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Red Ginseng Extract Improves Coronary Flow Reserve and Increases Absolute Numbers of Various Circulating Angiogenic Cells in Patients with First ST-Segment Elevation Acute Myocardial Infarction

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The effects of red ginseng extract on circulating angiogenic cell mobilization and improvement of microvascular integrity were evaluated in ST-elevation acute myocardial infarction (AMI) patients during 8-month follow-up. AMI patients ($n = 50$) were randomly assigned to the red ginseng group (3 g/day, $n = 25$) or the placebo group ($n = 25$) after coronary stenting. Coronary flow reserve (CFR) was measured at baseline and at 8 months with an intracoronary Doppler wire. Serial changes in the absolute numbers of circulating angiogenic cells such as $CD34^+$, $CXCR4^+$, $CD117^+$, $CD133^+$ and C-met^+ were measured at baseline, 1 day, 5 days and at 8 months.

CFR were similar between the two groups at baseline, and CFR was significantly higher in the red ginseng group than in the placebo group (2.80 ± 0.91 and 2.56 ± 0.77, $p < 0.05$, respectively) after 8 months of red ginseng administration. The absolute numbers of circulating $CD34^+$, $CXCR4^+$ and $CD117^+$ cells were significantly higher in the red ginseng group at 1 and 5 days after stenting. Significant positive correlations were found between the numbers of circulating angiogenic cells at day 1 and the changes from baseline in CFR for $CD34^+$, $CXCR4^+$, $CD117^+$ and C-met^+ cells. Red ginseng extract increased $CD34^+$, $CXCR4^+$ and $CD117^+$ circulating angiogenic cell mobilization and decreased inflammation in AMI patients, thereby improving CFR during the 8-month follow-up.

Keywords: red ginseng extract; acute myocardial infarction; coronary flow reserve; stem cell.

INTRODUCTION

Percutaneous coronary intervention in acute myocardial infarction (AMI) patients has reduced reinfarction and the number of deaths significantly (Grines *et al*., 1993). Coronary microvascular dysfunction in the territory of a recanalized infarct-related artery occurs in AMI patients, and various adjuvant pharmacotherapies have been shown to improve left ventricular function and coronary microvascular integrity (Cannon *et al*., 2004; Chen *et al*., 2009; Kober *et al*., 1995). Because coronary angiography does not adequately reflect the degree of microvascular integrity, coronary flow reserve (CFR) is used frequently to assess microvascular function after AMI. CFR, assessed with an intracoronary Doppler wire, reflects the integrity of the coronary microvascular system. Baseline CFR has been used as a predictor of improvement in left ventricular function after AMI (Cortigiani *et al*., 2009); however, to the best of our knowledge, baseline and 8-month follow-up CFR values have never been compared in ST-elevation AMI patients after red ginseng extract administration. Since the benefit of early revascularization in patients with non-ST-segment elevation MI has been inconsistent (Anderson *et al*., 2007), only patients with ST-segment elevation MI were enrolled in this study. Circulating angiogenic cells were defined as a heterogeneous population that includes endothelial progenitor cells, myeloid, mesenchymal and hematopoietic stem cells, which integrate into the endothelium of new or recovering vessels and promote vascular growth and remodeling through the production of angiogenic cytokines (Bosch-Marce *et al*., 2007; Jin *et al*., 2006; Yoder *et al*., 2007).

Three types of ginseng are currently available: fresh, white and red ginseng. White ginseng is made by peeling and drying fresh ginseng without steaming, and red ginseng, known as *Panax ginseng*, is made by steaming fresh ginseng (Kim *et al*., 2002). Chemical changes, which make red ginseng different from other ginsengs in terms of safety and therapeutic effects, occur during the steaming process. Red ginseng has been shown to improve endothelial function, decrease oxidative stress, stimulate angiogenesis, decrease thrombus formation, decrease low-density lipoprotein (LDL)-cholesterol and decrease blood pressure (Hwang *et al*., 2008; Jeon *et al*., 2000; Jin *et al*., 2007; Kim *et al*., 2002, 2007; Lee *et al*., 2009). Although previous studies have suggested that red ginseng has beneficial cardiovascular effects, a randomized controlled trial with red ginseng in patients with ST-elevation AMI has not been performed. Therefore, in this study, the effects of red ginseng extract on circulating angiogenic cell mobilization and

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improvement of microvascular integrity were compared in ST-segment elevation AMI patients during an 8-month follow-up period.

METHODS

Study patients. Patients aged 45–75 years with first ST-segment elevation AMI requiring stent implantations were eligible for participation in the study. Patients were prospectively enrolled in the study at Korea University Anam Hospital cardiovascular centers between April 2007 and March 2009. A total of 177 patients were screened for inclusion in the study. Patients were treated by primary percutaneous coronary intervention within 100 min of admission. Patients (n = 26) who did not fulfill the inclusion criteria or who had any of the exclusion criteria (n = 101) were excluded. Eligible patients (n = 50: 20 women and 30 men) were randomly assigned to receive either red ginseng extract (3 g/day) (red ginseng group, 25 patients) or placebo (placebo group, 25 patients) for 8 months (Fig. 1). A loading dose of clopidogrel 600 mg was administered on admission and a daily maintenance dose of 75 mg of clopidogrel was administered thereafter. Aspirin and clopidogrel were maintained in all patients during the 8-month follow-up. Beta blockers, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, and atorvastatins were added as needed. First ST-segment elevation AMI patients who presented within 6 h of symptoms with proximal coronary target lesions were included in the study. Patients with Killip Class IV MI, left main coronary lesions, additional lesions with a diameter stenosis >50% distal to the treated infarct-related artery, a prior history of interventional or surgical treatment for coronary artery disease, cerebrovascular disease, uncontrolled arrhythmia within 3 months, serum creatinine >2.0 mg/dL, or a life expectancy of <1 year were excluded. Also excluded were patients for whom complete baseline measurements of CFR could not be completed 3 to 5 days after stenting. The study was approved by the University Hospital Institute Review Board, and all participants or legal guardians provided written informed consent.

Coronary angiograms were obtained at baseline, immediately after stenting, 3 to 5 days after stenting, and after 8 months. Balloon angioplasty and sirolimus-eluting stent implantation were performed according to standard clinical practice as described previously (Hong *et al.*, 2007). The primary end points of the study were to determine the differences in CFR values and in absolute numbers of circulating angiogenic cells ($CD34^+$, $CD117^+$, $CD133^+$, $CXCR4^+$, C-met^+) during the 8-month follow-up period in both groups. Differences in inflammatory markers [interleukin (IL)-6, tumor necrosis factor (TNF)-α, soluble intercellular adhesion molecule-1 (sICAM-1), soluble vascular cell adhesion molecule-1 (sVCAM-1), high-sensitive C-reactive protein (hsCRP) and adiponectin] and major adverse cardiovascular events (cardiac death, MI, target lesion revascularization) during the 8-month follow-up period were also investigated. Lipid profiles and inflammatory markers such as hsCRP, IL-6, and TNF-α were measured as described previously (Hong *et al.*, 2007). sICAM-1 and sVCAM-1 were measured using a commercially available ELISA according to the manufacturer's instructions (R & D Systems, Minneapolis, Minnesota).

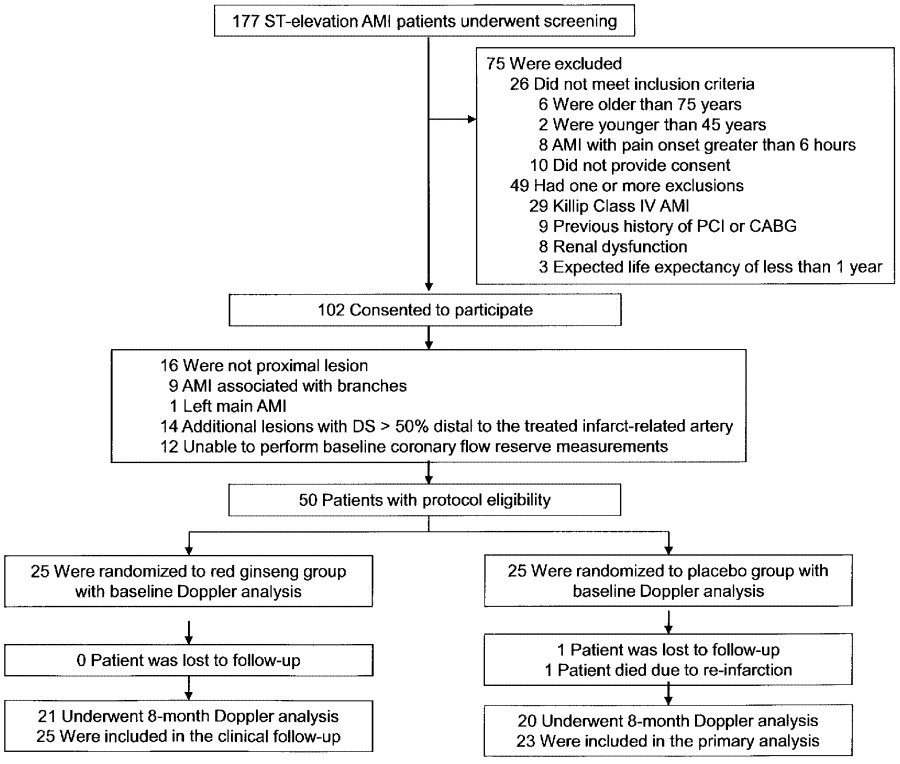

Figure 1. Eligible patients (n = 50) were randomly assigned to receive either red ginseng extract (25 patients) or placebo (25 patients), and red ginseng extract (3 g/day) was coadministered with 75 mg of clopidogrel and 100 mg of aspirin on a daily basis. AMI, acute myocardial infarction; PCI, percutaneous coronary intervention; CABG, coronary artery bypass surgery; DS, diameter stenosis.

Red ginseng extract preparation. Red ginseng extract was prepared by the Korea Ginseng Corporation (Daejeon, Korea) in a standardized and reproducible process. Red ginseng was made using the roots of a 6-year-old fresh *Panax ginseng* plant whose botanical identity had been verified. Red ginseng was extracted seven times with 10 volumes of distilled water at 85°C for 8 h followed by cooling. The aqueous extract was combined, and then concentrated twice under vacuum at 40°C. The ginsenoside content was measured by extracting the red ginseng with 10 volumes of 80% methanol at 85°C for 1 h. The alcohol extract was concentrated under vacuum at 40°C, dissolved in water and passed through a Sep-Pak C18 cartridge. Ginsenosides were eluted with 90% methanol and then analysed by high performance liquid chromatography (Jin *et al*., 2007). The total content of ginsenosides in the red ginseng extract was 13.5%, and the red ginseng extract contained the following quantities of major ginsenosides: -Rb1: 2.66 mg/g, -Rb2: 1.28 mg/g, -Rc: 1.31 mg/g, -Rd: 1.21 mg/g, -Re: 0.48 mg/g, -Rf: 1.20 mg/g, -Rg1: 0.33 mg/g, -Rg3: 5.07 mg/g, as well as other minor ginsenosides (Jin *et al*., 2007). Three grams of red ginseng extract was added to sealed containers with 75 mL of distilled water. The placebo comprised 75 mL of distilled water with ginseng fragrance and was provided in a sealed container.

Intracoronary Doppler measurements and coronary flow parameters. Coronary microvascular integrity was measured by an intracoronary Doppler wire 3 to 5 days after the percutaneous coronary intervention. After the target vessel was cannulated with a 5F catheter, 0.2 mg nitroglycerine was injected directly into the coronary circulation to achieve maximal epicardial coronary artery dilatation without significantly affecting coronary microcirculation. A Doppler guidewire (Flowire, Volcano Corp, Rancho Cordova, CA) was inserted into the infarct-related artery and positioned just distal to the previously implanted stent. The location of the Doppler wire tip was confirmed in two orthogonal views. Coronary flow was measured at baseline and during maximal hyperemia induced by an intracoronary bolus injection of 20 μg of adenosine for the right coronary artery and 30 μg of adenosine for the left coronary artery. Doppler measurements were repeated at least three times, and the average values were used. Intracoronary Doppler measurements were repeated 8 months later by identically positioning the tip of the Doppler wire just distal to the implanted stent.

The average peak flow velocity was measured at baseline and during maximal hyperemia induced by an intracoronary bolus injection of adenosine. Systolic retrograde flow was defined as retrograde peak velocity ≥10 cm/s and a duration of >60 ms (Schachinger *et al*., 2006). CFR was calculated by dividing the adenosine-induced average peak flow velocity by the average peak flow velocity at baseline.

Quantitation of circulating angiogenic cells. Peripheral blood samples (10 mL) were drawn into heparinized-tubes on admission (day 0), and 1 day, 5 days and 8 months after the procedure. Within 1 h of collection, peripheral blood mononuclear cells (PBMNCs) were isolated by density gradient centrifugation using Ficoll-Paque plus® (Amersham Biosciences Corporation, NJ, USA). For flow cytometric analysis, PBMNCs were fixed in 2% paraformaldehyde, stained with anti-CD34 (550760, BD Pharmingen, San Diego, CA, USA), anti-CD117 (555713, BD Pharmingen), anti-CD133 (130-090-422, Miltenyi Biotec, Bergisch Gladbach, Germany), anti-CXCR4 (MAB170, R & D Systems, Minneapolis, MN, USA), and anti-c-met (MAB358, R & D Systems) monoclonal antibodies for 20 min at 4°C. The cells were then stained with isotype-matched phycoerythrin (PE)-conjugated secondary antibody (349043, BD Biosciences, San Jose, CA, USA). A negative control incubated with buffer with no primary antibody for 20 min at 4°C was used to identify non-specific binding by the secondary antibody. The 10000 cells/sample were analysed on a FACS Vantage SE flow sorter (Becton Dickinson). Dead cells and debris were gated out using the scatter properties of the cells. Data were analysed using CellQuest Pro software (Becton Dickinson). Samples from five age-matched healthy volunteers were used as normal controls. The number of positive cells was calculated on the basis of absolute leukocyte count × percentage (%) of positive cells, and expressed as the absolute number of cells per 1 μL of whole blood.

Statistical analysis. Data are expressed as mean ± SD for continuous variables, and data for the categorical variables are expressed as the number and the percentage of patients. Fisher's exact test was used for categoric variables. Variables that did not show a normal distribution were log-transformed for subsequent analysis. Results between the two groups were compared by a Mann-Whitney *U*-test, and the comparisons between before and after treatment were analysed by a Wilcoxon signed rank test. The correlations between circulating angiogenic cells and changes from baseline in CFR were assessed with Spearman rank correlation test. The primary end points, which were measured repeatedly, were compared using two-way repeated-measures analysis of variance (ANOVA). Sample size was calculated from our preliminary data comparing 8-month CFR values between red ginseng (n = 7) and placebo (n = 7) groups (3.1 ± 1.6 and 2.3 ± 1.1, respectively), given that no similar study has been published. Using a 2-sided test for differences in independent binomial proportions with an alpha level of 0.05, it was calculated that 37 patients (19 patients in each group) would have to undergo randomization for the study to have 80% power to detect a difference in the CFR values between the two groups; therefore, at least 25 patients were enrolled in each group to account for the 25% loss in follow-up angiography. Angiographic analyses were performed according to the number of patients available for each analysis. All analyses were performed according to the intention-to-treat principle. A value of $p < 0.05$ was considered statistically significant. SPSS software (version 10.0) was used for analyses (SPSS Inc., Chicago, Illinois, USA).

RESULTS

Baseline patient and angiographic characteristics

Baseline clinical characteristics of patients in the red ginseng group (n = 25) and the placebo group (n = 25) were similar (Table 1). The rates of diabetes, hyperten-

Table 1. Baseline patient and angiographic characteristics

Variable	Red ginseng group (n = 25)	Placebo group (n = 25)	p Value
Age (years)	61.4 ± 12.4	59.5 ± 11.8	0.345
Male sex	16 (64.0%)	14 (56.0%)	0.564
Body mass index (kg/m^2)	24.8 ± 4.1	24.1 ± 3.0	0.394
Risk factors			
Diabetes mellitus	8 (32.0%)	7 (28.0%)	0.758
Hypertension	12 (48.0%)	13 (52.0%)	0.777
Hyperlipidemia	4 (16.0%)	5 (20.0%)	1.000
Current smoking	8 (32.0%)	10 (40.0%)	0.556
Family history of coronary artery disease	2 (8.0%)	3 (12.0%)	1.000
Past history of TIA or stroke	1 (4.0%)	2 (8.0%)	1.000
Time from onset of chest pain to angioplasty (h)	3.98 ± 1.63	4.21 ± 1.54	0.463
Door to balloon time (min)	67.1 ± 26.8	64.3 ± 22.7	0.791
LVEF (%)	41.1 ± 8.6	42.1 ± 9.1	0.881
Number of stents used	34	32	
Coronary artery disease			
One vessel	12 (48.0%)	10 (40.0%)	0.569
Two vessels	7 (28.0%)	10 (40.0%)	0.370
Three vessels	6 (24.0%)	5 (20.0%)	0.733
Infarct-related artery			
Left anterior descending artery	12 (48.0%)	11 (44.0%)	0.777
Left circumflex artery	5 (20.0%)	7 (28.0%)	0.508
Right coronary artery	8 (32.0%)	7 (28.0%)	0.758
TIMI flow grade			
0	15 (60.0%)	17 (68.0%)	0.556
1	6 (24.0%)	5 (20.0%)	0.733
2	3 (12.0%)	2 (8.0%)	1.000
3	1 (4.0%)	1 (4.0%)	1.000
Proximal tortuosity	2 (8.0%)	1 (4.0%)	1.000
Calcified lesion	3 (12.0%)	2 (8.0%)	1.000
Baseline			
Reference diameter (mm)	2.90 ± 0.45	2.91 ± 0.35	0.932
Minimal lumen diameter (mm)	0.39 ± 0.46	0.37 ± 0.47	0.747
Percentage of stenosis	86.6 ± 17.2	87.3 ± 18.4	0.734
Mean lesion length (mm)	22.4 ± 6.5	22.1 ± 5.9	0.878
Postprocedure			
Reference diameter (mm)	3.14 ± 0.47	3.11 ± 0.49	0.851
Minimal lumen diameter (mm)			
In segment	2.88 ± 0.47	2.89 ± 0.48	0.712
In stent	2.92 ± 0.44	2.94 ± 0.45	0.801
Percentage of stenosis			
In segment	8.3 ± 4.8	7.2 ± 4.5	0.399
In stent	7.1 ± 4.2	5.6 ± 3.9	0.201
Acute gain (mm)			
In segment	2.49 ± 0.63	2.52 ± 0.70	0.565
In stent	2.53 ± 0.60	2.59 ± 0.73	0.661
Mean stent diameter (mm)	2.9 ± 0.4	3.0 ± 0.5	0.489
Mean stent length (mm)	26.1 ± 7.7	25.6 ± 6.8	0.781
No of stents implanted per patient, range	1.33 ± 0.45 (1-2)	1.39 ± 0.46 (1-2)	0.686
Abciximab therapy	11 (44.0%)	13 (52.0%)	0.571
Postprocedure TIMI flow grade			
0	0 (0.0%)	0 (0.0%)	NA
1	0 (0.0%)	0 (0.0%)	NA
2	1 (4.0%)	1 (4.0%)	1.000
3	24 (96.0%)	24 (96.0%)	1.000
Maximal creatinine kinase MB (U/L)	199 ± 92	229 ± 99	0.501

Values represent number (%) or mean ± 1 SD.
LVEF, left ventricular ejection fraction; NA, not available.

sion, hyperlipidemia and current smoking at baseline were similar between the two groups. The use of various active medications at baseline was also similar between the two groups (Supplement Table 1). The main target vessel was the left anterior descending artery in more than 40% of patients in each group. Baseline and post-procedure reference diameter, minimal luminal diameter, percentage of stenosis and mean lesion length

Table 2. Eight-month angiographic and clinical outcomes

Variable	Red ginseng group (n = 25)	Placebo group (n = 25)	p Value
Number of patients with 8 months f/u (%)	21 (84.0%)	20 (80.0%)	0.713
Eight-month follow-up			
Reference diameter (mm)	3.07 ± 0.48	3.06 ± 0.52	0.898
Minimal lumen diameter (mm)			
In segment	2.50 ± 0.93	2.41 ± 0.92	0.712
In stent	2.54 ± 0.86	2.49 ± 0.89	0.802
Percentage of stenosis			
In segment	18.7 ± 25.6	21.2 ± 20.1	0.439
In stent	17.3 ± 24.6	18.6 ± 19.3	0.781
Late lumen loss (mm)			
In segment	0.39 ± 0.69	0.48 ± 0.64	0.319
In stent	0.38 ± 0.66	0.45 ± 0.59	0.461
Loss index			
In segment	0.16 ± 0.29	0.19 ± 0.30	0.611
In stent	0.15 ± 0.27	0.17 ± 0.33	0.822
Binary restenosis (%)			
In segment	2 (8.0%)	3 (12.0%)	1.000
In stent	2 (8.0%)	3 (12.0%)	1.000
Death (%)	0 (0.0%)	1 (4.0%)	1.000
Myocardial infarction (%)	0 (0.0%)	1 (4.0%)	1.000
Target vessel revascularization (%)	1 (4.0%)	2 (8.0%)	1.000
Target lesion revascularization (%)	1 (4.0%)	2 (8.0%)	1.000
Percutaneous coronary intervention	1 (4.0%)	2 (8.0%)	1.000
Coronary bypass	0 (0.0%)	(0.0%)	NA

Values represent number (%) or mean ± 1 SD.
NA, not available.

showed no significant differences between the two groups (Table 1). The 8-month CFR follow-up was performed in about 80% of patients in each group [21 patients (84.0%) in the red ginseng and 20 patients (80.0%) in the placebo groups, $p = 0.713$], and the 8-month angiographic and clinical outcomes demonstrated no significant differences between the two groups (Table 2). One patient with reinfarction due to subacute stent thrombosis was counted as a single case of cardiac death in the placebo group. Two cases (100%) of in-stent restenosis in the red ginseng group and the two cases (66.7%) of restenosis in the placebo group were of the focal type (Table 2).

Baseline and 8-month coronary flow measurements in the infarct-related artery

The CFR measurements at baseline were not significantly different between the two groups (2.01 ± 0.75 in the red ginseng group vs 1.98 ± 0.63 in the placebo group, $p = 0.897$) (Table 3). The increases from baseline in CFR at 8 months were significantly greater in the red ginseng group than in the placebo group (0.79 ± 0.59 vs 0.58 ± 0.43, $p = 0.038$, respectively) (Fig. 2, Table 3). Compared with the initial CFR measurements, CFR increased by 39.3% in the red ginseng and 29.3% in the placebo groups after 8 months (both $p < 0.05$ versus index admission). After 8 months, the diastolic deceleration time increased significantly from the index admission value (522 ± 271 to 723 ± 376 ms in the red ginseng group vs 531 ± 256 to 619 ± 245 ms in the placebo group, $p < 0.05$ for both groups). The percent increase in diastolic deceleration time from the index admission was significantly greater in the red ginseng group than in the placebo group (38.5 ± 16.7% and 16.6 ± 8.0%, $p < 0.05$, respectively). The rates of early systolic retrograde flow were similar between the two groups [two patients (8.0%) in the red ginseng vs two patients (8.0%) in the placebo groups, $p = 1.000$], and no early systolic retrograde flow was observed after 8 months in either group. Left ventricular ejection fraction as measured by echocardiogram demonstrated a significant improvement in ejection fraction in both groups (red ginseng group, 38.9 ± 7.8% at index admission and 44.0 ± 10.2% at 8 months, $p < 0.05$; placebo group, 39.3 ± 8.1% at index admission and 43.1 ± 7.2% at 8 months, $p < 0.05$), but the 8-month increases from baseline were not significantly different between the two groups (13.1 ± 10.0% in the red ginseng group versus 9.7 ± 7.9% increases in the placebo groups, $p = 0.208$).

Increases in the absolute number of various circulating angiogenic cells

Significantly more peripheral $CD34^+$, $CXCR4^+$, $CD117^+$, $CD133^+$ and C-met^+ cells were mobilized in both groups 1 day after the procedure than in the control (Fig. 3). The serial increases in the absolute number of $CD34^+$, $CXCR4^+$ and $CD117^+$ cells were significantly greater in the red ginseng group, especially 1 and 5 days after the procedure (two-way repeated-measures ANOVA: $p = 0.042$, $p = 0.026$ and $p = 0.047$, respectively) (Fig. 3A, B and C). The absolute numbers of all circulating angiogenic cells returned to levels similar to the control after 8 months in both groups. Significant positive correlations were found between the numbers of circulating

Table 3. Initial and 8-month follow-up coronary flow measurements in the infarct-related artery between the red ginseng and the placebo groups

	Red ginseng group (n = 25)		Placebo group (n = 25)	
	Baseline	After adenosine	Baseline	After adenosine
Initial measurements				
Peak diastolic velocity (cm/s)	45.8 ± 21.8	79.1 ± 28.3[a]	47.1 ± 22.9	77.3 ± 38.8[a]
Changes from baseline (cm/s)		33.3 ± 25.4		30.7 ± 22.5
Peak systolic velocity (cm/s)	22.3 ± 11.6	44.2 ± 22.4[a]	23.8 ± 14.1	46.3 ± 23.1[a]
Changes from baseline (cm/s)		22.8 ± 15.1		22.7 ± 17.2
Average peak flow velocity (cm/s)	25.1 ± 11.1	51.9 ± 22.3[a]	24.1 ± 13.2	46.3 ± 24.1[a]
Changes from baseline (cm/s)		25.5 ± 22.1		22.4 ± 16.3
Diastolic-systolic peak velocity ratio	2.07 ± 1.29	1.79 ± 0.91[a]	1.96 ± 0.88	1.68 ± 0.72[a]
Changes from baseline		−0.29 ± 0.69		−0.27 ± 0.45
Heart rate (bpm)	71.8 ± 10.9	79.1 ± 11.1[a]	73.1 ± 9.1	78.0 ± 10.4[a]
Changes from baseline (bpm)		7.1 ± 8.4		4.8 ± 11.2
PDVR		1.72 ± 0.75		1.65 ± 0.68
PSVR		1.99 ± 0.68		1.91 ± 0.84
Coronary flow reserve		2.01 ± 0.75		1.98 ± 0.63
8-month measurements				
Peak diastolic velocity (cm/s)	36.9 ± 24.2	80.1 ± 38.3[a]	39.1 ± 19.8	74.3 ± 33.5[a]
Changes from baseline (cm/s)		43.2 ± 33.6		35.3 ± 30.2
Peak systolic velocity (cm/s)	20.1 ± 14.5	48.1 ± 33.2[a]	22.4 ± 16.9	47.5 ± 28.1[a]
Changes from baseline (cm/s)		27.9 ± 23.6		25.0 ± 16.7
Average peak flow velocity (cm/s)	18.8 ± 10.7	52.6 ± 24.2[a]	19.3 ± 10.1	49.5 ± 21.2[a]
Changes from baseline (cm/s)		33.3 ± 19.8		30.3 ± 10.3
Diastolic-systolic peak velocity ratio	1.85 ± 0.96	1.67 ± 0.87[a]	1.75 ± 0.71	1.56 ± 0.69[a]
Changes from baseline		−0.18 ± 0.61		−0.19 ± 0.35
Heart rate (bpm)	68.9 ± 9.2	73.1 ± 10.1[a]	68.1 ± 8.0	72.6 ± 8.1[a]
Changes from baseline (bpm)		4.2 ± 7.7		4.4 ± 5.5
PDVR		2.16 ± 1.25		1.90 ± 0.92
PSVR		2.41 ± 0.93[b]		2.13 ± 0.69
Coronary flow reserve		2.80 ± 0.91[b]		2.56 ± 0.77

PDVR, adenosine/baseline peak diastolic velocity; PSVR, adenosine/baseline peak systolic velocity.

[a] $p < 0.05$ compared with baseline.

[b] $p < 0.05$ compared with the placebo group.

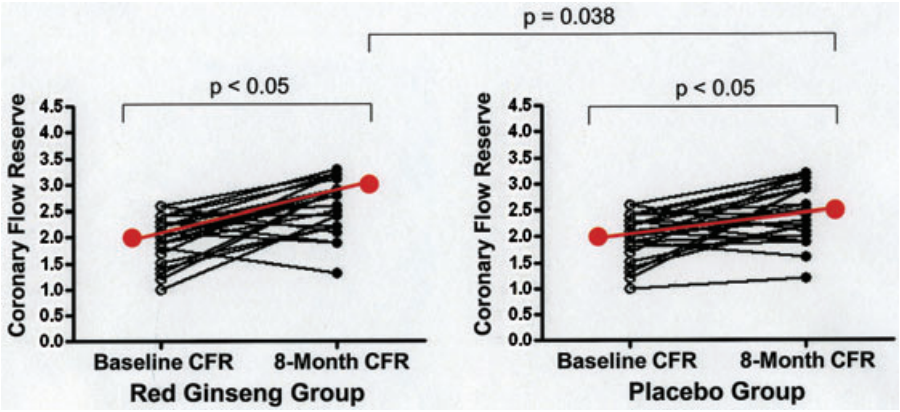

Figure 2. CFR values at baseline were not significantly different between the two groups. The increase in CFR values at 8 months compared with baseline was significantly greater in the red ginseng group than in the placebo group (0.79 ± 0.59 vs 0.58 ± 0.43, p = 0.038, respectively). Black dots indicate average baseline and 8-month CFR values. CFR, coronary flow reserve This figure is available in colour online at http://wileyonlinelibrary.com/journal/ptr.

angiogenic cells at day 1 and the changes from baseline in CFR: r = 0.600 ($p < 0.001$) for $CD34^+$ cells, r = 0.321 (p = 0.023) for $CXCR4^+$ cells, r = 0.286 (p = 0.044) for $CD117^+$ cells, r = 0.511 ($p < 0.001$) for C-met^+ cells (Fig. 4A–C, E).

Comparison of biochemical parameters at baseline and the 8-month follow-up

Levels of inflammatory markers such as IL-6 and hsCRP decreased significantly after 8 months compared

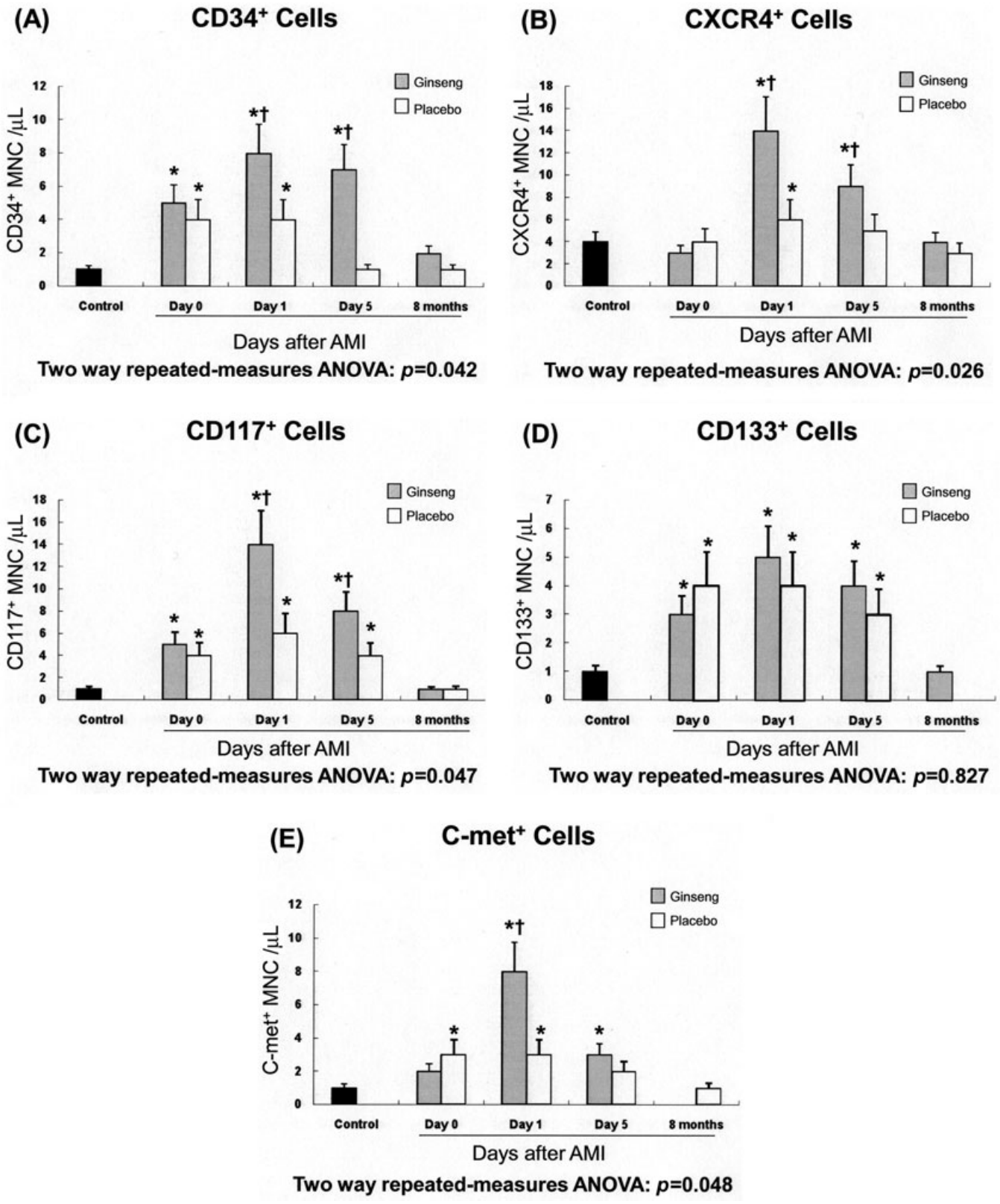

Figure 3. Absolute numbers of circulating angiogenic cells increased significantly in both groups compared with the controls 24 h after the procedure: (A) $CD34^+$ angiogenic cells; (B) $CXCR4^+$ angiogenic cells; (C) $CD117^+$ angiogenic cells; (D) $CD133^+$ angiogenic cells; (E) C-met^+ angiogenic cells (* $p < 0.05$ compared with control and † $p < 0.05$ compared with the placebo group). Two-way repeated measures ANOVA showed significant increases in $CD34^+$, $CXCR4^+$, $CD117^+$ and C-met^+ cells in the red ginseng group.

with the baseline in both groups. TNF-α, sICAM-1 and sVCAM-1 levels decreased significantly only in the red ginseng group (Table 4). Decreases in IL-6, TNF-α, sICAM-1 and sVCAM-1 from baseline levels were significantly greater in the red ginseng group than in the placebo group. Adiponectin levels increased significantly in both groups during the 8-month follow-up, but were not significantly different between the two groups.

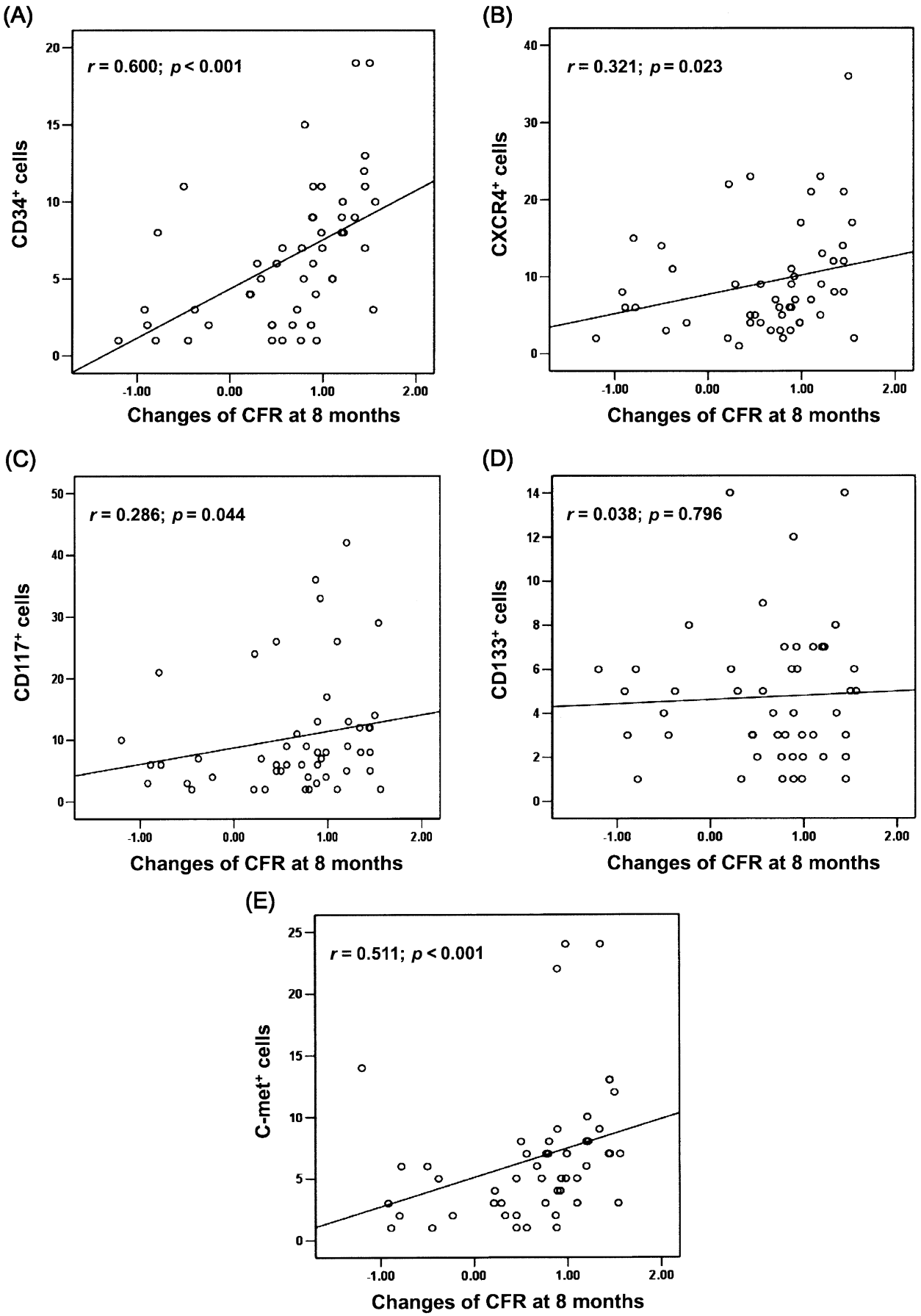

Figure 4. Correlation between absolute numbers of circulating angiogenic cells at day 1 and changes from baseline in CFR in ST-segment elevation AMI patients: (A) $CD34^+$ angiogenic cells; (B) $CXCR4^+$ angiogenic cells; (C) $CD117^+$ angiogenic cells; (D) $CD133^+$ angiogenic cells; (E) C-met^+ angiogenic cells. Significant positive correlations were found between the changes from baseline in CFR and the numbers of circulating angiogenic cells such as $CD34^+$ cells, $CXCR4^+$ cells, $CD117^+$ cells and C-met^+ cells.

Table 4. Comparison of biochemical parameters at baseline and 8-month follow-up

	Red ginseng group (n = 25)		Placebo group (n = 25)	
Variable	Baseline	8-month f/u	Baseline	8-month f/u
IL-6 (pg/mL)[c]	3.91 ± 3.12	1.45 ± 1.10[a]	3.56 ± 3.21	2.23 ± 1.71[a]
Changes from baseline (pg/mL)	–2.54 ± 2.32[b]		–1.34 ± 2.12	
TNF-α (pg/mL)[c]	5.71 ± 4.19	4.16 ± 2.56[a]	5.16 ± 3.41	4.99 ± 3.11
Changes from baseline (pg/mL)	–1.54 ± 1.51[b]		0.14 ± 1.12	
hsCRP (mg/L)[c]	4.89 ± 4.11	1.80 ± 1.57[a]	5.11 ± 4.22	1.89 ± 1.81[a]
Changes from baseline (mg/L)	–3.09 ± 3.01		–3.21 ± 2.98	
Adiponectin (μg/mL)[c]	5.29 ± 4.17	6.36 ± 4.14[a]	5.55 ± 3.85	6.49 ± 4.66[a]
Changes from baseline (μg/mL)	1.12 ± 1.09		0.98 ± 0.88	
sICAM-1 (ng/mL)[c]	430 ± 154	391 ± 133[a]	416 ± 131	422 ± 172
Changes from baseline (ng/mL)	–39 ± 52[b]		6 ± 72	
sVCAM-1 (ng/mL)[c]	1107 ± 344	954 ± 328[a]	1088 ± 429	1077 ± 401
Changes from baseline (ng/mL)	–154 ± 198[b]		–11 ± 356	
Total cholesterol (mmol/L)	5.69 ± 1.40	3.73 ± 1.07[a]	5.57 ± 1.13	3.64 ± 0.84[a]
Changes from baseline (mmol/L)	–1.96 ± 1.60		–1.93 ± 1.10	
LDL-cholesterol (mmol/L)	3.70 ± 1.89	1.89 ± 1.36[a]	3.78 ± 2.78	2.00 ± 1.02[a]
Changes from baseline (mmol/L)	–1.82 ± 1.02		–1.77 ± 1.09	
HDL-cholesterol (mmol/L)	1.14 ± 0.71	1.19 ± 0.47	1.04 ± 0.58	1.16 ± 0.32
Changes from baseline (mmol/L)	0.06 ± 0.22		0.11 ± 0.18	
Triglyceride (mmol/L)[c]	1.43 ± 0.80	1.24 ± 1.06	1.39 ± 0.84	1.28 ± 0.78
Changes from baseline (mmol/L)	–0.18 ± 0.79		–0.11 ± 0.67	

HDL, high-density lipoprotein; hsCRP, high-sensitive C-reactive protein; IL, interleukin; LDL, low-density lipoprotein; sICAM, soluble intercellular adhesion molecule; sVCAM, soluble vascular cell adhesion molecule; TNF, tumor necrosis factor.

[a] $p < 0.05$ compared with baseline.

[b] $p < 0.05$ compared with the placebo group.

[c] Geometric mean and SD values are given.

Total cholesterol and LDL-cholesterol levels decreased significantly in both groups, but were not significantly different between the two groups.

DISCUSSION

This is the first prospective, randomized, single-blind study to investigate the effect of red ginseng extract on the restoration of microvascular integrity in first ST-elevation AMI patients. Red ginseng extract significantly improved CFR in AMI patients during the follow-up period of 8 months, indicating restoration of microvascular function. This is the first study to compare index CFR with 8-month follow-up CFR after administration of either red ginseng extract or placebo in AMI patients. The improvement in microvascular integrity after administering red ginseng extract was correlated with an increase in absolute numbers of circulating angiogenic cells in AMI patients. Significant early increases in circulating angiogenic cells such as $CD34^+$, $CXCR4^+$, $CD117^+$ and C-met^+ cells together with significant decreases in various inflammatory markers such as IL-6, TNF-α, sICAM-1 and sVCAM-1 likely contributed to the improvement in microvascular integrity in ST-elevation AMI patients after red ginseng extract administration.

Red ginseng extract contains acidic polysaccharide proteins, aminoglycosides, complex carbohydrates, phytosterols, water-soluble vitamins, trace minerals, and ginsenosides, the latter of which are thought to be the active compounds responsible for ginseng's health benefits (Heo *et al*., 2008; Jang *et al*., 2008; Jin *et al*., 2007; Kim *et al*., 2002, 2007). Red ginseng extract has higher concentration of ginsenosides such as Rg3, Rg5 and Rh2 than other ginseng products (Heo *et al*., 2008; Jang *et al*., 2008; Jin *et al*., 2007; Kim *et al*., 2007). An increasing number of patients with cardiovascular diseases are using herbs such as red ginseng to improve treatment outcomes, often without notifying their physicians (Eisenberg *et al*., 1998), and many physicians have questioned the safety and efficacy of taking red ginseng in addition to conventional medical treatments (Angell and Kassirer, 1998). Although red ginseng has been studied in small trials for its cardiovascular safety and efficacy (Hwang *et al*., 2008; Jeon *et al*., 2000; Jin *et al*., 2007; Kim *et al*., 2002, 2007), a randomized, placebo-controlled, prospective clinical trial with standardized red ginseng extract was needed to provide convincing evidence that it does improve myocardial function in AMI patients. Investigating the efficacy and safety of red ginseng is complex, because red ginseng contains mixtures of compounds in various amounts. Therefore, 6-year-old ginseng was used in this study to produce red ginseng extract in a standardized manner by the Korea Ginseng Corporation. Other studies have shown high variability in terms of the amounts of active ingredients present because of different preparation methods and various root ages (Sievenpiper *et al*., 2006; Vuksan *et al*., 2000). Lack of quality control and of product standardization prevent the production of effective and reproducible doses of red ginseng extract.

It was demonstrated in this study that red ginseng extract increased the absolute numbers of circulating angiogenic cells such as $CD34^+$, $CD117^+$, and $CXCR4^+$

cells significantly 1 and 5 days after coronary stenting. Circulating angiogenic cells have been demonstrated to increase spontaneously within 7 days after AMI (Turan *et al*., 2007), which was similar to the increase in circulating angiogenic cells in both groups, 1 and 5 days after AMI in our study. It was demonstrated that the early increase in circulating angiogenic cells could be significantly potentiated by adding red ginseng extract to the conventional medical therapy. It was speculated that the early increases in circulating angiogenic cells had a greater impact on the restoration of microvascular integrity, which was reflected in the significant increases in CFR at 8 months. The exact mechanisms underlying the observed increase in mobilization of circulating angiogenic cells still need to be verified. Red ginseng extract may have sex hormone-like effects (Jang *et al*., 2008); sex hormones stimulate endothelial progenitor cell and hematopoietic stem cell migration and proliferation (Ray *et al*., 2008). Red ginseng extract also increases vascular endothelial growth factor levels, thereby stimulating angiogenic cell mobilization (Ray *et al*., 2008). The effects of red ginseng extract on LDL-cholesterol or total cholesterol concentrations could not be determined in this study because more than 90% of patients in both groups [96% ($n = 24$) in the red ginseng group and 92% ($n = 23$) in the placebo group, $p = 0.552$] received atorvastatin 10 mg as a standard medication after AMI.

Red ginseng extract reduced the levels of inflammatory markers such as IL-6, TNF-α, sICAM-1 and sVCAM-1 to a greater extent than the placebo. However, decreases in hsCRP and increases in adiponectin concentrations were similar between the two groups. The increases in IL-6 and TNF-α are responsible for boosting the synthesis of hsCRP from hepatocytes and coronary artery smooth muscle cells (Hong *et al*., 2006). IL-6 and TNF-α concentrations were presumably more directly affected by red ginseng extract than were hsCRP and adiponectin concentrations, and it was inferred that hsCRP and adiponectin concentrations were influenced by other unknown pathophysiological mechanisms and medications used in this study. Moreover, the increases from baseline in left ventricular ejection fraction measured by echocardiogram did not show significant differences between the two groups, suggesting that slight improvements in left ventricular function could not be compared with a conventional echocardiogram. Improvements in the 8-month follow-up CFR and diastolic deceleration time measurements in the red ginseng group indicate that these measurements may be more sensitive for detecting the restoration of microvascular integrity and myocardial function.

Rare but possible side-effects of red ginseng extract such as urticaria, rash and diarrhea was not found in any patients during the follow-up. However, one patient in the red ginseng group had an alanine aminotransferase level greater than 3 times the upper normal limit 5 days after randomization. Administration of red ginseng extract was maintained because the ALT level normalized during the follow-up period. Selected ginsenosides from red ginseng extract have been reported not to interfere with the metabolism of various cytochrome P450 isoforms when coadministered with other drugs (Henderson *et al*., 1999; Vuksan *et al*., 2000). Based on these findings, red ginseng extract can be safely and effectively used in AMI patients at least up to a dose of 3 g per day, although a study involving a larger number of AMI patients with long-term follow-up is required to confirm our safety findings. Although a good correlation between the increase of circulating angiogenic cells and the recovery of microvascular integrity was observed in this study, the causal link of these observations should be investigated in more detail. Moreover, the improvement of CFR is not reflected in 8-month clinical outcomes, so the clinical benefit of red ginseng extract remains to be determined in larger trials.

In conclusion, administration of 3 g of red ginseng extract per day improved CFR in first ST-elevation AMI patients during the 8-month follow-up. Greater decreases in inflammatory cytokines such as IL-6, TNF-α, sICAM-1 and sVCAM-1, together with greater increases in circulating angiogenic cells such as $CD34^+$, $CXCR4^+$, $CD117^+$, C-met^+ cells are thought to have expedited the recovery of microvascular integrity after acute ischemic injury in the red ginseng group. These observations suggest that 3 g per day of red ginseng extract for 8 months is an effective and safe treatment for first ST-elevation AMI patients.

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Conflict of Interest

The authors have declared that there is no conflict of interest.

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