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Nicorandil enhances the efficacy of mesenchymal stem cell therapy in isoproterenol-induced heart failure in rats

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ABSTRACT

Stem cell transplantation has emerged as a promising technique for regenerative medicine in cardiovascular therapeutics. However, the results have been less than optimal. The aim of the present study was to investigate whether nicorandil could offer an additional benefit over bone marrow-derived mesenchymal stem cell therapy in isoproterenol-induced myocardial damage and its progression to heart failure in rats. Isoproterenol was injected subcutaneously for 2 consecutive days at doses of 85 and 170 mg/kg/day, respectively. Nicorandil (3 mg/kg/day) was then given orally with or without a single intravenous bone marrow-derived mesenchymal stem cell administration. Electrocardiography and echocardiography were recorded 2 weeks after the beginning of treatment. Rats were then sacrificed and the ventricle was isolated for estimation of tumor necrosis factor-alpha, vascular endothelial growth factor and transforming growth factor-beta. Moreover, protein expressions of caspase-3, connexin-43 as well as endothelial and inducible nitric oxide synthases were evaluated. Finally, histological studies of myocardial fibrosis and blood vessel density were performed and cryosections were done for estimation cell homing. Combined nicorandil/bone marrow-derived mesenchymal stem cell therapy provided an additional improvement compared to cell therapy alone toward reducing isoproterenol-induced cardiac hypertrophy, fibrosis and inflammation. Notably, combined therapy induced significant increase in angiogenesis and cell homing and prevented isoproterenol-induced changes in contractility and apoptotic markers. In conclusion, combined nicorandil/bone marrow-derived mesenchymal stem cell therapy was superior to cell therapy alone toward preventing isoproterenol-induced heart failure in rats through creation of a supportive environment for mesenchymal stem cells.

1. Introduction

Despite improved clinical care and increased public awareness, myocardial infarction (MI) remains a leading cause of morbidity and mortality worldwide [1]. MI is an acute condition of necrosis that occurs as a result of imbalance between coronary blood supply and myocardial demand [2]. Isoproterenol (ISO)-induced MI is a well-standardized model to study the protective effects of many drugs on cardiac function because it mimics the clinical conditions of MI [3]. Administration of high doses of ISO primarily results in multiple disseminated myocardial necrosis and patchy areas of fibrosis rather than the fixed, zonal injury of MI in humans. However, both could result in progressive left ventricular (LV) enlargement and the development of heart failure [4]. The lesions produced by ISO are severe and consist of infarct areas of necrosis with myocyte loss, fibrosis, hyalinization of muscle fibers and inflammation [5].

Stem cell transplantation has emerged as a promising technique for regenerative medicine in cardiovascular therapeutics [6]. During the last decade, widespread experimental and clinical studies have shown the safety, feasibility and efficacy of stem cell based therapies for myocardial regeneration. However, the results have been less than optimal [7]. Several factors play a role in early cell death after engraftment in the ischemic myocardium including absence of survival factors in the transplanted heart, disruption of cell–cell interaction, insufficient vascular supply and elaboration of inflammatory cytokines resulting from ischemia and/or cell death [8].

Mesenchymal stem cells (MSCs) have received special attention for cardiomyoplasty due to their ability to differentiate into cardiomyocytes in several experimental studies both in vitro and in

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vivo [9,10]. The cardiac repair mediated by MSCs could rely on paracrine effects of cytokines and growth factors released by grafted cells leading to increased angiogenesis, decreased apoptosis and possibly induction of endogenous cardiomyocyte regeneration [11].

Various preclinical studies have investigated the effects of concomitant use of MSCs and pharmacological therapy on cardiac repair [12]. There are two trends for combining pharmacological drugs with stem cells either in vivo [10,13] or in vitro [14–16]. Pharmacological therapy may target enhancement of the survival of transplanted cells and activation of various paracrine signaling cascades for mobilization, recruitment, proliferation and differentiation of transplanted cells. This strategy was confirmed in a previous experimental study where in vivo treatment with simvastatin improved the therapeutic efficacy of MSCs transplantation in acutely infarcted hearts by promoting cell survival and cardiovascular differentiation [10].

Nicorandil is a unique drug with a dual mode of action that acts not only as mitochondrial K_{ATP} channel opener, but also as a nitric oxide (NO) donor [17]. Nicorandil has been effectively used in patients with acute heart failure as it reduces preload and afterload while increasing coronary perfusion [18]. NO also induces several differentiation processes including cardiomyogenesis [19]. A previous study demonstrated that NO treatment could significantly improve survival of transplanted cells in addition to its ability to repair liver fibrosis in mice [20]. Moreover, it was reported that preconditioning of MSCs with diazoxide, a mitochondrial K_{ATP} channel opener, improved the survival rate of transplanted MSCs, reduced infarct size and enhanced LV function in rats [14].

Therefore, the goal of the present study was to investigate whether nicorandil treatment could add an additional benefit over bone marrow-derived mesenchymal stem cell (BM-MSC) therapy in ISO-induced myocardial damage and its progression to heart failure in rats.

2. Methods

2.1. Animals

Male Wistar rats weighing 170–190 g were obtained from the animal facility of Faculty of Pharmacy, Cairo University. Rats were housed under controlled temperature ($25 \pm 2\,^{\circ}C$) and constant light cycle (12 h light/dark) and allowed free access to standard rodent chow diet and water. The investigation complies with the *Guide for Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 2011) and was approved by the Ethics Committee for Animal Experimentation at Faculty of Pharmacy, Cairo University (Permit Number: PT 733).

2.2. Chemicals

Isoproterenol hydrochloride and nicorandil were obtained from Sigma–Aldrich Company, USA and Adwia Pharmaceutical Company, Egypt, respectively.

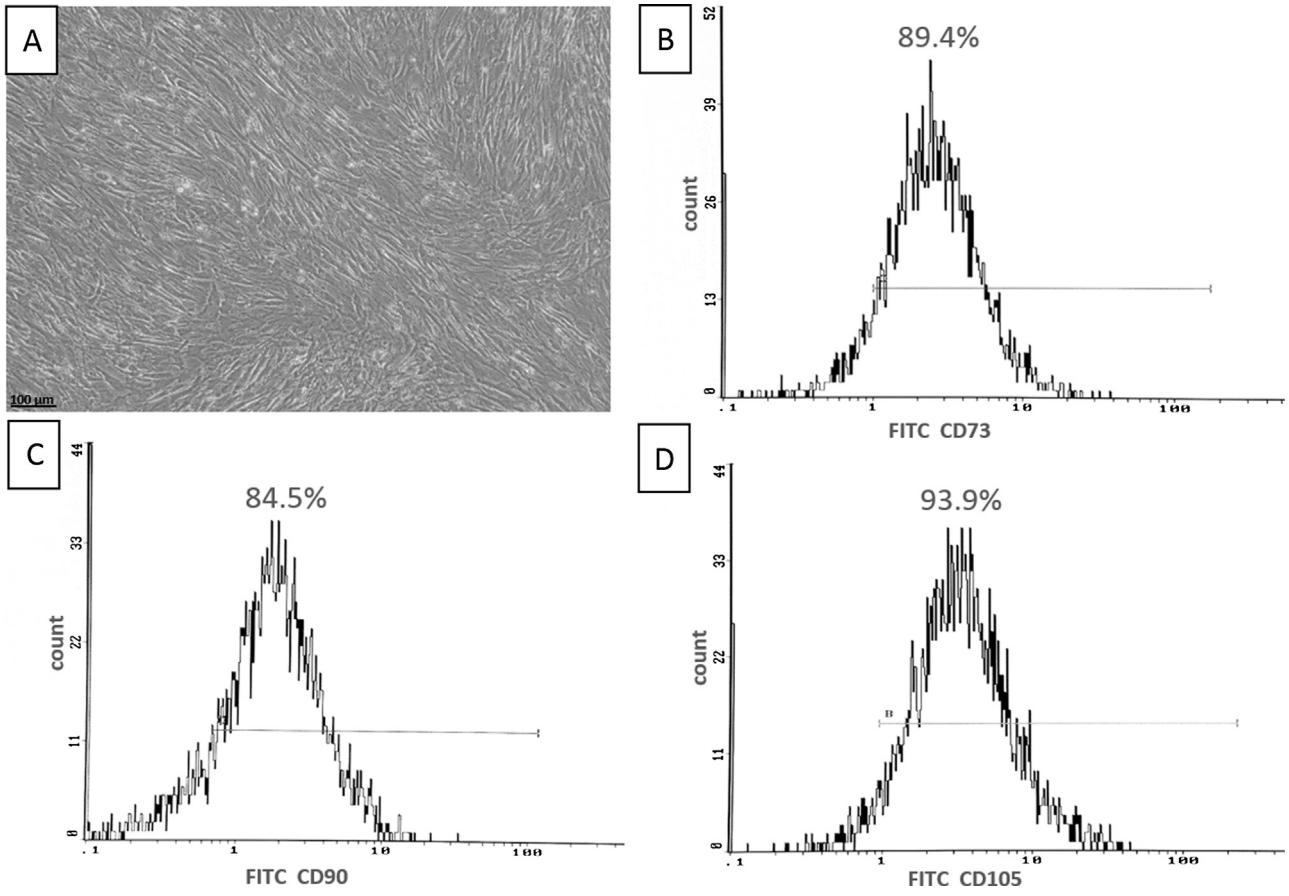

Fig. 1. Mesenchymal stem cell (A) spindle-shaped MSCs population in culture. (B–D) Flow cytometric immunophenotyping for identification and characterization of BM-MSC ($CD73^+$, $CD90^+$ and $CD105^+$).