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# Pioglitazone ameliorates systolic and diastolic cardiac dysfunction in rat model of angiotensin II-induced hypertension

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ABSTRACT

We previously showed that administration of angiotensin II to rats causes fibrosis and lipid accumulation in the heart. In the current study, we examined the effect of pioglitazone, an agonist of peroxisome proliferator activated receptor-γ, on angiotensin II-induced intracardiac lipid accumulation and cardiac dysfunction. Pioglitazone, given orally at a dose of 2.5 mg/kg/d, reduced cardiac triglyceride content and suppressed lipid deposition in the heart of angiotensin II-induced hypertensive rats without affecting angiotensin II-induced upregulation of lipogenic gene expression. Histological examination showed that pioglitazone reduced the area of cardiac fibrosis and iron deposition in the heart of angiotensin II-treated rats. Expression of an antioxidative molecule, heme oxygenase-1, was increased by angiotensin II infusion, and pioglitazone treatment preserved expression of HO-1. Angiotensin II increased the superoxide signals detected by dihydroethidium staining in myocardial cells with lipid deposition, and this increase was suppressed by pioglitazone. Cardiac function was analyzed in an ex vivo isolated cardiac perfusion system. It was found that pioglitazone improved both the systolic and diastolic cardiac performance, which was weakened by angiotensin II infusion, after transient ischemia and reperfusion. These findings collectively suggest that pioglitazone treatment ameliorated the histological and functional cardiac damage induced by angiotensin II infusion, the mechanism of which may be related to the antioxidative action of pioglitazone.



## 1. Introduction

Several different mechanisms are considered to underlie the functional and histological cardiac damage caused by activation of the renin–angiotensin system (RAS) [1]; these include development of myocardial hypertrophy, interstitial fibrosis [2], enhancement of oxidative stress [3], and activation of inflammatory signaling pathways [4]. Excessive accumulation of triglycerides may occur in various non-adipose tissues, and can cause tissue damage, termed lipotoxicity [5,6]. Accumulation of lipids in the myocardium is known to occur in the conditions of pressure overload, metabolic syndrome [7], and diabetes, which may enhance cardiac damage [8]. The finding that inhibition of RAS reduced myocardial lipid deposition and improved cardiac function in a diabetic animal model [9] suggested that lipotoxicity may be another mechanism underlying the cardiac damage exerted by activated RAS. In a recent study, we demonstrated that administration of angiotensin II (Ang II) caused accumulation of lipids in the heart and kidney in rat model [10,11]. Ang II-induced cardiac and renal accumulation of lipids was, in part, a pressor-independent phenomenon, and was accompanied by the altered expression of lipid metabolism-related genes, such as sterol-regulatory element binding protein (SREBP)-1c, peroxisome proliferator activated receptor-γ (PPARγ), and fatty acid synthase (FAS).

Several previous studies have shown that PPARγ agonists may act favorably in suppressing excess lipid accumulation [12] and preserving cardiac function in conditions that occur in response to certain noxious stimuli, such as myocardial ischemia [13,14] and abnormal glucose metabolism [15]. To this end, we here investigated whether administration of a PPARγ agonist, pioglitazone (Pio), would reduce the cardiac lipid content and suppress the cardiac injury induced by Ang II. Because increased superoxide production was demonstrated at the site of lipid deposition, we also examined whether the extent of superoxide production would be reduced by Pio in Ang II-treated animals.

## 2. Materials and methods

### 2.1. Animal models

The experiments were performed in accordance with the Guidelines for Animal Experimentation approved by the Animal Center for Biomedical Research, Faculty of Medicine, University of Tokyo. Ang II-induced hypertension was induced in male Sprague–Dawley rats (250 to 300 g) by subcutaneous implantation of an osmotic minipump (Alza Pharmaceutical) as described previously [16]. Briefly, Val5-Ang II (Sigma Chemical) was infused at doses of 0.7 mg/kg/day via a subcutaneously implanted osmotic minipump that was continued for 7 days unless stated otherwise. In some

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**Table 1**
Baseline conditions.

| Variables | Control | Pio | Ang II | Ang II + Pio |
|---|---|---|---|---|
| Weight | 328 ± 3 | 326 ± 2 | 265 ± 4† | 255 ± 5† |
| Systolic BP (mm Hg) | 112 ± 5 | 117 ± 2 | 202 ± 7† | 194 ± 7† |
| Total cholesterol (mg/dL) | 52 ± 2 | 48 ± 3 | 62 ± 4* | 54 ± 4 |
| Triglycerides (mg/dL) | 24 ± 2 | 25 ± 2 | 32 ± 2* | 24 ± 2 |
| Free fatty acids (μEq/L) | 486 ± 27 | 487 ± 45 | 674 ± 30† | 688 ± 43† |
| Plasma fasting Glucose (mg/dL) | 146 ± 5 | 151 ± 8 | 186 ± 8† | 159 ± 8 |
| Serum fasting insulin (mg/dL) | 1.1 ± 0.3 | 1.7 ± 0.5 | 1.4 ± 0.2 | 1.8 ± 0.5 |

Pio and Ang II indicate pioglitazone and angiotensin II, respectively.
Number of samples was ≥7 in each group.
* P<0.05 versus untreated control.
† P<0.01 versus untreated control.

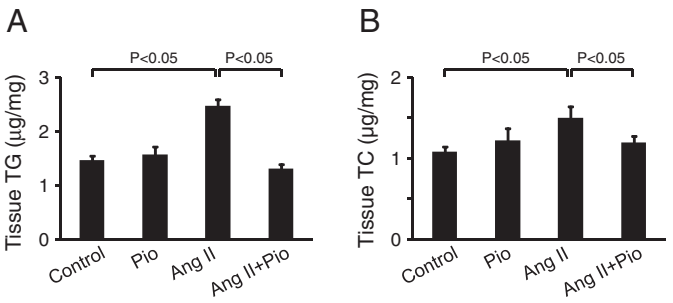


**Fig. 2.** Tissue content of lipids. Content of triglycerides (TG) (A) and total cholesterol (TC) (B) in the heart. Data represent the mean ± SEM of the results from 5 to 7 rats in each group.

angiotensin II-infused rats, Pio (Takeda Pharmaceutical Co., Tokyo, Japan) was given orally at a dose of 2.5 mg/kg/day. These treatments were continued until sacrifice.

### 2.2. Measurement of lipid content in serum and the heart

Serum levels of total cholesterol (TC) and triglycerides (TG), and nonesterified fatty acid (NEFA) were measured by enzymatic methods [10]. The amount of TG and TC in the heart tissue was measured in homogenated extracts by enzymatic colorimetric determination using the Triglyceride-E Test, Cholesterol-E Test, and Free cholesterol-E Test Wako, respectively (Wako Pure Chemicals).

### 2.3. Langendorff system and global ischemia and subsequent reperfusion

Cardiac function of the excised heart was investigated in rats treated with Ang II for 14 days, because neither systolic nor diastolic cardiac function was found to be significantly reduced by 7 days of Ang II treatment (data not shown). The excised heart was put into ice-cold modified Krebs buffer and quickly perfused in the Langendorff apparatus (ADInstruments) at a constant perfusion pressure of 100 cm $H_2O$ at 37 °C with modified Krebs–Henseleit buffer solution (NaCl 118.5 mmol/L, KCl 4.8 mmol/L, $KH_2PO_4$ 1.2 mmol/L, $MgSO_4$ 1.2 mmol/L, $CaCl_2$ 2.5 mmol/L, $NaHCO_3$ 25.0 mmol/L, and glucose 11 mmol/L, pH 7.4) that was continuously aerated with 95% $O_2$ + 5% $CO_2$. Systolic (+dP/dt) and diastolic (−dP/dt) left ventricular function was monitored and recorded continuously using a fluid-filled left ventricular balloon in line with a transducer (Powerlab, ADInstruments). The balloon volume was set to produce a left ventricular end diastolic pressure (LVEDP) of 5 mm Hg. Each heart was perfused for 15 min (control perfusion) and then subjected to 30 min of global ischemia by stopping the circulation, which was then followed by reperfusion for 60 min.

### 2.4. Histological analysis

Oil red O staining was performed on sections of unfixed, freshly frozen heart samples (3 μm in thickness). The areas of lipid deposition were calculated by using the image analysis software, Photoshop (Adobe), and semiquantification of the lipid deposition was performed as described elsewhere [17]. Prussian blue staining was used to detect iron deposition within the cardiac tissue. The fibrosis area was calculated in the heart of rats given Ang II for 14 days. For quantification of the fibrous areas, heart sections subjected to Mallory-Azan staining were photographed and digitalized, and the number of pixels of blue-color was counted by using a photoimaging system. The ratio of the area affected by fibrosis to the total cardiac area in each sample is expressed as the percentage of fibrosis [18]. Staining with the oxidative fluorescent dye dihydroethidium (DHE) was performed as described previously [10]. Images were obtained with a fluorescent microscope BX51 (Olympus, Tokyo, Japan), and the fluorescence intensity, which was determined from at least five fields for each section, is presented as the percentage of that of untreated controls.

### 2.5. Western blot analysis

Western blot analysis was performed as described previously [19]. Antibodies against total and phosphorylated forms of AMP-activated protein kinase (Cell Signaling Technology, Danvers, MA), and total and phosphorylated forms of acetyl-CoA carboxylase (ACC) (Cell Signaling Technology) were used at a dilution of 1/1000. Polyclonal antibodies against rat ferritin (Panapharm, Kumamoto, Japan) and heme oxygenase-1 (HO-1, StressGen, Victoria, BC, Canada), and monoclonal antibody against β-actin (Sigma) were used at dilutions of 1/1000, 1/2000 and 1/1000. The ECL Western blotting system (Amersham Life Sciences, Arlington Heights, IL) was used for detection. Bands were visualized by a lumino-analyzer (Fuji Photo Film, Tokyo, Japan). Band intensity was calculated and is expressed as a percentage of the control value.

### 2.6. Real time reverse transcription-polymerase chain reaction (RT-PCR)

Expression of mRNA of lipid metabolism-related genes was analyzed by real time quantitative PCR performed by a LightCycler coupled with hybriprobe technology (Roche Diagnostics). Expression of each target gene was normalized to that of an endogenous control, glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The target genes encoded the following proteins: SREBP-1c, fatty acid synthase (FAS), carnitine palmitoyltransferase (CPT)-1 PPARγ, and Nox1. The forward and backward primers used have been described elsewhere [11].

### 2.7. Statistical analysis

Data are expressed as the mean ± SEM. We used ANOVA followed by a multiple comparison test to compare raw data, before expressing the results as a percentage of the control value using the statistical analysis software SPSS Dr II. A value of $p<0.05$ was considered to be statistically significant.

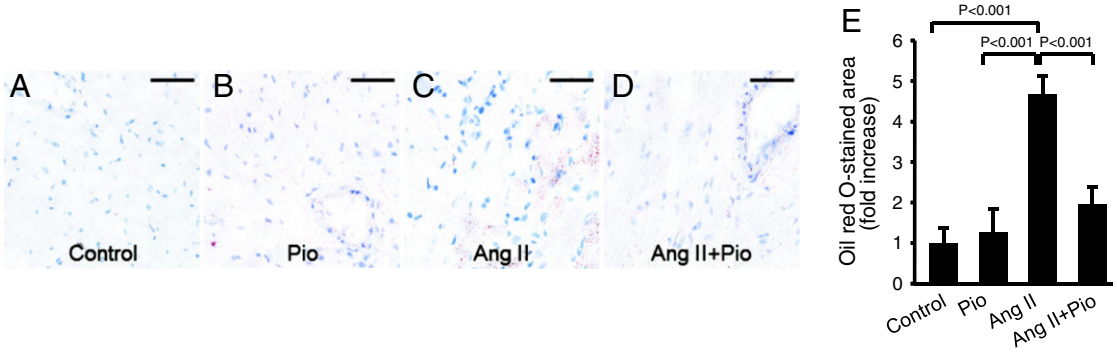


**Fig. 1.** Oil red O staining of the heart of rats given angiotensin II with or without pioglitazone administration. Shown are heart sections from a control rat (A), a rat given pioglitazone (Pio) (B), a rat given angiotensin II (Ang II) (C), and a rat given Ang II plus Pio (D). Original magnification, ×200. Scale bar indicates 100 μm. E. Semiquantification of the oil red O-stained area. Data represent the mean ± SEM of the results from 4 to 5 rats in each group.