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Cyclophosphamide Improves the Function of Post-Infarct Hearts by Reducing Old Infarct Area and Accelerating the Mobilization of CD34+ Cells

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Background Myelosuppressives such as cyclophosphamide (Cy) and 5-fluorouracil (5FU), which can increase circulating CD34+ cells, may have a beneficial effect in the post-infarct heart.
Methods and Results Twenty-four hours after 30-min ischemia–reperfusion, rabbits were intravenously treated with Cy (20 mg/kg), 5FU (15 mg/kg) or saline (S). Cy significantly improved cardiac function and remodeling and decreased the old infarct area 1 month after infarction, compared with those given S. The number of circulating CD34+ cells was higher and neovascularization and matrix metalloproteinase-1 was induced in the Cy group.
Conclusion Cy has potential for post-infarct treatment. (*Circ J* 2005; **69:** 763–765)

Key Words: CD34+ cell; Cyclophosphamide; 5-fluorouracil; Myocardial infarction

Hematologically, myelosuppressives such as cyclophosphamide (Cy) and 5-fluorouracil (5FU) transiently suppress bone marrow (BM), but then enhance its activity and mobilize stem/progenitor cells including BM-CD34+ cells into circulation[1] BM cell transplantation and G-CSF improve left ventricular (LV) remodeling and function after myocardial infarction (MI) via mobilization of the cells and/or repair-related cytokines.[2–5] Therefore, we investigated the therapeutic potency of Cy and 5FU for MI.

Methods

In male Japanese white rabbits (≈2.0 kg), MI was induced by 30-min ischemia–reperfusion as previously documented (Day 0).[5] The rabbits, which had been randomly assigned to 3 groups, were treated with 5FU (15 mg/kg), Cy (20 mg/kg), or saline (S, n=16 in each group) on Day 1. Ten rabbits from each group were killed by an overdose of pentobarbital sodium on Day 28. Meanwhile, arterial blood was collected before ischemia and again on Days 3, 7, 14, 21, and 28 for white blood cell count (WBC) and hemogram. From the blood samples obtained on Days 7, 14, 21, or 28, mononuclear cells were separated by ficoll gradient and incubated with anti-human FITC-conjugated CD34 according to the manufacturer's instruction (Serotec Ltd) in order to count the CD34+ cells by flow cytometer (EPICS XL, Beckman Coulter). The LV end-diastolic dimension (EDD/body weight (BW), mm/kg) and LV ejection fraction (EF, %) were echocardiographically measured before MI and on Day 28. After death, the LV was weighed, and stained immunohistochemically with α-sarcomeric actin to measure the LV wall area, infarcted and surviving areas, or with CD31 for counting the vessel density in the infarcted area.[5] Six rabbits from each group were killed on Day 7 for Western analysis of matrix metalloproteinase (MMP)-1 expression.[5]

Differences of all values, means ± SD, were assessed by two-way repeated measurements of analysis of variance (ANOVA) with post hoc test (Tukey-Kramer's test), and $p<0.05$ was considered significant.

Results

All rabbits in each group survived for 7 or 28 days. Echocardiographically, LVEF and EDD/BW on Day 28 was significantly improved in the Cy group (EF/(EDD/BW): 64±5%/4.2±0.4 mm/kg) compared with the values for S group [(EF/(EDD/BW): 53±4%/5.1±0.5 mm/kg)] (Fig 1). The area of old infarct was significantly smaller in the Cy group (9.2±2.4%) than in the S group (19.2±4.1%), but no significant difference between the 5FU (15.6±3.4%) and S groups. The number of circulating CD34+ cells was significantly increased in the Cy (155±81/μL) and 5FU groups (81±41/μL) than in the S group (31±7/μL), but the increase in Cy was greater than in 5FU on Day 7. These treatments did not reduce the WBC until Day 7, and conversely increased it on Days 14 and 21. Cy was superior to 5FU or S (Fig 2) for neovascularization and MMP-1 expression,

Discussion

The current study demonstrated that Cy, but not 5FU, improved cardiac function and remodeling and reduced scar area in the infarcted heart, by mechanisms that involved

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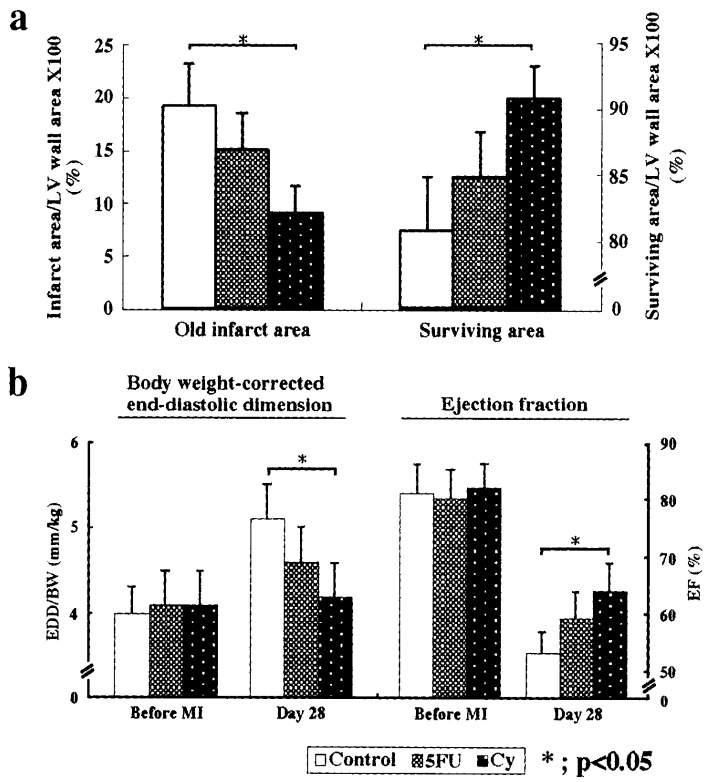

Fig 1. Cardiac function and size of the old infarct. In 28-day reperfused hearts, the cyclophosphamide-treated group (Cy; n=10) had a significantly reduced old infarct size and increased area of surviving myocardium, compared with the 5-fluorouracil-treated (5FU, n=10) or saline group (S, n=10). Accordingly, Cy significantly ameliorated left ventricular wall motion and remodeling.

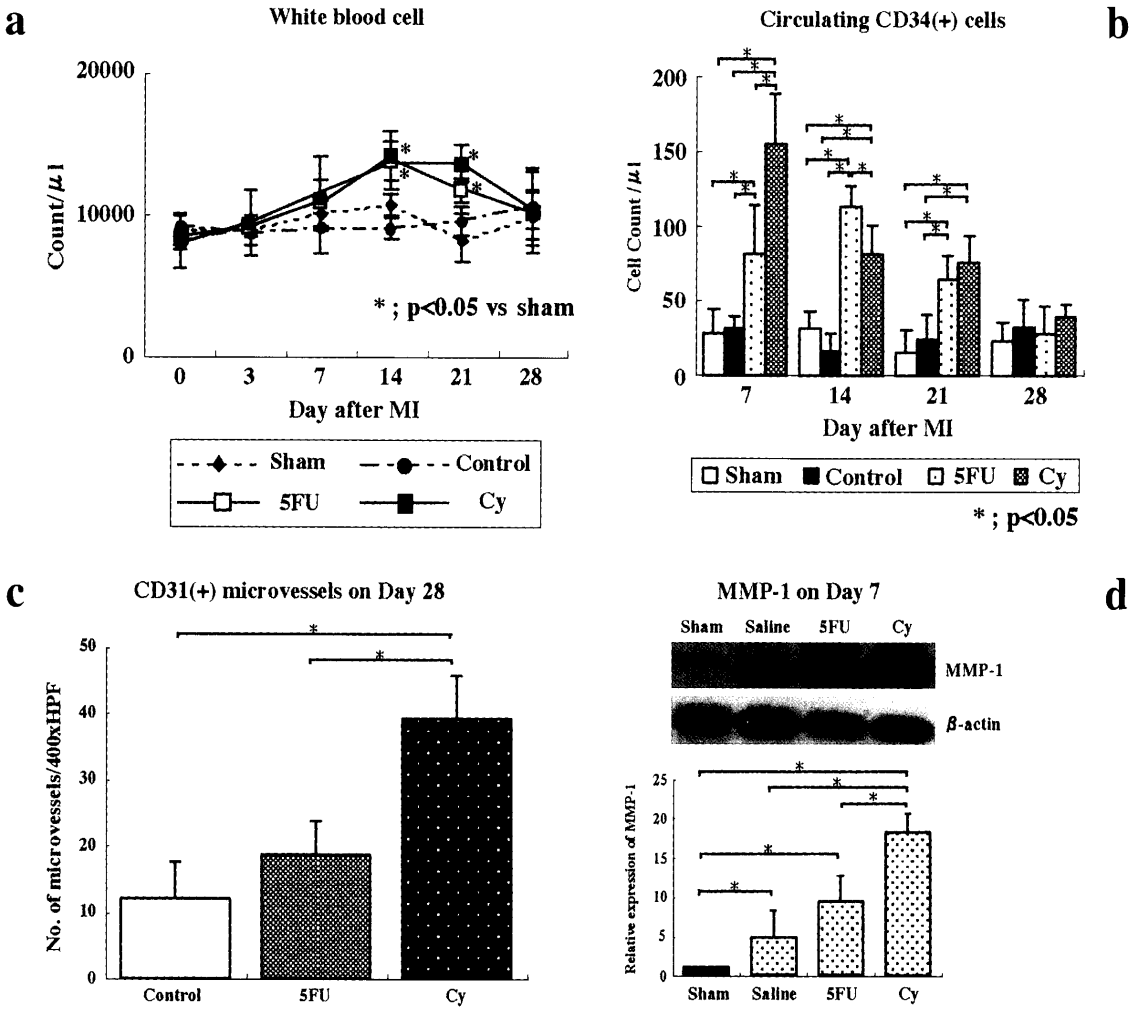

Fig 2. White blood cells (WBC) and CD34+ cells. Flow cytometry demonstrated a significant increase in the number of circulating CD34+ cells in the cyclophosphamide-treated group (Cy) peaking on Day 7, compared with the saline (S) group. The increase in CD34+ cells occurred later in the 5-fluorouracil-treated (5FU) group without a reduction in WBC as an adverse effect. Cy induced significant neovascularization and upregulation of matrix metalloproteinase-1 (MMP-1).

neovascularization and MMP-1 upregulation. To our knowledge, this is the first report of a beneficial effect of myelosuppressives in an infarcted heart. Cy and 5FU did not reduce the WBC until 7 days after MI, and it was increased thereafter, indicating mild myelosuppression followed by hyper-reaction. However, the increase in the number of circulating $CD34^+$ cells was greater for Cy than 5FU. $CD34^+$ cells facilitate regeneration of myocardium and/or secrete various cytokines that eventually beneficially affect cardiac repair.[3–5] Thus, the greater increase by Cy may explain the current differences in effect of Cy and 5FU. Anticancer treatment with myelosuppressives causes adverse effects such as marked myelosuppression, but in the current study, a dose of Cy caused mild myelosuppression that was beneficial for infarcted hearts, suggesting a potential for clinical application.

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