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Original Article

Role of PDGFs/PDGFRs signaling pathway in myocardial fibrosis of DOCA/salt hypertensive rats

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Abstract: This study aimed to investigate the role of PDGF/PDGFR signaling pathway in myocardial fibrosis of desoxycorticosterone (DOCA) induced salt-sensitive hypertensive rats and explore the influence of PDGF/PDGFR signaling pathway on fibroblasts and myofibroblasts in the heart. 60 male SD rats underwent right nephrectomy and bred with 1% sodium chloride and 0.1% potassium chloride for 4 weeks, and then randomly divided into 3 groups (CON group, DOCA group and DOCA+IMA group). Results showed that: 1) 14 and 28 days after intervention, the SBP in DOCA and DOCA+IMA group was significantly higher than that in CON group. At days 28, the severity of myocardial fibrosis and PVCA/VA ratio in DOCA group were significantly increased when compared with CON group. The severity of myocardial fibrosis and PVCA/VA ratio in DOCA+IMA group were markedly lower than those in DOCA group although they were higher than those in CON group. 2) At days 14, the mRNA expressions of PDGFRα and PDGFRβ in DOCA group were significantly higher than CON and DOCA+IMA group. At days 28, the mRNA expressions of PDGFRβ, FSP-1, α-SMA, procollagen I and procollagen III in DOCA group were significantly higher than those in CON group. In addition, in a specific group, the PDGFRβ mRNA expression was higher than the PDGFRα mRNA expression. In DOCA+IMA group, the mRNA expressions of PDGFRβ, FSP-1, α-SMA, procollagen I and procollagen III were markedly reduced when compared with DOCA group. 3) At 14 days, the protein expressions of PDGFRα and PDGFRβ in DOCA group were significantly higher than those in CON group. The PDGFRα protein expression in DOCA+IMA group was markedly lower than that in DOCA group. At days 28, the protein expressions of PDGFRα and PDGFRβ in DOCA group were significantly increased when compared with CON group. The protein expressions of PDGFRα and PDGFRβ in DOCA+IMA group were significantly lower than those in DOCA group. At day 28, the cardiac interstitium mainly contained vimentin positive fibroblasts, and α-SMA positive cells were less identified in CON group. In DOCA group, α-SMA positive fibroblasts (spindle-shaped) increased significantly, but the myofibroblasts reduced significantly in DOCA+IMA group when compared with DOCA group. 4) PDGFRα protein expression was observed in fibroblasts and myofibroblasts, but not in VSMCs. PDGFRβ protein expression was noted in not only fibroblasts and myofibroblasts but also VSMCs. Thus, During myocardial fibrosis of DOCA induced salt-sensitive hypertensive rats, PDGFRα acts at early stage, but PDGFRβ functions in the whole process. PDGFRα and PDGFRβ expressions increase in fibroblasts and myofibroblasts, suggesting that PDGF/PDGFR signaling pathway is involved in the myocardial fibrosis via stimulating fibroblasts to proliferate and transform into myofibroblasts.

Keywords: Platelet-derived growth factor, platelet-derived growth factor receptor, fibroblasts, myofibroblasts, desoxycorticosterone, imatinib, myocardial fibrosis

Introduction

Myocardial fibrosis (MF) is a pathological process with excessive proliferation of fibroblasts and imbalance between deposition and degradation of extracellular matrix (ECM) in myocardial interstitium and a terminal pathological manifestation of multiple cardiovascular diseases [1, 2]. Currently, the pathogenesis of myocardial fibrosis is still poorly understood. Previous work on myocardial fibrosis of desoxycorticosterone (DOCA) induced salt-sensitive hypertensive rats demonstrated that PDGF/PDGFR signaling pathway was involved in the myocardial fibrosis [3]. However, the ways, which bridge the PDGF/PDGFR signaling pathway and myocardial fibrosis, are still unclear. There is evidence showing that the fibroblasts

Table 1. Primers used in RT-PCR

Gene		Primer sequences
PDGFRα	Sense	GAGACCCTCCTTCTACCACCT
	Anti-sense	GTTGTCAGAGTCCACACGCAT
PDGFRβ	Sense	GCACCGAAACAAACACACCTT
	Anti-sense	ATGTAACCACCGTCGCTCTC
FSP-1	Sense	ACCTCTCTGTTCAGCACTTCC
	Anti-sense	GAACTTGTCACCCTCGTTGC
α-SMA	Sense	CCAGTCGCCATCAGGAACC
	Anti-sense	AGCAAAGCCCGCCTTACAG
procollagen I	Sense	ACGCATGAGCCGAAGCTAAC
	Anti-sense	AGGGACCCTTAGGCCATTGT
procollagen III	Sense	ATAGACCTCAAGGCCCCAAG
	Anti-sense	CCACCCATTCCTCCGACT
GADPH	Sense	TGGGAAGCTGGTCATCAAC
	Anti-sense	GCATCACCCCATTTGATGTT

in the heart have the potential to differentiate into myofibroblasts [4]. In study on myocardial fibrosis after myocardial infarction, investigators proposed that PDGF/PDGFR signaling pathway is involved in the pathogenesis of myocardial fibrosis via stimulating fibroblasts to proliferate and transform into myofibroblasts and to secret massive collagens [5]. However, this has not been confirmed in vivo. In the present study, the cells with PDGFR expression and the changes in fibroblasts and myofibroblasts of the heart were investigated in DOCA induced salt-sensitive hypertensive rats, aiming to explore the role of PDGF/PDGFR signaling pathway in the myocardial fibrosis.

Materials and methods

Animal model and grouping

Specific pathogen free male SD rats (n=60) weighing 200-250 g were purchased from the Experimental Animal Center of Affiliated Provincial Hospital of Anhui Medical University. Animals were anesthetized intraperitoneally with 10% chloral hydrate at 400 mg/kg and then received nephrectomy. At 1 week after surgery, animals were bred with 1% sodium chloride and 0.2% potassium chloride and randomly assigned into 3 groups (n=20 per group): 1) CON group: animals were subcutaneously treated with soybean oil once every 4 days and intragastrically treated with distilled water once daily; 2) DOCA group: animals were subcutaneously treated with DOCA at 60 mg/kg/4d and intragastrically treated with distilled water once daily; 3) DOCA+IMA group: animals were subcutaneously treated with DOCA at 60 mg/kg/4d and intragastrically treated with imatinib at 60 mg/kg/d once daily.

In CON group, the volume of intragastrical-distilled water was equal to that of intragastrical drugs in other two groups; in CON group, the volume of subcutaneous soybean oil was identical to that of subcutaneous drugs dissolved in soybean oil. Treatment was done for 28 days. Before and at 14 and 28 days after treatment, systolic blood pressure (SBP) was measured. At 14 and 28 days after treatment, animals were anesthetized with 10% chloral hydrate at 400 mg/kg and then sacrificed (n=10 at each time point). The heart was harvested and the atriums, major vessels and connective tissues were removed. The remaining ventricular tissues were divided into 3 parts: one was stored at -80°C for real-time fluorescence quantitative PCR; one was fixed in 4% paraformaldehyde for 24 h and embedded in paraffin followed by HE staining and immunohistochemistry; one was fixed in 4% paraformaldehyde for 24 h and dehydrated in 30% sucrose for frozen sectioning and subsequent immunofluorescence staining.

Main reagents

The following reagents were used in the present study: DOCA (Sigma, USA), imatinib (LC, labs; USA), sirius red dye (Beijing Haide Biotech Co., Ltd), phosphate buffer solution (PBS), polylysine coated slides (Wuhan Boster Biotech Co., Ltd), SP kit for immunohistochemistry, DAB, FITC conjugated goat anti-mouse secondary antibody, rhodamine red conjugated goat anti-rabbit secondary antibody (Beijing Zhongshan Golden Bridge Biotech Co., Ltd), rabbit anti-rat PDGFRα, rabbit anti-rat PDGFRβ, mouse anti-rat Vimentin and mouse anti-rat α-SMA primary antibodies (Abcam UK), rabbit anti-rat p-PDGFRβ primary antibody (Santa Cruz, USA), DAPI (Sigma, USA), citrate acid solution for antigen retrieval, anti-quencher (Beyotime Institute of Biotechnology), Jung embedding reagent for frozen sections (Leica, Germany), sodium chloride, potassium chloride and chloral hydrate (Sinapharm Chemical Reagent Co., Ltd).

The primers for FSP-1, α-SMA, PDGFR-α, PDGFR-β, procollagen I, procollagen III and GADPH (**Table 1**) were designed with Primer-Blast software of the National Center for Biotechnology Information (http://www.ncbi.

Table 2. SBP in different groups ($\overline{x}$±s, n=10 or n=20)

		CON	DOCA	DOCA+IMA
SBP (mmHg)	Day 0	128±12	138±11	137±11
	Day 14	137±5	158±5**	159±11**
	Day 28	134±6	190±7**	193±10**

**$P<0.01$ vs CON. n=20 per group at each time point.

nlm.nih.gov/tools/primer-blast) and synthesized in Shanghai Sangon Biotech. RNeasy Mini Kit for mRNA extraction (Qiagen Germany), PrimeScript™ RT reagent Kit with gDNA Eraser for reverse transcription, and SYBR® *Premix Ex Taq*™ II for real time quantitative PCR (Takara, Japan) were used for PCR. Sirius red dye, hematoxylin - eosin, formaldehyde, microtome (Leica), Cryostat for sectioning (Leica), and microscope camera system (Nikon eclipse 80i, Japan) were provided by the comprehensive laboratory of Basic Medicine of Anhui Medical University. Thermal cycler (Applied Biosystems Step One Plus System) was provided by the Department of Parasitology of Basic Medicine of Anhui Medical University.

Detection of myocardial fibrosis

The left ventricle was fixed in 4% paraformaldehyde for 24 h and then dehydrated. After embedded in paraffin, the left ventricle was sectioned (4 μm in thickness) onto polylysine-coated slides followed by heating. At 28 days, paraffin embedded sections were also prepared and stained with picric acid and Sirius red for collagen staining. Photographs were captured via the microscope camera system and analyzed with Image Pro plus 6.0. The severity of myocardial interstitial fibrosis was determined (area of myocardial interstitial collagen/area of total field and perivascular collagen volume area [PVCA]/vascular area [VA]).

Detection of mRNA expression of PDGFR, FSP-1, α-SMA, procollagen I and procollagen III by real time fluorescence quantitative PCR

mRNA expressions of target genes were measured by real time fluorescence quantitative PCR according to manufacturer's instructions. In brief, the heart tissues were homogenized, followed by extraction of mRNA with RNeasy Mini Kit. Then, mRNA was mixed with genomic DNA free gDNA Eraser followed by reverse transcription into cDNA with PrimeScript™ RT reagent Kit with gDNA Eraser kit. Amplification of cDNA was done on thermal cycler (Applied Biosystems Step One Plus System). The reaction mixture was 20 μl in volume, and amplification was performed according to manufacturer's instructions (SYBR® Premix Ex Taq™ II PCR kit and Applied Biosystems Step One Plus System). The reaction conditions were as follows: pre-denaturation at 95°C for 30 s and 40 cycles of 95°C for 5 s and 60°C for 30 s. The melt curve was employed to determine the specificity of products. The supporting software was used to analyze the Ct value of products. According to the following formula: $\Delta Ct=Ct_{target\ gene} - Ct_{internal\ reference}$, the ΔCt was calculated in two groups. The Ct value of target gene is negatively proportional to the copies of this gene, and thus, the larger the ΔCt, the lower the expression of a gene is. Then, $2^{-\Delta\Delta Ct}$ method was employed to calculate the relative mRNA expression of target genes.

Detection of protein expressions of PDGFRs, p-PDGFRβ, vimentin and α-SMA in heart by immunohistochemistry

The paraffin embedded sections were prepared as above mentioned and then deparaffinized. After antigen retrieval in citrate acid at 95°C for 10 min, sections were blocked in 3% H_2O_2 for 10 min at 37°C and then treated with 10% normal goat serum for 30 min at 37°C. Subsequently, these sections were independently treated with PDGFRα (1:400), PDGFRβ (1:200), p-PDGFRβ (1:200), vimentin (1:2500) and α-SMA (1:100) at 4°C overnight and then with biotinylated secondary antibody and HRP conjugated streptavidin at 37°C for 25 min followed by visualization with DAB. Washing was performed between procedures (5 min in each) and a final counterstaining was done with hematoxylin. After dehydration and transparentization, mounting was done. In the negative control group, the primary antibody was replaced with PBS. Under a light microscope, cells with brown cytoplasm were regarded as positive. Analysis was performed with Image Pro Plus 6.0, and the integrated optical density (OD) of PDGFRα, PDGFRβ and p-PDGFRβ was determined. The α-SMA positive spindle-shaped cells (myofibroblasts) were counted in each section and the average was calculated.

Detection of cells positive for PDGFRα and PDGFRβ by immunofluorescence staining

The heart tissues were fixed in 4% paraformaldehyde for 24 h and then in 30% sucrose at

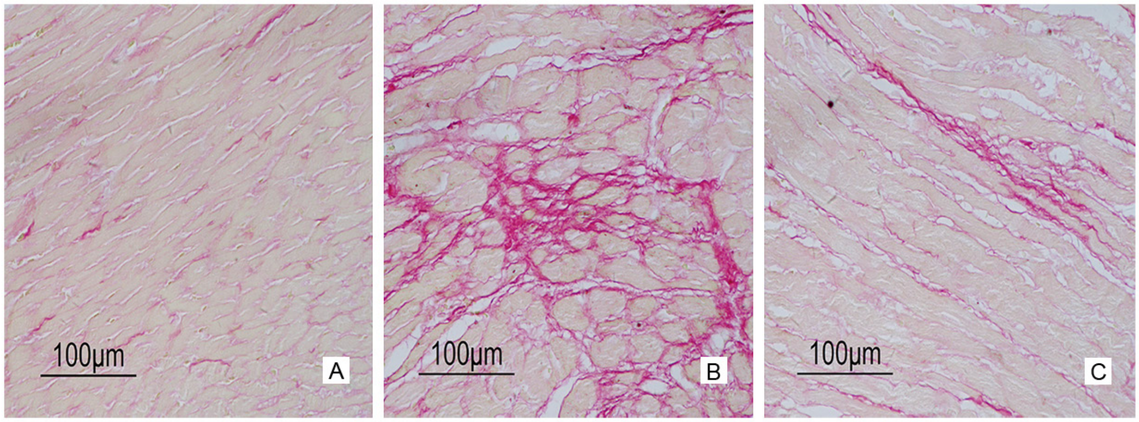


Figure 1. Sirius red staining of myocardial interstitium in different groups on day 28 (× 200). A: CON; B: DOCA; C: DOCA+IMA. Myocardial fibrosis was the most severe in DOCA group, but attenuated in DOCA+IMA group.

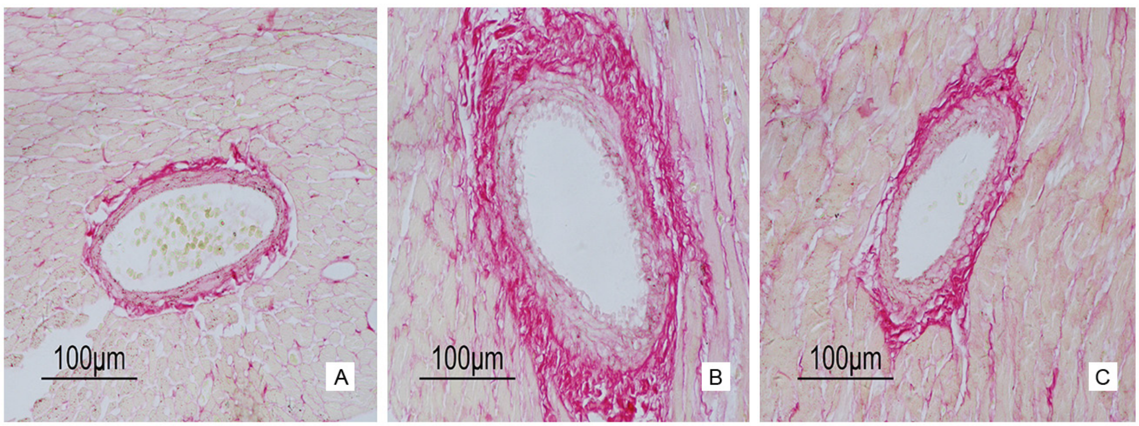


Figure 2. Sirius red staining of perivascular interstitium in different groups on day 28 (× 200). A: CON; B: DOCA; C: DOCA+IMA. Perivascular fibrosis was the most severe in DOCA group, but attenuated in DOCA+IMA group.

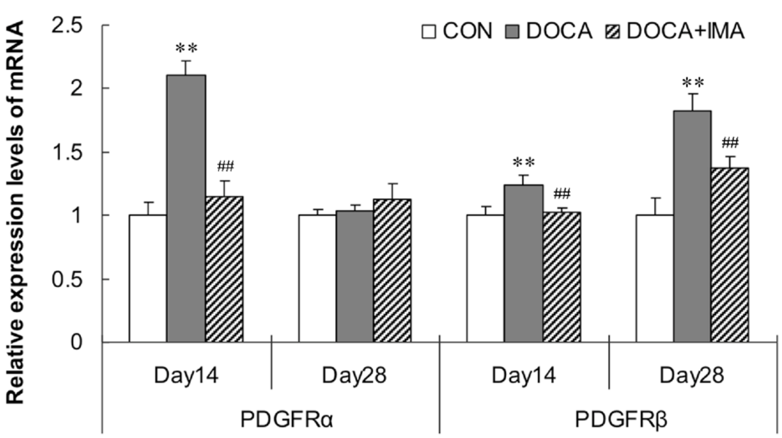


Figure 3. mRNA expressions of PDGFRα and PDGFRβ in different groups. $\overline{x}\pm s$, n=10. ***P<0.01* vs CON. ##*P<0.01* vs DOCA.

4°C until these tissues sank. After embedded in embedding reagent for frozen sections, 6-μm sections were obtained onto polylysine-coated slides. These slides were treated with acetone at 4°C for 15 min and then with PBS. After treatment with 0.5% Triton X-100 at 37°C for 30 min, sections were incubated with 10% normal goat serum at 37°C for 45 min. Subsequently, these sections were treated with primary antibody (α-SMA: 1:100; vimentin: 1:2500; PDGFRα: 1:200; PDGFRβ: 1:100) at 4°C overnight and secondary antibody (FITC conjugated goat anti-mouse antibody: 1:200; rhodamine red conjugated goat anti-rabbit antibody: 1:200; DAPI: 1:2000) at 37°C for 30 min. After washing in PBS 5 times (5 min

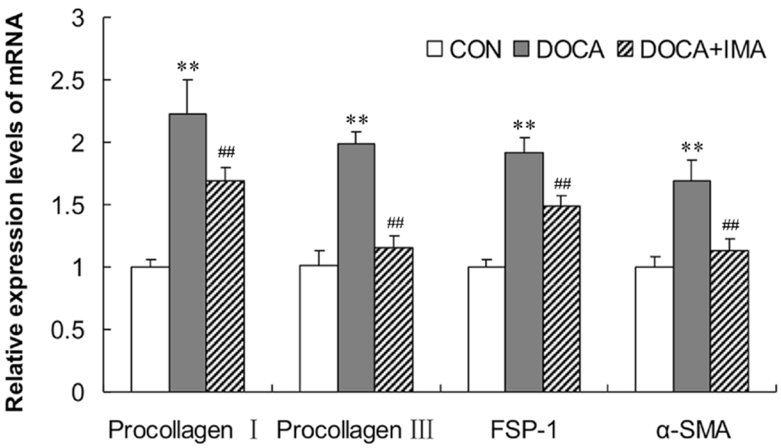


Figure 4. mRNA expressions of procollagen I, procollagen III, FSP-1 and α-SMA in different groups on day 28. $\bar{x}$±s, n=10. ***P<0.01* vs CON. ##*P<0.01* vs DOCA.

for each), mounting was done with anti-quencher, and sections were observed under a fluorescence microscope and photographed (× 200). Image J image analysis software was employed to analyze and merge these photographs.

Statistical analysis

Statistical analysis was done with SPSS version 19.0. Data were expressed as mean ± standard deviation (*x*±s). Comparisons of means between two groups were done with independent t test, and those of rates were performed with chi square test. A value of P<0.05 was considered statistically significant.

Results

Change in blood pressure in different groups

Before interventions, the SBP was comparable among groups (P>0.05). At 14 and 28 days after intervention, the SBP in DOCA group and DOCA+IMA group were markedly higher than those in CON group (P<0.01), but there was no significant difference between DOCA group and DOCA+IMA group (P>0.05) (**Table 2**).

Myocardial interstitial and perivascular fibrosis

Sirius red staining showed the collagens in myocardial interstitium were red and the myocardium was yellow. Results showed the myocardial fibrosis was the most severe, and the amount of collagens in myocardial interstitium was the highest in DOCA group. In addition, the ratio of myocardial interstitial collagen area to total field area was 27.23% and PVCA/VA ratio was 1.4676, which were markedly higher than those in CON group (2.56% and 0.4097, respectively; P< 0.01). In DOCA+IMA group, the ratio of myocardial interstitial collagen area to total field area was 3.05% and PVCA/VA ratio was 0.6841, which were significantly higher than those in CON group (P<0.05) but significantly lower than those in DOCA group (P<0.01) (**Figures 1** and **2**).

mRNA expressions of PDGFRs, FSP-1, α-SMA, procollagen I and procollagen III in heart

After real time PCR, the Ct value and number of cycles were employed for delineation and the amplification curve of mRNA was obtained. Results showed there was good repeatability and the amplification efficiency was consistent. $2^{-\Delta\Delta Ct}$ method was used to determine the relative expressions of target genes. Results revealed that the mRNA expressions of PDGFRα and PDGFRβ were 2.1012 and 1.2426, respectively, in DOCA group at 14 days, which were significantly higher than those in CON group (1.0045 and 1.0024, respectively; P<0.01). The mRNA expressions of PDGFRα and PDGFRβ were 1.1437 and 1.025, respectively, in DOCA+IMA group, which were dramatically lower than those in DOCA group (P<0.01). At 28 days after intervention, the mRNA expressions of PDGFRβ, FSP-1, α-SMA, procollagen I and procollagen III were 1.8283, 1.9155, 1.6853, 2.2209 and 1.9894, respectively in DOCA group, which were significantly higher than those in CON group (1.0070, 1.0013, 1.0028, 1.0014 and 1.0066, respectively; P<0.01). However, the PDGFRα mRNA expression was comparable between DOCA group and CON group (1.0358 and 1.001, P>0.05). In addition, the PDGFRβ mRNA expression was significantly higher than the PDGFRα mRNA expression in DOCA group (P<0.01). In DOCA+IMA group, the mRNA expressions of PDGFRβ, FSP-1, α-SMA, procollagen I and procollagen III were 1.3754, 1.4885, 1.1355, 1.6879 and 1.1506, respectively, which were markedly lower than those in DOCA group (P<0.01) (**Figures 3** and **4**).

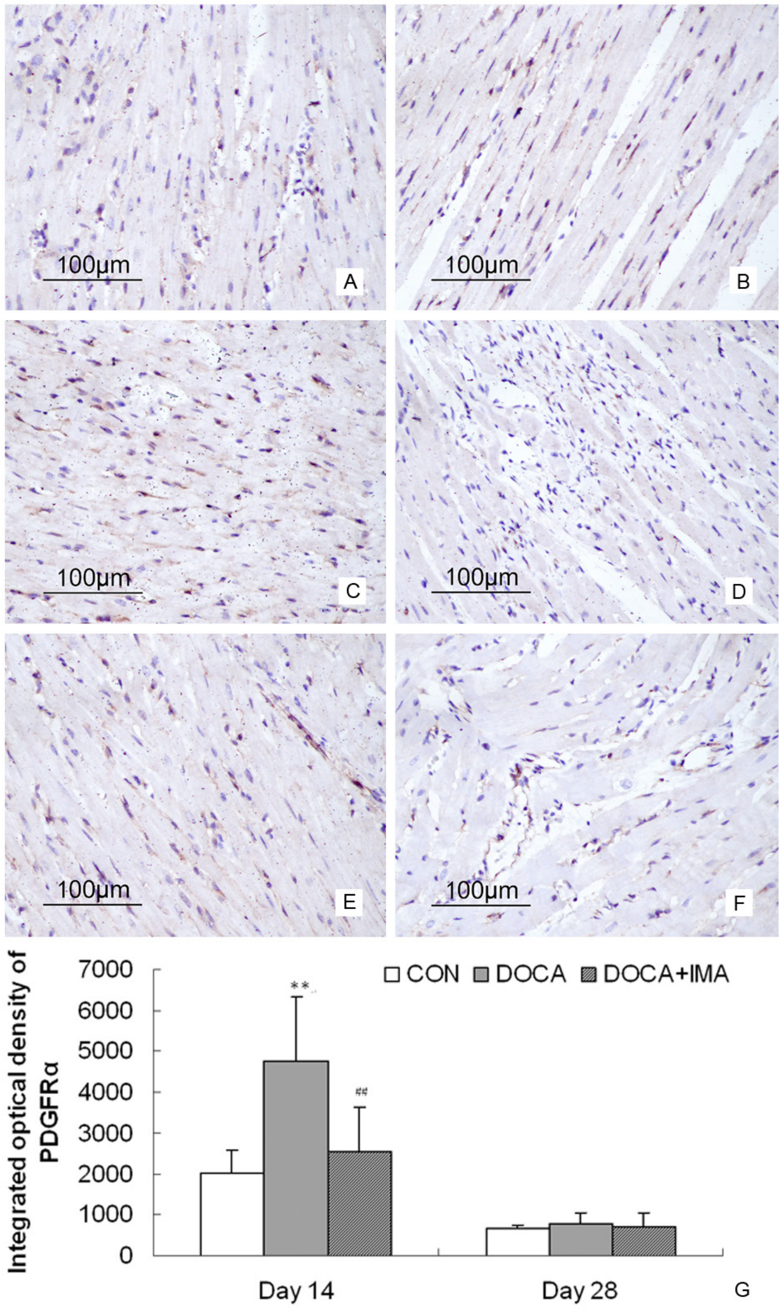


Figure 5. Immunohistochemistry for PDGFRα in different groups on days 14 and 28 (× 200). A: Day 14 CON; B: Day 14 DOCA; C: Day 14 DOCA+IMA; D: Day 28 CON; E: Day 28 DOCA; F: Day 28 DOCA+IMA. G, $\overline{x}$±s, n=10. ***P<0.01* vs CON. *##P<0.01* vs DOCA.

Protein expressions of PDGFRα, PDGFRβ, p-PDGFRβ, vimentin and α-SMA

Immunohistochemistry showed PDGFRα and PDGFRβ were mainly expressed in the myocardial interstitial cells. Image analysis revealed that the integrated ODs of PDGFRα and PDGFRβ were 4748.3-011 and 3213.6168, respectively, in DOCA group, which were markedly higher than those in CON group (2012.1771 and 1844.56-14, respectively, P<0.01). In DOCA+IMA group, the integrated ODs of PDGFRα and PDGFRβ were 2563.1-259 and 2175.8949, respectively, which were markedly lower than those in DOCA group (P<0.01). At 28 days after intervention, the integrated ODs of PDGFRβ and p-PDGFRβ were 116-12.0221 and 8787.1242, respectively, in DOCA group, which were significantly higher than those in CON group (2983.8478 and 1603.5756, respectively; P<0.01), but the PDGFRα protein expression was comparable between DOCA group and CON group (789.2215 and 659.8933, respectively; P>0.05). In DOCA+IMA group, the integrated ODs of PDGFRβ and p-PDGFRβ were 5702.9481 and 3060.2147, respectively, which were significantly lower than those in DOCA group (P<0.01). AT 28 days after intervention, the heart mainly had vimentin positive fibroblasts in CON group and less α-SMA positive cells were noted. In DOCA group, the number of α-SMA positive spindle-shaped cells (myofibroblasts) was 16.4 per field, which was higher than that in CON group (5.4 per field) (P<0.01). In DOCA+IMA group, the number of myofibroblasts was 13.1 per field, which was significantly smaller than that in DOCA group (P<0.05) (**Figures 5**-**7**).

Cells expressing PDGFRα and PDGFRβ

Under fluorescence microscope, vimentin or α-SMA positive cells presented with green fluo-

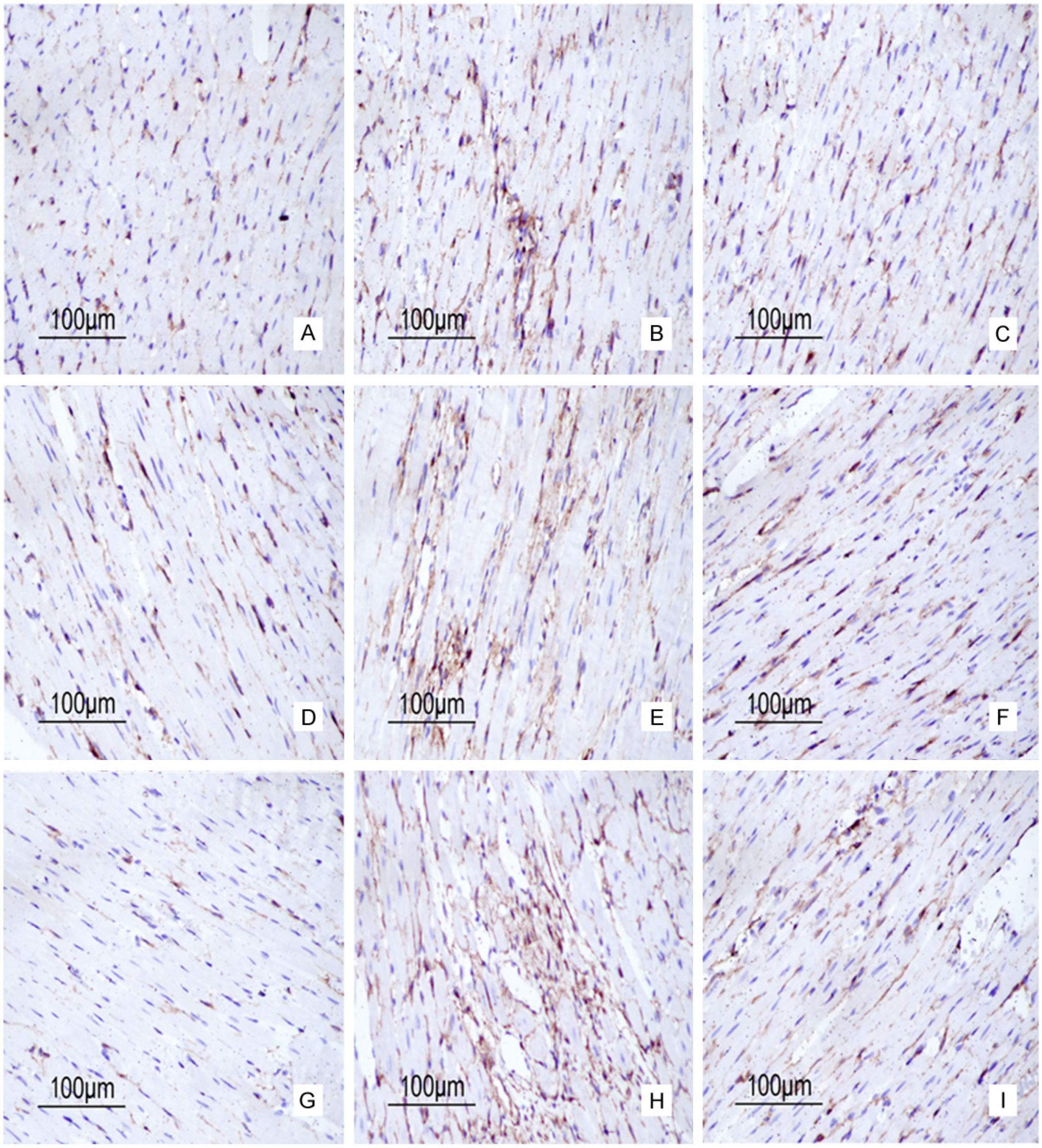


Figure 6. Immunohistochemistry for PDGFRβ and p-PDGFRβ in different groups on days 14 and 28 (× 200). A-F: PDGFRβ, A: Day 14 CON; B: Day 14 DOCA; C: Day 14 DOCA+IMA; D: Day 28 CON; E: Day 28 DOCA; F: Day 28 DOCA+IMA. G-I: p-PDGFRβ, G: Day 28 CON; H: Day 28 DOCA; I: Day 28 DOCA+IMA.

rescence mainly in the cytoplasm; PDGFRα or PDGFRα positive cells presented with red fluorescence mainly in the cytoplasm. The merged fluorescence was orange. DAPI positive nuclei presented with blue fluorescence. Results showed fibroblasts and myofibroblasts were positive for PDGFRα, which was mainly, found in the cytoplasm. In VSMCs, no PDGFRα expression was observed. In addition, PDGFRβ expression was observed in not only fibroblasts and myofibroblasts but also VSMCs (**Figure 8**).

Discussion

MF is a pathological process of excessive deposition of collages due to their abnormal metabolism and a major cause of heart failure. In multiple cardiovascular diseases at end stage, MF is a major pathological feature of my-ocardium.

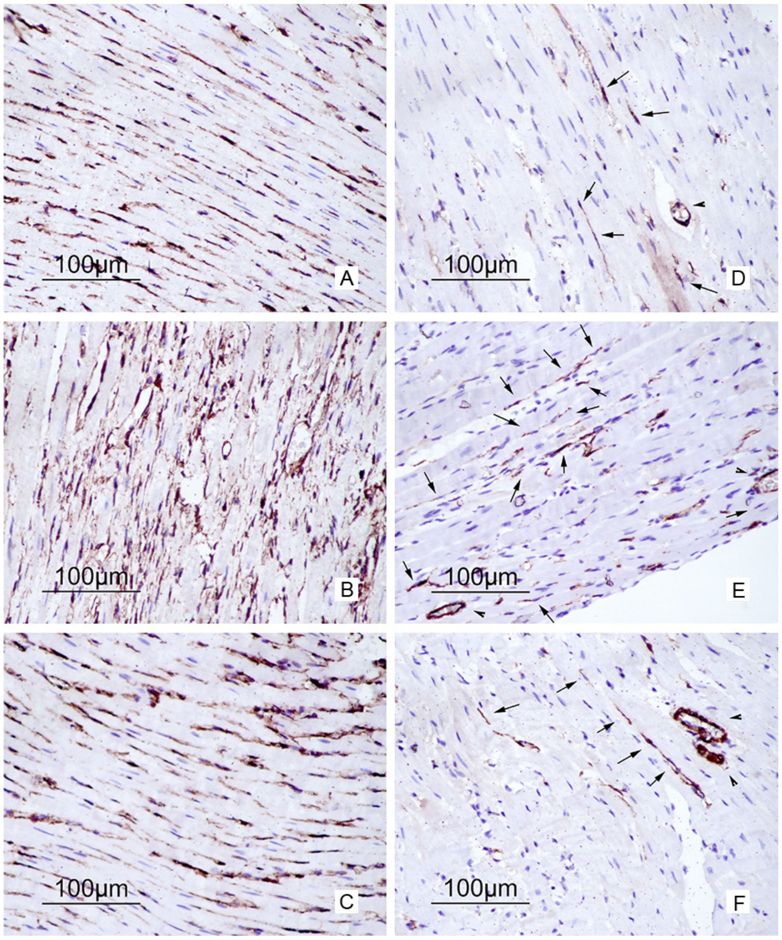
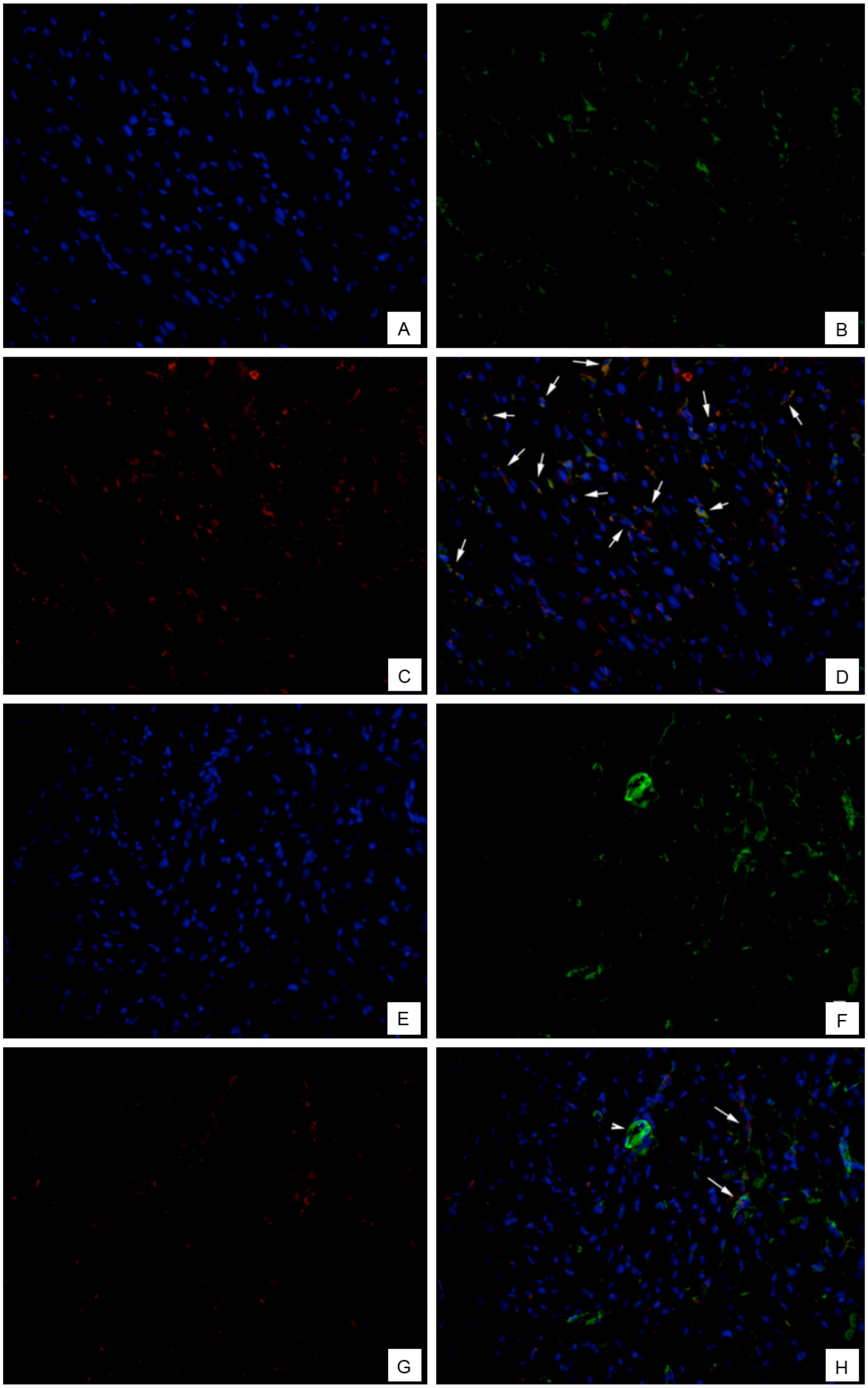


Figure 7. Immunohistochemistry for α-SMA and vimentin and α-SMA positive spindle-shaped cells in different groups on day 28 (× 200). A: Vimentin CON; B: Vimentin DOCA; C: Vimentin DOCA+IMA; D: α-SMA CON; E: α-SMA DOCA; F: α-SMA DOCA+IMA.

To date, some theories have been proposed for the mechanisms underlying the occurrence and development of MF, and the increase in mineralocorticoid has been reg-arded as a major cause of MF and become a hot topic in this field [6]. However, the specific pathogenesis of MF is still unclear. In recent years, studies reveal that PDGF/PDGFR signaling pathway is found to be involved in not only the MF after myocardial infraction and MF secondary to viral myocarditis [5, 7], but the DOCA/salt induced hypertensive MF. In study on DOCA/salt induced hypertensive rats, PDGF/PDGFR signaling pathway was found to promote the deposition of collagens and the inhibitor of PDGF/PDGFR signaling pathway could attenuate MF [3]. This suggests that PDGF/PDGFR signaling pathway is invo-lved in the occurrence and development of MF. PDGF is a potent factor that can promote the mitosis. After binding to PDGFR, PDGF can stimulate the growth, differentiation and migration of interstitial cells including fibroblasts and VSMCs [8, 9]. Zymek et al [5] found PDGFRβ signaling pathway in myocardial infarction animal model was a unique mechanism for the regulation of myocardial vasculature system, and PDGF-A/PDGFRα signaling pathway could promote the collagen deposition, but failed to regulate vascularization. In an in vitro study of Zhao et al, results showed PDGFR-D/PDGFRβ signaling pathway could promote the synthesis and secretion of collagens [10]. In the present study, the PDGFRα expression was higher than the PDGFRβ expression in rats with DOCA/salt induced hypertensive MF at early stage. However, the PDGFRα expression failed to increase markedly, and the expressions of PDGFRβ and p-PDGFRβ elevated markedly at late stage of MF. In addition, the myocardial interstitial collagen deposition increased dramatically. After treated with IMA, the p-PDGFRβ expression reduced, and the myocardial interstitial collagens decreased accordingly. These suggest that PDGFRα signaling pathway acts mainly at early stage of DOCA/salt induced hypertensive MF, but PDGFRβ functions in the whole process of MF.

In mammalians, myocytes, fibroblasts and vascular cells (VSMCs and endothelial cells) are the main cells in the heart. The myocytes and fibroblasts function synergistically to regulate the heart function and account for 90% of cells in the heart [11]. The fibroblast-like cells (fibroblasts and myofibroblasts) in the myocardial interstitium have been regarded as a major source of ECM [12], and collagen in ECM is a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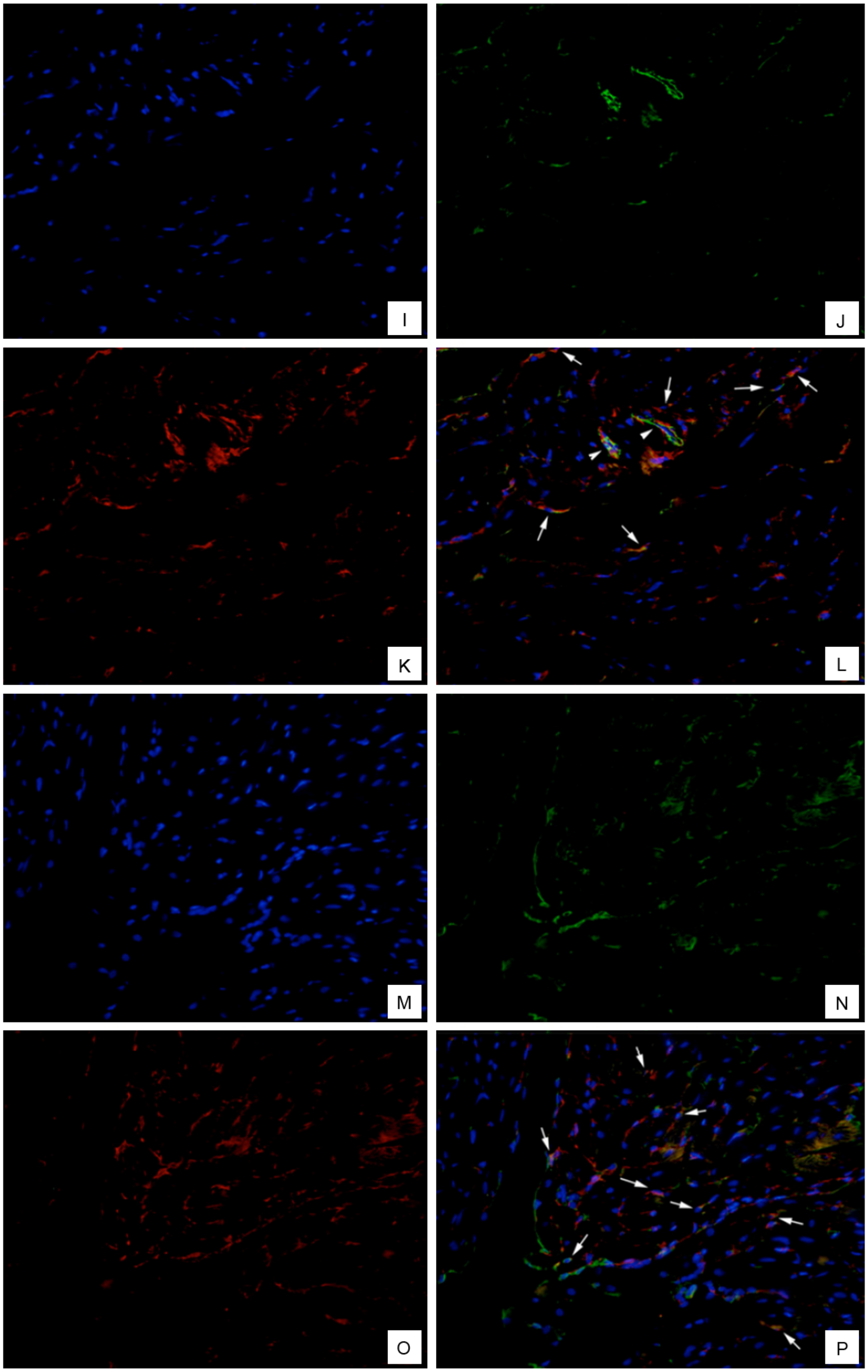
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Figure 8. Immunofluorescence staining of PDGFRα and PDGFRβ (× 200). PDGFRα was located in fibroblasts and myofibroblasts, but not in VSMCs. PDGFRβ was not only located in fibroblasts and myofibroblasts, but also in VSMCs. A: DAPI, B: Vimentin, C: PDGFRα, D: Merged; E: DAPI, F: α-SMA, G: PDGFRα, H: Merged; I: DAPI, J: α-SMA, K: PDGFRβ, L: Merged; M: DAPI, N: Vimentin, O: PDGFRβ, P: Merged.

main bearer of stress. The paracrine signals of lymphocytes and autocrine factors of fibroblasts can act synergistically to initiate and maintain the activation of fibroblasts [2]. The activated fibroblasts can generate a lot of factors involved in the process of MF, and these cells can also differentiate into myofibroblasts [13]. In normal myocardium, few myofibroblasts are observed [14]. Studies have confirmed that myofibroblasts are the direct effector cells in the MF and have the characteristics of fibroblasts and smooth muscle cells. Thus, myofibroblasts are regarded as smooth muscle like fibroblasts. Specific expression of α-SMA is an important feature of myofibroblasts [15]. In MF, the synthesis and degradation of collagens are mainly regulated by myofibroblasts [13]. In vitro study demonstrated that pro-fibrotic factors could promote the synthesis and secretion of collagens via increase the proliferation of fibroblasts and the differentiation of myofibroblasts [10]. The increased expression of pro-fibrotic factors as well as the proliferation and differentiation of non-cardiac cells may finally cause MF [16]. In the present study, few myofibroblasts were observed in the myocardium, but some fibroblasts were noted in control group. However, in DOCA/salt induced hypertensive MF rats, the myofibroblasts increased markedly when compared with CON group, but reduced dramatically after treatment with IMA, an inhibitor of tyrosine kinase. This change was consistent with the changes in expressions of procollagen I and procollagen III.

Our results showed PDGF/PDGFR signaling pathway was involved in the mineralocorticoid induced MF, and the myofibroblasts increased during the process of MF. Immunofluorescence staining showed both PDGFRα and PDGFRβ were expressed in fibroblasts and myofibroblasts, but VSMCs has only PDGFRβ expression. It is speculated that the activated monocytes/macrophages and fibroblasts secrete PDGF [13, 17], which bind to PDGFR on fibroblasts leading to the activation of fibroblasts via the PDGF/PDGFR signaling pathway. Overactivation of PDGF/PDGFR signaling pathway may stimulate the proliferation of fibroblasts and promote the transformation of these cells into myofibroblasts. Both fibroblasts and myofibroblasts can secret a large amount of collagens, which deposit in the myocardial interstitium, resulting in MF. After treatment with IMF, the PDGF/PDGFR signaling pathway is blocked, and the myofibroblasts reduced, leading to the attenuation of MF.

Taken together, in DOCA induced salt sensitive hypertensive rats, PDGFRα acts mainly at early stage of MF, but PDGFRβ functions in the whole process of MF. In addition, PDGFRβ can also act on vascular fibers. Both PDGFRα and PDGFRβ are expressed in fibroblasts and myofibroblasts, suggesting that PDGF/PDGFR signaling pathway can induce the massive collagen deposition via stimulating fibroblasts to proliferate and transform into myofibroblasts, resulting in MF.

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Disclosure of conflict of interest

None.

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