ACTA STEREOL 1999; 18/1: 33-37
ORIGINAL SCIENTIFIC PAPER

REMODELING OF VASCULAR SMOOTH MUSCLE CELLS IN THE RABBIT AFTER MULTIPLE GESTATION

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ABSTRACT

Remodeling and dilatation of the saphenous vein wall is a feature in the development of varicose vein disease. Because of the existence of high similarities between varicose vein disease and the normal vascular remodeling of veins of the lower extremity during pregnancy we tested the hypothesis that vascular smooth muscle cells of the saphenous vein from rabbits after multiple gestation changed their shape and size. We have measured the volume densities of smooth muscle cells of the saphenous vein wall of both multiparous and nulliparous rabbits by point counting. The number of cells per unit volume were counted and the cross diameters of the vascular smooth muscle cells were estimated by measuring their diameters perpendicular to the long axis of their nuclei. It appeared that the vascular smooth muscle cells of the saphenous vein from multiparous rabbits had an increased cellular volume and an enlarged cellular diameter. However, the length of the vascular smooth muscle cells of the saphenous vein from multiparous rabbits showed a decrease compared to the vascular smooth muscle cells of the saphenous vein from nulliparous rabbits. This suggests that after multiple gestation the vascular smooth muscle cells of the saphenous vein remain a change in shape and size. This remodeling of the vessel wall could therefore destroy cell-cell and cell-matrix attachments resulting in a modified compliance of the vein wall as also observed in varicose vein disease.

Key words: hypertrophy, saphenous vein, stereology, vein disease.

INTRODUCTION

A transformation of the extracellular matrix and vascular smooth muscle cells of the saphenous vein wall is associated to a lower contractile capacity in varicose veins. It has been described that during varicosity the venous wall has an increased content of collagen (Rose and Ahmed, 1986; Travers et al., 1996) and a decreased fraction of smooth muscle cells (Rose and Ahmed, 1986). Recently, we described that the volume density of smooth muscle cells and collagen did not changed between varicose and non-varicose veins (Kockx et al., 1998). Moreover, we demonstrated that the smooth muscle cells of the human varicose saphenous vein are hypertrophic and showed microherniations. These microherniations are vesicles that

bud from the smooth muscle cell and could be associated to local pericellular proteolysis. This transformation of the smooth muscle cells of the human saphenous vein could induce a weakness of the venous wall when compliance of the vessel wall is needed to compensate for an increased hemodynamic pressure. An induced weakness of the venous wall has been related to the blood flow turbulence by reflux observed in varicose vein disease (Leu et al., 1979; Miranda et al., 1993). Because pregnancy can induce varicose veins of the lower extremity (Schadeck and Vin, 1984; Skudder and Farrington, 1993), related to hormonal and hemodynamic alterations (Cordts and Gawley, 1996), we tested the hypothesis that vascular smooth muscle cells of the saphenous vein of rabbits after a third gestation remain hypertrophic and therefore comparable to the transformation of vascular smooth muscle cells found in human varicose veins.

MATERIAL AND METHODS

We studied the transformation of the smooth muscle cells in the saphenous vein from multiparous (n=5) and nulliparous (n=5) rabbits. The study was aged matched and the samples of the multiparous group were obtained three weeks after they had their third delivery. The length of the obtained saphenous veins samples varied from 1 cm to 1.5 cm. From each individual vein sample three systematically random regions were taken. These three randomly taken vein parts were fixed in 4% formaldehyde, embedded in paraffin and sectioned serially at 5 μm. The sixth slide was sectioned at 30 μm. The plane of section was perpendicular to the long axis of the saphenous vein. We used a Trichrome-Masson staining to assess fibrous and muscular components of the venous wall. From the slide, stained with Trichrome-Masson, the volume density (V_v) of the vascular smooth muscle cells of the saphenous vein were measured using a point counting frame. We used in total 30 counting frames selected systematically on the three vein segments of each animal. On each fourth slide a Verhoeff staining was performed for the observation of the elastin network. From the sixth slide, 30 μm thick and stained with HE, the number of smooth muscle cells per unit volume were counted using the optical disector. In total we used 45 counting frames systematically random selected from the three venous segments of each animal. The sampling volume of the disector was the distance between the two disector sections (10 μm) multiplied by the area of the test frame. The cellular volume of the smooth muscle cells were calculated by multiplying the total unit volume (of all disectors) by the volume density of the smooth muscle cells and then dividing by the total number of the counted smooth muscle cells. From each third section, stained with PAS, the cellular diameter of the vascular smooth muscle cells of the saphenous vein were measured perpendicular to the long axis of the selected nuclei. We used a PAS staining to recognize the border of the smooth muscle cells in the circular orientated layer of the vein wall. The average diameter of the smooth muscle cells was calculated from the individual measured diameters of smooth muscle cells obtained from 50 random taken counting frames. The length of the smooth muscle cells were calculated by dividing the cellular volumes of the smooth muscle cells by $1/4\Pi d^2$. Here d was the average diameter of the smooth muscle cells. For each animal, the values of the smooth muscle cell density, the cellular volume, and the diameter of the smooth muscle cells were collected from the different counting frames used. Thus the data given in the text are the averages of the individual animals plus or min the standard deviation. The differences between the saphenous vein wall of multiparous and nulliparous rabbits were compared using the Mann-Whitney-U-test using the values of the five individual animals. A value of $p=0.05$ was considered statistically significant.

RESULTS

The venous wall of the saphenous vein of rabbits did not contain stainable fibers of elastin. These findings differed with the observed elastin pattern found in the human saphenous vein wall. The V_V of the vascular smooth muscle cells of the saphenous vein differed significantly (p=0.0452) between the nulliparous (92.9 ± 5.1%) and multiparous rabbits (84.7 ± 1.6%)(fig 1A). The extracellular matrix of the saphenous vein from multiparous rabbits showed more collagen content between the layers of smooth muscle cells compared to the vessel wall of nulliparous rabbits using the Thrichrome-Masson staining. The thickness of the venous wall was increased in the saphenous vein of multiparous rabbits. More interestingly, the cellular volumes of the individual smooth muscle cells of the saphenous vein from multiparous rabbits showed a significant increase compared to the smooth muscle cells of the saphenous veins from nulliparous rabbits, respectively 3677 ± 248 μm^3 and 3183 ± 177 μm^3 (fig 1B). Like the smooth muscle cells of the human saphenous vein in varicose vein disease, the smooth muscle cells of the venous wall from multiparous rabbits were hypertrophic and contained microherniations. The diameter of the smooth muscle cells of the saphenous vein from multiparous rabbits showed a significant increase compared to the diameter of smooth muscle cells of the saphenous vein from nulliparous rabbits, respectively 11.32 ± 1.22 µm and 8.10 ± 1.45 µm (fig 1C). The length of the smooth muscle cells of the saphenous vein from multiparous rabbits showed, however, a significant decrease compared to the smooth muscle cells of the saphenous vein from nulliparous rabbits (fig 1D) suggesting that the cells of the multiparous rabbits became rounded.

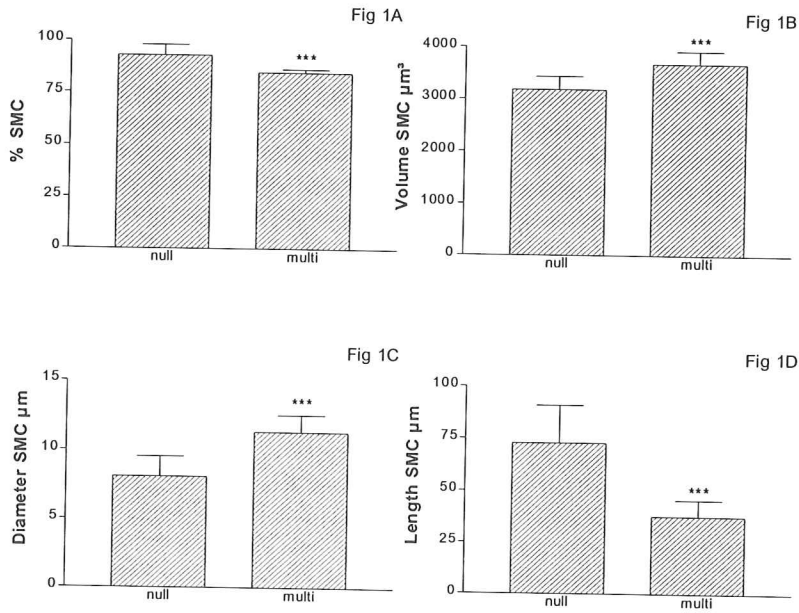

Fig. 1 *A) shows the average ± S.D. of the V_V of the smooth muscle cells in the saphenous vein from nulliparous (nulli) and multiparous (multi) rabbits (p=0.0452). B) shows the cellular volume of the individual smooth muscle cells (p=0.0163). C) shows the diameter of smooth muscle cells (p=0.0160) and D) shows the length of the smooth muscle cells of the saphenous vein from both nulliparous and multiparous rabbits. (p=0.0283).*

CONCLUSION

In general the remodeling of the saphenous vein wall of rabbits after multpile gestation can be compared to the changes of smooth muscle cells observed in the circular layer of the human saphenous vein wall during varicose vein disease. In both veins the smooth muscle cells transformed into hypertrophy of the smooth muscle cells. In the present study we demonstrated that the V_V of the smooth muscle cells differed significantly between the saphenous vein of nulliparous and multiparous rabbits. Recently, we could not observed a significant difference in V_V of vascular smooth muscle cells in the vessel wall of varicose and non-varicose human saphenous veins (Kockx et al., 1998). To compare the V_V of smooth muscle cells of the saphenous vein of nulliparous rabbits with those of multiparous rabbits is stereologically incorrect and could give inappropriate conclusions. The decreased V_V of the smooth muscle cells found in this study, could be the result of an increased collagen content and/or a decreased fraction of the vascular smooth muscle cells. The content of collagen did show an increase in staining pattern for Trichrome-Masson in the saphenous vein wall of the multiparous rabbits. This increased content of collagen could be secondary to the transformation of the smooth muscle cells. It appeared that the increased content of collagen is not related to the viscoelastic properties of the vessel wall suggesting that the remodeling of the venous wall in order to compensate its weakness is not due to a changed collagen content (Psaila and Melhuish, 1989). In the present study, we did not observed elastin patterns in the saphenous veins of nulliparous and multiparous rabbits. It was suggested that in varicose veins of the human saphenous vein the elastin patterns were significant changed and that disruption of the elastin-contractile units could be induced by these vesicles that bud from the smooth muscle cells (Kockx et al., 1998). Moreover, it appeared that there was a significant correlation between the appearance of vesicles, the change of elastin pattern, and the appearance of smooth muscle cell hypertrophy in the human saphenous veins during varicosity. The fact that rabbits do not have an elastin pattern similar to human limit the use of this animal model to evaluate if hypertrophy of smooth muscle cells could cause the disruption of elastin-contractile units. In the present study we observed a significant increase of cellular volume of the vascular smooth muscle cells of the saphenous vein from multiparous rabbits compared to the smooth muscle cells of the saphenous vein from nulliparous rabbits. In addition the vascular smooth muscle cells of the multiparous rabbits showed microherniations, i.e. vesicles that bud from the smooth muscle cells. Hypertrophy of smooth muscle cells can be induced by hormonal and systemic factors (Van Bilsen and Chien, 1993). This suggests that during the stage of pregnancy when hormonal and systemic factors are upregulated (Cordts and Cawley, 1996) hypertrophy of smooth muscle cells could be induced. This increased cellular volume of the smooth muscle cells in the saphenous vein from multiparous rabbits could transform the shape and size of these cells. We observed that the smooth muscle cells of the saphenous vein from multiparous rabbits increased their cellular diameter and decreased their total length. These findings confirm our suggestion that vascular smooth muscle cells of the saphenous vein had transformed their shape and size during multiple gestation. This remodeling of the vascular smooth muscle cells of the venous wall could influence the interactions between the smooth muscle cells as well as the extracellular matrix, possibly by pericellular proteolysis. Therefore, disruption of cell-cell and cell-matrix attachment could be the consequence of the appearance of smooth muscle cell hypertrophy resulting in a modified compliance of the vein wall. In addition, the remodeling of smooth muscle cells in the saphenous vein of multiparous rabbits due to cellular hypertrophy can be compared to the remodeling of smooth muscle cells in the circular layer of the human saphenous vein as observed in varicose vein disease.

Acknowledgement

This work is supported by the INOV foundation. Dr. MM Kockx is a holder of a fund for fundamental clinical research of the Flemish Fund for Scientific Research (FWO).

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Presented at the 7th European Congress for Stereology, Amsterdam, April 20th to 23rd, 1998.