Regenerative Therapy 9 (2018) 79–88

Contents lists available at ScienceDirect

Regenerative Therapy

journal homepage: http://www.elsevier.com/locate/reth

Original Article

Pretreatment with an angiotensin II receptor blocker abolished ameliorating actions of adipose-derived stem cell sheets on cardiac dysfunction and remodeling after myocardial infarction

Kenshiro Yamamoto [a], Yasutaka Kurata [b, *], Yumiko Inoue [a], Maya Adachi [a], Motokazu Tsuneto [a], Junichiro Miake [c], Kazuhide Ogino [d], Haruaki Ninomiya [e], Akio Yoshida [a], Yasuaki Shirayoshi [a], Yoshiko Suyama [f], Shunjiro Yagi [f], Motonobu Nishimura [g], Kazuhiro Yamamoto [c], Ichiro Hisatome [a]

[a] Division of Regenerative Medicine and Therapeutics, Department of Genetic Medicine and Regenerative Therapeutics, Tottori University Graduate School of Medical Science, 86 Nishi-cho, Yonago 683-8503, Japan
[b] Department of Physiology II, Kanazawa Medical University Faculty of Medicine, 1-1 Daigaku, Uchinada-machi, Kahoku-gun, Ishikawa 920-0293, Japan
[c] Division of Cardiovascular Medicine, Department of Molecular Medicine and Therapeutics, Tottori University Faculty of Medicine, Yonago, Japan
[d] Department of Cardiology, Tottori Red Cross Hospital, Tottori, Japan
[e] Department of Biological Regulation, Tottori University, Yonago, Japan
[f] Department of Plastic and Reconstructive Surgery, Tottori University Faculty of Medicine, Yonago, Japan
[g] Department of Cardiovascular Surgery, Tottori University Faculty of Medicine, Yonago, Japan

ARTICLE INFO

Article history:
Received 11 June 2018
Received in revised form
10 August 2018
Accepted 22 August 2018

Keywords:
Adipose-derived stem cell sheet
Myocardial infarction
Angiotensin II
Irbesartan
VEGF

ABSTRACT

Introduction: Cell sheets using myoblasts have been developed for the treatment of heart failure after myocardial infarction (MI) bridging to heart transplantation. Stem cells are supposed to be better than myoblasts as a source of cells, since they possess a potential to proliferate and differentiate into cardiomyocytes, and also have capacity to secrete angiogenic factors. Adipose-derived stem cells (ASCs) obtained from fat tissues are expected to be a new cell source for ASC sheet therapies. Administration of angiotensin II receptor blockers (ARBs) is a standard therapy for heart failure after MI. However, it is not known whether ARBs affect the cell sheet therapy. This study aimed to examine ameliorating effects of ASC sheets on heart failure and remodeling after MI, and how pretreatment with ARBs prior to the creation of MI and ASC sheet transplantation modifies the effects of ASC sheets.

Methods: ASCs were isolated from fat tissues of wild-type rats, and ASC sheets were engineered on temperature-responsive dishes. In *in vitro* studies using cultured cells, mRNA levels of vascular endothelial growth factor (VEGF) in ASCs were determined by RT-PCR in the presence of angiotensin II and/or an ARB, irbesartan, under normoxia and hypoxia; mRNA and protein levels of angiotensin II receptor type 1a (AT1aR), type 1b (AT1bR) and type 2 (AT2R) were also determined by RT-PCR and western blotting. In *in vivo* studies using a rat MI model, effects of transplanted ASC sheets and/or irbesartan on cardiac functions and remodeling after MI were evaluated by echocardiography, histological analysis and molecular biological techniques.

Results: In the *in vitro* studies, ASCs expressed higher levels of VEGF mRNA under hypoxia. They also expressed mRNA and protein of AT1aR but not AT1bR or AT2R. Under normoxia, angiotensin II increased the level of VEGF mRNA in ASCs, which was abolished by irbesartan. Under hypoxia, irbesartan reduced the level of VEGF mRNA in ASCs regardless of whether angiotensin II was present or not. In the *in vivo* studies, ASC sheets improved cardiac functions after MI, leading to decreased interstitial fibrosis and increased capillary density in border zones. These effects of ASC sheets were abolished by oral administration of irbesartan before MI and their transplantation.

Abbreviations: ANP, atrial natriuretic peptide; ARB, angiotensin receptor blocker; ASC, adipose-derived stem cell; AT1(2)R, angiotensin II receptor type 1(2); CRT, cardiac resynchronization therapy; EF, ejection fraction; FGF, fibroblast growth factor; FS, fractional shortening; HGF, hepatocyte growth factor; LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; MI, myocardial infarction; MSC, mesenchymal stem cell; RAS, renin–angiotensin system; VEGF, vascular endothelial growth factor; vWF, von Willebrand factor.

* Corresponding author.
E-mail address: yasu@kanazawa-med.ac.jp (Y. Kurata).

Peer review under responsibility of the Japanese Society for Regenerative Medicine.

https://doi.org/10.1016/j.reth.2018.08.005

Conclusions: ASC sheets ameliorated cardiac dysfunctions and remodeling after MI via increasing VEGF expression, which was abolished by pretreatment with irbesartan before the creation of MI and transplantation.

1. Introduction

Arteriosclerotic diseases such as myocardial infarction (MI) are the major cardiovascular problems associated with the westernization of Japanese lifestyle. Treatment of choice for acute MI is percutaneous coronary angioplasty or surgical bypass operation, both of which have decreased mortality and morbidity of patients with MI. Oxidative stress following MI causes cardiac remodeling to lead to ischemic heart failure; cardiac protective medicines that decrease oxidative stress are utilized against the ischemic heart failure, but do not sufficiently increase the survival rate of patients.

The renin–angiotensin system (RAS), as well as the sympathetic nervous system, has been reported to be activated in MI. These neurohumoral factors are involved in cardiac remodeling (e.g., cardiac hypertrophy, cell death and fibrosis), which leads to terminal heart failure [1]. To prevent the RAS from exaggerated activation, angiotensin II receptor blockers (ARBs) are used as the first-line medicine. However, with developing the drug resistant heart failure, either cardiac resynchronization therapy (CRT) or auxiliary artificial heart is applied, and eventually heart transplantation is conducted to cure the patients with terminal heart failure. Since the donor for heart transplantation is lacking, it is necessary to reduce the number of patients to whom heart transplantation should be applied. Therefore, an alternative way to bridge between medicine and heart transplantation is needed.

Cell-based regenerative medicine could improve blood supply to the damaged heart, and could minimize both the area of infarction and cardiac remodeling [2–4] through secretions of several cytokines such as hepatocyte growth factor (HGF) and vascular endothelial growth factor (VEGF) [5]. Direct injection of cells into heart is problematic because of a significant loss of live cells and proarrhythmic effects [6,7]. Tissue engineering using cell sheets has been developed to overcome these disadvantages; the cell sheet could have improved viability of cells [8,9] and prolonged secretion of cytokines [10]. Initially, sheets from skeletal myoblast cells were reported to improve cardiac functions in patients with severe heart failure [11]. However, collecting skeletal myoblasts from patients requires invasive procedures, and it takes a long time to obtain a sufficient number of myoblasts. In addition, myoblasts secrete low levels of cytokines and lack capability to differentiate into cardiomyocytes. Thus, an alternative source of stem cells for cell sheets is required.

Adipose-derived stem cells (ASCs) belong to the mesenchymal stem cells (MSCs). It has been reported that they secrete several cytokines such as VEGF, HGF, and fibroblast growth factor (FGF), and prevent cardiac dysfunction and remodeling after MI [12–14] by inducing neovascularization [15–18].

A local RAS is reported to operate both in hearts and in MSCs [19]. Long-term activation of the RAS induces progressive injury to hearts, leading to hypertrophy, fibrosis and inflammation [20]. It has also been reported that activation of the RAS could play a pivotal role in the secretion of VEGF by MSCs derived from bone marrow [21]; exposure of the MSCs to angiotensin II increased expression of VEGF mRNA, which could be suppressed by ARBs [22]. In addition, MSCs treated with ARBs have been reported to improve the efficiency of cardiomyogenic transdifferentiation and improve cardiac functions via angiogenesis [23].

Since ARBs are the first-line medicine for the treatment of patients who suffer from MI, cell sheet transplantation might be combined with administration of ARBs in the future. It has been reported that ARBs such as candesartan administered after the creation of MI significantly improve the function of hearts transplanted with MSCs after MI [23]. However, it has never been tested whether pretreatment with ARBs before the creation of MI and ASC sheet transplantation affects ASC sheet-induced improvement of cardiac functions after MI. In the present study, we evaluated effects of the pretreatment with an ARB, irbesartan, on functions of rat hearts transplanted with ASC sheets after MI.

2. Materials and methods

2.1. In vitro study

2.1.1. Engineering of ASC sheets

ASCs were enzymatically isolated from the inguinal subcutaneous fat tissue of rats and cultured as previously described [15]. To prepare cell sheets, ASCs (2–3 passage) were cultured on 35-mm temperature-responsive culture dishes ($2 \times 10^5/cm^2$) (UpCell, Cell Seed Inc., Tokyo, Japan) in an incubator at 37 °C. After the temperature-responsible culture dish was preincubated with PBS for 24 h, 1×10^6 cells were transferred on a dish, cultured for 48 h, and then maintained at 20 °C for 1 h to release them as an intact sheet.

2.1.2. Real-time RT-PCR analysis

ASC sheets were incubated at 37 °C under normal (21% O_2) or hypoxic (<2% O_2) conditions for 48 h using an AnaeroPack system (MITSUBISHI GAS CHEMICAL Inc., Tokyo, Japan). Total RNA was extracted from ASC sheets using RNeasy Mini kit (QIAGEN Inc., Valencia, CA, USA). Real-time RT-PCR analysis of HGF, VEGF, basic FGF (bFGF), hypoxia inducible factor-1α (HIF-1α), angiotensin II receptor type 1a (AT1aR), 1b (AT1bR) and 2 (AT2R), and β-actin was performed using 1 μg RNA with the ABI 7900HT Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). The primers shown in Tables 1 and 2 were designed to amplify their genomic regions, as described elsewhere [15]. Their mRNA levels were expressed as ratios to those of β-actin. The mRNA levels of VEGF, HGF, HIF-1α and bFGF as ratios to that of β-action were further normalized to the control MI group values, and the ratio of VEGF mRNA under hypoxia was also given as $2^{-\Delta\Delta Ct}$ using the comparative C_T method.

Table 1
PCR primers for angiogenic factors and beta-actin.

Gene name	Probe#	Sequence
Rattus β-actin	115	Forward: 5′-CTAAGGCCAACCGTGAAAAG-3′ Reverse: 5′-GCCTGGATGGCTACGTACA-3′
Rattus VEGF	4	Forward: 5′-TTAAACGAACGTACTTGCAGATG-3′ Reverse: 5′-TCTAGTTCCCGAAACCCTGA-3′
Rattus HGF	49	Forward: 5′-GATTGGATCAGGACCTTGTGA-3′ Reverse: 5′-CCATTCTCATTTTGTGTTGTTCA-3′
Rattus bFGF	7	Forward: 5′-TCTTCCTGCGCATCCATC-3′ Reverse: 5′-GCTTGGAGCTGTAGTTTGACG-3′