

## Effects of Carvedilol and BL-443 on Kidney of Rats with Cyclosporine Nephropathy

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**Abstract.** Effects of cyclosporine A on kidneys of rats and the effects of carvedilol or BL-443 on kidneys of rats with cyclosporine nephropathy were studied. Male rats (Wistar) were divided into four groups ( $n = 7$ ). Three groups of rats were treated in single oral daily doses of 45 mg cyclosporine A/kg body weight to cause cyclosporine nephropathy. Two of the treated groups were then medicated either with carvedilol or BL-443 in single daily doses of 10 mg/kg b.w., and 1 ml doses of saline were given daily i.p. to the third group of rats. Animals were treated and medicated for 17 days. The rats of intact group had no treatment and medication. L-lactate dehydrogenase isoenzymes LD(1–4) in the kidney extracts were determined by polyacrylamide gel electrophoresis. Significant differences of LD(1–4) pattern in kidneys between intact rats and each of the three groups of rats with cyclosporine nephropathy were found by F-test and  $t$ -test ( $p < 0.05$ ). Treatment with cyclosporine A affected the LD(1–4) pattern in kidneys. On the other hand, no significant differences of LD(1–4) pattern in kidneys between rats with non-treated cyclosporine nephropathy and rats with cyclosporine nephropathy medicated with carvedilol or BL-443 were found.

**Key words:** Consupren — Carvedilol — Cyclosporine nephropathy — L-lactate dehydrogenase isoenzymes

### Introduction

Cyclosporine A (CyA), a potent immunosuppressant, has been used not only in transplantology but also in treatment of autoimmune diseases. However, nephrotoxicity and hepatotoxicity of CyA have been recorded (Nitta et al. 1993; Zimmerhackl

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et al. 1997). They depend strongly on the vehicle used with the preparation (Jiang and Acosta 1993). Nephrotoxic effects of two CyA preparations, Sandimmune (Sandoz, Switzerland) and Consupren (Galena, Czech Republic), are comparable (Bohdanecká et al. 1994). Increased production of reactive oxygen intermediates of oxidative stress and vasoconstriction is an indirect consequence of CyA effects in the tissues (Buetler et al. 2000). Oxidative stress seems to be in reverse proportion to the concentration of glutathione, and CyA-induced toxicity is reduced by ascorbic acid in primary rat hepatocyte culture (Wolf et al. 1997). Beta-blockers have been studied and administered in therapy of heart, kidney and other tissue diseases (Laser et al. 1996; Nečas et al. 1997). Carvedilol represents one of potent antihypertensives combining in single substance action of  $\alpha_1$ - and beta-adrenoreceptor antagonists (Rittinghausen 1988; Strein and Sponer 1990). The butyl ester of 4-[(2-hydroxy-3-isopropylamino) propoxy] phenylcarbamic acids (compound BL-443) shows beta-adrenolytic activity (Račanská et al. 1990). Lactate dehydrogenase (LD, L-lactate : NAD oxidoreductase, EC 1.1.1.27) is released from the culture of endothelial bovine cells by CyA action (Zoja et al. 1986). The LD release from cells into medium was also evaluated (Andrés et al. 2000) as a parameter of CyA cytotoxicity that was higher in the older of two hepatocyte cultures.

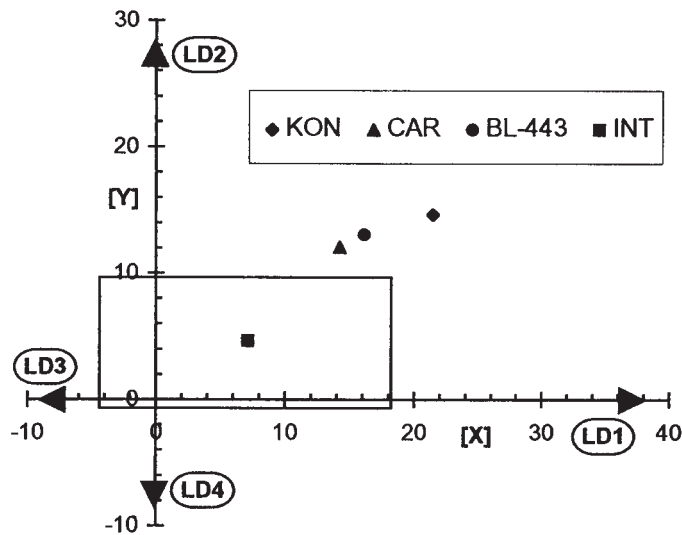
Five tetrameric LD isoenzymes: LD1 ( $H_4$ ), LD2 ( $HM_3$ ), LD3 ( $H_2M_2$ ), LD4 ( $H_3M$ ) and LD5 ( $M_4$ ) are formed after proteosynthesis of H, M polypeptides in most mammalian cells. LD isoenzymes in tissues are suggested as diagnostic aid in some studies (Yasminéh et al. 1978; Yasuda et al. 1989; Cobben et al. 1997; Gupta et al. 2000).

In this study, kidneys of healthy rats, rats with non-treated CyA nephropathy and rats with CyA nephropathy medicated with carvedilol or BL-443 were compared by LD(1–4) pattern to examine the effects of immunosuppressant CyA, beta blocker carvedilol and the drug BL-443 on rat kidneys.

## Materials and Methods

CyA (preparation Consupren<sup>®</sup>) was obtained from Galena (Opava, Czech Republic). Carvedilol substance, m.w. 404.49, was purchased from Boehringer (Mannheim, Germany). The compound BL-443 was kindly supplied by J. Csöllei from Pharmaceutical Faculty, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic.

Institutional guidelines for care and use of laboratory animals have been followed. Male Wistar rats weighting  $351 \pm 19$  g were divided into four groups ( $n = 7$ ), and were conventionally kept individually in glass metabolic cages. The rats of intact group (INT) obtained no treatment and medication. Three groups of rats (CAR, BL-443, KON) were treated for 17 d in single oral daily doses of 45 mg CyA/kg body weight to cause CyA nephropathy. Two of the treated groups were then medicated either with carvedilol or BL-443 in single daily doses of 10 mg/kg b.w., and 1 ml of saline was given in single i.p. daily doses to the third group of rats (KON). The animals were sacrificed by exsanguination on day 18 of experiment.



**Figure 1.** Mean LD(1–4) pattern in kidneys of intact rats (INT), rats with non-treated CyA nephropathy (KON) and rats with CyA nephropathy medicated with carvedilol (CAR) or BL-443 are expressed by points in the graph of the basic model SV4,LD(1–4). Position of a point is determined as the end point of resultant vector computed by summation of four constituent vectors, whose magnitudes reflect relative activities of LD(1–4) isoenzymes. The distribution of LD isoenzyme constituent vectors is shown. The point that represents mean LD(1–4) pattern in the kidneys of intact rats is equipped with a rectangle which corner points are defined by the equations:  $SV[\text{mean } x] \pm 2s_{[x]}$ ;  $SV[\text{mean } y] \pm 2s_{[y]}$ . The rectangle area contains the end point of the resultant vector whose LD pattern is in the reference interval for a healthy population ( $p < 0.05$ ).

One kidney of each animal was immediately transferred into liquid nitrogen and stored in freezer at  $-30^{\circ}\text{C}$ . Isoenzyme LD(1–4) pattern in the kidney extracts was determined by the conventional vertical polyacrylamide electrophoresis (Šalplachta and Nečas 2000). The following procedure was applied to evaluate experimental data. Relative activities of isoenzymes were converted to resultant vectors of basic model SV4,LD(1–4) under conditions stated earlier (Šalplachta 1997a,b). The basic model SV4,LD(1–4) uses a two-dimensional system of rectangular axes  $x$ ,  $y$  in the plane in which LD(1–4) isoenzymes are represented with four constituent vectors starting in the point of intersection of axes. Orientations of constituent vectors are shown in Fig. 1. Resultant vector is determined by summation of four constituent vectors so that the end point of a resultant vector represents its LD(1–4) pattern. The end point variables  $SV4[x;y]$  of the resultant vector were used to create the graphical presentation of the results and for statistical evaluation by two-tailed F-test and Student's  $t$ -test ( $p < 0.05$ ).

Results

We presumed that an evaluation of the effect of CyA on kidney of rats as well as the effects of carvedilol and BL-443 on rats with CyA nephropathy is possible by comparison of LD(1–4) pattern in kidneys of experimental rats. Mean LD(1–4) isoenzyme patterns in kidneys of four groups of rats are presented in Table 1.

**Table 1.** Mean isoenzyme LD(1–4) pattern in kidneys of intact rats (INT), rats with non-treated cyclosporine A nephropathy (KON) and rats with cyclosporine A nephropathy medicated with carvedilol or BL-443

groups <i>n</i> = 7	LD1 (%)	LD2 (%)	LD3 (%)	LD4 (%)	SV[x]	SV[y]
KON	37.1 ± 6.9	30.9 ± 1.8	15.6 ± 5.4	16.3 ± 2.1	21.5 ± 12.3**	14.6 ± 3.5**
CAR	32.7 ± 2.2	30.5 ± 1.3	18.4 ± 2.5	18.4 ± 1.7	14.2 ± 4.7**	12.1 ± 3.0**
BL-443	33.9 ± 2.6	30.7 ± 1.7	17.7 ± 1.8	17.7 ± 1.9	16.1 ± 4.2**	13.0 ± 3.2**
INT	29.1 ± 3.0	26.8 ± 0.7	21.9 ± 2.8	22.2 ± 2.1	7.1 ± 5.6	4.6 ± 2.8

Sum of relative activities of LD(1–4) isoenzymes is 100%. Data are presented as mean ± S.D. An end point of resultant vector is specified by co-ordinate variables SV[x], SV[y] in basic model SV4,LD(1–4). \*\* *p* < 0.05 – significantly different from intact rats.

Mean LD isoenzyme patterns are represented by end points of resultant vectors depicted in Fig. 1. Evaluation of mean data revealed the following. There was a clear increase in LD1 (+8%), LD2 (+4.1%) relative activity and a corresponding decrease in LD3 (–6.3%), LD4 (–5.9%) relative activity in the kidneys of rats treated with CyA. An increase in LD1 (+3.6%), LD2 (+3.7%) relative activity and a decrease in LD3 (–3.5%), LD4 (–3.8%) relative activity were found in the kidneys of rats treated with CyA and medicated with carvedilol. An increase in LD1 (+4.8%), LD2 (+3.9%) relative activity and a decrease in LD3 (–4.2%), LD4 (–4.5%) relative activity were found in the kidneys of rats treated with CyA and medicated with BL-443. Significant differences in LD(1–4) isoenzyme patterns in kidneys evaluated by variables SV[x], SV[y] between intact rats and each of the three groups of rats with cyclosporine nephropathy (KON, CAR, BL-443) were found by F-test and t-test (*p* < 0.05). Statistics sustained as non-significant the differences in LD(1–4) isoenzyme pattern in kidneys between the three groups of rats with CyA nephropathy (KON, CAR, BL-443). Data on LD(1–4) isoenzyme pattern in kidneys of rats medicated with carvedilol or BL-443 (CAR, BL-443) were found within the interval of LD(1–4) isoenzyme patterns of intact rats (INT) and rats with CyA nephropathy (KON). Relation  $INT \ll CAR \leq BL-443 \leq KON$  summarizes the differences in LD(1–4) pattern in kidneys between intact rats and each of the three groups of rats with cyclosporine nephropathy.

## Discussion

CyA nephrotoxicity is commonly exhibited by LD release from cells (Jiang and Acosta 1993; Nitta et al. 1993; Bohdanecká et al. 1994; Zimmerhackl et al. 1997; Buetler et al. 2000). We found that LD(1–4) pattern in kidneys of rats with CyA nephropathy differs from that in healthy rats. This makes possible to study the effects of drugs on rats with CyA nephropathy. However, medication with carvedilol or BL-443 did not show any significant impact on LD(1–4) pattern in kidneys of rats with CyA nephropathy. Further study is needed to explain whether an alteration of cell population and/or alteration of LD pattern in cell resulted in the increase of LD1, LD2 activity and a parallel decrease of LD3, LD4 activity in rat kidneys as a consequence of CyA treatment. CyA toxicity is partly given by an imbalance between antioxidative enzyme activities of catalase, and Mn- and Cu, Zn-superoxide dismutases (Andrés et al. 2000). We hypothesized that different sensitivity of cells to intracellular levels of peroxides and superoxide anion can result in alteration of the cell population.

The antihypertensive agent and beta blocker carvedilol protects the cells against reactive oxygen intermediates of oxidative stress (Yue et al. 1993). The drug shows a protective effect upon the cells of proximal renal tubule in the conditions of experimental alloxan diabetes, ischaemic-reperfusion renal damage and induced burn state of rats (Nečas et al. 1997; Bartošíková et al. 1997, 1998). However, negligible protective effects of carvedilol and newly tested drug BL-443 on rat kidneys with CyA nephropathy were found in our experiments.

The evaluation of LD pattern in tissues allows differentiation between the diseased and healthy part of population and it is used in the studies related to: muscular disorders in human (Yasminéh et al. 1978), pleural effusions of exudative and transudative origin (Cobben et al. 1997), liver diseases of cows (Yasuda et al. 1989) as well as in brain diseases caused by insecticides carbofuran and methyl parathion (Gupta et al. 2000). In conclusion, our study has found an alteration of LD(1–4) pattern both in kidneys of rats with non-treated CyA nephropathy and in kidneys of rats with CyA nephropathy medicated with carvedilol or BL-443.

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