Effect of antihypertensive treatment with candesartan or amlodipine on glutathione and its redox status, homocysteine and vitamin concentrations in patients with essential hypertension

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Objective To compare the effect of candesartan or amlodipine on concentrations of cellular markers of oxidative stress, plasma homocysteine and vitamins in hypertensive patients.

Methods Forty-nine middle-aged patients with untreated stage I–II essential hypertension were recruited in a randomized double-blind double-dummy study to receive a daily dose either of 8 mg candesartan (\(n=25\)) or 5 mg amlodipine (\(n=24\)) for 16 weeks. Blood pressure, reduced glutathione (GSH) and oxidized glutathione (GSSG), glutathione redox ratio (GSSG : GSH) in red blood cells, plasma homocysteine, vitamin B\textsubscript{12} and folic acid status were measured at baseline, at week 2 and at week 16. The same parameters were measured in 32 healthy age- and sex-matched controls. An increase in homocysteine of at least 2 \(\mu\text{mol/l}\) was considered significant.

Results Hypertensive patients had significantly greater oxidative stress and homocysteine concentrations than controls. In addition to a significant decrease in blood pressure, in both treatment groups GSSG decreased (\(P<0.03\)), GSSG : GSH had a tendency to decrease (\(P=0.054\)), but homocysteine did not change. An increase in homocysteine concentration of at least 2 \(\mu\text{mol/l}\) was found in 12 patients (five in the candesartan group, seven in the amlodipine group), with a significant decrease in folic acid concentration and no changes in cellular oxidative stress. In patients with no increase in homocysteine concentration, both GSSG (\(P<0.02\)) and GSSG : GSH (\(P=0.051\)) decreased. GSH and vitamin B\textsubscript{12} did not change in any of the groups studied.

Conclusion: Untreated hypertension is associated with disturbed glutathione redox status and increased plasma homocysteine concentrations. Both candesartan and amlodipine had favourable effects on cellular oxidative stress, but the oxidative stress status did not decrease in patients with adverse changes in homocysteine. J Hypertens 23:105–112 © 2005 Lippincott Williams & Wilkins.

Introduction

Essential hypertension remains a major modifiable risk factor for cardiovascular diseases. Its aetiology has not been fully elucidated, mostly because of the as yet unknown genetic variation and many non-hereditary factors that have important and modifiable influences on blood pressure [1].

It has been demonstrated that, in the presence of traditional cardiovascular risk factors, homocysteine may have a permissive role in endothelial damage, even when present within the traditionally used reference range of concentrations [2,3]. These effects seem to be related to increased oxidative stress [4,5]. Homocysteine concentrations depend on a series of intracellular metabolic reactions in which folic acid acts as a substrate and vitamin B\textsubscript{12} serves as a coenzyme. However, recent studies have suggested that the benefits of folic acid are not limited to homocysteine metabolism: it appears to interact with nitric oxide metabolism, to enhance the availability of tetrahydrobiopterin, to reduce superoxide anion generation and, hence, to improve endothelial dysfunction [6]. Besides being among the causes of hyperhomocysteinaemia, folate deficiency is associated with genomic instability, defective DNA repair and apoptosis [7].
High-grade oxidative stress and development of hypertension have been demonstrated in normal rats after glutathione depletion [8]. In humans, glutathione supplementation reverses endothelial dysfunctions and improves nitric oxide bioavailability [9]. Glutathione is a tripeptide synthesized from precursor amino acids glutamate, glycine and cysteine, the last of which is produced during trans-sulphuration of homocysteine; homocysteine metabolism provides about 50% of glutathione. High grade oxidative stress may also induce cellular suppression of the glutathione antioxidant defence system, and reduce homocysteine-derived glutathione synthesis [10], an observation supported by the findings of our previous study [11]. Cellular reduced glutathione (GSH) has a principal role in the protection of endothelial cells from excess oxygen free radicals, preventing endothelial dysfunction in the arteries exposed to profound oxidative stress [12,13]. The glutathione redox status ratio is the dominating factor in modulation of cell responses to redox changes. Expressed as the ratio of oxidized glutathione (GSSG, a relatively toxic compound) to GSH, it is crucial in maintaining cellular viability [14].

Drugs that alter GSH concentrations or the expression of any GSH-dependent enzyme may initiate deterioration of antioxidant defence; increases in homocysteine or decreases in cellular GSH concentrations are undesirable side effects of drugs. Antihypertensive drugs differ regarding their effect on homocysteine or GSH concentrations [15,16] and, to the best of our knowledge, there exist no human studies in which both of these markers have been evaluated jointly during antihypertensive treatment.

Both angiotensin II type 1 (AT1) receptor antagonists and calcium channel blockers are among the drugs of first choice for treatment of essential hypertension [17]. Drugs of both these groups are also known to express some antioxidant activity [18,19] and to improve endothelial function [18,20].

The aim of this study was to evaluate, in a homogeneous group of hypertensive patients, whether the AT1 receptor antagonist candesartan and the calcium channel blocker amlodipine affect cellular GSH and its redox status, and plasma homocysteine and vitamin concentrations during antihypertensive treatment.

**Patients and methods**

**Patients**

The study population consisted of 49 outpatients with untreated mild to moderate essential hypertension. All individuals who responded to an advertisement and in whom the inclusion criteria were met were recruited on a consecutive basis between September 2000 and December 2002 at the Department of Cardiology, University of Tartu, Estonia. The diagnosis of hypertension was established on the basis of systolic blood pressure > 140 mmHg, diastolic blood pressure > 90 mmHg, or both, measured during three different visits. For a diagnosis of hypertension, increased blood pressure values were required to be present at each visit. Patients who had previously received antihypertensive treatment had been free of medication for at least 2 months. Thirty-four patients (17 in each group) had never been treated for essential hypertension. All patients were clinically stable. We excluded those with diabetes (based on a glucose tolerance test), a history of cardiac or cerebrovascular disease, heart failure (left ventricular ejection fraction < 50%), hypercholesterolaemia (total cholesterol > 6.5 mmol/l), other systemic diseases, recent or current infection, anaemia and secondary hypertension. Routine clinical, haematological and radiological examinations excluded the secondary forms of hypertension.

The control group consisted of 32 healthy sex- and age-matched consecutive volunteers who responded to the advertisement. All controls demonstrated normal findings at physical and biochemical examinations, and had normal blood pressure values (systolic blood pressure less than 136 mmHg and diastolic blood pressure less than 88 mmHg).

All those recruited to the study were non-smokers with body mass index (BMI) < 30 kg/m². None of the patients had clinical evidence suggestive of coronary artery disease, based upon history, electrocardiography, exercise test and echocardiography. There was no left ventricular dysfunction on echocardiography or microalbuminuria on urine analysis (data not shown). Individuals who were taking any medical vitamin preparations or drugs were not included. No dietary restrictions were imposed.

The Ethics Committee of the Medical Faculty, University of Tartu approved the study procedure, and informed consent was obtained from all participants before the study.

**Study procedure**

During the 4-week run-in period, the patients did not receive any treatment and were seen for the performance of an exercise stress test, glucose tolerance test, echocardiography, ultrasound investigation of renal arteries and repeated measurements of blood pressure. The patients were recruited in a randomized, double-blind double-dummy study, to receive a daily dose of 8 mg candesartan or 5 mg amlodipine for 16 weeks. After week 2, the patients were seen at 4-week intervals during the study. Patients who did not respond (systolic blood pressure at least 140 mmHg, diastolic blood pressure at least 90 mmHg, or both) to
the above-mentioned doses of drugs at week 2 or 6 of treatment received a double dose of either drug for the remaining trial period. The blood samples were collected at baseline, week 2 and week 16.

The study participants were studied and the blood samples were collected between 0800 and 0900 h, after an overnight fast. Blood pressure was measured in both arms with the individual in the sitting position after 10 min of rest, using a conventional mercury sphygmomanometer and a normal size cuff: the mean of three readings at 2 min intervals was taken, with diastolic blood pressures at Korotkov phase V. Each individual’s height and weight were recorded, and their BMI calculated.

Blood samples and assays
Blood samples were drawn from the antecubital vein for the measurement of plasma homocysteine, serum creatinine, folic acid and vitamin B12, and red blood cell (RBC) folic acid, GSH and GSSG. The blood samples were processed within 30 min. Homocysteine was measured by enzyme immunoassay (Axis-Shield Diagnostics Ltd, Dundee, UK), for which blood was drawn into tubes containing EDTA, placed on ice and then centrifuged. The plasma samples were stored at –70°C until required for analysis. GSH and GSSG were measured by an enzymatic method (intra-assay precision 7.7%, total precision 9.4%) as described previously [11].

The group of patients studied was classified retrospectively according to the changes in homocysteine concentration during the 16 weeks of antihypertensive treatment. We considered an increase in homocysteine concentration of at least 2 µmol/l clinically meaningful, both because it is based on the finding in several studies [21,22] that the difference in homocysteine concentration between hypertensive patients and normotensive individuals is approximately 2 µmol/l and because, according to a consensus reported by D.A.Ch-Liga (German, Austrian and Swiss Homocysteine Societies) in 2003, 2 µmol/l is the cut-off point between values for healthy individuals and those patients with pre-existing cardiovascular disease or its risk. In addition, the intra-individual variability in homocysteine concentrations is very low: repeat measurements after 6–18 months in healthy volunteers showed good reproducibility of baseline values, with non-significant intra-individual variations of as little as 0.85–1.2 µmol/l [23].

The concentrations of folic acid and vitamin B12 in serum, and of folic acid in RBCs in both whole blood and serum were measured by chemiluminescence with the Immulite 2000 Analyzer (Diagnostic Products Corporation, Los Angeles, CA, USA). RBC folic acid (in ng/ml) was calculated according to the formula:

\[
RBC \text{ folic acid} = (R - \{S \times (100 - H)/100\} \times (100/H)
\]

where \(R\) denotes whole blood folic acid, \(H\) is the haematocrit and \(S\) is the patient’s serum folic acid concentration.

Statistical analysis
Normally distributed data are presented as mean ± SD; non-normally distributed data are presented as geometric mean with 95% confidence intervals. Differences between the control group and the treatment group at baseline were estimated by \(t\)-test for the normally distributed variables and by non-parametric Mann–Whitney \(U\)-test for non-normally distributed variables. Differences in the drug and other aspects at different time points were analysed by multiple linear regression modelling. Changes in blood pressure values and biochemical variables were calculated as the difference between the baseline values and the values at the end of the study. Multiple linear regression modelling was used for estimating the associations between the changes in systolic or diastolic blood pressures and the biochemical variables in treatment groups. To compare individuals with increases in homocysteine concentrations of at least 2 µmol/l and those without such changes, \(t\)-test was used for normally distributed data and Mann–Whitney \(U\)-test for non-normally distributed data. In both groups, divided according to changes in homocysteine concentration, changes in biochemical variables over time were estimated by paired \(t\)-test for normally distributed variables and Wilcoxon matched pairs test for non-normally distributed data. Logistic regression was used to assess the determinants of increases in homocysteine concentration of at least 2 µmol/l. All statistical analyses were conducted using the software R version 1.9.0. for Windows. The level of significance was defined as \(P < 0.05\) (two-tailed).

Results
Forty-nine patients with untreated hypertension (43 men, six women) were compared with 32 normotensive controls (27 men, five women). Three patients did not complete the study: two (one from either study group) left the study because of personal reasons unrelated to the study, and one patient in the amlodipine group discontinued treatment at week 6 because of a skin reaction; thus data from 46 patients were available for analysis. Twenty-four patients [22 men/two women, mean age 53.9 ± 6.9 years; BMI 27.0 ± 2.1 kg/m², duration of hypertension 16.0 years (range 0.5–35 years)] were treated with candesartan, and 22 patients [19 men/three women, mean age 50.9 ± 6.6 years; BMI 27.0 ± 2.1 kg/m², duration of hypertension 12.4 years (range 0.5–44 years)] were treated with amlodipine. There were no differences in demographic data between the groups.
The study variables for the hypertensive patients at baseline are compared with those of healthy controls in Table 1. At baseline, in addition to significantly greater blood pressure and BMI values, hypertensive patients had greater plasma homocysteine concentrations and cellular markers of oxidative stress (GSSG and GSSG:GSH) than did controls. Groups were similar with regard to serum creatinine, vitamin B₁₂ and both cellular and serum folic acid.

The changes in blood pressure and biochemical variables at baseline, week 2 and week 16 in patients treated with candesartan or amlodipine are shown in Table 2. Throughout the study, the treatment groups did not differ with regard to the parameters studied. Both drugs caused a highly significant decrease in both mean systolic and mean diastolic blood pressure (\(P < 0.0001\)), with no significant differences between the drugs. The decrease was most pronounced during the first 2 weeks. The further decrease in blood pressure in the course of the treatment was insignificant. Doubling of the dose was required for 12 patients in the candesartan group and for nine in the amlodipine group. In comparison with baseline, significant decreases were detected in serum creatinine (\(P < 0.002\)) and RBC GSSG (\(P < 0.03\)) concentrations at week 16 in both groups. The GSSG : GSH ratio decreased insignificantly (\(P = 0.054\)) during the treatment. Plasma homocysteine tended to increase in both treatment groups, but these changes also were not significant.

The changes in systolic blood pressure were associated with the changes in diastolic blood pressure and were not associated with the changes in the biochemical variables. The decrease in diastolic blood pressure was associated with the decrease in systolic blood pressure and the concentration of GSSG (a relatively toxic compound) and with the increase in cellular folic acid (Table 3).

A significant increase in plasma homocysteine concentration (Fig. 1) compared with the baseline values was detected in 12 patients (26%), five in the candesartan group and seven in the amlodipine group. These changes were not related to the antihypertensive efficacy of the drugs or to the need to increase the dose. Patients both with and without an increase in homocysteine concentration did not differ with regard to age (54.4 ± 6.4 and 51.1 ± 6.9 years), sex (11 men/one woman compared with 28 men/five women), BMI (27.4 ± 1.6 and 26.7 ± 2.2 kg/m²) or duration of hypertension [6.6 (4.1 to 17.1) years compared with 8.5 (10.9 to 20.7) years, respectively]. In those patients in whom there was an increase in homocysteine concentration, folic acid decreased significantly in the serum at week 2 (from 5.2 ± 2.5 to 3.7 ± 1.3 ng/ml, \(P < 0.03\)) and in RBCs at week 16 [from 267.3 (201.3 to 391.5) ng/ml at baseline to 200.9 (165.2 to 257.5) ng/ml at week 16; \(P < 0.01\)].

In the patients with no increase in homocysteine concentration, no changes in the biochemical variables were detected at week 2. Compared with the baseline, by the end of the study GSSG : GSH had decreased slightly [from 0.12 (0.11 to 0.16) μmol/l to 0.09 (0.08 to 0.15) μmol/l; \(P = 0.051\)], and GSSG and creatinine had decreased significantly [from 111.0 (101.9 to 144.4) μmol/l to 98.9 (75.8 to 122.0) μmol/l (\(P < 0.02\)) and from 88.2 ± 8.5 to 83.0 ± 12.4 μmol/l (\(P < 0.006\)), respectively]. Creatinine had decreased insignificantly also in the patients with an increase in homocysteine concentration (from 94.5 ± 11.9 to 91.7 ± 10.3 μmol/l), remaining significantly (\(P < 0.05\)) greater both at baseline and at the end of the study than that in patients.

### Table 1 Baseline characteristics of the hypertensive patients and the healthy controls

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients (n = 49)</th>
<th>Controls (n = 32)</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>52.5 ± 7.0</td>
<td>51.0 ± 5.5</td>
<td>0.30</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.0 ± 2.1</td>
<td>24.5 ± 2.7</td>
<td>0.0001</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>43/6</td>
<td>27/5</td>
<td>0.60</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>151.1 ± 10.3</td>
<td>117.9 ± 9.6</td>
<td>0.0001</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>96.3 ± 6.6</td>
<td>76.4 ± 7.4</td>
<td>0.0001</td>
</tr>
<tr>
<td>Serum creatinine (μmol/l)</td>
<td>90.8 ± 10.6</td>
<td>91.0 ± 11.9</td>
<td>0.92</td>
</tr>
<tr>
<td>Plasma Hcy (μmol/l)</td>
<td>10.1 (9.7 to 11.1)</td>
<td>8.4 (7.9 to 9.2)</td>
<td>0.001</td>
</tr>
<tr>
<td>Serum folic acid (ng/ml)</td>
<td>5.6 ± 2.8</td>
<td>5.4 ± 1.9</td>
<td>0.65</td>
</tr>
<tr>
<td>Folic acid in RBCs (ng/ml)</td>
<td>221.9 (215.0 to 309.5)</td>
<td>187.2 (161.3 to 248.8)</td>
<td>0.20</td>
</tr>
<tr>
<td>Serum vitamin B₁₂ (pg/ml)</td>
<td>305.9 (290.4 to 353.6)</td>
<td>282.3 (241.4 to 381.3)</td>
<td>0.1</td>
</tr>
<tr>
<td>RBC GSH (μmol/l)</td>
<td>924.6 ± 188.2</td>
<td>1061.9 ± 280.9</td>
<td>0.01</td>
</tr>
<tr>
<td>RBC GSSG (μmol/l)</td>
<td>121.1 (116.1 to 160.0)</td>
<td>78.0 (71.0 to 97.0)</td>
<td>0.0002</td>
</tr>
<tr>
<td>RBC GSSG/RBC GSH</td>
<td>0.13 (0.12 to 0.18)</td>
<td>0.09 (0.07 to 0.01)</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Normally distributed data are presented as mean ± SD; non-normally distributed data are presented as geometric mean (95% confidence interval). BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; Hcy, homocysteine; RBC, red blood cell; GSH, reduced glutathione; GSSG, oxidized glutathione.
The primary goal of antihypertensive treatment is not merely to decrease increased blood pressure, but also to prevent clinical complications. Any additional information about the responses of a particular patient to such treatment will contribute to the achieving of this goal.

### Table 2

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline Week 2</th>
<th>Week 16 Baseline</th>
<th>Week 2</th>
<th>Week 16</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SBP (mmHg)</strong></td>
<td>150.3 ± 12.3</td>
<td>135.2 ± 8.9</td>
<td>132.2 ± 7.8*</td>
<td>151.6 ± 8.4</td>
</tr>
<tr>
<td><strong>DBP (mmHg)</strong></td>
<td>96.6 ± 6.0</td>
<td>88.1 ± 6.9</td>
<td>86.3 ± 5.8*</td>
<td>96.5 ± 5.1*</td>
</tr>
<tr>
<td><strong>Serum creatinine (µmol/l)</strong></td>
<td>89.3 ± 9.6</td>
<td>90.9 ± 7.6</td>
<td>90.8 ± 6.0</td>
<td>90.5 ± 5.1*</td>
</tr>
<tr>
<td><strong>Plasma Hcy (µmol/l)</strong></td>
<td>10.3 (9.5 to 11.6)</td>
<td>10.8 (9.1 to 13.7)</td>
<td>10.7 (9.0 to 14.3)</td>
<td>10.1 (9.4 to 11.3)</td>
</tr>
<tr>
<td><strong>Serum folic acid (ng/ml)</strong></td>
<td>5.9 ± 2.9</td>
<td>6.0 ± 3.3</td>
<td>6.0 ± 2.5</td>
<td>5.8 ± 2.8</td>
</tr>
<tr>
<td><strong>Folic acid in RBCs (ng/ml)</strong></td>
<td>233.4 (202.7 to 360.2)</td>
<td>181.1 (155.0 to 275.1)</td>
<td>224.3 (167.1 to 293.9)</td>
<td>268.3 (230.9 to 351.5)</td>
</tr>
<tr>
<td><strong>Serum vitamin B12 (pg/ml)</strong></td>
<td>294.4 (261.0 to 376.8)</td>
<td>269.3 (230.9 to 351.5)</td>
<td>294.6 (264.7 to 362.9)</td>
<td>330.2 (296.4 to 354.1)</td>
</tr>
<tr>
<td><strong>RBC GSH (µmol/l)</strong></td>
<td>942.7 ± 197.4</td>
<td>986.5 ± 216.8</td>
<td>866.8 ± 145.7</td>
<td>886.3 ± 120.7</td>
</tr>
<tr>
<td><strong>RBC GSSG (µmol/l)</strong></td>
<td>119.9 (107.4 to 150.5)</td>
<td>114.8 (102.2 to 148.2)</td>
<td>98.4 (84.1 to 150.4)*</td>
<td>119.1 (102.2 to 180.8)</td>
</tr>
</tbody>
</table>

Normally distributed data are presented as mean ± SD; non-normally distributed data are presented as the geometric mean with 5% confidence intervals. SBP, systolic blood pressure; DBP, diastolic blood pressure; GSH, reduced glutathione; GSSG, oxidized glutathione; Hcy, homocysteine; RBC, red blood cells. *Significant time trend (P < 0.05).
Antihypertensive treatment with either candesartan or amlodipine did not alter the homocysteine concentration, but decreased the grade of cellular oxidative stress in most individuals studied. To date, data as to the effect of antihypertensive treatment on homocysteine have been inconsistent, and even conflicting. Sharabi et al. [21] found in their observational study that treatment with antihypertensive drugs did not affect plasma homocysteine concentrations, but patients who were receiving β-blockers, angiotensin-converting enzyme inhibitors, diuretics and nitrates tended to have lower concentrations of plasma homocysteine. Patients treated with α-blockers or calcium channel blockers tended to have higher homocysteine values than untreated patients. However, it was not stated whether the patients received monotherapy or a drug combination. In a randomized study in which captopril and hydrochlorothiazide were compared, 4 weeks of treatment with the angiotensin-converting enzyme inhibitor, captopril, induced an insignificant (0.8 μmol/l) increase in homocysteine concentration [24].

An important finding of our study was that one subgroup of the study population revealed adverse alterations with regard to homocysteine concentrations during antihypertensive treatment with either candesartan or amlodipine. Some patients (26%) exhibited an increase in homocysteine concentration, with a concurrent decrease in folate, first in the serum, and thereafter in RBCs by week 16. In the remaining 74% of the hypertensive patients, the concentrations of homocysteine and folate acid were not altered and there was a concomitant decrease in cellular oxidative stress. Homocysteine metabolism and folate acid metabolism are closely related: a folate acid deficit is the most common water-soluble vitamin deficiency in Europe, and a decrease in folate acid concentrations leads to an increase in homocysteine concentration [23]. No dietary restrictions were imposed on our patients, and a limitation of the study is that we did not record the dietary habits of those studied. Whether the decrease in folate acid concentration in some patients was the result of a limited use of fresh fruit and vegetables, or was associated with antihypertensive medication, is difficult to establish.

The effect of candesartan on homocysteine or GSH in uncomplicated non-diabetic hypertensive patients has not been studied before. There exist no data as to the relationship between treatment with amlodipine and the concomitant changes in homocysteine. In a randomized study of hypertensive patients with type 2 diabetes, no statistically significant changes were detected in homocysteine after 1 month (−0.3 μmol/l) or 12 months (−0.9 μmol/l) of treatment with candesartan [25]. In the Framingham Offspring Study, those individuals who used antihypertensive medication had greater plasma homocysteine concentrations than those who were not taking such medications. The increase in homocysteine was not likely to have resulted from impaired renal function because the association was completely unaffected by adjustment for serum creatinine concentrations [15]. The same is partially valid for our study. The patients with an increase in homocysteine concentration had significantly greater creatinine values than those in whom such changes in homocysteine were not recorded. However, creatinine decreased in both homocysteine groups. It should be noted that all the patients studied had normal creatinine values before entering the study.

Both drugs were also similar regarding their renal effect. A significant decrease in serum creatinine occurred in both study groups. An experimental study demonstrated that inhibition of angiotensin II by candesartan had protective effects on the glomerular damage which extended beyond the haemodynamic and involved down-modulation of glomerular inflammation, reduction of mesangial cell proliferation and a decrease in chemokine expression [26]. This seems to be a class effect, because in a randomized trial another AT1 receptor blocker, losartan, also appeared to be renoprotective irrespective of its antihypertensive action [27]. In a randomized placebo-controlled study in normotensive renal transplant recipients, amlodipine reduced serum creatinine only 8 weeks after treatment [28]. In animal experiments, the favourable effects of amlodipine have also been attributed to an increase in nitric oxide synthase activity [29], which was accompanied by an improvement in the parameters of the microvasculature [29,30].

The decrease in oxidative stress that we observed during antihypertensive treatment is consistent with the findings of several previous studies. In a randomized placebo-controlled study, significant reduction in oxidative stress, determined by malondialdehyde in the case of candesartan [18] and by products of lipid peroxidation, free radicals and hydroperoxides in addition to total antioxidant capacity for amlodipine [19], has been demonstrated during antihypertensive treatment.

To date, few and even conflicting data have been reported about the changes in cellular GSH concentration during antihypertensive treatment, although an association has been established between hypertension and cellular GSH [11,31–33]. It has been shown in a randomized study that long-term treatment (6 months) with enalapril reduces the GSH concentration significantly, whereas no change has been noted in patients treated with captopril [16]. The impact of antihypertensive treatment with AT1 blockers in humans has been evaluated in a randomized study of losartan, in which...
GSH increased significantly [33], but no such studies have been undertaken with candesartan. No human studies have been conducted on changes in cellular GSH occurring during antihypertensive treatment with amlodipine. In our study, GSH did not change in any of the groups studied.

The glutathione redox status is crucial in maintaining cellular function and viability [14]. Several case-control studies have reported an approximately twofold increased glutathione redox ratio in hypertensive patients compared with normotensive controls, indicating an imbalance/altered cellular glutathione system [11, 31–33]. This finding was also a feature of our study. Comparison of hypertensive patients in both treatment groups at baseline with normotensive controls showed that the hypertensive patients had a disturbed intracellular antioxidative status in addition to an increased plasma homocysteine concentration. In the present study, favourable changes in the glutathione redox ratio and in GSSG concentration were detected in both treatment groups. In the hypertensive individuals, GSSG concentrations almost attained values found in the normotensive controls. The decrease in GSSG concentration was associated with the decrease in diastolic blood pressure, but not with the decrease in systolic blood pressure. It should be noted that, when changes in homocysteine concentration according to our criteria were taken into account, favourable changes in cellular markers of oxidative stress were significant only in those patients in whom the homocysteine concentration did not increase.

Both candesartan and amlodipine were effective in decreasing blood pressure in patients with mild to moderate essential hypertension. This is consistent with the findings of a previous randomized study in which both these drugs were highly effective in controlling blood pressure in patients with essential hypertension [34]. Furthermore, it should be noted that neither the efficacy of antihypertensive treatment nor the need to double the treatment dose was related to the adverse changes in plasma homocysteine or folic acid concentrations. Whether such alterations were attributable to the genetic polymorphism of enzymes, important for homocysteine or GSH metabolism, or whether there exist some other mechanisms is not clear.

Another limitation of our study is that we did not include a placebo arm. Therefore, the question may arise as to whether the changes in the biochemical parameters were indeed attributable to the antihypertensive treatment. It is known that homocysteine has low intra-individual variability [23], and the same is valid for GSH and GSSG (intra-assay precision for GSH and GSSG is 7.7%, total precision 9.4%). All our patients were clinically stable and the majority of them had been untreated for several years. Thus we presume that changes in the parameters studied were not caused by factors other than the treatment used.

In conclusion, untreated hypertensive patients have disturbances of the cellular glutathione redox system and increased plasma homocysteine concentrations. Effective antihypertensive treatment with either candesartan or amlodipine did not alter the homocysteine concentration, but decreased the grade of cellular oxidative stress in most persons studied. Proceeding from the possible adverse alterations in homocysteine concentration, our study points to the need for an individual approach when antihypertensive treatment is being prescribed. It is possible that patients with increased homocysteine concentrations could benefit from the use of folic acid as an adjuvant to conventional antihypertensive treatment.

Acknowledgements This study was supported by the Estonian Scientific Foundation, grants No. 4442 and 5327. The authors thank AstraZeneca AB for providing the study drugs in a double-dummy formula.

References


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