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CHAPTER 1

The pathobiology of CTO

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Introduction

Chronic total occlusion (CTO) is defined as occlusion age of at least one month, with angiographic thrombolysis in myocardial infarction (TIMI) flow grade 0 or 1 [1]. CTOs are classified as "early chronic" and "late chronic" if their age is 1–3 months old and >3 months old respectively. The current understanding of CTO development is based on animal CTO models as well as on autopsy and imaging studies in humans. Recent progress in elucidating CTO pathobiology has led our group to identify several novel biological targets to facilitate guidewire crossing during percutaneous coronary intervention (PCI).

Current paradigm of CTO evolution

The development of CTOs includes several specific stages with unique histologic characteristics present at each stage. The initial acute event leading to the development of a CTO is in many cases a ruptured atherosclerotic plaque with bidirectional thrombus formation [2]. Clinically the arterial occlusion may develop insidiously with minimal symptoms or may present as an acute coronary syndrome. In patients with minimal or no symptoms, the timing of the occlusive event cannot be clearly identified. In fact, the age of approximately 60% of CTO cases cannot be reliably dated by symptoms [3]. In patients with ST segment elevation myocardial infarction (STEMI) not treated with reperfusion therapy, an occluded infarct related artery has been found in 87% of patients within 4 hours, in 65% within 12–24 hours, and in 45% at 1 month [4, 5]. Up to 30% of patients treated with thrombolytic therapy alone have a chronically occluded artery 3–6 months after MI [6]. In patients treated with percutaneous coronary intervention (PCI) during evolving acute myocardial infarction (AMI), approximately 6–11% will have chronic occlusion of an infarct related artery at 6 months, due to either initial treatment failure or late re-occlusion [7].

Characterization of CTO development in human studies is problematic since CTOs are often diagnosed at a very late stage, and data regarding initial stages in their evolution is lacking. Several animal models have been developed to systematically define the development stages of a CTO; however these models have certain characteristics that could potentially limit their relevance to humans, such as the lack of underlying atherosclerotic substrate or significant calcification. In this chapter we shall review the current understanding of CTO pathobiology.

Development of CTOs

Acute arterial occlusion due to atherosclerotic plaque rupture with thrombus formation seems to be a common initiating event, which then triggers an inflammatory reaction. The freshly formed thrombus contains platelets and erythrocytes within a fibrin mesh, which is followed by an invasion of acute inflammatory cells. Jaffe *et al.* [8] have recently shown that an acute inflammatory response during the first 2 weeks after the initial event is accompanied by patchy formation of a proteolycan-enriched extracellular matrix and myofibroblast infiltration into the thrombotic occlusion. At the initial part of the intermediate stage (6 weeks), there is marked negative arterial remodeling and disruption of the internal elastic lamina accompanied by intense intraluminal

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neovascularization and increased CTO perfusion. Total microvessel cross-sectional area increases 2-fold along with a nearly 3-fold increase in the size of individual intraluminal vessels. However, the latter intermediate stage (12 weeks) is characterized by decreasing microvessel formation and CTO perfusion which further declines at the advanced stage (18–24 weeks). A progressive decrease in the CTO perfusion coincides with gradual replacement of proteoglycans by collagen in the extracellular matrix. Accumulation of collagen and calcium characterize the later stages of CTO maturation (Figures 1.1 and 1.2). The density of the fibrocalcific tissue is highest at the proximal and distal ends of the lesion compared to the body. Thus, the composition of the CTO evolves over time with remarkable spatial variability along the length of the CTO. From a pathobiology standpoint, three specific regions of the CTO have been identified:

(1) The proximal fibrous cap is a thickened structure at the entrance (the proximal end) of the CTO containing particularly densely packed collagen. It usually contains types I, III, V, and VI of collagen. Type IV collagen has also been observed in calcified tissues [9]. This region represents a distinct physical barrier to accessing the CTO.

(2) The distal fibrous cap also contains densely packed collagen, but is commonly regarded (although not proven in studies) as a thinner and softer structure compared to the proximal cap. This has been part of the rationale for developing the retrograde approach to cross the CTO.

(3) The main body of CTO.

Human coronary artery autopsy studies [10] have shown that the lumen of the CTO in some cases contains organized thrombus. Recanalization channels were observed in nearly 60% of lesions. Unlike the preclinical rabbit femoral artery model, the frequency of lumen recanalization and sizes of the channels were similar in different CTO ages. The intimal plaques within the CTO contained collagen, calcium, elastin, cholesterol clefts, foam cells, giant cell atherophagocytes, mononuclear cells (lymphocytes, monocytes), and red blood cells. "Soft" or cholesterol-laden lesions were more prevalent in younger CTOs age (< 1 year); however the amount of cholesterol-laden and foam cells declined with advancing CTO age. Older age CTOs typically contained hard fibrocalcific lesions ("hard plaque"). Iron and hemosiderin depositions could be observed at sites of previous intimal plaque hemorrhage. Extensive recanalization of the intimal plaques by neovascular channels was frequently evident particularly within and adjacent to the sites infiltrated by inflammatory cells (lymphocytes and macrophages). In some cases, intimal neovascular channels directly communicate with adventitial vasa vasorum, while their communication with lumen recanalization channels was rarely observed. Neovascular channels were also observed in the vascular medial layer; the extent of medial neovascularization was proportional to the cellular inflammation in the intimal plaque. The adventitia of the vessel is usually extensively revascularized in CTOs of all ages.

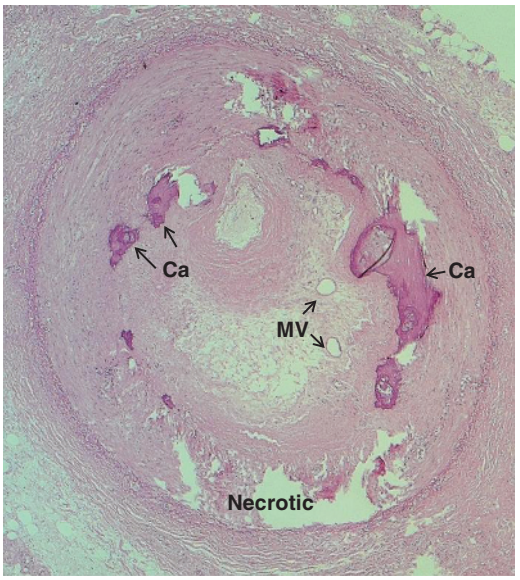

Figure 1.1 Hematoxylin-eosin stained human coronary CTO, demonstrating extensive collagen-rich fibrous tissue, several patches of calcification (Ca), two small microvessels (MV), and a large necrotic area (necrotic). (Courtesy of Dr. Jagdish Butany, University Health Network, Toronto, ON, Canada.)

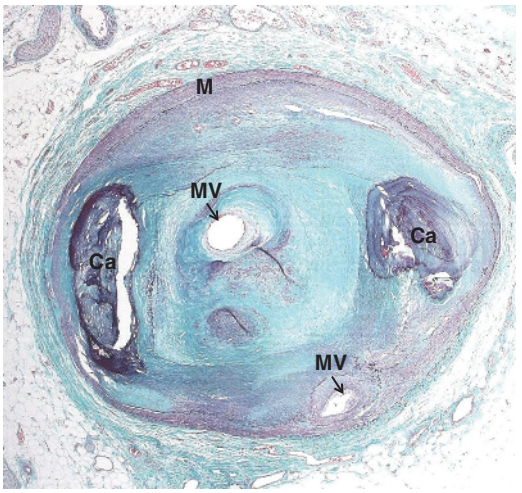

Figure 1.2 Elastin-trichrome stained human coronary CTO, demonstrating fibrous tissue (lighter staining material inside the lumen), with two distinct areas of calcification (Ca) and two microvessels (MV). M = media. (Courtesy of Dr. Jagdish Butany, University Health Network, Toronto, ON, Canada.)

Again, the extent of adventitial neovascularization is correlated to adventitial cellular inflammation. Munce *et al.* have shown in a rabbit peripheral artery CTO model that a large rise in extravascular vessels surrounding the occluded artery occurred at early time points, which was followed by a significant increase in intravascular vessels within the central body of the occlusion. The temporal and geographic pattern of microvessel formation and the presence of connecting microvessels support the thesis that the extravascular vessels may indeed initiate formation of the intravascular channels within the center of the occlusion. However, as the CTO matures beyond 6 weeks, a reduction in the size and number of central intravascular microchannels was demonstrated, suggesting that many of the vessels in this region become nonfunctional [11].

Neovascularization and angiogenesis

There are three types of microvessel formation in arteries with advanced atherosclerotic lesions. The first pattern occurs in the vasa vasorum, which is the fine network of microvessels in the adventitia and outer media. These vessels proliferate in atherosclerosis and in response to vascular injury such as angioplasty and stenting [10, 12, 13]. Hypoxia in the outer layers of the vessel wall appears to act as an important stimulus [13]. Occasionally in CTOs these adventitial blood vessels are well developed and can be recognized as "bridging collaterals." Such microchannels, which can recanalize the distal lumen, may result from thrombus derived from angiogenic stimuli [14], and can be recognized on an angiogram by the appearance of a well defined stump leading into the CTO. Second, neovascularization can develop within occlusive atherosclerotic intimal plaques, predominantly in response to chronic inflammation [15]. The localization of plaque vessels in so-called "hot spots" in the shoulders of atheromas may predispose these plaques to rupture and acute coronary events [16, 17]. The third type is the pattern of intraluminal microvessel formation or "recanalization channels." These microvessels generally range in size from 100 to 200 μm, but can be as large as 500 μm [10]. In contrast to the vasa vasorum which run in radial direction, these intimal microvessels run within and parallel to the thrombosed parent vessel [16], and therefore have particular relevance for crossing of CTOs as a pathway for guidewire crossing.

Angiogenesis within the CTO is a complex process which starts with recanalization of the thrombus through a mechanism that is dependent on the proteolytic activity of circulating mononuclear cells and engraftment of endothelial progenitor cells [17]. Angiogenesis within the arterial thrombi is modulated by pro-angiogenic molecules in the extracellular matrix, including perlecan [18], hyaluronan [19], and anti-angiogenic agents such as collagen type I [20] and decorin [21]. The process of angiogenesis is initiated by vasodilation and increased permeability of the existing microvessels. This is followed by coordinated proteolysis, resulting in the destabilization of the vessel wall and endothelial cell migration and proliferation with subsequent formation of primitive endothelial tubes [22, 23]. Maturation of these tubes includes recruitment of pericytes or smooth muscle cells and deposition of extracellular matrix [24, 25]. Various aspects of angiogenesis are regulated by multiple growth factors including vascular endothelial growth factor (VEGF) and its receptor VEGFR2; platelet derived growth factor (PDGF) and its receptor PDGFR-β [25, 26] , angiopoietin-1, angiopoetin -2, and TIE-2 receptor [21, 22, 24, 27], fibroblast growth factor-2 (FGF-2) [28], TGFβ [29], and endothelium derived nitric oxide [30].

Calcification

For non-CTO atherosclerotic plaques; calcification is correlated with chronic kidney disease, diabetes mellitus, and is a consequence of aging. Our understanding of the balance between promotion and inhibition of calcification in the CTO is much more limited.

Most CTOs contain calcification that ranges from minor to extensive, depending on several factors including the age of the occlusion [31]. Intimal plaque calcification is seen in 54% of CTOs aged 3 months or less, and reaches 100% in CTOs aged above 5 years. In contrast, insulin-dependent diabetes mellitus was more frequently observed in patients with predominantly cholesterol laden or mixed CTOs than in those with fibrocalcific CTOs [9]. The extent of the CTO calcification has repeatedly been identified as a negative predictor of PCI success due to failure to cross with guidewires [32, 33, 34].

The process of the CTO calcification is usually simplified into two mechanisms [35, 36]:

(1) Passive process: It was initially considered that calcium precipitation occurred when apoptotic cell fragments and cholesterol crystals served as a crystallization nidus and the calcium and phosphate concentration approached the salt solubility product in the presence of a lower concentration of local calcium-chelating molecules. The formation of hydroxyapatite crystals in this way is now regarded as a semi-regulated process, and the high phosphate levels might induce vascular smooth muscle cells to differentiate into an osteoblastic phenotype resulting in bone formation.

(2) Active osseous process: Recruitment of osteoblasts and osteoblast-like cells, which is triggered by immunomodulating cytokines (including bone morphogenetic proteins, osteogenic transcription factors etc.). Similar to skeletal bone, these bone/cartilage-like structures are subject to resorption by osteoclast-like cells.

Current research in CTO pathobiology

Identification of specific components of the CTO at various stages is critical to understanding CTO pathobiology and improving guidewire crossing success rates. Complementary information is obtained by several approaches:

Human CTO samples

Samples of CTO collected during autopsies, amputations, endarterectomies, and transplants provide an important but very infrequent opportunity to study these highly heterogeneous lesions. Different modalities of *ex vivo* CTO imaging is an important area of present and future studies.

Animal models of CTO

A challenge in developing animal models of CTO is the lack of spontaneous atherosclerosis in animals. Different approaches have included external arterial constriction, thermal injury, gas-drying of the artery, injection of authologous blood above a stenosis, copper stents, stents with occluded outflow, alcohol injection, and insertion of polymer plugs. We have developed a rabbit CTO model in which thrombin is injected into an isolated femoral artery segment [37]. This model was used in investigation of the natural history of CTO [8]. Due to the significant impact of arterial calcification on the success rate of percutaneous CTO revascularization, the creation of a calcified CTO model is important for the future research. Suzuki *et al.* [38] used apatite-coated bioabsorbable polymer sponges to produce calcified CTO lesions in rabbit and pig coronary arteries/peripheral arteries. These lesions were found to have microvascular channels and microcalcification, yet no significant osseous transformation was visible. Recently, we were able to develop a calcified CTO model in a common femoral artery of rabbit (unpublished results), which incorporates both passive and active calcification. The model is unique in that the CTO is heavily calcified and contains islands of bone/cartilage that are very similar to human pathology. Interestingly, we have also noticed that the patterns and time sequences of microvessel formation and underlying inflammatory responses appear to differ between animal models. The rabbit femoral artery had a very predictable pattern of microvessel formation and then regression during the initial 12 weeks of CTO formation [8]. In contrast, pig coronary arteries demonstrated a much more heterogeneous response of neovascularization during the same 12 week period (unpublished observations).

CTO imaging techniques

Coronary angiography remains the primary imaging technique for assessment of the CTO in clinical practice. However, other imaging modalities now provide an opportunity to identify specific components of CTO in patients and experimental models. Proposed imaging techniques for CTOs can be broadly categorized into large field of view and modest resolution (such as cardiac magnetic resonance imaging (MRI) and computed tomography (CT)), and small field of view and high resolution [39]. High-resolution methods include forward-looking adaptations of intravascular ultrasound (IVUS), optical coherence tomography (OCT), and intravascular MRI. Many of these imaging modalities can be coupled with interventional techniques, and thus improve upon the guidance provided by angiography during revascularization. The multi-slice or multi-detector CT coronary angiogram is rapidly gaining in popularity in the assessment of coronary lesions. It is especially useful in the assessment of the amount of CTO calcification, which has a negative predictive value for successful coronary intervention [32, 33, 34]. Three-dimensional micro-CT is a high resolution imaging technique for *ex vivo* samples that provides detailed rendering of complex microscopic vascular structures with a resolution down to 17 μm [11].

Cardiac MRI with contrast agents has a spatial resolution down to 200 μm in plane and about 1 mm through the plane and can determine composition of atherosclerotic plaque components within the CTO. We have used MR contrast agents such as gadolinium and clariscan to assess intraluminal perfusion in experimental CTO [8]. Another application of cardiac MRI that may have relevance to the assessment of CTO is direct thrombus imaging (MRDTI). MRDTI allows for the estimation of the extent and age of thrombi without the use of an exogenous contrast agent and is being refined for use towards coronary lesions.

Intravascular MRI can image soft tissue and may potentially guide therapeutic procedures without ionizing radiation or nephorotoxic agents. Early efforts in intravascular MRI development have been directed toward side-viewing orientations. Currently forward-looking intravascular MRI coils are also available [40]. The recent advent of 3.0 T magnets has

allowed a reduction in exogenous contrast dose without compromising overall imaging quality.

Intravascular ultrasound (IVUS) – both conventional (side-looking) and forward-looking – is a particularly appealing imaging modality for image guidance purposes due to its high resolution and reasonable penetration depth. IVUS based techniques such as elastography, radiofrequency tissue characterization, or virtual histology can be incorporated into the forward-looking IVUS system to identify the mechanical properties and composition of CTOs [11].

Compared to IVUS, optical coherent tomography (OCT) has higher resolution at the cost of poorer penetration. Forward-looking OCT has more than sufficient resolution to clearly depict microchannels and the different layers of the vessel wall.

Summary

In this chapter we have summarized the key components of CTOs and the impact of each on guidewire crossing and balloon compliance. We described innovative imaging modalities, such as forward-looking IVUS and OCT and CMR, which are in various stages of development, including evaluation in animal models. Better understanding of the CTO structure incorporating the above imaging techniques with advances in guidewires and other plaque modification strategies may enable significant improvements in CTO revascularization. The pathophysiology of collagen accumulation and calcification in CTO is now at the frontier of CTO translational to clinical research. These efforts will hopefully lead to a breakthrough in CTO revascularization success rates in the near future.

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