Pirfenidone mitigates left ventricular fibrosis and dysfunction after myocardial infarction and reduces arrhythmias

Duy T. Nguyen, MD, Chunhua Ding, MD, PhD, Emily Wilson, BS, Gregory M. Marcus, MD, Jeffrey E. Olgin, MD

From the Cardiac Electrophysiology and Cardiovascular Research Institute, University of California, San Francisco.

BACKGROUND Post–myocardial infarction (MI) complications include ventricular tachycardia (VT). Excessive non-MI fibrosis, involving the infarct border zone (IBZ) and beyond, is an important substrate for VT vulnerability.

OBJECTIVE This study assessed whether the antifibrotic agent pirfenidone can mitigate fibrosis in remodeling and determined its effects on myocardial function and VT susceptibility in a rodent MI model.

METHODS We studied 2 groups of rats undergoing MI 1 week prior to treatment: a control group (n = 15) treated with placebo and a pirfenidone group (n = 15). We performed serial echocardiograms, and after 4 weeks of treatment, we conducted electrophysiological and optical mapping studies as well as histology.

RESULTS There was less decline in left ventricular (LV) ejection fraction for pirfenidone-treated rats, 8.6% versus 24.3% in controls ($P < 0.01$). Pirfenidone rats also had lower rates of VT inducibility, 28.6% versus 73.3% in control rats ($P < 0.05$). Furthermore, pirfenidone-treated rats had faster conduction velocities in their IBZs compared with controls, at all pacing cycle lengths ($P < 0.05$). Rats treated with pirfenidone also had smaller infarct dense scar (8.9% of LV myocardium vs. 15.7% in controls, $P < 0.014$), less total LV fibrosis (15% vs. 30% in controls, $P < 0.003$), and less nonscar fibrosis (6.6% vs. 12.6% in controls, $P < 0.006$).

CONCLUSION Pirfenidone decreased total and nonscar fibrosis in a rat MI model, which correlated with decreased infarct scar, improved LV function, and decreased VT susceptibility. Directly targeting post-MI fibrotic substrates may have a role in limiting infarct-dense scar, improving LV function, and reducing VT vulnerability.

KEYWORDS Fibrosis; Optical mapping; Pirfenidone; Ventricular tachycardia; Remodeling

ABBREVIATIONS **APD** = action potential duration; **BDM** = butadione monoxime; **CV** = conduction velocity; **EDV** = end diastolic volume; **ESV** = end systolic volume; **IBZ** = infarct border zone; **LV** = left ventricle; **LVEF** = left ventricular ejection fraction; **MI** = myocardial infarction; **PCL** = pacing cycle length; **TGFβ1** = transforming growth factor β1; **VT** = ventricular tachycardia

(Heart Rhythm 2010;7:1438–1445)

Introduction

Despite advances in treating acute coronary syndromes, significant morbidity and mortality remain after a myocardial infarction (MI), including ventricular arrhythmias. Abnormal conduction in the infarct border zone (IBZ) is important in the pathogenesis of post-MI arrhythmias.[1] These abnormalities are due to remodeling in tissue architecture. An MI can be considered a healing wound, characterized by an initial inflammatory response, and followed by fibrosis development, thus minimizing infarct expansion and cardiac perforation.[2] However, ongoing and excessive fibrosis contributes to adverse cardiac remodeling. Fibrosis causes nonuniform anisotropic conduction that may lead to re-entry circuits and wave breaks predisposing to arrhythmogenesis.[3,4]

Fibrogenesis consists of redundant pathways and feedback loops, with transforming growth factor β1 (TGFβ1) signaling pathway being integral.[5] Pirfenidone is an oral antifibrotic agent that significantly diminishes and possibly reverses collagen formation by affecting TGFβ1-mediated fibrosis.[6] We therefore hypothesized that attenuation of post-MI fibrosis by pirfenidone will ameliorate arrhythmogenesis. To address this hypothesis, we studied ventricular tachycardia (VT) inducibility in an ischemia-reperfusion rat MI model after pirfenidone treatment. Furthermore, we assessed ventricular function, electrophysiological properties, and extent of fibrosis.

Methods

Animal model

This study was approved and monitored by the Laboratory Animal Resource Center at University of California at San Francisco, and conformed to *Guide for the Care and Use of Laboratory Animals* by the U.S. National Institutes of Health (Publication No. 85-23, revised 1996).

Drs. Nguyen and Ding contributed equally to this work. Supported by National Institutes of Health RO1-HL072854 (Dr. Olgin); National Institutes of Health F32HL090260 (Dr. Nguyen); and the Stephen and Nancy Grand Fund (Dr. Olgin). Dr. Olgin has received research funds from InterMune for separate research not reported here. **Address reprint requests and correspondence:** Dr. Jeffrey Olgin, 500 Parnassus Avenue, Box 1354, San Francisco, California 94143. E-mail address: olgin@medicine.ucsf.edu. (Received November 24, 2009; accepted April 23, 2010.)

1547-5271/$ -see front matter doi:10.1016/j.hrthm.2010.04.030

After baseline echocardiography, 30 male Sprague-Dawley rats, ages 6 to 10 weeks, underwent an ischemia-reperfusion MI, as previously described.[7] After a left thoracotomy and pericardiotomy were performed, a 7-0 Ticron suture was introduced into the myocardium, using the left atrial appendage and right outflow tract as landmarks. The depth of entry was 2 mm, which was slightly greater than the level of the left coronary artery. The suture was tightened to occlude the artery for 25 minutes and then removed to allow for reperfusion. The chest was then closed. After 1 week and repeat echocardiography, rats were randomized for 4 weeks to placebo feed (control, n = 15) or feed mixed with 1.2% pirfenidone (InterMune, Brisbane, California, n = 15).

Echocardiography

Serial echocardiography was performed at baseline and at 1 and 5 weeks after infarction, using a high-resolution echocardiographic system (Vevo 660, VisualSonics, Toronto, Canada) equipped with a 25-MHz mechanical transducer. Parasternal long-axis and short-axis views were acquired. Using the long-axis view, left ventricular (LV) end-systolic and end-diastolic volumes (ESV and EDV) as well as LV ejection fraction (LVEF) were calculated. The system software utilizes a formula based on a cylindrical-hemiellipsoid model (volume = 8 * area^2 ÷ 3 ÷ length).[8] LVEF was calculated using the formula: (EDV − ESV)/EDV * 100.

Optical mapping

Optical mapping was performed 5 weeks after MI (4 weeks of treatment). One animal in each group could not be mapped optically. After anesthesia, hearts were rapidly excised and arrested in cold cardioplegia solution. Aortas were cannulated and retrogradely perfused at a rate of 6 ml/min with 37°C modified Tyrode solution containing (in mmol/l): 130 NaCl, 20.0 $NaHCO_3$, 1.2 $MgCl_2$, 4.0 KCl, 5.6 glucose, and 1.8 $CaCl_2$, gassed with 95% O_2/5% CO_2. Cannulated hearts were then placed in a temperature-controlled optical recording chamber (maintained at 37°C) while electrocardiograms, perfusion rates, and temperatures were measured continuously. Before optical recordings, Tyrode solution containing voltage-sensitive dye Pittsburg-I (10 μl of 2.5 mM stock) was perfused through preparations.[9] During optical recordings, contractility was blocked with 15 mM butadione monoxime (BDM).

Optical mapping procedures were modified from previously described studies.[10] To summarize, 10,000 simultaneous optical action potentials were recorded with a 100 × 100 complementary metal oxide semiconductor (CMOS) camera (Scimedia, Costa Mesa, California) within a 19 × 19-mm mapping field on the epicardium of the LV anterior wall. Using a 1,000-W tungsten-halogen light source, fluorescence was excited with an excitation filter of 530 nm and transmitted with an emission long-pass filter of >630 nm. Pacing was performed from 1 site—an area of viable myocardium on the LV epicardium. Fluorescent optical maps were acquired at 1,000 Hz during ventricular bipolar pacing with pacing cycle lengths (PCLs) from 250 to 90 ms, and then with S1-S2 using a basic PCL of 200 ms and S2 decremented by 10 ms until the ventricular effective refractory period was reached. After this, 1 attempt of programmed stimulation, with up to 3 extrastimuli, and 2 attempts of burst pacing, from 90 ms to 60 ms (decremented by 2 ms), were performed to induce ventricular arrhythmias. Inducibility was defined as the ability to provoke sustained (≥30 seconds) VT or ventricular fibrillation.

Data analysis

Optical mapping data were analyzed using OMproCCD software (courtesy of Bum-rak Choi, Providence, Rhode Island). Raw fluorescence data were viewed as a video of normalized fluorescence intensity. Quantitative data were then obtained from optically derived action potentials for each of the 10,000 pixels of the CMOS camera. Activation time and action potential duration at 80% repolarization (APD80) were measured for each PCL. APD80 was measured as duration from activation time (start of action potential) to the time point where the action potential had recovered to 20% of maximal fluorescent signal. Rise time was calculated as time between takeoff (maximum value of the second derivative) and peak of the action potential.[11]

OMproCCD software was used to calculate conduction vectors representing local conduction velocities (CVs) and directions at each pixel.[12,13] To quantify conduction heterogeneity, phase differences were calculated as average differences with neighboring activation times at each site, as previously described.[14,15] Frequency histograms were then constructed for phase differences within a recorded area. These histograms were summarized as phase time at the 5th (P5), 50th (P50), and 95th percentiles (P95) of the phase distribution. Heterogeneity index was defined as (P95 − P5)/P50.

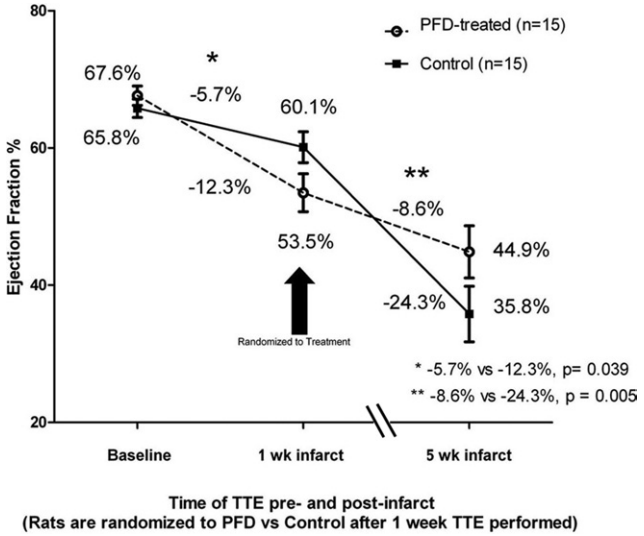

Figure 1 Control vs. pirfenidone serial ejection fractions. Rats were randomized to pirfenidone (n = 15) or control (n = 15) at 1 week postinfarction. At 5 weeks postinfarction (after 4 weeks of treatment), the pirfenidone group **(dotted line)** had significantly less decline in its ejection fraction, 8.6%, compared with the control group, which had a decrease of 24.3% in ejection fraction. TTE = transthoracic echocardiogram.