

# THE EFFECT OF FOSINOPRIL AND LERCANIDIPINE ON OXIDATIVE STRESS IN ELDERLY PATIENTS WITH ISOLATED SYSTOLIC HYPERTENSION

Ioana Dana Alexa, Adrian Covic, Florentina Palade, Larisa Panaghiu, Paul Gusbeth-Tatomir, Gabriel Ungureanu

## REZUMAT

**Obiective:** Urmărirea efectului tratamentului cu inhibitori ai enzimei de conversie ai angiotensinei (IECA) (Fosinopril) și cu blocanți ai canalelor de calciu (BCC) (Lercanidipină) asupra stress-ului oxidativ (factor pro-aterogenic) la pacienții vârstnici cu hipertensiune arterială sistolică izolată. **Metode:** Am efectuat un studiu prospectiv ce a cuprins 128 de pacienți cu vârsta  $\geq 65$  ani, care au fost internați în clinica noastră în ultimul an cu diagnosticul de hipertensiune arterială sistolică izolată ( $TA_s \geq 160$  mmHg și  $TA_d < 90$  mmHg). Pacienții au fost împărțiți în 3 loturi: lotul I (N = 43), care a primit tratament cu Fosinopril, 10 mg/zi, lotul II (N = 45), la care s-a administrat Lercanidipină, 10 mg/zi și lotul III (N = 40), care a primit ambele medicamente. Markerii stress-ului oxidativ urmăriti au fost: glutathionul (GSH), glutathion-peroxidaza (GSH-Px), superoxid-dismutaza (SOD) și peroxizii lipidici (PL). Determinările s-au efectuat la intrarea în studiu, la 6 și la 12 săptămâni de la începerea tratamentului. **Rezultate:** Cele trei loturi au fost omogene din punct de vedere a parametrilor demografici și a valorilor TA. Tratamentul cu IECA a determinat creșteri semnificative ale GSH și GSH-Px: GSH a crescut de la  $1,56 \pm 0,09$  la  $2,04 \pm 0,13$  (+ 25%) după 6 săptămâni și la  $2,68 \pm 0,17$  (+ 56%) după 12 săptămâni; GSH-Px ( $\mu\text{mol GSSG/ml/min}$ ) a crescut de la  $0,167 \pm 0,036$  la  $0,221 \pm 0,039$  (+ 30%) și respectiv la  $0,304 \pm 0,023$  (+ 75%). Tratamentul cu BCC a indus creșterea semnificativă a activității SOD (U/Ht), de la  $0,338 \pm 0,009$  la  $0,551 \pm 0,012$  (+ 48%) după 6 săptămâni și la  $0,724 \pm 0,005$  (+ 70%) după 12 săptămâni. Asocierea celor două medicamente a indus creșterea tuturor celor trei antioxidanți: GSH ( $\mu\text{g/ml}$ ) de la  $1,58 \pm 0,008$  la  $2,385 \pm 0,14$  (+ 50%) după 6 săptămâni și la  $2,68 \pm 0,17$  (+ 62%) după 12 săptămâni, GSH-Px ( $\mu\text{mol GSSG/ml/min}$ ) de la  $0,148 \pm 0,031$  la  $0,224 \pm 0,045$  (+ 52%) și la  $0,298 \pm 0,018$  (+ 78%), SOD (U/Ht) de la  $0,402 \pm 0,011$  la  $0,590 \pm 0,009$  (+ 49%) și la  $0,776 \pm 0,008$  (+ 72%); în plus am înregistrat o scădere semnificativă a valorilor serice ale PL ( $\mu\text{mol MDA/ml}$ ) de la  $3,26 \pm 0,02$  la  $2,64 \pm 0,036$  (- 49%) după 6 săptămâni și la  $1,96 \pm 0,028$  (- 64%) după 12 săptămâni de tratament. **Concluzii:** Rezultatele noastre confirmă ipoteza conform căreia tratamentul cu IECA și BCC determină creșterea activității antioxidante, studiul nostru fiind unul din puținele care au studiat acest efect la persoanele vârstnice. Mecanismul prin care se realizează aceste efecte este incomplet cunoscut. **Cuvinte cheie:** hipertensiune arterială, vârstnic, IECA, BCC, stress oxidativ

## ABSTRACT

**Background:** High oxidative stress appears to be related to high cardiovascular risk particularly in populations like elderly patients with isolated systolic hypertension (ISH). Few data are available on the effect of angiotensin-converting enzyme inhibitors (ACEI) and calcium-channel blockers (CCB) in this population. We aimed to examine the effect of treatment with fosinopril and/or lercanidipine on several parameters of oxidative stress as proatherogenic factors in elderly patients with ISH. **Methods:** A prospective study of 168 patients aged  $\geq 65$  years admitted in our clinic in 2003 for ISH (systolic BP  $\geq 160$  mmHg and diastolic BP  $< 90$  mmHg), was conducted. Patients were divided into four groups: Group I (N = 43) treated with Fosinopril, 10 mg/day, Group II (N = 45) treated with Lercanidipine, 10 mg/day, Group III (N = 40) treated with both drugs and Group IV (N = 40), the control group treated with diuretics. Markers of oxidative stress (glutathione - GSH, glutathione-peroxidase - GSH-Px, superoxid dismutase - SOD and lipid peroxides - LP) were studied during the antihypertensive treatment. Assessment was performed at baseline, + 6, +12 weeks. **Results:** There was no difference in demographics and in BP levels between the four groups. Fosinopril significantly increased the GSH complex: GSH ( $\mu\text{g/ml}$ ) from  $1.56 \pm 0.09$  to  $2.04 \pm 0.13$  (+ 25%) at 6 weeks and to  $2.68 \pm 0.17$  (+ 56%) at 12 weeks and GSH-Px ( $\mu\text{mol GSSG/ml/min}$ ) from  $0.167 \pm 0.036$  to  $0.221 \pm 0.039$  (+ 30%) at 6 weeks and to  $0.304 \pm 0.023$  (+ 75%) at 12 weeks. Lercanidipine significantly increased SOD activity (U/Ht) from  $0.338 \pm 0.009$  to  $0.551 \pm 0.012$  (+ 48%) at 6 weeks and to  $0.724 \pm 0.005$  (+ 70%) at 12 weeks. The association of both drugs increased all three antioxidants: GSH ( $\mu\text{g/ml}$ ) from  $1.58 \pm 0.008$  to  $2.385 \pm 0.14$  (+ 50%) at 6 weeks and to  $2.68 \pm 0.17$  (+ 62%) at 12 weeks, GSH-Px ( $\mu\text{mol GSSG/ml/min}$ ) from  $0.148 \pm 0.031$  to  $0.224 \pm 0.045$  (+ 52%) at 6 weeks and to  $0.298 \pm 0.018$  (+ 78%) at 12 weeks, SOD (U/Ht) from  $0.402 \pm 0.011$  to  $0.590 \pm 0.009$  (+ 49%) at 6 weeks and to  $0.776 \pm 0.008$  (+ 72%) at 12 weeks and decreased serum level of LP ( $\mu\text{mol MDA/ml}$ ) from  $3.26 \pm 0.02$  to  $2.64 \pm 0.036$  (- 49%) at 6 weeks and to  $1.96 \pm 0.028$  (- 64%) at 12 weeks. These results suggested the additive effect of the association of ACEI and CCB. There were no significant modifications of oxidative stress markers in the control group. **Conclusions:** Our results support the hypothesis that fosinopril and lercanidipine increase antioxidant activity in elderly patients, our study being one of the few that studied this effect in elderly. The mechanisms underlying these effects remain unknown

**Key Words:** hypertension, elderly, ACEI, CCB, oxidative stress

Department of Internal Medicine-Nephrology, Parhon University Hospital, University of Medicine and Pharmacy Gr.T. Popa Iasi, Romania

Correspondence to:  
Prof.dr. Adrian Covic, MD, PhD, Nephrology Clinic and Dialysis and Transplantation Center, C.I. Parhon University Hospital, Iasi, 6600, Romania,  
Fax: +40-232-210940, Email: acovic@xnet.ro

Received for publication: Jul. 8, 2005. Revised: Oct. 3, 2005.

## **INTRODUCTION**

The prevalence of hypertension in the elderly is clearly increasing, currently reaching a figure of 35% to 45%.<sup>1</sup> Apart from the total increase in subjects aged > 65 years, a number of additional factors are responsible for the growth of the elderly hypertensive population, particularly the problem of definition and the specific metabolic features associated with old age.<sup>2</sup> Screening studies have revealed that increasing age is more frequently accompanied by isolated systolic hypertension (ISH).<sup>3,4</sup> Many studies have stressed the need for appropriate treatment of ISH in order to reduce cardiovascular and cerebrovascular risk.<sup>5-8</sup>

Oxidative stress is the result of the imbalance between pro-oxidant factors (reactive oxygen species – ROS, lipid peroxides – LP) and antioxidant factors. The free radical theory of aging considers that senescence-related loss of function is caused by the accumulation of damage inflicted on biomolecules by ROS and LP. The “oxidative-modification hypothesis” of atherosclerosis (ATS) considers that atherogenesis is initiated by oxidation of the lipids.<sup>9,10</sup>

Lipid peroxidation is a process initiated and maintained by ROS, generated by the atherogenic cells themselves. The endothelial aggression in ATS is accompanied by endothelial dysfunction, which is due to the toxic effects of free radicals that influence the ATS process at different levels and by several mechanisms.<sup>10,11</sup>

The neutralization of these toxic effects is performed by the individual antioxidant status, which is influenced by environmental factors as well as by genetic determinants. The antioxidant therapy, controversial at the moment, represents an associated therapy in endothelial dysfunction in ATS.

The aim of the study was to investigate the effects of ACEI (fosinopril) and CCB (lercanidipine) on oxidative stress in elderly patients with ISH, considering that these patients are more exposed to sudden death, myocardial infarction, renal dysfunction and stroke.

## **SUBJECT AND METHODS**

A prospective study of 168 patients aged  $\geq 65$  years admitted in our clinic between 01.01.2003 and 31.12.2003 for ISH (systolic BP  $\geq 160$  mm Hg and diastolic BP  $< 90$  mm Hg), was conducted. Patients were blindly randomized into four groups: Group I of 43 patients treated only with an ACEI (fosinopril, 10 mg o. d.), Group II of 45 patients treated only with a CCB (lercanidipine, 10 mg o. d.), Group III of 40

patients treated with both drugs and Group IV (N = 40), the control group, treated only with a diuretic (hydrochlorothiazide, 25 mg o.d. or furosemide, 40 mg o.d.). All patients were on a low salt diet and adequate physical activity.

Blood pressure values were based on the average of three sphygmomanometric measurements obtained after 5 min of rest, through the use of the first and the fifth Korotkoff sounds to identify systolic and diastolic values, respectively.

Admission criteria in the study were:

- informed consent;
- patients with ISH without any other previous or concomitant antihypertensive treatment;
- patients with no other concomitant diseases which could increase oxidative stress: diabetes mellitus, cirrhosis, renal disease, chronic obstructive pulmonary disease, neurological disorders.

Exclusion criteria from the study were:

- significant renal failure defined as serum creatinine  $> 1.4$  mg/100 ml;
- acquired renal insufficiency after therapy with fosinopril (increase of serum creatinine with 30% compared to baseline after 72 h of treatment);
- altered hepatic function (ALT and AST twice the upper normal values);
- secondary hypertension;
- malignant hypertension;
- myocardial infarction, revascularization or stroke within the previous 6 months;
- contraindications or hypersensitivity to the drugs employed in the study.

Two patients from Group I and five patients from group III were lost from follow up. Therefore 161 patients were finally included in the study analysis. There were no significant differences in the entire population.

Patients were seen at a screening visit, at which a complete medical history, physical examination and standard laboratory examinations were obtained. If they were under antihypertensive treatment, they were seen after a 15-day washout period. They were randomly assigned to one of the four groups. Follow-up visits were made at 3 and 6 weeks. At each visit, BP was measured as for the baseline visit.

Oxidative stress was evaluated at baseline and after 6 and 12 weeks of antihypertensive treatment by measuring the following markers:

- the antioxidant enzymatic system represented by glutathione (GSH), glutathione-peroxidase (GSH-Px), and superoxid dismutase (SOD) as major scavengers in lipid peroxidation;

- lipid peroxides (LP) as markers of pro-oxidative stress, toxic compounds that induce endothelial damage, increase peripheral vasoconstriction and thromboxane synthesis.

GSH levels were measured spectrophotometrically using the method described by Ellman; GSH-Px levels were measured spectrophotometrically using the method described by Flohe measurement of SOD activity was performed by a colorimetric method in which formazan production was measured spectrophotometrically at 560 nm using the method described by Sun; LP levels were measured using the method described by Yagi, based on the spectrophotometric determination of malondialdehyde (MDA), which is the result of the reaction between PL with thiobarbituric acid.<sup>12-15</sup> All measurements were performed on total fresh blood drawn on heparin after 12 hours of fasting.

Results were statistically processed using SPSS 12.0 software package. The comparison between the four groups of change from baseline measurements at planned visits was made by means of Student's paired and unpaired „t” test. Results were expressed as mean values  $\pm$  SD. A significant difference was considered at  $p < 0.05$ .

## RESULTS

The demographic structure of the four groups is represented in Table 1.

**Table 1.** Demographic parameters in the three groups. The results are expressed as mean values  $\pm$  SD; \* =  $p < 0.05$ .

Parameter	Group I (N = 43)	Group II (N = 45)	Group III (N = 40)	Group IV (N = 40)
<b>Age</b>	72 $\pm$ 1.2	70 $\pm$ 1.4	71 $\pm$ 1.1	70 $\pm$ 1.1 (ns)
<b>Sex (M/F)</b>	20/23	21/24	18/22	19/21 (ns)
<b>SBP (mmHg)</b>				
Baseline	179 $\pm$ 11	172 $\pm$ 7	180 $\pm$ 5	177 $\pm$ 4
+ 6 w	147 $\pm$ 9* (-18%)	138 $\pm$ 5* (-20%)	130 $\pm$ 2* (-28%)	147 $\pm$ 5* (-18%)
+ 12 w	135 $\pm$ 6* (-25%)	132 $\pm$ 6* (-24%)	125 $\pm$ 3* (-31%)	145 $\pm$ 4* (-19%)
<b>DBP (mmHg)</b>				
Baseline	82 $\pm$ 7	80 $\pm$ 3	79 $\pm$ 3	77 $\pm$ 4
+ 6 w	80 $\pm$ 4 (ns)	80 $\pm$ 2 (ns)	75 $\pm$ 4 (-10%)	78 $\pm$ 2 (ns)
+ 12 w	81 $\pm$ 3 (ns)	79 $\pm$ 2 (ns)	74 $\pm$ 2 (-11%)	77 $\pm$ 2 (ns)
<b>HR (beats/min)</b>	76 $\pm$ 9	74 $\pm$ 4	75 $\pm$ 6	74 $\pm$ 2
<b>Smokers</b>	9.8%	11.2%	10.4%	10.6%
<b>Ex-smokers</b>	16.3%	14.5%	14.9%	16.1%

As shown in Table 1, patients were often older than 70 years. SBP was on average quite high, i.e. close to 180 mm Hg. All patients' other characteristics were well matched between the four groups. We obtained very good control of BP after 6 and 12 weeks of treatment in all four groups, especially in group III where the

association ACEI + CCB proved very efficient due to a synergic effect of the two drugs.

The parameters of oxidative stress in Group I are presented in Table 2.

**Table 2** Oxidative stress in patients treated with ACEI (group I). The results are expressed as mean values  $\pm$  SD; \* =  $p < 0.05$ .

Parameter	Initial values	After 6 weeks	After 12 weeks
GSH ( $\mu$ g/ml)	1.56 $\pm$ 0.09	2.04 $\pm$ 0.13* (+ 25%)	2.68 $\pm$ 0.17* (+ 56%)
GSH-Px ( $\mu$ mol GSSG/ ml/min)	0.167 $\pm$ 0.036	0.221 $\pm$ 0.039* (+30%)	0.304 $\pm$ 0.023* (+75%)
SOD (U/Ht)	0.447 $\pm$ 0.013	0.541 $\pm$ 0.007* (+ 25%)	0.568 $\pm$ 0.01* (+ 40%)
LP ( $\mu$ mol MDA/ml)	2.82 $\pm$ 0.06	2.46 $\pm$ 0.042 (- 13%)	2.23 $\pm$ 0.034* (- 20%)

The parameters of oxidative stress in Group II are presented in Table 3.

**Table 3.** Oxidative stress in patients treated with CCB (group II). The results are expressed as mean values  $\pm$  SD; \* =  $p < 0.05$ .

Parameter	Initial values	After 6 weeks	After 12 weeks
GSH ( $\mu$ g/ml)	1.68 $\pm$ 0.11	1.94 $\pm$ 0.16* (+ 22%)	2.28 $\pm$ 0.09* (+ 49%)
GSH-Px ( $\mu$ mol GSSG/ ml/min)	0.186 $\pm$ 0.024	0.212 $\pm$ 0.017* (+ 23%)	0.278 $\pm$ 0.009* (+ 49%)
SOD (U/Ht)	0.388 $\pm$ 0.009	0.551 $\pm$ 0.012* (+ 48%)	0.724 $\pm$ 0.005* (+70%)
LP ( $\mu$ mol MDA/ml)	2.76 $\pm$ 0.08	2.34 $\pm$ 0.052 (- 13%)	2.16 $\pm$ 0.044* (- 24%)

Treatment with lercanidipine had the same effect as fosinopril regarding the antioxidant levels and LP levels: GSH levels increased with 49%, GSH-Px with 49% and SOD with 70% and the LP levels decreased with 24% after 12 weeks of treatment. As seen from Table 2 and 3, fosinopril had a major effect on the

GSH system while lercanidipine had a bigger effect on SOD levels.

The parameters of oxidative stress in Group III are presented in Table 4.

**Table 4.** Oxidative stress in patients treated with ACEI and CCB (group III). The results are expressed as mean values  $\pm$  SD; \* =  $p < 0.05$ .

Parameter	Initial values	After 6 weeks	After 12 weeks
GSH ( $\mu\text{g/ml}$ )	1.58 $\pm$ 0.008	2.385 $\pm$ 0.14* (+ 50%)	2.68 $\pm$ 0.17* (+62%)
GSH-Px ( $\mu\text{mol GSSG/ ml/min}$ )	0.148 $\pm$ 0.031	0.224 $\pm$ 0.045* (+ 52%)	0.298 $\pm$ 0.018* (+78%)
SOD (U/Ht)	0.402 $\pm$ 0.011	0.590 $\pm$ 0.009* (+ 49%)	0.776 $\pm$ 0.008* (+ 72%)
LP ( $\mu\text{mol MDA/ml}$ )	3.26 $\pm$ 0.02	2.64 $\pm$ 0.036* (- 49%)	1.96 $\pm$ 0.028* (- 64%)

In Group III, all the parameters were significantly influenced after 6 weeks of treatment and even more after 12 weeks: GSH and GSH-Px increased by 62% and 78%; SOD activity increased by 72% after 12 weeks of antihypertensive treatment. Moreover, serum levels of LP were significantly decreased (- 64%), which implies that the two drugs had a synergic effect not only over the BP values but also over the oxidative stress levels.

The parameters of oxidative stress in Group IV are presented in Table 5.

**Table 5.** Oxidative stress in the placebo control group (group IV).

Parameter	Initial values	After 6 weeks	After 12 weeks
GSH ( $\mu\text{g/ml}$ )	1.54 $\pm$ 0.006	1.60 $\pm$ 0.09 (ns)	1.64 $\pm$ 0.11 (ns)
GSH-Px ( $\mu\text{mol GSSG/ ml/min}$ )	0.143 $\pm$ 0.028	0.151 $\pm$ 0.018 (ns)	0.154 $\pm$ 0.016 (ns)
SOD (U/Ht)	0.396 $\pm$ 0.009	0.401 $\pm$ 0.007 (ns)	0.406 $\pm$ 0.008 (ns)
LP ( $\mu\text{mol MDA/ml}$ )	3.19 $\pm$ 0.04	3.14 $\pm$ 0.028 (ns)	3.04 $\pm$ 0.022 (ns)

There were no significant modifications of the oxidative stress parameters in Group IV. There was a slight increment of antioxidant enzymes (GSH + 7%, GSH-Px +8%, SOD + 3%) and slight decrement of LP levels (LP - 6%) after 12 weeks of diuretic treatment, possibly due to the very good control of BP.

## DISCUSSION

Our study is one of the few that studied the antioxidant effects of ACEI (Fosinopril) and CCB (Lercanidipine) in elderly patients with ISH. We chose patients aged > 65 years because they are more exposed to severe cardiovascular complications such as myocardial infarction, renal disease, stroke and sudden death and because they rarely are the object

of independent trials. Moreover, for many years, it was assumed that elderly do not benefit of primary or secondary cardiac prevention because they have imuable ATS lesions. Our study tried to prove that elderly respond at least as well as middle-aged patients to drugs that prevent ATS development.

The results of this study showed that fosinopril and lercanidipine induced not only a very good control of systolic BP values without noticing side effects but also a significant increase in the antioxidant system; fosinopril had a very good effect on the GSH complex and lercanidipine determined a significant increase of the SOD activity. Not surprisingly, association of both drugs displays the same very good results on both antioxidant systems and, moreover, induced a significant decrease in serum levels of LP, proving a synergic activity.

The biological activity of ROS depends upon their relative balance in relation to intracellular antioxidant defenses. One of the major protective systems against oxidant damage is the glutathione redox cycle, composed of GSH-Px and the co-substrates GSH and NADPH. GSH is the most abundant nonproteic intracellular thiol, its concentration frequently being in the millimolar range. Aging has been associated with low glutathione levels in the blood and tissues of several animals as well as in human blood. In addition, GSH supplementation has been shown to reverse the age-associated decline in immune responsiveness in mice and to improve survival in *Drosophila* and in mice. However, despite numerous experimental and clinical studies, the mechanism by which these agents exert their effect is not fully understood.<sup>16</sup>

ACEI are widely recommended for the treatment of hypertension and congestive heart failure. Evidence from various reports suggests the potential of ACEI and CCB as antiatherogenic agents.<sup>17-20</sup> Overall, ACEI produce a dramatic decrease in cardiovascular events independent of the effect on left ventricular function, and blood pressure lowering, supporting the idea that this intervention may have direct effects on vascular function. Particularly ACEI have been shown to retard the development of atherosclerosis in experimental models and to improve endothelial dysfunction in patients with coronary artery disease.<sup>20,21</sup>

The most relevant evidence for such a mechanism was provided by the observation that quinapril treatment for six months improved coronary endothelial function in patients with coronary artery disease.<sup>22</sup> Various experimental evidences support the involvement of hemodynamic effects and/or the stimulation of cytoprotective prostaglandins.

Additionally, the potentiation of a free radical scavenger action by ACEI has also been postulated. ACEI induce an augmentation of tissue GSH content in different tissues: brain, lung, and particularly erythrocytes.<sup>23</sup> In a previous study in mice, it was found that chronic administration of enalapril attenuates age-associated myocardial and glomerular sclerosis and increases survival. Subsequent work showed that 11-wk enalapril or captopril treatments increase antioxidant enzymes and nonenzymatic antioxidant defenses in several mouse tissues.<sup>24,25</sup>

Given the link between the renin-angiotensin system and vascular NAD(P)H oxidase activity, ACEI may act as “antioxidants” in part by limiting angiotensin II-mediated superoxide production by NAD(P)H oxidase at its source and, thus, preventing superoxide-mediated inactivation of endothelin-derived nitric oxide (EDNO). ACEI may also affect downstream effects of superoxide limiting hydrogen peroxide formation and vascular proliferation, consistent with observations that ramipril reduced progression of carotid intimal thickening. ACEI therapy may also limit peroxynitrite generation and lipid peroxidation, and down regulate activation of redox-sensitive proinflammatory signals.<sup>24</sup>

This study brings new evidence in humans that supports the hypothesis that ACEI increase antioxidant activity, especially the GSH complex. Treatment with fosinopril controlled ISH without major side effects and increased significantly the levels of GSH (+56%) and GSH-Px (+75%) as postulated by Pool and co.<sup>26</sup> This effect was well established after 6 weeks of treatment and was maximal after 12 weeks of treatment. Fosinopril also induced an elevation of the SOD activity (+40%) and decreased PL levels (-25%), but the effect was less important.

Numerous experimental and clinical studies suggested that CCB induce the suppression of smooth muscle cell proliferation and migration and connective tissue secretion, all processes dependent on calcium as an intracellular second messenger.<sup>27</sup> Moreover, they block the cholesterol-induced increase in smooth muscle cell proliferation at concentrations, which had no effect on membrane calcium permeability, suggesting that their antiatherogenic activity is independent of its action on calcium channels. The dihydropyridine ring can donate electrons to the propagating radicals to reduce them to a non-reactive form and prevent low-density lipoprotein (LDL) oxidation, at least in vitro.<sup>28,29</sup>

The reason for choosing lercanidipine for this study was the very few data on the antioxidant effects of this drug.<sup>30</sup> Our results showed that lercanidipine

increased significantly SOD activity (+70%) while controlling efficiently ISH. It also increased GSH (+49%) and GSH-Px levels (+49%) and decreased serum levels of LP (-29%). The most interesting findings were provided by Group III, where the association of the two drugs induced not only an excellent control of BP but also a marked increment of both GSH complex (GSH = +68%, GSH-Px = +78%) and SOD activity (+72%) and a marked decrement of LP levels (-64%), suggesting that there is a synergic activity. These findings might be explained by their different mechanisms of influencing oxidative status.

In conclusion, considering the relevance of the glutathione system and of SOD activity as cellular antioxidant mechanisms, the present findings suggest that by increasing antioxidant defenses, fosinopril and lercanidipine can protect cells from ROS-mediated damage. The increment of antioxidant defenses might explain, at least in part, the ancillary beneficial effects shown by ACEI in various pathologies as well as during aging. This effect was as important in elderly as proven to be in middle-aged persons from other trials which suggested that aged persons should benefit from primary and secondary cardiovascular prevention as well.<sup>26-28</sup>

The association between fosinopril and lercanidipine proved to have additive effects not only in controlling BP but also in modifying the oxidative stress, possibly because the two drugs have different paths in interfering the oxidative mechanisms of atherosclerosis and endothelial damage. These findings encourage further research on the CCB and ACEI-induced increase of human antioxidant defenses and the underlying mechanisms. The elucidation of those mechanisms might help to develop new strategies aimed at increasing the endogenous antioxidant defenses, as opposed to less effective non-dietary antioxidant supplementation. The achievement of a sustained elevation of endogenous antioxidant agents could be useful in delaying the progression of degenerative conditions related to oxidant-induced damage.

**Conflict of interest:** none declared.

## REFERENCES

1. Franklin SS, Jacobs MJ, Wong ND, et al. Predominance of isolated systolic hypertension among middle-aged and elderly US hypertensives: analysis based on National Health and Nutrition Examination Survey (NHANES) III. *Hypertension* 2001,37(3): 869-74.
2. Hazzard WR, Blass JP, Ettinger WH Jr, et al. *Principles of Geriatric Medicine and Gerontology*, William R Hazzard et al eds, 4<sup>th</sup> edition, 1998

3. Safar ME. Systolic blood pressure, pulse pressure and arterial stiffness as cardiovascular risk factors. *Curr Opin Nephrol Hypertens* 2001;10(2):257-61.
4. Waeber B. Trials in isolated systolic hypertension: an update. *Curr Hypert Rep* 2003;5:329-36.
5. Vetter W. Treatment of senile hypertension. The fosinopril in old patients study (FOPS). *Am J Hypertens* 1997;10:255-61S.
6. Wang JG, Staessen JA. Antihypertensive drug therapy in older patients. *Curr Opin Nephrol Hypertens* 2001;10(2):263-9.
7. Kaplan NM. What is goal blood pressure for the treatment of hypertension? *Arch Int Med* 2001;161(12):1480-2.
8. Keaney JF Jr, Oxidative Stress and Vascular Disease. Keaney JF (ed), New York, Kluwer Academic Publishers
9. SHEP Cooperative Group: Prevention of stroke by antihypertensive drug treatment in older persons with isolated systolic hypertension. Final results of the Systolic Hypertension in the Elderly Program (SHEP). *JAMA* 1991;265:3255-64.
10. Cai H, Harrison DG. Endothelial dysfunction in cardiovascular disease. The role of oxidant stress, *Circ Res* 2000;87:840-4.
11. Channon KM. Oxidative stress and coronary plaque stability. *Arterioscler Thromb Vasc Biol* 2002;22:751-2.
12. Motchnik PM, Frei B, Ames BN. Measurements of antioxidants in human blood plasma. *Method Enzymol* 1994;234:269-79.
13. Flohe L, Gunzler WA. Assays of glutathione peroxidase. *Methods Enzymol* 1984;105:114-21.
14. Sun Y, Oberley LW, Ying L. A simple method for clinical assay of superoxide dismutase. *Clin Chem* 1988;34:497-500.
15. Yagi K. Assay from serum lipid peroxide level and its clinical significance. In: Yagi K. (ed.): *Lipid Peroxides in Biology and Medicine*. New York, Academic Press, 1982.
16. Bourdel-Marchasson I, Delmas-Beauvieux M, et al. Antioxidant defences and oxidative stress markers in erythrocytes and plasma from normally nourished elderly Alzheimer patients, *Age and Aging*, 2001.
17. Schulze PC, Lee RT. Oxidative stress and atherosclerosis. *Curr Atheroscler Rep* 2005;7:242-8.
18. Cominacini L, Garbin U, Pasini Af, et al. Oxidised low-density lipoprotein increases the production of intracellular reactive oxygen species in endothelial cells. Inhibitory effect of lacidipine. *J Hypertens*, 1998;16:1913-9.
19. Campbell RC. The rennin-angiotensin system: a 21<sup>st</sup> Century Perspective. *J Am Soc Nephrol* 2004;15:1963-4.
20. Thomas M, Keaney JF Jr. Are ACE Inhibitors a "Magic Bullet" Against Oxidative Stress? *Circulation* 2001;104:1571-4.
21. Yusuf S, Sleight P, Pogue J, et al. Effects of an angiotensin-converting-enzyme inhibitor, ramipril, on cardiovascular events in high-risk patients. The Heart Outcomes Prevention Evaluation Study Investigators. *NEJM* 2000;342:145-53.
22. Mancini GB, Henri GC, Macaya C, et al. Angiotensin-converting enzyme inhibition with quinapril improves endothelial vasomotor dysfunction in patients with coronary artery disease. The TREND Study. *Circulation* 1996;94:258-65.
23. Espinel CH, Bruner DE, Davis JT, et al. Enalapril and verapamil in the treatment of isolated systolic hypertension in the elderly. *Clin Ther* 1992;14:835-44.
24. Djordjevic VB, Pavlovic D, et al. Changes of lipid peroxides and antioxidative factors levels in blood of patients treated with ACE inhibitors. *Clin Nephrol* 1997;47:243-7.
25. De Cavanagh EMV, Insera F, Ferder L, et al. Enalapril and captopril enhance glutathione-dependent antioxidant defences in mice tissues. *Am J Physiol* 2000;278:R572-77.
26. Pool JL. Antihypertensive effect of fosinopril, a new angiotensin converting enzyme inhibitor, findings of the Fosinopril Study Group II. *Clin Ther* 1990;12:520-533.
27. Napoli C, Salomone A et al. 1,4-Dihydropyridine calcium channel blockers inhibit plasma and LDL oxidation and formation of oxidation-specific epitopes in the arterial wall and prolong survival in stroke-prone spontaneously hypertensive rats. *Stroke*. 1999;30:1907-15.)
28. Tulenko TN, Brown J, Khan M, et al. Atheroprotection with amlodipine: Cells to lesions and the PREVENT trial. Prospective Randomized Evaluation of the Vascular Effects of Norvasc Trial. *J Cardiovasc Pharmacol* 1999;33(Suppl 2):17-22.
29. Baykal Y, Yilmaz M, et al. Effects of antihypertensive agents, alpha receptor blockers, beta blockers, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers and calcium channel blockers, on oxidative stress. *J Hypert* 2003;21(6):1207-11.
30. Incandela L, Belcaro G, et al. Oxygen-free radical decrease in hypertensive patients treated with lercanidipine. *Int Angiol* 2001;20(2):136-40.