

Effect of Captopril and Melatonin on Fibrotic Rebuilding of the Aorta in 24 Hour Light-Induced Hypertension

K. REPOVÁ-BEDNÁROVÁ¹, S. AZIRIOVÁ¹, J. HRENÁK¹, K. KRAJČÍROVIČOVÁ¹, M. ADAMCOVÁ⁴, L. PAULIS¹, F. ŠIMKO^{1,2,3,5}

¹Department of Pathophysiology and ²Third Clinic of Medicine, School of Medicine, Comenius University, Bratislava, Slovak Republic, ³Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovak Republic, ⁴Department of Physiology, Faculty of Medicine in Hradec Kralove, Charles University in Prague, Hradec Kralove, Czech Republic, ⁵Centrum of Excellence NOREG, Slovak Republic

Received March 15, 2013

Accepted June 18, 2013

Summary

Chronic continuous light exposure leads to melatonin deficiency along with complex neurohumoral activation resulting in hypertension development in rats. The aim of this study was to show, whether continuous light induces fibrotic rebuilding of the aorta and whether the treatment with melatonin or angiotensin converting enzyme inhibitor captopril can prevent these potential alterations. In a six-week experiment, 3-month-old Wistar rats were divided into 4 groups (ten per group): controls, rats exposed to continuous light, exposed to continuous light plus treated with captopril (100 mg/kg/24 h) and exposed to continuous light plus treated with melatonin (10 mg/kg/24 h). Systolic blood pressure (SBP) and collagen type I and III in the media of thoracic aorta were measured. Continuous light induced hypertension and fibrotic rebuilding of the aorta in terms of enhancement of collagen I and III concentration in the aortic media. Both captopril and melatonin prevented SBP rise and reduced collagen III concentration in the aorta. However, only melatonin reduced collagen I and the sum of collagen I and III in the aortic tissue. We conclude that in continuous light-induced hypertension, administration of melatonin, along with SBP reduction, decreases collagen I and III concentration in the aorta. It is suggested that antifibrotic effect of melatonin may reduce the stiffness of the aorta and small arteries and beneficially influence the nature of the pulse wave and peripheral vascular resistance.

Key words

Continuous light • Hypertension • Fibrosis • Melatonin • Captopril • Collagen I and III

Corresponding author

Fedor Simko, Department of Pathophysiology, School of Medicine, Comenius University, Sasinkova 4, 813 72 Bratislava, Slovak Republic. Fax: +421-(0)2-59357601.

E-mail: fedor.simko@fmed.uniba.sk

Introduction

Fibrotic rebuilding of the heart, aorta, small arteries or kidneys in the condition of chronically increased blood pressure represents the principal tissue damage resulting in deterioration of organ function and worsening of prognosis. It is generally assumed that attenuation of the pathological remodeling of the heart and large vessels diminishes cardiovascular risk. This stimulates the search for new approaches and drugs against the consequences of hemodynamic overload-induced cardiovascular remodeling in variable models of experimental hypertension and in clinical conditions (Paulis *et al.* 2009, 2010b, Pechanova *et al.* 2007, Rezzani *et al.* 2010, Schiffrin 2012, Simko *et al.* 2004, 2009, 2010).

Melatonin was proven to express antihypertensive action in several animal models of hypertension (Li *et al.* 2009, Paulis *et al.* 2010a, Paulis and Simko 2007, Simko *et al.* 2009, Simko and Paulis 2007, Simko *et al.* 2010, Xia *et al.* 2008) and in normotensive and hypertensive humans (Grossman *et al.* 2006, 2011, Paulis *et al.* 2012, Rehcinski *et al.* 2010, Scheer *et al.* 2004, Simko and Pechanova 2009) and the

deficit of melatonin results in blood pressure rise (Jonas *et al.* 2003, Simko *et al.* 2013, Zeman *et al.* 2005). The model of continuous illumination-induced hypertension represents a melatonin-deficient blood pressure elevation as a result of reduced nocturnal melatonin secretion and complex neurohumoral activation (Simko *et al.* 2010, 2013). Previously it has been shown in our laboratory that continuous light-induced increase in blood pressure was associated with left ventricular hypertrophy and fibrosis and the administration of melatonin prevented oxidative stress and fibrosis of the left ventricle (Simko *et al.* 2010).

Melatonin exerts manifold protection on cardiovascular system. This indolamine has a remarkable antioxidant and scavenging effect both at extra- and intracellular level (Reiter *et al.* 2007, Tengattini *et al.* 2008). Furthermore, melatonin has also antihypertensive, antiproliferative and sympatholytic effects (Simko and Paulis 2007, 2012, 2013). Similarly, angiotensin converting enzyme (ACE) inhibitors, including captopril, decrease blood pressure, remodeling of the heart and vessels, and neurohumoral activation, resulting in cardiovascular protection (Simko *et al.* 2003).

In this experiment we investigated, whether light-induced hypertension induces (or not) fibrosis of the aorta and whether this potential excess of collagen can be modified by melatonin or angiotensin converting enzyme inhibitor captopril.

Methods

Animals and treatment

3-month-old male Wistar rats were randomly divided into four groups (n=10 each): controls (c), rats exposed to continuous light (24), exposed to continuous light plus treated with captopril (100 mg/kg/24 h) (24C) (Egis Pharmaceuticals Ltd., Budapest, Hungary) and exposed to continuous light plus treated with melatonin (10 mg/kg/24 h) (24M) (Sigma Chemical Co., Deisenhofen, Germany). All treatment drugs were administered perorally dissolved in drinking water each day during the six weeks of experiment. The concentration of these drugs was adjusted to daily water consumptions to ensure the correct dosage. Melatonin containing solutions were protected from light. All rats were housed at a temperature of 22-24 °C in individual cages and were freely fed a regular pellet diet in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National

Institutes of Health (NIH publication no. 8523, revised 1985).

Systolic blood pressure (SBP) was measured each week by noninvasive tail-cuff plethysmography (Hugo-Sachs Elektronik, Freiburg, Germany). After six weeks of experiment the animals were sacrificed by decapitation and the samples of thoracic aorta (approximately 50 mg) were used for the analysis of collagen type I and III in the media.

Collagen type I and III in aortic media

Samples from the thoracic aorta were fixed in 4 % formaldehyde (Sigma Chemie, Germany) for 24 h, embedded in paraffin, cut in 5 µm slices. Deparaffinized and rehydrated 5 µm thick slices were stained with picrosirius red as follows: the slides were submerged in 0.2 % phosphomolybden acid for clearing the cytoplasm, then the slides were stained with 0.1 % sirius red F3BA in a saturated water solution of picric acid for 90 min. The slides were washed 2 min in 0.01 N HCl, dehydrated and mounted.

10 randomly selected places of aorta on each slide were viewed by microscopy under polarized light and documented with a digital photographic camera PowerShot S5 IS (Canon, Japan). The size of each taken picture of aorta was 4.2 x 3.1 mm. Then, the parts of aortic media sized 0.58 x 0.58 mm (1 px = 0.39 µm) from each picture were selected and evaluated by histomorphometry using the ImageJ software v.1.43u (National Institutes of Health, Bethesda, USA). Threshold values were determined for the particular colors of spectrum, red to yellow for thick collagen I and green for thin collagen III, the numbers of pixels of each color were counted and the percentage of the picture's area was calculated.

Statistical analysis

For statistical analysis the software GraphPad InStat version 3.06 (GraphPad Software Inc, San Diego, CA, USA) was used. The results were expressed as mean ± standard error of mean (SEM). One-way, two-tailed analysis of variance (ANOVA) and the Bonferroni post-test were used. Differences were considered significant if the P value was less than 0.05.

Results

During the six weeks of treatment, the average SBP in controls and in 24-group were

126.06±0.25 mmHg and 143.03±1.86 mmHg, respectively (increased by 13 %, $P<0.05$). Systolic blood pressure decreased significantly ($P<0.05$) after both captopril (14 %) and melatonin (9 %) treatment (Fig. 1).

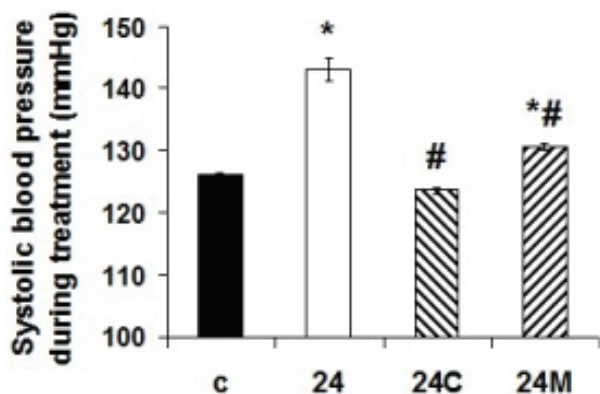


Fig. 1. The effect of captopril (24C) and melatonin (24M) on average systolic blood pressure during 6 weeks of experiment in 24 h continuous light-induced hypertension (24). c, Wistar controls. * $P<0.05$ vs. c; # $P<0.05$ vs. 24

The level of collagen I in aortic media was 7.77 ± 0.73 % and 29.04 ± 1.77 % in controls and 24-group, respectively (increased by 274 %, $P<0.05$). Only melatonin significantly reduced collagen I (by 39 %, $P<0.05$) (Fig. 2A, 3). The level of collagen III in aortic media was 0.0012 ± 0.0003 % and 0.0319 ± 0.0069 % in controls and in 24-group, respectively ($P<0.05$). Both captopril (47 %) and melatonin (88 %) decreased collagen III level ($P<0.05$) (Fig. 2B, 3). The sum of collagen I and III in aortic media was 7.77 ± 0.73 % and 29.35 ± 1.95 % in controls and in 24-group, respectively (increased by 277 %, $P<0.05$). Only melatonin reduced the sum of collagen I and III by 39 % ($P<0.05$) (Fig. 2C, 3).

Discussion

Continuous application of 24 h/day light during six weeks resulted in hypertension development and fibrotic rebuilding of the aorta in terms of enhancement of collagen I and III concentration in the media. Both captopril and melatonin prevented blood pressure rise and reduced collagen III concentration in the aorta. However, only melatonin reduced collagen I and the sum of collagen I and III in the aortic tissue.

There are two experimental approaches that severely compromise melatonin production and disturb its regulatory role in daily rhythmicity, i.e. pinealectomy and continuous light exposure (Simko and Paulis 2013).

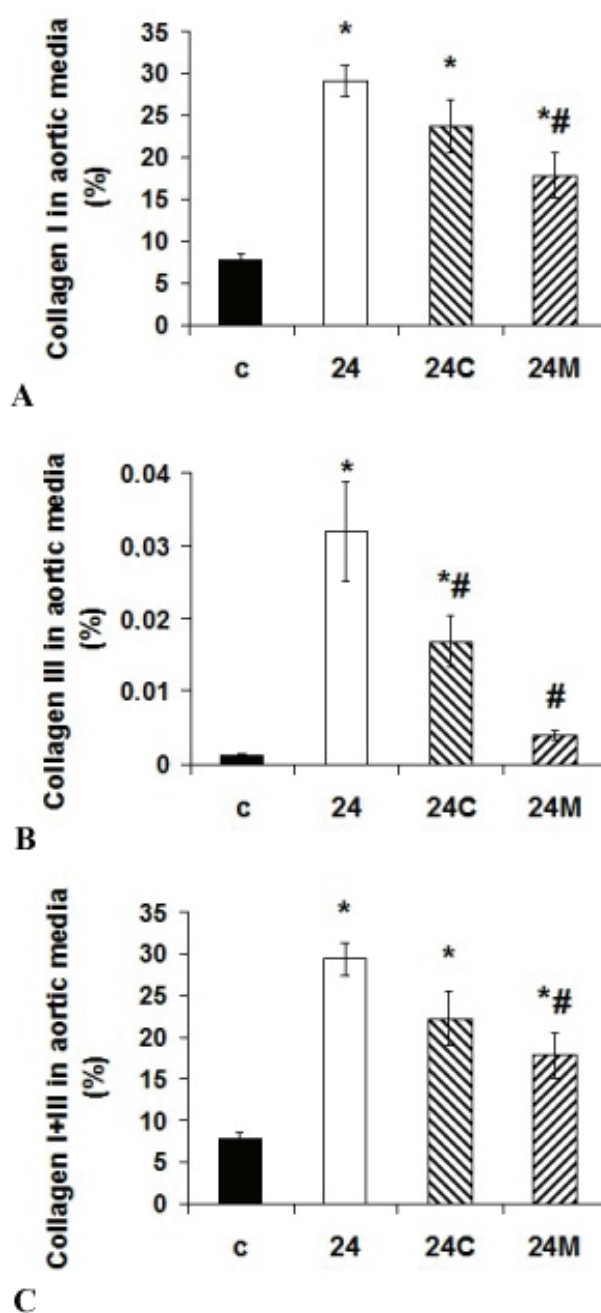


Fig. 2. The effect of captopril (24C) and melatonin (24M) on the concentration of collagen I (A), III (B) and the sum of collagen I and III (C) in the aortic media in 24 h continuous light-induced hypertension (24). c, Wistar controls. * $P<0.05$ vs. c; # $P<0.05$ vs. 24

However, in the light-induced “melatonin-deficient” hypertension development, several neurohumoral mechanisms seem to participate, including increased epinephrine synthesis in the adrenal gland (Ivanisevic-Milovanovic *et al.* 1995), the shortage of sympatholytic effect of melatonin (Arangino *et al.* 1999), increased expression of angiotensin II level in the left ventricle (Simko *et al.* 2010), or activation of adrenocorticotropic

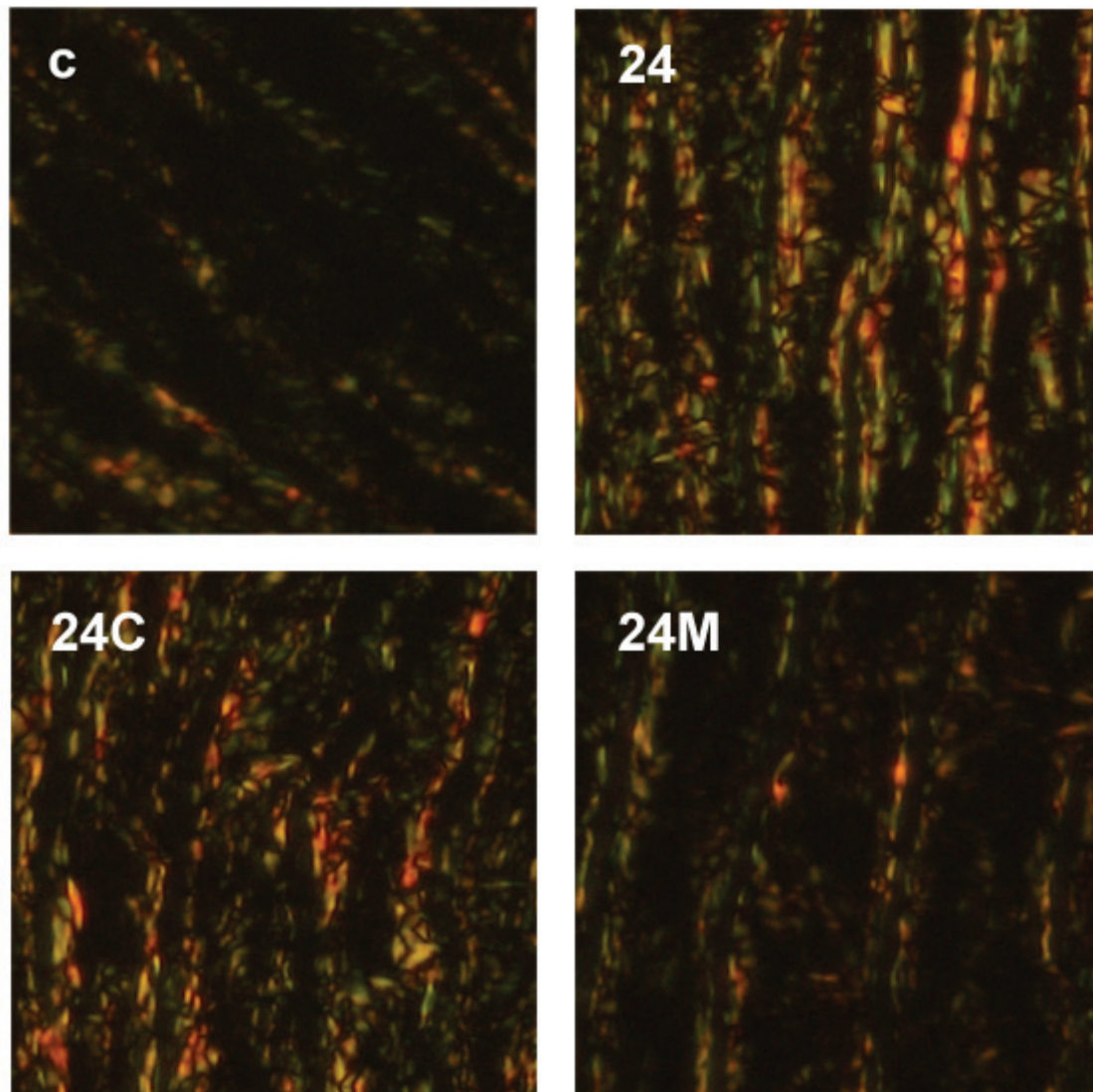


Fig. 3. The effect of captopril (24C) and melatonin (24M) on the concentration of collagen in the aortic media in 24 h continuous light-induced hypertension (24). Specimens are stained with picrosirius red and evaluated in polarized light: red to yellow color represents collagen I and green color represents collagen III. c, Wistar controls

hormone – glucocorticoid axis (Milosevic *et al.* 2005). It is suggested that melatonin deficiency in continuous light exposed animals triggers a sequence of neurohumoral imbalances, which may contribute to blood pressure alterations and target organ damage *via* variable indirect (vasoconstrictive and circulating fluid enhancing) or direct mechanisms (Simko and Paulis 2013).

In our previous experiments it has been shown that light-induced hypertension stimulated hypertrophy and fibrosis development in the left ventricle (Simko *et al.* 2010). Both captopril and melatonin prevented blood pressure increase. However, only melatonin decreased the concentration of insoluble and the total collagen in the left ventricle, although only captopril reduced left ventricular hypertrophy development (Simko *et al.* 2010).

Similarly, melatonin reduced left ventricular fibrosis in L-NAME-induced hypertension (Paulis *et al.* 2009) and in spontaneously hypertensive rats (Simko *et al.* 2009).

It has been shown that melatonin improves endothelial function in terms of enhancement of nitric oxide synthesis and reduction of its degradation (Dominguez-Rodriguez *et al.* 2010, Paulis *et al.* 2010a, Rezzani *et al.* 2010, Tengattini *et al.* 2008) and attenuates the remodeling of smaller arteries (Paulis *et al.* 2010b). However, according to our knowledge, the data referring to the protective effect of melatonin in the aorta and large conduit arteries are missing. In this experiment melatonin prominently reduced the collagen I and III concentrations. Within the cardiovascular tissues at least twelve types of collagen were disclosed, however,

collagen I and III seems to play the dominant role (Pelouch *et al.* 1995, Simko and Paulis 2012). Collagen I fibers have an extremely high tensile strength (Pelouch *et al.* 1995, Weber 1989) and in a decisive manner determine the stiffness of the myocardium or aorta. Type III collagen is more distensible than collagen I and seems to maintain the structural integrity of the network (Weber 1989). These collagen types together are responsible for tensile strength, but also contribute, together with elastin, somewhat to the extensile properties of the aorta (Dobrin and Mrkvicka 1994). Collagen linking to vascular smooth muscle cells significantly contributes to the stiffness of the aorta and conduit arteries (Schiffrin 2012), and enhancement of the aortic stiffness seems to be prognostically unfavorable (Schiffrin 2012). Several antihypertensive agents, including those interfering with angiotensin II production or effect, aldosterone receptor blockers or calcium channel blockers, were shown to reduce vascular stiffness, potentially through their inhibition of the production of growth factors resulting in the attenuation of proliferation and fibrosis (Schiffrin 2012).

The potent antifibrotic effect of melatonin in the aorta shown in our experiment, which was more prominent than that of captopril, may hardly be determined purely by melatonin's hemodynamic effect, since the SBP reduction, although significant, is relatively weak compared to captopril. More plausible explanation resides in the prominent antioxidative effect

of melatonin. This indolamine acts as both antioxidant and scavenger not just extracellularly but also intracellularly, including the nucleus (Reiter *et al.* 2007, 2010). Moreover, melatonin increases the bioavailability of nitric oxide with its antiproliferative effect (Paulis *et al.* 2009, Rezzani *et al.* 2010, Simko *et al.* 2010) and interferes with several neurohumoral systems including sympathetic and renin-angiotensin systems (Simko *et al.* 2013).

We conclude that in continuous light-induced hypertension, administration of melatonin, along with blood pressure reduction, decreases collagen I and III concentration in the media of aorta. This action of melatonin could improve the elasticity of aorta and other conduit arteries. We speculate that the antifibrotic effect of melatonin in the aorta and potentially in smaller arteries may beneficially influence the nature of the pulse wave and peripheral vascular resistance and could mitigate the undesirable alterations of the vascular system in conditions of hypertension, aging or chronic neurohumoral activation.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

This study was supported by grants for scientific research of VEGA No 1/0227/12, 1/0831/11 and 2/0183/12, of APVV-0205-12 and by the programme PRVOUK P37/5.

References

- ARANGINO S, CAGNACCI A, ANGIOLUCCI M, VACCA AM, LONGU G, VOLPE A, MELIS GB: Effects of melatonin on vascular reactivity, catecholamine levels, and blood pressure in healthy men. *Am J Cardiol* **83**: 1417-1419, 1999.
- DOBRIN PB, MRKVICKA R: Failure of elastin or collagen as possible critical connective tissue alterations underlying aneurysmal dilatation. *Cardiovasc Surg* **2**: 484-488, 1994.
- DOMINGUEZ-RODRIGUEZ A, ABREU-GONZALEZ P, SANCHEZ-SANCHEZ JJ, KASKI JC, REITER RJ: Melatonin and circadian biology in human cardiovascular disease. *J Pineal Res* **49**: 14-22, 2010.
- GROSSMAN E, LAUDON M, YALCIN R, ZENGIL H, PELEG E, SHARABI Y, KAMARI Y, SHEN-ORR Z, ZISAPEL N: Melatonin reduces night blood pressure in patients with nocturnal hypertension. *Am J Med* **119**: 898-902, 2006.
- GROSSMAN E, LAUDON M, ZISAPEL N: Effect of melatonin on nocturnal blood pressure: meta-analysis of randomized controlled trials. *Vasc Health Risk Manag* **7**: 577-584, 2011.
- IVANISEVIC-MILOVANOVIC OK, DEMAJO M, KARAKASEVIC A, PANTIC V: The effect of constant light on the concentration of catecholamines of the hypothalamus and adrenal glands, circulatory hadrenocorticotropin hormone and progesterone. *J Endocrinol Invest* **18**: 378-383, 1995.
- JONAS M, GARFINKEL D, ZISAPEL N, LAUDON M, GROSSMAN E: Impaired nocturnal melatonin secretion in non-dipper hypertensive patients. *Blood Press* **12**: 19-24, 2003.

- LI HL, KANG YM, YU L, XU HY, ZHAO H: Melatonin reduces blood pressure in rats with stress-induced hypertension via GABAA receptors. *Clin Exp Pharmacol Physiol* **36**: 436-440, 2009.
- MILOSEVIC V, TRIFUNOVIC S, SEKULIC M, SOSIC-JURJEVIC B, FILIPOVIC B, NEGIC N, NESTOROVIC N, MANOJLOVIC STOJANOSKI M, STARCEVIC V: Chronic exposure to constant light affects morphology and secretion of adrenal zona fasciculata cells in female rats. *Gen Physiol Biophys* **24**: 299-309, 2005.
- PAULIS L, SIMKO F: Blood pressure modulation and cardiovascular protection by melatonin: potential mechanisms behind. *Physiol Res* **56**: 671-684, 2007.
- PAULIS L, PECHANOVA O, ZICHA J, KRAJCIROVICOVA K, BARTA A, PELOUCH V, ADAMCOVA M, SIMKO F: Melatonin prevents fibrosis but not hypertrophy development in the left ventricle of NG-nitro-L-arginine-methyl ester hypertensive rats. *J Hypertens Suppl* **27**: S11-S16, 2009.
- PAULIS L, PECHANOVA O, ZICHA J, BARTA A, GARDLIK R, CELEC P, KUNES J, SIMKO F: Melatonin interactions with blood pressure and vascular function during L-NAME-induced hypertension. *J Pineal Res* **48**: 102-108, 2010a.
- PAULIS L, PECHANOVA O, ZICHA J, LISKOVA S, CELEC P, MULLEROVA M, KOLLAR J, BEHULIAK M, KUNES J, ADAMCOVA M, SIMKO F: Melatonin improves the restoration of endothelium-derived constricting factor signalling and inner diameter in the rat femoral artery after cessation of L-NAME treatment. *J Hypertens* **28** (Suppl 1): S19-S24, 2010b.
- PAULIS L, SIMKO F, LAUDON M: Cardiovascular effects of melatonin receptor agonists. *Expert Opin Investig Drugs* **21**: 1661-1678, 2012.
- PECHANOVA O, ZICHA J, PAULIS L, ZENEBE W, DOBESOVA Z, KOJSOVA S, JENDEKOVA L, SLADKOVA M, DOVINOVA I, SIMKO F, KUNES J: The effect of N-acetylcysteine and melatonin in adult spontaneously hypertensive rats with established hypertension. *Eur J Pharmacol* **561**: 129-136, 2007.
- PELOUCH V, MILEROVA M, OSTADAL B, HUCIN B, SAMANEK M: Differences between atrial and ventricular protein profiling in children with congenital heart disease. *Mol Cell Biochem* **147**: 43-49, 1995.
- REHCINSKI T, TRZOS E, WIERZBOWSKA-DRABIK K, KRZEMINSKA-PAKULA M, KURPESA M: Melatonin for nondippers with coronary artery disease: assessment of blood pressure profile and heart rate variability. *Hypertens Res* **33**: 56-61, 2010.
- REITER RJ, TAN DX, TERRON MP, FLORES LJ, CZARNOCKI Z: Melatonin and its metabolites: new findings regarding their production and their radical scavenging actions. *Acta Biochim Pol* **54**: 1-9, 2007.
- REITER RJ, TAN DX, FUENTES-BROTO L: Melatonin: a multitasking molecule. *Prog Brain Res* **181**: 127-151, 2010.
- REZZANI R, PORTERI E, DE CIUCEIS C, BONOMINI F, RODELLA LF, PAIARDI S, BOARI GE, PLATTO C, PILU A, AVANZI D, RIZZONI D, AGABITI ROSEI E: Effects of melatonin and Pycnogenol on small artery structure and function in spontaneously hypertensive rats. *Hypertension* **55**: 1373-1380, 2010.
- SCHEER FA, VAN MONTFRANS GA, VAN SOMEREN EJ, MAIRUHU G, BUIJS RM: Daily nighttime melatonin reduces blood pressure in male patients with essential hypertension. *Hypertension* **43**: 192-197, 2004.
- SCHIFFRIN EL: Vascular remodeling in hypertension: mechanisms and treatment. *Hypertension* **59**: 367-374, 2012.
- SIMKO F, PAULIS L: Melatonin as a potential antihypertensive treatment. *J Pineal Res* **42**: 319-322, 2007.
- SIMKO F, PAULIS L: Hypertensive heart disease: bone marrow as a significant player in pathologic remodelling? *J Hypertens* **30**: 1702-1705, 2012.
- SIMKO F, PAULIS L: Antifibrotic effect of melatonin – perspective protection in hypertensive heart disease. *Int J Cardiol* **168**: 2876-2877, 2013.
- SIMKO F, PECHANOVA O: Potential roles of melatonin and chronotherapy among the new trends in hypertension treatment. *J Pineal Res* **47**: 127-133, 2009.
- SIMKO F, SIMKO J, FABRYOVA M: ACE-inhibition and angiotensin II receptor blockers in chronic heart failure: pathophysiological consideration of the unresolved battle. *Cardiovasc Drugs Ther* **17**: 287-290, 2003.
- SIMKO F, MATUSKOVA J, LUPTAK I, KRAJCIROVICOVA K, KUCHARSKA J, GVOZDJAKOVA A, BABAL P, PECHANOVA O: Effect of simvastatin on remodeling of the left ventricle and aorta in L-NAME-induced hypertension. *Life Sci* **74**: 1211-1224, 2004.

- SIMKO F, PECHANOVA O, PELOUCH V, KRAJCIROVICOVA K, MULLEROVA M, BEDNAROVA K, ADAMCOVA M, PAULIS L: Effect of melatonin, captopril, spironolactone and simvastatin on blood pressure and left ventricular remodelling in spontaneously hypertensive rats. *J Hypertens Suppl* **27**: S5-S10, 2009.
- SIMKO F, PECHANOVA O, PELOUCH V, KRAJCIROVICOVA K, CELEC P, PALFFY R, BEDNAROVA K, VRANKOVA S, ADAMCOVA M, PAULIS L: Continuous light and L-NAME-induced left ventricular remodelling: different protection with melatonin and captopril. *J Hypertens* **28** (Suppl 1): S13-S18, 2010.
- SIMKO F, REITER RJ, PECHANOVA O, PAULIS L: Experimental models of melatonin-deficient hypertension. *Front Biosci-Landmark* **18**: 616-625, 2013.
- TENGATTINI S, REITER RJ, TAN DX, TERRON MP, RODELLA LF, REZZANI R: Cardiovascular diseases: protective effects of melatonin. *J Pineal Res* **44**: 16-25, 2008.
- WEBER KT: Cardiac interstitium in health and disease: the fibrillar collagen network. *J Am Coll Cardiol* **13**: 1637-1652, 1989.
- XIA CM, SHAO CH, XIN L, WANG YR, DING CN, WANG J, SHEN LL, LI L, CAO YX, ZHU DN: Effects of melatonin on blood pressure in stress-induced hypertension in rats. *Clin Exp Pharmacol Physiol* **35**: 1258-1264, 2008.
- ZEMAN M, DULKOVA K, BADA V, HERICHOVA I: Plasma melatonin concentrations in hypertensive patients with the dipping and non-dipping blood pressure profile. *Life Sci* **76**: 1795-1803, 2005.
-