Glutathione attenuates coronary constriction to acetylcholine in patients with coronary spastic angina

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Glutathione attenuates coronary constriction to acetylcholine in patients with coronary spastic angina. Am J Physiol Heart Circ Physiol 280: H264–H271, 2001.—This study examined the effect of reduced glutathione (GSH), an important antioxidant that restores intracellular redox imbalance and prevents inactivation of endothelial-derived nitric oxide, on the abnormal vasomotor reactivity in spastic coronary arteries. The responses of epicardial diameter of the left coronary arteries to intracoronary infusion of acetylcholine (ACh; 50 μg/min) were measured by quantitative coronary angiography before and during combined intracoronary infusion of GSH (50 mg/min for 6 min) or saline as a placebo in 24 patients with coronary spastic angina and in 28 control patients. All of the spastic coronary arteries showed constrictor response to ACh, whereas the control coronary arteries as a whole showed only minimal diameter changes to ACh. GSH infusion suppressed constrictor response of epicardial diameter to ACh in patients with coronary spastic angina, whereas it had no significant effect in control subjects. Saline infusion did not have any effects. The results indicate that GSH attenuated the constrictor response to ACh in epicardial coronary arteries of patients with coronary spastic angina. GSH may have an important role in the regulation of coronary vasomotor function in patients with coronary spastic angina.

METHODS

Study patients. The study initially recruited 29 consecutive patients with coronary spastic angina (mean age 62 yr, ranging from 37 to 72; 16 men and 13 women) who underwent cardiac catheterization in our hospital. The patients with coronary spastic angina fulfilled all of the following inclusion criteria: 1) spontaneous attacks of chest pain associated with S-T segment elevation or depression on 12 lead ECG, 2) spontaneous attacks of chest pain associated with ST segment elevation or depression on 12 lead ECG, 3) spontaneous attacks of chest pain associated with ST segment elevation or depression on 12 lead ECG, 4) spontaneous attacks of chest pain associated with S-T segment elevation or depression on 12 lead ECG, 5) spontaneous attacks of chest pain associated with ST segment elevation or depression on 12 lead ECG, and 6) spontaneous attacks of chest pain associated with S-T segment elevation or depression on 12 lead ECG. The study was approved by the institutional review board of Kumamoto University Hospital. All patients gave informed consent.

Coronary vasospasm; endothelial function; free radicals

IMPAIRMENT OF ENDOTHELIUM-DEPENDENT VASODILATION AS WELL AS HYPERCONTRACTILE RESPONSE OF SMOOTH MUSCLE IN CORONARY ARTERIES may play an important role in the genesis of coronary spasm (4, 5). Although coronary risk factors and atherosclerotic changes are also known to increase coronary arterial tone (2, 7, 6, 11, 16), only smoking is among the risk factors associated with patients with coronary spastic angina (19). Furthermore, spasm frequently occurs in the angiographically normal coronary arteries rather than in arteries with organic stenosis (4, 5, 13). Thus coronary spasm seems to have a unique feature from the vascular disorders distinct from atherosclerotic coronary artery disease, and the precise mechanisms by which coronary spasm occurs remain to be determined. We and others (4, 10) have recently shown that oxidative stress is increased in patients with coronary spastic angina and that vitamins C and E, antioxidants, restored the abnormal arterial reactivity in patients with coronary spastic angina. These previous studies suggest that oxygen-derived free radicals may cause endothelial dysfunction or inactivate endothelial-derived nitric oxide, leading to the increase in vasomotor reactivity in coronary arteries of patients with coronary spastic angina.

Vitamins C and E are thought to exert their beneficial effects through scavenging reactive oxygen molecules and increasing the availability of intracellular reduced glutathione (GSH) and thiols (9, 12). GSH is a major naturally occurring antioxidant, and it has an important role in the regulation of intracellular redox state and protects cells from oxidative injury in systems that scavenge radicals, eliminate lipid peroxidation products through GSH peroxidases (GPx), and repair oxidant damage (9). We and others (6, 15) have recently shown that intracoronary infusion of GSH restored arterial endothelial dysfunction in patients with coronary risk factors. However, these previous studies did not show any data regarding effects of GSH on oxidative state. This study thus examined the effects of exogenous addition of GSH on the coronary vasomotor reactivity in patients with coronary spastic angina compared with those effects in control subjects.

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Table 1. Characteristics of study patients

<table>
<thead>
<tr>
<th></th>
<th>CSA (n = 24)</th>
<th>Control Subjects (n = 28)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>60 ± 3</td>
<td>59 ± 3</td>
<td>NS</td>
</tr>
<tr>
<td>Sex, female/male</td>
<td>12/11</td>
<td>16/12</td>
<td>NS</td>
</tr>
<tr>
<td>Current smoker</td>
<td>10 (42)</td>
<td>6 (21)</td>
<td>NS</td>
</tr>
<tr>
<td>Total cholesterol, mg/dl</td>
<td>198 ± 4.2</td>
<td>204 ± 3.8</td>
<td>NS</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>6 (25)</td>
<td>5 (18)</td>
<td>NS</td>
</tr>
<tr>
<td>Hypertension</td>
<td>4 (17)</td>
<td>5 (18)</td>
<td>NS</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>4 (17)</td>
<td>4 (14)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data presented are number of patients or means ± SE. Values in parentheses are % of total. Smoker, ≥10 cigarettes/day for 10 yr; hypercholesterolemia, total cholesterol ≥240 mg/dl or a lipids-lowering medication; hypertension, blood pressure ≥140/90 mmHg or on an antihypertensive medication; diabetes mellitus, fasting blood glucose ≥126 mg/dl or on an antidiabetic medication. CSA, coronary spastic angina; NS, not significant.

electrocardiograms (ECG) or ambulatory ECG at rest, usually in the middle of the night or early morning; 2) no organic stenosis (<10% stenosis) in coronary arteries angiographically; 3) coronary artery spasm (total or subtotal occlusion) in the left coronary arteries, demonstrated angiographically during the anginal attack of chest pain, with the S-T segment changes after the intracoronary infusion of acetylcholine (ACh), as reported previously (also described in Study protocol and RESULTS) (4, 5, 13).

This study also included 28 control patients with atypical chest pain (mean age 59 years, ranging from 48 to 78; 16 men and 12 women) who underwent cardiac catheterization during the same study periods as the patients with coronary spastic angina. These control patients were selected to match the risk factors for coronary artery disease to those in patients with coronary spastic angina. The control patients fulfilled all of the following inclusion criteria: 1) no significant S-T segment changes during the chest pain on 12-lead ECG and ambulatory ECG; 2) neither chest pain nor S-T segment changes during the treadmill test; 3) angiographically normal coronary arteries (<10% stenosis) and no coronary spasm (<50% decrease of the coronary diameter from the baseline) during the intracoronary infusion of ACh (4, 5).

Clinical characteristics of these patients are shown in Table 1. All medications except sublingual nitroglycerin were withdrawn at least 3 days before the study. None of the study patients had pharmacological doses of antioxidants at least 1 mo before the study. No study patient had taken nitroglycerin within 6 h of the study. No patient had previous myocardial infarction, congestive heart failure, cardiomyopathy, valvular heart disease, or other serious diseases. Written informed consent was obtained from all patients before the study. The study was conducted in agreement with the guidelines approved by the ethics committee at our institution.

Study protocol. The study protocol is schematically shown in Fig. 1. Coronary angiographic study was performed with the Judkins’ technique using contrast material (Iopromide, Schering-AG) in the morning when the patients were fasting. After the baseline measurements of systemic hemodynamics, we perform an angiography of the left and right coronary arteries. ACh was then infused into the left coronary artery (50, 100 μg/min) and subsequently into the right coronary artery (20, 50 μg/min) until coronary spasm was induced or the maximal doses were reached in all patients of both groups recruited. The infusion of ACh was performed for 1 min with a 5-min interval between the different doses of ACh. The measurements of systemic hemodynamics and the angiography of the coronary arteries were repeated at the end of each infusion. Coronary spasm induced by this method resolved spontaneously within 2–3 min without use of nitrate and allowed further studies in all patients with coronary spastic angina in this study.

Of the 29 patients with coronary spastic angina initially recruited, 24 patients with coronary spastic angina, who had spasm in the left coronary arteries after the infusion of ACh at 100 μg/min but not 50 μg/min (described in RESULTS), were subsequently examined for effects of GSH or saline on the coronary reactivity to the ACh infusion at 50 μg/min. Also, all of the 28 control subjects were further examined. Fifteen minutes after the completion of the intracoronary infusion of ACh, by which time the coronary diameter had nearly returned to the baseline level as confirmed by coronary angiography, measurements of systemic hemodynamic parameters and the control angiography of the left coronary arteries were performed. GSH (50 mg/min at a rate of 2 ml/min for 6 min; Yamanouchi, Tokyo, Japan) was infused by an infusion pump into the left coronary artery through the Judkin’s catheter in 13 patients with coronary spastic angina and in 14 control subjects, and saline (0.9%) as a placebo for GSH was infused in the remaining 11 patients with coronary spastic angina and 14 control subjects in an otherwise identical manner as GSH. This dose of GSH improved coronary endothelial vaso-motor dysfunction in humans (6), and we expected −1 mmol/l of GSH plasma concentration in the coronary circulation (6), concentrations of which were reported to increase intracellular GSH level by twofold in cultured endothelial cells (20). During the last 1 min of GSH or saline infusion, ACh (50 μg/min) was simultaneously infused into the left coronary arteries in the same manner as performed before the infusion of GSH or saline. The measurements of systemic hemodynamics and the coronary angiography were performed before and at the end of the infusion. After an additional 10 min, isosorbide dinitrates (1 mg) were injected into the left coronary arteries, and the coronary angiography was performed in multiple projections. All drugs were dissolved in 0.9% saline in a sterile manner and kept at 37°C. The effects of GSH or saline on the coronary reactivity to the ACh infusion

Fig. 1. Schematic representation of the infusion protocol. CSA, patients with coronary spastic angina; control, control subjects; ACh, acetylcholine; GSH, reduced glutathione; ISDN, isosorbide dinitrate.
RESULTS

Provocation of coronary spasm. In all patients with coronary spastic angina, spasm occurred in the coronary arteries into which ACh was infused in association with both chest pain and ischemic ST segment changes. Four LAD and two LCx arteries in five patients with coronary spastic angina had spasm during the intracoronary infusion of 50 μg ACh. The remaining 24 patients had spasm in 23 LAD and 11 LCx arteries during the intracoronary ACh infusion at 100 but not 50 μg/min. Total occlusion occurred at the proximal segment of 3 coronary arteries, and subtotal occlusion occurred diffusely in at either the proximal or distal segments in the remaining 31 coronary arteries. On the other hand, the intracoronary infusion of ACh did not induce coronary spasm associated with signs of myocardial ischemia in any of the control patients.

Responses of epicardial coronary arteries to ACh. In patients with coronary spastic angina, the coronary diameter responses to the ACh infusion alone and in combination with GSH or saline were examined in the left coronary arteries at the sites with the ACh-induced spasm (Table 2), and this analysis was performed at the concentration of 50 μg ACh, which did not provoke spasm in the patients with coronary spastic angina, because the diameter of the coronary arteries during total or subtotal occlusion due to coronary spasm cannot be accurately measured. Therefore, this study excluded the five patients with coronary spastic angina who had spasm in the left coronary arteries after the ACh infusion at 50 μg/min. In control patients, the diameter responses of 28 LAD and 28 LCx arteries to ACh at 50 μg concentration were analyzed as referenced control coronary arteries (Table 2). All of the spastic coronary arteries showed strong constrictor response to the subthreshold concentration of 50 μg ACh, whereas the control coronary arteries as a whole showed minimal diameter changes to 50 μg ACh, as shown in Fig. 2. As shown in Table 3, heart rates and mean blood pressure at baseline and after ACh infusion in patients with coronary spastic angina were not significantly different from those in control patients.

Table 2. Coronary segments in left coronary arteries analysed for vasomotor reactivity

<table>
<thead>
<tr>
<th>Patients with GSH infusion</th>
<th>LAD Proximal</th>
<th>LAD Distal</th>
<th>LCx Proximal</th>
<th>LCx Distal</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSA (n = 13)</td>
<td>11</td>
<td>9</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>Control (n = 14)</td>
<td>14</td>
<td>14</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Patients with saline infusion</td>
<td>5</td>
<td>11</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>CSA (n = 11)</td>
<td>14</td>
<td>14</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Control (n = 14)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are number of coronary segments; n is the number of patients. LAD: left descending coronary arteries, LCx: left circumflex coronary arteries. Coronary vasomotor reactivity was measured in all coronary segments with spasm provoked by ACh at 100 but not 50 μg in patients with CSA and in all of proximal and distal coronary segments in control patients.
Responses to GSH alone and in combination with ACh. The effects of the GSH infusion on the coronary luminal diameters were analyzed in all segments with spasm in the left coronary arteries in 13 patients with coronary spastic angina, i.e., 32 spasm sites in 19 proximal (11 LAD and 8 LCx arteries) and 13 distal segments (9 LAD and 4 LCx arteries) of the 21 spasm arteries (Table 2). The analysis was also performed at 28 proximal (14 LAD and 14 LCx arteries) and 28 distal segments (14 LAD and 14 LCx arteries) of the 28 control arteries in 14 control patients (Table 2).

The epicardial coronary diameters were not significantly changed after the infusion of GSH alone in both spastic and control coronary arteries (diameters at proximal segment of spastic arteries (19 segments): 2.4 ± 0.2 mm before GSH vs. 2.4 ± 0.1 mm after GSH, P = not significant (NS); diameters at proximal segments of control arteries (28 segments): 2.8 ± 0.2 mm before GSH vs. 2.8 ± 0.1 mm after GSH, P = NS). However, the constrictor response to ACh (50 μg) in spastic coronary arteries was significantly attenuated by the combined infusion of GSH (Fig. 3), whereas the response to ACh in control coronary arteries was not affected by GSH [percent changes of the distal segments from baseline, spastic arteries (12 segments): ACh alone −28 ± 6% vs. ACh plus saline −28 ± 7%, P = NS; control arteries (28 segments): ACh alone −2 ± 4% vs. ACh plus saline −2 ± 4%, P = NS]. The saline infusion did not significantly affect systemic hemodynamics (data not shown).

Responses to combined infusion of saline with ACh. The effects of the saline infusion on the response of the coronary luminal diameters to the ACh infusion were analyzed in all of the distal coronary segments with spasm in patients with coronary spastic angina, i.e., 12 spasm sites at the distal segments (11 LAD and 1 LCx arteries) (Table 2). The analysis was also performed at 28 distal segments (14 LAD and 14 LCx arteries) of the 28 control arteries in 14 control patients (Table 2). The intracoronary infusion of saline did not affect the response of the epicardial coronary diameters to ACh in both spastic and control coronary arteries [percent changes of the distal segments from baseline, spastic arteries (12 segments): ACh alone −28 ± 6% vs. ACh plus saline −28 ± 6%, P = NS; control arteries (28 segments): ACh alone −2 ± 4% vs. ACh plus saline −2 ± 4%, P = NS]. The saline infusion did not significantly affect systemic hemodynamics (data not shown).

Baseline coronary diameter and response to nitrate. The baseline diameters of the spastic arteries in patients with coronary spastic angina were significantly smaller compared with the respective diameters at the control arteries in control patients [2.4 ± 0.2 mm (16 segments) vs. 2.7 ± 0.2 mm (28 segments), P < 0.05 at the proximal segment, and 1.4 ± 0.1 mm (20 segments) vs. 1.7 ± 0.1 mm (28 segments), P < 0.05 at the distal segment, respectively, in LAD arteries]. The percent

Table 3. Systemic hemodynamic parameters

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>ACh (50 μg)</th>
<th>GSH alone</th>
<th>GSH + ACh (50 μg)</th>
<th>ISDN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, beats/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CSA</td>
<td>67 ± 13</td>
<td>66 ± 14</td>
<td>66 ± 14</td>
<td>67 ± 13</td>
<td>71 ± 14</td>
</tr>
<tr>
<td>Control</td>
<td>70 ± 12</td>
<td>68 ± 11</td>
<td>71 ± 12</td>
<td>69 ± 12</td>
<td>75 ± 12</td>
</tr>
<tr>
<td>Mean blood pressure, mmHg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CSA</td>
<td>101 ± 13</td>
<td>99 ± 12</td>
<td>100 ± 12</td>
<td>99 ± 13</td>
<td>85 ± 13a</td>
</tr>
<tr>
<td>Control</td>
<td>102 ± 10</td>
<td>98 ± 11</td>
<td>103 ± 11</td>
<td>100 ± 12</td>
<td>86 ± 14a</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SD. ISDN, isosorbide dinitrate. *P < 0.01 vs. baseline value.
increase in the coronary diameter after nitrate was significantly greater in the spastic arteries than the control arteries at the proximal segments, as shown in Fig. 4. The coronary diameters of the spastic coronary arteries after nitrate were not significantly different from those of the control coronary arteries at either the proximal or distal segment (data not shown). Thus the basal tone of the epicardial coronary arteries was increased in the spastic arteries compared with that in the control arteries, a result that is consistent with previous reports (4, 5). The dilator response of the epicardial diameter to nitrate after the GSH infusion was significantly greater than that after the saline infusion in control coronary arteries (Fig. 4). In spastic coronary arteries, the dilator response to nitrate was not significantly different between after GSH and saline infusions, as shown in Fig. 4. The difference between the coronary diameter responses to the infusion of ACh alone and ACh plus GSH, reflecting the magnitude of the GSH-induced improvement of vasomotor reactivity to ACh, was not significantly correlated with the basal coronary diameter, dilator response to nitrate, and constrictor response to ACh in spasm coronary arteries [the difference between the coronary diameter responses of distal segments to 50 µg ACh alone and 50 µg ACh + GSH (13 segments): $r = -0.12$ vs. the basal diameter, $P = NS$; $r = 0.22$ vs. the dilator response to nitrate, $P = NS$; $r = 0.33$ vs. the constrictor response to 50 µg of ACh alone, $P = NS$].

**TBARS levels and GPx activity in coronary circulation.** There was no significant difference in TBARS plasma levels in the aortic root at baseline and each of the infusions between patients with coronary spastic angina and control patients (data not shown). The difference in TBARS levels between the coronary sinus and aortic root (calculated by TBARS plasma level in coronary sinus minus that in the aortic root), reflecting the generation of lipid peroxidation in the coronary circulation, was comparable at baseline between patients with coronary spastic angina and control patients, as shown in Fig. 5. It was significantly increased from the baseline values during the ACh infusion in both patients groups, but the magnitude of the increase was greater in patients with coronary spastic angina than control patients during the infusion of ACh even at the lower concentration (50 µg/min), which did not provoke coronary spasm. The coronary sinus-arterial difference of the TBARS levels was not significantly changed from the baseline by the infusion of GSH alone in both groups (data not shown). However, the combined infusion of GSH significantly suppressed the increase in the coronary sinus-arterial difference of the TBARS level during the ACh infusion in both patients groups to the comparable values (Fig. 5), whereas the combined infusion of saline with ACh had no effect in both groups (data not shown).

The coupled enzymatic assay with use of GSH showed comparable activities of GPx among the plasma samples tested, as shown in Table 4. The activity was dependent on the amount of plasma (10–100 µl) added and the GSH concentrations (0.1–10 mmol/l) used in the assay (data not shown). The assay without use of GSH showed significant activity only in the samples from the coronary sinus during the GSH infusion but no detectable activity in the other samples, as shown in Table 4.

**DISCUSSION**

The present study showed that the intracoronary infusion of GSH attenuated the constrictor response of the epicardial spastic coronary arteries to ACh, whereas it had no effect on the response to ACh in control coronary arteries. Furthermore, GSH had no significant effect on the dilator response of spastic coronary arteries to nitrate, an endothelium-independent relaxant, suggesting that the effect of GSH on the...
constrictor response of spastic arteries to ACh may be partly mediated by an improvement in endothelium-dependent dilation. The present study also showed that the beneficial effect of GSH on spastic coronary arteries was associated with the suppression of the increase in the production of TBARS, a marker of lipid peroxidation, in the coronary circulation during the ACh infusion. Therefore, it is possible that suppression of oxidative stress in coronary arteries may contribute to the GSH-induced attenuation of the constrictor response to ACh in spastic coronary arteries.

GSH protects cells from oxidative damage through scavenging radicals and serving as a substrate of GPx and GSH S-transferases, which detoxify lipid-derived peroxides (9, 22). GPx is shown to exist in plasma, but no peroxidase activity was observed in plasma unless it was supplemented with exogenous GSH as a substrate of plasma GPx (22). This may be due to a lower extracellular concentration of GSH (~0.3 μmol/l) as a substrate of GPx (1). GPx activity is usually assayed by coupling the peroxidase reaction with the reduction of oxidized glutathione by glutathione reductase with NADPH in the presence of millimolar concentrations of GSH (14), because millimolar concentrations of GSH are present in intracellular spaces. The present coupled enzymatic assay without use of GSH, however, showed that plasma from the coronary sinus during the GSH infusion had a weak but significant GPx activity, whereas the assay without GSH showed no detectable activity in samples from either the aortic root or coronary sinus at baseline. This indicates that the increase in plasma GSH concentration (~1 mmol/l) during its infusion induced plasma GPx activation for reduction of hydrogen peroxide in the coronary circulation. The induction of GPx activity during the GSH infusion could at least partly contribute to the reduction of TBARS in the coronary sinus in association with improvement of endothelial vasomotor dysfunction. Furthermore, exogenously added GSH is likely to undergo extracellular breakdown, serving as a source of substrates for endogenous intracellular synthesis of GSH (1, 9, 20). This leads to a substantial increase in intracellular GSH concentration and a severalfold increase in the concentration of intracellular and extracellular cysteine in cultured endothelial cells and in humans (1, 20). The extracellular and intracellular GSH and cysteine might also scavenge oxygen-derived free radicals as antioxidants in the coronary circulation. Although we previously showed (6) that the GSH infusion improved endothelial vasomotor dysfunction in patients with coronary risk factors or atherosclerotic changes, the study did not provide the effects of the GSH infusion on oxidative state in the coronary circulation. Coronary artery spasm is likely to have several different features from atherosclerotic coronary artery disease (13, 19), but oxidative stress is shown to play a common role in the mechanisms of hyperreactive coronary arterial tone in both vascular diseases according to the present and previous studies (2, 4, 6, 11, 15, 21). A number of reports (6, 10, 15, 21) have shown that antioxidants improved endothelial vasomotor dysfunction in patients with risk factors or atherosclerotic changes, whereas there was only one report (4) showing attenuation of constrictor response of spasm coronary arteries by an antioxidant. The present report is the first study to show the beneficial effects of GSH infusion on spastic coronary arteries partly through reduction of oxidative stress, and this report thus provides important and novel information that would contribute to elucidation of the mechanisms of coronary artery spasm.

The beneficial effect of GSH is unlikely to be due to the spontaneous change of the response to the repeated infusion of ACh, since the saline infusion otherwise in the same protocol as the GSH infusion had no effects on the response of the epicardial diameter in the spasm coronary arteries to ACh. Also the effect of GSH was not due to the higher basal tone and the hypercontractile response to ACh of spasm coronary arteries, since the attenuation with GSH was not significantly correlated with the basal coronary diameter, the dilator response to nitrate, and the contractile response to ACh.

We showed that oxidative stress is increased in patients with coronary spastic angina in the present and previous studies (4, 10). However, we did not define the sources of oxidative stress in patients with coronary spastic angina. Several lines of clinical studies from our (7, 11) and other (2, 17) laboratories indicate that oxidative stress is associated with cigarette smoking, which is known to be highly prevalent in patients with coronary spastic angina (19). The myocardial ischemia-reperfusion, which is known to generate oxygen-derived free radicals in the coronary endothelium and myocardium (8, 23), is repeated by coronary spasm and is one of the characteristic features in patients with coronary spastic angina. Cigarette smoking and repeated ischemia-reperfusion could have consumed the antioxidants in plasma and the intracellular antioxidant defenses in vascular cells of spastic coronary arteries. This may partly contribute to the higher levels of TBARS.

### Table 4. GPx activity in plasma using the coupled enzymatic assay

| Assay without GSH | Control | (n = 14) | ND | ND | 0.03 ± 0.01 | ND |
| Assay without CSA | (n = 13) | ND | ND | 0.04 ± 0.01 | ND |
| Assay with GSH | Control | (n = 14) | 0.24 ± 0.02 | 0.21 ± 0.01 | 0.24 ± 0.02 | 0.23 ± 0.01 |
| Assay with CSA | (n = 13) | 0.23 ± 0.01 | 0.23 ± 0.01 | 0.24 ± 0.01 | 0.22 ± 0.01 |

Values (in U/ml) are expressed as means ± SD; n is the number of plasma samples. GPx, GSH peroxidase; ND, not detectable.
levels of TBARS production in spastic coronary arteries than control coronary arteries in the coronary circulation during the ACh infusion. The endothelial generation of oxygen-derived free radicals by the increase in blood flow during the ACh infusion (4) may play a critical role in the abnormal vasomotor reactivity of the spastic coronary arteries to ACh because the infusion of GSH alone had no effect on the baseline diameter of the spastic coronary arteries.

The present study showed that GSH infusion potentiated dilator response to nitrate in control arteries, a result that is consistent with a previous report (6) and may be possibly explained by stabilization of nitric oxide derived from nitrate and/or by augmentation of guanylate cyclase activation in smooth muscle (3, 18). On the other hand, greater dilation to nitrate after GSH did not reach significance compared with that after saline in spastic coronary arteries. Enhancement of dilator response to nitrate, which has been shown in spastic coronary arteries (4, 13), may possibly overwhelm the potential effect of GSH on dilator response to nitrate in spastic coronary arteries.

There are several limitations in the present study. It was hard to demonstrate direct evidence that the intracoronary infusion of GSH increased in intracellular GSH levels and improved redox state in the coronary endothelium in the present in vivo human study. It also remains to be determined in the present study whether GSH treatment could prevent anginal attack in patients with coronary spastic angina. TBARS measurement is susceptible to artifacts caused by variations in sample lipid content and iron contamination of reagents. Further studies are needed for evaluation of the potential therapeutic value of long-term oral administration of GSH or L-2-oxothiazolidine-4-carboxylic acid, a cell-permeable precursor of GSH (21), in patients with coronary spastic angina.

In conclusion, the results indicate that exogenous addition of GSH improved the abnormal vasomotor reactivity in response to ACh in epicardial coronary arteries of patients with coronary spastic angina through reduction of oxidative stress. GSH may have an important role in the regulation of coronary endothelial vasomotor function in patients with coronary spastic angina.

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