CARDIOVASCULAR

Therapeutic Effect of Midkine on Cardiac Remodeling in Infarcted Rat Hearts

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Background. **Midkine is expressed in the developing fetus and in adult organs stressed by ischemia, but its physiologic role in ischemic organs is poorly understood. Here we investigated the effect of midkine on cardiac remodeling after ischemia caused by myocardial infarction.**

Methods. **The expression pattern of the endogenous midkine gene in rat heart was evaluated by real-time polymerase chain reaction for 2 weeks after myocardial infarction. To investigate its effect, recombinant midkine was injected into hearts 2 weeks after myocardial infarction, and cardiac functions were monitored by echocardiography. Six weeks later, the hearts were removed, and the areas of infarcted and viable tissue and the extent of cardiomyocyte hypertrophy were determined histologically.**

Results. **The midkine gene was strongly upregulated in the infarcted myocardium, but this upregulation lasted less than 2 weeks. Cardiac remodeling was significantly and dose-dependently attenuated by midkine treatment. The midkine treatment also increased collagen accumulation and facilitated angiogenesis in the infarcted area, and the viable muscle area after myocardial infarction dose-dependently increased. Despite this increase of viable muscle area, the midkine-treated hearts showed significantly less cardiomyocyte hypertrophy than vehicle-treated hearts, suggesting midkine had prevented chronically ischemic cells from dying and dropping out by angiogenesis.**

Conclusions. **Our results indicate midkine can attenuate cardiac remodeling after myocardial infarction, and suggest midkine has therapeutic potential for subacute myocardial infarction.**

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Progress in the treatment of myocardial infarction (MI) has significantly reduced acute mortality from ischemic cardiomyopathy. As a result, however, a large number of patients suffer from chronic heart failure caused by the loss of the infarcted myocardium. Patients with postinfarction heart failure account for nearly half of the candidates for cardiac transplantation [1]. Currently, heart transplantation is the only curative procedure for end-stage heart failure. However, cardiac transplantation is limited by donor heart availability. Therefore, an important goal for treating MI is to protect the heart by preventing the cardiac remodeling induced by the loss of myocardium.

Several cytokines, including granulocyte colony-stimulating factor, hepatocyte growth factor, insulinlike growth factor-1, and leukemia inhibitory factor have beneficial effects on cardiac remodeling after myocardial infarction [2–6]. These cytokines mobilize bone-marrow–derived cells to the injured heart after MI and induce the regeneration of myocardium or prevent the activation of cell death in the viable myocardium after infarction, limiting the ventricular dilation and myocardial loading.

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Midkine (MDK) is a developmentally regulated cytokine and is highly conserved from Drosophila to human. Midkine has been implicated in neural development, neurodegenerative diseases, and certain cancers. Midkine is expressed in the radial glial processes of the embryonic brain, along which neural stem cells migrate and differentiate [7, 8]. Midkine shows mitotic, antiapoptotic, and angiogenic activities, which are the basic property required for the regeneration of injured tissues, not only in developing neural tissues but also in the adult brain [7–9]. Furthermore, some reports suggest that MDK is expressed in and contribute to the repair of injured extraneural organs, including the liver and heart [10–13]. In addition, MDK stimulates collagen by enhancing the expression of transforming growth factor-β_1 [14].

Here, we examined the expression of MDK in rat hearts after MI and tested whether MDK could have therapeutic effects on subacute MI heart through its stimulation of collagen expression and their angiogenic, antiapoptotic, and growth-promoting activities.

Material and Methods

Animals and Surgical Procedures

Male Lewis rats were used at 8 weeks of age (weight, 220 to 250 g [Seac Yoshitomi, Fukuoka, Japan]). All animals received humane care under the Guidelines on Animal Experiments of Osaka University Graduate School of

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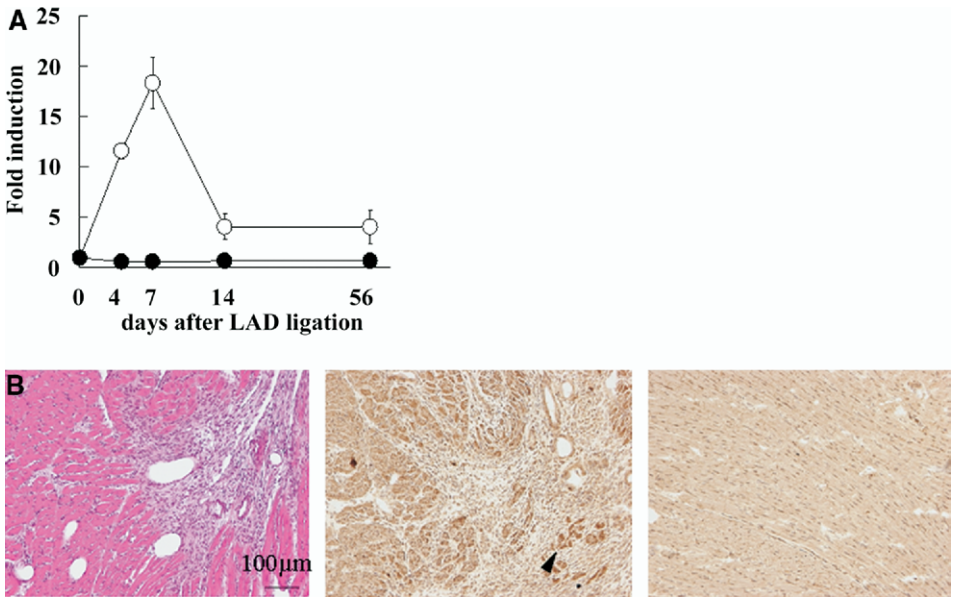

Fig 1. (A) Expression of midkine (MDK) mRNA in infarcted rat hearts. Open symbols represent the infarcted left ventricular myocardium, and closed symbols represent the noninfarcted myocardium of ligated left anterior descending artery (LAD) Lewis hearts. (B) Immunohistochemical staining for MDK protein showed MDK in the surviving cardiomyocytes of the border zone and infarcted area (arrowhead) but not in the noninfarcted area of the heart after myocardial infarction. Left, border-zone area in the hematoxylin-eosin–stained heart; middle, border-zone area in the anti-MDK–stained heart; right, noninfarcted area in the anti-MDK-stained heart.

Medicine and the Japanese Animal Protection and Management Law. The rats were anesthetized with ketamine (90 mg/kg) and xylazine (10 mg/kg); and MI was produced by ligation of the left anterior descending coronary artery (LAD) under mechanical ventilation, as described [15]. Two weeks after the ligation, baseline cardiac functions were measured, and the infarcted rats were treated by injecting various doses of recombinant human MDK (R&D Systems, Minneapolis, Minnesota) into the border-zone myocardium at four sites. The injections were performed by reopening the left thorax of the animals under the same anesthesia. The cytokine was given in doses of 1, 5, or 25 μg in 200 μL collagen gel using a 30G needle, as previously described [16]; and the control rats received collagen gel alone (n = 10 for each subgroup). Collagen gel was used to avoid the loss of the protein when it was injected into the heart.

Time Course of MDK Gene Expression After MI

Infarcted and noninfarcted left ventricular (LV) myocardium from the LAD-ligated rats was removed 4, 7, 14, or 56 days after the ligation and immediately stored in RNA*later* solution (QIAGEN, Hilden, Germany) until use (n = 5 for each time point). The infarcted border was included in the infarcted area. Native myocardium from 15 normal rats was also removed and used as reference samples. Total RNA was extracted with the RNeasy mini kit (QIAGEN), and the relative levels of transcripts from the MDK genes were measured by the real-time quantitative polymerase chain reaction technique using the ABI PRISM 7700 sequence detection system [17]. The nucleotide sequences for the forward primers, reverse primers, and TaqMan probes were as follows. Rat MDK: forward, CGG ATG GTC TCC TGG CAC; reverse, AGC AAG GAC TGC GGC ATG; and probe, GCC ACA CGC CCC CCA GCT. The average copy number of gene transcripts in each sample was normalized to GAPDH, and the fold induction was calculated using the formula: Fold induction = normalized value in a sample after MI divided by average of the normalized value from the 15 reference myocardium samples.

Table 1. Cardiac Functions of Infarcted Rat Hearts After Midkine (MDK) Injection

	LVEDA	LVESA	EF	HR	Mean BP
Control at baseline	60.4 ± 2.4	38.7 ± 2.8	47.3 ± 1.6	369.5 ± 6.5	74.5 ± 3.0
Six weeks after injection	73.5 ± 4.0[a]	48.2 ± 3.7[a]	39.7 ± 1.7[a]	357.5 ± 12.5	81.9 ± 4.4
MDK 1 μg at baseline	61.0 ± 2.2	39.9 ± 2.5	46.8 ± 1.6	374.4 ± 6.0	74.0 ± 4.3
Six weeks after injection	74.6 ± 5.7[b]	50.2 ± 5.5[b]	35.4 ± 3.0[b]	377.0 ± 13.7	70.0 ± 6.4
MDK 5 μg at baseline	56.8 ± 2.2	35.6 ± 1.8	49.1 ± 2.9	361.8 ± 13.0	90.8 ± 3.8
Six weeks after injection	58.2 ± 3.0	35.3 ± 2.7	53.8 ± 3.9	362.9 ± 11.9	77.9 ± 3.4
MDK 25 μg at baseline	65.4 ± 3.0	41.3 ± 2.4	48.9 ± 1.1	371.2 ± 12.6	79.7 ± 3.3
Six weeks after injection	66.7 ± 4.4	40.9 ± 3.9	51.4 ± 2.8	355.9 ± 10.5	72.3 ± 4.4

[a] $p < 0.01$ versus baseline in the same group. [b] $p < 0.05$ versus baseline in the same group.

N = 10 for each group.

BP = blood pressure; EF = ejection fraction; HR = heart rate; LVEDA = left ventricular end-diastolic area; LVESA = left ventricular end-systolic area.