Protective Effect of Captopril, Olmesartan, Melatonin and Compound 21 on Doxorubicin-Induced Nephrotoxicity in Rats

J. HRENÁK[1], K. ARENDÁŠOVÁ[1], R. RAJKOVIČOVÁ[1], S. AZIRIOVÁ[1], K. REPOVÁ[1], K. KRAJČÍROVIČOVÁ[1], P. CELEC[1,2], N. KAMODYOVÁ[2], A. BÁRTA[3,7], M. ADAMCOVÁ[4], L. PAULIS[1], F. ŠIMKO[1,5,6,7]

[1]Department of Pathophysiology, School of Medicine, Comenius University, Bratislava, Slovak Republic, [2]Institute of Molecular Biomedicine, School of Medicine, Comenius University, Bratislava, Slovak Republic, [3]Institute of Normal and Pathological Physiology, Bratislava, Slovak Republic, [4]Department of Physiology, Faculty of Medicine in Hradec Kralove, Charles University in Prague, Hradec Kralove, [5]Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovak Republic, [6]Third Clinic of Medicine, School of Medicine, Comenius University, Bratislava, Slovak Republic, [7]Centre of Excellence NOREG, Bratislava, Slovak Republic

Summary

Chronic kidney disease (CKD) represents a serious public health problem with increasing prevalence and novel approaches to renal protection are continuously under investigation. The aim of this study was to compare the effect of melatonin and angiotensin II type 2 receptor agonist compound 21 (C21) to angiotensin converting enzyme inhibitor captopril and angiotensin II type 1 receptor blocker olmesartan on animal model of doxorubicin nephrotoxicity. Six groups of 3-month-old male Wistar rats (12 per group) were treated for four weeks. The first group served as a control. The remaining groups were injected with a single dose of doxorubicin (5 mg/kg i.v.) at the same day as administration of either vehicle or captopril (100 mg/kg/day) or olmesartan (10 mg/kg/day) or melatonin (10 mg/kg/day) or C21 (0.3 mg/kg/day) was initiated. After four week treatment, the blood pressure and the level of oxidative stress were enhanced along with reduced glomerular density and increased glomerular size. Captopril, olmesartan and melatonin prevented the doxorubicin-induced increase in systolic blood pressure. All four substances significantly diminished the level of oxidative burden and prevented the reduction of glomerular density and modestly prevented the increase of glomerular size. We conclude that captopril, olmesartan, melatonin and C21 exerted a similar level of renoprotective effects in doxorubicin-induced nephrotoxicity.

Corresponding author

Fedor Simko, Department of Pathophysiology, School of Medicine, Comenius University, Sasinkova 4, 811 08 Bratislava, Slovak Republic. E-mail: fedor.simko@fmed.uniba.sk

Introduction

Chronic kidney disease (CKD) is a progressive pathological condition which can result in renal failure or end-stage renal disease. CKD represents a serious public health problem and its prevalence is steadily increasing worldwide (Stenvinkel 2010).

The CKD is characterized by progressive decline in renal function due to the decrease in number of nephrons. Irrespective to the variable etiologic factors, the pathogenesis of chronic kidney disease involves persistent inflammation and necrotic or apoptotic progressive cell death. This is followed by reparatory processes associated with glomerulosclerosis, tubular atrophy, and tubulointerstitial fibrosis (Schlondorff 2008). The therapeutic effort is aimed on the prevention or reduction of proteinuria, fibrosis and the associated structural and functional alterations of the kidney in terms

of loss of nephrons and residual nephron enlargement. Although the agents interfering with the renin-angiotensin system such as angiotensin converting enzyme inhibitors (ACEI) and angiotensin AT_1 receptor blockers (ARBs) represent the cornerstone of CKD treatment, their benefit is only partial and new approaches to the renal protection are continuously under investigation (Schlondorff 2008, Maione *et al.* 2011).

Doxorubicin-induced nephropathy is a well established and frequently used animal model of CKD (Lee and Harris 2011). The aim of this study was to investigate, whether the first non-peptide agonist of angiotensin II type 2 receptors, compound 21 (C21) (Wan *et al.* 2004, Steckelings *et al.* 2011, Paulis *et al.* 2012) or the pineal hormone melatonin could prevent the doxorubicin-induced renal damage. Moreover, we aimed to compare the potential protective effect of these substances with the ACEI captopril and the ARB olmesartan. C21, olmesartan, captopril and melatonin were administered at doses successfully tested in our previous experiments (Simko *et al.* 2010, Paulis *et al.* 2012).

Methods

Animals and treatment

72 male Wistar rats, three months old, were randomly divided into six groups of 12 animals each. The first group served as a control (CTRL). The remaining five groups were intravenously injected with a single dose of doxorubicin (5 mg/kg). Control animals were injected with 0.9 % NaCl in corresponding volume. Starting from the day of the doxorubicin application the oral administration of either vehicle (doxorubicin control group, DOX), or melatonin (DOX+MEL, 10 mg/kg/day), or captopril (DOX+CAP, 100 mg/kg/day), or olmesartan medoxomil (DOX+OLM, 10 mg/kg/day) or compound 21 (DOX+C21, 0.3 mg/kg/day) was initiated. All substances were given for four weeks. Melatonin and captopril were administered in tap water. Olmesartan and C21 were mixed in an emulsion and were applied *via* a gavage once daily. All other animals were daily gavaged with placebo to ensure same handling conditions for all animals in the experiment.

All animals were housed in a controlled environment with 12/12 light–dark rhythm at a temperature of 22-24 °C in individual cages and fed with a regular pellet diet *ad libitum*. The investigation conformed to the Guide for the Care and Use of Laboratory Animals published by US National Institutes of Health (NIH Publication No 8523, revised 1985).

The systolic blood pressure (SBP) was weekly measured by non-invasive tail-cuff plethysmography. After four weeks, the animals were sacrificed by decapitation. The body weight (BW) and left kidney weight (KW) were measured. Samples of the left kidney were used for the morphometric and biochemical analysis.

Glomerular morphometry

Kidneys were removed and weighed. Renal cortex samples were routinely fixed in formaldehyde, sectioned at 5 μm thick slices and stained with hematoxylin-eosin. Morphometric evaluation was performed on Nikon-119 (Japan) light microscope connected to high-resolution CCD camera (GKB, CC8706S, Taiwan) equipped with a two-dimensional image analyzer (Impor Pro, Kvant, s.r.o., Slovakia).

To measure the *glomerular tuft area (AG)* 10 glomeruli per animal (120 per group) were analyzed in a blinded manner. In each glomerulus a line was manually drawn around the boundary of the glomerular tuft. The area inside the line in μm^2 was calculated by computerized morphometry.

The *glomerular numerical density per area (N)* was determined by observing 10 microsopic fields per animal (120 per group). Glomeruli were counted in a frame of $1\,mm^2$, only considering well-preserved structures and not counting any that were not completely within our pre-defined $1\,mm^2$ area (Pechanova *et al.* 2006).

Markers of oxidative stress

Markers of oxidative stress were measured in 10 % homogenates of renal cortex. *Total antioxidant capacity (TAC)* was measured using 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid (Erel 2004). *Thiobarbituric acid reacting substances (TBARS)* levels were measured spectrophotometrically using a high-throughput 96-well plate method (Behuliak *et al.* 2009). *Advanced glycation end-products (AGEs)* were determined by fluorescence estimation (Munch *et al.* 1997). The *ferric reducing ability of plasma (FRAP)* was measured by automated FRAP assay (Benzie and Strain 1996).

Hydroxyproline concentration

The samples of left kidney were dried 24 hrs at

100 °C and then hydrolyzed in 6 mol.dm^{-3} HCl. Hydroxyproline concentrations were measured spectrophotometrically at 550 nm (Reddy and Enwemeka 1996).

Statistical analysis

The results are expressed as mean ± S.E.M. Differences were considered significant if the p-value was less than 0.05. For statistical analysis, one-way analysis of variance (ANOVA) and the Bonferroni test were used.

Results

Body weight and left kidney weight

After four weeks of treatment, the body weight of the animals in all experimental groups was significantly lower, when compared to controls (p<0.05). There was no significant difference in the left kidney weight between the groups (Table 1).

Systolic blood pressure

After four weeks of treatment, SBP was (122.9±2.77 mmHg) in control rats and (156.3±5.72 mmHg) in doxorubicin group (increase by 27 %, p<0.05). Blood pressure was significantly (p<0.05) lower in the captopril (28 %), olmesartan (25 %) and melatonin (22 %) group. There was no statistically significant difference in SPB between the DOX and DOX-C21 groups (Fig. 1).

Table 1. Body weight, relative left kidney weight and renal hydroxyproline (Hyp) concentration after four weeks of treatment.

	CTRL	DOX	DOX+CAP	DOX+OLM	DOX+C21	DOX+MEL
BW (g)	363.33±8.94	296.67±10.9*	268.64±8.61*	292.00±13.44*	316.50±9.52*	264.50±11.14*
LKW/BW (mg/kg)	2.64±0.34	4.45±0.28	4.40±0.53	4.15±0.35	3.75±0.49	4.44±0.27
Hyp (mg/g)	0.146±0.013	0.159±0.018	0.129±0.011	0.161±0.018	0.163±0.010	0.184±0.015

CTRL, control; BW, body weight; DOX, doxorubicin; DOX+CAP, DOX plus captopril; DOX+OLM, DOX plus olmesartan; DOX+C21, DOX plus compound C21; DOX+MEL, DOX plus melatonin; LKW, left kidney weight. * P<0.05 vs. controls
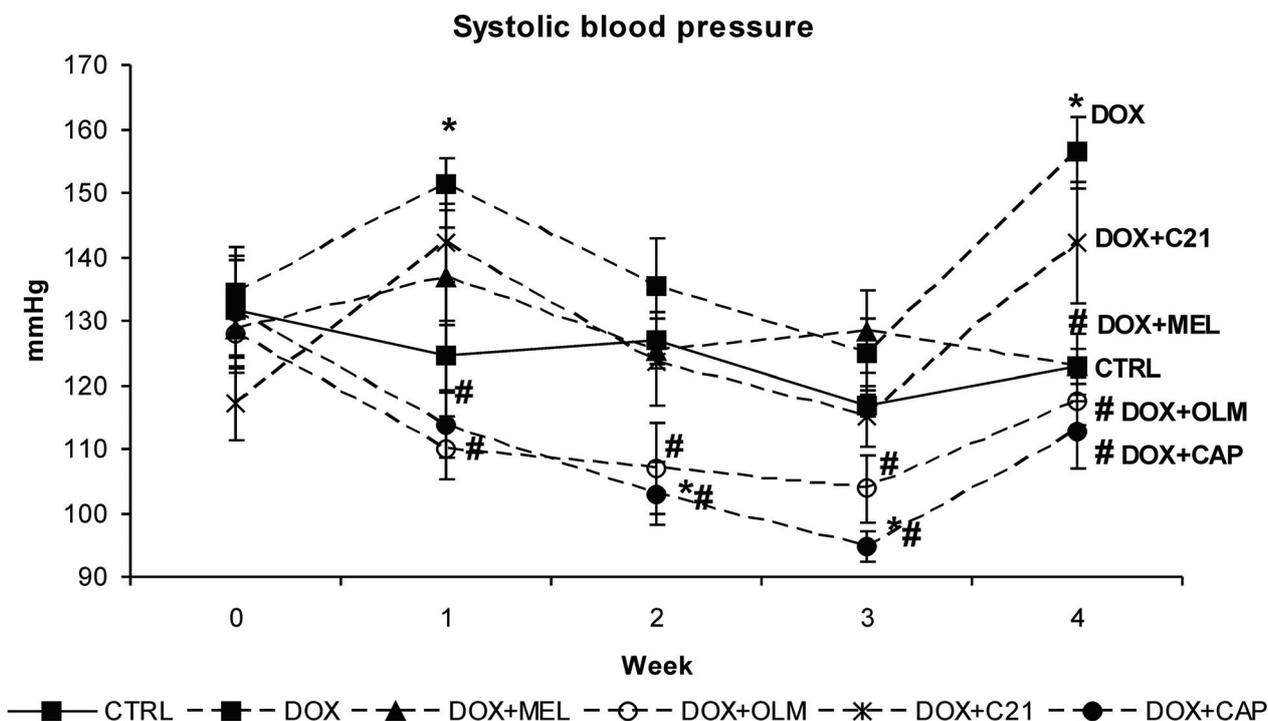
Fig. 1. The effect of captopril (DOX+CAP), olmesartan (DOX+OLM), compound C21 (DOX+C21) and melatonin (DOX+MEL) on systolic blood pressure in doxorubicin (DOX)-treated rats. CTRL, control; * P<0.05 vs. CTRL, # P<0.05 vs. DOX

A Glomerular numerical density per area (N)

B Glomerular tuft area (AG)
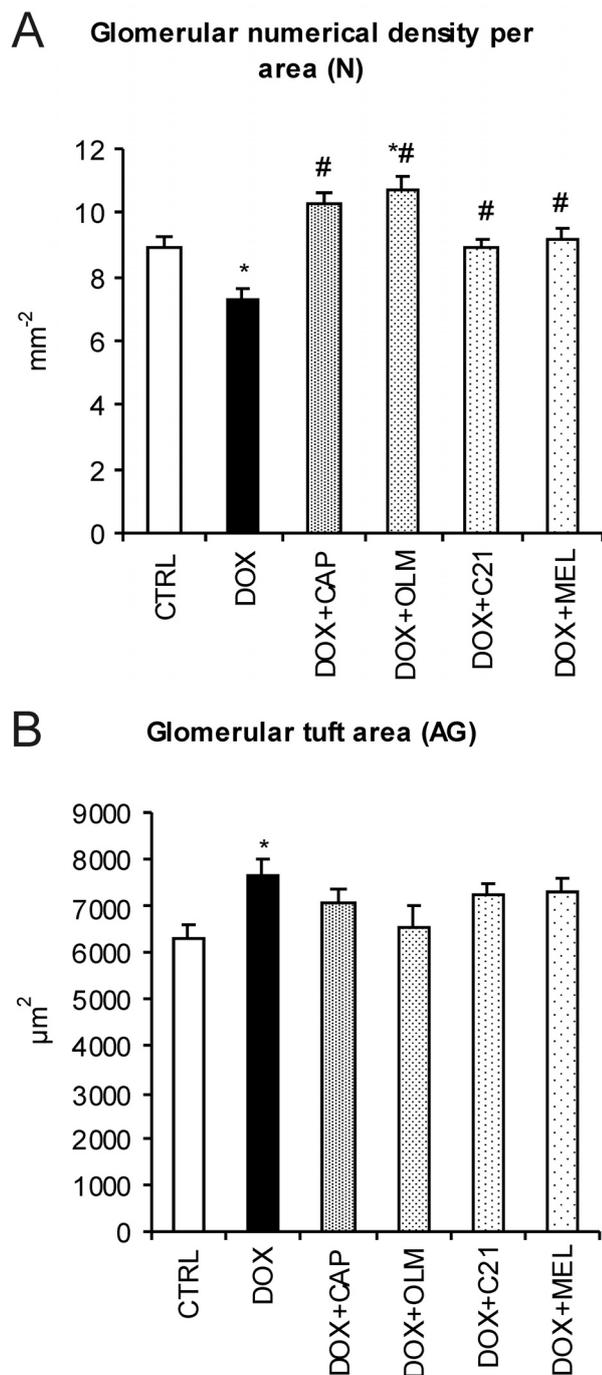
Fig. 2. The effect of captopril (DOX+CAP), olmesartan (DOX+OLM), compound C21 (DOX+C21) and melatonin (DOX+MEL) on glomerular numerical density per area (**A**) and glomerular tuft area (**B**) in doxorubicin (DOX) treated rats. CTRL, control; * P<0.05 vs. CTRLs, # P<0.05 vs. DOX

Glomerular morphometry

After four weeks of doxorubicin treatment the glomerular numerical density per area in renal cortex was 8.93±0.31 mm^{-2} in control rats and 7.27±0.34 mm^{-2} in DOX (reduction by 19 %, p<0.05). The treatment with captopril, olmesartan, melatonin and C21 completely

prevented this effect of doxorubicin (p<0.05) by 41 %, 47 %, 25 % and 23 %, respectively (Fig. 2A).

Doxorubicin induced an increase in glomerular tuft area (7661±368 μm^2) in comparison with controls (6595±285 μm^2) by 16 %. All the applied substances only slightly prevented these changes (Fig. 2B).

Markers of oxidative stress

The level of TAC was 46.69±4.28 μmol/g prot in the control group. The application of doxorubicin was associated with a significantly lower TAC levels (23.14±2.9 μmol/g prot) by 50 % (p<0.05) in the renal tissue when compared to controls. All of the applied substances prevented this alteration (there was no significant difference compared to the control group) (Fig. 3A).

The concentration of AGEs was 0.071±0.006 g/g prot (Fig. 3B) and of TBARS 2.099±0.186 μmol/g prot (Fig. 3C) in the control group and doxorubicin increased it by 68 % and 97 %, respectively (p<0.05). The treatment with captopril, olmesartan, melatonin and C21 prevented the increase of AGEs and TBARS in the kidney (p<0.05). There was no statistically significant difference in FRAP levels among the individual groups (Fig. 3D).

Hydroxyproline concentration

There was no statistically significant difference in hydroxyproline concentration in the kidney between the individual groups (Table 1).

Discussion

In this experiment, 4 weeks after doxorubicin administration blood pressure and the markers of oxidative load were elevated, the number of glomeruli was reduced and the glomerular area was increased. Captopril, olmesartan and melatonin prevented blood pressure increase. All four substances – captopril, olmesartan, melatonin and C21 prevented the augmentation of the oxidative load and the reduction of glomerular density.

Doxorubicin and related anthracycline antibiotics with an antineoplastic potential including daunorubicin or epirubicin are widely used in the oncologic medicine. However, the risk of cardiotoxicity largely limits the effective use of these cytostatics (Simunek *et al.* 2009). Despite an abundant usage of these drugs, the pathophysiological background of their toxic effect in not completely elucidated.
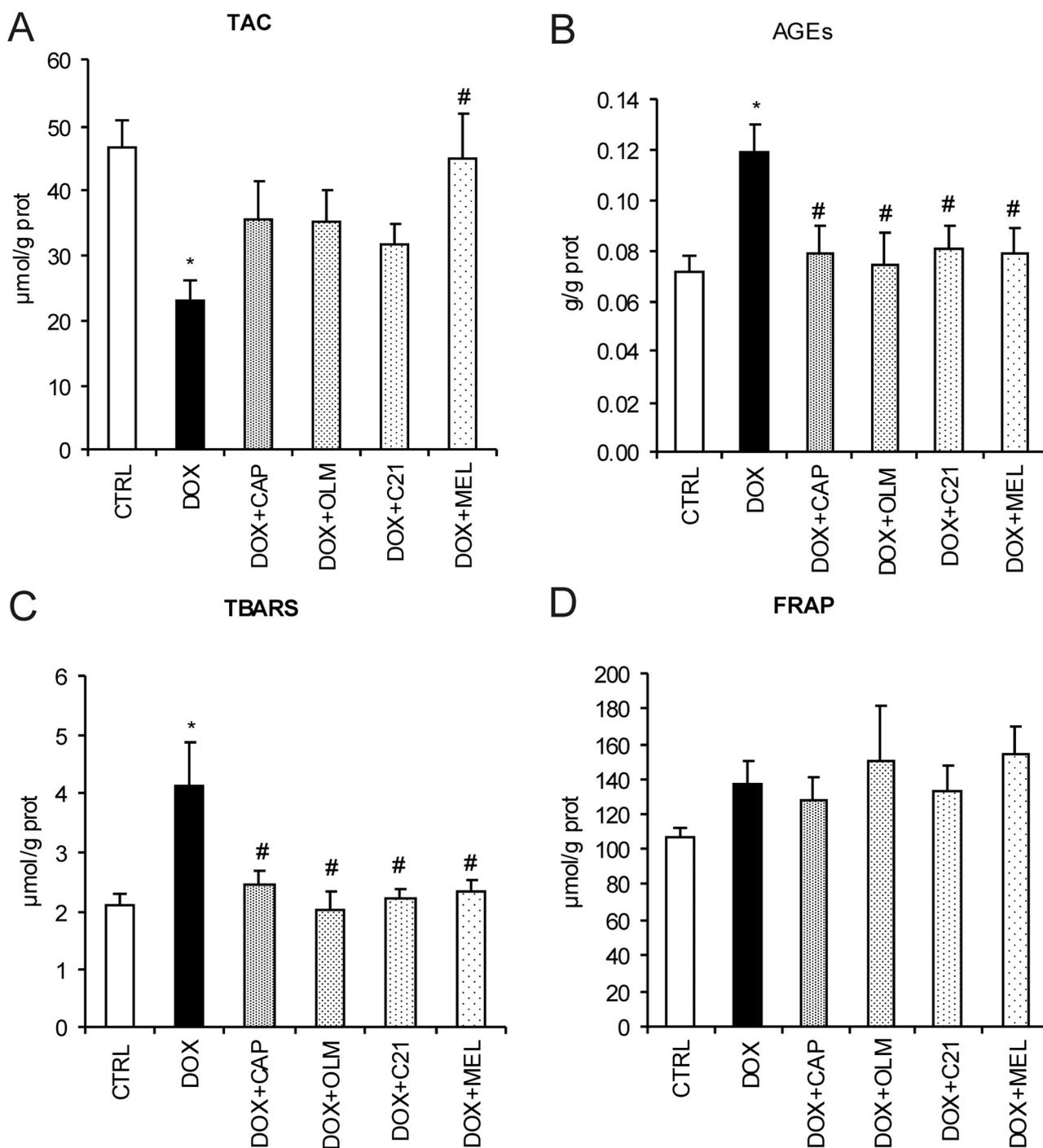
Fig. 3. Markers of oxidative stress in the kidney. The effect of captopril (DOX+CAP), olmesartan (DOX+OLM), compound C21 (DOX+C21) and melatonin (DOX+MEL) on total antioxidant activity (TAC) (**A**), advanced glycation end-products (AGEs) (**B**), thiobarbituric acid reacting substances (TBARS) (**C**) and ferric reducing ability of plasma (FRAP) (**D**) in doxorubicin (DOX) treated rats. CTRL, control; * P<0.05 vs. CTRLs, # P<0.05 vs. DOX

In our experiment we have observed an increased blood pressure in doxorubicin-treated rats. This finding might be surprising given the fact that doxorubicin-induced cardiotoxicity is associated with structural and functional myocardial rebuilding. The development of systolic and diastolic dysfunction can be expected to present rather with a decline than an increase in blood pressure. However, in some heart failure patients a paradoxical blood pressure augmentation is observed, probably resulting from concomitant neurohumoral activation or endothelial dysfunction and the subsequent peripheral vasoconstriction (Gheorghiade and Pang 2009).

Some studies have also demonstrated elevated

plasma renin activity in doxorubicin-treated rats and suggested that vasoconstriction triggered by increased renin activity might be responsible for blood pressure increase in these animals (Rashikh *et al.* 2013). This hypothesis is supported by the prevention of blood pressure increase by both captopril and olmesartan in doxorubicin-treated rats in our experiment. On the other hand, increased blood pressure does not seem to be the crucial factor in the development of doxorubicin-induced nephrotoxicity as in most reports nephrotoxicity develops without blood pressure elevation (e.g. Colak *et al.* 2012) and in our experiment blood pressure rise prevention was not necessary for the prevention of glomerular density reduction in the C21-treated group.

The major cause of the doxorubicin toxicity has been attributed to the oxidative injury evoked by a formation of doxorubicin-iron complexes. However, also other factors including suppression of mitochondrial biogenesis seem to be involved (Jirkovsky *et al.* 2012). The complex pathogenesis of doxorubicin toxicity may be underlined by the fact that doxorubicin affects other organs besides the heart, such as the gastrointestinal tract, bone marrow or kidney. Doxorubicin nephropathy is characterized by podocyte injury followed by progressive sclerosis of glomeruli and tubulointerstitial fibrosis; thus it is considered to be a model of focal segmental glomerulosclerosis by some authors (Lee and Harris 2011).

The dose and mode of application of doxorubicin differs in variable experiments. It is to be expected that higher doses and repeated application may be associated with more prominent kidney damage. However, our aim was to estimate the dose, which induces detectable renal alterations without prominent enhancement of mortality. The 5 mg/kg i.v. dose fitted best to these demands. Analogically, many different drugs were also shown to induce chronic kidney disease in variable doses and schemes of application, such as cisplatin (6.5 mg/kg in a single i.p., dose) (Sener *et al.* 2000), methotrexate (7 mg/kg body weight i.p. for three days) (Abraham *et al.* 2010), gentamycin (100 mg/kg/day i.v.) (Lee *et al.* 2012) or cyclosporine (15 mg/kg of body weight per day s.c. for 14 days) (Chang *et al.* 2004).

Monotherapy with ACEIs or ARBs has reduced proteinuria and postponed end-stage renal disease in patients with albuminuria and cardiovascular risk factors (Maione *et al.* 2011). Reduction of the proliferative, profibrotic, inflammatory and oxidative burden stimulating effect of angiotensin II and aldosterone is considered to play a key role in the renoprotective effects of ACEIs and ARBs (Simko *et al.* 2003).

However, both, melatonin and C21 exert antioxidant, anti-inflammatory and antiproliferative properties as well. Melatonin, besides being an extremely effective antioxidant and free radical scavenger (Simko and Paulis 2007, Reiter *et al.* 2009a, b), improves endothelial function, reduces neurohumoral activation (Paulis and Simko 2007, Simko and Pechanova 2009, Simko *et al.* 2013) and helps to preserve the daily physiological rhythms (Dominguez-Rodriguez *et al.* 2010, Reiter *et al.* 2011, Simko 2012). In our experiment, melatonin attenuated blood pressure increase, prevented the augmentation of the oxidative load and the decline in glomerular numerical density. These results are in agreement with the findings of other authors reporting that in doxorubicin-induced kidney damage melatonin reduced the level of lipid peroxidation, increased the antioxidant renal capacity, reduced proteinuria and improved renal function and structure (Montilla *et al.* 1997, Agapito *et al.* 2001, Dziegiel *et al.* 2002, Oz and Ilhan 2006). In the clinical conditions the protective data of melatonin in CKD are sparse. In hemodialyzed patients with end-stage renal disease the chronobiologic action of melatonin reduced sleep disturbances caused potentially by melatonin deficit (Koch *et al.* 2009, 2010a, b).

C21, the first non-peptide AT_2 receptor agonist was reported to exert anti-inflammatory, antiproliferative and antioxidant actions (Steckelings *et al.* 2010, 2011, Steckelings and Unger 2012) with cardioprotective (Kaschina *et al.* 2008, Rompe *et al.* 2010), renoprotective (Steckelings *et al.* 2009) or neuroprotective (Namsolleck *et al.* 2013) implications. In line with these data, in our experiment, C21 prevented the reduction of glomerular number, reduced free radical burden, yet without any effect on increased blood pressure. Similarly to the reported cardioprotective and vasculoprotective effects (Kaschina *et al.* 2008, Paulis *et al.* 2012, Rehman *et al.* 2012) the renoprotective effects observed in our study seem to be blood pressure independent. Without affecting blood pressure, C21 reduced monocyte/macrophage infiltration in the kidney and T-lymphocyte infiltration in the renal cortex of spontaneously hypertensive rats (Rehman *et al.* 2012) and prevented early renal inflammatory changes along with reduction of TNF-α, IL-6, TGF-β1 levels and an increase in NO and cGMP levels in 2 kidney 1 clip hypertensive rats (Mattavelli *et al.* 2011). Therefore we suppose that the major mechanism responsible for the renoprotective effects of

C21 is the inhibition of inflammation and NF-κB (Rompe *et al.* 2010).

We conclude that captopril, olmesartan and melatonin prevented increase in oxidative load, loss of glomeruli and blood pressure increase in doxorubicin-induced nephrotoxicity; while, C21 showed antioxidant effects and prevented the decline in numerical glomerular density, yet without prevention of increased blood pressure. We suggest that both melatonin and C21 might offer some renoprotective effect in chronic kidney disease.

Limitations of the Work

It is very probable that the changes in the number and size of nephrons could be associated with functional alterations such as changes in plasma urea and creatinine or increased urinary albumin excretion and with histopathological alterations. However, to perform these investigations was beyond the scope and possibilities of this study.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

References

ABRAHAM P, KOLLI VK, RABI S: Melatonin attenuates methotrexate-induced oxidative stress and renal damage in rats. *Cell Biochem Funct* **28**: 426-433, 2010.

AGAPITO MT, ANTOLIN Y, DEL BRIO MT, LOPEZ-BURILLO S, PABLOS MI, RECIO JM: Protective effect of melatonin against adriamycin toxicity in the rat. *J Pineal Res* **31**: 23-30, 2001.

BEHULIAK M, PALFFY R, GARDLIK R, HODOSY J, HALCAK L, CELEC P: Variability of thiobarbituric acid reacting substances in saliva. *Dis Markers* **26**: 49-53, 2009.

BENZIE IF, STRAIN JJ: The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal Biochem* **239**: 70-76, 1996.

CHANG EJ, MUN KC: Effect of melatonin on the malondialdehyde level of neutrophils in cyclosporine-treated rats. *Transplant Proc* **36**: 2165-2166, 2004.

COLAK MC, PARLAKPINAR H, TASDEMIR S, SAMDANCI E, KOSE E, POLAT A, SARIHAN E, ACET A: Therapeutic effects of ivabradine on hemodynamic parameters and cardiotoxicity induced by doxorubicin treatment in rat. *Hum Exp Toxicol* **31**: 945-954, 2012.

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.

DZIEGIEL P, SUDER E, SUROWIAK P, JETHON Z, RABCZYŃSKI J, JANUSZEWSKA L, SOPEL M, ZABEL M: Role of exogenous melatonin in reducing the nephrotoxic effect of daunorubicin and doxorubicin in the rat. *J Pineal Res* **33**: 95-100, 2002.

EREL O: A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. *Clin Biochem* **37**: 277-285, 2004.

GHEORGHIADE M, PANG PS: Acute heart failure syndromes. *J Am Coll Cardiol* **53**: 557-573, 2009.

JIRKOVSKY E, POPELOVÁ O, KRIVAKOVÁ-STANKOVA P, VÁVROVA A, HROCH M, HASKOVA P, BRCAKOVÁ-DOLEZELOVA E, MICUDA S, ADAMCOVA M, SIMUNEK T, CERVINKOVA Z, GERSL V, STERBA M: Chronic anthracycline cardiotoxicity: molecular and functional analysis with focus on nuclear factor erythroid 2-related factor 2 and mitochondrial biogenesis pathways. *J Pharmacol Exp Ther* **343**: 468-478, 2012.

KASCHINA E, GRZESIAK A, LI J, FORYST-LUDWIG A, TIMM M, ROMPE F, SOMMERFELD M, KEMNITZ UR, CURATO C, NAMSOLLECK P, TSCHOPE C, HALLBERG A, ALTERMAN M, HUCKO T, PAETSCH I, DIETRICH T, SCHNACKENBURG B, GRAF K, DAHLOF B, KINTSCHER U, UNGER T, STECKELINGS UM: Angiotensin II type 2 receptor stimulation: a novel option of therapeutic interference with the renin-angiotensin system in myocardial infarction? *Circulation* **118**: 2523-2532, 2008.

KOCH BC, NAGTEGAAL JE, HAGEN EC, VAN DER WESTERLAKEN MM, BORINGA JB, KERKHOF GA, TER WEE PM: The effects of melatonin on sleep-wake rhythm of daytime haemodialysis patients: a randomized, placebo-controlled, cross-over study (EMSCAP study). *Br J Clin Pharmacol* **67**: 68-75, 2009.

KOCH BC, VAN DER PUTTEN K, VAN SOMEREN EJ, WIELDERS JP, TER WEE PM, NAGTEGAAL JE, GAILLARD CA: Impairment of endogenous melatonin rhythm is related to the degree of chronic kidney disease (CREAM study). *Nephrol Dial Transplant* **25**: 513-519, 2010a.

KOCH BC, NAGTEGAAL JE, HAGEN EC, TER WEE PM, KERKHOF GA: Different melatonin rhythms and sleep-wake rhythms in patients on peritoneal dialysis, daytime hemodialysis and nocturnal hemodialysis. *Sleep Med* **11**: 242-246, 2010b.

LEE IC, KIM SH, LEE SM, BAEK HS, MOON C, KIM SH, PARK SC, KIM HC, KIM JC: Melatonin attenuates gentamicin-induced nephrotoxicity and oxidative stress in rats. *Arch Toxicol* **86**: 1527-1536, 2012.

LEE VW, HARRIS DC: Adriamycin nephropathy: a model of focal segmental glomerulosclerosis. *Nephrology* **16**: 30-38, 2011.

MAIONE A, NAVANEETHAN SD, GRAZIANO G, MITCHELL R, JOHNSON D, MANN JF, GAO P, CRAIG JC, TOGNONI G, PERKOVIC V, NICOLUCCI A, DE COSMO S, SASSO A, LAMACCHIA O, CIGNARELLI M, MANFREDA VM, GENTILE G, STRIPPOLI GF: Angiotensin-converting enzyme inhibitors, angiotensin receptor blockers and combined therapy in patients with micro- and macroalbuminuria and other cardiovascular risk factors: a systematic review of randomized controlled trials. *Nephrol Dial Transplant* **26**: 2827-2847, 2011.

MATAVELLI LC, HUANG J, SIRAGY HM: Angiotensin AT_2 receptor stimulation inhibits early renal inflammation in renovascular hypertension. *Hypertension* **57**: 308-313, 2011.

MONTILLA P, TUNEZ I, MUNOZ MC, LOPEZ A, SORIA JV: Hyperlipidemic nephropathy induced by adriamycin: effect of melatonin administration. *Nephron* **76**: 345-350, 1997.

MUNCH G, KEIS R, WESSELS A, RIEDERER P, BAHNER U, HEIDLAND A, NIWA T, LEMKE HD, SCHINZEL R: Determination of advanced glycation end products in serum by fluorescence spectroscopy and competitive ELISA. *Eur J Clin Chem Clin Biochem* **35**: 669-677, 1997.

NAMSOLLECK P, BOATO F, SCHWENGEL K, PAULIS L, MATHO KS, GEURTS N, THONE-REINEKE C, LUCHT K, SEIDEL K, HALLBERG A, DAHLÖF B, UNGER T, HENDRIX S, STECKELINGS UM: AT2-receptor stimulation enhances axonal plasticity after spinal cord injury by upregulating BDNF expression. *Neurobiol Dis* **51**: 177-191, 2013.

OZ E, ILHAN MN: Effects of melatonin in reducing the toxic effects of doxorubicin. *Mol Cell Biochem* **286**: 11-15, 2006.

PAULIS L, SIMKO F: Blood pressure modulation and cardiovascular protection by melatonin: potential mechanisms behind. *Physiol Res* **56**: 671-684, 2007.

PAULIS L, BECKER ST, LUCHT K, SCHWENGEL K, SLAVIC S, KASCHINA E, THÖNE-REINEKE C, DAHLÖF B, BAULMANN J, UNGER T, STECKELINGS UM: Direct angiotensin II type 2 receptor stimulation in Nω-nitro-L-arginine-methyl ester-induced hypertension: the effect on pulse wave velocity and aortic remodeling. *Hypertension* **59**: 485-492, 2012.

PECHANOVA O, MATUSKOVA J, CAPIKOVA D, JENDEKOVA L, PAULIS L, SIMKO F: Effect of spironolactone and captopril on nitric oxide and S-nitrosothiol formation in kidney of L-NAME-treated rats. *Kidney Int* **70**: 170-176, 2006.

RASHIKH A, PILLAI KK, AHMAD SJ, AKHTAR M, NAJMI AK: Aliskiren alleviates doxorubicin-induced nephrotoxicity by inhibiting oxidative stress and podocyte injury. *J Renin Angiotensin Aldosterone Syst* **14**: 14-22, 2013.

REDDY GK, ENWEMEKA CS: A simplified method for the analysis of hydroxyproline in biological tissues. *Clin Biochem* **29**: 225-229, 1996.

REHMAN A, LEIBOWITZ A, YAMAMOTO N, RAUTUREAU Y, PARADIS P, SCHIFFRIN EL: Angiotensin type 2 receptor agonist compound 21 reduces vascular injury and myocardial fibrosis in stroke-prone spontaneously hypertensive rats. *Hypertension* **59**: 291-299, 2012.

REITER RJ, PAREDES SD, MANCHESTER LC, TAN DX: Reducing oxidative/nitrosative stress: a newly-discovered genre for melatonin. *Crit Rev Biochem Mol Biol* **44**: 175-200, 2009a.

REITER RJ, TAN DX, FUENTES-BROTO L: Melatonin: a multitasking molecule. *Prog Brain Res* **181**: 127-151, 2009b.

REITER RJ, ROSALES-CORRAL S, COTO-MONTES A, BOGA JA, TAN DX, DAVIS JM, KONTUREK PC, KONTUREK SJ, BRZOZOWSKI T: The photoperiod, circadian regulation and chronodisruption: the requisite interplay between the suprachiasmatic nuclei and the pineal and gut melatonin. *J Physiol Pharmacol* **62**: 269-274, 2011.

ROMPE F, ARTUC M, HALLBERG A, ALTERMAN M, STRÖDER K, THÖNE-REINEKE C, REICHENBACH A, SCHACHERL J, DAHLÖF B, BADER M, ALENINA N, SCHWANINGER M, ZUBERBIER T, FUNKE-KAISER H, SCHMIDT C, SCHUNCK WH, UNGER T, STECKELINGS UM: Direct angiotensin II type 2 receptor stimulation acts anti-inflammatory through epoxyeicosatrienoic acid and inhibition of nuclear factor kappaB. *Hypertension* **55**: 924-931, 2010.

SCHLONDORFF DO: Overview of factors contributing to the pathophysiology of progressive renal disease. *Kidney Int* **74**: 860-866, 2008.

SENER G, SATIROGLU H, KABASAKAL L, ARBAK S, ONER S, ERCAN F, KEYER-UYSA M: The protective effect of melatonin on cisplatin nephrotoxicity. *Fundam Clin Pharmacol* **14**: 553-560, 2000.

SIMKO F: Chronobiology of blood pressure: emerging implications of melatonin. *Eur J Clin Invest* **42**: 1252-1254, 2012.

SIMKO F, PAULIS L: Melatonin as a potential antihypertensive treatment. *J Pineal Res* **42**: 319-322, 2007.

SIMKO F, PECHANOVA O: Recent trends in hypertension treatment: perspectives from animal studies. *J Hypertens* **27** (Suppl 6): S1-S4, 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, 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* **18**: 616-625, 2013.

SIMUNEK T, STERBA M, POPELOVA O, ADAMCOVA M, HRDINA R, GERSL V: Anthracycline-induced cardiotoxicity: overview of studies examining the roles of oxidative stress and free cellular iron. *Pharmacol Rep* **61**: 154-171, 2009.

STECKELINGS UM, UNGER T: Angiotensin II type 2 receptor agonists – where should they be applied? *Expert Opin Investig Drugs* **21**: 763-766, 2012.

STECKELINGS UM, ROMPE F, KASCHINA E, UNGER T: The evolving story of the RAAS in hypertension, diabetes and CV disease: moving from macrovascular to microvascular targets. *Fundam Clin Pharmacol* **23**: 693-703, 2009.

STECKELINGS UM, WIDDOP RE, PAULIS L, UNGER T: The angiotensin AT2 receptor in left ventricular hypertrophy. *J Hypertens* **28** (Suppl 1): S50-S55, 2010.

STECKELINGS UM, LARHED M, HALLBERG A, WIDDOP RE, JONES ES, WALLINDER C, NAMSOLLECK P, DAHLÖF B, UNGER T: Non-peptide AT2-receptor agonists. *Curr Opin Pharmacol* **11**: 187-192, 2011.

STENVINKEL P: Chronic kidney disease: a public health priority and harbinger of premature cardiovascular disease. *J Intern Med* **268**: 456-467, 2010.

WAN Y, WALLINDER C, PLOUFFE B, BEAUDRY H, MAHALINGAM AK, WU X, JOHANSSON B, HOLM M, BOTOROS M, KARLEN A, PETTERSON A, NYBERG F, FANDRIKS L, GALLO-PAYET N, HALLBERG A, ALTERMAN M: Design, synthesis, and biological evaluation of the first selective nonpeptide AT2 receptor agonist. *J. Med Chem* **47**: 5995-6008, 2004.