Low-Dose Captopril and Antioxidant Combination as Adjunct Therapy in Type-2 Diabetic Patients with Coronary Artery Disease: A preliminary study

1Hosny Elewa, 2Zeinab Al-Kasaby Zalat, 3Ghaleb Oriquat, 4Rowaida Rifaat and 5Wessam El-Hadidy

1Faculty of Pharmacy, Clinical and Hospital Pharmacy Department, Taibah University, Madena Al Menawra City, KSA.
2Faculty of Pharmacy, Department of Pharmaceutics, Girls branch, Azhar University, Cairo, Egypt.
3Al-Ahliyya Amman University, Amman, Jordan.
4Medical Research Institute, Alexandria University, Alexandria, Egypt.
5Arab International University, Damascus, Syria.

ABSTRACT

The atherosclerotic complications constitute the main cause of mortality among diabetes patients, in general, and in type-2 diabetics, in particular. Although the atherosclerotic process is indistinguishable from that affecting the non-diabetic population, it begins earlier and may be severe. The accelerated atherosclerosis in diabetes involves a multitude of mechanisms including oxidative modification of low density lipoproteins through the oxidative stress accompanying the pathology of diabetes. Homocysteine, which causes autoxidation of LDL and the reaction between glucose and protein or lipoproteins leading to glycated products in arterial walls are also involved. Angiotensin II is known to be a potent stimulator of ROS production in endothelial cells and vascular smooth muscle cells, and ACE inhibitors increase endogenous oxidant scavengers, and enhance glutathione-dependent antioxidant defense. The aim of the present study was to compare the possible role of supplementation with either a low dose of the ACE inhibitor captopril or a combination of antioxidants to the regular treatment regimens of type 2 diabetic patients with CAD on some markers of atherosclerosis. Thirty subjected of the same socioeconomic class were recruited into the study, and were divided into 3 groups with similar age and gender distribution: Group I (controls) included 10 healthy non-obese individuals, Group II and group III subjects (10 patients in each group) were type 2 diabetics with atherosclerotic coronary artery disease (CAD). At the beginning of the study, treatment of patients in group II was supplemented by once daily tablet containing antioxidant combination. Supplementation in group III patients consisted of a low daily dose (12.5 mg) of the ACE inhibitor captopril. At the start of the study and after one and three months blood samples were withdrawn to assess glycemic control by estimating the fasting glucose and the glycated hemoglobin. Total glutathione and its reduced and oxidized fractions, as well as the thiobarbituric acid reactive substances were assayed as a measure of oxidative stress. The serum levels of homocysteine and autoantibodies against oxidized LDL (ox-LDL Ab) were also assayed. At the start of the study, diabetic patients showed hyperglycemia, elevated glycated hemoglobin, increased oxidative stress, depressed antioxidant defense, and elevated serum levels of autoantibodies against oxidized LDL and hyperhomocysteinemia, as compared to non-diabetic controls. The results of a three month follow-up of type 2 diabetic patients indicated that adjunct treatment with antioxidants or low-dose captopril improved all parameters tested, including glycemic control, oxidative stress, and hyperhomocysteinemia. However, it seems that the titer of circulating ox-LDL may not be a good prognostic indicator for atherosclerosis in the studied patients. The clinical improvement and the observed stepwise shift in the disease indices toward normal levels make the use of the suggested adjuvant therapy in type 2 diabetics with cardiovascular disease worth pursuing in a larger clinical study.

Key words: Mortality, diabetes, atherosclerotic, antioxidant, Captopril

Introduction

The pathology of diabetes mellitus is associated with an increased incidence of macrovascular complications including coronary artery disease (CAD) (Boyle, 2007). It is well established that diabetes is one of the major risk factors for atherosclerosis and diabetic patients have a two- to four-fold higher risk of coronary heart disease than non-diabetic individuals (Stumvoll et al., 2005). These atherosclerotic complications constitute the main cause of mortality among diabetes patients, in general, and in type-2 diabetics, in particular.

Corresponding Author: Hosny Elewa, Department of Clinical and Hospital Pharmacy, Faculty of Pharmacy, Taibah University, Madena Al Menawra City, KSA.
E-mail: Hosnyelewa1960@yahoo.com
(Kesavulu et al., 2001). Although the atherosclerotic process is indistinguishable from that affecting non-diabetic population, it begins earlier and may be severe (Zachary, 2002).

The accelerated atherosclerosis in diabetes involves a multitude of mechanisms. Oxidative modification of low density lipoprotein (LDL) has been implicated as a major factor in the pathogenesis of coronary atherosclerosis (Berlinger and Heinecke, 1996). A correlation has also been established between the level of circulating ox-LDL and the extent of CAD in type 2 diabetic patients (El-Bassiouni et al., 2007). Once formed, the native properties of oxidized LDL are altered and may contribute to rapid progression of atherosclerosis by a multitude of mechanisms (Jessup and Kritihardies, 2000; Xin et al., 2010). Homocysteine causes autooxidation of LDL through generation of the superoxide radical, reduction of the antioxidant status, and affecting nitric oxide production, which could also injure vascular endothelial cells (Prasad, 1999 and Lonn et al., 2006). Another important factor responsible for accelerated atherosclerosis in diabetes is the non-enzymatic reaction between glucose and proteins or lipoproteins leading to glycated products in arterial walls (Boyle, 2007).

Reactive oxygen species (ROS) provide a link between angiotensin II and atherosclerosis. Angiotensin II is a potent stimulator of ROS production in endothelial cells and vascular smooth muscle cells (Griendling and Ushio-Fukai, 2000). Angiotensin converting enzyme inhibitors (ACEI) therapy was found to reduce oxidant stress in the blood vessel wall, as measured by decreased oxLDL or improved endothelial NO-dependent vasorelaxation (De Cavangh et al., 2002).

The aim of the present study is to compare the possible role of supplementation with either a low dose of captopril or a combination of antioxidants to the regular treatment regimens of type 2 diabetic patients with CAD on some markers of atherosclerosis.

Subjects, Materials and Methods

Participants in the study were normotensive subjects of the same socio-economic class and were divided into three different groups. Group I (controls) included 10 healthy non-obese individuals aged 41-71 years. Group II and group III subjects (10 patients in each group) were type 2 diabetics with atherosclerotic coronary artery disease (CAD). Their ages ranged from 46 to 72 years. These patients were diagnosed, treated and followed-up in the outpatient clinic of the cardiology unit of the Medical Research Institute of Alexandria University. The reported duration of their diabetes ranged between 2 and 8 years. Their hepatic and renal functions (particularly microalbuminuria) were within the clinically acceptable range. Criteria for exclusion from these two groups included a history of ketoacidosis, severe renal or liver dysfunction, malignancy, endocrinical problems other than diabetes, smoking and the use of antioxidant supplements.

At the time of the study, all patients in groups II and III were treated with diet control and oral antidiabetic agents. Most (16 out of 20 patients) were treated with a combination of sulphphonylurea, either chlorpropamide (100-250 mg daily) or glyburide (2.5-5 mg daily), and metformin (500 mg 2-3 times daily). One patient was on gluburide (5 mg daily) as monotherapy, while 3 were on roziglitazone (2-4 mg three times daily). Prescribed dosages were individualized according to patient requirement. The study was approved by the ethics committee of the Medical Research Institute and informed written consent was obtained from each participant before enrollment.

At the beginning of the study, treatment of each of the 10 patients in group II was supplemented by once daily antioxidant tablet (Antox®; Farco Pharmaceuticals, Alexandria) containing 30 mg vitamin E, 100 mg ascorbic acid, 5.54 mg vitamin A acetate, 50µg selenium and 105 mg medical yeast. Supplementation in group III patients consisted of a 12.5 mg daily dose of the angiotensin converting enzyme inhibitor captopril (Capoten™, Squibb Egypt, Cairo).

Biochemical assays

Fasting plasma glucose level was assayed by the glucose oxidase method (Trinder, 1969). Glycated hemoglobin (HbA1c) was determined using a turbidimetric inhibition immunoassay for hemolyzed whole blood (Karl, 1993). The enzymatic method described by Griffith (Griffith, 1980) was used to measure the plasma total glutathione (tGSH) and the reduced (rGSH) and oxidized (GSSG) fractions. The Nernst equation was used to calculate the plasma redox potential (Scalfar and Buettner, 2001). The lipid peroxidation was measured as thiobarbituric acid reactive substances (TBARS) and calculated as malondialdehyde (MDA) (Inoue, 2001). Serum level of autoantibodies against oxidized low density lipoproteins was estimated using an enzyme immunoassay kit (Biomedica, USA) (Nishikawa et al., 2000) and serum homocysteine was determined by an immunochemical assay using a commercial kit (Axis Biochemicals).

Statistical analysis

All data are presented as mean ± SD. One-way analysis of variance (ANOVA) was performed on each variable and the Bonferroni statistics employed to compare the mean values from the different groups. Paired t-
test was used to assess the effect of adjunct therapies used at one and three months. Differences were considered significant at \( P<0.05 \). All statistical analyses were performed using SPSS statistical software (version 10).

**Results and Discussion**

The results of the control of glycemia and other clinical chemistry parameters in the different studied groups are summarized in Table 1. As may be expected, diabetic patients showed higher fasting glucose levels than did the controls. Compliance with antidiabetic treatment was reasonably good, as judged by HbA1c levels. However, in diabetic patients with CAD, the plasma homocysteine levels, at the beginning of the study, were between 60% and 70% higher as compared with the control group. Besides, the antibodies against oxidized LDL (oxLDL-Ab) were also elevated by 50-60%.

In general, addition of the antioxidant combination or captopril to the treatment regimens of type 2 diabetic patients with CAD improved their glycemic status. Better control in the form of a stepwise decline in fasting blood glucose was observed, which could be detected even after the first month of the study period. With the antioxidant combination, the average fasting blood glucose level showed statistically significant decreases to 10.2 ± 1.39 mmol/L after one month and to 8.7 ± 1.83 mmol/L at the end of the study period of 3 months. However, the changes in the glycated hemoglobin were not as prominent, which was expected, showing declines to 6.50 ± 0.80% and to 6.00 ± 0.73% after 1 and 3 months of initiating adjunct antioxidant therapy respectively. Such changes in HbA1c were without statistical significance. The low dose of captopril had a relatively milder effect on glycemic control. The fasting blood glucose declined by 10.3% to 9.83 ± 1.01 mmol/L and by 22.1% to 8.54 ± 1.27 mmol/L, while the HbA1c showed slight declines down to 6.74 ± 1.08% and to 6.30±0.9% after one and three months respectively.

<table>
<thead>
<tr>
<th>Diabetics with CAD</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Antioxidants)</td>
<td>n=10</td>
<td>n=10</td>
<td>n=10</td>
</tr>
<tr>
<td>Age range (years)</td>
<td>41-70</td>
<td>44-71</td>
<td>42-69</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>6/4</td>
<td>7/3</td>
<td>7/3</td>
</tr>
<tr>
<td>FBG (mmol/L)</td>
<td>4.8 ± 0.54</td>
<td>13.7 ± 2.94&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.0 ± 1.46&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.0 ± 0.51</td>
<td>6.9 ± 0.89&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.0 ± 1.08&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Homocysteine (μmol/ml)</td>
<td>11.6 ± 2.08</td>
<td>18.9 ± 2.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.7 ± 3.09&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>oxLDL-Ab (mU/ml)</td>
<td>304.1 ± 61.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>478.2 ± 172.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>457.9 ± 94.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Data presented as mean ± SD. Significantly different from control group by one-way ANOVA, FBG: Fasting blood glucose, HbA1c: glycated hemoglobin, ox-LDL Ab: oxidized low-density lipoprotein antibodies

Increased production of TBARS and perturbed glutathione system representing excessive oxidative stress was clear in the diabetic patients (Table 2). The blood levels of TBARS were about twice as high as the average of controls. This was coupled with lower levels of total and reduced GSH. The decrease was more pronounced in reduced GSH reaching 83.7% and 73.6% below control in groups I and II respectively. In contrast to the reduced GSH, plasma concentration of GSSG was higher in diabetics, being more than three-fold higher than the control value. The large decrease in GSH/GSSG ratio was indicative of the oxidative stress in diabetic patients. From the obtained glutathione results, the calculated redox potential confirmed the shift in the redox environment of the plasma of the diabetic patients toward a more oxidative state.

<table>
<thead>
<tr>
<th>Oxidative stress and antioxidant parameters in the plasma of the groups studied.</th>
<th>Control</th>
<th>CAD + antioxidants</th>
<th>CAD + captopril</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Group II</td>
<td>Group III</td>
<td></td>
</tr>
<tr>
<td>TBARS (mmol/L)</td>
<td>2.52 ± 0.46</td>
<td>5.08 ± 1.68&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.87 ± 1.30&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>rGSH (mmol/mL)</td>
<td>2.99 ± 0.48</td>
<td>1.25 ± 0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.37 ± 0.21&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>rGSH (mmol/mL)</td>
<td>2.76 ± 0.49</td>
<td>0.45 ± 0.26&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.33 ± 0.13&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>GSSG (mmol/mL)</td>
<td>0.12 ± 0.01</td>
<td>0.40 ± 0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.42 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>GSH/GSSG</td>
<td>23.9 ± 5.4</td>
<td>1.2 ± 0.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.7 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Redox potential (mV)</td>
<td>-139 ± 4.7</td>
<td>-78 ± 2.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-66 ± 4.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Data presented as mean ± SD. Significant difference from control value by one-way ANOVA. TBARS: thiobarbituric acid reactive substances calculated as malondialdehyde (MDA), rGSH: total glutathione, rGSH: reduced glutathione, GSSG: oxidized glutathione*

Adjunct treatment with antioxidant combination was effective in alleviating the stressful condition in diabetic plasma (Table 3). Even in the short follow-up period of three months, TBARS slowly but steadily declined towards the normal control level. Although the mean blood concentration declined by 31.1% from that of base line at the end of the follow-up period, it was still 51.6% higher than control. Improvement in the redox environment was also manifest in the changes of the glutathione values. The increase in total GSH was coupled
with a relative large elevation in reduced GSH and a decrease in GSSG, but the GSH/GSSG ratio was still far removed from that of control. A gradual improvement in the blood redox potential was evident, showing only 17.3% difference from control value. Adjunct treatment with captopril gave a similar qualitative pattern, but the quantitative improvement was less. This could be seen in the results of the different fractions of glutathione and the calculated redox potential at the end of the study period (Table 4), which was less prominent than those of the antioxidant treatment.

Table 3: Effect of adjunct treatment with antioxidant combination on oxidative stress and antioxidant parameters in type 2 diabetic patients with CAD (Group II)

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>1 month</th>
<th>3 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBARS (nmol/mL)</td>
<td>5.08 ± 1.6</td>
<td>4.40 ± 1.4</td>
<td>3.50 ± 0.9*</td>
</tr>
<tr>
<td>tGSH (nmol/mL)</td>
<td>1.52 ±0.06</td>
<td>1.79 ± 0.3</td>
<td>2.39 ± 0.39**</td>
</tr>
<tr>
<td>rGSH (nmol/ml)</td>
<td>0.45 ± 0.26</td>
<td>1.04 ± 0.26*</td>
<td>1.75 ± 0.30**</td>
</tr>
<tr>
<td>GSSG (nmol/mL)</td>
<td>0.40 ± 0.06</td>
<td>0.38 ± 0.11</td>
<td>0.32 ± 0.07**</td>
</tr>
<tr>
<td>GSH/GSSG</td>
<td>1.2 ± 0.6</td>
<td>3.0 ± 1.2*</td>
<td>5.4 ± 1.3*</td>
</tr>
<tr>
<td>Redox potential (mV)</td>
<td>-78 ± 8.2</td>
<td>-100 ± 7.3*</td>
<td>-115 ± 3.8**</td>
</tr>
</tbody>
</table>

Data presented as mean ± SD, *Significantly different from baseline value by paired t-test (p<0.05). **Significantly different from one month treatment by paired t-test (p<0.05). Number in parentheses represents percentage deviation from baseline value . TBARS: thiobarbituric acid reactive substances calculated as MDA, GSH: total glutathione, rGSH: reduced glutathione, GSSG: oxidized glutathione

Table 4: Effect of adjunct treatment with low-dose captopril on oxidative stress and antioxidant parameters in type 2 diabetic patients with CAD (Group III)

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>1 month</th>
<th>3 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBARS (nmol/mL)</td>
<td>4.87±1.21</td>
<td>4.69±1.30</td>
<td>3.99±1.02</td>
</tr>
<tr>
<td>tGSH (nmol/mL)</td>
<td>1.57±0.21</td>
<td>1.82±0.38</td>
<td>2.33±0.28**</td>
</tr>
<tr>
<td>rGSH (nmol/ml)</td>
<td>0.73±0.13</td>
<td>0.88±0.30*</td>
<td>1.61±0.28**</td>
</tr>
<tr>
<td>GSSG (nmol/mL)</td>
<td>0.42±0.05</td>
<td>0.40±0.05</td>
<td>0.36±0.04*</td>
</tr>
<tr>
<td>GSH/GSSG</td>
<td>1.7 ± 0.3</td>
<td>2.6 ± 0.4</td>
<td>4.3 ± 1.1*</td>
</tr>
<tr>
<td>Redox potential (mV)</td>
<td>-66±4.0</td>
<td>-75 ± 4.2*</td>
<td>-88 ± 4.9**</td>
</tr>
</tbody>
</table>

Data presented as mean ± SD, *Significantly different from baseline value by paired t-test (p<0.05). **Significantly different from one month treatment by paired t-test (p<0.05) Number in parentheses represents percentage deviation from baseline value . TBARS: thiobarbituric acid reactive substances calculated as MDA, tGSH: total glutathione, rGSH: reduced glutathione, GSSG: oxidized glutathione

The changes in the serum levels of the ox-LDL Ab following the adjunct therapy are illustrated in Figure 1. The oxidized LDL antibodies showed a strong response to the administration of the antioxidant combination represented by a 23.1% decline after one month and practically reaching mean control value at the end of 3 months. Again the effect of captopril was qualitatively similar with somewhat less quantitative response. After one month of treatment, the serum level of the ox-LDL Ab decreased by 12.6%, and by 26.4% after 3 months, being only 10.8% higher than the control value.

The results of adjunct therapy on the serum concentration of homocysteine are presented in Figure 2. The decline in the elevated homocysteine values in diabetic patients paralleled what was seen with ox-LDL Ab. However, the rate of decline was slower, reaching values at the end of the study still significantly higher than the control value of 11.58 μmol/ml. With the antioxidant combination, the mean homocysteine concentration in plasma, which was 18.88 ± 2.08 μmol/ml at baseline decrease by23.4% after one month and by 32.0% after 3 months of therapy to reach 12.86 ± 1.37 μmol/ml, which was 11.1% higher than the control value. The decline with captopril was somewhat slower. It decreased from a baseline value of 19.69 ± 3.09 μmol/ml to 15.89 ± 1.98 μmol/ml after one month and to 13.83 ± 2.96 μmol/ml at the end of the study; which was still 19.4% higher than control.
Fig. 1: Changes in plasma homocysteine concentration in type 2 diabetic patients with CAD following addition of antioxidant combination or low dose captopril to the basic antidiabetic treatment regimen.

Fig. 2: Changes in plasma oxidized LDL-Ab concentration in type 2 diabetic patients with CAD following addition of antioxidant combination or low dose captopril to the basic antidiabetic treatment regimen.

Discussion

Atherosclerosis has been characterized as an inflammatory disease of the blood vessel wall resulting from an initial injury that increases local oxidative stress. All the cardiovascular risk factors can initiate cellular events that lead to endothelial dysfunction by altering the redox state in the vessel wall. Complications of diabetes can be traced back to vascular origins. Hyperglycemia induces a large number of alterations in vascular tissue that potentially promote accelerated atherosclerosis. Among the sequel of hyperglycemia, oxidative stress has been suggested as a potential mechanism for atherosclerosis. A major mechanism of oxidative stress appears to be the hyperglycemia-induced intracellular ROS generated by mitochondrial electron transport chain leading to increased production of superoxide radicals (Rodenburg et al., 2006). Another mechanism involves the autoxidation of free glucose, which is catalyzed by transition metals yielding superoxide anion and hydrogen peroxide (Preisleben and Packer, 2009).

The results of the present study clearly indicated that there was a definite overproduction of free radicals and excessive exposure to oxidative stress in diabetic patients. The lipid peroxidation index, TBARS, was significantly higher in diabetic patients with CAD than in healthy individuals. Increased production of TBARS, which was coupled with depressed levels of total glutathione, depletion of reduced GSH, and lower levels of the redox potential in type 2 diabetics with CAD, clearly indicated that these patients suffered a strong oxidative stress, compared to non-diabetic controls. The addition of antioxidants or captopril to the treatment regimen of the diabetic patients resulted in improvement in their glycemic status. Such improvement, represented by statistically significant decreases in the fasting glucose and HbA1c levels, probably reflects a tendency towards
overall improvement in general health and tissue metabolic status and alleviation of oxidative stress (El-Bassiouni et al., 2007). The better control of the glycemic status seen by the end of the first month of the study probably indicates a very rapid action of the adjunct therapy. It has been reported that scavenging antioxidants in combination act synergistically (Giugliano et al., 2001), while captopril has been shown to increase endogenous oxidant scavengers in mouse tissue (De Cavangh et al., 2002).

Many reactions associated with hyperglycemia may acutely and chronically increase the production of free radicals, resulting in an oxidant/antioxidant imbalance (Preisleben and Packer, 2009; Jeremy et al., 2001). Glutathione is the dominant intracellular non-protein thiol and the single largest source of reducing equivalents accounting for about 90% of these equivalents (Rosen et al., 2005). Therefore, the depletion of reduced glutathione could affect the overall redox potential significantly. The present study found decreased levels of total and reduced glutathione, with increased GSSG. Many investigations have reported a lower concentration of GSH in the plasma of diabetic patients (El-Bassiouni et al., 2007; Draper et al., 2003). In addition to the oxidant/antioxidant imbalance, the decreased level of glutathione could be influenced by decreased activity of certain enzymes such as γ-glutamylcysteine synthase and glutathione reductase, possibly because of their glycation by hyperglycemia (Jialal et al., 1995). In view of the observed decreased HbA1c, as indication of protein glycation, it is possible that the recovery of the glycated glutathione metabolizing enzymes will depend more on the turn-over rate of their proteins, and this could be one reason for the relative rapid increase in total and reduced glutathione.

By inspecting the calculated redox potential in the present study, it became clear that redox potential for the diabetic patients with CAD was shifted towards the oxidizing side, and the supplementation with the antioxidant combination or captopril for three months partially corrected the balance of GSH/GSSG to restore the reducing potentials.

Oxidative modification of LDL has been implicated as a major factor in the pathogenesis of coronary atherosclerosis. Some studies have demonstrated that ox-LDL levels are significantly higher in patients with diabetes mellitus than in control subjects, and that the high levels of circulating ox-LDL can serve as an independent and significant predictor for future cardiac events in type 2 diabetic patients (Berlinger and Heinecke, 1996; El-Bassiouni et al., 2007). The levels of circulating ox-LDL antibodies were found to be significantly higher in patients with type 2 diabetes than in control subjects. Such higher levels were taken as indirect indication that the levels of ox-LDL were increased. The definite and steady decline in the circulating ox-LDL antibodies was clear and is indicative of improvement in the atherosclerosis condition. Vitamin E, a component of the antioxidant mixture used and the major fat soluble antioxidant present in the LDL particle, is believed to protect LDL from oxidative damage by acting as a chain breaking antioxidant and preventing lipid peroxidation of polyunsaturated fatty acids and modification of proteins by ROS (Porkkala-Sarataho et al., 1996). Among the endogenous plasma antioxidants, ascorbic acid is particularly active in inhibiting lipid peroxidation induced by different types of oxidative stress. Supplementation with vitamin E and vitamin C has a potential role in boosting antioxidant defense (Packer et al., 2004). Moreover, decreasing the synthesis and/or blocking the action of ANGII improves endothelial dysfunction and slows the progression of atherosclerosis in diabetic patients. However, the response of the circulating ox-LDL Ab occurred early the short study period of three months, there was no equivalent reciprocal effect on oxidative stress and others parameters tested. It has been suggested that ox-LDL levels but not ox-LDL Ab titer may serve as an independent indicator for evaluation of atherosclerosis in type 2 diabetic patients (El-Bassiouni et al., 2007). The results of the present study seem to strengthen this notion.

A strong relation has been demonstrated between homocysteine and ox-LDL, since homocysteine autoxidation has been shown to support the oxidation of LDL, not only through generation of the superoxide anion radical, but also by reducing the antioxidant status and affecting nitric oxide production, which could injure vascular endothelial cells (Prasad, 1999 and Lonn et al., 2006). In the present study, the adjunct treatment with antioxidants or captopril was accompanied by a parallel decrease in the elevated levels of homocysteine. The response was rapid, reaching levels close to the mean control values at the end of the study period. Such lower levels may contribute to the protection against the injury of endothelium leading to slowing of the progress of the atherogenic process.

The shift in all oxidative stress indices, observed in the present study, towards normal values and the possible slowing down of the progress or even the regression of the process of atherosclerosis make the use of antioxidants or low-dose captopril as adjunct therapy in patients with type 2 diabetes worth pursuing in a larger clinical study. It is possible that the indices for atherosclerosis could be brought down to and maintained within normal values with sustained use of the suggested adjunct therapies.

References


Jeremy Lyons, MD; Astrid Rauh-Pfeiffer, MD; and; Yong Ming-Yu, MD, 2001. Cysteine metabolism and whole blood glutathione synthesis in septic pediatric patients. Faseb J., 29: 69-83.


