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Full paper

The effects of dipeptidyl peptidase-4 on cardiac fibrosis in pressure overload-induced heart failure

Masanori Hirose [a], Hiroyuki Takano [b, *], Hiroshi Hasegawa [a], Hiroyuki Tadokoro [a], Naoko Hashimoto [b], Genzo Takemura [c], Yoshio Kobayashi [a]

[a] Department of Cardiovascular Medicine, Chiba University Graduate School of Medicine, Chiba 260-8670, Japan
[b] Department of Molecular Cardiovascular Pharmacology, Chiba University Graduate School of Pharmaceutical Sciences, Chiba 260-8675, Japan
[c] Department of Internal Medicine, Asahi University School of Medicine, Mizuho 501-0296, Japan

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ABSTRACT

Dipeptidyl peptidase-4 (DPP-4) inhibitors are hypoglycemic agents. DPP-4 inhibitor has cardioprotective effects after transverse aortic constriction (TAC), but role of DPP-4 on cardiac fibrosis after TAC is not well known. Our aim was to determine the effects of DPP-4 on cardiac fibrosis in murine TAC model. Wild-type mice and DPP-4 knockout mice were subjected to TAC. Wild-type mice were then treated with vehicle or DPP-4 inhibitor. DPP-4 activities in serum and heart tissue were significantly increased at 2 weeks after TAC, but they were significantly decreased by DPP-4 inhibitor treatment. The inhibition of DPP-4 did not affect left ventricular hypertrophy, but improved cardiac function and decreased myocardial and perivascular fibrosis after TAC. The inhibition of DPP-4 decreased the collagen type III/I ratio in myocardium. These results suggest that DPP-4 inhibition ameliorates the progression of heart failure after TAC by changing the quality and quantity of cardiac fibrosis.

1. Introduction

Recently, the number of patients suffering from heart failure has been increasing[1] in advanced countries including Japan. Since recent therapies for chronic heart failure are effective, heart failure is still one of the leading causes of death in many countries.[2,3] Heart failure is a complex syndrome that consists not only of left ventricular (LV) dysfunction but also of metabolic and neurohumoral alterations.[4] LV hypertrophy is induced by an adaptive response to increased afterload such as hypertension and aortic valve stenosis,[5,6] but continuous LV hypertrophy results in LV dysfunction leading to various cardiovascular events including heart failure and fatal arrhythmia.

Dipeptidyl peptidase-4 (DPP-4) inhibitors are oral hypoglycemic agents that block DPP-4 enzyme activity.[7] As DPP-4 degrades and inactivates incretin hormones such as glucagon-like peptide 1 (GLP-1) and gastric inhibitory polypeptide (GIP), DPP-4 inhibitors lower blood glucose level. Many basic researches reported that DPP-4 inhibitors have protective effects on various organs including pancreas, kidney, brain, and heart.[8–13] Those papers report that DPP-4 inhibitors-induced increases in GLP-1 and stromal cell-derived factor-1α (SDF-1α), which is also a substrate of DPP-4, contribute to the protective effects of DPP-4 inhibitors. We reported that DPP-4 inhibition has direct protective effects on post-myocardial infarction (MI) heart by inducing an antiapoptotic effect and inhibiting a decrease in vessel number through SDF-1α/CXCR4-mediated STAT3 signaling pathway.[14]

The murine transverse aortic constriction (TAC) model[15,16] is widely used to elucidate the pathophysiology of transition from LV hypertrophy to heart failure.[17,18] After TAC, LV hypertrophy progresses, and then LV function deteriorates with the symptoms of heart failure.[19] Recent studies have shown that there is a myocardial inflammatory reaction and a fibrosis response after TAC.[20] Cardiac fibrosis is one of the detrimental factors that contribute to heart failure during pressure overload conditions. DPP-4 inhibitor has been reported to improve the progression of heart failure after TAC,[21] but little is known about its effects on the fibrosis in the myocardium.

* Corresponding author. 1-8-1 Inohana, Chuo-ku, Chiba 260-8675, Japan. Fax: +81 43 226 2883.
E-mail address: htakano-cib@umin.ac.jp (H. Takano).
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The purpose of the present study was to determine the effects of DPP-4 on cardiac fibrosis and the progression from LV hypertrophy to heart failure in murine TAC model.

2. Materials and methods

2.1. Animals

Wild-type male C57BL/6 mice (8-week-old, weighting 22–24 g) were purchased from Japan SLC (Shizuoka, Japan). DPP-4 (CD26) KO mice were kindly provided by Chikao Morimoto (Juntendo University, Tokyo, Japan). The construction and characterization of DPP-4KO mice has been described previously.[22] All experimental procedures were performed according to the guidelines established by Chiba University for experiments in animals.

2.2. Induction of TAC

Mice were anesthetized by inhalation of isoflurane (2.5%) and artificially ventilated with respirator. Either TAC or sham-operation was performed as previously described.[23] In brief, the transverse aortic arch was surgically accessed and constricted by a 7-0 silk suture ligature tied firmly against a 27-gauge needle, followed by prompt removal of the needle. After aortic constriction, the chest was closed and mice were allowed to recover from anesthesia. Sham-operated mice underwent the same procedure except that aortic constriction was not done. After the operation, Wild-type (WT) mice were randomized to treatment with vehicle alone (WT-TAC group) or with vehicle plus 3 mg/kg/day of DPP-4 inhibitor MK-0626 (an analog of des-fluorositagliptin) (WT-TAC + DPP-4i group). MK-0626 was provided by Merck & Co (New Jersey, US). DPP-4KO mice were subjected to TAC operation similarly, and vehicle (DPP-4KO-TAC group).

2.3. DPP-4 activity

At 2 weeks after TAC, the activities of DPP-4 in serum and myocardium were measured using a Luciferase-based assay kit (DPP-4-Glo protease Assay, Promega).[24]

2.4. Echocardiography

Transthoracic echocardiography was performed on 0, 2 and 4 weeks after TAC with a VisualSonics (Vevo 660; VisualSonics, Toronto, Canada) equipped with a 30-MHz imaging transducer. We determined the LV end-diastolic diameter (LVEDD), the LV end-systolic diameter (LVESD), anterior wall thickness at end-diastole (AWT) and posterior wall thickness at end-diastole (PWT) in the M-mode recordings.

2.5. Histological analysis and immunostaining

Cardiac tissue was fixed by perfusion with 10% formaldehyde. The fixed samples were embedded in paraffin and sectioned at 4 μm thickness for immunostaining. For measurement of the cardiomyocyte cross-sectional area, 50 randomly selected cardiomyocytes in an LV cross-section were measured. To determine the degree of myocardial fibrosis, we selected 5 fields at random and calculated the ratio of Masson trichrome staining fibrosis area to total myocardium area.[25] The degree of perivascular fibrosis of arteries was evaluated from short-axis images of myocardial arteries and arterioles on Masson trichrome staining. The perivascular fibrosis was defined as the ratio of the fibrosis area surrounding the artery to the area occupied by the artery.[26] Collagen content was calculated as a percentage of the area of total myocardium. To determine the degree of myocardial fibrosis, we selected 8 fields at random and calculated the ratio of picrosirius red staining fibrosis area to total myocardium area. First, we resolved the subtracted image into its hue, saturation and brightness. Only the hue component was retained and a histogram of hue frequency was obtained from the resolved 8-bit hue images, which contain 255 colors. We used the following hue definitions; red 0–19 and 195–255, yellow 20–48, green 49–118. The hue range 119–194 consisted of interstitial space and non-birefringent tissue elements such as red blood cells. The number of pixels within each hue range was determined and expressed as a percentage of picrosirius red staining fibrosis area to total myocardium area.[27,28]

2.6. Transmission electron microscopy (TEM)

Electron microscopy was performed on 4 weeks after TAC. Cardiac tissue from left ventricle was quickly cut into 1 mm-cubes, immersion-fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) overnight at 4 °C, and postfixed in 1% buffered osmium tetroxide. The specimens were then dehydrated through a graded ethanol series and embedded in epoxy resin. Ultrathin slice (90 nm), which were double-stained with uranyl acetate and lead citrate, were examined using an electron microscopy (H-800, Hitachi, Tokyo, Japan).[29]

2.7. Statistics

All data are shown as mean ± SEM. Multiple group comparison was performed by two-way ANOVA followed by Bonferroni's procedure for comparison of means. Comparison between two groups was analyzed by two-tailed Student's *t* test. Values of $P < 0.05$ were considered statistically significant.

3. Results

3.1. Effects of DPP-4 inhibition on DPP-4 activities in serum and myocardium

We first assessed effects of DPP-4 inhibition on DPP-4 activities in serum and myocardium to confirm the effects of MK-0626. At 2 weeks after TAC, DPP-4 activity was significantly increased in WT-TAC group, but it was significantly decreased in WT-TAC + DPP-4i group (serum: WT-Sham, 3.9 ± 1.4IU/L; WT-TAC, 9.5 ± 2.1IU/L; WT-TAC + DPP-4i, 1.4 ± 0.8IU/L; $P < 0.05$; myocardium: WT-Sham, 6735 ± 955IU/mg protein; WT-TAC, 9765 ± 1544IU/mg protein; WT-TAC + DPP-4i, 3014 ± 646IU/mg protein; $P < 0.05$) (Fig. 1A and B).

3.2. Effects of DPP-4 inhibition on cardiac function and hypertrophy

We assessed echocardiographic parameters to confirm pressure overload-induced cardiac hypertrophy and heart failure. There were no significant differences in physiological and echocardiographic parameters between 4 groups before TAC (Table 1). LV hypertrophy was recognized in WT-TAC, WT-TAC + DPP-4i and DPP-4KO-TAC groups at 1 week after TAC. There was no significant difference in LV hypertrophy between 3 groups at 2 and 4 weeks after TAC (Table 1). At 4 weeks after TAC, % fractional shortening (FS) was significantly decreased in WT-TAC group, but it was significantly improved in WT-TAC + DPP-4i and DPP-4KO-TAC groups. (WT-Sham, 44.5 ± 0.2%; WT-TAC, 31.5 ± 2.9%; WT-TAC + DPP-4i, 40.4 ± 1.6%; DPP-4KO-Sham, 44.9 ± 0.1%; DPP-4KO-TAC, 41.8 ± 0.7%; $P < 0.05$) (Table 1) The cell surface area of cardiomyocyte was significantly increased in WT-TAC, WT-TAC + DPP-4i and DPP-4KO-TAC groups compared with corresponding Sham

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