Enhanced exercise capacity in mice with severe heart failure treated with an allosteric effector of hemoglobin, myo-inositol trispyrophosphate

Andrea Biolo, Ruth Greferath, Deborah A. Siwik, Fuzhong Qin, Eugene Valsky, Konstantina C. Fylaktakidou, Srinivasa Pothukuru, Carolina D. Duarte, Richard P. Schwarz, Jean-Marie Lehn, Claude Nicolau, and Wilson S. Colucci

A major determinant of maximal exercise capacity is the delivery of oxygen to exercising muscles. myo-Inositol trispyrophosphate (ITPP) is a recently identified membrane-permeant molecule that causes allosteric regulation of Hb oxygen binding affinity. In normal mice, i.p. administration of ITPP (0.5–3 g/kg) caused a dose-related increase in the oxygen tension at which Hb is 50% saturated (p50), with a maximal increase of 31%. In parallel experiments, ITPP caused a dose-related increase in maximal exercise capacity, with a maximal increase of 57 ± 13% (P = 0.002). In transgenic mice with severe heart failure caused by cardiac-specific overexpression of Gαq, i.p. ITPP increased exercise capacity, with a maximal increase of 63 ± 7% (P = 0.005). Oral administration of ITPP in drinking water increased Hb p50 and maximal exercise capacity (+34 ± 10%; P < 0.002) in normal and failing mice. Consistent with increased tissue oxygen availability, ITPP decreased hypoxia inducible factor-1α mRNA expression in myocardiurn. It had no effect on myocardial contractility in isolated mouse cardiac myocytes and did not affect arterial blood pressure in vivo in mice. Thus, ITPP decreases the oxygen binding affinity of Hb, increases tissue oxygen delivery, and increases maximal exercise capacity in normal mice and mice with severe heart failure. ITPP is thus an attractive candidate for the therapy of patients with reduced exercise capacity caused by heart failure.
half-life of RBC in mice, suggests that the effect of ITTP on Hb is relatively long-lived.

**ITTP Suppresses Hypoxia-Inducible Factor (HIF) 1α in Myocardium in Vivo.** To further test the ability of ITTP to increase oxygen delivery to tissues in vivo, we measured the expression of HIF 1α mRNA in myocardium from normal mice. In normal mice, ITTP administration (2 g/kg, i.p.) decreased the level of myocardial HIF mRNA, measured 3 days after administration, from 5.5 ± 1.4 to 1.7 ± 0.6 arbitrary units (P = 0.06; n = 3 per group). These data are consistent with the thesis that the ITTP-induced decrease in Hb oxygen affinity, as reflected by the increase in p50, results in increased availability of oxygen at the tissue level.

**ITTP Increases Exercise Capacity in Normal Mice.** Because ITTP should increase the availability of oxygen at the tissue level, we hypothesized that ITTP would increase exercise capacity in vivo in mice. We therefore measured the maximal exercise capacity of normal mice by using a progressive workload motorized treadmill with air puff motivation to ensure maximal effort. Maximal exercise capacity was determined as the maximal distance run (meters) until exhaustion on a motorized treadmill by using air-puff stimulation. Bars represent distance run at baseline (B) and 16–24 h after i.p. administration of ITTP (T) in doses ranging from 0.5 to 3 g/kg or placebo. *, P < 0.05 vs. baseline; n = 22 in baseline group, 4–7 in each treatment group.

It has recently been reported that, in mice, bone marrow transplantation with a Hb variant having a low oxygen affinity or

animals with heart failure, we used transgenic mice with dilated cardiomyopathy caused by cardiac-specific overexpression of Gαq, as described (17). These mice have severe left ventricular (LV) dilatation (LV end-diastolic dimension = 4.3 ± 0.4 mm vs. 3.0 ± 0.1 mm in normal mice; P < 0.001; n = 10) and a markedly reduced LV fractional shortening (29 ± 4% vs. 62 ± 2% in normal mice; P < 0.001; n = 10). Baseline maximal exercise capacity is consequently severely depressed to ~60% of that in normal mice (Fig. 3A). ITTP administration caused striking dose-related increases in maximal exercise capacity of 34% and 71% for the 1 and 2 g/kg doses, respectively, whereas placebo had no effect (Fig. 3A). The magnitude of the ITTP-induced increase in exercise capacity was related to the increase in Hb p50 (Fig. 3B).

**ITTP Improves Exercise Function in Mice with Severe Heart Failure.** To test the hypothesis that ITTP would increase exercise capacity in
with a parallel, dose-related increase in maximal exercise capacity in both normal mice and mice with reduced exercise capacity caused by severe myocardial failure. These observations demonstrate that allosteric modulation of Hb oxygen binding affinity can exert clinically meaningful effects on maximal exercise capacity. Accordingly, ITPP is an attractive therapeutic candidate to alleviate symptoms in patients with reduced exercise capacity caused by low cardiac output heart failure. ITPP may also enhance physical performance of otherwise healthy individuals, in particular, under extreme conditions such as high altitude or intense physical exercise.

**Methods**

**Synthesis of ITPP.** This membrane-permeant allosteric effector of Hb was synthesized as reported (15), by using an improved procedure. Briefly, IP₆ dodecasodium salt (Sigma) was converted via its perprotonated form, obtained by passage over Dowex 50 resin (Sigma–Aldrich) in its H⁺ form, into its triethylammonium salt and then, upon heating with dicyclohexylcarbodiimide (Sigma–Aldrich) in acetonitrile/water 2/1 solution, triply cyclized to give the trimethylammonium salt of ITPP. Thereafter, an aqueous solution of the latter was passed over Dowex Marathon C Na⁺ resin (Sigma–Aldrich) until cation exchange of trimethylammonium against sodium was complete. Evaporation of the aqueous solution gave the sodium salt of ITPP in high purity (~90% yield).

**p50 Measurements.** The p50 value (P₂₅, at which 50% of Hb is saturated with O₂), a measure of the affinity of Hb for oxygen, was determined by using a HEMOX Analyzer (TCS Scientific), by constructing Hb dissociation curves based on dual wavelength spectrophotometry as described (16).

**RT-PCR for HIF-1α mRNA.** Total RNA was extracted from mouse hearts by using a Total RNA Purification System (Invitrogen). Quantitative RT-PCR was performed by using cybergreen and the i-cycler iQ (RT-PCR) (Bio-Rad). The primers for HIF-1α were: 5′-TCAAGTCAGCAACTGGAAGACG-3′ and 5′-TATCGAAAGCTG- TGTGACGCTG-3′.

**Exercise Testing.** Maximum exercise capacity was measured by using a rodent treadmill equipped with an air puff motivator (Columbus instruments) as described (23). Animals were trained on the treadmill for 1 week at baseline levels. Age-matched controls were bred by using heterozygote Gqq male mice with WT females obtained from Charles River. Animals were studied at 12–14 weeks of age. The institutional Animal Care and Use Committee at Boston University School of Medicine approved all study procedures and use of animals.

**Statistical Analysis.** Data are shown as mean ± SEM. Group comparisons were performed by using Student’s t test (2 groups) or 1-way ANOVA (multiple groups). Baseline versus treatment comparisons were performed by paired t test. All tests were 2-tailed, and P < 0.05 was considered significant.

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