Smooth Muscle Changes in the Cephalic Vein of Renal Failure Patients before Use as an Arteriovenous Fistula (AVF)

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Abstract

Complications in arteriovenous fistula (AVF) occur in up to 35% of renal failure patients on hemodialysis. The most frequent complication is thrombosis, usually from stenotic lesions in the venous outflow system. To study the pre-existing smooth muscle changes in the cephalic vein of these patients, we prospectively collected a total of 17 cephalic vein specimens from 3 normal controls and 14 renal failure patients undergoing primary AVF construction on the chosen limb. After preparation, ultrathin sections were stained with uranyl and lead acetate and were examined under the transmission electron microscope (TEM). Compared with the normal controls, abnormal fibrous infiltration of the intima and the media and varying degrees of smooth muscle degenerative changes were observed in all the cephalic vein sections of renal failure patients. Smooth muscle cells (SMCs) lost their normal fusiform shape and were widely separated by increased amount of irregularly disposed, extracellular collagen fibers. Other cellular abnormalities included irregular cell membrane, granular cytoplasm, Peri- and Para-nuclear vacuoles and mega mitochondria. SMCs also showed morphological expression of phagocytosis of collagen and elastic fibers as a sign of remodeling of the vein wall. In conclusion, pre-existing wall and smooth muscle changes were observed in all the cephalic vein sections of renal failure patients, which may contribute to the later complications of AVFs.

Key words: arteriovenous fistula (AVF), renal failure, hemodialysis, smooth muscle cells (SMCs), transmission electron microscope (TEM)

Introduction

Long-term hemodialysis remains the most important support for patients with end-stage renal disease (ESRD) and reliable vascular access is an essential component of this management plan (McEwen and Audie, 1994). The native arteriovenous fistula (AVF) with its long-term patency rate and low complication profile is usually the first choice procedure for
vascular access creation (Chia et al., 1999). However, as survival among patients with renal failure improves, vascular access becomes more difficult, and preservation of a functioning access increases in importance (Myers and Mukherjee, 2000).

Complications associated with the vascular access for hemodialysis present one of the most important causes of morbidity among patients on renal replacement therapy (Neumann et al., 2001), and a major contribution to hemodialysis cost (De Marchi et al., 1996). The cost of this morbidity among end-stage renal disease (ESRD) patients in the United States (US) today will soon exceed $1 billion per year (Feldman et al., 1996). While complications are less frequent with arteriovenous fistula (AVF) than with synthetic grafts, they account for 15% of hospital admissions among US hemodialysis patients (Hirth et al., 1996). In the study by Bagolan et al., 35% of AVFs had either immediate or late complications and thrombosis was the most frequent complication observed (Bagolan et al., 1998), which usually results from stenotic lesions in the venous outflow system (De Marchi et al., 1996). Other complications of AVF include non-function, heart failure, infection, aneurysmal dilatation and bleeding (Yeboah et al., 1982). In the study by Anastassov, serious surgical aneurysmal complications of AVF were observed in more than 40% of the cases (Anastassov, 1992).

Existing methods to assess the suitability of the cephalic vein for arteriovenous fistula (AVF) construction are clinical examination, venography and color flow Doppler ultrasound. This study was conducted to evaluate the pre-existing ultrastructural changes in the cephalic vein of renal failure patients undergoing primary arteriovenous fistula (AVF) construction, with emphasis on the changes in the smooth muscle cells (SMCs).

**Material and Methods**

During the period between December 2000 and April 2001, we collected a total of 17 cephalic vein specimens from 3 young trauma patients who underwent repair of their upper limb arterial injuries and 14 renal failure patients who underwent primary, direct arteriovenous fistula (AVF) construction at Asir Central Hospital, Abha, Kingdom of Saudi Arabia. The trauma patients, who acted as normal controls, were 3 males with a mean age of 24 ± 3.6 (range 20–27 years). The renal failure patients were 5 males and 9 females with a mean age of 49 ± 19.9 (range 20–80 years). None of the patients had an existing or previously documented history of deep vein thrombosis or superficial thrombophlebitis. Renal failure patients underwent preoperative physical examination and, if required, upper limb venography to assess the presence of a suitable cephalic vein at either the wrist or the elbow. None of these patients had a previously constructed vascular access on the chosen limb. At the time of surgery, a small circumferential segment was excised from the right cephalic vein in 5 patients and from the left one in 12 patients. These were collected from the cephalic vein at the wrist in 11 patients and at the elbow in 6 patients.

Specimens were immediately put in small, labeled test tubes containing 2.5% glutaraldehyde solution and were 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 then kept in the refrigerator at 4°C for two hours. Samples were
later post-fixed in 1% osmium tetroxide in sodium cacodylate buffer, dehydrated in ascending concentration series of ethyl alcohol and embedded in Spur’s resin. Semithin sections were prepared using diamond knives, stained with toluidine blue and examined under the light microscope. Ultrathin sections were also prepared using diamond knives, but were stained with uranyl acetate and lead acetate and then examined at 80 kV under the transmission electron microscope “TEM” [Jeol® 1200 EX, Japan].

Results

Semithin sections stained with toluidine blue from both the control group and the renal failure patients, were examined under the light microscope. The tunica media of the control veins showed regular, circumferential layers of the smooth muscle cells (SMCs) separated by a normal amount of extracellular matrix. The tunica media of the cephalic vein from renal failure patients undergoing arteriovenous fistula (AVF) operation, showed excessive invasion by collagen fibers with subsequent wide separation of the SMCs. Compared with the control cephalic vein, there was thickening of the intima and media due to both hyperplasia and hypertrophy of the intimal and medial SMCs and the simultaneous increase in the extracellular fibrous tissue. This resulted in an apparent disruption of the normal, regular circular pattern of SMCs.

At the transmission electron microscope (TEM) level, the normal SMCs appeared fusiform in shape. Each cell was enclosed in an intact, regular cell membrane and contained a single nucleus with close cell-to-cell apposition (Fig. 1a). At higher magnification, regular lattice-like network containing actin and myosin filaments formed the intracellular content of each cell in addition to cisterns of rough endoplasmic reticulum, polysomes and few mitochondria (Fig. 1b). Sections of the cephalic vein from renal failure patients, showed wide separation of the medial SMCs with loss of the normal cell-to-cell contact. There was also severe disruption of the cell membrane of some SMCs, bringing disorganized, extracellular collagen fibers into direct contact with the intracellular contents (Fig. 1c). Most SMCs of the tunica intima and tunica media were irregular in shape and showed degeneration and vacuolation both around the nucleus (peri-nuclear) and along the periphery of the cell (Fig. 1d).

A characteristic observation was the presence of both collagen and elastic fibers inside SMCs. SMCs extended pseudopodia-like projections around these extracellular fibers and completely surrounded them (Fig. 2a). Fragments of internal elastic lamina and collagen fibers were seen inside irregular-shaped SMCs (Fig. 2b). Multiple and single strands of collagen fibrils were found in intracellular vacuoles (Fig. 2c) or simply fused with intracellular contents of SMCs (Fig. 2d). Another very important finding was the aggregation of increased number of mitochondria both around the nuclei and along the periphery of the SMCs (Fig. 3a). Some mitochondria coalesced together producing what is commonly referred to as “mega mitochondria” (Fig 3b). Other mitochondria showed degeneration of both their matrix and the intra-mitochondrial ridges (Fig. 3c). With further cellular degeneration, increased number of pleomorphic, dense lipofuscin granules were seen freely scattered in both the intra- and the extra-cellular matrix (Fig. 3d). These granules are often observed in degenerating cells.
Fig. 1. Normal and pre-access cephalic vein smooth muscle cells. a. Three normal smooth muscle cells (SMCs) showing regular fusiform shape, normal cell membrane (arrows) and a single nucleus (N). Note the intra-cellular lattice-like network (stars) and normal cell-to-cell contact (× 8,636). b. Higher magnification of a normal smooth muscle cell (SMC) showing intact cell membrane (arrow), few peri-nuclear mitochondria (m) and a single nucleus (N) containing a nucleolus (nu) (× 25,500). c. Damaged smooth muscle cell (SMC) of a pre-access cephalic vein showing loss of cell membrane (arrows) and disorganized collagen fibers (C) in direct contact with the intra-cellular SMC content (× 20,000). d. Degenerating SMC of pre-access cephalic vein showing irregular cell membrane (arrows), peri-nuclear degeneration (star) and no apparent organelles. Note the amorphous extracellular connective tissue (C) (× 8,250).
Fig. 2. Phagocytic activity of medial smooth muscle cells in pre-access cephalic vein. a. Extension of pseudopodia-like projections (arrow) surrounding or engulfing extracellular collagen fibers (C) (× 20,000). b. Grossly irregular smooth muscle cell surrounding extracellular elastin (E) and collagen (C) fibers, probably in a process of phagocytosis (× 9,000). c. Intracellular, collagen-containing vacuole (V) with extracellular, immature collagen fibers (C) (× 20,000). d. Abnormal presence of free lying, intracellular collagen fibers (C) (× 24,000).
Fig. 3. Mitochondrial changes. a. Abnormal increase in the number of peri-nuclear mitochondria (m) and their peripheral disposition (white arrow). Note the damaged cell membrane (black arrow) (×10,000). b. Abnormal conglomeration of mitochondria forming mega mitochondria (m). Note the chain of ghost bodies (stars) forming cytoplasmic extensions (×16,667). c. Damaged mitochondria (m) migrating towards the cell membrane and out of the cell (×12,444). d. A large number of pleomorphic lipofuscin granules (white arrows) freely scattered both inside and outside a completely damaged SMC (×10,000).
With the progression of the cellular degeneration, SMCs showed accumulation of different sizes of electron-dense granules and lipid droplets within their cytoplasm (Fig. 4 a & b). With further degeneration, SMCs lost their normal lattice-like network structure and showed granular appearance (Fig. 4c). They were later reduced to membrane-bound, structureless bodies and vesicles, marking the end-stage of the degenerative process (Fig. 4d). All the changes described were found in the cephalic vein sections from renal failure patients, but with varying degrees of severity. No evidence of venous wall calcification was found in any of the specimens.

**Discussion**

Myointimal thickening of a different degree was observed in all cephalic vein sections of renal failure patients. De Marchi et al. (1997) found that hemodialysis patients, compared with healthy subjects, had elevated levels of platelet-derived growth factor (PDGF), monocyte chemoattractant protein-1 and interleukin-6; three proteins that might be involved in the neointima formation, regulating the proliferation of vascular smooth muscle cells. In addition, these patients had numerous endothelial and hemostatic abnormalities that indicated a thrombophilic state (De Marchi et al., 1997). The same authors found that patients with AVF dysfunction, compared with those with a functioning AVF, had higher serum levels of monocyte chemoattractant protein-1 and interleukin-6; two cytokines that regulate the proliferation of vascular smooth muscle cells, which is the key mechanism in the pathogenesis of AVF stenosis (De Marchi et al., 1996).

The intracellular collagen fibrils seen inside some SMCs had the usual periodicity of collagen. The intracellular sheets of elastin observed in some SMCs also showed the same morphological appearance as those in normal control veins. This is probably a morphological expression of phagocytosis and decomposition of extracellular collagen and elastin by SMCs. Arterial SMCs (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). Vascular smooth muscle cells (SMCs) are also 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 a part of this cellular basis for collagen breakdown in the remodeling of the blood vessel wall (Jurukova and Milenkov, 1982). The lysosomal theory of collagen resorption is now widely acceptable; this theory proposes that collagen fibrils are first fragmented in the intercellular spaces by the collagenase (non-lysosomal) enzyme and then engulfed by the phagocytic cells where they undergo further degradation in the lysosomes (Woessner, 1968).

Vein wall distensibility is controlled by collagen, elastin and smooth muscle. Smooth muscle in tunica media is responsible for wall tone, which is influenced by autonomic nerves and circulating stimulants. Passive tone is provided by collagen and elastin (Traverse et al., 1996). Defects in any or all of these wall components could lead to loss of tone of the vein wall. Therefore, accumulation of collagen fibers in place of smooth muscle cells (SMCs) would cause a decrease in the elasticity of the vein wall. Also, as suggested by Rose and Ahmed (1986),
Fig. 4. Advanced degeneration. a. Degenerating smooth muscle cell (SMC) showing electron-dense granules (arrows) and loss of the normal cell texture (stars). The cell is surrounded by amorphous extracellular material (e) ($\times$ 16,000). b. Degenerating fragmented smooth muscle cell (SMC) showing lipid droplets (L), electron-dense granules (arrows) and loss of the cell texture ($\times$ 10,800). c. Severely damaged smooth muscle cell (SMC) with almost complete loss of the cellular lattice-like network (star) ($\times$ 11,280). d. Final stage of SMC degeneration showing complete loss of intracellular components. The cells are reduced to structure-less, membrane-bound vesicles (V), which are widely separated by amorphous extracellular material (stars) ($\times$ 7,077) (Compare with Fig. 1a).
Smooth muscle changes in the cephalic vein before AVF 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, as “effective contraction cannot occur unless individual cells are in communication with each other”. 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, could contribute to the elongation and aneurismal dilatation of the veins once they are used as AVFs.

Numerous studies have shown that changes occur in the saphenous vein after implantation as bypass grafts (Viodaver and Edwards, 1971; Lawrie and Brawley, 1976; Batayias et al., 1977; Lie et al., 1977; Spray and Roberts, 1977; Smith and Geer, 1983), and these have been regarded as acquired, representing their adjustment to the arterial circulation (Giannoukas et al., 1997). However, evidence of unsuspected pre-existing venous wall changes in otherwise macroscopically normal saphenous veins prior to bypass has been presented, and the morphological similarities of these changes to the intimal hyperplasia seen in vein grafts has lead to the hypothesis that they may be related (Marín et al., 1991). Cheanvechai et al. (1975) described phlebosclerosis in unused segments of long saphenous veins from patients undergoing coronary artery bypass surgery. They described intimal plaques composed of acellular collagen and hypertrophy of the tunica media in approximately one quarter of veins. Thiene et al. (1980) described acellular intimal plaques, luminal narrowing, and changes in the medial longitudinal muscle layer in unused segments of the long saphenous vein in patients undergoing coronary artery bypass surgery. Spray and Roberts (1977) noted the presence of phlebosclerosis in unused segments of veins used for coronary artery grafts and noted that pre-existing intimal fibrosis bore a remarkable resemblance to post-grafting intimal changes. Batayias et al. (1977) reported that unused segments of long saphenous vein from 14 out of 50 patients examined showed varying degrees of sclerosis, consisting of a mild degree of relatively acellular intimal thickening, associated with slight to moderate fibrosis and hyalinization.

The changes that occur in veins after grafting or use as an arteriovenous shunt have been termed arterializations. Now that some changes are documented in veins before grafting or anastomosis to arteries, i.e. in the absence of arterial pressure, the term arterialization may not be appropriate. The changes recorded in these veins may have important consequences once the vein is used as a shunt. Their presence may form the basis for further lesions once the vein is used as an AVF, leading to further fibrosis. Our results suggest a pre-existing defect in the structure of the cephalic vein of renal failure patients, but it is not known whether SMCs separate because of an intrinsic muscle abnormality (which is followed by collagen infiltration) or abnormal collagen fiber production. According to Rose and Ahmed (1986), SMCs can transform into connective tissue cells, secreting collagen. However, the stimulus for fibrous infiltration is unknown. The study of the metabolism of SMCs in the vein wall should, therefore, be an important focus of future research (Venturi et al., 1996).

The high incidence of pre-existing smooth muscle changes in our series, suggests that it is rather unusual to find a cephalic vein without changes. This might reflect either the pre-existing vascular changes in renal failure patients or the fact that most of the patients undergoing AVF construction for hemodialysis are elderly. The mean age for the patients in this study was 49 years and it has been previously shown that age-linked changes in the venous wall appear
between 50 and 60 years of age (Bouissou et al., 1991). The presence of these changes may have significance for the outcome of the vein grafts in regards to the development of stenosis or occlusion (Marin et al., 1993). However, the inevitable limitation in our study, as is usual in similar studies, was that the whole length of the cephalic vein was not available for microscopic examination.

**Conclusion**

In conclusion, the observed structural changes may have important implications in early and late AVF failure and may well determine the future behavior of the vein as a shunt. The changes described form the basis for further study of cephalic vein pathology in renal failure patients.

**Acknowledgements**

All the patients gave informed consent for the use of the specimens obtained from them for the purpose of the histopathological investigations and the authors were appreciated for the agreement of the use of these specimens for the present study.

**References**


Smooth muscle changes in the cephalic vein before AVF


(Received July 10, 2002: Accepted July 29, 2002)