Xuezhikang, an Extract of Cholestin, Protects Endothelial Function Through Antiinflammatory and Lipid-Lowering Mechanisms in Patients With Coronary Heart Disease

Shui Ping Zhao, MD, PhD; Ling Liu, MD, PhD; Yan Chun Cheng, MD, PhD; Mehdii H. Shishehbor, DO; Ming Hui Liu, MD; Daoquan Peng, MD, PhD; Yu Ling Li, MD

Background—Endothelial dysfunction is associated with inflammation and postprandial hypertriglyceridemia. Xuezhikang, an extract of Cholestin, a dietary supplement, has lipid-modulating and antiinflammatory effects. We explored the effects of xuezhikang on endothelial function and high-sensitivity C-reactive protein (hs-CRP) in patients with coronary heart disease (CHD).

Methods and Results—We prospectively randomized 50 CHD patients to xuezhikang 1200 mg/d or placebo for 6 weeks. Fasting hs-CRP concentrations, flow-mediated vasodilation (FMD) at 0 and 4 hours, and lipid parameters at 0, 2, 4, and 6 hours were monitored after a high-fat meal (800 calories; 50 g fat) in all patients. All patients underwent a high-fat meal test at the beginning of the study and after 6 weeks of treatment. Postprandial FMD was significantly worse at 4 hours after a high-fat meal ($P<0.05$), and this was associated with the area under the triglyceride curve (TG-AUC) ($r=0.345, P<0.01$). After 6 weeks of xuezhikang, fasting hs-CRP levels and TG-AUC ($P<0.001$ for each) decreased. Furthermore, preprandial and postprandial FMD significantly improved ($P<0.001$). There were no significant changes in serum lipids and FMD in the placebo arm. In multivariable regression analysis, changes in TG-AUC and fasting hs-CRP levels were predictive of improvement in preprandial FMD ($P<0.05$).

Conclusions—Xuezhikang effectively improved preprandial and postprandial endothelial function through its potent antiinflammatory and lipid-lowering effects. (Circulation. 2004;110:915-920.)

Key Words: postprandial period ■ vasodilation ■ blood flow ■ coronary disease ■ xuezhikang
were given a high-fat meal. Blood samples were drawn at 0, 2, 4, and 6 hours. Fasting CRP concentrations and serum total cholesterol, triglyceride, HDL cholesterol (HDL-C), and LDL cholesterol (LDL-C) concentrations during fasting and postprandial states were measured. Endothelial function was evaluated at baseline and 4 hours after the high-fat meal.

Subsequently, patients were randomly divided into 2 groups: 25 patients were treated with xuezhikang (300 mg Cholestin per capsule, WBL Peking University Biotech Co, Ltd) 1200 mg/d, and the rest were given a matching placebo (routine group) for 6 weeks. In addition, all patients were subjected to dietary control and were treated with aspirin (100 mg/d), metoprolol, fosinopril, and nitrates. At the end of the 6 weeks, each subject had the same high-fat meal test again, and the following parameters were measured: fasting CRP, total cholesterol, HDL-C, LDL-C, and triglyceride concentrations during fasting and postprandial states. Endothelial function was also assessed again at baseline and 4 hours postprandially.

Oral High-Fat Tolerance Test
The oral high-fat tolerance test was undertaken as described previously by a nutritionist.1,13 The high-fat meal consisted of 800 calories with 50 g of fat (345 mg of cholesterol), 28 g of protein, and 60 g of carbohydrates.

Brachial Artery Vasodilation Measurement
Endothelial function was measured by a previously described non-invasive technique.1,13 All imaging was performed by a single, highly skilled sonographer who was unaware of the study assignment. Brachial artery diameter was imaged with a 10-MHz linear high-frequency probe (Acuson Sequoia) by an experienced ultrasonographer who was unaware of the study assignment. Brachial artery diameter was imaged with a 10-MHz linear high-frequency probe (Acuson Sequoia) by an experienced ultrasonographer who was unaware of the study assignment. At baseline (D0) and after reactive hyperemia (D1) and sublingual nitroglycerine (D2) were recorded. The FMD [(D1−D0)/D0×100%] was used as a measure of endothelium-dependent vasodilation. The nitroglycerine-induced vasodilatation (NID) [(D1−D0)/D0×100%] was used as a measure of endothelium-independent vasodilatation. Arterial blood flow was measured as Doppler flow velocity multiplied by the cross-sectional area (πr²).

Laboratory Assays
Blood samples were separated at 4°C and stored at −20°C. Serum total cholesterol, triglyceride, HDL-C, and LDL-C concentrations were measured on a Hitachi 7170A analyzer by a specialist who was unaware of the study assignment.

High-sensitivity CRP (hs-CRP) was measured at 550 nm with the use of the Particle Enhanced Immunoturbidimetric Assay (Orion Diagnostica).

### Statistical Analysis
Data were analyzed with the use of SPSS (version 10.0) and are presented as mean±SD unless otherwise indicated. Log transformation was made for distribution-dependent analyses. Differences between intragroup and intergroup means were analyzed by t test or 1-way ANOVA. Coefficients of correlation (r) were calculated by the Pearson correlation analysis. Multiple stepwise regression analysis was used to define the influence of the changes of serum lipid and hs-CRP levels on the change of FMD. Postprandial triglyceride area under the curve (TG-AUC) over the fasting concentration was calculated by the trapezoidal method. Statistical significance was assumed at P<0.05.

### Results
Baseline characteristics of the patients are shown in Table 1. Both groups were similar in regard to age, gender, body mass index, blood pressure, and use of medications such as β-blockers, diuretics, calcium channel blockers, and nitrates. Furthermore, there were no significant differences in fasting lipids, hs-CRP concentrations, and TG-AUC at baseline between the 2 groups (Table 2). Similarly, fasting NID, FMD, and postprandial worsening of FMD were comparable between the xuezhikang group and the placebo group at baseline (Figure 1).

### Table 1. Baseline Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Xuzhikang Group (n=25)</th>
<th>Routine Group (n=25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>58.2±4.2</td>
<td>59.1±6.3</td>
</tr>
<tr>
<td>Gender, male/female</td>
<td>15/10</td>
<td>14/11</td>
</tr>
<tr>
<td>Smoker, %</td>
<td>36</td>
<td>40</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>25.2±3.4</td>
<td>25.7±2.1</td>
</tr>
<tr>
<td>Blood pressure, mm Hg</td>
<td>126/82</td>
<td>127/80</td>
</tr>
<tr>
<td>Fasting glucose, mmol/L</td>
<td>5.62±0.48</td>
<td>5.51±0.52</td>
</tr>
<tr>
<td>History of hypertension, % (n)</td>
<td>20 (5)</td>
<td>20 (5)</td>
</tr>
<tr>
<td>Medication history, % (n)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-Blocker</td>
<td>20 (5)</td>
<td>20 (5)</td>
</tr>
<tr>
<td>Diuretic</td>
<td>8 (2)</td>
<td>12 (3)</td>
</tr>
<tr>
<td>Calcium channel blocker</td>
<td>12 (3)</td>
<td>16 (4)</td>
</tr>
<tr>
<td>Nitrate</td>
<td>24 (6)</td>
<td>20 (5)</td>
</tr>
</tbody>
</table>

### Table 2. Baseline and 6-Week Lipid and hs-CRP Levels in the Xuezhikang and Placebo Groups

<table>
<thead>
<tr>
<th></th>
<th>Xuzhikang Group (n=25)</th>
<th>Routine Group (n=25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>5.37±0.51</td>
<td>5.37±0.46</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.15±0.20</td>
<td>1.15±0.14</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>3.2±0.38</td>
<td>3.35±0.35</td>
</tr>
<tr>
<td>Triglyceride, mmol/L</td>
<td>1.7±0.48</td>
<td>1.7±0.35</td>
</tr>
<tr>
<td>TG-AUC, mmol/L (0.6 h)</td>
<td>6.15±2.78</td>
<td>6.13±2.27</td>
</tr>
<tr>
<td>hs-CRP, mg/L</td>
<td>2.70 (1.70, 4.13)</td>
<td>2.70 (1.95, 3.65)</td>
</tr>
<tr>
<td>log(hs-CRP)</td>
<td>0.40±0.25</td>
<td>0.39±0.24</td>
</tr>
</tbody>
</table>

Values are mean±SD except that hs-CRP is shown on the original scale (median [lower, upper quartile]) and on the log scale (mean±SD).

*P<0.001, †P<0.05 compared with baseline.
The serum total cholesterol, LDL-C, and HDL-C concentrations did not change significantly in the postprandial period (data not shown), whereas the postprandial triglyceride concentrations increased significantly at 2, 4, and 6 hours \((P<0.05)\) (Figure 2).

FMD decreased significantly at 4 hours after a high-fat meal \((7.91\pm3.16\% \text{ versus } 5.34\pm2.78\%; \ P<0.05; \ n=50)\). Baseline artery diameter, blood flow, reactive hyperemia flow (data not shown), and NID were not affected by the high-fat meal (Figure 1). After 6 weeks of treatment, the fasting triglyceride, total cholesterol, and LDL-C concentrations decreased, whereas HDL-C concentration increased significantly in response to xuezhikang \((P<0.001)\) (Table 2). The postprandial serum triglyceride concentrations at all time points (2, 4, and 6 hours) (Figure 2) and TG-AUC decreased significantly in the xuezhikang group \((P<0.001)\), accompanied by a 50% reduction in the fasting hs-CRP concentration (Table 2 and Figure 3). Placebo therapy had no significant effect on the fasting lipids concentrations. Patients treated with xuezhikang had a

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**Figure 1.** Effects of a high-fat meal on FMD and NID in CHD patients after 6 weeks of xuezhikang treatment vs placebo (routine group). Values are presented as mean±SEM. *\(P<0.05\) compared with preprandial FMD within 1 day; †\(P<0.05\) compared with preprandial FMD at baseline; ‡\(P<0.05\) compared with postprandial FMD at baseline.

**Figure 2.** Changes in serum triglyceride concentrations in response to a high-fat meal in the xuezhikang group and placebo (routine) group before and after 6 weeks. Values are presented as mean±SEM. †\(P<0.05\) compared with fasting triglyceride concentration; ‡\(P<0.001\) compared with baseline triglyceride concentration at the same time point in the xuezhikang group. X indicates xuezhikang group; R, routine group.

**Figure 3.** Changes of serum hs-CRP concentrations in CHD patients after 6-week xuezhikang treatment vs placebo (routine group).
significantly greater reduction of hs-CRP concentration than
the routine group (50.0% versus 25.4%; P < 0.05). There was
a significant correlation between changes in hs-CRP concen-
tration and TG-AUC in the xuezhikang group (r = 0.441,
P < 0.001), whereas no significant correlation was seen be-
tween the changes in hs-CRP and other lipid parameters (data
not shown).

Both preprandial and postprandial FMD significantly im-
proved compared with baseline (P < 0.05), and no significant
worsening of FMD after a high-fat meal was observed in the
xuezhikang group. However, individuals in the routine group
had no significant change in FMD after 6 weeks, and postpran-
dial impairment was still present in this group. NID did not
change during xuezhikang or placebo therapy (Figure 1).

When we analyzed all 50 patients, worsening of postpran-
dial FMD was significantly correlated with TG-AUC (r = 0.345,
P < 0.01), and improvement of postprandial FMD was correlated with the decrement of TG-AUC (r = 0.455,
P < 0.01).

The improvement of preprandial FMD was significantly
 correlated with decrements of TG-AUC (r = 0.427,
P < 0.01), hs-CRP (r = 0.388, P < 0.05), and total cholesterol (r = 0.390, P < 0.01) concentrations and the increment of HDL-C concentration (r = 0.356, P < 0.05). In multiple
stepwise regression analysis, only the decrements of TG-
AUC and hs-CRP concentration independently and signif-
icantly predicted the improvement of preprandial FMD
(P < 0.05) (Figure 4).

Discussion

The postprandial state plays a critical role in atherogenesis.
Acutely impaired endothelium-dependent vasodilation after a
high-fat meal has been found to be associated with postpran-
dial hypertriglyceridemia.2,13,14 Furthermore, postprandial
hypertriglyceridemia may induce endothelial dysfunction
through the direct effects of triglyceride-rich lipoproteins3,4
and increasing oxidative stress in circulation.1,15,16 These data
indicate that lowering postprandial triglyceride concentration
may lead to improvement of endothelial function.

In this randomized, placebo-controlled study, we showed
that xuezhikang, an extract of Cholestin, significantly de-
creased postprandial triglyceride concentrations. Further-
more, patients in the xuezhikang group were protected from
postprandial endothelial dysfunction, as measured by FMD.
In addition, xuezhikang led to a significant reduction in the
inflammatory marker hs-CRP.

The effect of lipid-lowering treatment on endothelial func-
tion has been controversial. Fibrates improved postprandial
endothelial function in type 2 diabetes.17 However, this effect
was not observed in healthy subjects,14 even though postpran-
dial hypertriglyceridemia was attenuated and fasting FMD
was improved.18 Recently, evidence showed that statins
reduced remnant-like particles–cholesterol14 and serum tri-
glyceride15 after an oral fat load. In addition, statin therapy
attenuated postprandial endothelial dysfunction in healthy
volunteers and in diabetic patients.14,15 Because xuezhikang
contains statin-like components, similar results were ob-
erved in our study. Xuezhikang decreased postprandial
hypertriglyceridemia and protected preprandial and postpran-
dial endothelial function. These data support the hypothesis
that xuezhikang improves endothelial function by eliminating
triglyceride-rich lipoprotein and other harmful factors asso-
ciated with postprandial hypertriglyceridemia. Furthermore,
xuezhikang may exert a direct protective effect on endothelial
cells and maintain nitric oxide bioactivity by its antioxidant
properties.19

Endothelial dysfunction is closely related to systemic
inflammation in addition to dyslipidemia. Postprandial
hypertriglyceridemia can activate nuclear factor-κB by
postprandial triglyceride-rich lipoproteins.20–22 Therefore,
repeated postprandial hypertriglyceridemia could conceiv-
ablely lead to a chronic inflammatory state, which can
contribute further to endothelial dysfunction. For example,
CRP, a marker and a mediator of inflammation, directly
inhibits the activity and expression of endothelial nitric
oxide synthase23 and increases endothelin-1 expression24
in endothelial cells. In previous studies, CRP concentration
was an independent predictor for endothelium-dependent
vasodilation in patients with coronary artery disease25 and
in healthy children.26 Furthermore, children with higher

Figure 4. Correlations between change in preprandial FMD and
changes in TG-AUC and log(hs-CRP) after 6-week xuezhikang
treatment in all CHD patients.
CRP levels have worse endothelial function as assessed by FMD.26 Interestingly, reducing CRP levels over time is associated with improvement of endothelium-dependent vasodilation in patients with coronary artery disease.25,27 In this study, xuezhikang had a potent effect on lowering CRP, which was associated with improvement of fasting FMD. This supports the hypothesis that xuezhikang may protect endothelial function partly through an antiinflammatory mechanism. It is known that statins exert potent antiinflammatory effects independent of their lipid-lowering properties. Xuezhikang contains naturally occurring statin-like elements. Nevertheless, we found a significant correlation between the reductions of hs-CRP and TG-AUC in the xuezhikang group. Therefore, the reduction in hs-CRP levels observed in this study could be secondary to decreased postprandial triglyceride levels as well as the direct antiinflammatory effect of xuezhikang.

Patients in the placebo arm also had a mild reduction in CRP concentration. Patients in this arm received dietary control and other agents such as aspirin, nitrates, metoprolol, and fosinopril. Although a low dose (80 mg/d) of aspirin, which is near the dose of aspirin in the present study, seemed to have no effect on CRP levels in healthy volunteers,28 β-blockers and angiotensin-converting enzyme inhibitors inhibit cytokines synthesis and lower CRP concentration effectively in patients with CHD.29–31 Furthermore, a low-energy diet could reduce vascular inflammatory factors, including CRP.32 Therefore, the reduction seen in CRP levels in the placebo arm could be related to dietary control and the combination of medications these patients were taking.

Although a small decrease in CRP levels were seen in the placebo arm, FMD did not improve in these patients. Previous studies have shown that aspirin at moderate doses (162 mg/d) for 8 weeks effectively restores endothelium-dependent dilation in hypertensive patients33; however, a lower dose of aspirin (100 mg/d for 6 weeks) seemed to be ineffective in the same population. In our study, patients received low-dose aspirin, which could explain the lack of improvement in FMD in the placebo group. Furthermore, compared with previous studies, our patients were a sicker group of patients with multiple coronary risk factors.

In summary, xuezhikang significantly decreased hs-CRP levels in addition to its lipid-lowering effects. Furthermore, xuezhikang significantly improved FMD during preprandial and postprandial states. Taken together, these results suggest that xuezhikang protects endothelial function through its potent systemic antiinflammatory and lipid-lowering effects. Future large randomized trials are needed to examine the protective effects of xuezhikang in preventing cardiovascular events.

Acknowledgments

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References


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