EXPERIMENTAL STUDY

Dual inhibition of angiotensin converting enzyme and neutral endopeptidase produces effective blood pressure control in spontaneously hypertensive rats

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Abstract

Background: **The synergistic effects of the combined ACE and NEP inhibition is based both on the blockade of angiotensin II synthesis and degradation of vasoactive peptides and NEP substrates (ANP, arginine, endothelial cells, guanylat cyclase etc.), including bradykinine and the natriuretic peptides, which contribute to vasodilatation, diuresis and improvement of myocardial function.**
Objectives: **This study was undertaken to asses the hypotensive effect of a dual ACE/NEP inhibitor (omapatrilat) in comparison to a NEP inhibitor (candoxatril) and ACE inhibitor (enalapril) in SHRS.**
Methods: **The study was performed in 130 male spontaneously hypertensive rats (SHRS) that were divided into 4 groups and treated orally by a gastric tube for 14 days according to the following dosage regimen: omapatrilat (40 mg/kg b.w./24 h); candoxatril (30 mg/kg b.w./24 h); enalapril (20 mg/kg b.w./ 24 h) and control (water). Systolic blood pressure values were determined at the beginning of the study by the tail-cuff pletysmographic method, at the 7th and 14th day of the treatment, as well as 14 days after the end of the drug administration. For evaluation of the effect of omapatrilat, candoxatril and enalapril on the investigated parameters (plasma atrial natriuretic peptide and serum ACE), 10 animals from the control group were sacrificed at the beginning of the study, and afterwards 10 animals from each group were also sacrificed on the 7th and 14th day of the treatment, as well as 14 days after the end of the drug administration (28th day).**
Results: **The dual ACE/NEP inhibitor, omapatrilat and the ACE inhibitor, enalapril lowered SBP more effectively than the NEP inhibitor, candoxatril at all time points of the experiment ($p<0.01$). Omapatrilat was slightly more effective than the enalapril treatment.**
Conclusions: **Two-week treatment with the dual ACE/NEP inhibitor omapatrilat caused a significant decrease of the SBP, inhibition of the serum ACE activity and increase of the plasma ANP values, and therefore it should be considered as a new potential therapeutic agent in blood pressure management *(Tab. 3, Fig. 2, Ref. 20)*.**
Key words: omapatrilat, candoxatril, enalapril, hypertension, inhibition, SHR.

Recent tools in the management of hypertension include several antihypertensive groups of drugs: diuretics, antiadrenergic drugs, antihypertensive vasodilators, renin-angiotensin-aldosteron system blockers (RAAS) and angiotensin II receptor antagonists. Perspective new drugs in the treatment of arterial hypertension are the combined or dual vasopeptidase inhibitors of angiotensin converting enzyme (ACE) and neutral endopeptidase (NEP).

The mechanism of action of ACE inhibitors (enalapril, lisinopril, captopril etc.) is explained with the inhibition of conversion of inactive angiotensin I to highly active angiotensin II (potent vasoconstrictor) thus changing the RAAS activity and inhibiting the biological effects of angiotensin II (increase of the blood pressure and aldosterone secretion, decrease of the rennin secretion and natriuresis, increase of the sympathetic nerve activity, cell proliferation and hypertrophy) (1).

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Neutral endopeptidase (NEP) catalyzes the degradation of vasodilator peptides (ANP, BNP, CNP, adrenomedullin, substance R and bradykinin) thus potentiating the effects of vasoconstrictor peptides (endothelin-1 and angiotensin II). NEP also takes part in the metabolism of kinine peptides, chemotaxic peptide, encephalines and amiloid beta-peptide (2). It's interesting to point out that in the presence of ACE inhibition, NEP takes a dominant role in metabolism of bradykinin. Experimental studies suggest that the reduction of ischemia and perfusion impairments after NEP inhibition are mediated through kinine mediators (3). Due to their vasodilatator and protective properties, NEP inhibitors (candoxatril, ecadotril, tiorphan) provide a basis for development of a new therapeutic approach for potentiating the natriuretic peptide system by the selective inhibition of NEP (4).

There is a complex interaction between the ACE activity and NEP activity. The synergistic effects of the combined ACE and NEP inhibition is based both on the blockade of angiotensin II synthesis and degradation of vasoactive peptides and NEP substrates (ANP, arginine, endothelial cells, guanylat cyclase etc.), including bradykinine and the natriuretic peptides, which contribute to vasodilatation, diuresis and improvement of myocardial function (5). Thus, the development of the dual vasopeptidase inhibitors (omapatrilat, sampatrilat, fasidotril) is an attractive therapeutic approach in the treatment of hypertension (6). The blockade of ACE and NEP activity can be achieved by concomitant administration of substances that act on these enzymes separately or by application of a single substance that exhibits a dual inhibition at a same time (7).

Omapatrilat is a potent orally active dual ACE/NEP inhibitor, which causes a long-lasting hypotensive effect in animal models of hypertension in comparison to the effects that occur after a selective inhibition of only one of these two enzymes (8). The potent dual ACE/NEP inhibitors could be efficient and of great interest in the therapy of hypertensive syndromes. This study was undertaken to asses the hypotensive effect of a dual ACE/NEP inhibitor (omapatrilat) in comparison to a NEP inhibitor (candoxatril) and ACE inhibitor (enalapril) in SHRS.

Material and methods

Experimental design

The study was performed in 130 male spontaneously hypertensive rats (SHRS), weighing from 200 g to 300 g (16-week old) obtained from the Animal Facility of the Department of Preclinical and Clinical Pharmacology and Toxicology, Medical Faculty, Skopje. Animals were kept in cages, maintained under controlled light and temperature conditions, fed a normal rat chow and had a free access to tap water. The animals were divided into 4 groups and treated according to the following dosage regimen:

– 1st group (n=30): omapatrilat (40 mg/kg b.w./24 h)
– 2nd group (n=30): candoxatril (30 mg/kg b.w./24 h)
– 3rd group (n=30): enalapril (20 mg/kg b.w./24 h)
– 4th group (n=40): control (water)

The drugs were orally administered by a gastric tube for 14 days, once in the morning. Baseline systolic blood pressure (SBP) values were determined at the beginning of the study by the tail-cuff pletysmographic method. SBP was also measured at the 7th and 14th day of the treatment, as well as 14 days after the end of the drug administration. For evaluation of the effect of omapatrilat, candoxatril and enalapril on the investigated parameters (plasma atrial natriuretic peptide and serum ACE), 10 animals from the control group were sacrificed at the beginning of the study, and afterwards 10 animals from each group were also sacrificed on the 7th and 14th day of the treatment, as well as 14 days after the end of the drug administration (28th day). The blood of the anesthetized rats was withdrawn from the abdominal aorta (6–8 ml) and collected into tubes with EDTA.

Blood pressure measurement

The SBP was measured in conscious animals by tail-cuff plethysmographic method (IITC, Life Science, California, USA). Animals were maintained at a temperature of 26±1 °C during blood pressure recording sessions and trained to stay in restraining holders for 1 week before starting the experiment. Recordings were taken once before drug treatment and at weekly intervals within 3 hours after the morning dose. To validate the tail-cuff method for blood pressure (BP) measurement, four rats were implanted with femoral arteries catheters and underwent direct BP measurement method. The mean value of direct systolic blood pressure (SBP) compared with the mean value of indirect measurement showed correlation of 90 %.

Determination of angiotensin converting enzyme (ACE) activity in serum

A sensitive photometric procedure was used for the assay of ACE in serum. Serum (10ul) was incubated for 30 minutes with hippuryl-glycyl-glycine. After a deproteinization, the liberated glycyl-glycine is derivatized with a borate-buffered (pH 9.3) trinitrobenzenesulfonate sodium (60 mmol/l) to form trinitrophenyl-glycylglycine, the absorbance of which is read at 450 nm versus a serum blank. The calibration curve was in the concentration range of 67–1333 ACE U/L (one unit of ACE activity is defined as the amount of enzyme required to release 1 umol of hippuric acid per minute per liter of serum) (9). The photometric measurements were performed with Wallac 1420 VICTOR 2.

Plasma atrial natriuretic peptide (ANP) concentration

Atrial natriuretic peptide in plasma was determined with a commercially available RIA kit (S-2039 ANP (I^{125}) assay system) supplied from Peninsula Laboratories Inc, USA, that offers measurment of ANP in the range of 0.1–64 pg/tube. The extraction was made by the use of Sep-Pak C18 cartridge, while the elution was performed with 60 % acetonitrile containing 1 % trifluoroacetic acid (TFA, HPLC Grade).

Statistical analysis

Statistical evaluation of the results was performed using the computer statistical programme Statistica for Windows 5.0. Results were expressed as means±SD. Comparisons were made using the Student „t" test and one way analysis of variance

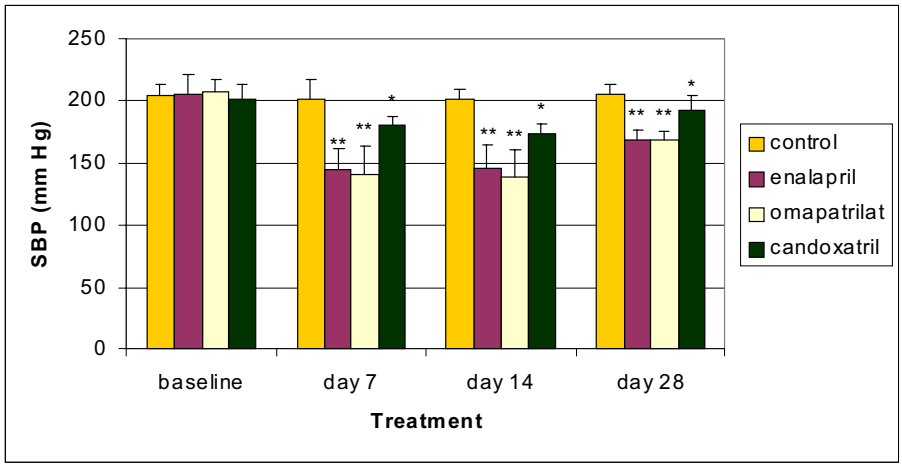

Fig. 1. The effect of enalapril, omapatrilat and candoxatril treatment on the systolic blood pressure (SBP) at different time points vs baseline levels. ** p<0.01; * p<0.05

ANOVA. Correlations between systolic blood pressure and ACE activity as well as ANP values were expreseed by Pearson coefficient of correlation (r). A p value <0.05 was considered to indicate a statistical significance.

Results

Our results show that drug treatments (enalapril, omapatrilat, candoxatril), decreased SBP levels in SHRs (Tab. 1, Fig. 1). The decrease was statistically significant after 7 and 14 days of the treatment, as well as 14 days after the end of the drugs administration (28th day) in comparison to the baseline SBP levels ($p<0.01$). On the other hand no statistical significance ($p>0.05$) was found between the values of SBP determined in day 14 compared with day 7 in enalapril and omapatrilat treated SHRs.

The dual ACE/NEP inhibitor, omapatrilat and the ACE inhibitor, enalapril lowered SBP more effectively than the NEP inhibitor, candoxatril at all time points of the experiment ($p<0.01$). Omapatrilat was slightly more effective than the enalapril treatment.

Two-week treatments with omapatrilat and particularly with enalpril, produced significant decrease in ACE activity in serum, measured after 7 and 14 days from the beginning of the drugs administration, which was maintained throughout 28th day of the study comparing to baseline values ($p<0.01$) (Tab. 2). In our experiment, there couldn't be found a statistically significant difference in ACE activity in serum at all time points, in candoxatril treated SHRs ($p>0.05$).

After comparing the values of ACE activity in serum in the omapatrilat treated SHRs at all time points of the experiment

Tab. 1. The effect of enalapril, omapatrilat and candoxatril treatment on the systolic blood pressure (mmHg) at different time points vs baseline levels.

Treatment	Baseline	Day 7	Day 14	Day 28
Control	203.5±10.12	201.2±15.32	202±8.35	204.6±9.28
Enalapril	205.28±15.95	145.29±16.63**	146±19.55**	169.17±7.36**
Omapatrilat	208.06±9.26	140.63±22.94**	139±21.83**	168.23±7.4**
Candoxatril	201.39±12.58	180.28±7.37*	173.33±8.35*	191.67±12.91*

** p<0.01; * p<0.05

Tab. 2. The effect of enalapril, omapatrilat and candoxatril treatment on the serum ACE activity (U/L) at different time points vs baseline levels.

Treatment	Baseline	Day 7	Day 14	Day 28
Control	95.6±7.79	97.5±6.58	94.3±8.82	96.2±6.46
Enalapril	97.5±7.25	48.52±8.34**	37.05±6.67**	70.15±9.05**
Omapatrilat	94.3±8.67	67.0±6.20**	57.38±7.39**	74.84±7.80*
Candoxatril	96.2±6.49	89.66±8.11	90.90±8.05	97.70±6.91

** p<0.01; * p<0.05

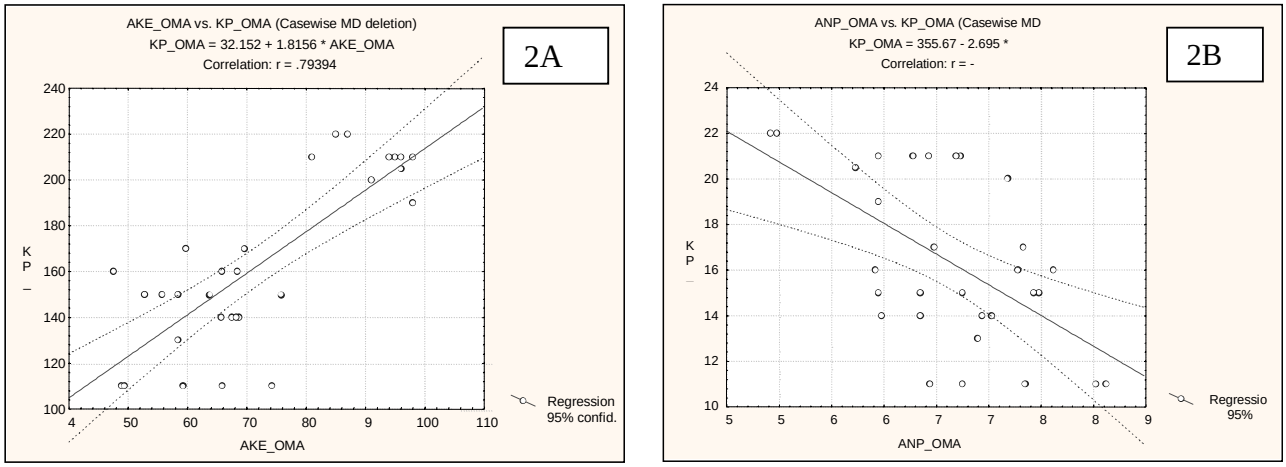

Fig. 2. Correlation between systolic blood pressure and: serum ACE activity (Fig. 2A) and plasma ANP concentrations (Fig. 2B) during the treatment with omapatrilat.

with the SBP levels, a statistically significant positive correlation was established (r=0.85) (Fig. 2A).

As shown in Table 3 plasma ANP concentrations obtained from the candoxatril tretated SHRs were increased during the all study period comparing to the control values measured at the beginning of the study, in particular after 14 days from the beginning of candoxatril administration when we measured statistically significant increase of plasma ANP concentrations compared with the control group ($p<0.01$). Slight increase ($p>0.05$) in plasma ANP concentrations was found in omapatrilat treated SHRS after 7 days from the beginning of the candoxatril administration. On the other hand we measured statistically significant increase of plasma ANP concentrations ($p<0.05$) after 14 days of omapatrilat treatment.

Contrary, enalapril had no effect on plasma ANP concentrations during the all study periods comparing to the baseline levels ($p>0.05$).

Comparing the values of plasma ANP concentrations in omapatrilat treated SHRs during the all time points of the experiment with the SBP levels, a significant negative correaltion was determined (r=-0.57) (Fig. 2B).

Discussion

Omapatrilat, a mercaptoacyl-based fused dipeptide mimetic, is the most developed vasopeptidase inhibitor used in the management of hypertension. It has balanced inhibitory effects on neutral endopeptidase and angiotensin converting enzyme (10). The end result is blockade of angiotensin II formation and inhibition of the catabolism of vasodilatatory hormones, such as natriuretic peptides, bradikynin and adrenomedullin. Some of the pharmacologic effects include vasodilatation, natriuresis and diuresis, witch may be beneficial in the management of various cardiovascular diseases, such as hypertension (11). In the present study, we have shown that two-weeks treatment with omapatrilat exerted superior hypotensive effect than enalapril and candoxatril treatment in SHRs. Similar results were observed in several experiments where omapatrilat was administered for a longer period of time in various laboratory species (12, 13, 14).

In our study, omapatrilat caused a significant decrease of the serum ACE activity that was most expressed after 14-teen days of the treatment. The inhibition of the ACE activity caused by omapatrilat (39 %) was lower in comparison to the ACE inhibition produced by enalapril (63 %), whereas candoxatril had no effect on the ACE inhibition. These results confirm the mechanism of action of the investigated drugs, and correlated with the decrease of the SBP, and correspond to the findings of other studies (15, 16).

The degree of NEP inhibition was evaluated through determination of plasma ANP concentrations in SHRs. The obtained results show that the most potent NEP inhibitor is candoxatril.

Tab. 3. The effect of enalapril, omapatrilat and candoxatril treatment on the plasma ANP concentrations (pmol/L) at different time points vs baseline levels.

Treatment	Baseline	Day 7	Day 14	Day 28
Control	65.3±6.29	67.2±7.68	66.8±8.53	64.3±7.91
Enalapril	66.14±4.13	61.74±8.17	59.37±11.11	62.20±10.02
Omapatrilat	65.84±7.36	72.74±6.02	75.73±7.03*	69.59±8.60
Candoxatril	63.50±8.36	75.02±5.32*	79.14±6.42**	71.04±5.79*

** $p<0.01$; * $p<0.05$

Omapatrilat caused a less pronounced NEP inhibition, i.e. higher ANP values, while enalapril didn't cause any changes on these parameter. The correlation between the SBP and the degree of NEP inhibition confirm the mechanism of action of candoxatril and omapatrilat that is accomplished through a complete or partial NEP inhibition, respectively.

Chronic treatment with NEP inhibitors increases the effects of ANP and decreases blood pressure in hypertension. Effects on the blood pressure due to the selective endopeptidase inhibition depend on the relative effects of vasodilator (including ANP) and vasoconstrictor (RAA and sympathicus) systems (17).

In two large multicentric studies: OPERA (Omapatrilat in Persons with Enhanced Risk of Atherosclerotic Events, Stage I Isolated Systolic Hypertension, Placebo Controlled) and OCTAVE (Omapatrilat Cardiovascular Treatment Assessment Versus Enalapril) the combined vasopeptidase inhibitor omapatrilat has confirmed its efficacy in patients with moderate hypertension. There are other ongoing clinical studies, where beside the proven efficacy of omapatrilat in lowering the blood pressure, its safety and tolerability have also been investigated. In order to replace or continue the traditional therapeutic concept, the new cardiovascular drugs should manifest an appropriate therapeutic action and acceptable tolerability and safety. The dual vasopeptidase inhibitors have demonstrated their efficacy in the treatment of arterial hypertension and chronic heart failure, even in conditions associated with diabetes, endothelial dysfunction or renal insufficiency (18, 19, 20).

We can conclude that a two-week treatment with the dual ACE/NEP inhibitor omapatrilat caused a significant decrease of the SBP, inhibition of the serum ACE activity and increase of the plasma ANP values, and therefore it should be considered as a new potential therapeutic agent in blood pressure management.

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