Prevalence of Fetal-Type Smooth Muscle Cells in the Media of Microvessels From Hypertensive Patients

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Abstract—Significant structural and functional changes in smooth muscle cells (SMCs) of microvessels (diameter 30 to 300 μm) occur in hypertension. However, in microvessels of hypertensive patients, the differentiation pattern of SMCs underlying such changes remains undefined. To analyze the differentiation pattern of SMCs (adult, postnatal, or fetal), 49 muscle biopsies (rectus abdominis) were analyzed: 16 from children (aged 11 months to 11 years), 15 from normotensive adults (aged 55 to 74 years), 18 from hypertensive adults (aged 55 to 74 years). Transverse cryosections of specimens were studied by immunocytochemistry using monoclonal antibodies SM-E7 and NM-F6, which recognize smooth muscle myosin heavy chain (MyHC) and A pla-like nonmuscle MyHC, respectively. The total number of microvessels was assessed via SM-E7 staining. The number of NM-F6 positive (fetal-type SMC) or negative (adult-type SMC) microvessels was assessed. The number of microvessels per area unit was considerably lower (P<0.0005) in normotensive adults (0.22±0.17) than in children (0.98±0.61). Even more significant reduction was found in hypertensive adults compared with control adults (P=0.013) and children (P<0.0005). The qualitative immunocytochemistry analysis by NM-F6 revealed 2 differentiation patterns of the media layer of microvessels: positive or negative. In hypertensive subjects, the percentage of microvessels positive to NM-F6 was 49.8±35.6%, close to that found in children (50.6±12.6%), whereas in normotensive subjects it was significantly lower (24.4±21.1%). The following conclusions were drawn. (1) The medial layer of microvessels is heterogeneous in terms of SMC differentiation. (2) In hypertension, a prevalence of fetal-type SMCs takes place in microvessels, resembling that of children. Compared with children, a rarefaction of microvessels is present in normotensive adults that is even more remarkable in hypertensive adults. (Hypertension. 2004;44:191-194.)

Key Words: muscle, smooth ■ hypertension, arterial ■ arterioles

It is well known that structural and functional changes in arterioles and small resistance arteries (lumen diameter ≈30 to 300 μm) are associated with hypertension.1,2 These alterations include a decreased luminal diameter and an increased medial thickness leading to an increased media-lumen ratio.3–5 The media-lumen ratio in microvessels can increase as a consequence of eutrophic remodeling (ie, a rearrangement of otherwise normal material around a narrowed lumen), or because of hypertrophic remodeling (either hypertrophy or hyperplasia of vascular smooth muscle cells).6,7 These processes would imply a profound change in the differentiation pattern of medial smooth muscle cells (SMCs)8–10 that has not yet been defined.

Analysis of the expression of different myosin heavy chain (MyHC) isoforms has identified, in rabbit and in human aorta, 3 maturation steps of SMCs: fetal, postnatal, and adult. In previous studies,8,9,11 we have shown that fetal-type SMCs coexpressed anti-smooth muscle (SM)1/2-MyHC, along with the 2 anti-nonmuscle (NM)-MyHC isoforms A pla and A pla. Postnatal SMCs expressed SM1/2-MyHC and NM-MyHC A pla. The adult-type SMC was characterized by the expression of SM-MyHC isoforms only. In adult rabbit aorta, 2 SMC populations have been identified: cells showing either the adult or the postnatal pattern, whereas in the prenatal period, fetal-type SMCs predominated. A similar picture was observed in the human aorta.11 The SMC phenotype has been reported to range in a continuum from “contractile SMCs” to “synthetic SMCs,” according to a decreasing contractile capacity paralleled by an increasing capability of replicating and metabolizing proteins and lipoproteins. Taking into account the expression of our markers during development and in vascular disease,8,10–12 the fetal-type SMCs of our nomenclature would overlap the synthetic SMC phenotype, whereas the adult-type would correspond to the contractile one. Accordingly, intimal SMC proliferation is accompanied by the expression of a fetal pattern of MyHC isoform and by

Received January 28, 2004; first decision February 20, 2004; revision accepted May 18, 2004.

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Hypertension is available at http://www.hypertensionaha.org

DOI: 10.1161/01.HYP.0000133692.47754.e5

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a marked increase in the number of immature-type SMCs in the media underlying the atherosclerotic plaque.\textsuperscript{8,10,12,13}

A previous study of microvessels (MVs) from heart and skeletal muscle of renovascular hypertensive rabbits showed that a prevalence of fetal-type SMC is present in this model, which is poorly influenced by raised blood pressure levels.\textsuperscript{9}

Data on SMC differentiation in MVs of hypertensive subjects is lacking. Therefore, the goal of our study was to define the potential changes in SMC types of MVs (diameter range 30 to 300 μm) from human skeletal muscle of patients with essential hypertension and of children.

Methods

We studied 16 children (mean age 3.5 years, range 1 month to 11 years), 15 normotensive subjects (mean age 63.9 years, range 55 to 74 years), and 18 hypertensive patients (mean age 66.4 years, range 55 to 74 years) undergoing abdominal surgery. We defined hypertension according to World Health Organization guidelines (systolic blood pressure \(\geq 140\) mm Hg or diastolic blood pressure \(\geq 90\) mm Hg).\textsuperscript{14}

Exclusion criteria were secondary hypertension, diabetes mellitus, older than 74 or younger than 55 years. All hypertensive patients were being treated with calcium antagonists and/or angiotensin-converting enzyme inhibitors, except 1 who was taking β-blockers. There was no difference between normotensive and hypertensive adults as far as demographic and anthropometric data are concerned, including total plasma cholesterol, triglycerides, and glycemia. In our hypertensive subjects, the average blood pressure level was 150.6±15.5 mm Hg systolic and 85.9±7.8 mm Hg diastolic.

Forty-nine skeletal muscle biopsies (rectus abdominis) were analyzed. Immediately after surgery, each sample was placed in an OCT compound (Tissue Tek), frozen in liquid nitrogen, and stored at \(-80^\circ\text{C}\). Transverse cryosections (8 μm) of specimens were studied by immunocytochemistry using monoclonal antibodies. The following antibodies were used: SM-E7 SM-MyHC and NM-F6 NM-MyHC. SM-E7 binds selectively to SM-type MyHC 1 and 2 isoforms, whereas NM-F6 identifies a specific antigenic epitope localized in the platelet-type MyHC isoform MyHC-Apla1 and reacts neither with B-type NM-MyHC nor SM-MyHC.\textsuperscript{10}

The combined reactivity to SM-E7 and NM-F6 antibodies identifies fetal-type SMCs, whereas reactivity to SM-E7 is typical of the entire SMC population independent of the phenotype.\textsuperscript{10}

The number of MVs was assessed by SM-E7 staining. Moreover, we evaluated the number and relative percentage of MVs stained by NM-F6. Nuclei were revealed with the use of the bis-benzimide stain (Hoechst 33258). The number of MVs counted on each section was normalized according to the area unit (1 mm\(^2\)). Measurements of MVs were carried out in blind on 3 sections per sample. Tissue masses were comparable among the 3 groups. Calculation of tissue area, as well as counting of MVs, was performed with a computerized image analysis system (Software QWIN, Leica Microsystems).

For statistical analysis, ANOVA plus Tukey post hoc test was used.

Results

The number of MVs per area unit was considerably lower in normotensive adults than in children (0.22±0.17 versus 0.98±0.61, respectively, \(P<0.0005\)). An even more remarkable decrease was found in hypertensive patients (0.10±0.08, \(P<0.0005\) versus children, \(P=0.013\) versus normotensive adults; Figure 1A).

The qualitative immunocytochemistry analysis by NM-F6 revealed 2 differentiation patterns of the media layer of MVs: positive or negative (Figure 2). Details of positive and negative vessels, respectively, are shown in Figure 3. Based on staining of nuclei, lack of reactivity to the NM-F6 antibody was not related to a decreased number of cells into the media layer because the same cell density was always observed by staining of nuclei (Figure 3). There was no correlation between positivity to the NM-F6 and the arterial diameter (not shown).

We also assessed the percentage of NM-F6 positive vessels per tissue area (Figure 1B). In hypertensive patients, there were many more NM-F6–positive MVs compared with normotensive subjects (49.8±35.6% versus 24.4±21.1%, respectively, \(P=0.021\)). In specimens from children, the prevalence of NM-F6–positive microvessels was close to that of hypertensive adults (50.6±12.6%).

Discussion

Differentiation and subsequent growth of SMCs have an important impact on the biology of common vascular diseases [...]
such as atherosclerosis and restenosis after revascularization.\textsuperscript{10,12,15} The basic feature of these pathologies is represented by the proliferation/migration of medial SMCs to the intima, which is accompanied by marked morphological, biochemical, and functional changes of the SMCs found in the normal, adult arterial wall.\textsuperscript{15,16} Several studies have stressed the pathogenetic role of SMCs showing a fetal phenotype because of their great capacity of proliferating, migrating, synthesizing collagen, and metabolizing lipoproteins. These cells do, indeed, represent the bulk of atherosclerosis and of restenosis lesion\textsuperscript{12} and the way whereby hypertrophy of the tunica media of large vessels is achieved during hypertension.\textsuperscript{8,13}

This study shows that the media layer of human MVs is heterogeneous in terms of SMC differentiation. In particular, an increase in MVs showing a prevalence of fetal-type SMCs takes place in hypertension, which resembles the pattern found in children. This would indicate that in hypertensive MVs a type of ontogenetic recapitulation occurs that would enable MVs to face the new environmental conditions dictated by high blood pressure and the subsequent need for wall remodeling via cell proliferation and collagen deposition. A different picture came out from the study of MVs in hypertensive rabbits,\textsuperscript{9} because in this animal model fetal-type SMCs predominate even in normotensive MVs from controls. This species-related difference is not surprising\textsuperscript{10,17} and may partly explain the peculiar time-course of experimental hypertension in these animals.

In agreement with previous studies,\textsuperscript{18–20} a rarefaction in MVs was observed in specimens from hypertensive adults. A similar rarefaction has also been described during normal development and in the early stages of hypertension.\textsuperscript{20} Although both rarefaction and luminal narrowing play a key role in increasing vascular resistance and, hence, blood pressure level, their biological relationship remains elusive and deserves further studies. On the whole, our study demonstrates that hypertensive MVs are characterized by prevalence of fetal-type SMCs in the tunica media, which may serve as the biological basis for wall thickening and decreased luminal diameter. Unfortunately, it was not possible to investigate whether or not the prevalence of fetal-type SMCs was related to structural remodeling of MVs. In fact, under our experimental conditions (frozen muscle specimens), it was not possible to reliably assess the wall-to-lumen ratio. Apparently, there was no relationship between SMC phenotype and MV diameter. An appropriate approach to clarify this issue is to undertake specific studies on isolated MVs.

The changes we observed in MVs from hypertensive adults can play a pathophysiological role in the occurrence and maintenance of high blood pressure. Because changes in the differentiation pattern of SMCs precede cell proliferation and the achievement of new functional properties, we propose that antihypertensive drugs should also be evaluated in terms of their efficacy in preventing or reversing changes in the differentiation pattern of SMCs of MVs. In fact, under our experimental conditions (frozen muscle specimens), it was not possible to reliably assess the wall-to-lumen ratio. Apparently, there was no relationship between SMC phenotype and MV diameter. An appropriate approach to clarify this issue is to undertake specific studies on isolated MVs.

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In conclusion, this study shows for the first time that in the medial layer of MVs from hypertensive subjects an increase in fetal-type SMCs takes place, resembling that of children, along with vascular rarefaction. Several aspects remain to be

\begin{figure}[h]
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\includegraphics[width=\textwidth]{image1.png}
\caption{Micrograph of skeletal muscle (transverse 8-μm-thick cryosection) showing the differentiation pattern of microvessels. In microvessels, we found 2 different patterns of tunica media staining with the NM-F6 monoclonal antibody: positive (·) or negative (○). (×10).}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{image2.png}
\caption{Seriate, transverse cryosections from skeletal muscle of a control subject. A and B, Immunocytochemistry with the NM-F6 monoclonal antibody. Microvessels negative (A) or positive (B) to staining are shown. C and D, Hoechst nucleus staining. Negativity of staining with the NM-F6 in A was not because of lack of medial cells, but to absent reactivity to the antibody itself. (×20).}
\end{figure}
investigated, particularly the extent and the time course of such changes in secondary hypertension.

**Perspectives**

By further investigating the modifications occurring in SMC phenotype of MVs from hypertensive patients, it will be possible to better understand the time course of changes in the biological adaptation to high blood pressure levels and hence, of the basic processes leading to MV remodeling. In fact, SMC rearrangement implies a previous change in phenotype, which would impact on several structural and functional properties of MV wall, including extracellular matrix production and expression of various molecules such as cytokines and growth factors. Moreover, it will be possible to shed a new light onto the impact of antihypertensive medication on the molecular properties of SMCs of the MV wall. The evaluation of the basic features that are upstream to changes in structure and function of MVs could also disclose a new opportunity to identify normotensive subjects who are potentially prone to rearrangement of the shape and the function of their MV wall before they become hypertensives.

**References**

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Hypertension. 2004;44:191-194; originally published online June 28, 2004; doi: 10.1161/01.HYP.0000133692.47754.e5
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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