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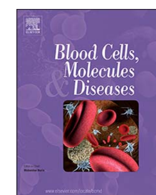

Moderate exercise training decreases inflammation in transgenic sickle cell mice

Emmanuelle Charrin[a,b,c], John J. Dubé[d,g], Philippe Connes[a,b,e], Vincent Pialoux[a,b,e], Samit Ghosh[f], Camille Faes[a,b], Solomon F. Ofori-Acquah[c,f,1], Cyril Martin[a,b,*,1]

[a] Interuniversity Laboratory of Human Movement Biology EA7424, Vascular biology and Red Blood Cell team, University Claude Bernard Lyon 1, Villeurbanne, France
[b] Laboratory of Excellence "GR-Ex", Paris, France
[c] Division of Hematology/Oncology, Department of Medicine, University of Pittsburgh, Pittsburgh, PA 15261, USA
[d] Division of Endocrinology and Metabolism, University of Pittsburgh, PA 15261, USA
[e] Institut Universitaire de France, Paris, France
[f] Center for Translational and International Hematology, Vascular Medicine Institute, University of Pittsburgh, Pittsburgh, PA 15261, USA
[g] Department of Biology, Chatham University, Pittsburgh, PA 15232, United States

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ABSTRACT

Chronic systemic inflammation is a pathophysiological feature of sickle cell disease (SCD). Considering that regular exercise exerts multiple beneficial health effects including anti-inflammatory actions, we investigated whether a treadmill training program could minimize the inflammatory state in transgenic sickle cell (SS) mice. To test this hypothesis, SS mice were subjected to a treadmill training protocol of 1 h/day, 5 days a week for 8 weeks. Exercise training increased the percent of venous oxyhemoglobin and sharply decreased the percent of carboxyhemoglobin suggesting that exercise training may limit the proportion of erythrocytes that were deoxygenated in the venous circulation. Exercise training attenuated systemic inflammation as attested by a significant drop in white blood cell (WBC) count and plasma Th1/Th2 cytokine ratio. There was reduction in interleukin-1β and endothelin-1 mRNA expression in trained sickle mice. The spleen/body mass ratio was significantly decreased in trained sickle mice and there was a strong correlation between the magnitude of congestion and the relative spleen mass in all animals (trained and untrained). We conclude that moderate intensity exercise training, without any noticeable complications, may be associated with limited baseline blood deoxygenation and inflammation in sickle cell mice, and reduce sequestration of sickle erythrocytes/congestion in the spleen.

1. Introduction

Sickle cell disease (SCD) is a genetic disorder characterized by the production of an abnormal hemoglobin (HbS) that forms intracellular polymers under deoxygenated conditions [1]. The ensuing damage to sickle red blood cells (RBCs) results in poor deformability, intravascular hemolysis and occlusion of small vessels [2]. Intravascular hemolysis is accompanied by a release of cell-free HbS and other dangerous molecules into the bloodstream that promote oxidative stress and damage the endothelium [3]. The subsequent inflammatory response and endothelial activation triggers the recruitment of adherent leucocytes, as well as RBCs and platelets, on the vascular wall. Increased blood cell adhesion to the endothelium disturbs microvascular blood flow dynamics and accentuates sickle RBCs transit time into the microcirculation [4]. Therefore, homozygous SCD patients exhibit persistent inflammation as indicated by higher circulating levels of inflammatory mediators [5–7] resulting in severe organ damage that could lead to the death of the patient [8,9].

Acute bouts of exercise can cause a temporary increase in inflammatory markers, adhesion molecules and oxidative stress markers [10]. The acute inflammatory response is necessary for muscle healing and repair process that initiate performance related adaptations [11]. SCD patients are usually advised to not participate in aggressive sporting activities largely due to fears of causing painful vasoocclusive crisis [12–14]. However, several recent studies have demonstrated that short acute submaximal exercises are not associated with clinical events among SCD patients [14,15]. Importantly, biological responses to exercise were not different among healthy subjects and SCD patients

* Corresponding author at: Interuniversity Laboratory of Movement Biology LIBM EA7424, 27–29 boulevard du 11 novembre 1918, 69622 Villeurbanne Cedex, France.
E-mail address: cyril.martin@univ-lyon1.fr (C. Martin).
[1] Equivalent Place.

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[15–17]. Liem and colleagues demonstrated that a maximal cardiopulmonary exercise testing (CPET) did not exacerbate endothelial activation and inflammation in children with SCD [18]. Thus, acute aerobic exercise appears to be a safe intervention for SCD patients.

Exercise training promotes anti-inflammatory responses in healthy people [19] as well as in several cardiovascular, metabolic and respiratory diseases [20–25]. We have previously described reduced plasma soluble vascular adhesion molecule (sVCAM-1) in physically active sickle cell trait (SCT) individuals at rest and 24 h after an incremental maximal exercise [26]. Furthermore, there is a decrease in pulmonary p-selectin and VCAM-1 expression in SAD sickle cell mice (mild SCD phenotype) following 8-weeks of volunteer aerobic training [27]. Hitherto, the potential anti-inflammatory benefits of exercise therapy in SCD have not been investigated. Hence, to test the hypothesis that regular exercise may improve inflammatory state of SCD, we subjected humanized transgenic sickle mouse model expressing exclusively human sickle hemoglobin to an 8-week moderate and individualized exercise training.

2. Material & methods

2.1. Animals

A colony of Townes sickle mice was established using breeding pairs purchased from the Jackson Laboratory (Bar Harbor, ME, USA). Hb genotype and phenotype were confirmed by PCR and Hb gel electrophoresis respectively. Townes mice have both human α- and β-globin genes knocked into the mouse locus, allowing the generation of littermates AA (healthy) and SS (homozygous SCD) by intercrossing AS mice. Mice were used and maintained on a 12-hour light-dark cycle with food and water ad libitum. The Institutional Animal Care and Use Committee (IACUC) at the University of Pittsburgh (Protocol #13102567) approved all mice studies.

2.2. Peak exercise protocol

Seven-week old male SS (n = 17) mice were subjected to a 3-day acclimation with progressively increased intensity and duration of treadmill exposure (RM Exer-3/6 Open Treadmill with Manual Incline; Columbus Instruments). For the peak test, mice ran on an enclosed single-lane treadmill (Molecular Enclosed Metabolic Treadmill for Mice; Columbus Instruments) attached to an Oxymax/Comprehensive Laboratory Animal Monitoring System (CLAMS; Columbus Instruments) that allowed real-time measurements of oxygen consumption ($\dot{V}O_2$) and carbon dioxide output ($\dot{V}CO_2$). Mice were fasted for 4 h with testing being completed between 9 and 11 AM. Mice went through a warm-up (5 m/min for 5 min) before they ran at fixed velocities of 9, 12 and 15 m/min at 15° inclination for 5 min at each level [28]. Thereafter, treadmill speed was increased by 2 m/min every 2 min until exhaustion, which was defined as the inability to return to treadmill running after 10 s. The speed at which $\dot{V}O_2$ reaches a plateau ($\dot{V}O_2$peak) is defined as the maximal aerobic speed (MAS).

2.3. Treadmill training protocol

For the investigations of prolonged training adaptations, mice were rested for 7 days following the peak exercise and were then randomized into either treadmill running (trained: T-SS) or sedentary control groups (untrained: UT-SS) at 8 weeks of age. Trained mice ran on a motorized treadmill (RM Exer-3/6 Open Treadmill with Manual Incline; Columbus Instruments) at 15° inclination, 1 h/day, 5 days/week for 8 weeks. The training protocol was designed as follows: for the first 2 weeks the intensity was fixed at 40% of MAS and running times were increased by 15 min every 2–3 days until the animals ran for 1 h per day; the intensity was then increased by 5% every week until it reached 60% MAS. This intensity was maintained during the rest of the 8-week training period [29]. UT-SS mice were exposed to the treadmill 1 days/week and handled daily in a similar fashion as T-SS mice. Mice were exposed to brief periods of electric shock if they failed to keep up with the speed of the treadmill. If a mouse absolutely refused to run, the animal was removed from the treadmill for the day. Overall, for the entire 8-week training period, SS mice ran an average of 48 ± 8 min per session.

2.4. Tissue sampling

Mice were anesthetized with isoflurane (Isothesia®, Henry Schein, Dublin, OH, USA) 72 h after the last exercise session and blood was collected by a retro-orbital venipuncture into EDTA tubes for hematological analysis. Mice were then sacrificed by cervical dislocation. Heart, liver, spleen and kidneys were collected, weighed and immediately frozen in liquid nitrogen for oxidative stress, western blot and qRT-PCR analyses.

2.5. Whole blood CO-oximetry and hematology

Total Hb concentration and percent of O_2Hb, COHb and MetHb were measured in venous whole blood using a portable CO-oximeter (AVOXImeter 4000, ITC). Complete blood count (CBC) was assessed using a HemaTrue hematology analyzer (Heska). The percentage of reticulocytes was determined by flow cytometry using thiazole-orange staining. According to Dill and Costill, plasma volume change was calculated [30]. Correction was then made to plasma cytokines and blood cell concentrations (data not shown).

2.6. Plasma analysis

Freshly collected EDTA blood samples were centrifuged at 1200*g* at room temperature for 15 min to collect plasma. Total plasma hemoglobin, total plasma heme and LDH activity were quantified using colorimetric assay kits (BioAssay Systems, #DIHB-250, #DIHM-250 and #D2DH-100).

2.7. Luminex assay on plasma Th1/Th2 cytokines

Plasma Th1/Th2 cytokines were determined with the BioPlex Pro™ mouse cytokine, chemokine, and growth factor 23-plex assay (BioRad, Hercules, CA, USA). The assay was performed according to the manufacturer's instructions.

2.8. qRT-PCR

For antioxidant enzymes mRNA level, total mRNA from organs was isolated using RNeasy mini kit (Qiagen, Venlo, Netherlands) according to the manufacturer's instructions. 500 ng per sample of total mRNA were reverse transcribed to cDNA with the high-capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. The reactions were incubated in a thermal cycler for 10 min at 25 °C, 2 h at 37 °C 5 min at 85 °C and then held at 4 °C. Real-time qPCR analysis was performed on a StepOne Realtime PCR system (Applied Biosystems, Foster City, CA, USA). All experiments were done in triplicate with TaqMan fast advanced master mix (Applied Biosystems, Foster City, CA, USA). Expression level was calculated by the ddCt method and normalized to ribosomal 18S RNA [31]. The TaqMan probes used were: *Heme oxygenase-1* (Hmox-1; Mm00516005_m1), *Nadph quinone oxidoreductase-1* (Nqo-1; Mm01253561_m1), *Superoxide dismutase-1* (Sod-1; Mm01344233_g1).

For cytokines mRNA level, total mRNA from organs was isolated using Trizol reagent (Tri Reagent LS, Euromedex, Souffelweyersheim, France) according to the manufacturer instructions, purified with DNase I (EN0525, ThermoFisher scientific, Waltham, MA, USA) and concentrated at 80 ng/μL. 1000 ng per sample of total mRNA were reverse transcribed to cDNA with the reverse transcriptase RNase

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