COLLAGEN–ELASTIN IMBALANCE AND EXTRACELLULAR MATRIX REMODELING OF LEG ARTERIES IN EXPERIMENTAL METABOLIC SYNDROME

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Abstract

The study aimed to characterize extracellular matrix (ECM) remodeling of the leg arteries in experimental metabolic syndrome (MS), focusing on the balance between the elastic and collagen components of the arterial wall. 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 using hematoxylin–eosin and pentachrome staining with digital morphometry (NanoZoomer Hamamatsu, QuPath). As early as day 30, the volume fraction of elastic fibers decreased by 13.1–18.6% and the internal elastic lamina (IEL) became 8.3–15.2% thinner, while the collagen fiber fraction increased by 24.1–28.9%. By day 90, elastic fibers had decreased by 33.9–46.2% and IEL thickness by 24.1–38.8%, whereas collagen increased by 72.3–79.3% and adventitial thickness by 36.3–64.6%. Elastolytic and fibrotic changes were most pronounced in the distal (popliteal and tibial) segments. Collagen content correlated strongly and negatively with endothelial CD34 expression ($r=-0.86$) and positively with α-SMA expression ($r=0.78$). Progressive collagen–elastin imbalance is thus a key structural mechanism of peripheral arterial remodeling in MS.

Keywords: metabolic syndrome, leg arteries, extracellular matrix, collagen fibers, elastic fibers, internal elastic lamina, arterial fibrosis, digital morphometry.

Introduction

Metabolic syndrome is one of the most widespread cardiometabolic conditions, and its global prevalence continues to rise [1, 2]. Insulin resistance, hyperglycemia, dyslipidemia and oxidative stress affect not only the endothelium but the whole arterial wall, including smooth muscle cells and the ECM [3–5]. In the arterial wall, elastic fibers provide recoil and distensibility, whereas collagen fibers limit overstretching and largely determine wall stiffness. Impaired elastic properties and structural remodeling of arteries have been documented in experimental models of diabetes and hypertension [6–9].

ECM architecture differs along the arterial tree [10], so proximal and distal leg arteries may respond differently to metabolic stress. However, the time course and

segmental distribution of collagen–elastin remodeling in the leg arteries in MS remain insufficiently quantified.

The aim of the study was to evaluate the dynamics of the elastic and collagen components of the leg artery wall in experimental MS and to relate these changes to endothelial (CD34) and smooth muscle (α-SMA) immunohistochemical markers.

Materials and methods

The study was performed on 90 sexually mature male white laboratory rats weighing 180–220 g, kept under standard vivarium conditions of Tashkent State Medical University. The animals were divided into control and experimental groups. Control rats 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 harvested. Serial paraffin sections 4–5 μm thick were stained with hematoxylin and eosin for general histology and with Masson's pentachrome method to differentiate collagen, elastic fibers and other ECM components. Slides were digitized with a NanoZoomer (Hamamatsu) scanner and analyzed in QuPath software.

The following parameters were measured: internal and external diameters; total wall, intima, media and adventitia thickness; IEL thickness; volume fractions of collagen and elastic fibers; and the number of smooth muscle cell (SMC) layers. Changes are expressed as percentages relative to the control values of the corresponding artery; ranges reflect variation among the arteries studied. Data are presented as M±m; intergroup differences were assessed with Student's t-test, and $p<0.05$ was considered significant. Relationships between ECM parameters and CD34 and α-SMA expression, obtained by immunohistochemistry in the same experimental series, were assessed by correlation analysis.

Results and discussion

Experimental MS caused a progressive collagen–elastin imbalance in all studied leg arteries, detectable as early as day 30 (Table 1).

Table 1

Changes in ECM-related parameters of the leg arteries in experimental MS (change relative to control, %)

Parameter	Day 30	Day 60	Day 90
Elastic fiber volume fraction	−13.1 to −18.6	−21.0 to −30.5	−33.9 to −46.2
Collagen fiber volume fraction	+24.1 to +28.9	+42.0 to +47.2	+72.3 to +79.3
Total wall thickness	+11.3 to +21.8	+19.3 to +35.8	+32.9 to +60.4

Day 30. The IEL thinned by 8.3–15.2% and the elastic fiber fraction fell by 13.1–18.6%, while the collagen fraction rose by 24.1–28.9%. These shifts developed together

with endothelial activation, intimal thickening by 22.4–47.9% and an increase in the number and density of SMC layers. ECM remodeling therefore starts at the early stage of MS rather than being only a late consequence of wall damage.

Day 60. Elastic fibers decreased by 21.0–30.5% and the IEL continued to thin, while collagen increased by 42.0–47.2%. SMCs became hypertrophic and showed features consistent with a transition to the synthetic phenotype. Synthetic SMCs are an important source of collagen matrix [4, 5], which may explain the parallel growth of collagen content and medial thickness at this stage.

Day 90. Elastic fibers had decreased by 33.9–46.2% and IEL thickness by 24.1–38.8%, while collagen had increased by 72.3–79.3%. Fibrosis extended to all wall layers: media thickness increased by 30.6–56.1% and adventitial thickness by 36.3–64.6%. Collagen accumulation accelerated in the late period: its excess over control grew by about 18% of the control value between days 30 and 60, but by 30–32% between days 60 and 90. Elastic fiber loss accelerated in the same way (by 8–12% and 13–16% in these intervals).

Segmental features. Elastolytic and fibrotic changes were most pronounced in the distal segments. The maximal values on day 90 — elastic fiber loss of 46.2%, IEL thinning of 38.8% and collagen gain of 79.3% — were recorded in the popliteal and tibial arteries. Lumen narrowing was also greatest distally: the internal diameter of the anterior tibial artery decreased by 26.5%, versus 12.3% in the external iliac artery. Distal arteries have a thinner wall (60.7±2.1 μm versus 116.2±3.4 μm in the control external iliac artery), which may limit their elastic reserve and increase their susceptibility to metabolic injury.

Relationship with cellular markers. Collagen content correlated negatively with endothelial CD34 expression ($r=-0.86$) and positively with α-SMA expression ($r=0.78$). Elastic fiber content showed the mirror pattern: positive correlation with CD34 ($r=0.83$) and negative with α-SMA ($r=-0.76$; all $p<0.001$). Loss of endothelial integrity and activation of medial SMCs were thus accompanied by replacement of elastic structures with collagen, indicating a single endothelium–SMC–matrix axis of remodeling.

The shift from elastic to collagen components forms a morphological substrate for increased arterial stiffness, in line with the impaired elastic properties and microstructural remodeling described in diabetic arteries [6, 7]. Because the mechanical properties of the vessels were not measured directly, this interpretation requires biomechanical confirmation.

Conclusions and recommendations

1. Experimental MS causes early and progressive collagen–elastin imbalance in the leg arteries: elastic fiber loss increases from 13.1–18.6% on day 30 to 33.9–46.2% on day 90, while collagen accumulation increases from 24.1–28.9% to 72.3–79.3%.

2. Fibrotic remodeling accelerates between days 60 and 90 and extends to all wall layers, including the adventitia (+36.3–64.6%).

3. The distal popliteal and tibial arteries are the most vulnerable segments, showing maximal elastic degradation, collagen accumulation and lumen narrowing.

4. ECM changes are tightly linked to endothelial injury (CD34) and SMC activation (α-SMA), reflecting a coupled endothelium–SMC–matrix mechanism of remodeling.

Recommendations: the volume fractions of collagen and elastic fibers and IEL thickness should be included as quantitative markers when assessing peripheral arterial remodeling in experimental MS. Distal leg arteries are recommended as the most sensitive segments for such studies. Since ECM changes are already detectable on day 30, preventive and therapeutic interventions should be evaluated from this early stage.

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