*Original Article*

# Early and Delayed Tranilast Treatment Reduces Pathological Fibrosis Following Myocardial Infarction

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*Background:* **Tranilast has been shown to inhibit TGFβ1-related fibrosis and organ failure in various disease models. We sought to examine the effects of tranilast on left ventricular (LV) remodelling post-MI.**

*Methods:* **Following coronary artery ligation, Sprague Dawley rats were randomised to receive tranilast (300 mg/kg/d, p.o.) or vehicle control over one of two treatment periods: (1) from 24 h until seven days post-MI, (2) from seven days to 28 days post-MI. Cardiac tissue was harvested for molecular, immunohistochemical and cell culture analyses.**

*Results:* **Tranilast treatment of MI rats from 24 h until seven days post-MI reduced myocardial collagen content, α1 (I) procollagen, TGFβ1 and CTGF mRNA transcripts, monocyte/macrophage infiltration and exacerbated infarct expansion compared with vehicle-treatment. Delaying the commencement of tranilast treatment to seven days post-MI attenuated myocardial fibrosis, gene expression of α1(I) procollagen, α1(III) procollagen, fibronectin, TGFβ1 and CTGF mRNA transcripts, and monocyte/macrophage infiltration at 28d compared to vehicle-treatment, without detriment to infarct healing. Extended post-MI also preserved LV infarct size. In cultures of rat cardiac fibroblasts, tranilast attenuated TGFβ1-stimulated fibrogenesis.**

*Conclusion:* **Tranilast inhibits myocardial TGFβ1 expression, fibrosis in rat post-MI and collagen production in cardiac fibroblasts. While tranilast intervention from 24 h post-MI exacerbated infarct expansion, delaying the commencement of treatment to seven days post-MI impeded LV remodelling.**

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## Introduction

Cardiac fibrosis is an important determinant of left ventricular (LV) dysfunction and remodelling following myocardial infarction (MI). Whist reparative pathological fibrosis at the site of infarct preserves the structural integrity of the heart; progressive fibrosis of the non-infarcted myocardium may contribute to contractile dysfunction by enhancing myocardial stiffness and electrical isolation of surviving myocytes. Inhibition of myocardial fibrosis post-MI is associated with improved structural and functional outcomes in animal models of heart failure and may represent a significant mechanism by which current heart failure therapies, such as ACE inhibitors and beta-blockers, exert their beneficial effects [1]. Thus, pharmacological strategies which attenuate myocardial fibrosis are likely to constitute an important element in post-MI left ventricular dysfunction treatment and management.

Previous studies provide strong evidence that transforming growth factor β1 (TGFβ1) plays a causative role in myocardial fibrosis in a variety of conditions, including heart failure of hypertensive and ischaemic origin [2]. As well, TGFβ binds to two different serine/threonine kinase receptors (TβR), termed type I and type II. The activated TβR type I kinase phosphorylates Smad2 and Smad3 (p-smads2/3). P-Smad2 is a primary step and intracellular signalling effector for the mediation of intracellular signalling of TGFβ [3]. Following MI chronic activation of this

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pathway, as evidenced by persistently elevated myocardial TGFβ1 protein levels, receptor binding and downstream signalling, is temporally and spatially coincident with regions of fibrosis during the pathogenesis of heart failure. TGFβ1 triggers a cascade of profibrotic events in the heart, which include amplification of its own signal *via* autocrine and paracrine pathways in resident cardiac fibroblasts, proliferation and activation of the myofibroblast phenotype, enhancement of collagen synthesis and inhibition of collagen degradation [2]. In addition, TGFβ1 may act as a common profibrotic pathway for other neuroactive peptides, such as angiotensin II [4]. Together the data suggest that TGFβ1 is a key profibrogenic peptide in the heart and represents a promising target for therapeutic intervention in the post-MI context.

The pharmacological agent, *N*(3,4-dimethoxyciannamoyl) anthranilic acid (tranilast), has been shown to exhibit a number of inhibitory actions against TGFβ1, including down regulation of TGFβ1 synthesis, gene expression and activation for a variety of cell types [5,6]. These actions are recognised as important mechanisms underpinning its clinical use in the treatment of allergic indications such as bronchial asthma [7]. However given the diverse profile of action of TGFβ1, broader applications of tranilast have been investigated. Of particular interest are the studies which report the beneficial effects of tranilast in disease states characterised by TGFβ1-driven extracellular matrix (ECM) expansion, inflammation, cellular proliferation and related organ failure; features that characterise heart failure disease progression post-MI. Tranilast treatment in animal models of hypertensive heart failure inhibits myocardial fibrosis and inflammatory cell infiltration, reduces myocyte size, improves LV function, attenuates LV remodelling and improves survival [8–11]. Moreover tranilast inhibits TGFβ1-induced extracellular matrix production in skin [12], vascular smooth muscle cells [13]. As well as, an antifibrotic effects on renal and cardiac interstitial fibroblasts has been demonstrated [14,15].

The current literature suggests that tranilast treatment may inhibit the accumulation of ECM proteins post-MI, which may translate into beneficial effects on LV remodelling and dysfunction. In this study we examined the effect of tranilast following coronary artery ligation in rats on reparative fibrosis, which occurs in the acute phase post-MI, and on reactive fibrogenesis, which occurs in regions remote from the infarct from one week post-MI [16]. In addition, we performed cell culture experiments to investigate the *direct* effects of tranilast on fibrogenesis in cardiac fibroblasts, by measuring collagen synthesis, expression of fibrogenic genes and SMAD signalling in the absence of any haemodynamic influences that may occur in the *in vivo* setting.

## Methods

### *Animal Studies*

Animal studies were conducted with the approval of the Animal Ethics Committee of the Alfred Monash Research and Education Precinct and comply with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

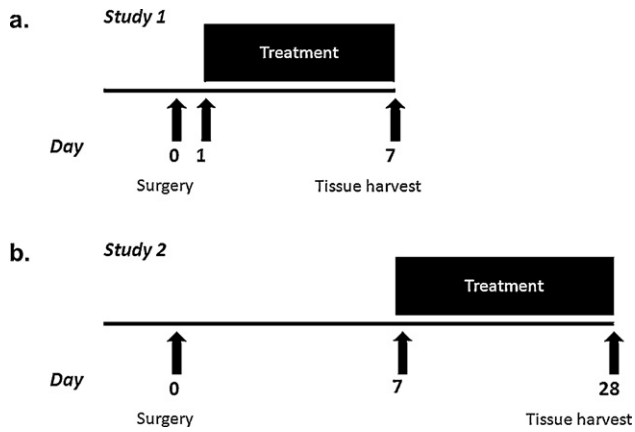


**Figure 1.** *The treatment protocols used in this study to examine: (1) reparative fibrosis, and (2) reactive fibrosis.*

### *Coronary Artery Ligation*

Male Sprague-Dawley rats aged 8–10 weeks were anaesthetised with isoflurane (5%/95% oxygen) and underwent coronary artery ligation to induce MI as described previously [17].

### *Drug Administration*

Two treatment periods were examined: [1] intervention from 24 h to day 7 post-MI, [2] intervention from day 7 to day 28 post-MI (Fig. 1). During each treatment period, one group received tranilast by daily gavage ($n = 8$; 300 mg/kg [18,19]) while the second group received an equivalent volume of methylcellulose as vehicle control (Sigma; $n = 8$). Sham operated control animals also received vehicle ($n = 8$).

### *Tissue Harvesting*

At the end of the seven and 28 days treatment periods, hearts were excised, rinsed in cold 0.9% saline, and sectioned transversely. For hydroxyproline assays and Northen blot analysis, the necrotic anterior wall was dissected away from the viable LV and each region was snap-frozen and stored at −80 °C for separate analyses. Corresponding regions of the LV were obtained from sham-operated hearts. For immunohistochemistry transverse sections of the heart were frozen in OCT embedding compound (Sakura Finetek, Torrance, CA). Formalin-fixed tissues were paraffin-embedded for Masson's Trichrome staining.

### *Hydroxyproline Assay*

Hydroxyproline assays were performed as previously described [8]. Briefly, snap frozen tissues were lyophilized to a constant weight then hydrolyzed in 6 M HCl at 110 °C over 16 h. The supernatant was evaporated and Erlich's reagent was added to complex with hydroxyproline present in the sample. The optical density of each