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BITC induces cardiomyocyte proliferation and heart regeneration

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Abstract

Mammalian cardiomyocytes have the ability to proliferate from the embryonic stage until early neonatal stage, with most of them being arrested from the cell cycle shortly after birth. Therefore, adult mammalian heart cannot regenerate myocardial injury. Despite much attention, pharmacological approaches for the induction of cardiomyocyte proliferation and heart regeneration have yet to be successful. To induce cardiomyocyte proliferation by drug administration, we focused on benzyl isothiocyanate (BITC). Firstly, we showed that BITC induces cardiomyocyte proliferation both in vitro and in vivo through the activation of the cyclin-dependent kinase (CDK) pathway. In addition, we demonstrated that BITC treatment induces heart regeneration in the infarcted neonatal heart even after the regeneration period. Furthermore, we administered BITC to adult mice in parallel with mild hypoxia (10% O2) treatment and showed that a combination of BITC administration and mild hypoxia exposure induces cell cycle reentry in the adult heart. The present study suggests that pharmacological activation of the CDK pathway with BITC concurrently with the activation of hypoxia-related signaling pathways may enable researchers to establish a novel strategy to induce cardiac regeneration in patients with heart disease.

Main

Mammalian cardiomyocytes have the ability to proliferate from the embryonic stage until early neonatal stage, with most of them being arrested from the cell cycle shortly after birth[1]. Therefore, adult mammalian heart cannot regenerate myocardial injury, resulting in the fact that ischemic heart disease is the biggest cause of death in the world. As such, enhancement of cell cycle reentry for endogenous cardiomyocytes is of great interest for the replacement of the injured fibrotic scar tissue with healthy myocardium[2]. However, despite much attention, pharmacological approaches for the induction of cardiomyocyte proliferation and heart regeneration have yet to be successful.

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To induce cardiomyocyte proliferation by drug administration, we focused on benzyl isothiocyanate (BITC), which is known to regulate the cell cycle in a dose-dependent manner[3]. First, we subcutaneously administered 20 mg/kg/day of BITC to neonatal mice from postnatal day 0 (P0) to P6, and examined the number of proliferating cardiomyocytes at P7. The number of phosphorylated histone H3 (pH3)-positive cardiomyocytes was significantly increased at P7 in the BITC-treated mice compared with control DMSO-treated ones (Figure A). Interestingly, cardiomyocyte proliferation did not change upon treatment with 50 or 100 mg/kg/day of BITC (data not shown), suggesting that BITC promotes cell proliferation of neonatal cardiomyocytes in a dose-dependent manner. Next, in an attempt to identify the target of BITC, we examined cyclin-dependent kinases (CDKs) because a previous study revealed that CDKs are important for cardiomyocyte proliferation[4]. Phosphorylated CDK1 in myocardial nuclei was increased in the BITC-administered heart at P7, indicating that BITC induces the activation of CDK1, which may well contribute to cell cycle progression. Furthermore, after treatment with 50 μM BITC, about 70% of neonatal murine ventricular cardiomyocytes (NMVCs) were positive for pH3 compared with control (Figure C). Similarly, the same treatment with BITC on human iPS cell-derived cardiomyocytes (hiPSC) resulted in a tendency toward increased cell proliferation compared with control, although this enhanced proliferation was not statistically significant, most likely due to a variability among hiPSC batches (Figure C). These results indicate that BITC directly induces cell proliferation in cardiomyocytes, rather than via an indirect systemic effect.

Based on these results, we hypothesize that BITC treatment enables heart regeneration after myocardial infarction (MI) through enhanced cardiomyocyte proliferation. To test this hypothesis, we induced MI in P7 mice by permanent ligation of the left anterior descending coronary artery (LAD), and subsequently administered 20 mg/kg/day of BITC or control DMSO (Figure D, upper panel) from P8 to P14. At P28, BITC-treated mice showed significantly recovered ejection fraction (EF) as well as significantly decreased fibrotic scar compared with DMSO-treated mice (Figure D), suggesting that BITC administration induces cardiomyocyte proliferation, thereby allowing heart regeneration after MI in neonatal mice even after the regenerative period. From a therapeutic standpoint, it is important to know whether cell cycle reentry is induced by BITC administration in the adult heart. In order to evaluate the effect of BITC treatment on adult cardiomyocytes, we administered 20 mg/kg/day of BITC intraperitoneally to 2-month-old ICR mice for 14 days. In parallel, we placed these mice in a 10% O_2 environment based on findings from a previous study showing that a hypoxic environment aids cell cycle reentry in the adult heart[5]. pH3-positive cardiomyocytes were almost absent in the DMSO- or BITC-treated heart under the normal oxygen condition, nor were they present in the DMSO-injected heart in the 10% O_2 condition in consistent with previous findings (Figure E). In contrast, pH3-positive cardiomyocytes were significantly increased after BITC administration in the hypoxic environment (Figure E), indicating

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that a combination of BITC administration and mild hypoxia exposure induces cell cycle reentry in the adult heart.

Taken together, our data reveal a beneficial effect of BITC on heart regeneration. BITC treatment enhances cardiomyocyte proliferation through the activation of CDK1 in the neonatal heart. In addition, BITC administration in combination with mild hypoxia in adult mice is sufficient to induce cardiomyocyte cell cycle reentry. The present study suggests that pharmacological activation of the CDK pathway with BITC concurrently with the activation of hypoxia-related signaling pathways may enable researchers to establish a novel strategy to induce cardiac regeneration in patients with heart disease.

Figure legends

(A) To quantify the number of pH3-positive cardiomyocytes, BITC- (SIGMA 252492, 20 mg/kg) or DMSO-administered heart sections were stained with pH3 (green, Thermo Fisher 06-570, 1:100) and cardiac troponin T (cTnT, red, Thermo Fisher MS295P, 1:100) antibodies (N=3 each).

(B) To quantify the number of pCDK1-positive cardiomyocyte nuclei, we used immunofluorescence of pCDK1 (red, abcam ab201008, 1:400) and cTnT (green) in BITC- or DMSO-administered heart at P7 (N=3 each).

(C) Primary culture of NMVCs (left) and hiPSC (right) were treated with 50 μM BITC or DMSO, and the number of pH3-positive cardiomyocytes was quantified.

(D) After MI surgery at P7, BITC or DMSO was administered intraperitoneally once a day from P8 to P14, and the ejection fraction (EF) was measured by echocardiography blindly at P28 (top and bottom left panel). Representative images of the histological sections of MI heart stained with Masson's trichrome staining (SCY TRM-1) and quantification of fibrotic area are shown (N=3 each).

(E) Adult ICR mice were exposed to 10 % O_2 or normal conditions and injected with BITC or DMSO once a day for two weeks. pH3 positive cardiomyocytes were quantified as shown in the right panel.

All protocols were approved by the Institutional Animal Care and Use Committee of RIKEN Kobe Branch. All experiments were performed on age-matched mice. Data are means ± SEM, the *P* values were determined with student's t test, and one-way ANOVA was applied for multiple comparison. All scale bars represent 20 μm.

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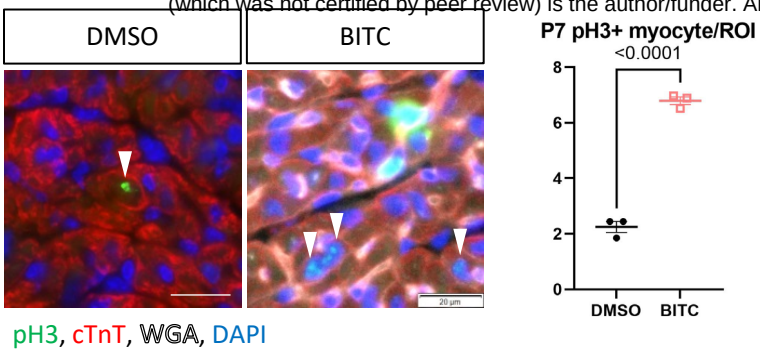
(A)



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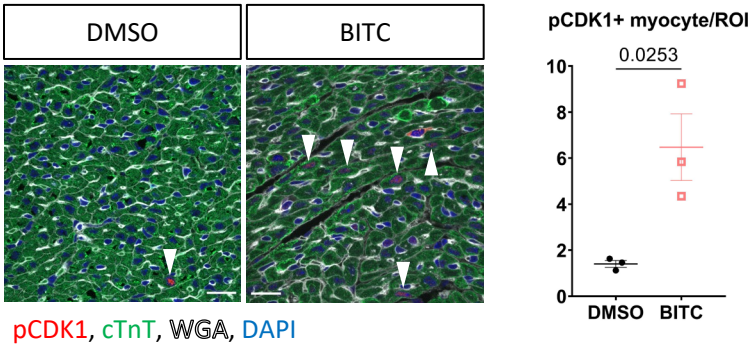

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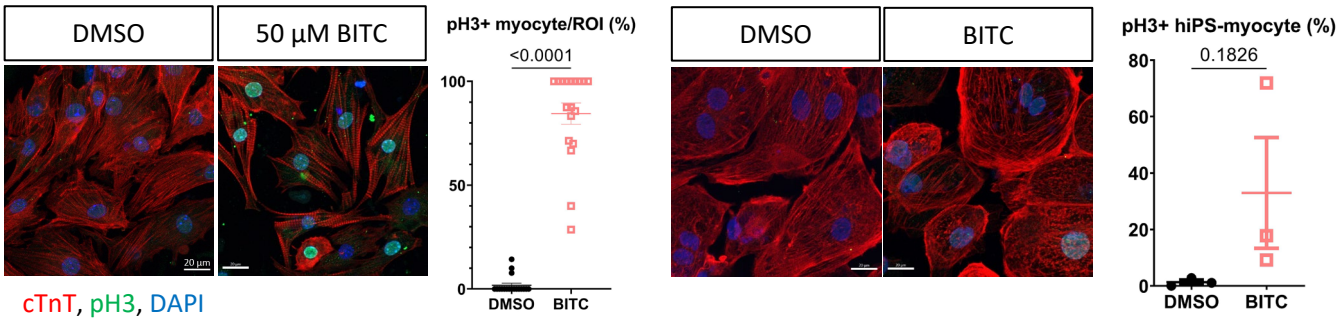

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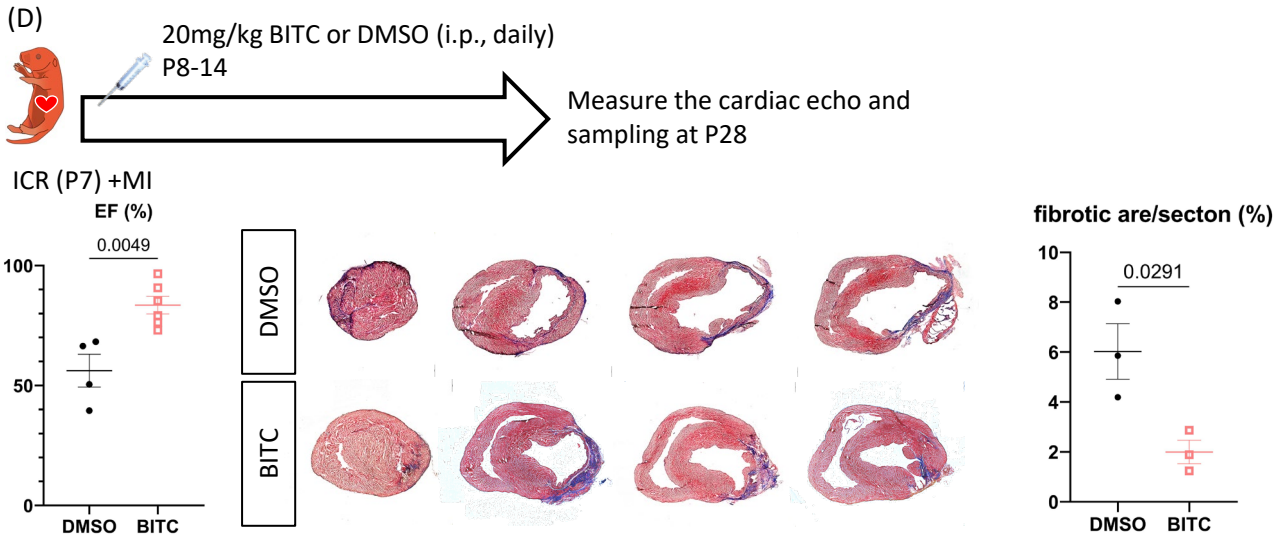
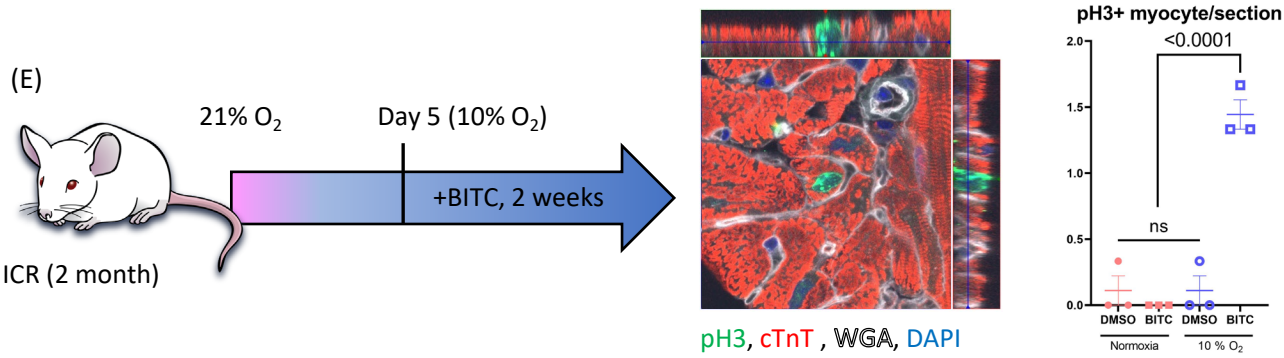
(E)