoprotein B (the most atherogenic of the apolipoproteins), to

apolipoprotein mutations, to the regulation of lipoprotein metabolism through receptor mechanisms. Recent evidence has suggested that the presence of a large fraction of small, dense low-density lipoprotein particles in conjunction with elevations of triglycerides and low levels of high-density lipoprotein predisposes to coronary disease. One mutation that could produce this disorder is the heterozygous state for lipoprotein lipase deficiency. Postprandial lipemia, low levels of high-density lipoprotein, and hypertriglyceridemia are discussed from the points of view of basic research and clinical manifestations.

No specific marker of apolipoproteins using the restriction fragment-length polymorphism (RFLP) technique has yet been shown to be predictive in coronary disease in population groups. However, a number of interesting observations have been made from RFLP studies regarding apolipoprotien mutations, which was the subject of one symposium presentation.

Another chapter examines the etiology of hypertension in a strain of genetically hypertensive rats. The way in which hypertension and hyperlipidemia interact to induce atherosclerosis is not known.

The volume closes with a current and future perspective on clinical trials with lipid-lowering agents.

The meeting from which this book was derived also fostered much productive discussion not included in this volume, since participants were able to interact informally as well to discuss their findings. This opportunity – within a spectacular program of social activities – was made possible through the generosity of Princess Lilian of Belgium and the Princess Lilian Cardiology Foundation.

July 1991 *Antonio M. Gotto, Jr, MD, DPhil*

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Chapter 1

Morphological Findings in the Coronary Arteries in Fatal Coronary Artery Disease

W. C. Roberts

Introduction

Atherosclerotic coronary artery disease (CAD) is the most common cause of death in the Western world. One American dies every minute because of atherosclerotic CAD. In the USA alone, about 6 million persons have symptomatic myocardial ischemia because of atherosclerotic CAD. About 250 000 coronary artery bypass grafting operations were performed in 1990 in the USA and about 300 000 coronary angioplasty procedures. The cause of atherosclerosis is now clear. The evidence is overwhelming that atherosclerosis is a cholesterol problem. The higher the blood total cholesterol level (specifically the low-density lipoprotein level) the greater the chance of developing symptomatic CAD, the greater the chance of having fatal CAD, and the greater the extent of the atherosclerotic plaques. Furthermore, lowering the blood total cholesterol level decreases the chances of having symptomatic or fatal CAD and the greater the chance that some atherosclerotic plaques will actually become smaller, i.e. regress. Although the coronary arteries have been examined by visual inspection at necropsy for over 100 years, only in recent years has the extent of the atherosclerotic process in the coronary arteries in patients with symptomatic or fatal CAD become appreciated. This chapter initially reviews the status of the major epicardial coronary arteries in various subsets of patients with fatal atherosclerotic CAD. It then describes the effects of angioplasty on these arteries, some observations in patients having thrombolytic therapy and coronary bypass, and then various complications of myocardial ischemia.

Number of Major Epicardial Coronary Arteries Severely Narrowed in the Various "Coronary Events"

The most common method for describing the severity of CAD in patients with clinical evidence of myocardial ischemia is by the number of major epicardial coronary arteries narrowed >50% in luminal diameter by angiogram. Thus, patients are divided into groups of 1-vessel, 2-vessel, 3-vessel and "left main" CAD. Because a 50% diameter reduction in general is equivalent to a 75% cross-sectional area narrowing, the cut-off point of "significant" as opposed to "insignificant" luminal narrowing at necropsy is the 75% cross-sectional area point. Physiologically, there is no obstruction to arterial flow until the lumen is narrowed >75% in cross-sectional area.

Table 1.1 summarizes the number of major (right, left main, left anterior descending, and left circumflex) epicardial coronary arteries narrowed >75% in cross-sectional area by atherosclerotic plaque alone in patients with fatal CAD (Roberts 1989). Among the 129 patients with fatal CAD studied at necropsy, 516 major epicardial coronary arteries were examined and of them 345 (67%) were narrowed at some point 76%–100% in cross-sectional area by atherosclerotic plaque. In contrast, of 40 control subjects, mainly victims of acute leukemia, and without clinical evidence of myocardial ischemia during life, 160 major epicardial coronary arteries were examined and of them 60 (37%) were narrowed at some point >75% in cross-sectional area by plaque. Among the 129 coronary patients, only 11 (8%) had a single coronary artery severely narrowed (controls = 23%); 37 (29%) had two arteries so narrowed (controls = 13%); 64 (50%) had three arteries severely narrowed (controls = 5%) and 17 patients (13%) had all four major arteries so narrowed (controls = 0). Thus, of the four major coronary arteries in the coronary patients an average of 2.7 were narrowed >75% in cross-sectional area by plaque, and among the control subjects 0.7 of four.

Table 1.1. Number of major (right, left main, left anterior descending and left circumflex) coronary arteries narrowed >75% in cross-sectional area by atherosclerotic plaque in fatal coronary artery disease

Coronary event	Pts (*n*)	Mean age (yrs)	No. of four arteries/Pt > 75% ↓ in CSA by plaque				
			4	3	2	1	Mean
Sudden coronary death	31	47	3	20	6	2	2.8
Acute myocardial infarction	27	59	3	14	10	0	2.7
Healed myocardial infarction							
Asymptomatic	18	66	0	7	7	4	2.2
Chronic CHF without aneurysm	9	63	0	3	5	1	2.2
Left ventricular aneurysm	22	61	1	12	6	3	2.5
Angina pectoris/unstable	22	48	10	8	3	1	3.2
Total (%)	129	56	17 (13)	64 (50)	37 (29)	11 (8)	2.7
Controls (%)	40	52	0 (0)	5 (5)	12 (13)	21 (23)	0.7

CHF = congestive heart failure; CSA = cross-sectional area

The numbers of major coronary arteries severely narrowed by atherosclerotic plaque among the various subsets of coronary patients was relatively similar except for the unstable angina patients (Table 1.1). Among the 31 *sudden coronary death* patients (Roberts and Jones 1979) all of whom died outside the hospital, usually within a few minutes of onset of symptoms of myocardial ischemia, an average of 2.8 of the four major arteries were severely narrowed, a number virtually identical to that of the 27 patients with *transmural acute myocardial infarction* (Roberts and Jones 1980), all of whom died in a coronary care unit. Only two of the 31 sudden death victims and none of the 27 acute myocardial infarction victims had only a single coronary artery ("1-vessel disease") severely narrowed.

The *healed myocardial infarction* group was divided into three subgroups. One consisted of patients who had had an acute myocardial infarction in the past and it had healed and thereafter there was never evidence of myocardial ischemia clinically, and these patients died from a non-cardiac cause, usually cancer (Virmani and Roberts 1981). Nevertheless, the average number of major coronary arteries severely narrowed at necropsy was 2.2 of four. Another subgroup consisted of patients who had chronic congestive heart failure after healing of an acute myocardial infarction but in the absence of a left ventricular aneurysm (Virmani and Roberts 1980). This group might be called *ischemic cardiomyopathy*. The average number of major coronary arteries severely narrowed in them also was 2.2 of four. The other subgroup of healed myocardial infarction patients had a true left ventricular aneurysm (Cabin and Roberts 1980). The average number of major coronary arteries severely narrowed in them was 2.5 of four.

The final subgroup consisted of 22 patients with *unstable angina pectoris* and all of them had had coronary artery bypass grafting procedures within seven days of death (Roberts and Virmani 1979). Preoperatively, all had normal left ventricular function and none had had a clinically apparent acute myocardial infarct or congestive heart failure at any time. The average number of major coronary arteries severely narrowed by plaque was 3.2 of four, and 10 of the 22 patients had severe narrowing of the left main coronary artery as well as severe narrowing of the other three major coronary arteries ("4-vessel disease"). (Another study (Bulkley and Roberts 1976) has indicated that severe narrowing of the left main coronary artery usually is an indicator that the other three major arteries also are severely narrowed.) The unstable angina group thus had the largest average number of major coronary arteries severely narrowed of any of the subgroups but nevertheless this group of patients had excellent left ventricular function.

Quantitative Approach to Atherosclerotic Coronary Artery Disease: Amounts of Narrowing in Each 5-mm Segment of Each of the Four Major Coronary Arteries

Although the 1-, 2-, 3-, and 4-vessel disease approach has been useful clinically, this type of severity analysis might be thought of as a *qualitative* approach, and differences in degrees of coronary narrowing in the various subsets of coronary

patients is usually not discernible by this approach. To obtain a better appreciation of the extent of the atherosclerotic process in patients with fatal CAD, several years ago my colleagues and I began examining each 5-mm long segment of each of the four major coronary arteries (Roberts 1989). In adults, the average length of the right coronary artery is 10 cm; the left main, 1 cm; the left anterior descending, 10 cm, and the left circumflex, 6 cm. Thus, 27 cm of major epicardial coronary artery are available for examination in each adult. Because each 1 cm is divided into two 5-mm long segments, an average of 54 5-mm segments is available to examine in each heart. This approach not only allows one to ask how many of the 5-mm segments are narrowed 76%–100% in cross-sectional area, but also how many are narrowed 51%–75%, 26%–50%, and 0%–25%. This approach, in contrast to the 1-, 2-, 3-, 4-vessel disease approach, might be considered a *quantitative* one.

The same patients previously described by the qualitative approach also were examined at necropsy by the quantitative approach and the findings are summarized in Table 1.2. A total of 6461 5-mm segments were sectioned and later examined histologically. The sections were stained by the Movat method to delineate the internal elastic membrane. The findings in the 129 coronary patients were compared to those in 1849 5-mm segments in 40 control subjects. In each coronary subgroup the 5-mm segments from each of the four major coronary arteries were pooled together so by this approach the amount of narrowing in an individual patient was not discernible. The percentage of 5-mm segments narrowed 76%–100% in cross-sectional area by atherosclerotic plaque was 35% for the coronary patients and 3% for the control subjects; the percentage narrowed 51%–75% was 36% for the coronary patients and 22% for the control subjects. Thus, 71% of the 5-mm segments in the coronary patients were narrowed >50% in cross-sectional area by atherosclerotic plaque and 25% in the control subjects. In contrast, only 29% of the 5-mm segments in the coronary patients were narrowed <50% and only 8% even approached normal, i.e. narrowed 25% or less in cross-sectional area. In contrast, 75% of the 5-mm segments in the control subjects were narrowed <50% and 31% of them were normal or nearly normal. Thus, in the coronary patients 92% of the 6461 5-mm segments of the four major epicardial coronary arteries were narrowed >25% in cross-sectional area by atherosclerotic plaque. Accordingly, the coronary atherosclerotic process is a diffuse one in patients with fatal CAD. To believe that the atherosclerotic process is a focal one in patients with fatal CAD is to believe a myth.

Among the various subsets of coronary patients, those with *sudden coronary death* (Roberts and Jones 1979) and *acute myocardial infarction* (Roberts and Jones 1980) had similar percentages of 5-mm segments narrowed 76%–100% in cross-sectional area by plaque (36% and 34%, respectively); patients with *healed myocardial infarction* (Virmani and Roberts 1980, 1981; Cabin and Roberts 1980) as a group had the least severe narrowing (31% of segments narrowed >75%), and the patients with *unstable angina pectoris* (Roberts and Virmani 1979) had the most severe narrowing of all (48% of the 5-mm segments were narrowed >75% by plaque).

In an attempt to provide a single number for the amount of coronary arterial narrowing in each patient, a score system was utilized. A segment narrowed 0%–25% was assigned a score of 1; a segment narrowed 26%–50%, 2; a segment narrowed 51%–75%, 3; and one narrowed 76%–100%, a score of 4.