Alpha-Lipoic Acid Ameliorates Oxidative Stress by Increasing Aldehyde Dehydrogenase-2 Activity in Patients with Acute Coronary Syndrome

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Aldehyde dehydrogenase-2 (ALDH2) is the main enzyme responsible for acetaldehyde oxidation in ethanol metabolism and also provides protection against oxidative stress. Alpha-lipoic acid (α-LA), a natural dithiol compound with antioxidant properties, has been reported to increase ALDH2 activity in cultured cells. We analyzed the therapeutic efficacy of α-LA in 63 patients with confirmed acute coronary syndrome (ACS). These patients (52 men and 11 women, with age range 49-72 years) were randomized into two groups: untreated group (n = 30) and α-LA group (n = 33). Patients in the α-LA group were given an intravenous injection of 600 mg α-LA every day for 5 days while the patients in the untreated group were given saline. An isoprostane, 8-iso-prostaglandin F2α (8-iso-PGF2α), one product of arachidonic acid metabolism, was measured as a marker for oxidative stress. The serum levels of 8-iso-PGF2α and ALDH2 activity were determined at admission to the hospital (time 0), and at 24 hours and 1 week after treatment. At 24 hours and 1 week after treatment, ALDH2 activity was significantly higher in the α-LA group than in the untreated group (P < 0.05), whereas the levels of 8-iso-PGF2α were significantly lower in the α-LA group than in the untreated group (all P < 0.05). Importantly, the decrease of 8-iso-PGF2α levels correlated with the increased ALDH2 activity at both 24 hours (r = 0.6234, P < 0.001) and 1 week after treatment (r = −0.3941, P = 0.0014). α-LA may ameliorate oxidative stress through up-regulating ALDH2 activity in patients with ACS.

Keywords: acute coronary syndrome; aldehyde dehydrogenase-2; alpha lipoic acid; antioxidant; oxidative stress

Acute coronary syndrome (ACS) is related to the vulnerability of atherosclerotic plaques. Plaque rupture with thrombosis and vasospasm is the most important cause of ACS (Fuster et al. 1992). Oxidative stress plays an important role in the instability of atherosclerotic plaques, as well as in atherogenesis (Rajagopalan et al. 1996; Azumi et al. 2002). Studies have confirmed that oxidative stress levels are markedly increased in patients with ACS compared with patients with stable angina pectoris (Azumi et al. 2002; Szuldrzyński et al. 2010). Oxidative stress is a condition characterized by elevated levels of intracellular reactive oxygen species (ROS), which are produced by activated platelets, vascular smooth muscle cells, and various inflammatory cells in a range of conditions, such as tissue hypoxia (Maritim et al. 2003). Moreover, increased ROS production may play a major role in modulating plaque instability (Rajagopalan et al. 1996; Channon 2002). ROS production can promote formation of oxidized low-density lipoprotein (oxLDL), allowing scavenger receptor-mediated uptake of oxLDL into macrophages, leading to formation of activated foam cells (Azumi et al. 2002). In addition, oxidation of arachidonate by ROS generates a large variety of derivatives known as isoprostanes (IsoPs), which are isomers of prostaglandins. IsoPs are bioactive products of arachidonic acid metabolism, and markers of oxidative stress. One of the most stable IsoPs, 8-iso-prostaglandin F2α (8-iso-PGF2α), has become the “gold standard” measure of oxidative stress in vivo (Morrow 2005).

Alpha-lipoic acid (α-LA), a natural dithiol compound with excellent antioxidant properties, is known to be a cofactor for mitochondrial dehydrogenase. There are reports that α-LA is able to reduce myocardial injury and preserve cardiac function during ischemia-reperfusion injury (IRI) (Schonheit et al. 1995; Wang et al. 2011), and these mecha-
nisms are involved in the inhibition of IRI-induced oxidative stress by α-LA. However, to date, most studies of α-LA have focused on IRI, whereas the effect and mechanism of α-LA on myocardial ischemia without reperfusion is not fully understood.

Recently, α-LA was reported to increase the activity of mitochondrial aldehyde dehydrogenase-2 (ALDH2) by ~ 60% in isolated rat heart mitochondria (Wenzel et al. 2007). ALDH2, the main enzyme responsible for acetaldehyde oxidation in ethanol metabolism, is thought to be responsible for oxidation and detoxification of aromatic and aliphatic aldehydes (Bosron and Li 1986; Ohsawa et al. 2003; Vasiliou and Nebert 2005; Bian et al. 2010). In a previous study, we found that the activity and expression of ALDH2 in the diabetic rat heart could be inhibited by hyperglycemia-induced oxidative stress, and that administration of antioxidants ameliorated these changes (Wang et al. 2011). Other authors have reported that over-expression of ALDH2 can down-regulate ROS generation and provide protection against oxidative stress (Choi et al. 2011; Hu et al. 2011). We therefore hypothesized that α-LA, by up-regulating ALDH2 activity, could inhibit ischemia-induced oxidative stress in patients with ACS. To test this hypothesis, we selected ACS patients without receiving a revascularization strategy. We first evaluated the effect of α-LA on ALDH2 activity and 8-iso-PGF2α levels, and then applied correlation analysis to determine the association between the change in ALDH2 activity and the 8-iso-PGF2α levels.

**Methods**

**Study population**

We enrolled 63 consecutive patients (52 men and 11 women, age range 49-72 years) who were admitted to the emergency department of Qilu Hospital of Shandong University from September 2011 to March 2012. Inclusion criteria were: symptoms suggestive of cardiac ischemia plus electrocardiographic changes (ST depression or transient elevation of ≥ 1 mm or T-wave changes in ≥ 2 leads), or positive results for troponin I at admission and/or on serial testing. Patients with ACS who underwent percutaneous coronary intervention (PCI) within 1 week after admission were excluded from this study. Other exclusion criteria were as follows: acute infection; use of oral antioxidants, warfarin, immunosuppressants, cytotoxic drugs, or anti-inflammatory drugs (for example, non-steroidal anti-inflammatory drugs (NSAIDs)); and serious diseases such as malignancies, autoimmune disorders, renal failure, hepatic failure, and heart failure (New York Heart Association class III/IV).

The patients were randomized into two groups: the untreated group (n = 30) and the α-LA group (n = 33). Patients in the α-LA group were given an intravenous injection of 600 mg α-LA every day for 5 days, while patients in the untreated group were given a saline injection. Both groups of patients were also given the routine treatment according to the updated guidelines for ACS in China, including aspirin (100 mg/day), clopidogrel (75 mg/day), and statins (atorvastatin 20 mg/day).

The study was approved by the ethics committee of Qilu Hospital of Shandong University, and informed consent was obtained from all patients.

**Measurements of blood biomarkers**

Blood samples from all patients were collected on admission to the hospital (time 0), and at 24 hours and 1 week after treatment initiation. Blood was collected by venipuncture into two foil-wrapped tubes containing 5 mL EDTA. The tubes were placed in a centrifuge at 4°C and spun at 1,500 rpm for 10 minutes. The serum was removed into 2 mL cryovials, and stored at ~80°C.

Serum 8-iso-PGF2α levels were measured by a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) kit (Shanghai Westang Bio-Tech Inc., Ltd., Shanghai, China) according to the manufacturer’s protocol. The serum levels of high sensitivity C-reactive protein level (hs-CRP) were measured by an immunonephelometric assay on the BNII ProSpec nephelometer (Dade Behring, Siemens Healthcare Diagnostics, Germany).

**Enzymatic activity of ALDH2**

ALDH2 activity was assayed using a commercial kit (GenMed Sciences Inc., Wilmington, DE, USA), according to the manufacturer’s instructions. The activity of ALDH2 was determined by monitoring the formation of NADH from NAD+ at 340 nm in a spectrophotometer (Beckman Coulter, Chaska, MN, USA). The assay mixture (0.2 mL) contained 100 mmol/L Tris-HCl (pH 8.5), 1 mmol/L NAD+, 1 mmol/L 4-methylpyrazole, and 50 μg protein. The reaction was started by the addition of 1 mmol/L acetaldehyde or propionaldehyde to the cuvette. Enzyme-specific activity was expressed as nmol NADH per min per mg protein.

**Statistical analysis**

All statistical analyses were performed with the SPSS software package (version 16.0; SPSS Inc, Chicago, IL, USA), and data are presented as mean ± SEM. For comparisons between the two groups, the unpaired Student t-test was used as appropriate, and χ² analysis was used to compare categorical data. The correlations between two variables were assessed by Pearson’s or Spearman’s correlation analysis. P < 0.05 (two-tailed) was considered statistically significant.

**Results**

**General characteristics of enrolled patients**

There were no significant differences between the two groups with regard to age, gender, blood pressure, diabetes, smoking or drinking habits, family history of cardiovascular disease (CVD), or medication during hospitalization (Table 1). There was also no significant difference in baseline levels of serum lipid, glucose, troponin I, alanine transaminase and creatinine between the two groups.

**ALDH2 activity and blood biomarkers**

At baseline (time 0), there were no significant differences between the α-LA and the untreated group in ALDH2 activity (4.31 ± 1.79 vs. 4.23 ± 2.28 nmol NADH/min/mg, respectively; p > 0.05) or 8-iso-PGF2α (1.347.30 ± 215.37 vs. 1.276.03 ± 240.10 μmol/L; p > 0.05). However, ALDH2 activity was significantly higher in the α-LA group compared with the untreated group at 24 hours after treatment (9.21 ± 2.41 vs. 6.66 ± 2.20 nmol NADH/min/mg protein, respectively; p < 0.01) and at 1 week after treatment (7.26 ± 1.56 vs. 5.39 ± 2.27 nmol NADH/min/mg protein; p < 0.05) (Fig. 1). By contrast, the levels of 8-iso-PGF2α were sig-
α-LA Inhibits Oxidative Stress in Patients with ACS

Table 1. Demographics of patient population at initial admission, and details of drugs given during hospitalization.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Untreated group (n = 30)</th>
<th>α-LA group (n = 33)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>61 ± 9</td>
<td>60 ± 10</td>
<td>NS</td>
</tr>
<tr>
<td>Males, n (%)</td>
<td>25 (83.3)</td>
<td>27 (81.8)</td>
<td>NS</td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td>11 (36.7)</td>
<td>12 (36.4)</td>
<td>NS</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>8 (26.7)</td>
<td>9 (27.3)</td>
<td>NS</td>
</tr>
<tr>
<td>Smoking, n (%)</td>
<td>10 (33.3)</td>
<td>11 (33.3)</td>
<td>NS</td>
</tr>
<tr>
<td>Drinking, n (%)</td>
<td>10 (33.3)</td>
<td>11 (33.3)</td>
<td>NS</td>
</tr>
<tr>
<td>Family history of CVD, n (%)</td>
<td>4 (13.3)</td>
<td>6 (18.2)</td>
<td>NS</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>129 ± 15</td>
<td>139 ± 19</td>
<td>NS</td>
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<tr>
<td>Diastolic BP (mmHg)</td>
<td>74 ± 10</td>
<td>78 ± 15</td>
<td>NS</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>1.64 ± 0.94</td>
<td>1.57 ± 0.94</td>
<td>NS</td>
</tr>
<tr>
<td>CHO (mmol/L)</td>
<td>4.17 ± 1.07</td>
<td>4.61 ± 1.62</td>
<td>NS</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.03 ± 0.22</td>
<td>1.24 ± 0.29</td>
<td>NS</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>2.59 ± 0.83</td>
<td>2.81 ± 1.37</td>
<td>NS</td>
</tr>
<tr>
<td>Troponin I (ng/ml)</td>
<td>1.09 ± 1.62</td>
<td>1.67 ± 1.56</td>
<td>NS</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>6.18 ± 2.77</td>
<td>6.63 ± 2.83</td>
<td>NS</td>
</tr>
<tr>
<td>Alanine transaminase (U/L)</td>
<td>27.9 ± 16.7</td>
<td>29.5 ± 18.3</td>
<td>NS</td>
</tr>
<tr>
<td>Creatinine (μmol/L)</td>
<td>67.7 ± 16.9</td>
<td>69.6 ± 19.6</td>
<td>NS</td>
</tr>
<tr>
<td>Medicine</td>
<td></td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Aspirin, n (%)</td>
<td>30 (100)</td>
<td>33 (100)</td>
<td></td>
</tr>
<tr>
<td>Clopidogrel, n (%)</td>
<td>30 (100)</td>
<td>33 (100)</td>
<td></td>
</tr>
<tr>
<td>Statins, n (%)</td>
<td>30 (100)</td>
<td>33 (100)</td>
<td></td>
</tr>
<tr>
<td>B-blockers, n (%)</td>
<td>10 (33.3)</td>
<td>11 (33.3)</td>
<td></td>
</tr>
<tr>
<td>Nitrates, n (%)</td>
<td>28 (93.3)</td>
<td>29 (87.9)</td>
<td></td>
</tr>
</tbody>
</table>

CVD, cardiovascular diseases; NS, not significant.

Fig. 1. Time-dependent increase in serum ALDH2 activity in the α-LA group.
Shown are ALDH2 activities (nmol NADH/min.mg protein) in the untreated group and the α-LA group. #p < 0.01 vs. untreated, *p < 0.05 vs. untreated.

Significantly lower in the α-LA group than in the untreated group at 24 hours (1,007.86 ± 195.11 vs. 1,138.68 ± 208.03, respectively ρ/pg/L; p < 0.05) and 1 week after treatment (852.09 ± 200.29 vs. 1,002.29 ± 184.44 ρ/pg/L; p < 0.05) (Fig. 2).

Spearman correlation analysis showed that the decrease in 8-iso-PGF2α levels correlated negatively with the increased ALDH2 activity both at 24 hours (r = −0.6234, p < 0.001) (Fig. 3A) and at 1 week after treatment (r = −0.3941, p = 0.0014) (Fig. 3B). No correlation between hs-CRP and ALDH2 activity was observed.

At 24 hours after α-LA treatment, no significant differ-
Fig. 2. Time-dependent decrease in serum levels of 8-iso-PGF2α in the α-LA group. Shown are serum levels of 8-iso-PGF2α (ρ/pg.L⁻¹) in the untreated group and the α-LA group. *p < 0.05 vs. untreated.

Fig. 3. Relationship of ALDH2 activity with 8-iso-PGF2α levels at different times. The decrease in 8-iso-PGF2α levels was negatively associated with the increase in ALDH2 activity over the same period, including (A: between 0 and 24 hours) and (B: between 0 hours and 7 days).
ence was found in hs-CRP levels between the α-LA and the untreated group (12.13 ± 3.70 vs. 14.46 ± 4.14 μg/mL, respectively; p > 0.05) (Fig. 4), but at 1 week after treatment, the hs-CRP levels were significantly decreased in the α-LA group compared to the untreated group (3.48 ± 2.02 vs. 5.53 ± 3.39 μg/mL; p < 0.01) (Fig. 4).

**Discussion**

This study compared oxidative stress, estimated by levels of 8-iso-PGF2α, between ACS patients with and without α-LA treatment. The major findings of the present study are: 1) α-LA was able to significantly lower 8-iso-PGF2α levels and up-regulate ALDH2 activity in patients with ACS; and 2) there was a significant negative correlation between decreased 8-iso-PGF2α levels and increased ALDH2 activity, which indicates that the effect of α-LA on oxidative stress may be mediated through improvement in ALDH2 activity.

α-LA is a natural compound that is found in most foods. In humans, α-LA can be synthesized by the liver and other tissues, and it functions as a co-factor for a number of metabolic enzymes, such as pyruvate dehydrogenase and α-keto-glutarate dehydrogenase (Dudek et al. 2008; Ghibu et al. 2009a). α-LA and its reduced dithiol form, dihydrolipoic acid, are powerful antioxidants (Ghibu et al. 2009b). Most studies on antioxidants have failed to show any benefits for clinical outcome in patients with stable angina pectoris, but their possible effect in ACS is still under discussion. In this study, we selected ACS patients without receiving any revascularization strategy, in order to exclude the effect of ischemia-reperfusion injury, and investigated the effect and mechanisms of α-LA on the elevated levels of 8-iso-PGF2α in patients with ACS (Azumi et al. 2002; Szuldrzyński et al. 2010). 8-iso-PGF2α is not only a marker of oxidative stress but also a biologically active molecule. It promotes atherosclerosis and attenuates angiogenesis by activating the thromboxane receptor (Benndorf et al. 2008), which contributes to platelet activation (Davi and Patrono 2007). Moreover, increased levels of 8-iso-PGF2α were found to be an independent risk factor of atherosclerosis (Schwedhelm et al. 2004). Our results show that α-LA can significantly lower 8-iso-PGF2α levels in patients with ACS, indicating that α-LA could provide more protection against oxidative stress in ischemic myocardium.

Measurement of hs-CRP is an established tool for detecting systemic inflammation, and a number of studies have shown that hs-CRP level is associated with future adverse cardiac events in patients with ACS. However, in contrast to 8-iso-PGF2α, the levels of hs-CRP levels could not be decreased by α-LA until after 1 week of treatment. This result suggests that the effect of α-LA on inflammation is slower than its effect on oxidative stress, which might be the result of inhibited oxidative stress.

In our previous study, we found that the activity and expression of ALDH2 in the diabetic rat heart could be inhibited by hyperglycemia-induced oxidative stress (Wang et al. 2011). Others have shown that over-expression of ALDH2 can downregulate ROS generation and protect against oxidative stress (Hu et al. 2011; Choi et al. 2011). Studies have also demonstrated that α-LA could increase ALDH2 activity by ~ 60% in isolated rat heart mitochondria (Wenzel et al. 2007). Our results from the present study showed that α-LA was able to significantly reduce serum levels of 8-iso-PGF2α, which was accompanied by up-regulation of ALDH2 activity. Importantly, the decrease in 8-iso-PGF2α levels correlated negatively with the increased ALDH2 activity, which indicates that the regulatory effect of α-LA acts through a mechanism involving ALDH2 activity improvement. These results are in accordance with a recent study by He et al., who showed that IRI in rats is also modulated by lipoic acid through a mechanism involving ALDH2 activation, and that the regulatory effect of lipoic acid on ALDH2 activity is dependent on the protein kinase Ce signaling pathway (He et al. 2012).
An important limitation of the present study was that it was a single-center, small-scale study. Additional investigations in a multicenter setting and with larger study populations are needed. We also did not use any control group for patients without ACS in our study. The use of other treatments (e.g., statins, aspirin, and clopidogrel) recommended by the updated guidelines in China could also have affected oxidative stress (Rubba 2007; Hu et al. 2011; Chen et al. 2012). However, this treatment plan was strictly implemented during hospitalization for all patients; thus all patients received the same drugs (statins, aspirin, and clopidogrel) at the same dose. Finally, the clinical events of these patients are still being followed up, and are not reported in this study.

**Conclusion**

In summary, our results have demonstrated that α-LA, a potent antioxidant, may reduce oxidative stress in patients with ACS, which might be through a mechanism involving improvement in ALDH2 activity.

**Acknowledgments**

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**Conflict of Interest**

The authors have no conflict of interest to declare.

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