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Natural progression of atherosclerosis from pathologic intimal thickening to late fibroatheroma in human coronary arteries: A pathology study

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ABSTRACT

Objective: Smooth muscle cells, macrophage infiltration and accumulation of lipids, proteoglycans, collagen matrix and calcification play a central role in atherosclerosis. The early histologic changes of plaque progression from pathologic intimal thickenings (PIT) to late fibroatheroma lesions have not been fully characterized.

Methods: A total of 151 atherosclerotic coronary lesions were collected from 67 sudden death victims. Atherosclerotic plaques were classified as PIT without macrophage infiltration, PIT with macrophages, and early and late fibroatheromas. Presence of macrophages and proteoglycans (versican, decorin and biglycan) were recognized by specific antibodies while hyaluronan was detected by affinity histochemistry. Lipid deposition was identified by oil-red-O, and calcification was assessed following von Kossa and alizarin red staining.

Results: Lesion progression from PIT to late fibroatheroma was associated with increase in macrophage accumulation ($p < 0.001$) and decreasing apoptotic body clearance by macrophages (ratio of engulfed-to-total apoptotic bodies) ($p < 0.001$). Lipid deposition in lipid pool of PIT had a microvesicular appearance whereas those in the necrotic core were globular in nature. Overall, the accumulation of hyaluronan ($p < 0.001$), and proteoglycan versican ($p < 0.001$) and biglycan ($p = 0.013$) declined along with lesion progression from PIT to fibroatheromas. Microcalcification was first observed only within areas of lipid pools and its presence and size increased in lesions with necrotic core.

Conclusions: PIT to fibroatheroma lesions are accompanied by early lipid accumulation, followed by macrophage infiltration with defective clearance of apoptotic bodies along with decrease in proteoglycan and hyaluronan in lipid pools that convert to necrotic cores. Calcification starts in PIT and increases with plaque progression.

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1. Introduction

The natural history of atherosclerosis in humans is a dynamic process involving the progression of early lesions to advanced plaques that are responsible for the majority of acute ischemic cardiovascular events. The early development of atherosclerotic lesion in coronary arteries starts in industrialized nations in the second decade of their life [1]. However, the early atherosclerotic lesions do not always progress to late stage to cause catastrophic acute vascular events such as myocardial infarction or sudden cardiac death [2–4]. Since the mouse models do not mimic the progression of atherosclerotic plaque seen in man, it is essential that lesion progression be studied in greater detail in human

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arteries to advance our knowledge such that new diagnostic modalities can be developed to identify lesions that are precursors of the lesions that lead to catastrophic events of thrombosis.

The earliest feature of progressive atherosclerosis as described by the American Heart Association (AHA) classification is pathologic intimal thickening (PIT), which is characterized by extracellular lipid accumulation (lipid pools) that are rich in proteoglycans and hyaluronan. Inflammation plays a pivotal role in the progression and destabilization of atherosclerotic lesions [5]. The infiltration of macrophages is a distinctive characteristic of progression of PIT to fibroatheromas; however, the processes involved are poorly understood. It has been reported both in animal models and in man that macrophages apoptosis is an integral process of plaque progression [6,7]. Furthermore, increase in macrophage apoptosis, but also a decreased uptake of apoptotic bodies is thought to be of utmost importance in necrotic core formation in fibroatheroma [8].

Extracellular matrix (ECM) molecules such as proteoglycans and collagen play an important role in plaque mass but also govern lipid accumulation and the trafficking of inflammatory cells within the plaque. Many different proteoglycans and hyaluronan, which are non-fibrillar components of the ECM, have been identified in atherosclerotic plaques and are synthesized by vascular smooth muscle cells (SMCs) and influenced by growth factors [9–11]. The response-to-retention hypothesis in early atherogenesis states that atherogenic lipoproteins are retained in the intima by binding to ECM proteoglycans [12]. This hypothesis further states that lipoprotein–proteoglycan complexes exhibit increased susceptibility to oxidation and lead to uptake by macrophages to form foam cells. Proteoglycans have been reported to play a fundamental role in cellular and extracellular events associated with the pathogenesis of vascular lesions, such as thrombosis [13], lipid metabolism [14], and vascular SMC proliferation and migration [11]. Nakashima et al. [15] showed that in early human coronary atherosclerotic lesions, biglycan and decorin were distributed in the outer layer of diffuse intimal thickening when no lipids were detected. In PIT, decorin co-localized with the lipid in some instances but much less than biglycan; however, the presence and localization of hyaluronan and versican were not evaluated in their study.

In the current study, we not only studied the accumulation of hyaluronan and other proteoglycans in progression of plaque from PIT to late fibroatheroma but also showed how these relate to the presence of lipid, macrophage and apoptosis along with calcification to further our understanding of their relationship to one another. Elucidating the involvement of these pivotal processes of atherosclerotic progression, through which lipid pool convert to the necrotic core, is of utmost importance to prevent or slow the progress of advanced lesion development.

2. Methods

2.1. Study population

The coronary arteries for this study were obtained from the hearts of 67 patients (60 men, mean age = 47.6 ± 11.1 years) who had died suddenly of coronary causes (n = 41 cases) or non-coronary causes (n = 26 cases) (Table 1). A total of 151 coronary plaques of various phenotypes from early atherosclerosis lesions, PIT to fibroatheroma, were selected for further analysis. We selected 1 section from 1 lesion to represent the lesion morphology. The 151 lesions included 128 paraffin-embedded and 23 frozen sections. Morphometric analysis was performed in all paraffin-embedded lesions. Select 60 paraffin-embedded lesions were used for immunostaining for macrophages (CD68), T-lymphocytes (CD45RO), vasa vasorum (CD31/34), and nick-end in situ labeling for apoptosis, while 40 paraffin-embedded lesions were submitted for additional proteoglycan staining (10 of 40 lesions had been used in previous report [16]). In addition, 28 non-decalcified paraffin-embedded sections with lesion morphology PIT or early fibroatheroma were used for von Kossa and alizarin red staining. The additional 23 frozen sections were used for oil red O staining (Table 1, Supplemental Fig. 1).

Table 1
Patient and lesion characteristics.

Patient (n = 67)	
Age (years)	47.6 ± 11.1
Male gender	60 (90%)
Hypertension	39 (58%)
Hyperlipidemia	14 (21%)
Diabetes mellitus	9 (13%)
Smoking	13 (19%)
Prior myocardial infarction	20 (30%)
Cause of death	
Sudden coronary death	
Plaque rupture	15 (22%)
Plaque erosion	6 (9%)
Severe CAD without acute thrombus	20 (30%)
Non-cardiac death	26 (39%)
Lesions (n = 151)	
Paraffin embedded sections for morphometry (n = 60)	
PIT without macrophages	10 (16%)
PIT with macrophages	18 (30%)
Early fibroatheroma	19 (32%)
Late fibroatheroma	13 (22%)
Paraffin embedded sections for proteoglycan staining (n = 40)	
PIT without macrophages	10 (25%)
PIT with macrophages	10 (25%)
Early fibroatheroma	10 (25%)
Late fibroatheroma	10 (25%)
Paraffin embedded non-decalcified sections for calcium staining (n = 28)	
PIT without macrophages	7 (25%)
PIT with macrophages	9 (32%)
Early fibroatheroma	12 (43%)
Frozen sections for oil red O staining (n = 23)	
Adaptive intimal thickening	3 (13%)
Fatty streak	3 (13%)
PIT without macrophages	3 (13%)
PIT with macrophages	4 (17%)
Early fibroatheroma	4 (17%)
Late fibroatheroma	6 (26%)

Values are expressed as means ± SD or n (%).
PIT = pathologic intimal thickening.

2.2. Classification of lesions

Early coronary plaques were classified using our modified AHA classification, to include adaptive intimal thickening (AIT), fatty streak, PIT, and fibroatheromas [17]. AIT consists mainly of SMCs and proteoglycan matrix with little or no infiltration of inflammatory cells. Fatty streak represent lesions primarily composed of foamy macrophages and to a lesser extent lipid laden SMCs within the intima. PIT is characterized by the presence of SMCs interspersed within ECM towards the lumen and areas of extracellular lipid accumulation with absence of SMCs (lipid pool) close to the media, and were further divided into 2 groups based on the presence or absence of macrophage infiltration (PIT without macrophages and PIT with macrophages). Fibroatheromas were characterized by a dense fibrous cap overlying a necrotic core, and this lesion was further divided into 2 different stages; early and late fibroatheromas [18]. Early fibroatheroma was defined as a lesion consisting of macrophage infiltration into the lipid pool forming focal areas of necrosis, with loss of proteoglycans as assessed by the Movat pentachrome staining, and few free cholesterol crystals. Late fibroatheroma consists of necrotic core with discrete collections of

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