Part II

Therapeutic perspectives

Chapter 5

Combined endothelin converting enzyme and neutral endopeptidase (ECE/NEP) inhibition has no anti-proteinuric and anti-glomerulosclerotic effect in 5/6 nephrectomized rats

A short communication

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Submitted

Abstract

Introduction Vaso-active peptide systems such as the endothelin system and the natriuretic peptide system are important pathways in the pathophysiology of renal and cardiovascular diseases. Early intervention with an ECE/NEP inhibitor appeared to be reno-protective. The aim of the study was to evaluate the reno-protective effects of the ECE/NEP inhibitor SLV306 in a rat model for advanced renal damage.

Methods and Results Six weeks after 5/6 nephrectomy, proteinuria averaged 123 ± 13 mg/day. Thereafter, rats were treated for 6 weeks with: vehicle (n=7); ECE/NEPi (30 and 200mg/kg/day, n=8 and n=10), and ACEi (lisinopril 2,5mg/kg/day, n=7). Proteinuria in the vehicle group further increased to 256 ± 36 mg/day. ECE/NEPi in both dosages had no effect on proteinuria (263 ± 47 and 389 ± 72 mg/day respectively, P= NS), while ACEi reduced proteinuria to 124 ± 19 mg/day ($P < 0.05$). In the vehicle group, glomerulosclerosis was 35 ± 7 AU. ECE/NEPi, at 30 and 200mg/kg/day, and ACEi had no significant effect on glomerulosclerosis (26 ± 7, 37 ± 8 and 15 ± 4 AU, respectively).

Conclusion These are the first preliminary data evaluating combined ECE/NEP inhibition in advanced renal damage after 5/6 nephrectomy. No reno-protective effects were observed, while ACEi was shown to be effective, demonstrating the responsiveness of the model.

Introduction

Inhibition of several vasoactive hormone systems is known to improve the outcome in chronic renal and cardiovascular diseases. For a substantial number of patients, proteinuria is not completely reduced by ACEi, while optimizing proteinuria reduction appears to improve renal and cardiovascular outcome even further[1;2]. Beside the RAAS, intervention in other hormone systems, like the endothelin and natriuretic peptide systems, has been proposed to lower proteinuria and therefore prevent further progression of renal damage[3-7].

The ECE/NEP inhibitor SLV306 showed beneficial renal effects in a diabetic model for renal damage[5], as well as cardiovascular protective effects in different animal models of heart failure[8]. However, these protective effects were established during the development of renal and/or cardiac failure. In the human situation, organ damage is most often already present before an intervention can be started. No data are available on reno-protective effects of ECE/NEP inhibition given in advanced renal failure, or in non-diabetic renal disease. The primary aim of the present study was to investigate the effects of the ECE/NEPi SLV306 in 5/6 nephrectomized rats on advanced renal damage. The ACEi lisinopril was used as positive control. We also tested, as a secondary aim, whether SLV306 had any effect on cardiac function in this model, since previous studies demonstrated impaired cardiac function to precipitate renal function loss[9].

Methods

At baseline, 40 male Wistar rats were subjected to 5/6 nephrectomy[17]. Six weeks thereafter, rats were randomly assigned to: vehicle (n=7); ECE/NEPi-30 (30mg/kg/day SLV-306; Solvay Pharmaceuticals GmbH, Hannover, Germany; n=8); ECE/NEPi-200 (200mg/kg/day SLV306, n=10), and ACEi (2.5mg/kg/day lisinopril; Sigma Chemical Co., St. Louis, MO, USA; n=7). The group ECE/NEPi-200 started with 100mg/kg/day SLV-306, which was switched after two weeks to 200mg/kg/day SLV-306 because proteinuria levels were still comparable to vehicle (204 ± 38 *versus* 203 ± 22 mg/day) while ACEi already caused a reduction in proteinuria (93 ± 11 mg/day). To be sure that the lack of effect of ECE/NEPi was not caused by non-responsiveness of the animals to antiproteinuric treatment, animals with a proven effect on ACEi were switched after 6 weeks to a 3 weeks treatment period with ECE/NEPi-30 (n=7).

Urinary protein concentration was determined using nephelometry (Dade Behring BNII, The Netherlands). Plasma renin activity was determined with an enzyme-kinetic method[18]. Creatinine clearance was determined using photometric determination with the Jaffe method (Ecoline Mega, DiaSys Diagnostic Systems GmbH, Holzheim, Deutschland). SBP was measured by the tail cuff method (IITC Life Sciences, Woodland Hills, CA, USA). Cardiac performance was measured by means of a pressure transducer catheter under isoflurane anesthesia (Micro-Tip 3French; Millar Instruments, Houston, TX, USA). The degree of focal glomerulosclerosis was semi-quantitatively assessed in PAS-stained renal sections[19]. Data were expressed as mean ± SEM. ANOVA and a Dunnett post hoc test were used to identify groups different from the vehicle. Statistical significance was accepted as $P < 0.05$.

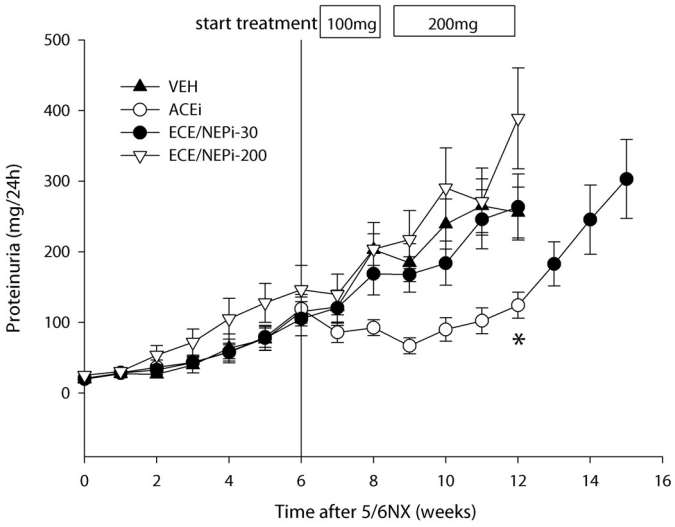

***Figure 1**. Effect of treatment on proteinuria after 5/6NX. ▲: VEH, ○: ACEi, ●: ECE/NEPi-30, ▽: ECE/NEPi-200, dose given in the upper bars. VEH, vehicle treated rats; ACEi, angiotensin converting enzyme inhibitor treated rats; ECE/NEP, endothelin converting enzyme/neutral endopeptidase inhibitor treated rats * : P< 0.05 versus VEH.*

Table 1. Effect treatment on renal characteristics

	Time point (weeks)	VEH	ACEi	ECE/NEPi-30	ECE/NEPi-200	ACEi-ECE/ NEPi-30
N		7	7	8	10	7
Creatinine clearance (ml/min/kg)	6	3.8 ± 0.7	3.3 ± 0.5	3.7 ± 0.5	3.6 ± 0.3	4.0 ± 0.3
	12	3.5 ± 0.4	3.7 ± 0.4	3.9 ± 0.4	2.1 ± 0.3 [ab]	4.3 ± 0.3
Renal blood flow (ml/min/kg)	12	4.0 ± 0.6	2.8 ± 0.1	2.5 ±0.3	2.7 ± 0.4	2.0 ± 0.4
Kidney weight (g)	12	3.07 ± 0.23	2.29 ± 0.15[a]	2.82 ± 0.22	3.23 ± 0.15	2.40 ± 0.15
FGS (AU)	12	35 ± 7	15 ± 4	26 ± 7	37 ± 8	22 ± 5
PRA (ngAI/ml/h)	12	3.7 ± 0.6	20.6 ± 5.0 [a]	4.5 ± 1.0	1.9 ± 0.4	8.6 ± 2.1

Data expressed as mean ± SEM. VEH, vehicle tratead rats; ACEi, angiotensin converting enzyme inhibitor treated rats; ECE/NEP, endothelin converting enzyme/neutral endopeptidase inhibitor treated rats; FGS, focal glomerulosclerosis; PRA, plasa renin activity. [a]: P< 0.05 *versus* vehicle, [b]: P< 0.05 versus randomization.

Table 2. Effect treatment on cardiac characteristics

	VEH	ACEi	ECE/NEPi-30	ECE/NEPi-200	ACEi-ECE/ NEPi-30
N	7	7	8	10	7
LVPSP (mmHg)	154 ± 9	120 ± 7[a]	154 ± 10	173 ± 8	117 ± 6[a]
LVEDP (mmHg)	11.7 ± 1.7	11.5 ± 1.1	12.1 ± 1.7	11.7 ± 0.8	6.3 ± 1.1[a]
$+dP/dt_{max}$ (s^{-1})	95 ± 5	107 ± 4	93 ± 5	94 ± 3	105 ± 3
$-dP/dt_{max}$ (s^{-1})	-84 ± 3	-92 ± 3	-92 ± 2	-89 ± 3	-90 ± 2
Heart weight (g)	1.57 ± 0.03	1.31 ± 0.06[a]	1.50 ± 0.05	1.60 ± 0.05	1.34 ± 0.0 [a]

Data in mean ± SEM. VEH, vehicle tratead rats; ACEi, angiotensin converting enzyme inhibitor treated rats; ECE/NEP, endothelin converting enzyme, neutral endopeptidase inhibitor treated rats. [a]: P< 0.05 *versus* vehicle.

Results

A dose dependent increase of the active metabolite of SLV306, KC12615, was measured in plasma: 30mg/kg/day: 67 ± 31 nM, 100mg/kg/day: 268 ± 67 nM, 200mg/kg/day: 454 ± 122 nM.

Proteinuria increased from 21 ± 1 to 123 ± 13 mg/day, 6 weeks after 5/6NX (figure 1). In vehicle, proteinuria further increased to 256 ± 36 mg/day. ECE/NEPi, at 30 and 200mg/kg/day, had no effect on proteinuria (263 ± 47 and 389 ± 72 mg/day, P= NS), while ACEi reduced proteinuria to 124 ± 19 mg/day (P< 0.05). After switching animals on ACEi to ECE/NEPi, proteinuria increased to 303 ± 56 mg/day.

Six weeks after 5/6NX, creatinine clearance was decreased from 6.6 ± 0.2 to 3.7 ± 0.2 ml/min/kg. In ECE/NEPi 200mg/kg/day, a further decrease in creatinine clearance was observed (P< 0.05), while no effect was seen in the ECE/NEPi 30/mg/kg, ACEi and vehicle (table 1). No significant effect of all treatment groups was observed on renal blood flow.

ECE/NEPi showed similar kidney weight compared to vehicle, while ACEi showed a significant lower kidney weight (table 1). ECE/NEPi, at doses of 30 and 200mg/kg/day, and ACEi, at a dose of 2.5 mg/kg/day, had no significant effect on glomerulosclerosis compared to vehicle. In the group that was as well treated with ACEi and switched to ECE/NEPi, also no significant reduction was observed.

The plasma renin activity (PRA) after 6 weeks treatment with ECE/NEPi, 30 and 200mg/kg/day, was comparable to vehicle, while in ACEi PRA was higher (P< 0.05, table 1). After switching from ACEi to ECE/NEPi, PRA was comparable to vehicle.

Systolic blood pressure (SBP) increased to 160 ± 4 mmHg 6 weeks after 5/6Nx. SBP further increased in the vehicle group to 174 ± 7 mmHg. ECE/NEPi, at 30 and 200mg/kg/day, did not affect SBP (159 ± 12 and 184 ± 8 mmHg, P= NS), while ACEi decreased SBP (124 ± 4 mmHg, P< 0.05, figure 2A). After switching from ACEi to ECE/NEPi, the antihypertensive effect of ACEi was significantly diminished by 25 mmHg compared to ACEi (figure 2B).

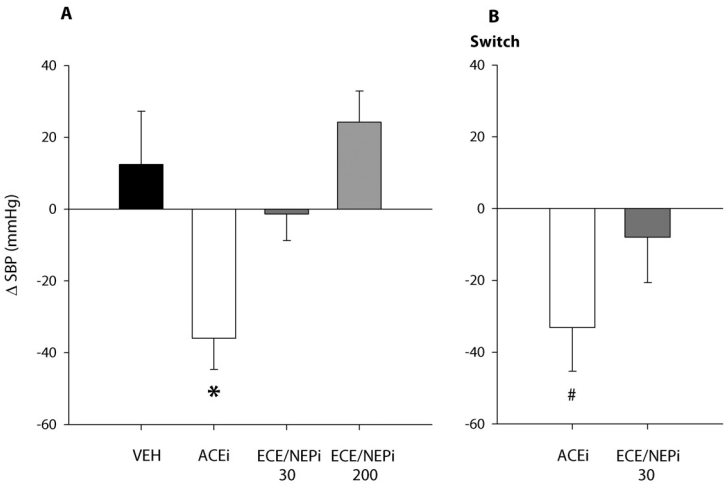

Figure 2. *Effect of treatment on systolic blood pressure. Panel A: Δ SBP between week 6 and 12, panel B: Δ SBP between week 6 and 12 for ACEi and between 12 and 15 after switching from ACEi to ECE/NEPi-30. VEH, vehicle tratead rats; ACEi, angiotensin converting enzyme inhibitor treated rats; ECE/NEP, endothelin converting enzyme/neutral endopeptidase inhibitor treated rats. * : P< 0.05 versus VEH, # : P< 0.05 versus ACEi treatment period.*

In all the treatment groups, cardiac left ventricular end diastolic pressure (LVEDP) was comparable to vehicle (table 2), except in the group which was switched from ACEi to ECE/NEPi. In the latter group a decrease to 6.3 ± 1.1 mmHg was observed ($P < 0.05$). In this group, left ventricular peak systolic pressure (LVPSP) was, at the end of the experiment, comparable to ACEi. Cardiac contractility ($+dp/dt_{max}$ and $-dp/dt_{max}$)did not improve under ECE/NEPi-and ACEi-therapy. ECE/NEPi, at 30 and 200mg/kg/day did not significantly affect heart weight, while in ACEi, heart weight was lower than in the vehicle ($P < 0.05$). After switching from ACEi to ECE/NEPi-30, this lower heart weight remained present.

Discussion

The objective of the study was to evaluate renal and cardio-hemodynamic effects of ECE/NEPi in advanced renal damage after 5/6Nx. We conclude that late intervention therapy with ECE/NEPi did not affect proteinuria and focal glomerulosclerosis. ACEi effectively reduced proteinuria but also did not significantly prevent focal glomerulosclerosis.

The endothelin system is activated after 5/6NX[10;11] and intervention in the endothelin system with endothelin receptor blockade prevented progression of renal failure in some experiments[3;12;13], although not in all[14]. Not much is known about ECE/NEPi in renal damage, although some beneficial effects have been shown in prevention of diabetic renal damage[5;6]. We could not confirm these findings in a late intervention protocol in advanced renal damage.

Beneficial cardiovascular effects have been shown in different animal models of heart failure[8]. In the time frame used, 30mg/kg/day SLV306 was ineffective on cardiovascular parameters, although after 5/6NX, the endothelin system is up regulated[3;11] and cardiac hypertrophy develops due to hypertrophy of cardiomyocytes, expansion of interstitial tissue, arteriolar thickening and diminished capillary supply[15;16]. Because these alterations in response to an activated endothelin system develop already early after 5/6NX, intervention with ECE/NEPi 6 weeks after 5/6NX might be too late. The discrepancy between the current finding and previous cardiovascular protection could be caused by an ineffective plasma concentration of the drug or unresponsiveness of the animals to antiproteinuric therapy. The doses we used should be effective. Previous studies showed that 30mg/kg/day SLV306 was an orally active dose in the rat[8]. In our experiments, this dose and higher doses (up to 200mg/kg/day) were not effective. However, the duration of treatment was shorter (6 weeks) in our experiments than that reported from other authors[5]. The lack of response could have been caused by unresponsiveness of the animal model. However, this appears to be unlikely, since when animals with a proven response to ACEi were switched to ECE/NEPi, the beneficial effects of ACEi on proteinurea were abrogated. Regarding the cardiac parameters, the beneficial effects of ACEi sustained under ECE/NEPi. This has to be interpreted with caution, because the initial values at the time of the switch were not measured.

Conclusion

These are some indications that the ECE/NEPi in advanced renal damage after 5/6 nephrectomy has no overt beneficial effects on proteinuria, focal glomerulosclerosis, blood pressure and cardiac structure and function. Due to the study limitations these data needs to be confirmed in further studies.

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