Smooth Muscle Changes in Varicose Veins: An Ultrastructural Study

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Abstract

In order to understand the pathology of varicose veins, we prospectively collected a total of 23 vein specimens both from the normal proximal thigh long saphenous vein (LSV) in 3 young trauma patients and from the unstripped proximal LSV near the sapheno-femoral junction and the distal calf blowouts in 10 primary varicose veins patients. Ultra-thin sections were examined under the transmission electron microscope (TEM). Compared with the normal control LSV, varicose vein sections showed increase in the diameter of the lumen, hypertrophy of the wall and elongation and invagination of the intima. Smooth muscle cells (SMCs) lost their normal fusiform shape and were widely separated by increased amounts of extra-cellular collagen fibers. The cells underwent marked degeneration, vacuolization and disintegration into fiber-like material and small separated fragments. SMCs were seen in the subintimal tissue and some of them were lost into the lumen. SMCs also showed marked phagocytic activity, engulfing not only collagen and elastic fibers, but also other smooth muscle cells. Although these changes were more marked and advanced in the distal calf blowouts, they were also present in the proximal, clinically non-dilated LSV. In conclusion, SMCs of varicose veins show severe degeneration in both the distal calf blowouts and the proximal, clinically non-varicose LSV. It appears that they both form and phagocytose collagen and elastic fibers and play a major role in the pathogenesis of varicose veins.

Key words: varicose veins, long saphenous vein, calf varicosities, smooth muscle cells, electron microscope

Introduction

Varicose veins affect up to 20% in the western world (Lofgren, 1979). Roughly 10% of those affected have complications such as superficial thrombophlebitis, pigmentation, lipodermatosclerosis, hemorrhage, ulceration and increased risk of deep vein thrombosis (Travers et al., 1996). The exact mechanism by which primary varicosis occurs in the lower
limb in susceptible individuals has yet to be determined (Clarke et al., 1961). Heredity is an important factor as more than half of those affected have a positive family history, suggesting a congenital weakness of the vein wall (Travers et al., 1996). The weak wall theory with secondary valvular incompetence has been proposed as the most likely cause of primary varicose veins (Psaila & Melhuish, 1989). Primary wall weakness explains why varicosities are often found below competent valves (Rose and Ahmed, 1986). Recent histological studies performed on varicose vein walls have led to several hypotheses. Contradictory evidence exists on the pathology of smooth muscle in varicose veins. Several studies have reported an increase in smooth muscle, or its activity (Prerovsky, 1981; Browse et al., 1988; Obitsu, 1990), whereas others report reduced amounts of smooth muscle due to replacement by connective tissue (Rose and Ahmed, 1986; Browse et al., 1988). Rose and Ahmed suggested that separation of muscle cells by fibrous infiltration prevents them from acting as a unified whole, with subsequent alterations in wall tone leading to pathological dilatation (Rose and Ahmed, 1986). Other studies have failed to demonstrate any difference in the wall muscle content of control and varicose veins (Lees et al., 1992a; Lees et al., 1992b). In the earlier report by Khan et al. (2000), the general cellular and extracellular changes in varicose veins were compared with normal controls at the intimal level only.

Because of the conflicting reports on the smooth muscle contents of varicose veins and their postulated role in varicosis, this study aims to look at the ultrastructural morphology and changes of smooth muscle cells in both the proximal, unstripped clinically non-dilated long saphenous vein and the distal calf varicosities which represent the end-stage of the disease.

**Material and Methods**

A total of 23 vein specimens of the long saphenous vein (LSV) and the distal calf varicosities were collected from 13 patients at Asir Central Hospital (ACH), Abha, Saudi Arabia, during the period from February to September 1997. Three young trauma patients, with a mean age of 27 years, underwent repair of their arterial injuries and 10 patients underwent surgery for their primary varicose veins. The patients were 7 males and 6 females with a mean age of 35 ± 11.55 years (± SD, 13–51 years). The trauma patients had no clinical evidence of varicose veins. The varicose veins patients underwent both physical and continuous wave Doppler (CWD) examination to assess the extent of their varicosities and the presence of sapheno-femoral junction (SFJ), sapheno-popliteal junction (SPJ) and/or LSV incompetence. On Doppler examination, all varicose veins patients had sapheno-femoral junction (SFJ) incompetence, five had saphenopopliteal junction (SPJ) incompetence and nine patients had LSV incompetence. Based on these findings, varicose veins patients underwent SPJ and/or SFJ ligation, LSV stripping as appropriate, and multiple stab avulsions of the distal calf varicosities.

In the three trauma patients, specimens were collected from the normal proximal thigh LSV after it was harvested for use as an arterial graft, and were used as normal controls. In all the varicose veins patients, specimens were taken from both the proximal, unstripped long saphenous vein, distal to the tied SFJ and before insertion of the vein stripper, and also from the avulsed distal calf varicosities. Specimens were immediately put in small, labeled test tubes.
containing 2.5% glutaraldehyde solution and sent to the Electron Microscopy (EM) laboratory. Each specimen was then cut into 2–3 mm cubes and immediately fixed in 2.5% glutaraldehyde solution in 0.1 M sodium cacodylate buffer, pH 7.2, and kept at 4°C for two hours. Samples were later post-fixed in 1% osmium tetroxide in sodium cacodylate buffer, dehydrated in an ascending series of ethyl alcohol and embedded in Spurr's resin. Semi thin sections were prepared using diamond knives, stained with toluidine blue and examined under the light microscope. Ultra thin sections were also prepared by diamond knives, stained with uranyl acetate and lead citrate and then examined at 80 KV under the transmission electron microscope “TEM” (jeol® 1200 EX, Japan).

**Results**

Compared with the normal saphenous vein, varicose veins semithin sections stained by toluidine blue and examined under the light microscope showed increase in the diameter of the lumen and the thickness of the wall with elongation and invagination of the intima. The increased thickness of the wall was due mainly to disproportionate increase in the thickness of the tunica intima, showing marked intimal hypertrophy, with minimal increase in the thickness of the tunica media. There was hyperplasia and hypertrophy of both the intimal and medial smooth muscle cells (SMCS) with simultaneous increase in the extracellular fibrous tissue. The increase in this extracellular fibrous tissue was much more marked in the tunica intima and contributed largely to its increased thickness. Under transmission electron microscope (TEM), the normal SMCs appeared fusiform in shape. Each cell was enclosed by an intact plasmalema and contained a single nucleus with close cell-to-cell contact. Regular lattice-like network containing actin and myosin filaments formed the intracellular content of each cell together with cisterns of rough endoplasmic reticulum, polyribosomes and few mitochondria (Fig. 1A).

In varicose vein sections of the tunica intima and tunica media, there was wide separation of the SMCs by an increased amount of extra-cellular matrix and variable bands of collagen fibers. The changes described below were present in both the medial and intimal SMCs. They were abnormal in shape and lost their regular fusiform appearance, with extension of plasmalemal projections, some of which separated completely from the cell surface (Fig. 1B). With the loss of their cellular integrity, some SMCs split into fiber-like material, possibly collagen fibers, in a process of fraying (Fig. 1C). With further destruction and degeneration, cells broke down into small fragments that were widely scattered in the extracellular space (Fig. 1D). There was also loss of the normal lattice-like network structure, with the appearance of vacuoles and vesicles in both the perinuclear region and in the periphery of the cell (Fig. 2A). These vacuoles and vesicles increased in size and number and coalesced to occupy most of the cell, compressing the normal cytoplasm into a thin rim along the periphery of the cell (Fig. 2B). This process progressed until complete degeneration and disintegration of the SMCs (Fig. 2C). SMCs also contained myelin bodies as an indication of the degenerative process (Fig. 2D).

We believe there is a sequence of events in the process of formation of ghost bodies. First, vacuoles appeared along the periphery of the cell (Fig. 3A), after which they projected and extended from the cell surface on a narrow pedicle or stalk (Fig. 3B & C). Later on, they
Fig. 1. Normal and varicose veins smooth muscle cells:
A. Normal smooth muscle cells (SMC) with an intact plasmalema (arrow), a single nucleus (N), few mitochondria (m), cisterns of rough endoplasmic reticulum (RER) and polyribosomes (double arrows). Note the normal cell to cell contact and the lattice-like network (star) of actin and myosin filaments. (× 10000)
B. Abnormal-shaped smooth muscle cell (SMC) of proximal long saphenous vein showing plasmalemal projections (arrows); some separating from the cell surface (double arrows). (× 8333)
C. Damaged smooth muscle cell (SMC) of a distal calf blowout splitting into fiber-like material (arrows) and surrounded by electrolucent, structureless material (star) and fragmented collagen fibers (C). (× 9333).
D. Fragments of a completely damaged smooth muscle cell (SMC) scattered between collagen fibers (C) in a distal calf varicosity. (× 3929).
Fig. 2. Severely damaged smooth muscle cells of distal calf varicosities:
A. Perinuclear (star) and peripheral (asterix) degeneration in a smooth muscle cell (SMC). The lower cell shows different-size vesicles (arrows). (× 6800).
B. Destruction of the lattice-like network of a smooth muscle cell (SMC). The oval, small, pyknotic nucleus (N) shows chromatin margination. (× 8750)
C. Severely damaged smooth muscle cell (SMC) showing unclear lattice-like network, and increased number of vacuoles (V) and vesicles (arrow). (× 7130).
D. Severely damaged smooth muscle cell (SMC) showing increased number of myelin figures (stars) and focal damage of its plasmalema (arrow). (× 12000)
Fig. 3. Formation of ghost bodies
A. A clear, electrolucent, peripheral part (star) of a smooth muscle cell (SMC), which contains few microfibrils (arrows). ($\times 6000$).
B. & C. Electro-lucent cytoplasmic projections (stars) on a narrow stalk (arrows) extending from the surface of a smooth muscle cell (SMC). ($\times 9500 \& \times 7500$).
D. Membrane-bound “ghost bodies” (stars), containing electro-lucent material and filling the extracellular space. ($\times 5400$).
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Separated completely from the cell surface to form the membrane-bound, structureless, electro-lucent, extracellular bodies known as ghost bodies, which are reminiscent of the degenerated smooth muscle cells (Fig. 3D).

Another very important finding was the ability of SMCs to phagocytose not only collagen and elastic fibers but also other damaged SMCs. SMCs were seen in close proximity to large amounts of extra-cellular collagen fibers (Fig. 4A). Later on, they extended pseudopodia-like projections around these fibers and engulfed them. Collagen fibers were seen inside the SMCs, both in the center and in the periphery, indicating the progress and continuity of this process (Fig. 4B). Elastic fibers were seen in phagocytic vacuoles inside SMCs (Fig. 4C). SMCs were also seen to extend pseudopodia around other neighboring SMCs in an apparent phagocytic process (Fig. 4D).

Discussion

During the past decade, experimental and clinical evidence has accumulated to suggest that the cause of dilatation of varicose veins is in the wall. The valves of normal and varicose veins are similar in number, morphology and function (Leu et al., 1979). Varicosities in the lower limbs may even occur without valvular incompetence (Rose and Ahmed, 1986). Furthermore, when a normal vein was exposed to high pressures (i.e., when a deep perforator is incompetent, or the vein is used for arterial bypass), it becomes hypertrophic, rather than dilated or grossly varicose. Finally, the alternating apparently normal and abnormally dilated segments observed in varicose veins are not related to the site and the function of valves: macroscopically dilated segments are often found below valves that appear competent at Doppler examination (Rose and Ahmed, 1986). The question whether the primary defect is the incompetent valve or an abnormal structure of the vein wall is not merely academic. In fact, if the primary defect is in the vein wall, the rational surgical approach would be to remove the whole pathologic vein (Venturi et al., 1996). Despite the evidence of a primary defect in the vein wall, the pathogenesis of the vein dilatation remains obscure.

In the present study, we tried to compare sections taken from the proximal, unstripped long saphenous vein (ISV) near the sapheno-femoral junction, which were clinically non-dilated, with sections taken from the distal calf varicosities, which represent the end-stage of the varicose veins disease. Our study showed that similar changes in the wall morphology and in the smooth muscle were present in both areas, but with varying degrees of severity, being more advanced in the distal calf blowouts. Varicose vein sections showed dilatation of the lumen compared with the control, with greater cross-sectional areas of the intima, media and whole wall. There was marked elongation of the intima, which was thrown into invaginations, probably due to the increase in the diameter of the lumen with increased surface area. These findings support others, which have shown by combined light and electron microscopy that there are significant alterations of wall morphology at proximal and distal sites, and in different tunics of varicose veins. Varicose veins were also larger with a greater cross-sectional areas (and hence volumes) of tunica intima and tunica media (Travers et al., 1996).

These changes, however, were not accompanied by changes in the wall thickness, which
Fig. 4. Phagocytic activity of varicose veins smooth muscle cells:
A. Smooth muscle cell (SMC) of a proximal long saphenous vein extending pseudopodia-like projections (arrows) around adjacent collagen fibers (C). (× 4000)
B. Smooth muscle cell (SMC) of a distal calf varicosity containing collagen fibers (C) in a central autophagic vacuole (V) and in some peripheral vacuoles (double arrows). (× 3517)
C. Smooth muscle cell (SMC) containing engulfed collagen (black arrow) and elastic (white arrow) fibers with extra-cellular damaged or immature collagen (C) and elastic (e) fibers. Local damage of plasmalema (double arrows) exposes the cell content to the extra-cellular fibers. (× 13500).
D. A Smooth muscle cell (SMC) extending pseudopodia (arrows) around another SMC (star) in a phagocytic process. (× 7600).
suggest that in varicosis the dilatation which occurs, is not accompanied by a generalized wall thinning. Thickening elsewhere compensates any thinning which occurs, and this is effected, in part at least, by the production of new wall material, mainly collagen. As previously noticed by Travers et al. (1996), we also found increased variation in varicose vein wall thickness between different sites, possibly reflecting different stages of the varicose process. Within a varicose vein, regions of thinning (blowouts) were often compensated by areas of thickening, whereas other regions were normal and unaffected by fibrosis (Travers et al., 1996). Blowouts consisted mainly of thinned out collagen fibers, which often showed fibrillar degeneration, lined by de-epithelialized intima and sub-endothelial tissues. The muscles had degenerated, broke down into fragments or else disappeared completely. Muscle fibers were found in the subintimal plane, and some were already lost into the lumen.

In general, the longitudinal and circular muscle layers of the tunica media were thicker than normal and had lost their regular sheet-like organization. This was due not only to the increase in the muscle bulk, but also to an increase in the intervening connective tissue, and the wide separation of the smooth muscle cells by increased amount of collagen. There was also frequent association between the pathological changes in both the muscle layer and the intima, which suggests that both have a common cause. These findings agree with those of Milroy et al. (1989). The conflicting findings in the literature of increases, no changes and decreases in concentrations of smooth muscle in varicose veins, must be treated warily because all could be accompanied by increases in absolute amounts (Travers et al., 1996). Stereological studies showed a highly significant increase in muscle density within the wall of varicose veins, but the biochemical studies showed no increase in the total protein content of the walls of varicose veins (Travers et al., 1996). The apparent increase in muscle density of varicose veins is, therefore, due to a non-protein component of the muscle, most likely the water content, which makes it a process of hypertrophy of the smooth muscle and not hyperplasia.

The varicose samples in this study were found to have an atrophied elastic network and a disorganized muscular structure that invaginated and divided the layers of increased collagen, which is in agreement with previous studies (Bouissou et al., 1988; Goldman and Froncek, 1989). Scattered elastic fibers, which in some areas formed large clumps, were also visible in the media, lying between disrupted muscle layers. Fibrosis was particularly evident in the inner longitudinal and circular muscle layers of the media. Areas of fibrous infiltration were not uniformly distributed and in some sections involved only part of the vein wall, as previously described by other workers (Travers et al., 1996). The separation and disruption of muscle cells caused by increased collagenous material may lead to diminished tone and vein wall dilatation, as “effective contraction cannot occur unless individual cells are in communication with each other” (Rose and Ahmed, 1986). Consequently, the pathological abnormality in varicosis may not be a deficiency of smooth muscle, but the inability of muscle to provide the necessary tone in vein walls due to the break up of its regular structure by fibrous tissue (Travers et al., 1996). Rose and Ahmed (1986) suggested that fibrous tissue invades muscle layers, disrupting their regular cellular pattern. While muscle hypertrophy in varicose vein walls with disorganization of connective tissue support has been suggested (Svejcar et al., 1963; Browse et al., 1988; Travers et al., 1992), others in contrast have reported muscle loss with replacement by fibrous
tissue (Rose and Ahmed, 1986; Dovel, 1992). In this study, we observed wider muscle layer in the media of varicose sections, but the amount of smooth muscle was proportionately less than in control sections.

However, the main finding was the degeneration, vacuolization and break up of smooth muscle cells. It may also be that the large proportional increase in collagenous tissue, causing dense fibrosis, contributes to the break up of muscle layers. The relaxation of the muscle cells, together with their gradually increasing decay, as well as the decreased tensile strength and elasticity of the connective tissue fibers are direct reasons for the elongation and the lateral blowouts of the veins (Lengyel and Acsady, 1990). The weakness is due to a thinning of the muscular layer of the venous wall causing the dilatations, which we call varices. The tortuosity, frequently observed, is probably due to short, comparatively normal segments lying between varicose areas (Mashiah et al., 1991).

Although the results suggest a primary defect in wall structure, it is not known whether muscle cells separate because of an intrinsic muscle abnormality (which is followed by collagen infiltration) or abnormal collagen fiber production (Travers et al., 1996). According to Rose and Ahmed, smooth muscle cells can transform into connective tissue cells, secreting collagen (Rose and Ahmed, 1986). However, in the present study we could not identify synthetic smooth muscle cells in the vein wall, but we demonstrated some SMCs phagocytozing collagen fibers with the presence of intracellular collagen-containing vesicles. The stimulus for fibrous infiltration is unknown. Genetic factors may be involved and an individual could inherit a wall weakness due to abnormal muscle cells or abnormal collagen fibers (Travers et al., 1996). These findings show that varicosis is related to increased production of collagenous tissue, which is in excess of (disproportionate to) the increase in intima, media and wall area (Travers et al., 1996). There are no essential differences in collagen composition between apparently healthy and dilated portions. These results confirm the histological investigations, which indicate that anomalies of structure and organization of the wall are identical in the dilated and apparently healthy segments of the varicose vein (Bouissou et al., 1988).

Arterial smooth muscle cells (myofibroblasts) migrate from the media into the intima, where they produce elastic and collagen fibers in arterial lesions, in response to platelet-derived growth factor (PDGF). It is likely that similar cells in veins can do the same (Milroy et al., 1989). The endothelium plays an important role in this migration in that damage to the endothelium is thought to cause the release of PDGF and similar substances (Munro and Cotran, 1988). Denuding the endothelium of rats aortas has been shown to result in the migration of smooth muscle cells from the media into the intima (Tada and Reidy, 1987). Results of electron microscopical studies by Unni and associates revealed the proliferated cells of the intima to be smooth muscle cells (Unni et al., 1974). They believed that many of the cells had vacuoles containing lipid. Vlodaver and Edwards have also characterized intimal fibroplasias as a phenomenon responding to hemodynamic stress (Vlodaver and Edwards, 1971).

Human vascular SMC have a capacity for phagocytosis and decomposition of mature collagen. As previously observed by Jurukova and Milenkov (1982), the cells of the hyperplastic venous intima containing collagen fibrils had all the features of SMC: the myofilaments within
the cytoplasm, the attachment bodies, the distinct limiting membrane, and the micropinocytic vesicles along the cell membrane. As also previously noted by the same workers, the intracellular collagen in SMC differed from that observed in rapidly collagen-synthesizing cells in that (a) the cytoplasmic collagen resembled the extra-cellular fibrils in size, staining pattern, and electron density; (b) intracellular collagen fibrils were often packed together in small groups; and (c) collagen-containing SMCs displayed a significant number of lysosomes (Jurukova and Milenkov, 1982). Staubesand described collagenous fibrils in intimal SMCs in a case of human varicose veins, assuming them to represent a heterophagic enclosure of fibril material (Staubesand, 1977). The lysosomal theory of collagen resorption is now widely accepted: this theory proposes that collagen fibrils are first fragmented in the inter-cellular spaces by the collagenase (non-lysosomal) and then engulfed by the phagocytic cells, where they undergo further degradation in the lysosomes (Woessner, 1968). Vascular SMCs are capable of phagocytosis and disintegration of their own product, collagen. Thus, they resemble fibroblasts and monocytes, which are known to be involved in collagen phagocytosis of tissues undergoing resorption (Ten Cate, 1972). Vascular SMCs may be part of the cellular basis for collagen breakdown in the remodeling of the blood vessel wall (Jurukova and Milenkov, 1982). In support of our finding that smooth muscle cells phagocytose elastic fibers, Robert found that they also have elastolytic activity (Robert, 1986). The primary defect could in fact be in the muscle cells of the vein wall, which are responsible for the synthesis and catabolism of both collagen and elastin (Venturi et al., 1996).

Contrary to the study by Bouissou et al. (1991), most of the control and varicose veins samples in our study, came from relatively young patients who were younger than 50 years of age and most of the changes described cannot, therefore, be attributed to age. Future studies should concentrate on the metabolism of smooth muscle cells of both the normal and varicose veins.

**Conclusion**

In conclusion, our study suggests that the inadequacy of the vein wall, which renders it susceptible to dilatation and varicosis under normal or raised venous pressure, is due not to the deficiency of the connective tissue components, but to the separation, degeneration, and fragmentation of smooth muscle cells, with the break-up of their regular sheet-like structure. Contrary to the view of Mashiah et al. (1991), our results also confirm that surgery for varicose veins should include stripping of the long saphenous vein in addition to removal of the varicosities themselves as documented changes occur in the proximal, clinically non-varicose saphenous vein. In agreement with Venturi et al. (1996), the study of the metabolism of smooth muscle cells in the vein wall should be an important focus of future research.

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