References

Bačakova L , Švorčik V , Rybka V , Miček I , Hnatowicz V , Lisa V , Kocourek F (1996) Adhesion and proliferation of cultured human vascular smooth muscle cells on polystyrene implanted with N^+, F^+ and Ar^+ ions Biomaterials **17,** 1121—1126

Lowry O H , Rosebrough N J , Farr A L , Randall R J (1951) Protein measurement with the Folin phenol reagent J Biol Chem **193,** 265—275

Margiotta M S , Robertson F S , Greco R S (1992) The adherence of endothelial cells to Dacron induces the expression of the intercellular adhesion molecule (ICAM-1) Ann Surg **216,** 600—604

Pellicciari C , Bottone M G , Schaack V , Manfredi A A , Barni S (1996) Etoposide at different concentrations may open different apoptotic pathways in thymocytes Eur J Histochem **40,** 289—298

Skalli O , Ropraz P , Trzeciak A , Benzonana G , Gillessen D , Gabbiani G (1986) A monoclonal antibody against alpha-smooth muscle actin a new probe for smooth muscle differentiation J Cell Biol **103,** 2787—9276

Švorčik V , Rybka V , Hnatowicz V , Bačakova L , Lisa V , Kocourek F (1995) Surface properties and biocompatibility of ion implanted polymers J Mater Chem **5,** 27—30

Different Responsiveness of Male and Female Rat Aortic Smooth Muscle Cells (SMCs) to Repeated Passaging in Culture

L Bačáková[1], C Pellicciari[2], M-G Bottone[2], V Lisá[1] and V Mareš

1 *Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic*

2 *Dipartimento di Biologia Animale, Universita di Pavia, Pavia, Italy*

Abstract. The smooth muscle cell (SMC) cultures were prepared from the aorta of male and female 8-week-old rats and used at passage 5–7 or 40 45 On day 1, low-passaged cells of both sex groups adhered to growth supports at similar numbers while after repeated passaging the adherence of female-derived cells was higher These cells had also higher total protein content and contained more of the SMC specific α-actin, vimentin and α_v integrins Compared to the male type of cultures, the high passaged cells of female origin cycled at a slower rate and were undergoing massive polyploidization Male-derived cells remained of the same morphology, ploidy and the differentiation status at all passages Their passage response consisted mainly in faster cycling and growth to higher population densities The data could be of importance for explanation of different incidence of hyperplastic vascular diseases in males and females

Key words: Smooth muscle cells — Cell adhesion — Cycling and polyploidization — Sex-related differences

Correspondence address L Bačáková, Institute of Physiology, Academy of Sciences of the Czech Republic, Vídeňska 1083, 142 20 Praha 4 Krč, Czech Republic E-mail lucy@biomed.cas.cz

Hyperplasia of the smooth muscle cell (SMC) population accompanies formation of atherosclerotic plaques, hypertension-caused vessel wall thickening and origin of restenosis after vascular surgery Moreover, these changes occur more often in male organisms It was found earlier that migration and proliferation potential of vascular SMCs in cultures prepared from male donor rats was higher than in those from female donors Moreover, the growth curves measured in cultures prepared from both sex-donors suggested that this difference was more pronounced in repeatedly passaged population (Bačáková *et al* 1997) In this study we compare passage-dependent differences in cell kinetics and chemodifferentiation in male- and female-derived SMC populations

The SMC cultures were prepared from the thoracic aorta of male and female Wistar SPF 8-week-old rats The cells in passage 5 to 7 or 40 to 45 were seeded in 5 ml of Dulbecco Minimum Essential Medium (15,000 cells/cm^2) with fetal calf serum (10%) and gentamicin (40 μg/ml) Their growth was assessed by counting of cells in Burker haemocytometer after trypsinization and by flow cytometry of BrdU incorporation and propidium iodide stained DNA content (Pellicciari *et al* 1996, Bačáková *et al* 1997) The volume of cells was calculated from the diameters measured by an ocular microscale in living suspensions of cells prepared by mild trypsinization (0 1 % trypsin, 5 to 10 min) The number of chromosomes was determined in the cells blocked in mitosis by Colcemid (Ciba, 5 μg/ml, 120 min) The content of α-actin, vimentin and α_v integrins was determined by ELISA and the total content of protein by the colorimetric Lowry's method (Lowry *et al* 1951)

The number of cells that adhered to growth supports at 24 h post-seeding interval was in the low-passaged cultures (5–7) similar in both male and female-derived populations At passage 40–45, the number of adhered cells was significantly higher in female-derived cultures (12,500 ± 1500 vs 8500 ± 1200 cells/cm^2, $p < 0\ 05$) In addition, the high-passaged female-derived cells were more flat and polygonal, whereas the male SMCs were spindle-shaped and bulging After reaching confluence (days 4 to 7), the volume of the low-passaged female-derived SMCs was 1 5 times higher than in the male-type cultures (2480 ± 3 vs 1640 ± 2 μm^3, p < 0 001) This difference has further increased at higher passages The total protein content per cell was similar in low-passaged cultures from both sex donors, while at high passages it became higher in the female-derived cultures (0 23 ± 0 01 vs 0 16 ± 0 01 mg/10^6 cells, p < 0 02) As shown by ELISA, the total content of α-actin per cell, a marker of vascular SMC differentiation, was in the female derived cultures higher by 7% and 27% in the low- and high- passaged populations, respectively

Table 1 Content of cytoskeletal and adhesion molecules in cultured male and female aortic SMCs measured by ELISA[1]

Molecule	Passage 5–7			Passage 40 45		
	males		females	males		females
α-actin per cell	100%	$p < 0\ 05$	107 ± 3%	100%	$p < 0\ 001$	127 ± 4%
per mg protein	100%	n s	92 ± 4%	100%	n s	105 ± 4%
vimentin per cell	100%	$p < 0\ 05$	105 ± 2%	100%	$p < 0\ 05$	113 ± 6%
per mg protein	100%	n s	98 ± 8%	100%	n s	111 ± 7%
α_v integrins per cell	100%	$p < 0\ 05$	122 ± 11%	100%	n s	116 ± 17%
per mg protein	100%	n s	92 ± 3%	100%	n s	103 ± 3%

[1]Absorbances of female derived samples in % of values obtained in male derived cells Means ± S E M Measured in 7 day old cultures

(Table 1, $p < 0\ 05$ and $p < 0\ 001$, resp) Similar differences were observed in the content of vimentin As revealed by immunofluorescence, the α-actin containing microfilaments in male-derived SMCs formed longitudinal parallel bundles, whereas in female-originated cells, they were arranged in a mesh-like network Expression of α_v integrins was significantly higher in the female derived cultures at lower passages (+22%, $p < 0\ 05$) The flow cytometry revealed that low-passaged cells of both sex-donors were mainly diploid At higher passages, more than 70% cells of the female-derived population contained 4C DNA (Fig 1) In 30% of mitoses of female-type cells treated by Colcemid we found tetraploid chromosome numbers The proliferation capacity of SMCs of both sex donors was similar at lower passages while in high passage cultures, the male-derived SMCs had a significantly shorter doubling time (12 2 ± 0 8 vs 17 1 ± 1 8 h in female-derived specimens, $p < 0\ 05$), higher S-phase fraction (15–18% vs 5–6%) and a higher population density on day 3 after seeding (131,400 ± 7200 vs 87,700 ± 6200 cells/cm^2, $p < 0\ 001$)

The study showed that the response of SMCs of male and female origin to repeated passaging was qualitatively different At higher passages the cells of female origin were binding to the extracellular matrix molecules more firmly and the cells were reaching higher degree of chemodifferentiation Better adhesion of female-derived SMCs could be partly due to a higher expression of α_v integrins i e receptors for vitronectin adsorbed on

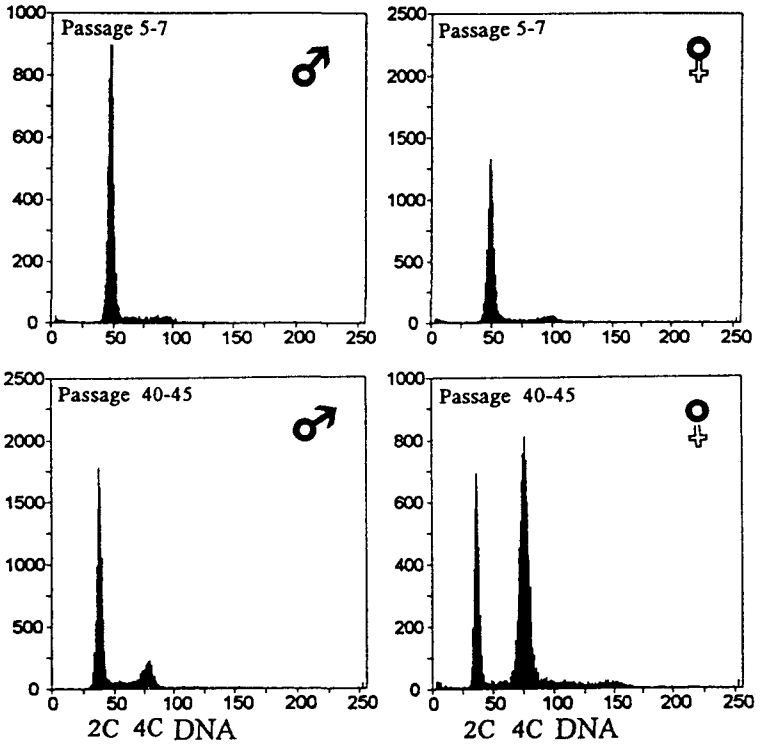

Figure 1. DNA histogram of male and female SMCs at passage 5–7 and 40–45 Abscissa DNA content in a u Ordinate Number of cells 2C, 4C Diploid and tetraploid content of DNA, respectively

the culture dishes from the culture medium With the increased number of passages, the cells of female origin became more prone to develop of a block in cyto- and karyokinesis resulting in a massive formation of tetraploid cells In contrast, the cells of male origin remained diploid and cycling at a higher rate A small number of 4C DNA cells is present also in the vascular wall *in situ* under physiological conditions and it increases with age and during hypertension (Rosen *et al* 1985, Owens *et al* 1988) Earlier studies performed *in vitro* showed that endomitosis followed by formation of 4C DNA cells occur in cultures of SMCs treated with angiotensin, arginin-vasopressin, catecholamines and TGF-beta in absence or very low concentrations of serum-provided mitogens (Yamori *et al* 1987, Owens *et al* 1988) In our study, repeated passaging could therefore induce different sensitivity of the male and female SMCs to the molecules responsible for the completion of karyokinesis resulting in a different number of 4C DNA cells in both sex-derived populations

Acknowledgements. Supported by the Grant No IAA7011908 of the Grant Agency of the Academy of Sciences of the Czech Republic and from the Fondo di Ateneo per la Ricerca (University of Pavia, FAR 1998) Cytometric measurements were taken at the Centro Grandi Strumenti (University of Pavia)

References

Bačakova L , Mareš V , Lisa V , Bottone M G , Pellicciari C , Kocourek F (1997) Sex related differences in the adhesion, migration, and growth of rat aortic smooth muscle cells in culture In Vitro Cell Develop Biol. **33,** 410—413

Lowry O H , Rosebrough N J , Farr A L , Randall R J (1951) Protein measurement with the Folin phenol reagent J Biol Chem **193,** 265—275

Owens G K , Geisterfer A A T , Yang Y W -H , Komoriya A (1988) Transforming growth factor-β-induced growth inhibition and cellular hypertrophy in cultured vascular smooth muscle cells J Cell Biol. **107**, 771—780

Pellicciari C , Bottone M G , Schaack V , Manfredi A A , Barni S (1996) Etoposide at different concentrations may open different apoptotic pathways in thymocytes Eur J Histochem. **40,** 289—298

Rosen E , Goldberg I D , Shapiro H M , Zoller L C , Myrick K V , Levenson S E , Halpin P A (1985) Growth kinetics as a function of ploidy in diploid, tetraploid, and octaploid smooth muscle cells derived from the normal rat aorta J Cell Physiol **125,** 512—520

Yamori Y , Mano M , Nara Y , Horie R (1987) Catecholamine induced polyploidization in vas cular smooth muscle cells Circulation **75** (Suppl I), I-92—I95

Ultrastructural Response of the Nuclear Envelope (NE) of C6-Glioma Cells to Cisplatin-Induced Apoptosis

D Krajčí[1], V Mareš[2] and V. Lisá[2]

1 *Department of Anatomy, Faculty of Medicine, Kuwait University, Kuwait,*
2 *Institute of Physiology, Academy of Sciences, Prague, Czech Republic*

Abstract. The early response of the nuclear envelope of C6-glioma cells ($t \leq 24$ h), treated with a cytostatic dose of cisplatin in culture (5 μg/ml) included formation of slim

Correspondence address V Mareš, Institute of Physiology, Academy of Sciences, Vídeňská 1083, 142 00 Prague, Czech Republic