PROTECTIVE EFFECTS OF β-BLOCKER CARVEDILOL BY EXPERIMENTALLY INDUCED SOLAR BURN IN RATS

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Abstract

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Evidence that free oxygen radicals may be responsible for tissue damage following skin thermal injury supports a theory of interventional use of antioxidants in therapy. Carvedilol (CVD) is used for treatment of hypertension and chronic heart failure. It also shows a potent scavenger property in pathological conditions in which free oxygen radicals (FOR) take part.

We studied whether carvedilol can reduce renal damage induced by solar thermal effect in a 17 - day-long experiment. A solar emittor was used to develop solar erythema within the range 30% of the total body surface of narcotised experimental rats. The animals were randomly assigned to two equal groups (n = 7). Group 1 was treated with CVD at a dose of 10mg/kg/day administered in 1ml of saline i.p. Group 2 (placebo group) was given 1 ml of saline i.p./day. Protein and malonyldialdehyde (MDA) concentrations were determined in serum samples obtained on days 1, 8, and 17. Proteinuria and total protein loss per day were measured in urine samples daily.

Serum protein levels of the two experimental groups did not differ significantly during the period under study. Serum MDA of the placebo group surpassed significantly that of the treated group on days 8 and 17 ($p < 0.05$). Protein concentrations in urine of CVD-treated animals were lower than those of the placebo group during the entire experimental period except for days 4 and 5 ($p < 0.05$ and $p < 0.01$). Total protein loss/day in urine shows similar differences between both groups ($p < 0.05$, $p < 0.01$). The results of our study show that in experimentally induced thermal injury carvedilol apparently protects renal function, by increased renal blood flow and by its antioxidant effect.

Carvedilol, burns, renal function, free oxygen radicals

It is known that high temperature does not cause only a local skin damage but impairs also the integrity of the organism with resulting functional affections of many organs and systems – systemic inflammatory response syndrome (SIRS) (Zogovic et al. 1996). Lungs, heart, kidneys, liver and the coagulation system are the most commonly affected organs in burns. There are many factors during thermal injury that may harm renal functions. The most important are the decreased cardiac output, the respiratory failure with hypoxia and acidosis, toxaemia and sepsis. Hypovolemia with hypoperfusion, infection and endotoxaemia also play an important role in renal failure as part of the multiple organ failure syndrome (MOFS).

Carvedilol is a multiple-action antihypertensive drug with selective α-adrenergic and nonselective ß-adrenergic blocking activity for treatment of mild to moderate hypertension. On the other hand, carvedilol inhibits the lipid peroxidation in myocardium, kidneys and the brain initiated by oxygen free radicals (Yue et al. 1992).

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Materials and Methods

Fourteen laboratory male Wistar rats of the same age, weighing 230 ± 15 g were used in the experiment. In total anaesthesia, an acute solar erythema, covering approximately 30% of total body surface was induced by a solar lamp. This injury resulted in a series of pathophysiological alterations**,** both local and distant tissue and organ injury. The animals were randomised into two equal groups and placed in metabolic cages. The treatment with carvedilol was started one hour after the burn.

The project was approved and monitored by the Local University Ethical Committee.

Group 1 - treated with CVD at a dose of 10 mg/kg/day in 1ml of saline administered once daily i.p. for 17 consecutive days.

Group 2 - placebo was given 1ml of saline administered once daily i.p. for 17 days.

Blood samples were collected from jugular vein on days 1, 8 and 17, and serum protein and malonyldialdehyde (MDA) concentrations were determined. For the assessment of free oxygen radicals concentration the thiobarbituric acid reacting substances (TBARS) test was used. Urine samples were taken daily and protein concentration was determined using Bio-La-Test Lachema Diagnostica Brno. The total daily protein excretion/day was then calculated as magnification of protein concentration in urine and diuresis per 24 hours.

Statistics

To carry out statistical evaluation, the UNISTAT 5.1 programme was used. Statistical analysis was performed as indicated in the figure legends, significant difference was accepted at $p < 0.05$ or/and $p < 0.01$.

Results and Discussion

Total protein values in serum of experimental animals do not show statistically significant differences between therapeutic group and placebo group (Fig. 1). The MDA values in serum show statistically significant differences between therapeutic group and placebo group on the 8th and 17th day ($p < 0.05$), see Fig. 2. Protein concentrations in urine increased in the first five days of experiment in both groups. Since the 6th day the protein levels in the therapeutic group were significantly lower than in the placebo group ($p < 0.05$, $p < 0.01$), see Fig. 3. The quantity of the total excretion of proteins in urine per 24 h (multiplication of diuresis ml/day by the protein content in 1ml) show a permanent statistical difference between therapeutic and placebo group from the 5th day till the end of the experiment ($p < 0.05$, $p < 0.01$), see Fig. 4.

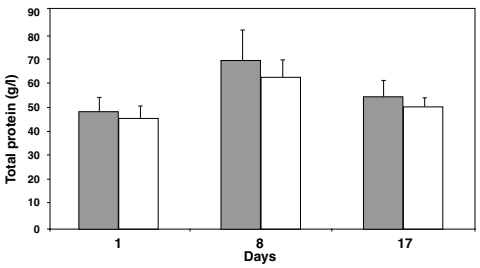

Fig. 1: Total protein values in the rat serum with solar burn syndrome. First column: treated animals (n = 7, mean ± SD), second column: placebo animals (n = 7, mean ± SD.

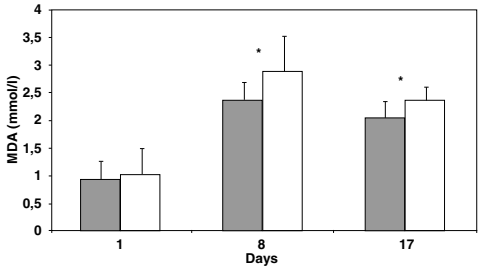

Fig. 2: Values of the MDA in the rat serum with solar burn syndrome. Effects of Carvedilol on TBARS'-reactive products. First column: treated animals (n = 7, mean ± SD), second column: placebo animals (n = 7, mean ± SD), *$p < 0.05$.

Thermal, chemical, or electrical injury may cause burns. Thermal injuries result from exposure to direct flames, hot liquids or radiation. Burn syndrome is a phenomenon consisting of a hypovolemic cardiovascular component and a cellular component. Researches have documented the release of many mediators – prostaglandins, thromboxanes, histamine and serotonin (Zogovic et al. 1996). The cellular response to burn injury falls into two categories: metabolic response and response of immune system.

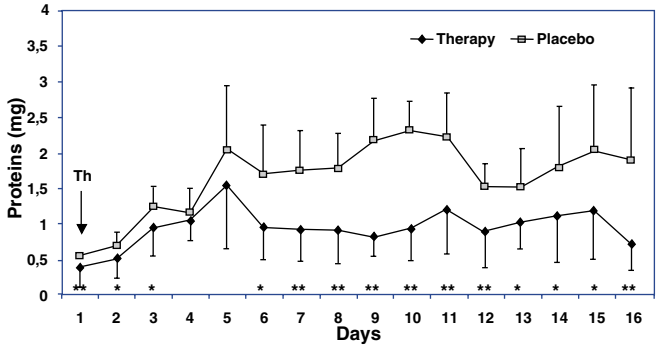

Fig. 3: Protein concentrations in the rat urine with solar burn syndrome. Upper curve: placebo animals (n = 7, mean ± S.D.), lower curve: treated animals (n = 7, mean ± S.D.) *$p < 0.05$; **$p < 0.01$.

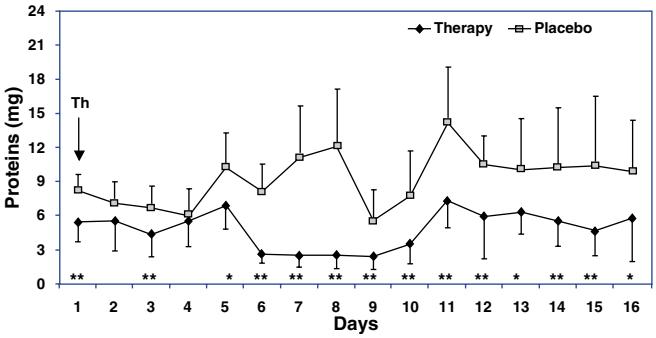

Fig. 4: Total excretion of proteins per 24 hours in the rat urine with solar burn syndrome, upper curve: placebo animals (n = 7; mean ± S.D.), lower curve: treated animals (n = 7; mean ± S.D.); *$p < 0.05$; **$p < 0.01$.

Burn serum contains an inhibitor of C3 conversion that leads to decreased opsonisation and polymorphonuclear (PMN) neutrofil dysfunction (Bjornson et al. 1977). Free oxygen radicals (FOR) are under these conditions released from PMN. Also the mesangial apparatus play a role in pathogenesis of renal damage, because oxidising agents formed by means of myeloperoxidase system can act as direct mediators of kidney damage. Further, the oxidising agents can inactivate antiproteases and so expose substrates as basal membranes to a larger influence of proteolytic reactions. Reduction of FOR production and blocking their effects on cells represent some of possible favourable influence on the kidney damage. Recently, a great number of substances were reported to exhibit the ability to scavenge FOR.

One of the most potent scavengers of FOR is carvedilol (Yue et al. 1992). It inhibits lipoperoxidation caused by FOR, prevents oxidation of LDL caused by macrophages and inhibits release of superoxides from PMN cells.

We have studied antioxidative effects of carvedilol under the condition of experimentally induced solar erythema in laboratory rats. Carvedilol was administered for 17 days once daily at a dose of 10 mg/kg/day in 1 ml of saline i.p.

These findings are in correlation with the statistically significant differences in serum MDA levels of treated and placebo group. The used dose of 10 mg/kg/day of carvedilol has resulted in significantly lower protein concentration in the rat urine of the treated group in comparison with the placebo group. Significant differences have been found also in the total daily protein excretion, whereas the treated group had also significantly lower values than the placebo group.

The half-life of disintegration of FOR is very short due to their high reactivity. For the scavenger to be efficient is, therefore, absolutely necessary that they are present on places of origin and action of FOR. Carvedilol meets this condition because is bound to plasmatic proteins and is secreted by kidney. Renal proximal tubules are the side of the protective effect of carvedilol. It is corresponding with our findings that plasma protein concentrations after burn trauma do not show significant difference in both groups. However, principal changes occur in the renal function. There is statistically significant difference in both urine protein concentrations and total protein excretion between treated and placebo group. Both examinations produce better results in CVD treated group. Another fundamental assignments are morphological alterations in renal tissue in placebo group in comparison with less serious damage in the CVD group. In terms of these facts it is possible to state that carvedilol prevents the functional and the morphological alterations in kidney. As mentioned above there are two principle mechanisms of action – vasodilator and antioxidant (Cameron and Cotter 1995). Its renal vasodilator effects result in preservation of renal blood flow and so renal perfusion and renal function are maintained well. These effects are enhanced by CVD scavenging property and were proved in many scientific works (Dupont 1992; Leeman et al. 1993; Vacek and Husek 1994; Kumar et al. 2000).

The principal question is, therefore, if our experimental results can be useful in analogical conditions in human medicine.

Studie protektivního efektu β-blokátoru carvedilolu u experimentálně vyvolané solární popáleniny

Poznání, že volné kyslíkové radikály (VKR) mohou být odpovědné za tkáňové poškození, navazující na popápeninový syndrom, podporuje teorii možného terapeutického použití antioxidačních látek v terapii těchto stavů. S ohledem na tuto skutečnost jsme sledovali možný protektivní efekt carvedilolu u experimentálně vyvolané solární popáleniny. Carvedilol je selektivní α-1 antagonista a neselektivní β-antagonista adrenergního systému, používaný k terapii lehké a střední formy hypertenze a chronického srdečního selhávání. Carvedilol také vykazuje silný antioxidační efekt u patologických stavů, ve kterých se účastní VKR.

V 17-denním experimentu jsme sledovali, zda carvedilol může redukovat poškození ledvin způsobené solární spáleninou. U experimentálních potkanů byl v narkoze navozen solární erytém v rozsahu 30% povrchu těla. Zvířata byla rozdělena náhodným výběrem do dvou skupin (n = 7). První skupina byla léčena carvedilolem v celkové dávce 10 mg/kg/den v 1 ml fyziologického roztoku, druhá skupina (n = 7) dostávala placebo - 1 ml fyziologického roztoku – bez účinné látky. Proteiny a malonyldialdehyd (MDA) v seru byly stanoveny 1. 8. a 17. den, denně byla stanovena proteinurie. Rozdíly v hladinách proteinů v seru byly statisticky nevýznamné ve všech měřeních, MDA vykazoval statisticky významný rozdíl 8. a 17. den pokusu ($p < 0.05$). Proteinurie, respektive celková ztráta bílkovin močí/den vykazuje statisticky významný rozdíl mezi léčenou a placebo skupinou v období 7. – 12. den, respektive 6. – 12. den experimentu ($p < 0.01$).

Výsledky naší práce ukazují, že carvedilol v podmínkách experimentálně navozeného solárního popáleninového syndromu chrání funkci ledvin, a to zřejmě dvojím způsobem: jednak zvyšuje průtok krve ledvinou a současně působí antioxidačním efektem.

Acknowledgements

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