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Ultrastructure of Evolving Deep Venous Collaterals

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ABSTRACT

Objective: To explore the structure of obstructive venous collaterals.

Design: A total of 25 rats underwent unilateral ligation of the distal common femoral vein. Bilateral (control and test) vein segments with collaterals were harvested and studied with conventional light microscopy and electron microscopy at 2-week intervals for 10 weeks post-ligation.

Results: Obstructive collaterals were quite unlike normal controls throughout the study. Initially, post-obstructive collateral walls showed disorganization of collagen, elastin, smooth muscle cells, and adventitia, while endothelial cells became more rounded and compact. The dense protein subendothelial deposits noted early became organized and moved more deeply into the wall at subsequent study intervals. Minimal motivation of smooth muscle cells, coalescence of elastic lamina, condensation of collagen and some organization of the wall were noted.

Conclusion: Inability of deep collaterals to function with normal wall properties is likely to be secondary to the disruption of connective tissue and sustained disorganization of the vein wall noted throughout the evolution of collateral formation.

Keywords: Collaterals; Ultrastructure; Vein

Introduction

The incidence of obstructive venous insufficiency is estimated to be 10–30% of patients suffering a deep vein thrombosis (DVT) [1]. The aetiology of obstruction is overwhelmingly post-thrombotic in nature. Among the compensatory mechanisms that occur to normalize obstructive haemodynamics are the formation of collaterals and recanalization of the thrombolytic segment. Little information on the nature and function of venous collaterals is available in the literature. Venographic studies have shown that both superficial and deep venous collaterals exhibit changes in luminal structure. We investigated the evolving ultrastructural alterations in post-obstructive deep venous collaterals in a rat model. Deep post-obstructive collateral walls exhibit distinct compositional wall changes that evolve over the period of their development. The disruption of connective tissue and the sustained disorganization of the vein wall throughout the formation and life of the deep collateral may result in abnormal compliance. Compliance has a fundamental role in the determination of post-exercise pressure [2].

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Methods

A total of 25 rats underwent unilateral ligation of the distal common femoral vein, leaving the contralateral side as a control system. Ascending venographic studies were performed bilaterally to confirm proper collateralization on the obstructed side. Bilateral (control and test) vein segments were harvested after sacrifice according to approved laboratory guidelines at 2-week intervals for 10 weeks post-ligation. All vein segments were studied with conventional light microscopy and transmission electron microscopy (TEM). Electron-microscopic specimens were initially flushed with 5% glutaraldehyde solution, cross-sectioned and fixed in a glutaraldehyde solution buffered at a pH of 7.4 for 24–48 h. The specimens were immersed in osmium tetroxide buffered at pH 7.4 for 1–2 h. After embedding in epoxy resin, the specimens were stained with uranyl acetate

Reynold's lead citrate. A Phillips 301 transmission electron microscope and photographic enlargement were used to examine the specimens.

Results

Thigh segments of the deep venous systems with collaterals were examined in the post-obstructive limbs and compared with normal controls on the contralateral side. The normal control limb veins consistently displayed a well-organized wall consisting of two layers of smooth muscle, with cells in the contracted, non-synthetic state. The endothelial cells displayed a normal nucleus to cytoplasm ratio and were flattened in apperance with normal junctions. Extracellular proteinaceous deposits were confined to the subendothelial area. The collagen in the adventitia and the wall elastin deposits were well organized (Fig. 1).

Obstructed Venous Segments

On the obstructed side, collaterals had already developed at 2 weeks. There was evident disorganization of the newly developed collaterals. The smooth muscle cells were less developed and in a synthetic phase. The endoplasmic reticulum was swollen and filled with protein. Collagen was less abundant and less organized with multidirectional orientations. The cell junctions appeared looser than in the normal controls. Suben-

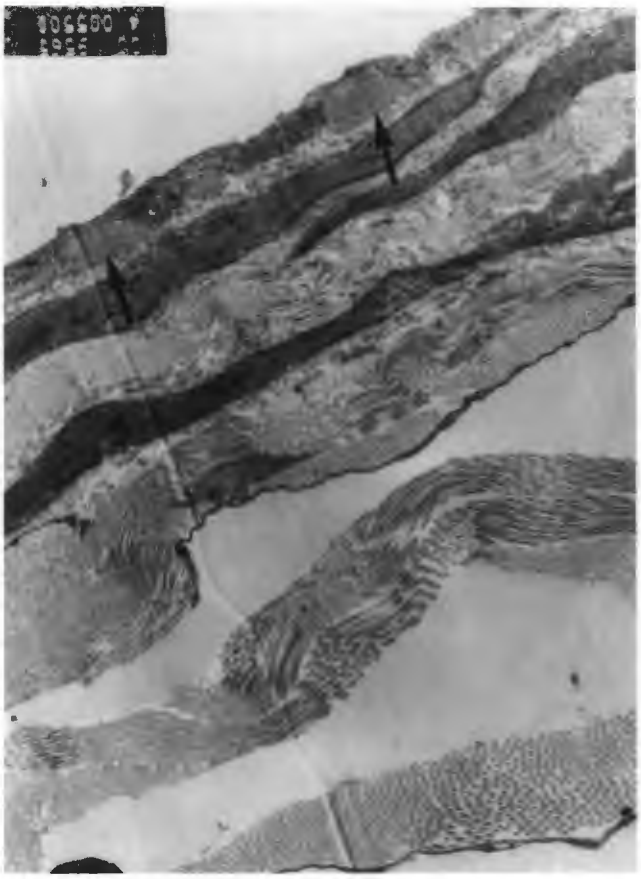

Fig. 1. Normal vein with two layers of smooth muscle, a normal nucleus/cytoplasmic ratio, well-organized collagen, and laminar organization. Well-circumscribed subendothelial deposits are seen (arrows).

Fig. 2. Two weeks post-obstruction. Smooth muscle cells are in active synthesis with swollen endoplasmic reticulum. Increased subendothelial deposits are seen (arrow).

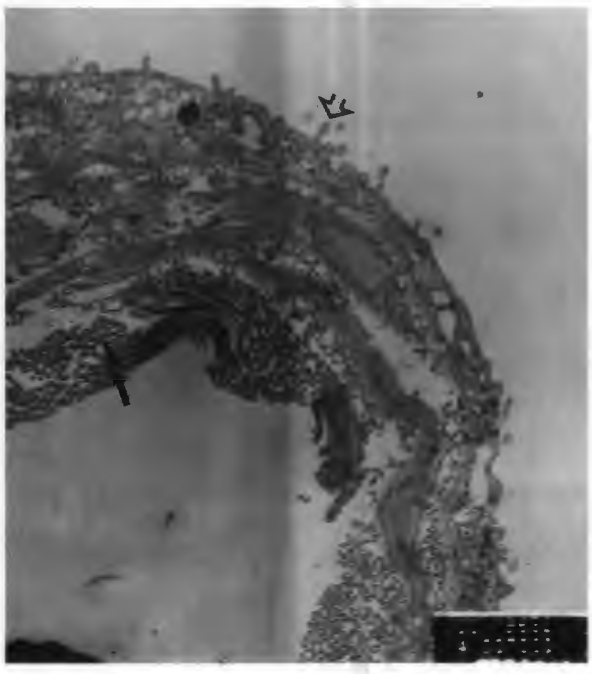

Fig. 3. At 4 weeks the collateral wall is disorganized, with collagen broken into single bundles (solid arrow). Increased pinocytic vesicles are seen in the endothelial cells along with increased vacuolization (open arrow).

dothelial proteinaceous deposits were clumped, globular and disorganized with loss of homogeneity (Fig. 2).

At 4 weeks, the collateral wall was even less organized. The collagen had broken into single bundles. The endothelial cells had increased vacuolization and there was an increased number of pinocytic vesicles with a vertical orientation of the cell nucleus. The smooth muscle cells remained in an active synthetic state, although there was less activity and less protein content in the endoplasmic reticulum. The subendothelial deposits remained clumped, but there appeared to be minimal dispersion throughout the wall (Fig. 3).

At 6 weeks the collateral wall depicted more organization. The smooth muscle cells were evolving to a more contracted, dormant state. The endothelial cells remained vertically oriented and less flattened in appearance but were less vacuolated and more organized. The proteinaceous deposits that earlier were confined to the subendothelial area were now dispersed throughout the vein wall, particularly immediately below the smooth muscle cells. Although the wall showed a movement toward a more mature state, it was not organized in the usual laminar fashion as noted in the controls (Fig. 4).

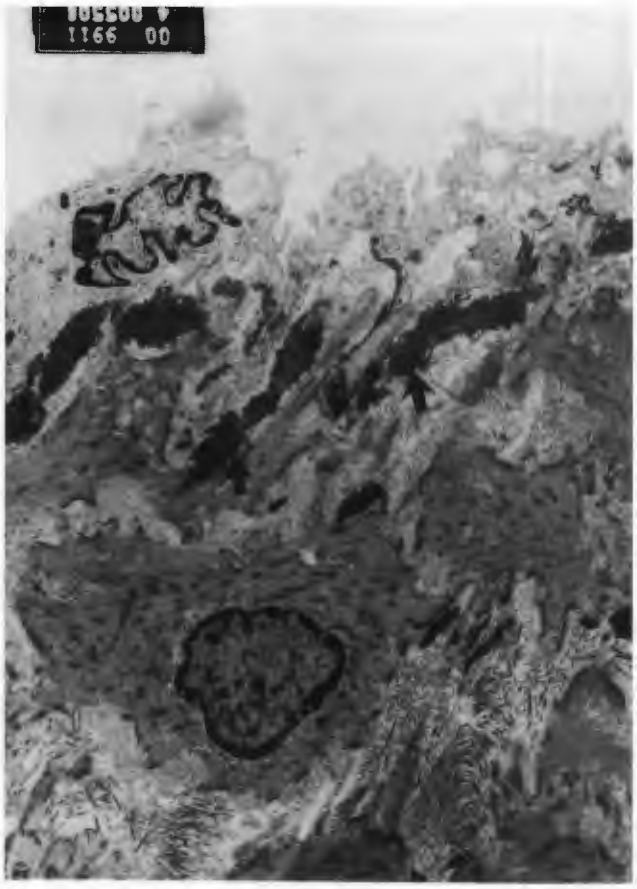

Fig. 4. The 6-week collateral wall is becoming more organized, with smooth muscle cells in a more contracted state and endothelial cells slightly more flattened. The subendothelial deposits are now dispersing throughout the wall (arrows).

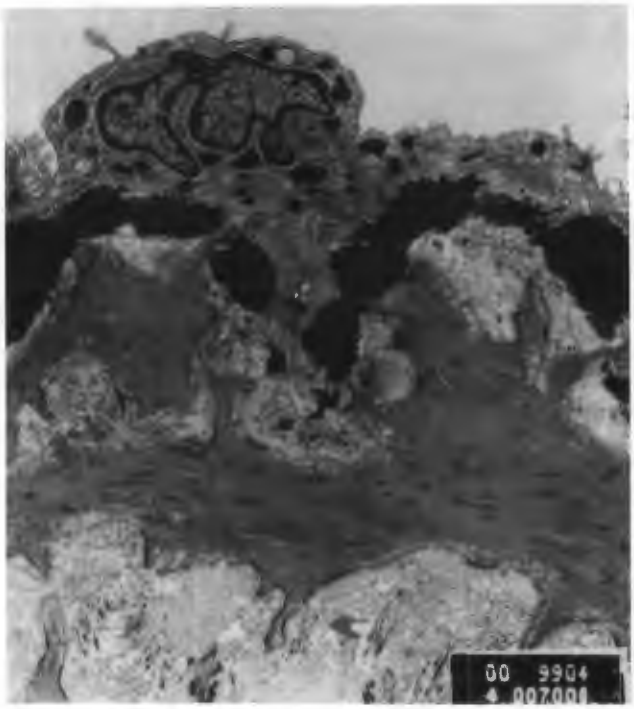

Fig. 5. At 10 weeks the wall of the collateral is more mature in appearance, with flattened endothelial cells with less vacuolization and the smooth muscle cells contracted. Coalescence of the elastic lamina and collagen is noted. Normal laminar organization is not attained.

At 8 weeks the collateral wall was largely unchanged from the appearance at 6 weeks. However, there appeared to be slightly more organization, with muscle cells in a more contracted state. The endothelial cells remained vertically oriented.

Finally, at 10 weeks the collateral wall had achieved a more organized state. The endothelial cells, although still globular, were more flattened and much less vacuolated. The proteinaceous deposits were once again localized to the subendothelial area. The smooth muscle cells were in the contracted state. Coalescence of the elastic lamina and condensation of collagen were noted. Although the wall was more organized, the normal laminar organization was yet to be achieved (Fig. 5).

Discussion

The post-phlebitic syndrome was well described by Bauer in 1942 [3]. Ascending venography has been the gold standard for diagnosing acute DVT and the presence of post-obstructive changes including the development of deep collaterals and recanalization of veins. Pathophysiological implications of this disease process have been described previously [2]. The major focus of such studies after venous obstruction has

concentrated on the valvular incompetence that develops [4]. Thulesius [5] examined the wall changes noted in non-obstructive superficial varicosities. He proposed that the superficial wall system was dysfunctional owing to disruption of the connective tissue network and its interaction with smooth muscle cells. The ability of the wall to display adequate responses to changes in pressure, neural–hormonal stimulation and other factors was impaired in these superficial varicosities. Psaila and Melhuish [6] noted that the viscoelastic properties in normal veins differed from those seen in saphenous varicosities that develop following deep venous obstruction. The degree of elasticity and collagen content also differed in the two groups. It has been postulated that prolonged exposure to increased hydrostatic pressures without significant periods of repair can cause irreversible wall changes, which are reflected in abnormal venous function studies. To our knowledge a careful evaluation of the walls of evolving post-obstructive deep collaterals has not been undertaken previously.

During the initial phase after proximal venous ligation (0–4 weeks) the wall of medium-sized thigh collaterals showed structural damage, with a total disruption of the laminar organization seen in normal veins. This disruption probably results from more than a change in hydrostatic pressure, as initially proposed in studies of superficial varicosities; the rat does not have erect posture. Most importantly, the smooth muscle cells in the wall immediately take on a synthetic state with an increase in the endoplasmic reticulum and an increase in the production of proteins presumed to be cellular wall components. Collagen is immature and disorganized in appearance, as in a reproductive and reparative mode. Even the endothelial cells give the appearance of a more active state with a higher nuclear/cytoplasmic ratio, an increase in pinocytotic vesicles and increased vacuolization. There is a clumping of proteinaceous deposits below the endothelial cells. New elastin appears to be forming. Overall the wall is very dissimilar to the normal vein of comparable size. The wall properties are also presumed to be dissimilar.

Over the next 4–6 weeks the wall evolves toward a more organized state with maturation of collagen and elastin and a dispersion of subendothelial proteinaceous deposits. The smooth muscle cells appear to be in a less synthetic state. By 10 weeks the wall approaches normalcy with flattening of endothelial cellular components, more contraction of smooth muscle cells, and a coalescence of elastic lamina and condensation of the collagen. The wall never achieved the laminar organization of a normal vein, however, during the 10 weeks of our study. The connective tissue components did not resume normal spatial organization and are presumed to be less functional. The infiltration of the wall by subendothelial deposits seen immediately post-obstruction is believed to preclude development of correct orientation of the connective components as well as their proper 'networking' with the smooth muscle components of the wall as described by Thulesius [5] in the superficial varicosities.

Reduction in vein wall elasticity has been noted in both superficial and deep venous disease [7]. This condition is thought to precede valvular incompetence in chronic venous disease. Immediate post-obstructive wall changes seen in these collaterals suggest reduced elasticity. The initial synthetic phase results in a hypertrophy of the wall; however, the disruption of components likely results in an inability to achieve proper contractility even after the wall hypertrophies. This may explain the inability of deep venous collaterals to function with normal wall parameters, contributing to the pathophysiology of the post-phlebitic syndrome.

Ethics: The care and use of all laboratory animals were in compliance with approved laboratory guidelines.

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