Carvedilol Protects against Cyclosporine Nephropathy in Rats

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Abstract

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The aim of our experimental work was to study whether carvedilol is able to protect renal tissue from cyclosporine toxic effect in animal model of cyclosporine nephropathy. The study was performed on twenty Wistar rats divided in two experimental groups: control (treated with placebo) and carvedilol (treated with p.o. dose 10mg/kg/day in 1 ml solution). Cyclosporine in oral dose of 15 mg/kg/day was administered to all animals during 15 days of experiment. Urine was collected daily for the assessment of diuresis, proteinuria, and determination of urea and creatinine levels. Serum collected at the end of the experiment (day 15) was used for the determination of urea and transferrin levels. The level of renal tissue damage was evaluated by the Jones method for basal membranes, glomeruli and tubuli impregnation, and by the Kossa method for calcium impregnation. For the determination of paranuclear inclusions presence we used chromanilinblue (CAB) method. Statistically significant differences between total protein levels in urine on day 7 of the experiment and urea levels in serum at the end of the experiment in the control group and the carvedilol-treated group indicate a protective effect of carvedilol on renal tissue, which is supported also by the results of a histological examination of renal tissue. Significant increase in the serum transferrin level was registered in the carvedilol-treated group and no significant changes were noted in ceruloplasmin serum levels. In conclusion, our pilot study showed that carvedilol has the ability to protect renal tissue from cyclosporine induced nephropathy in rats.

Carvedilol, cyclosporine nephropathy, antioxidative effect, histological examination

Carvedilol – (*R/S*)-1-(9*H*-Carbazol-4-yloxy)-3-(2-methoxy-benzylamino)-propan-2-ol is a cardiovascular drug with a wide therapeutic potential. It is a non selective blocker of α_1 and β-adrenergic receptors without intrinsic sympathomimetic activity, with membrane stabilising effects. Carvedilol, commonly used in therapy of hypertension and in patients with blood failure (Bultas 1998) combines several important pharmacological properties in one molecule. Beside the above mentioned effects, it also exerts an antioxidant potential, which was described in experiments both *in vitro* and in animal models. In a culture of endothelial cells, carvedilol protects from cell damage induced by oxygen radicals (Kumar et al. 2000; Ma et al. 1996). Carvedilol also has the ability to trap superoxid and hydroxyl radicals in both aqueous and lipid environment and interferes with the atherogenesis process (Ma et al. 1996). Because of its capability to trap free oxygen radicals (FOR), it inhibits oxidation of low density lipoproteins (LDL) and thus inhibits oxidized LDL formation and development of atherosclerotic plaque (Bultas 1998; Silbernagl and Lang 1998; Asbrink et al. 2000). Moreover, this compound mitigates the unfavourable effect of ischemia and successive reperfusion, where supposedly its ability to scavenge FOR, restore myocardial blood flow by vasodilatation, protect endothelial functions, inhibit adhesion and activation of polymorphonuclears and protect from accumulation of lipids in the aorta wall is exerted (Bultas 1998; Ma et al. 1996). Carvedilol was tested in conditions of the burn

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syndrome (Nečas et al. 2001) and alloxan diabetes (Bartošíková et al. 1998); propitious effect was most notable in proximal renal tubules, where it decreased the disintegration of tubular epithelia and inhibited development of oedematous changes. The antioxidant effect of carvedilol protects kidneys from gentamicin induced nephrotoxicity, reduces creatine clearance, proteinuria and increase of serum urea and creatinine, and it also limits development of glomerulosclerosis (Kumar et al. 2000).

In cyclosporine-induced nephrotoxicity, toxic effect of cyclosporine is given connected with oxidative stress (Pecháň and Dobišová 2003). Cyclic undecapeptide cyclosporine A is used in the immunosuppressive treatment of patients after organs transplantation and in some autoimmune diseases (Lüllmann et al. 2004). The aim of our experimental work within preclinical tests of carvedilol antioxidative effects was to elicit whether this compound is able to protect renal tissue from cyclosporine toxic effect in the animal model of cyclosporine nephropathy.

Materials and Methods

For the purpose of this study we used 20 laboratory Wistar rats, male gender, of the same age and weight 208 ± 15 g. The animals came from a conventional breeding colony (Faculty of Medicine, Masaryk University, Brno) and were housed under standard conditions (temperature 20-24 °C, humidity 40-60 %, 12:12 L:D cycles with lighting up to 200 lux maximum). Cyclosporine (Sandimmun® sol., Novartis International AG, Basel, Switzerland) at a dose of 15 mg/kg/day was administered *per os* (p. o.) to all animals during 15 days of experiment.

Animals were divided in two experimental groups:

1st group – control: treated with placebo - p.o. dose 1 ml of 0.9 % NaCl /day

2nd group – carvedilol: treated with p.o. dose 10mg/kg/day (in 1 ml solution)

During the experiment the animals were placed separately in metabolic boxes, fed a standard diet (Diet for small laboratory animals M_1) and given water *ad libitum*. Urine was collected daily for the assessment of diuresis, proteinuria, and determination of urea and creatinine levels. Immediately after the end of the experiment, animals were euthanized by exsanguination, and blood was prepared for biochemical examination. Serum was used for the determination of urea and transferrin levels. Biochemical evaluation was realised on the automatic analyser Hitachi® with commercial kits of the BioVendor company (Brno, Czech Republic).

Samples of renal tissue were taken for histopathological examination. The level of renal tissue damage was evaluated by the Jones method for basal membranes, glomeruli and tubuli impregnation, and the Kossa method for calcium impregnation. For the determination of paranuclear inclusions presence we used the chromanilinblue (CAB) method, which represents blue trichrom modified method (according to Department of Pathology and Anatomy, St. Anna Faculty Hospital methodology).

CAB (chromanilinblue) method:

1. Weigert hematoxilin
2. 1% phosphomolybden acid
3. CAB mixture (anilin blue 0,5g/100 ml, chromotrop 2R (Fluka) 2g), in distilled water, pH 1.05 (using HCl conc.)

The methodology of this study was approved by the Central Committee for Animal Protection and monitored by the Ethical Committee of the University of Veterinary and Pharmaceutical Sciences Brno.

The Stat Plus® program was used for the statistical evaluation; values were compared by an unpaired *t*- test. Values with $p < 0.05$ were considered significant.

Results and Discussion

Hydrogen peroxide is toxic to the glomerular and tubulointerstitial renal system either directly or through the hydroxyl radical formation which is catalyzed by iron. In physiological conditions cells form hydrogen peroxide, but it is easily trapped by antioxidizing systems. When cyclosporine is administered, renal tissue is exposed to oxidative stress where antioxidizing systems are depleted. FOR and lipid peroxidation importantly participate in development of nephrotoxicity in patients taking cyclosporine A (Kumar et al. 2000). It is assumed that FOR are not formed directly from cyclosporine A molecule, but their formation is conditioned by the involvement of cell enzymatic systems (Kumar et al. 2000). Outcomes of biochemical examination and histological findings confirm the induction of cyclosporine nephropathy in our experiment in the control group

of rats by administration of cyclosporine A at the dose of 15 mg/kg/day, as shown by statistically significant differences ($p < 0.01$ and $p < 0.05$) between levels of total protein in urine observed in the control group on days 7 and 15 of the experiment compared with day 2 (Table 1), and medium-severe insult of renal proximal tubules epithelia in the control group (Plate III, Fig. 1). Morphologic signs of cyclosporine nephrotoxic effect are marked in proximal tubules. In epithelial cytoplasm, large vacuoles of the same size are formed (isometric vacuolization). In histological sections cytoplasm shows a bright pattern and swelling. Formation of paranuclear inclusions is also detectable by the CAB methodology. It is a blue trichom modified method, used in section examination of e.g. hepatic biopsy. Paranuclear inclusion formation is explained by a marked enlargement of plasmatic organelles, mitochondria in particular.

Table 1. Total protein values assessed in urine of rats during experiment

	total protein [mg/l] in urine		
	day 2 of experiment	day 7 of experiment	day 15 of experiment
control	0.42 ± 0.16	1.13 ± 0.21**	1.00 ± 0.18*
carvedilol	0.54 ± 0.13	0.39 ± 0.08*	0.73 ± 0.09

Significant differences between levels of total protein in urine were observed in the control group on days 7 and 15 of experiment, compared with day 2 at the level of * $p < 0.05$ and ** $p < 0.01$, respectively. Statistically significant differences between levels of total protein in urine were observed in the control group and the carvedilol-treated group on day 7 at the level of * $p < 0.05$.

FOR are very reactive, have very short half-life and in most cases act at the point of origin. If scavengers of FOR are supposed to be effective, they must be present in the place of FOR activity. Carvedilol is partially eliminated by kidneys that guarantee its presence in point of FOR origin and activity (Jonsson et al. 1999). Lipid peroxidation seriously impairs membrane functions, modifies membrane fluidity, participates in its disintegration associated with calcium ions transfer in cytoplasm, decreases membrane potential and allows membrane permeability for ions (Štípek 2000). In the course of oxidizing stress, carvedilol protects from the collapse of plasmatic membrane, cell lyses and its subsequent death, impairment of lysosomes and cell nucleus, and maintains the structural and functional integrity of mitochondria (Santos and Moreno 2001). Statistically significant differences ($p < 0.05$) between total protein levels in urine on day 7 of the experiment (Table 1) and urea levels in serum at the end of the experiment (Table 2) in the control group and the carvedilol treated group indicate a protective effect of carvedilol on renal tissue. Also the results of our histological examination of renal tissue in carvedilol-treated group show its protective effect (Plate III, Fig. 2).

Table 2. Urea values assessed in serum of rats at the end of experiment

	urea [mmol/l] in serum
control	9.62 ± 1.9
carvedilol	7.50 ± 1.6*

Significant differences between levels of urea in serum were observed in the control group and the carvedilol-treated group at the level of * $p < 0.05$.

In our experiment we registered a significant increase in the serum transferrin level in the carvedilol-treated group (Table 3). Some studies do not put down the attenuation ability of carvedilol on lipoperoxidation and LDL oxidation to its scavenger activity, but to its ability to trap ferric ions. These ions are inactivated by binding with a molecule of carvedilol and

Table 3. Transferrin values in serum of rats at the end of experiment

	transferrin [g/l] in serum
control	1.61 ± 0.2
carvedilol	1.88 ± 0.1*

Significant differences between levels of transferrin in serum were observed in the control group and the carvedilol-treated group at the level of * $p < 0.05$

cannot take part in radical reactions and insult tissue (15). Ceruloplasmin is blood protein partaking in the copper transfer in system plasma, which is probably important for plasmatic Fe^{2+} oxidation; however only little of copper-bounded ceruloplasmin is transferred in tissues (Silbernagl and Lang 1998). Because part of the carvedilol protective effect is likely to be associated with its ability to bind iron, but not copper ions, we could assume that the increase in the ceruloplasmin serum level in the carvedilol-treated group would not be present. Hence, our experiment did not show statistically significant differences between levels of serum ceruloplasmin in the control group and the carvedilol-treated group (Table 4).

Table 4. Ceruloplasmin values serum of rats at the end of experiment

	ceruloplasmin [mmol/l] in serum
control	0.021 ± 0.003
carvedilol	0.024 ± 0.005

No significant differences between levels of ceruloplasmin in serum were observed in the control group and the carvedilol-treated group

Concluding our results, we can say that cyclosporine administered at the mentioned doses leads to an experimentally induced nephropathy model in rats, which can be applied to determine a possible protective effect of various substances to tissue damage. In our pilot study carvedilol demonstrated the ability to protect renal tissue of Wistar rats from cyclosporine-induced nephropathy. This ability can be documented by results of biochemical and histological examination. This suggests the conclusion that the protective effect of carvedilol is of great importance in cyclosporine induced nephropathy and this effect might be utilised also in clinical use.

Protektivní efekt carvedilolu na modelu cyclosporinové nefropatie u potkanů

Cílem naší experimentální práce bylo v rámci preklinického testování antioxidačních vlastností karvedilolu zjistit, zda má tato látka schopnost protektivně působit na ledvinovou tkáň při modelu cyklosporinové nefropatie. Pokus byl proveden na dvaceti laboratorních potkanech kmene Wistar, rozdělených do dvou skupin: kontrolní, které bylo podáno placebo 1 ml NaCl 0.9 % p.o./den, a skupiny léčené karvedilolem v dávce10mg/kg/den p.o. (v 1 ml roztoku).Všem zvířatům byl p.o. aplikován cyklosporin v dávce 15 mg/kg/den po celou dobu pokusu (15 dní). Denně byla odebírána moč, stanovena diuréza, provedeno vyšetření proteinurie, stanovení hladiny močoviny a kreatininu. Sérum, získané na konci experimentu, bylo použito pro vyšetření hladiny močoviny a transferinu. Pro histopatologické vyšetření byla odebrána ledvinná tkáň, pro posouzení stupně poškození ledvinné tkáně byla použita metodika Jonesova na impregnaci bazálních membrán, glomerulů a tubulů, metodika Kossa na průkaz vápníku, a hodnocena přítomnost paranukleárních inkluzí barvením metodou CAB (chromanilinblue). Statisticky významný rozdíl hodnot celkové bílkoviny v moči 7. den trvání pokusu a hladiny močoviny v séru mezi skupinou léčenou karvedilolem a skupinou kontrolní potvrzují protektivní účinek karvedilolu na ledvinou tkáň, který je vyjádřen i výsledky histologického vyšetření ledvinné tkáně. Bylo pozorováno statisticky

významné zvýšení hladiny transferinu v séru u skupiny léčené karvedilolem, v hodnotách sérového ceruloplasminu nebyl zjištěn statisticky významný rozdíl. Naše pilotní studie ukázala schopnost protektivního účinek carvedilolu na ledvinnou tkáň potkanů s experimentálně navozenou nefropatií.

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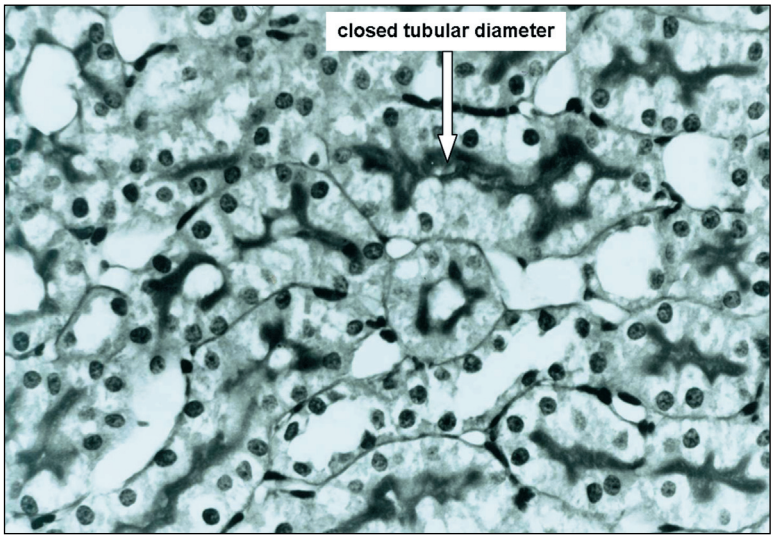

Fig. 1. Control – placebo-treated group – semiquantitatively evaluated cytoplasm microvacuolisation of proximal tubules epithelia. The damage is classified as medium-severe and affects 20 – 50% of tissue. The result of histopathological examination represents three histological samples. The figure shows a damage insult of renal tubular epithelia by isometric vacuolization. The extent of swelling of cytoplasm is so large that most tubules have fully closed diameters. Normally appearing tubules are not present.

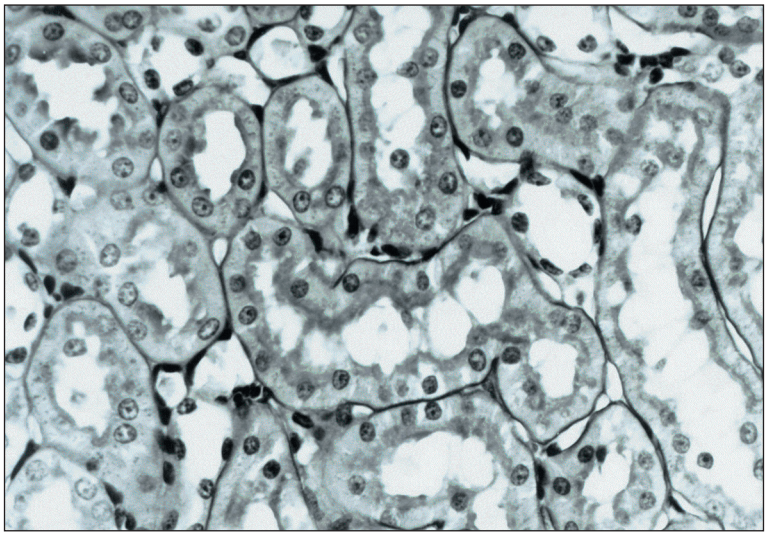

Fig. 2. Carvedilol-treated group – semiquantitatively evaluated cytoplasm microvacuolisation of proximal tubules epithelia. The insult is classified as small to medium and affects 20% of tissue at most. The result of histopathological examination represents three histological samples. Changes after the treatment are less intensive; the swelling of cytoplasm is not as marked as in Fig. 1. Tubular diameters stays wide open.