Intracoronary Injection of Granulocyte Colony-Stimulating Factor Ameliorates the Progression of Left Ventricular Remodeling After Myocardial Ischemia/Reperfusion in Rabbits

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Background Although granulocyte colony-stimulating factor (G-CSF) is known to prevent left ventricular (LV) remodeling after acute myocardial infarction (AMI), the best method of administration is unknown.

Methods and Results A rabbit ischemia/reperfusion model was created and G-CSF was administered into the coronary artery immediately after reperfusion. The LV size and contraction were determined by echocardiography, and the extent of infarcted myocardium was measured by Masson-Trichrome staining. The benefits of intracoronary injection of G-CSF on LV remodeling were similar to subcutaneous injection.

Conclusions Direct intracoronary G-CSF injection may become a new therapy for AMI with lower adverse effects. (Circ J 2006; 70: 942–944)

Key Words: Granulocyte colony-stimulating factor; Ischemia/reperfusion; Remodeling

We and others have reported that granulocyte colony-stimulating factor (G-CSF) prevents left ventricular (LV) remodeling after acute myocardial infarction (AMI)^{1–5} and improves limb ischemia.⁶ We recently demonstrated that it inhibits the progression of LV remodeling through direct activation of the Jak2/STAT3 pathway, which has anti-apoptotic effects and angiogenic effects² in cardiomyocytes. Because leukocytosis may have a deleterious effect on atherosclerosis and the infarcted myocardium, the most effective method of administering G-CSF with less activation of bone marrow (BM) and more stimulation on cardiomyocytes is necessary. In the present study, we investigated the effects of direct intracoronary (IC) administration of G-CSF using a rabbit ischemia/reperfusion model.

Animal Models

The male Japanese white rabbit ischemia/reperfusion model was used⁵ and all protocols were approved by the Institutional Animal Care and Use Committee of Chiba University. Under anesthesia, the carotid artery was cannulated with a 4-F wedge-pressure catheter and the tip of the catheter was placed at the supra-aortic valve. Next, left thoracotomy was performed and the large coronary arterial branch coursing down the middle of the anterolateral surface of the LV was ligated for 30 min. After release of the ligature, the ascending aorta was clamped by the balloon in-

flation of the wedge-pressure catheter and the rabbits were then randomized into 3 groups: (1) control group (CONT): saline injection into the coronary artery (n=12); (2) G-CSF IC group (IC): direct IC injection with recombinant human G-CSF (100 µg/kg, rhG-CSF, Kirin Brewery Co Ltd, Tokyo, Japan, n=15) for 30 s; and (3) G-CSF SC group; subcutaneous (SC) injection of G-CSF (10 µg/kg per day × 5 days, n=10).

Measurements

The number of circulating white blood cells (WBC) was measured at pre-operation and 1, 3, 5, 7 and 14 days after operation. Echocardiography was performed at pre-operation and 14 days after operation. At 14 days, rabbits were killed and the extent of infarcted myocardium in relation to total LV (%area) was measured by Masson-Trichrome staining.

Statistics

All measurements are expressed as group mean ± SEM, and changes in cardiac function and infarct size were analyzed by 2-way repeated-measures ANOVA. Statistical differences were considered significant if the p-value was <0.05.

One CONT rabbit died during the study period, but none of the IC or SC rabbits (mortality rate; CONT: 6.6%; IC: 0%; SC: 0%) died. None of the groups showed loss of body weight or other behavioral problems. The number of WBC was more increased in the SC rabbits, and peaked at 7 days compared with the CONT rabbits, which peaked on day 1 (Table 1). In the IC rabbits, the WBC peak was on day 1 and decreased rapidly thereafter.

LV dilatation and dysfunction were less in the IC and SC rabbits than in CONT rabbits (Figs 1A,B). Although the area at risk measured by Evan blue staining was the same

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Table 1 Number of WBCs in Peripheral Blood

	No. WBC (μ L)					
	Pre	1 day	3 days	5 days	7 days	14 days
CONT	7,950 $\pm$ 890	10,500 $\pm$ 1,400	10,000 $\pm$ 1,860	9,800 $\pm$ 1,570	9,720 $\pm$ 1,360	7,010 $\pm$ 390
IC	7,720 $\pm$ 360	21,450 $\pm$ 2,860*	12,910 $\pm$ 2,580	13,010 $\pm$ 2,070	12,180 $\pm$ 1,670	7,200 $\pm$ 400
SC	9,850 $\pm$ 1,110	12,500 $\pm$ 1,670	15,900 $\pm$ 2,950*	23,450 $\pm$ 3,730*	24,520 $\pm$ 3,420*	10,440 $\pm$ 960*

Results are mean $\pm$ SEM. * p <0.05 vs CONT.

WBC, white blood cell; CONT, control group; IC, intracoronary; SC, subcutaneous.

Fig 1. Effect of intracoronary granulocyte colony-stimulating factor (G-CSF) administration on (A) left ventricular end-diastolic diameter (LVDd) and end-systolic diameter (LVDs) and (B) left ventricular fractional shortening determined by echocardiography. (C) Effect of intracoronary G-CSF administration on myocardial infarction size measured by Masson-Trichrome staining. Results are expressed as mean $\pm$ SEM. * p <0.05. CONT, control group; IC, intracoronary; SC, subcutaneous.

in the 3 groups (CONT: 32.4 $\pm$ 2.3%; IC: 35.7 $\pm$ 2.6%; SC: 33.4 $\pm$ 2.9%), the %area of infarcted myocardium was smaller in the IC and SC hearts compared with the CONT hearts (Fig 1C).

In this study using a rabbit ischemia/reperfusion model, one injection of G-CSF directly into the coronary artery prevented LV remodeling and reduced the infarct size by a degree similar to that with SC injection. Moreover the survival rate was also improved in relation to the inhibition of LV remodeling. Because the level of leucocytosis was less in the IC rabbits compared with the SC rabbits, the leucocytosis-induced systemic side effects are also thought to have been low.

The effects of G-CSF on the AMI-affected heart are mainly anti-apoptotic and angiogenic through direct activation of the Jak2/STAT3 pathway in cardiomyocytes? In our previous study, the beneficial effects of G-CSF on LV remodeling were dose dependent and significantly reduced by a delayed start of treatment? Because the main effect of G-CSF on the AMI heart is the protection of cardiomyocytes, but not the mobilization of BM cells, earlier administration and a higher dose of G-CSF will conceiva-

bly protect cardiomyocytes more effectively after AMI. To more effectively protect infarcted myocardium with less systemic side effects, IC administration of G-CSF may be a better method of treating AMI.

Because most AMI patients are treated with percutaneous coronary intervention (PCI), it is very easy and reasonable to inject G-CSF into the coronary artery immediately after PCI. This treatment may become a new therapy for AMI patients and a more detailed study using large animals and a clinical study are awaited.

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