Intracoronary Infusion of Reduced Glutathione Improves Endothelial Vasomotor Response to Acetylcholine in Human Coronary Circulation

Kiyotaka Kugiyma, MD; Masamichi Ohgushi, MD; Takeshi Motoyama, MD; Osamu Hirashima, MD; Hirofumi Soejima, MD; Kenji Misumi, MD; Michihiro Yoshimura, MD; Hisao Ogawa, MD; Seigo Sugiyama, MD; Hirofumi Yasue, MD

Methods and Results—Responses of epicardial diameter and blood flow of the left anterior descending coronary artery to intracoronary infusion of acetylcholine (ACh, 50 μg/min) were measured by quantitative coronary angiography and Doppler flow-wire technique, respectively, before and during combined intracoronary infusion of GSH (50 mg/min) or saline in 26 subjects with no significant coronary stenosis. GSH infusion suppressed the constrictor response of epicardial diameter to ACh and enhanced the increase in blood flow response to ACh. Furthermore, GSH potentiated the coronary dilator effect of nitroglycerin. A beneficial effect of GSH on the epicardial diameter response to ACh was observed in a subgroup of subjects with ≥1 coronary risk factors but not in a subgroup without risk factors. Saline infusion did not have any effects.

Conclusions—The results indicate that GSH improved coronary endothelial vasomotor function, particularly in subjects with coronary risk factors, and it potentiated the vasodilator effect of nitroglycerin in human coronary arteries. (Circulation. 1998;97:2299-2301.)

Key Words: antioxidants free radicals endothelium-derived factors acetylcholine

Intracellular reduced glutathione (GSH) has an important role in protection of endothelial cells from oxygen free radicals, leading to prevention against endothelial dysfunction in arteries exposed to oxidative stress. Although extracellular GSH is not effectively transported into cells, exogenous addition of GSH is shown to cause a substantial increase in intracellular GSH concentration and a severalfold increase in concentration of intracellular and extracellular cysteine, a potent antioxidant, in cultured endothelial cells and in humans. Both GSH and cysteine can react with NO to form S-nitrosothiols, which stabilize NO derived from endothelium and organic nitrates and increase the vasodilator action of NO. The present study was thus conducted to assess the effect of intracoronary infusion of GSH on vasomotor reactivity in human coronary arteries.

Methods

Study Subjects
This study comprised 26 consecutive subjects (mean age, 62 years; range, 46 to 72 years; 14 men and 12 women) who underwent diagnostic cardiac catheterization for evaluation of atypical chest pain. All of the study subjects had angiographically normal coronary arteries (<10% stenosis) and no coronary spasm during intracoronary infusion of acetylcholine (ACh). All medications were withdrawn ≥3 days before study. None of the study subjects had taken pharmacological doses of antioxidants for at least 1 month before the study. No subject had previous myocardial infarction, congestive heart failure, cardiomyopathy, valvular heart disease, or other serious diseases. Written informed consent was obtained from all subjects before study. The study was conducted in agreement with the guidelines approved by the ethics committee at our institution.

Study Protocol
ACh (50 μg/min) was infused directly into the left coronary artery through a Judkins catheter for 1 minute. Fifteen minutes after ACh infusion, GSH (50 mg/min at a rate of 2 mL/min for 6 minutes; Yamanouchi Co) was infused into the left coronary artery through a Judkins catheter in 14 subjects (GSH group), and saline (0.9%) as a placebo for GSH was infused in the remaining 12 subjects (saline group) in an otherwise identical manner as GSH. This dose of GSH yielded 1.2 ± 0.1 mmol/L of GSH plasma concentration in coronary sinus, concentrations that were reported to increase intracellular GSH level by ∼2-fold in cultured endothelial cells. During the last minute of GSH or saline infusion, ACh (50 μg/min) was simultaneously infused into the left coronary arteries in the same manner as before the infusion of GSH or saline. After an additional 10 minutes, nitroglycerin (250 μg) was intravenously injected, and coronary angiography was performed in multiple projections. Coronary angiography and measurements of coronary blood flow and hemodynamics were performed before and at the end of each infusion. All drugs were dissolved in 0.9% saline in a sterile manner and kept at 37°C. Risk factors, age, and sex were matched between GSH (62 ± 4

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From the Division of Cardiology, Kumamoto University School of Medicine, Kumamoto, Japan.
Correspondence to Kiyotaka Kugiyma, MD, Division of Cardiology, Kumamoto University School of Medicine, Honjo 1-1-1, Kumamoto City, Japan 860-8556. E-mail kiyo@gpo.kumamoto-u.ac.jp

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years old, 8 men and 6 women) and saline (61±3 years old, 6 men and 6 women) groups. Seven subjects in the GSH group and 6 in the saline group had ≥1 coronary risk factor, defined as history of current smoking, hypercholesterolemia (>240 mg/dl), hypertension (>140/90 mm Hg or antihypertensive medication), and diabetes mellitus.

Quantitative Coronary Angiography and Measurement of Coronary Blood Flow

A quantitative coronary angiographic study was performed in the same manner as described in previous reports.8,9 In brief, the trunk of the left anterior descending coronary artery (LAD) was divided into proximal and distal segments of equal length. The luminal diameter at the center of each segment was measured quantitatively with the use of a computer-assisted coronary angiographic analysis system (Cardio 500, Kontron Instruments) by 2 observers blinded to the study protocol. Blood flow velocity was measured in 6 subjects in the GSH group and 10 in the saline group by use of a 0.014-in wire equipped with a Doppler crystal at its tip (Flow Wire, Cardiometrics) that was advanced through the Judkins catheter and carefully positioned in a straight proximal segment of the LAD.9 Coronary blood flow was calculated from blood flow velocity and arterial diameter.9 Responses of coronary artery diameter and flow to infusions of ACh, GSH, and saline were expressed as percentage changes from baseline coronary diameter and flow measured just before each infusion, respectively.

Statistical Analysis

Data are expressed as mean±SEM unless otherwise indicated. Differences between 2 means were compared by paired or unpaired Student’s t test. For comparison of coronary luminal diameters in subgroups with and without risk factors, 2-way ANOVA for repeated measures, followed by Bonferroni’s multiple comparison test, was used. A value of $P<0.05$ was considered statistically significant.

Results

Response to GSH and Saline Alone and in Combination With ACh

Infusion of GSH alone did not significantly change epicardial coronary diameters or blood flow. However, the combined infusion of GSH and ACh suppressed the constrictor response of epicardial coronary diameter to ACh (percentage change of distal diameter from baseline [minus sign denotes constriction], −7.7±2.9% before GSH versus −0.9±2.1% during GSH; $P<0.01$; Figure 1). Furthermore, GSH enhanced the increase in coronary flow response to ACh (percentage increase from baseline, 98±16% before GSH versus 140±20% during GSH; n=6; $P<0.05$). GSH did not significantly affect heart rate or mean blood pressure. Saline infusion alone or in combination with ACh did not have any effect (percentage change of distal diameter from baseline in response to ACh, −8.2±2.2% before saline versus −9.6±2.8% during saline, $P=NS$; percentage increase in blood flow from baseline in response to ACh, 92±14% before saline versus 98±12% during saline, $P=NS$). Coronary diameter and flow at baseline and their responses to ACh alone were each comparable between subjects treated with GSH and those given saline.

We also examined whether the presence of coronary risk factors altered the effect of GSH on the epicardial arterial diameter response to ACh. A beneficial effect of GSH on epicardial diameter response to ACh was observed in a subgroup of 7 subjects with ≥1 coronary risk factor (smoking, hypercholesterolemia, hypertension, or diabetes mellitus), whereas it was not observed in a subgroup of the remaining 7 subjects without risk factors, as shown in Figure 2. Age and sex were matched between the subgroups with and without risk factors. The subgroup with risk factors showed significantly greater constriction of epicardial coronary arteries in response to ACh alone than the subgroup without risk factors (Figures 1 and 2).

Response to Nitroglycerin

The dilator response of epicardial diameter to nitroglycerin was significantly greater in subjects treated with GSH than in subjects treated with saline (percentage increase in proximal diameter from baseline, 28±3% in the GSH group versus 19±2% in the saline group; $P=0.02$). The coronary dilator effect of nitroglycerin in both the GSH and saline groups was comparable between subgroups of subjects with and without risk factors (percentage increase in proximal diameter from baseline, 26±3% in the GSH group with risk factors versus 28±3% in the GSH group without risk factors, $P=NS$; 18±2% in the saline group with risk factors versus 19±2% in the saline group without risk factors, $P=NS$).

Discussion

This is the first study to show that intracoronary infusion of GSH suppresses constriction of epicardial arterial diameter...
and/or converts constriction to dilation in response to ACh in human coronary arteries. Furthermore, GSH enhances the increase in coronary blood flow response to ACh. The effects of GSH may be explained by at least 3 possible mechanisms: (1) improvement of redox state in endothelium, leading to restoration of endothelial functions, including NO synthase1,2; (2) formation of nitrosothiols with endothelium-derived NO and prevention of NO inactivation by oxygen free radicals6; and (3) augmentation of guanylate cyclase activation in smooth muscle.7

It has been shown10,11 that oxygen free radicals cause an imbalance in intracellular redox state in endothelial cells and inactivate endothelium-derived NO, leading to endothelial dysfunction and impairment of endothelium-dependent vasorelaxation in arteries of patients with coronary risk factors and in atherosclerotic arteries. In fact, the present study demonstrated that endothelium-dependent dilation of epicardial coronary arteries in response to ACh was impaired in subjects with risk factors compared with those without risk factors. The present study showed that the beneficial effect of GSH on the response of epicardial diameter to ACh was observed in subjects with risk factors but not in those without risk factors. Furthermore, the present study showed that GSH potentiated the coronary dilator response to nitroglycerin. However, GSH-induced potentiation of the dilator action of nitroglycerin, which shares a final common pathway through guanylate cyclase activation with endothelium-derived NO, was comparable between subjects with and without risk factors. Taken together, effects of GSH on the pathway of ACh-induced vasorelaxation, prior to guanylate cyclase activation in smooth muscle may be involved in mechanisms of GSH-induced improvement of the epicardial vasomotor response to ACh in subjects with risk factors. Thus, it is suggested that GSH improved endothelium-dependent dilation in response to ACh in part through restoration of intracellular redox imbalance and prevention of NO inactivation in endothelium. GSH administration may be useful in patients with coronary artery disease, both as a result of improvement of endothelial dysfunction and augmentation of nitroglycerin-induced vasodilation and antiplatelet activity.

In conclusion, intracoronary infusion of GSH improved the endothelial vasomotor response to ACh, particularly in subjects with coronary risk factors, and it potentiated the vasodilator effect of nitroglycerin in human coronary arteries.

References
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