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Ultrastructural Characteristics of Aortic Endothelial Cells in Borderline Hypertensive Rats Exposed to Chronic Social Stress

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Summary

Genetic predisposition and social stress may represent important risk factors in etiology of hypertension associated with endothelial dysfunction. Perturbations of endothelial structural integrity are also critical for the pathogenesis of vascular diseases. We examined effect of chronic social stress on structure of aortic endothelium in borderline hypertensive (BHR) and normotensive Wistar rats. Male BHR – offspring of Wistar mothers and SHR fathers and age-matched W were exposed to 6-week crowding stress (5 rats/cage, 200 cm^2/rat). Aortic tissue was processed for electron microscopy and NO synthase activity measurement. Crowding stress significantly increased blood pressure in BHR compared to basal values (140±3 mm Hg vs. 130±3 mm Hg, $p<0.05$) and reduced enzyme activity by 37 % ($p<0.01$) in the aorta of BHR. Local slight structural alterations of endothelium were found in non-stressed BHR ($p<0.001$) when compared with Wistar rats. Chronic stress caused marked ($p<0.005$) subcellular injury of endothelial cells in aorta of BHR characterized by mitochondrial damage, presence of vacuoles, increased number of lysosomes, Weibel-Palade bodies, changes of intercellular connections and local disruption of endothelium, while only slight changes were seen in Wistar rats. Results suggest increased sensitivity of aortic endothelium of BHR to chronic crowding that may contribute to acceleration of arterial dysfunction.

Key words

Social Stress • Hypertension • Endothelium • Ultrastructure • Rat

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Introduction

It is now apparent that endothelial dysfunction referring mainly to impaired NO-dependent vasodilatation precedes clinical symptoms of cardio-vascular disease. Besides, endothelial dysfunction is also accompanied with a microscopically visible cell injury as well as disruption of endothelial barrier lining. These structural alterations are implicated as important pathogenic factors contributing to vascular disease states as they affect the permeability of endothelium, allow the passage of circulating blood cells, macromolecules and inflammatory fluid from blood stream to the underlying tissues (Vallet 2003).

Hypertension is a major risk factor of cardiovascular diseases associated with endothelial dysfunction. Structural alterations of endothelium in vessels observed in hypertensive animals (Kristek *et al.* 1997, Okruhlicová *et al.* 2000, 2005, Tribulová *et al.* 2000) indicated their potential contribution to the development or maintenance of the high blood pressure. Hypertension is multifactorial disease, in many cases resulting from complex interaction of genetic and social factors. The use of chronic psychosocial stress in borderline hypertensive rats (BHR) resulted in the development of hypertension, heart hypertrophy and significant cardiac pathology (Lawler *et al.* 1981). Psychosocial stress is associated with cardiovascular alterations like endothelial dysfunction, inflammation, metabolic and hematological abnormalities, and increased activity of sympathetic nervous system and renin-angiotensin-aldosterone system (McCarty and Gold 1996, Esch *et al.* 2002). In addition, it was demonstrated in our previous study that altered vascular NO synthesis

might also be involved in chronic social stress-induced changes in vascular function and blood pressure in adult borderline hypertensive rats (Bernátová and Csizmadiová 2006).

Although there is some evidence from clinical and experimental studies indicating the impairment of the endothelium-dependent relaxation in hypertension (Török *et al.* 2006) and chronic stress (Sherwood *et al.* 1999), there is no available information about the fine structure of endothelium in borderline hypertension exposed to chronic stress. Therefore, we studied the effect of psychosocial stress on endothelial structural integrity of aorta of rats with genetic predisposition to hypertension.

Methods

Animals

BHR used in the study were born in our certified animal facility as F_1 offspring of normotensive (Wistar) dams and spontaneously hypertensive sires. All animals BHR and Wistar were housed at 22-24 ^{o}C on a 12:12-h dark-light cycle (07.00-19.00 lights on) and maintained on a pellet diet and tap water *ad libitum*. After weaning (25th day), male rats were kept in groups of 4 rats per cage (35/55/20 cm, 480 cm^2/rat). The investigation conforms to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication NO. 85-23, revised 1996).

Stress model

Adult male rats, 15 weeks old, were randomly divided into two groups. Control normotensive and borderline hypertensive rats were kept in groups of 4 rats/cage (35/55/20 cm, 480 cm^2/rat). Rats exposed to crowding stress were kept in groups 5 rats/cage (25/49/15 cm), where their living space was reduced to 200 cm^2/rat, for 6 weeks (Bernátová and Csizmadiová 2006).

Blood pressure measurement

Two weeks before the experiment, the rats were handled and accustomed to the procedure of blood pressure recording, using non-invasive tail-cuff plethysmography. Blood pressure was determined at the beginning and at the end of experiment after 6 weeks.

NO synthase activity

NO synthase activity was determined in crude homogenates of aorta by determination of [^{3}H]-L-citrulline formation from [^{3}H]-L-arginine (Amersham, UK), as described previously (Bernátová *et al.* 2002).

Transmission electron microscopy

For electron microscopic examination the heart was retrogradely fixed *via* thoracic aorta with 2.5 % glutaraldehyde in 100 mmol/l cacodylate buffer (pH 7.4). Thoracic aorta was cut into 3 mm long rings and additionally fixed by immersing in 2.5 % glutaraldehyde for 3 h at 4 ^{o}C. After washing in cacodylate buffer, the tissue was postfixed in 1 % OsO_4, dehydrated via ethanol series, infiltrated with propylene oxide and embedded in Epon 812. Ultrathin sections cut on ultramicrotome LKB Huxley (London, Great Britain) were counterstained with uranyl acetate and lead citrate and examined in electron microscope Tesla 500 (Brno, Czech Republic).

Statistical analysis

For the quantitative scoring, the electron micrographs of aortic endothelium were given a random number and scored blindly by three people. The pictures were scored from zero to three based on the level of structural changes seen in mitochondria, nucleus, vacuoles, lysosomes, Weibel-Palade bodies and intercellular connections. Score “zero” represents normal cell architecture, “one” slight change in cell structure, “two” moderate, and “three” represents severe injury of cell structure. The three independent scores were averaged together for each slide.

Differences were assigned between individual groups using Student’s t-test. Values were considered to be different significantly when $p<0.05$. All data are expressed as mean ± SEM.

Results

Blood pressure measurement

The chronic stress did not affect the blood pressure in stressed normotensive Wistar rats when compared with non-stressed rats (111±2 mm Hg vs. 113±2 mm Hg). On the other hand, blood pressure was significantly increased in BHR exposed to chronic crowding compared to basal values (130±2 mm Hg vs. 140±3 mm Hg, $p<0.05$).

NO synthase activity

The NO synthase activity was 7.75±0.80 pmol/min/mg in aorta of control BHR. Chronic stress reduced the enzyme activity in stressed BHR (3.32±0.52 pmol/min/mg, $p<0.01$). Crowding stress did not affect the

Tab. 1. Quantitative score of subcellular alterations in endothelial cells of the aorta.

	Control	Stress
W	0.23±0.01	0.93±0.02
BHR	1.06±0.02^{+}	2.06±0.02*

* p<0.005, BHR vs. BHRs; $^{+}$p<0.001, W vs. BHR. W – Wistar rats, BHR – borderline hypertensive rats. Subcellular alterations included changes in mitochondria, nucleus, vacuoles, lysosomes, Weibel-Palade bodies and intercellular connections.

NO synthase activity in aorta of Wistar rats compared to non-stressed rats (5.25±0.44 pmol/min/mg vs. 5.22±0.79 pmol/min/mg).

Transmission electron analysis

The electron microscopic examination showed classic architecture of aortic endothelial cells in control normotensive Wistar rats and in BHR. However, in BHR the endothelial cells locally displayed slight till moderate subcellular injury (Fig. 1) manifested by heterogeneous density of cell cytoplasm, presence of vacuoles, increased amount of lysosomes and Weibel-Palade bodies and electronlucent chromatin. Intercellular connections contained interdigitating complexes, overlapping clefts and end-to-end connections containing gap junctions and occasionally irregularly widened tight junctions.

The chronic stress resulted in structural alteration of endothelial cells of aorta of Wistar rats resembling those in non-stressed BHR (Fig. 2). Ultrastructural analysis of aortic endothelial cells of stressed BHR showed more severe subcellular injury compared to control BHR (Fig. 3). The cells were edematous, contained injured mitochondria, numerous vacuoles, and electronlucent chromatin. The integrity of the endothelial lining was locally destroyed. Quantitative scoring of subcellular alterations of endothelial cells is shown in Table 1. In addition, smooth muscle cells located in subendothelial media also locally displayed subcellular injury (Fig. 3).

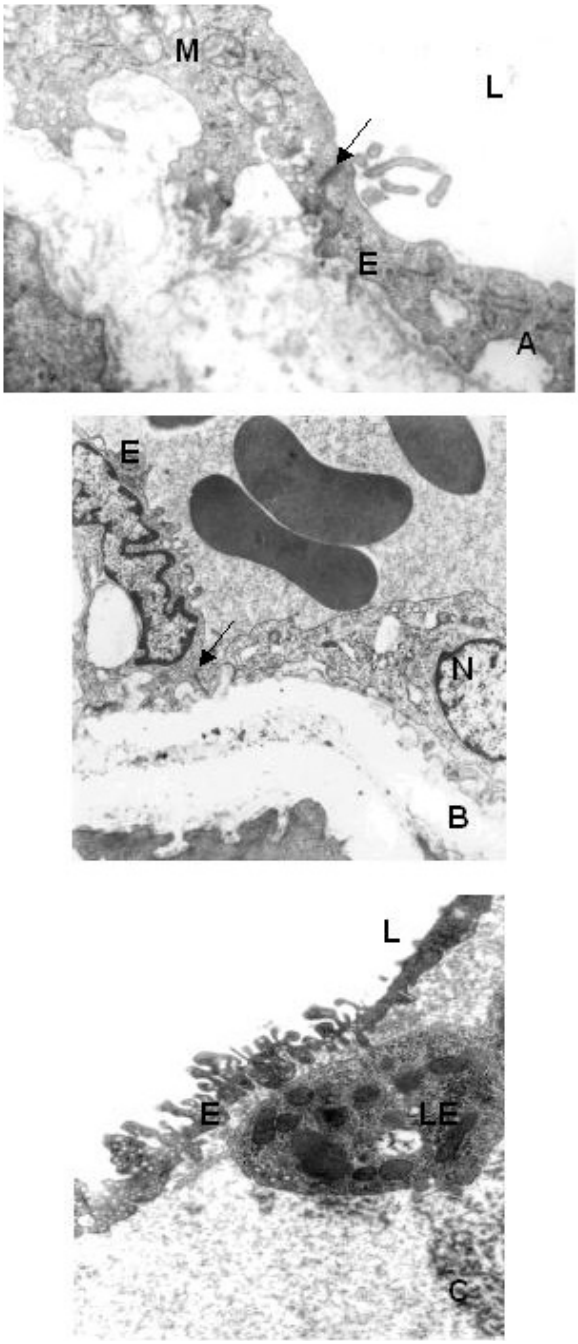

Fig. 1. Electron micrographs showing subcellular injury of aortic endothelium of control borderline hypertensive rats. Magnification: A x16 000, B x15 000, C x11 000.

Discussion

The vascular endothelium is an active, dynamic tissue that controls many important functions, including regulation of vascular tone, maintenance of blood circulation, fluidity, coagulation, and inflammatory responses (Galley and Webster 2004). Therefore, the impairment of endothelial structure may contribute to endothelial dysfunction that may initiate or contribute to changes in cell adhesion, lipid deposition, and other early steps leading to vascular diseases.

In the present study we have examined the effect of chronic social stress on the structure of aortic endothelial cells of rats with a genetic predisposition to hypertension. The most important finding of this study was that chronic crowding caused marked subcellular injury of endothelium in BHR and also slight one in

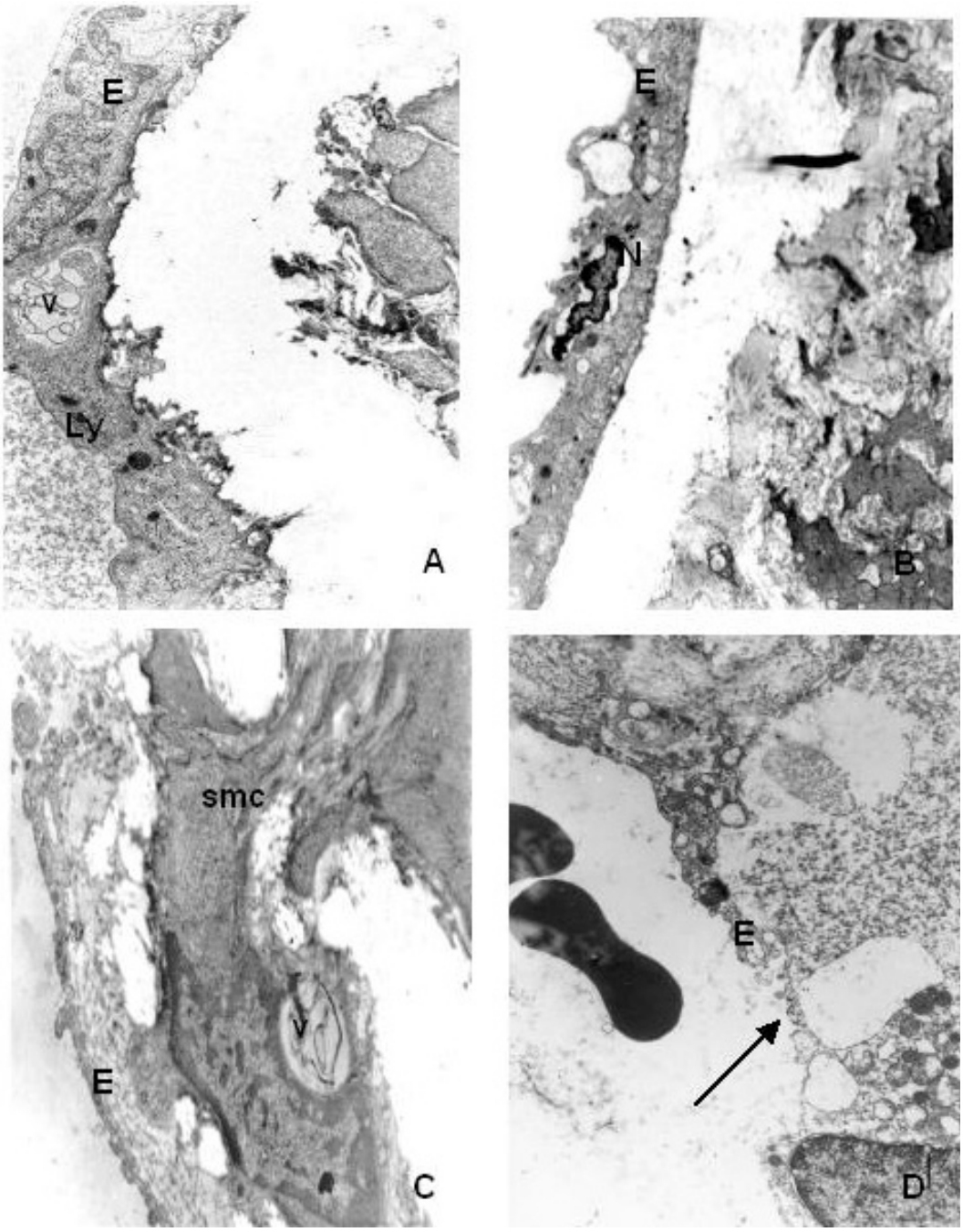

Fig. 2. Chronic stress-induced subcellular injury of aortic endothelium and smooth muscle cells in borderline hypertensive rats. Magnification: A x12 000, B x10 000, C x10 000, D x14 000.

stressed normotensive Wistar rats. Important is also the fact that even borderline hypertension was associated with focal subcellular damage of endothelial cells.

In our experiments we used a less traditional model of borderline hypertension with a normotensive mother and SHR father to prevent any effect of different maternal behavior and gestation environment of the hypertensive dams on blood pressure of their offspring (Woodworth *et al.* 1990, Porter *et al.* 2004). The model of borderline hypertension is appropriate for the investigation of stress-cardiovascular interactions since BHR are more sensitive to behavioral stress than normotensive and they do not develop age-dependent hypertension, while SHR do (Lawler *et al.* 1981). Basal blood pressure of BHR in the present work was in the range 130-150 mm Hg and was comparable to that reported by Woodworth *et al.* (1990). Impaired endothelial-dependent vascular relaxation was observed

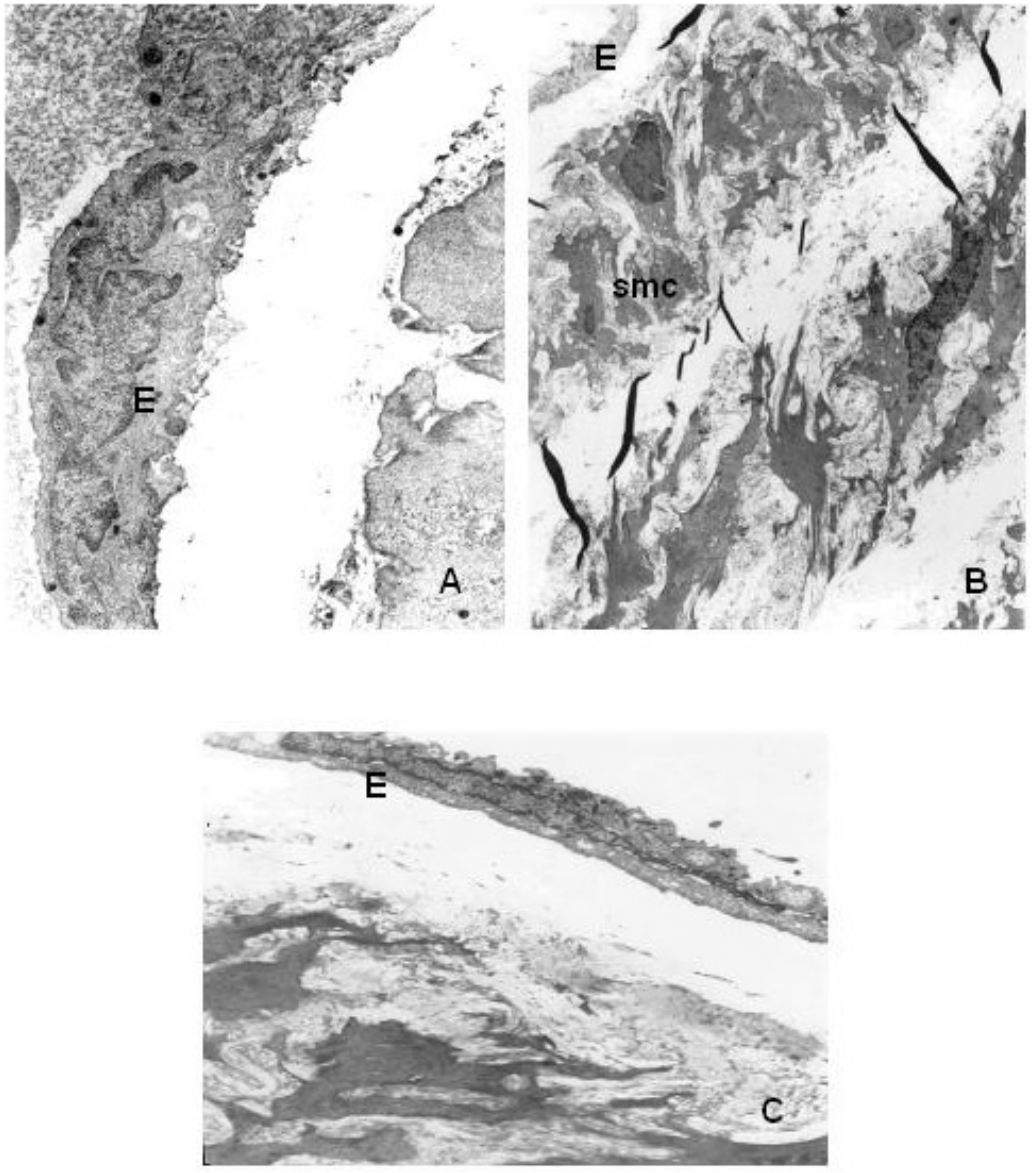

Fig. 3. Electron micrographs of endothelium and smooth muscle cells in control Wistar rats (A, B). Crowding stress-induced subcellular injury of aortic endothelium in stressed Wistar rats. Magnification: A x15 000, B x10 000, C x10 000. E – endothelium, L – lumen, LE – subendothelial leukocyte, Ly – lysosomes, M – mitochondria, N - nucleus, arrow – intercellular connection, arrow head – endothelial lining disruption, smc – smooth muscle cell, v – vacuoles.

in offspring of hypertensive patients (Esch *et al.* 2002) and animals as well (Liu *et al.* 2002). Our results showing local damage of endothelial cells of the aorta of control BHR indicated that it might affect endothelial integrity and permeability and activate inflammatory processes, which can initiate atherogenesis in a vessel (Schächinger and Zeiher 2002). Since no such prominent structural alterations were demonstrated in the aortic endothelium of control Wistar rats, it is possible to eliminate the effect of processing on the cell structure in BHR. It can indicate genetic origin of alterations due to interaction of some peculiar genetic network present in the hypertensive subject including related genes of the renin-angiotensin-aldosterone system (Corvol *et al.* 1999), angiotensin-converting enzyme (Fornage *et al.* 1998), activation of extracellular dual phosphorylated (Thr202/tyr204) extracellular-signal activated protein kinases (ERK) (Barančík *et al.* 2007) as well as activation of sympathetic nervous system.

The endothelial cells display the remarkable heterogeneity in the vascular tree (Garlanda and Dejana 1997). Despite that several studies demonstrated similar structural injury of endothelial monolayer in resistant and conduit vessels of hypertensive animals (Kristek *et al.* 1997, Tribulová *et al.* 2000, Okruhlicová *et al.* 2005), indicating pronounced sensitivity of endothelium to

increased blood pressure. Ultrastructural changes of endothelium were also observed in NO-deficient hypertension (Okruhlicová *et al.* 2000, Tribulová *et al.* 2000) accompanied with structural remodeling of vessel wall (Kristek *et al.* 1996, Cebová *et al.* 2006), associated with reduced NO production *per se* rather than with hypertension (Holécyová *et al.* 1996) and with attenuated vasodilatation.

Social chronic stress in a modern world represents an important risk factor for development of the cardiovascular disease. Its deleterious effects depend on the critical period of exposure, duration and type – as they all may alter functions of the basic autoregulatory stress response components: the hypothalamic-pituitary-adrenal axis and the sympathoadrenal medullar system activating renin-angiotensin-aldosterone system and sympathetic nervous system (McCarty and Gold 1996, Esch *et al.* 2002). Crowding evokes social stress reactions with prominent psychosocial components mimicking emotional state alterations (Bugajski 1999). Although crowding is a relatively weak stressor, chronic exposure may induce behavioral changes (Dubovický *et al.* 1999) and may affect the function of cardiovascular system, especially in individuals exhibiting higher responses to stressful conditions including genetic predisposition to hypertension. Our results demonstrated reduced activity of NO synthase in the aorta of stressed BHR. Ultrastructural injury of endothelial cell mitochondria of stressed BHR suggests that NO production may also be associated with an imbalance of defense antioxidative stress system that contributes to the reduction of NO in the cells. In addition, mitochondrial injury indicates also changes in intracellular ATP levels. The endothelial dysfunction is also accompanied with inflammation and procoagulation processes. In endothelial cells of stressed BHR we observed increased amount of Weibel-Palade bodies producing von Willenbrand factor that is known as a marker of adhesive, coagulative and thrombotic processes (Wannamethee *et al.* 2006); furthermore there were seen vacuoles and lysosomes representing structural markers of cell degradation processes, and altered intercellular connections providing functional coordination and communication between endothelial cells (Isakson *et al.* 2006). All these subcellular alterations may indicate considerable changes in adhesive, and permeable properties of the endothelium that can facilitate the transmigration of leukocytes into subendothelial area and accelerate progression of inflammation and atherosclerotic injury.

In conclusion: our results indicate that borderline hypertension is associated with the subcellular injury of aortic endothelial cells that may contribute to higher sensitivity of the endothelium to chronic stress and accelerate the arterial dysfunction.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

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