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Original article

Inhibition of protein kinase C reduces left ventricular fibrosis and dysfunction following myocardial infarction

Andrew J. Boyle [a], Darren J. Kelly [a], Yuan Zhang [a], Alison J. Cox [a], Renae M. Gow [a], Kerrie Way [a], Silviu Itescu [a], Henry Krum [b], Richard E. Gilbert [a,*]

[a] *Department of Medicine, St Vincent's Hospital, University of Melbourne, Melbourne, Princes Street, Fitzroy 3065, Vic., Australia*
[b] *Departments of Epidemiology and Preventive Medicine, Alfred Hospital, Monash University, Melbourne, Australia*

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Abstract

Despite current therapies, chronic heart failure (CHF) remains a major complication of myocardial infarction (MI). The pathological changes that follow MI extend to regions remote from the site of infarction (non-infarct zone, NIZ) where fibrosis is a prominent finding. Although the mechanisms underling this adverse remodeling are incompletely understood, activation of protein kinase C has recently been implicated in its pathogenesis. MI was induced in Sprague–Dawley rats by ligation of the left anterior descending coronary artery. One week post-MI, animals were randomized to receive the PKC-inhibitor, ruboxistaurin (LY333531) for 4 weeks, or no treatment. When compared with sham-operated animals, post-MI rats showed a 33 ± 7% reduction in fractional shortening over a 4 weeks period, that was attenuated by treatment with ruboxistaurin (6 ± 11%, $P < 0.05$). Increased matrix deposition was noted in the NIZ, particularly in the subendocardial region of post-MI rats, in association with elevated expression of the profibrotic growth factor, transforming growth factor-beta. These findings were also significantly reduced by ruboxistaurin. PKC-inhibition with ruboxistaurin led to attenuation in both the pathological fibrosis and impaired cardiac function that follow experimental MI, suggesting a possible role for this agent in preventing post-infarction heart failure.

Keywords: Protein kinase C; Fibrosis, Remodeling; Myocardial infarction; Collagen

1. Introduction

Chronic heart failure (CHF) is a major complication of myocardial infarction (MI) that substantially worsens prognosis. Although there have been major therapeutic advances in the management of MI, post-infarction CHF remains a common cause of morbidity, hospitalization and premature death [1].

The ischemic necrosis of MI is followed by a complex sequence of structural changes involving the left ventricle, referred to as post-infarction remodeling [1]. These changes include progressive chamber dilatation, eccentric hypertrophy and fibrosis [2]. Although, there has been substantial investigation into the roles of hypertrophy and dilatation, more recent studies have highlighted the importance of fibrosis, remote from the site of infarction, in the pathogenesis of post-infarction cardiac dysfunction [3]. Indeed, the predilection for fibrosis in the subendocardium of the non-infract zone (NIZ) is viewed as a major contributor to both mechanical dysfunction [4] and the propensity to dysrhythmia [5] following MI.

A range of neurohumoral, as well as mechanical factors have been associated with the maladaptive remodeling that occurs in response to cardiac injury. While seemingly diverse, factors such as angiotensin II, endothelin, pro-inflammatory cytokines and mechanical stretch all activate common intracellular signaling pathways that include the activation of protein kinase C (PKC) [6]. Indeed, recent studies have shown that, following MI, a number of P KC isoenzymes are activated, including alpha, beta, epsilon and delta [7,8].

Ruboxistaurin (LY 333531) is an inhibitor of PKC that is currently in Phase II clinical trials, where its investigation to date has been confined to the complications of diabetes [9].

* Corresponding author. Tel.: +61 3 9288 2580; fax: +61 3 9288 2581.
E-mail address: gilbert@medstv.unimelb.edu.au (R.E. Gilbert).

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Ruboxistaurin, inhibits PKC activity with an IC50 for the beta isoforms of 4.7 nM for beta I and 5.9 nM for beta II, compared with 200 nM for PKC-alpha and more than 1 μM for other PKC isoforms and non-PKC kinases [10]. However, despite its relative specificity for PKC-beta, there is approximately 50-fold more PKC-alpha in the left ventricle of the rat than PKC-beta [7], such that in the cardiac setting, ruboxistaurin may be viewed as inhibiting both PKC beta and alpha isoenzymes.

As in post-MI remodeling and heart failure, the pathological accumulation of excess matrix is also a feature of diabetic nephropathy where ruboxistaurin has been shown to reduce the expression of the profibrotic growth factor, transforming growth factor-beta (TGF-beta) with a concomitant decrease in kidney fibrosis [11]. Accordingly, the aims of the present study were twofold. We firstly sought to firstly determine the effects of PKC-inhibition on the cardiac dysfunction that develops post-MI and secondly to examine the pathological fibrosis and TGF-beta overexpression that occurs in this setting.

2. Materials and methods

2.1. Animal model

MI was induced in 15 male Sprague–Dawley rats, aged 10 weeks, by ligation of the left anterior descending (LAD) coronary artery, as previously described [12]. Animals were anesthetized with xylazine 1 mg/100 g, ketamine 7.5 mg/100 g and atropine 0.006 mg/100 g intraperitoneally (IP) and were given subcutaneous carprofen 5 mg/kg for analgesia. Sham animals underwent thoracotomy and incision of pericardial sac, but not LAD ligation. One week post-operatively, animals underwent echocardiography and infarcted animals were then randomized to treatment with ruboxistaurin (20 mg/kg body weight, kind gift of C Vlahos, Eli Lilly and Co., IN) or untreated chow. Four weeks later, repeat echocardiography was performed and the animals sacrificed. Lungs, LV, right ventricle (RV) and atria were separated, blotted dry once and weighed. The LV was then sectioned immediately and tissue was either frozen fresh, stored frozen in OCT or fixed in neutral buffered formalin for subsequent analyses. To exclude any effect of ruboxistaurin on sham-operated animals, a group of eight animals that did not undergo LAD ligation were also administered ruboxistaurin for at least 4 weeks. All experimental procedures adhered to the guidelines of the National Health and Medical Research Council of Australia's Code for the Care and Use of Animals for Scientific Purposes and were approved by the Animal Ethics Committee of the St Vincent's Hospital Melbourne, Australia.

2.2. Echocardiography

Transthoracic echocardiography was performed under light anesthesia (xylazine 0.5 mg/100 g, ketamine 3.75 mg/100 g), without atropine at 1 week post MI and 4 weeks later, prior to sacrifice. Sonographic images were obtained using the Vivid Seven system with a 10 MHz phased array probe (GE, Vingmed Ultrasound, Horton, Norway). Short axis views were obtained at the mid-level of the papillary muscle and linear dimensions were analyzed offline by a single investigator masked to the treatment group. Fractional shortening (FS%) was calculated according to the formula: FS% = (EDD – ESD)/EDD × 100, where EDD and ESD are end-diastolic diameter and end-systolic diameter, respectively, as previously described [13]. Three consecutive cardiac cycles were averaged for all analyses.

2.3. Infarct size and mortality

The Masson's trichrome-stained slides were examined under light microscopy and digitized, then analyzed using image analysis (AIS, Analytical imaging Station Version 6.0, Ontario, Canada). Infarct size was assessed morphologically and calculated as the ratio of scar average circumferences of the endocardium and the epicardium to LV average circumferences of the endocardium and the epicardium, as previously described [14].

2.4. Extracellular matrix deposition

To examine extracellular matrix deposition sections were stained with Masson's modified trichrome, as previously described [15]. All tissues were assessed with the examiner masked to the experimental groups. The accumulation of matrix within the non-infarct zone (NIZ) was then quantified using a modification of the technique described by Lal et al. [16]. Briefly, stained sections from the mid left ventricle were digitally captured in their entirety with a standard polarizing filter, and then loaded onto a Pentium III IBM computer. To isolate the NIZ from the infarct and the peri-infarct zone, the infarct and a 2 mm zone on either side of it were excluded from analysis. The remaining myocardium composed the NIZ, and was analyzed using computer-assisted image analysis, as previously described [17,18] using image analysis software (AIS, Analytical imaging Station Version 6.0, Ontario, Canada). The whole NIZ was used for quantification of ECM in order to prevent possible bias from using selected fields. An area of blue on a trichrome-stained section, representing extracellular matrix, was selected for its color range. For sham animals, the ECM content of the entire LV was quantitated by the same method, as described above.

2.5. In situ hybridization

Quantitative in situ hybridization autoradiography which permits the assessment of gene expression equivalent to Northern blot analysis [19] was used to determine the magnitude of gene expression in the subendocardial region of the NIZ. A ^{33}P-labeled anti-sense riboprobe for rat TGF-beta 1 (cDNA gift of Dr. Qian, NIH, Bethesda) was generated from