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Baicalein attenuates monocrotaline-induced pulmonary arterial hypertension by inhibiting endothelial-to-mesenchymal transition

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ABSTRACT

Aims: Endothelial-to-mesenchymal transition (EndoMT) was shown to lead to endothelial cell (EC) dysfunction in pulmonary arterial hypertension (PAH). Baicalein was reported to inhibit epithelial-to-mesenchymal transition (EMT), a biological process that has many regulatory pathways in common with EndoMT. Whether it can attenuate PAH by inhibiting EndoMT remains obscure.

Main methods: PAH was induced by a single subcutaneous injection of MCT (60 mg/kg) in male Sprague Dawley rats. Two weeks after MCT administration, the rats in the treatment groups received baicalein orally (50 or 100 mg/kg/day) for an additional 2 weeks. Hemodynamic changes and right ventricular hypertrophy (RVH) were evaluated on day 28. Cardiopulmonary interstitial fibrosis was detected using Masson's trichrome, Picrosirius-red, and immunohistochemical staining. The reactivity of pulmonary arteries (PAs) was examined *ex vivo*. The protein expresson of EndoMT molecules, bone morphogenetic protein receptor 2 (BMPR2), and nuclear factor-κB (NF-κB) was examined to explore the mechanism of protective action of baicalein.

Key findings: Baicalein (50 and 100 mg/kg) significantly alleviated MCT-induced PAH and cardiopulmonary interstitial fibrosis. Furthermore, baicalein treatment enhanced PA responsiveness to acetylcholine (ACh) in PAH rats. The upregulation of EndoMT molecules (N-cadherin, vimentin, Snail, and Slug) strongly suggest that EndoMT participates in MCT-induced PAH, which was reversed by baicalein (50 and 100 mg/kg) treatment. Moreover, baicalein partially reversed MCT-induced reductions in BMPR2 and NF-κB activation in the PAs.

Significance: Baicalein attenuated MCT-induced PAH in rats by inhibiting EndoMT partially via the NF-κB-BMPR2 pathway. Thus, baicalein might be considered as a promising treatment option for PAH.

1. Introduction

Pulmonary arterial hypertension (PAH) is a lethal disorder characterized by extensive obstruction of small and midsized pulmonary arteries (PAs) and a sustained increase in pulmonary arterial pressure (PAP), which ultimately results in right ventricular hypertrophy (RVH) and failure [1, 2]. The pathogenesis of PAH is multifactorial, and a significant number of reports have highlighted the contribution of endothelial cell (EC) dysfunction in the development of PAH [3–5]. Injury to the endothelium may result in an imbalance of endothelial vasoactive mediators, vasoconstriction, disordered EC proliferation, and loss of small PAs [6]. Aberrant EC proliferation leads to the formation of characteristic plexiform lesions in PAH [3, 7]. Therefore, improving endothelial function may be the most effective treatment for PAH.

Endothelial-to-mesenchymal transition (EndoMT) has been reported to cause endothelial dysfunction in human PAH and experimental models of PAH [8]. During EndoMT, ECs acquire markers of mesenchymal cells and express α-smooth muscle actin (α-SMA), while concurrently losing their endothelial marker proteins [9]. EndoMT-derived cells gain a migratory and invasive capacity and promote the formation and progression of occlusive intimal lesions in PAH patients and animal models of the disease [8, 10, 11]. EndoMT plays a vital role not only in the pathobiology of PAH, but also in the pathogenesis of fibrotic lung disease [8, 11–13]. Indeed, fibrosis is one of the pathological features of PAH [14]. Thus, inhibiting EndoMT and improving endothelial function might be a novel and efficacious therapeutic strategy for PAH.

Baicalein, a natural flavonoid extracted from *Scutellaria baicalensis*,

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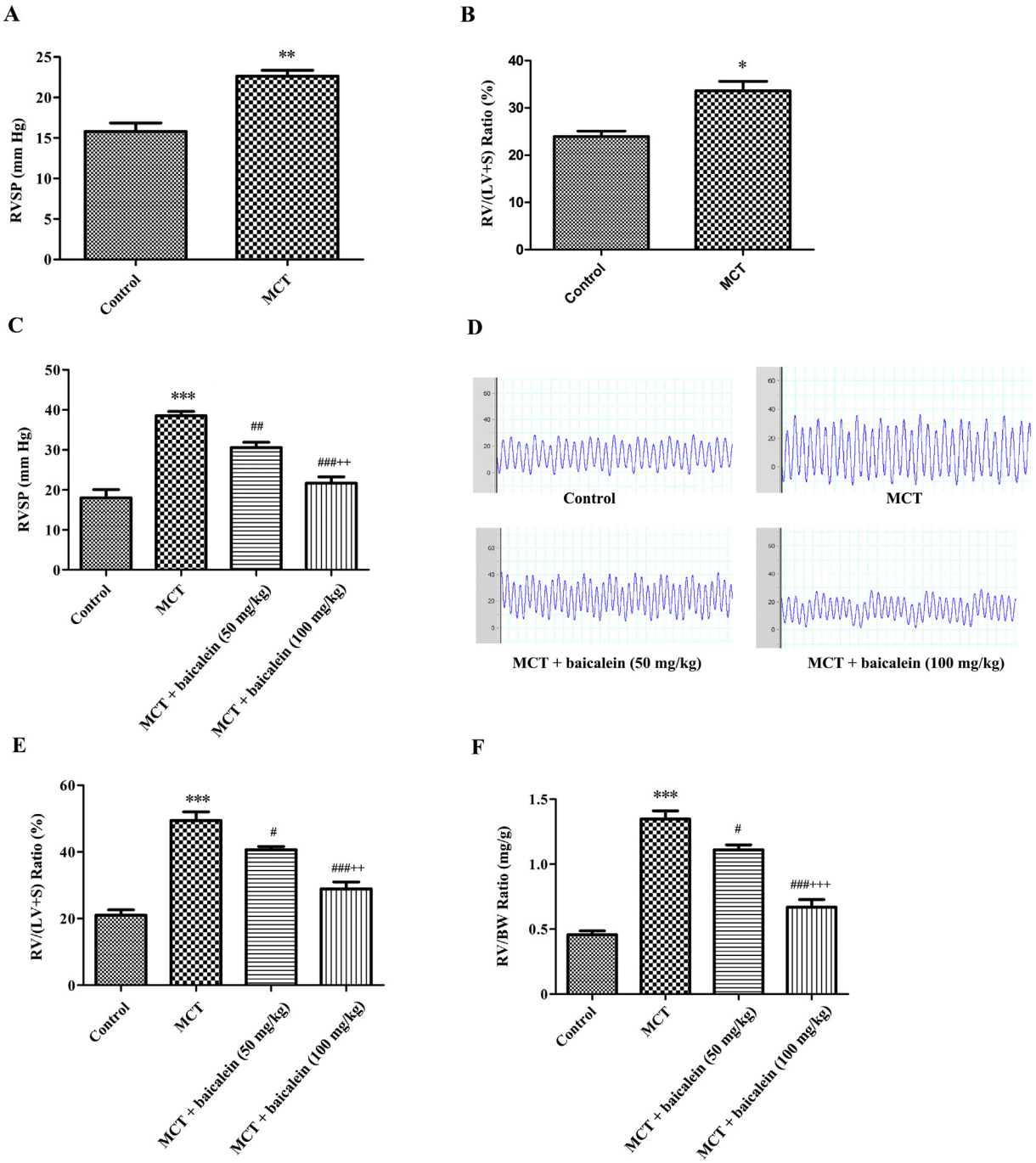

Fig. 1. Baicalein alleviated increased RVSP and RVH induced by MCT in rats. RVSP (A) and the ratio of RV/(LV + S) (B) were determined 2 weeks after MCT or normal saline injection. C Baicalein treatment (50 and 100 mg/kg) for 2 weeks reduced the MCT-induced increase of RVSP. D Representative pictures of RVSP waves in each group. The effects of baicalein (50 and 100 mg/kg) on RVH were assessed by the ratio of RV/(LV + S) (E) and RV/BW (F). n = 6 per group. Data are expressed as means ± S.E.M. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ versus control group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$ versus MCT-exposed group, $^{++}P < 0.01$ and $^{+++}P < 0.001$ versus 50 mg/kg/day baicalein-treated group.

possesses many pharmacological activities, such as antibacterial, antiviral, anti-inflammatory, anticancer, hypolipidemic, antiatherogenic, antithrombotic, and immunoregulatory effects [15–19]. Our previous study revealed that baicalein remarkably attenuated rat PAH induced by monocrotaline (MCT) [20]. However, its exact mechanism remains elusive. Baicalein has been demonstrated to exert a direct vasculoprotective effect by ameliorating vascular injury in diabetic animal models [21]. Baicalein effectively prevented endothelial dysfunction in spontaneously hypertensive rats [22]. A recent study demonstrated that baicalein attenuated vinorelbine-induced vascular EC injury in a rabbit model of phlebitis [23]. Furthermore, several reports have shown that baicalein could inhibit epithelial-to-mesenchymal transition (EMT), a biological process that has many regulatory pathways in common with EndoMT [24, 25]. Based on these findings, we hypothesized that baicalein would inhibit EndoMT and improve endothelial function to exert beneficial effects in MCT-induced rat PAH.