RECIPROCAL DYNAMICS OF CD34 AND α-SMA EXPRESSION AS MARKERS OF STAGED LEG ARTERY REMODELING IN EXPERIMENTAL METABOLIC SYNDROME

Otamurodov A.N.[1], Khoshimov B.L.[2]

[1]Termez Branch of Tashkent State Medical University, Termez, Uzbekistan

[2]Kimyo International University in Tashkent, Tashkent, Uzbekistan

Abstract

The study aimed to evaluate the combined dynamics of the endothelial marker CD34 and the smooth muscle marker α-SMA in the leg arteries in experimental metabolic syndrome (MS) and to substantiate a staged model of arterial remodeling. MS was induced in 90 sexually mature male white laboratory rats by a high-calorie diet combined with a 10% fructose solution as drinking water for 30, 60 and 90 days. The external iliac, femoral, popliteal, anterior and posterior tibial arteries were examined by immunohistochemistry and digital morphometry. The proportion of CD34-positive endothelial cells decreased from 87.53% in controls to 70.19%, 58.42% and 46.78% on days 30, 60 and 90, respectively. In contrast, the α-SMA-positive area of the media increased from 68.40% to 76.80%, peaked at 89.56% on day 60 and remained high (85.42%) on day 90. The two markers were strongly inversely correlated ($r=-0.81$; $p<0.001$). The α-SMA/CD34 ratio, calculated from group means, rose from 0.78 in controls to 1.09, 1.53 and 1.83, i.e. 2.3-fold by day 90. Three successive stages of remodeling were distinguished: endothelial–intimal (day 30), medial hypertrophic (day 60) and fibrotic (day 90). Combined assessment of CD34 and α-SMA determines the stage of peripheral arterial remodeling in MS more precisely than either marker alone.

Keywords: metabolic syndrome, leg arteries, CD34, α-SMA, endothelium, vascular smooth muscle cells, immunohistochemistry, arterial remodeling.

Introduction

The prevalence of MS continues to grow worldwide, and peripheral arteries of the lower limbs are among its important vascular targets [1, 2]. Endothelial injury is regarded as an initiating event of vascular remodeling, whereas smooth muscle cells (SMCs) shape the subsequent structural changes of the wall through phenotypic plasticity, shifting from a contractile to a proliferative and synthetic state [4, 5]. These two components are usually studied separately, which makes it difficult to establish their sequence and interrelation.

CD34 is a transmembrane glycoprotein expressed by vascular endothelial cells and endothelial progenitors [3] and is widely used to visualize the endothelial lining. α-Smooth muscle actin (α-SMA) is a component of the SMC contractile apparatus, and its expression changes during phenotypic modulation [4, 5]. Parallel assessment of both markers in the same arteries may therefore reveal the order in which the intima and media become involved.

The aim of the study was to analyze the reciprocal dynamics of CD34 and α-SMA expression in the leg arteries over 90 days of experimental MS, to relate them to morphometric parameters and to substantiate the stages of arterial remodeling.

Materials and methods

The study was performed on 90 sexually mature male white laboratory rats weighing 180–220 g, kept under standard vivarium conditions. Control animals received a standard laboratory diet. In the experimental group, MS was modeled by a high-calorie diet combined with a 10% fructose solution as drinking water for 30, 60 and 90 days.

The external iliac, femoral, popliteal, anterior tibial and posterior tibial arteries were examined. Immunohistochemistry was performed on paraffin sections with monoclonal antibodies against CD34 and α-SMA. For CD34, the proportion of immunopositive endothelial cells and staining intensity were assessed; for α-SMA, the

positive area and the proportion of positive cells in the media. Morphometry was carried out with a NanoZoomer (Hamamatsu) digital scanner and QuPath software and included internal diameter, intima, media and total wall thickness, intima/media index, number and density of SMC layers, and volume fractions of collagen and elastic fibers.

As an auxiliary integral index, the ratio of the α-SMA-positive area (%) to the proportion of CD34-positive endothelial cells (%) was calculated from the group means. Data were processed by variation statistics using Student's t-test and correlation analysis; $p<0.05$ was considered significant.

Results and discussion

CD34 and α-SMA expression changed in opposite directions throughout the experiment (Table 1).

Table 1

CD34 and α-SMA expression in the leg arteries in experimental MS (group means)

Period	CD34-positive endothelial cells, %	α-SMA-positive area, %	α-SMA/CD34 ratio
Control	87.53	68.40	0.78
Day 30	70.19	76.80	1.09
Day 60	58.42	89.56	1.53
Day 90	46.78	85.42	1.83

The rates of change of the two markers differed. CD34 fell most sharply during the first 30 days (by 17.34 percentage points) and then by 11.77 and 11.64 points in the following intervals. α-SMA, in contrast, rose most between days 30 and 60 (by 12.76 points versus 8.40 in the first interval) and then declined slightly (by 4.14 points). The maximal endothelial response therefore preceded the maximal medial response, which allowed three stages of remodeling to be distinguished.

Stage I — endothelial–intimal (day 30). CD34-positive cells made up 70.19% (537 of 765 endothelial cells). Focal loss of CD34 positivity was accompanied by endothelial swelling, nuclear enlargement, widened intercellular spaces and single foci of desquamation. Intima thickness increased by 22.4–47.9% and the intima/media index by 10.8–19.2%. α-SMA rose only moderately (76.80±2.5% versus 68.40±2.3% in controls; $p<0.05$), reflecting early functional activation of SMCs.

Stage II — medial hypertrophic (day 60). α-SMA reached its maximum (89.56%), with strong circumferential staining of the media, SMC hypertrophy and medial thickening. CD34 decreased further to 58.42%, and marker-negative areas of the endothelium expanded. Total wall thickness increased by 19.3–35.8%, and the internal diameter of the anterior tibial artery decreased by 20.4%. At this stage, the media becomes the leading site of remodeling.

Stage III — fibrotic (day 90). CD34 fell to 46.78%, with foci of almost complete loss of expression and multiple areas of desquamation. α-SMA remained high (85.42%) but became heterogeneous, with foci of reduced intensity. These changes coincided with an increase in collagen fibers by 72.3–79.3%, a loss of elastic fibers by 33.9–46.2% and adventitial thickening by 36.3–64.6%. The slight decline and heterogeneity of α-SMA amid massive collagen accumulation suggest a partial shift of SMCs from the contractile toward a synthetic, matrix-producing state, in which contractile proteins are down-regulated [4, 5].

Correlations. CD34 correlated positively with internal diameter ($r=0.82$) and elastic fiber content ($r=0.83$) and negatively with intima thickness ($r=-0.84$), intima/media index ($r=-0.79$), total wall thickness ($r=-0.81$) and collagen fraction ($r=-0.86$). α-SMA correlated positively with media thickness ($r=0.88$), number of SMC layers ($r=0.85$), SMC density ($r=0.80$) and collagen fraction ($r=0.78$), and negatively with elastic fiber content ($r=-0.76$; all $p<0.001$). The two markers were also inversely correlated with each other ($r=-0.81$; $p<0.001$). Both markers are thus tied to the same

matrix parameters with opposite signs, which makes them complementary rather than redundant.

Value of the combined index. Judged by α-SMA alone, medial remodeling would appear to stabilize between days 60 and 90 (89.56% → 85.42%). However, the α-SMA/CD34 ratio kept rising (1.53 → 1.83) because of progressive endothelial loss, in agreement with the ongoing fibrotic changes on day 90. The combined index therefore reflects the cumulative imbalance between the intima and media better than either marker separately. Since the index was derived from group means and the decline of CD34 may have several mechanisms, its diagnostic value requires validation on individual data.

Conclusions and recommendations

1. In experimental MS, CD34 and α-SMA expression in the leg arteries change reciprocally: CD34-positive endothelial cells decrease from 87.53% to 46.78% by day 90, while the α-SMA-positive area increases from 68.40% to a maximum of 89.56% on day 60 and remains high (85.42%) on day 90 ($r=-0.81$ between markers).
2. Arterial remodeling proceeds in three successive stages: endothelial–intimal (day 30), medial hypertrophic (day 60) and fibrotic (day 90).
3. The α-SMA/CD34 ratio increases 2.3-fold by day 90 and continues to reflect progression of remodeling when α-SMA alone reaches a plateau.

Recommendations: CD34 and α-SMA should be assessed together and combined with morphometric parameters of the intima, media and extracellular matrix when evaluating peripheral arterial remodeling in experimental MS. After validation on individual data, the α-SMA/CD34 ratio may serve as an auxiliary index for staging. The endothelial–intimal stage (day 30) is the most appropriate window for testing preventive interventions.

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