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PRECLINICAL RESEARCH

Reduction in Hemoglobin–Oxygen Affinity Results in the Improvement of Exercise Capacity in Mice With Chronic Heart Failure

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Objectives This study examined whether a reduction in hemoglobin–oxygen affinity improves exercise capacity in mice with heart failure.

Background Exercise intolerance is a major determinant of quality of life in patients with chronic heart failure. One of the major goals of the treatment for chronic heart failure is to improve quality of life.

Methods Four weeks after left coronary ligation, we transplanted bone marrow cells isolated from the transgenic mice expressing a hemoglobin variant with low oxygen affinity, Presbyterian, into the lethally irradiated mice with heart failure or administered a synthetic allosteric modifier of hemoglobin. The mice were then exercised on a treadmill.

Results Four weeks after the left coronary artery ligation, mice showed cardiac dysfunction and chamber dilation, which were characteristics of heart failure. The transplantation led to a reduction in hemoglobin–oxygen affinity and an increase in oxygen supply for skeletal muscle without changes in muscle properties. The transplanted mice showed improved running performance on a treadmill despite impaired cardiac contractility. Furthermore, administration of the synthetic allosteric modifier of hemoglobin showed a similar effect.

Conclusions Allosteric modification of hemoglobin represents a therapeutic option for improving exercise capacity in patients with chronic heart failure. One mechanism of improvement in exercise capacity is enhanced oxygen delivery in the skeletal muscle. (J Am Coll Cardiol 2008;52:779–86) © 2008 by the American College of Cardiology Foundation

The 3 major goals of treatment for chronic heart failure (CHF) are to reduce symptoms, prolong survival, and improve quality of life (1). A nearly universal manifestation of CHF is a reduction in exercise capacity, which diminishes quality of life. The pathophysiology of exercise intolerance in this syndrome is incompletely understood, but it is believed to be multifactorial.

The oxygen (O_2) affinity of hemoglobin (Hb), a tetrameric protein consisting of α- and β-globin subunits, is critical for gas exchange in the lung and O_2 delivery in the peripheral tissues. A variety of mutations in the genes of α- and β-globin have been reported, one of which, hemoglobin Presbyterian (Hb^{Pres}), carries lysine at asparagine-108 of β-globin and shows a low Hb–O_2 affinity in vitro (2). We previously reported that mutant mice, carrying the Presbyterian mutation generated with a targeted knock-in strategy, also showed a low Hb–O_2 affinity (2). The mutant mice

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Abbreviations and Acronyms

BMC = bone marrow cell
BMT = bone marrow cell transplantation
CHF = chronic heart failure
Hb = hemoglobin
Hb^{Pres} = hemoglobin Presbyterian
LCA = left coronary artery
MI = myocardial infarct/infarction
SDH = succinate dehydrogenase
WT = wild type

showed various phenotypes, including mild anemia, respiratory acidosis, enhanced tissue oxygenation, increased O_2 consumption, a higher ratio of type IIA/IIB muscle fibers, and increased spontaneous physical activity.

The 2-[4-[(3,5-dimethylanilino carbonyl)-methyl]phenoxy]-2-methylpropionic acid, known as RSR13, is a synthetic allosteric modifier of Hb that acts to increase the release of O_2 from Hb to the surrounding tissues (3,4). This agent markedly shifts the Hb–O_2 curve rightward in both in vitro and in vivo rodent models through the stabilization of deoxyhemoglobin in a manner similar to the natural allosteric effecter of Hb, 2, 3-diphosphoglycerate.

In the present study, we investigated whether a reduction in Hb–O_2 affinity could improve exercise capacity of CHF mice by transplanting bone marrow cells (BMCs) obtained from the Hb^{Pres} mice or by the administration of RSR13. We showed that a reduction in Hb–O_2 affinity leads to improved running performance on a treadmill despite impaired cardiac function.

Methods

This study was carried out under the supervision of the Animal Research Committee in accordance with the Guidelines on Animal Experiments of Osaka University and the Japanese Government Animal Protection and Management Law (no. 105).

BMC transplantation. The BMCs were obtained by flushing the bone marrow cavity of femurs of 6-week-old heterozygous Presbyterian mice with a C57Bl6/J background (2) and C57Bl6/J (wild-type [WT]) mice. The 5×10^6 cells were intravenously injected into WT mice irradiated with a single whole-body dose of 10 Gy (5).

Detection and quantification of β^{Pres}-globin and red blood cell oxygen dissociation curve. Erythrocytes lysate sample containing 50 μg Hb was subjected to reversed-phase high-performance liquid chromatography, as previously described (6). Oxygen dissociation curve measurements were performed at pH 6.9 and 7.4 at 37°C as we previously reported (7).

Surgical procedure and in vivo assessment of cardiac functions. Eight-week-old male WT mice were anesthetized with sodium pentobarbital (50 mg/kg, intraperitoneally), and silk thread (7-0 type) was passed around the left coronary artery (LCA) about 1 mm distal to the LCA origin (8). Echocardiography and hemodynamic measurements were performed on mice as we previously described (8,9).

Training and exercise protocol. Exercise was performed on a treadmill (10). Four weeks after bone marrow cell transplantation (BMT), mice were initially trained 3 times daily for 10 min for 3 days. The velocity of the treadmill was 10 m/min. Then, the exercise test was performed. The mice underwent 1 exercise session, which consisted of running on a treadmill at 10 m/min at a gradient of 0° for 30 min. The running of mice was video recorded for measuring the periods for running and resting.

Analysis of tissue O_2 and characterization of muscle fiber. Four weeks after BMT, an O_2 electrode with a thermocouple (Needle-type electrode, Laboratory and Medical Supplies, Tokyo, Japan) was inserted into the left gastrocnemius muscle to measure tissue O_2 in a double-chamber plethysmograph (2).

Muscle fiber type classification and succinate dehydrogenase (SDH) activity were examined as previously reported (2). Total SDH activity was measured as previously described (11). Capillary density from tibialis anterior muscle was measured using von Willebrand factor antibody.

Physical activity after RSR13 intravenous injection. Twelve-week-old male WT mice were initially trained 3 times per day for 10 min. The velocity of the treadmill was 15 m/min. The mice were then exercised 5 times per week for 30 min at 15 m/min for 3 weeks. The RSR13 was synthesized (3), dissolved in dimethyl sulfoxide and 2-amino-2-hydroxymethyl-1,3-propanediol/4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (pH 7.4), and injected intravenously (150 mg/kg) (12). Thirty minutes after the injection, the exercise test was performed. The exercise test consisted of running at 15 m/min for 30 min on a level surface, during the next 30 min on a 5° incline, and for the last 30 min on a 10° incline. The run was calculated every 30 min. To examine the effect of RSR13 on exercise capacity in myocardial infarct (MI) mice, male mice were used 4 weeks after LCA ligation. The mice were initially trained 3 times daily for 10 min for 3 days at a treadmill velocity of 10 m/min, followed by the exercise test. The graded exercise test was performed for 90 min at a constant speed of 12.5 m/min for first 30 min, followed by 30 min at an incline of 5°, and another 30 min at an angle of 10°.

Statistical analysis. All experiments were performed in a blinded fashion. Results are shown as mean ± SEM. Paired data were evaluated by the Student t test. A one-way analysis of variance with the Bonferroni post hoc test was used for multiple comparisons. For the analysis of exercise capacity, data were evaluated by nonparametric methods. A value of $p < 0.05$ was considered statistically significant.

Results

Analysis of peripheral blood cells in the mice transplanted with Presbyterian BMCs. The BMC obtained from femurs of heterozygous Presbyterian or WT mice were transplanted into WT mice. Reversed-phase high-performance liquid chromatography profiles of purified Hb