Imatinib Mesylate Attenuates Myocardial Remodeling Through Inhibition of Platelet-Derived Growth Factor and Transforming Growth Factor Activation in a Rat Model of Hypertension

Sung-Won Jang, Sang-Hyun Ihm, Eun-Ho Choo, Ok-Ran Kim, Kiyuk Chang, Chan-Seok Park, Hee-Yeol Kim, Ki-Bae Seung

Abstract—Imatinib mesylate is a specific tyrosine kinase inhibitor that may block the platelet-derived growth factor and transforming growth factor pathways. These pathways are known to provoke fibroblast activation. We evaluated whether imatinib, by inhibiting these pathways, prevents diastolic dysfunction and attenuates myocardial remodeling using spontaneously hypertensive rats (SHRs). Eight-week-old male SHRs were randomly assigned to either imatinib treatment group (30 mg/kg per day; n=10; SHR-I) or hypertensive control group (distilled water, n=10; SHR-C). Wistar–Kyoto rats were used as normal controls (n=10). At 16 weeks, all rats underwent hemodynamic studies and Doppler echocardiography and then were euthanized. Their hearts were extracted for histopathologic, immunoblotting, and quantitative reverse transcriptase polymerase chain reaction analyses. Although imatinib did not affect blood pressure, it markedly reduced perivascular and interstitial fibrosis in the hearts of SHR. Echocardiogram showed that imatinib significantly reduced the left ventricular wall thickness (septal/posterior wall; SHR-C versus SHR-I, 18±1/19±2 versus 15±1/15±1 mm; P<0.001) and increased the E/A ratio (SHR-C versus SHR-I, 1.59±0.11 versus 1.84±0.16; P=0.001). Also, imatinib significantly reduced the mRNA expression of collagen type I, III, and platelet-derived growth factor receptor-β phosphorylation in the hearts of SHR. In addition, imatinib reduced collagen production by inhibiting the phosphorylation of c-abl and platelet-derived growth factor receptor-β in rat cardiac fibroblasts. In conclusion, these results suggest that imatinib could attenuate myocardial remodeling and improve left ventricular diastolic dysfunction in a hypertensive rat model by affecting platelet-derived growth factor and transforming growth factor-β1 pathway without the blood pressure–lowering effect. (Hypertension. 2014;63:1228-1234.) • Online Data Supplement

Key Words: imatinib ■ platelet-derived growth factor ■ rats, inbred SHR ■ transforming growth factor beta ■ ventricular remodeling

Hypertension is a major risk factor for left ventricular (LV) hypertrophy and heart failure. Chronic hypertension leads to LV hypertrophy, which enables the heart to maintain a normal stroke volume, despite the increase in afterload. However, the heart undergoes pathological changes, which resulted in ventricular failure. The hallmark of pathological LV hypertrophy is reactive myocardial fibrosis, a progressive increase in the collagen content of the heart, which also plays an important role in heart failure. Although molecules responsible for myocardial fibrosis have not been clearly elucidated, angiotensin II, aldosterone, and cytokines, including transforming growth factor-β (TGF-β), interleukin-1, tumor necrosis factor-α, and platelet-derived growth factor (PDGF), are known to provoke fibroblast activation within the connective tissue. These molecules are potential targets for downregulation of fibroblast activation. Indeed, the use of angiotensin-converting enzyme inhibitors, β-blockers, and aldosterone receptor antagonists has been associated with a decrease in myocardial fibrosis in experimental heart failure models. PDGF stimulates fibroblasts to contract collagen matrices and differentiate into myofibroblasts. These isoforms act via 2 receptor tyrosine kinases: PDGF receptors-α (PDGFR-α) and β (PDGFR-β). Imatinib mesylate inhibits not only BCR/ABL-mediated phosphorylation but also other tyrosine kinase, such as c-abl, c-kit, and PDGFR. The spontaneously hypertensive rat (SHR) is a genetically hypertensive rat model used to study human essential

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hypertension. The SHR develops hypertension at 2 to 6 weeks, followed by progressive myocardial fibrosis, cardiac hypertrophy, and eventually heart failure. The purpose of this study was to evaluate whether imatinib prevents cardiac fibrosis and LV hypertrophy and thus improves LV diastolic dysfunction in SHR. Furthermore, we investigated whether imatinib mediates inhibition of PDGFR-β or c-abl tyrosine kinase activity in the aspect of attenuating myocardial remodeling.

Methods

Animal Preparation

This investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication No. 85-23, revised 1996). All procedures were performed in accordance with the protocols approved by the Institutional Animal Care and Use Committee in the School of Medicine, The Catholic University of Korea, Seoul, Republic of Korea.

Eight-week-old male SHR and nonhypertensive Wistar–Kyoto (WKY) control rats were purchased via the Central Animal Laboratory at our institution. Study subjects were divided into normal controls (WKY; n=10), hypertensive controls treated with distilled water (SHR-C; n=10), and SHR treated with imatinib mesylate (Gleevec; Norvatis Pharmaceuticals, Basel, Switzerland) dissolved in distilled water (SHR-I; 30 mg/kg per day; n=10). Treatment with placebo (distilled water) or imatinib was administered orally via gastric gavage starting at 8 weeks and continued until 16 weeks. Rats were caged individually and received normal rat chow and tap water in a temperature-controlled environment under a 12-hour artificial light and dark cycle. Other experimental protocols are presented in the online-only Data Supplement.

Results

Imatinib Did Not Affect Blood Pressure and Heart Weight in SHR

The body weight of the SHR was significantly lower than that of the WKY control rats, but the blood pressure of the SHR was significantly higher than that of the WKY (Table 1). Imatinib did not have an effect on the body weight change or systolic blood pressure. Heart weight and heart weight for the tibia length ratio were significantly higher in the SHR group than those in the WKY group. There were no significant differences in heart weight and heart weight/tibia ratio between SHR-C and SHR-I.

Table 1. General Characteristics

<table>
<thead>
<tr>
<th>Variables</th>
<th>WKY (n=10)</th>
<th>SHR-C (n=10)</th>
<th>SHR-I (n=10)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre–body weight, g</td>
<td>205±10</td>
<td>184±10*</td>
<td>182±10</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Post–body weight, g</td>
<td>377±21</td>
<td>338±17*</td>
<td>344±16*</td>
<td>0.001</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>115±7</td>
<td>168±13*</td>
<td>169±11*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Heart weight, g</td>
<td>1.2±0.1</td>
<td>1.4±0.2*</td>
<td>1.5±0.3*</td>
<td>0.004</td>
</tr>
<tr>
<td>Tibia length, mm</td>
<td>5.2±0.4</td>
<td>4.8±0.3*</td>
<td>4.8±0.3*</td>
<td>0.045</td>
</tr>
<tr>
<td>Heart/tibia ratio, g/mm</td>
<td>0.23±0.02</td>
<td>0.29±0.04*</td>
<td>0.31±0.03*</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SD. BP indicates blood pressure; SHR-C, spontaneous hypertensive rat control group; SHR-I, SHR treated with 30 mg/kg per day of imatinib; and WKY, Wistar–Kyoto rat. *P<0.05 vs WKY.

Imatinib Decreased LV Wall Thickness and Improved Diastolic Function in SHR

Figure 1 depicts M-mode and Doppler echocardiography of the study subjects. SHR-C showed thicker LV wall, lower E/A ratio, and longer deceleration time than those of WKY (Table 2). Imatinib was shown to decrease the LV wall thickness and deceleration time and increase the E/A ratio significantly. There was no significant difference in fractional shortening between WKY and SHR-C, and imatinib had no effect on fractional shortening.

Imatinib Decreased LV Hypertrophy and Interstitial Fibrosis in the Hearts of SHR

Heart sections were stained with Masson trichrome and observed under a light microscope. SHR-C displayed LV hypertension when compared with WKY, and imatinib attenuated ventricular hypertrophy in SHR rats (Figure S1 in the online-only Data Supplement). Interstitial and perivascular fibrosis was increased in SHR-C when compared with that in WKY, and it was decreased in SHR-I when compared with that in SHR-C (Figure 2). In addition, quantitative measurements using collagen area fraction demonstrated that imatinib significantly decreased the amount of fibrosis in the interstitial tissue (SHR-C versus SHR-I, 9.8±4.1% versus 1.3±0.2%; P<0.01).

Imatinib Attenuated Myocardial mRNA Expression of Collagen Type I, III, and TGF-β1 in the Hearts of SHR

The myocardial mRNA expressions of collagen type I, III, and TGF-β1 were examined with quantitative reverse transcriptase...
polymerase chain reaction. These were significantly increased in SHR when compared with that in WKY and decreased by imatinib treatment (Figure 3).

**Imatinib Attenuated Myocardial PDGFR Activity in the Hearts of SHR**

To access the myocardial phosphorylation state of PDGFR-β, we performed Western blot for the phosphorylated protein on their tyrosine residue and for PDGFR-β in LV. The myocardial phosphorylation of the PDGFR-β was increased in SHR, but it was significantly blunted by imatinib treatment (Figure 4A and 4B). The total expression of PDGFR-β was also increased in the LV of SHR, and it was significantly decreased by imatinib treatment (Figure 4A and 4C).

**Imatinib Attenuated mRNA Expression of Collagen Type III by Blocking the Phosphorylation of PDGFR-β and c-abl in Rat Cardiac Fibroblasts**

Incubation of cultured rat cardiac fibroblast with PDGF-BB increased the phosphorylation of PDGFR-β and mRNA expression of collagen type III. These were decreased by imatinib administration (Figure 5). Incubation of cultured rat cardiac fibroblast with TGF-β1 increased the phosphorylation of c-abl and mRNA expression of collagen type III, and these were also decreased by imatinib administration (Figure 6). In addition, incubation of cultured rat cardiac fibroblast with PDGF-BB or TGF-β1 increased the production of collagen III in the culture medium as well, and the administration of imatinib decreased the production of collagen type III (Figure S2).

**The Effect of Imatinib on c-abl Phosphorylation in PDGF-BB–Treated Rat Cardiac Fibroblast**

Phosphorylation of c-abl was increased when PDGF-BB was added in the rat cardiac fibroblast, and this was attenuated by imatinib administration (Figure S3).

**Discussion**

This study demonstrated that imatinib mesylate, a tyrosine kinase inhibitor, decreased myocardial fibrosis and resulted in improved LV diastolic dysfunction in a hypertensive rat model. Treatment with 30 mg/kg per day of imatinib in SHR significantly reduced the fibrosis of interstitial (A) and perivascular areas (C). Quantitative measurement using collagen area fraction shows that imatinib significantly decreased the amount of fibrosis in interstitial areas (B) and perivascular areas (D). Results are shown as mean±SD of 8 different rats. *P<0.05 vs WKY; †P<0.05 vs SHR-C. LV indicates left ventricular; and SHR-I, spontaneously hypertensive rats treated with imatinib.
In chronic hypertension, activated fibroblasts in the connective tissue are responsible for elevated extracellular matrix production, resulting in fibrosis. TGF-β1 and PDGF are key driving forces in fibroblast activation. The best-characterized signaling pathway used by TGF-β1 involves activation of the cellular smad 2/3 pathway. However, recent studies have demonstrated the important roles of non-smad (smad 2/3 independent) signaling pathways in mediating TGF-β1 responses. In the non-smad pathway, c-abl plays a significant role in the regulation of cardiac fibrosis. C-abl is also a novel mediator of TGF-β1–induced fibroblast growth, morphological transformation, and matrix gene expression. PDGF upregulates the expression of TGF-β1 in a rat cardiac allograft and c-abl in fibroblast. In addition, PDGF also directly stimulates fibroblasts to contract collagen matrices and differentiate into myofibroblasts.

The strength of our study lies in several points. Our study showed that imatinib effectively inhibited phosphorylation of c-abl and reduced collagen production by blocking TGF-β1/c-abl pathway in rat cardiac fibroblasts. Imatinib also attenuated the phosphorylation of PDGFR and decreased the production of collagen type III in rat cardiac fibroblasts. In addition, TGF-β1/c-abl pathways are affected by PDGF. We observed that c-abl phosphorylation was increased when PDGF-BB was added in the rat cardiac fibroblast, which was attenuated by imatinib administration. Therefore, it can be stated that PDGF promotes fibrosis both on PDGFR and on TGF-β1/c-abl, and imatinib attenuates fibrosis by blocking both pathways. We used SHR as a model for human essential hypertension and observed that imatinib decreased the LV wall thickness and attenuated LV diastolic dysfunction without the blood pressure–lowering effect. This observation substantiates the idea that targeted
inhibition of the growth factor system by imatinib may have beneficial effects on cardiac fibrosis and LV remodeling and eventually heart failure. To our knowledge, this is the first study demonstrating the attenuation of myocardial remodeling by imatinib through blocking the phosphorylation of PDGFR-β and inhibiting the TGF-β1 pathways in a hypertensive rat model.

The safety issue related to imatinib use in experimental strategy is still being disputed. Imatinib has been reported to be associated with cardiac toxicity, including congestive heart failure and asymptomatic LV dysfunction in the treatment of patients with chronic myeloid leukemia. In contrast to pre-clinical findings based on cellular and animal studies,27,28 a long-term follow-up study of patients receiving imatinib for...
chronic myeloid leukemia did not show any increase in the rates of serious adverse events.29,30

There are several potential limitations to our study. First, we did not adopt Tissue Doppler images, a useful technique for evaluating ventricular diastolic function, but we assumed that E/A ratio and deceleration time gave information that proved to be useful in assessing LV diastolic function. Furthermore, we obtained echocardiographic data at similar heart rates to maximize consistency. Second, although we observed reduction in LV wall thickness, heart weight and heart weight/tibia ratio did not differ after imatinib treatment. We speculate that wall thickness is more sensitive than heart weight when assessing LV hypertrophy, and that a sufficient number of animal subjects might have helped to achieve statistical significance on weight changes. Third, we could not demonstrate the effect of imatinib on the phosphorylation of c-abl in our hypertensive rat model (in vivo study, data not shown). We thought that the amount of c-abl proteins might be insufficient in the rat myocardium. In vitro study using rat cardiac fibroblast, however, demonstrated that imatinib blunted the phosphorylation of c-abl, and this was associated with decreased collagen production.

In conclusion, this study is the first one demonstrating that imatinib attenuates myocardial remodeling and improves LV diastolic dysfunction in a hypertensive rat model by blocking the phosphorylation of PDGFR-β and inhibiting the TGF-β1 pathway without the blood pressure–lowering effect.

Perspective

This study showed that imatinib reduced cardiac fibrosis, LV wall thickness, and improved LV diastolic dysfunction in a hypertensive rat model. The reduction of collagen production by imatinib was associated with decreased phosphorylation of PDGFR-β and c-abl. These results suggest that imatinib could attenuate myocardial remodeling and improve LV diastolic dysfunction by affecting the action of PDGF and TGF-β1-induced c-abl kinase. Additional work is required to confirm whether imatinib can be a potential therapeutic approach to prevent myocardial fibrosis and diastolic dysfunction in hypertensive heart diseases.

Sources of Funding

This study was partially supported by research grants from the Korean society of hypertension.

Disclosures

None.

References

Novelty and Significance

What Is New?
- Imatinib prevents diastolic dysfunction and attenuates myocardial remodeling using spontaneously hypertensive rats.
- Imatinib blocks the phosphorylation of c-abl kinase in rat cardiac fibroblast.

What Is Relevant?
- The platelet-derived growth factor and transforming growth factor-β1 pathways are known to provoke fibroblast activation.

Summary
Imatinib attenuates myocardial remodeling in a hypertensive rat model probably by blocking the phosphorylation of platelet-derived growth factor receptor-β and by inhibiting the transforming growth factor-β1/c-abl kinase activity, in addition.
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A running title: Imatinib Attenuates Cardiac Fibrosis.

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Methods

Weight, blood pressure, and echocardiography
Body weight was measured at the ages of 8 and 16 weeks. Blood pressure and heart rate were measured using tail plethysmography (BP-2000, Visitech system, Apex, NC, USA) at the age of 16 weeks. After sedation with 10mg/kg of xylazine and 100mg/kg of ketamine hydrochloride intramuscularly, echocardiographic measurement was performed at the age of 16 weeks. Standard views were obtained in the 2D mode and M-mode by transthoracic echocardiography with a 15 MHz transducer (Acuson Sequoia 256, Acuson, Mountain Views, CA, USA). LV dimensions were measured using echocardiography in the M-mode, and fractional shortening was calculated. Pulsed wave Doppler was used to measure the peak mitral flow velocity (E), peak velocity of the late filling wave due to atrial contraction (A), and deceleration time. All measurements were performed 3 times and the average value was used for the analysis.

Tissue preparation quantitative measurement
Rats were euthanized at 16 weeks by intraperitoneal injection of pentobarbital (100 mg/kg, Halim Pharm. Co. Ltd., Republic of Korea), and the adequacy of anesthesia was confirmed with the absence of pedal reflex. Hearts were taken out and weighed. Normal saline was infused into the LV which was then divided vertically into three pieces. The midsection containing the papillary muscle was immersed in 10% formalin for 24 hours, after which it was embedded in paraffin. LV sections were cut at 5 µm and stained with Masson’s trichrome to visualize collagen. Perivascular and interstitial areas were observed with a light microscope (Olympus ZX70 TR62A02, Olympus, Tokyo, Japan) at x100 and x200. LV interstitial collagen was quantified using Image pro plus software (ver. 4.5, Media Cybernetics, Silver Spring, MD, USA). Collagen area fraction was calculated as the percentage of positive staining area in the total myocardial tissue. The rest of the tissue was frozen for quantitative polymerase chain reaction (qPCR) and western blot.

RNA extraction and quantitative reverse transcription polymerase chain reaction (qRT-PCR)
Total RNA was extracted from the cardiac tissue using TRIZOL solution (Invitrogen, Carlsbad, CA, USA). Reverse transcription of RNA was performed with Omniscript RT kit (QIAGEN, Hilden, Germany) according to the manufacturer’s protocol. PCR was performed with QIAGEN multiplex PCR master mix (QIAGEN). Primer pairs were as follows: collagen type I (Col, type I, alpha2 NM_053356: sense, 5’-CCT GGT CCT CAA GGT TTC C-3’ and antisense, 5’-CCA CGA GCA CCA GCA CTG-3’), collagen type III (Col, type III, alpha1 NM_032085: sense, 5’-GCC AGT CCC ATG ACT GTC CCG CGG-3’ and antisense, 5’-AGT GCA GCC ATC CTC TAG AAC TGT G-3’), rat GAPDH (sense, 5’-AAT GCA TCC TGC ACC ACC AA-3’ and antisense, 5’-GTA GCC ATA TTC ATT GTC ATA-3’) and TGF-β1 (sense, 5’-ATC GAC ATG GAG CTG GTG A-3’ and antisense, 5’-TTG GCA TGG TAG CCC TTT G-3’). The expression of collagen type I, III and TGF-β1 was normalized to the expression of GAPDH.

Western blotting
Frozen heart tissues were dissolved in radioimmunoprecipitation assay (RIPA) buffer containing 0.1% SDS, 0.5% sodium deoxycholate, 1% NP 40, 150 mM NaCl, 5 mM EDTA, 50 mM Tris-HCl pH8.0, and 10% protease inhibitor cocktail (Sigma–Aldrich) for 30 minutes
at 4°C. The homogenates were centrifuged at 13,000 rpm for 3 minutes at 4°C. The supernatant was extracted and the protein concentration was measured with Bio-Rad Protein assay reagent (Bio-Rad, Regents Park, NSW, Australia). Heart tissue lysates were resolved on SDS–polyacrylamide gel electrophoresis according to standard protocols. After being transferred to membranes, the samples were immunoblotted with primary antibodies, followed by secondary antibodies conjugated with horseradish peroxidase. Bands were revealed by the use of an enzyme-linked chemiluminescence detection kit (Amersham Biosciences, Piscataway, NJ, USA). Western blotting was performed on heart tissues to identify phosphorylated PDGFR-β and PDGFR-β. Western blotting was also performed on cultured rat cardiac fibroblast to identify p-PDGFR-β, PDGFR-β, phosphorylated c-abl and c-abl.

Cell Culture
DMEM and FBS were purchased from GIBCO BRL (Rockville, NY, USA). We used third passage cells from the incubated rat cardiac fibroblasts (R306K, Cell Applications Inc., CA, USA). The cells were maintained in DMEM supplemented with 10% FBS along with 0.1% gentamycin, incubated at 37°C in a humidified chamber, and grown to confluence before synchronization in low-serum medium (0.1% FBS) 24 hours before the experiments.

For transactivation experiments, rat cardiac fibroblasts were treated with imatinib (1 or 10 µM) or placebo 30 minutes before the addition of PDGF-BB (20 ng/mL) or TGF-β1 (10 ng/mL) for 24 hours. After the experiments, media were removed and the cells were prepared for western blotting and qRT-PCR. The production of collagen type III were measured in the culture media after experimental treatment by using a commercial ELISA kit (Rat Collagen Type III alpha 1, Sunred bio, Shanghai, China), according to the protocols provided by the manufacturer.

Statistics
Data are expressed as means ± SD. Differences between two or multiple groups were analyzed by the student t-test or ANOVA, where appropriate. All comparisons were two-sided and a p-value of less than 0.05 was considered statistically significant.
Results

**Figure S1.** Vertical section of the study subject’s heart stained with Masson’s trichrome. SHR-C presented left ventricular hypertrophy compared with WKY. Treatment with imatinib attenuated left ventricular hypertrophy. Myocardial area was calculated by subtracting LV endocardial tracing from the epicardial tracing using image pro plus software. The graph demonstrated that the myocardial area of SHR-C and SHR-I were normalized to WKY group values. WKY, Wistar Kyoto rats; SHR-C, spontaneously hypertensive rats treated with normal saline; SHR-I, spontaneously hypertensive rats treated with 30 mg/kg/day of imatinib. *p<0.05, n=4 (WKY), n=5 (SHR-C and SHR-I).
**Figure S2.** Effect of imatinib on production of collagen type III in rat cardiac fibroblast culture medium after coinoculation of PDGF-BB or TGF-β1. The administration of imatinib decreased the production of collagen type III after coinoculation of PDGF-BB (A) or TGF-β1 (B). *p < 0.05 vs. PDGF-BB (-) or TGF-β1 (-) & Imatinib (-), †p < 0.05 vs. PDGF-BB (+) or TGF-β1 (+) & Imatinib (-), imatinib (+) : 1 µM, imatinib (++) : 10 µM, n=4.
Figure S3. Effect of imatinib on c-abl phosphorylation in PDGF-BB treated rat cardiac fibroblasts. Incubation of cultured rat cardiac fibroblasts with PDGF-BB increased the phosphorylation of c-abl. Coincubation of the cells with imatinib attenuated the c-abl phosphorylation. *p < 0.05 vs. PDGF-BB (-) & Imatinib (-), †p < 0.05 vs. PDGF-BB (+) & Imatinib (-), n=5