Expression of cytoskeletal proteins and ATPase activity in bovine femoral artery and vein intima

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Summary. Intimal cells play an important role in the biology of the vascular wall. Variability in the metabolic activity of intimal smooth muscle cells (SMC), as well as the differential expression of cellular cytoskeletal proteins depend on factors such as degree of differentiation, aging, atherosclerosis, etc. Myosin ATPase activity and cytoskeletal proteins were studied in the intima of bovine arterial and venous vessels, during ageing (Printseva et al., 1987; Jurukova, 1989; Marinov et al., 1989; Nanaev et al., 1990; Jurukova, 1989; Hajisiski et al., 1991; Sukhova et al., 1991; Bochaton Piallat et al., 1992).

It has to be noted also that abnormal proliferation and alterations in the metabolic activity of SMC are important features in describing processes of intimal thickening in vessels undergoing certain pathological changes. Along with that SMC show morphological and biochemical heterogeneity related to species, vessel type and local haemodynamic status (Gayele et al., 1982; Schmid et al., 1982; Price and Lazarides, 1983; Sakata et al., 1990; Nanaev et al., 1990; Nakamura et al., 1992; Durmowicz et al., 1995).

The aim of the present study was to examine the distribution of intermediate filament protein immunoreactivity and ATPase activity in bovine femoral artery and vein tunica intima in relation to possible causes of expression and development of SMC heterogeneity.

Materials and methods

Femoral arteries and veins were obtained from eight healthy mature animals of Bubalus bubalis species immediately after sacrifice. They were then fixed in 10% formaldehyde and embedded in paraffin. For enzyme cytochemistry unfixed frozen sections (12 μm) were fixed in formaldehyde-calcium solution for 10 min at room temperature. Myosin ATPase was investigated by the calcium-cobalt method of Padykula and Herman (1955) at pH 9.2, modified according to the protocol of Lojda et al. (1979). Serial controls were used and the specificity of the reaction was tested by incubation of sections without substrate for the evaluation of the enzyme activity; heating of the sections in distilled water at 80 °C for 10 minutes (before incubation) to test non-enzymatic hydrolysis of the substrate; incubation in equimolar concentration of 2-glycerophosphate to examine nonspecific phosphomonoesterases; and treatment with L-cystein to inhibit alkaline phosphatase.

Paraffin sections were processed for immunocytochemistry by the streptavidin-biotin immunolocalization
system (Histostain SP-kit, ZYMED Laboratories Ltd.,
South San Francisco USA). Prediluted antibodies to
desmin (No 08-0016), vimentin (No 09-0052) and
cytokeratin (No 08-0059) (ZYMED, USA, kindly
donated by Dr. A. Bella) were used. Controls were
performed by omission of the primary antibody,
replacement of the primary antibody with non-immune
serum and application of an inappropriate antibody. All
controls were negative. Positive controls for cytokeratin
were also performed on bovine and human placenta and
skin. Structural peculiarities of the respective vessels
were assessed by hematoxylin-eosin, orcein, Azan, Van
Gieson and Goldner staining.

Results

In the intima of six of the eight animals studied no
obvious structural differences from those accepted as
«normal» were noted in arteries (Fig. 1); in veins intimal
structure was normal in all animals (Fig. 6). Intimal
SMC of these vessels were predominantly longitudinally
oriented. In two of the femoral arteries, however,
uniformly thickened tunica intima, with two distinct
layers - inner-elastic hyperplastic (EHL) and outer-
musculo-elastic (MEL) were observed (Figs. 2-5, 8).
Elastic laminae were situated between these layers
and also at the borders with the media. In one of these
altered vessels we also encountered massive calcium
depositions on the inner surface of the lamina elastica
interna, as well as at the borders of media-adventitia
(Fig. 5).

ATPase enzyme histochemistry

Endothelial cells of femoral arteries and veins
showed positive reaction to ATPase (Figs. 7, 9). Enzyme

![Fig. 1. Femoral artery-immunoreactivity to
vimentin (A) and desmin (B). Endothelial cells
(arrows) are vimentin-positive and desmin-
negative; smooth muscle cells of tunica intima
show similar immunoreactivity. x 80]
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Fig. 2. Femoral artery with thickened tunica intima with EHL (A) and MEL (B). Goldner stain. x 40

Fig. 3. Femoral artery with thickened tunica intima. Desmin immunoreactivity in EHL and MEL as well as in tunica media (M). x 100
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Activity was observed in all SMC in arteries with «normal» intimas, as well as in all veins (Figs. 7, 9).

Differentiated enzymatic expression was observed in femoral arteries of those animals exhibiting intimal thickenings (Table 1). In the EHL SMC were ATPase negative, while those in the MEL were ATPase positive, like those in medial SMC (Fig. 8).

Immunohistochemistry

Endothelial cells (EC) of all vessels were immunopositive to vimentin (Figs. 1A, 4, 5, 6, 8) and negative to desmin and cytokeratin. In arteries with unchanged intimas, as well as in all veins, the longitudinal SMC were vimentin positive, and desmin and cytokeratin negative (Fig. 1B). In cases with intimal thickening we

Fig. 4. Femoral artery with thickened tunica intima. Vimentin immunoreactivity in EHL (A) and MEL (B). M: tunica media. x 100
observed differential distribution of immunolabelling for desmin and vimentin (Table 1). Desmin-positive cells were very rare in the EHL (Fig. 3, 5A) while those positive to vimentin were numerous and uniformly distributed around the circumference of the vessel (Figs. 4, 5B). In the MEL desmin-positive SMC were numerous and uniformly distributed (Fig. 3, 5A). Vimentin-positive SMC were rare and non-uniformly arranged. They occupied mainly its innermost part (Fig. 4). No cytokeratin immunoreactivity was encountered.

Discussion

The results from the present study show that EC of arteries and veins are vimentin positive, i.e. their cytoskeletal intermediate filaments are vimentin in nature. These results are consistent with other findings (Schmid et al., 1982) and our earlier data in different vertebrates (Dikranian et al., 1993). At the same time EC exhibit high ATPase activity, thus showing that they possess the capacity to utilise high energy phosphates for maintaining various energy-dependent processes such as synthesis, secretion and intercellular transport (Firth and Stranks, 1981; Cannon et al., 1982).

Vimentin- and desmin-immunoreactivity in intimal SMC is similar to that in the aorta and vena cava of different classes of vertebrates (Schmid et al., 1982; Dikranian et al., 1983) as well as in arteries and some veins in humans (Troshova and Dikranian, 1991).

The distribution of both desmin and vimentin cytoskeletal proteins in the thickened bilayered arterial intima as expressed by immunocytochemistry is of particular interest. Similar distribution has been observed in the process of ageing in human aortas (Rekhter et al., 1991). As it was already noted, desmin- and vimentin-immunopositive SMC were specifically distributed. This could also be interpreted in terms of their functional status. Desmin-immunoreactive SMC were predominantly situated in the MEL and may...
correspond to the so-called «elongated myocytes» observed in this layer in other vessels (Rekhter et al., 1991). The predilectional disposition of vimentin-immunoreactive SMC of the EHL suggests that they may correspond to the «stellate» SMC of this layer (Ross et al., 1984; Rekhter et al., 1991) although light microscopy does not give evidence for that. We also suppose that desmin-immunoreactive SMC of the intima originate from migrating medial myocytes, which were also intensely stained for desmin. This has also been observed in age- and disease-related states (Gayele et al., 1982; Ross et al., 1984; Jurukova, 1989; Guirato et al., 1991). In our opinion these cells could greatly contribute to intimal hyperplasia. The existence of vimentin-immunoreactive SMC of the EHL could be the result of phenotypic modulation from contractile to a secretory type and could also be caused by age-determined or other as yet unknown stimuli. Such a modulation has been shown in vitro as well as in the initial stages of SMC proliferation (Ross et al., 1984; Glukhova et al., 1985; Printseva et al., 1987; Andreeva et al., 1992).

However, possible colocalization of desmin and vimentin in this SMC population cannot be excluded.

Taken together our results of the differential immunopositivity of SMC in intimal thickenings could be explained following the principle that vimentin-immunoreactivity is predominantly characteristic of the
early stages of vessel wall structuring, while desmin-immunoreactivity is a marker of more advanced or age-determined stages of vessel differentiation in vivo and in vitro (Price and Lazarides, 1983; Glukhova et al., 1985; Nikkari et al., 1988, 1990; Absher et al., 1989; Nanaev et al., 1990; Bochaton Piallat et al., 1992; Nakamura and Sakurai, 1992).

It seems that intimal SMC classified as those of synthetic connective tissue cells, contributing to age and disease-determined pathological secretion of the intimal matrix (Jurukova, 1989; Hajiiski et al., 1991; Desmoliere and Gabbiani, 1992), can express different intermediate filament proteins depending on their functional status and/or degree of differentiation. Intimal SMC show intensive ATPase activity and this may contribute to the energy supply of the metabolic aspects of the processes of phenotypic modulation (Nikolov et al., 1988; Hajiiski et al., 1991) accompanying intermediate protein expression changes.

Judging from these results we assume that bovine femoral arteries showing proliferation kinetics in apparently non-diseased conditions could serve as an interesting natural model for investigations of the arterial system.

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References


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