Synergistic Enhancement of Sickle Red Blood Cell Adhesion to the Endothelium by Hypoxia and Low Nitric Oxide Bioavailability

Diana R. Gutsaeva, PhD, James B. Parkerson, Shobha D. Yerigenahally, Tohru Ikuta, MD, PhD, C. Alvin Head, MD

Anesthesiology and Perioperative Medicine, Medical College of Georgia, Augusta, Georgia

Introduction

The mechanisms underlying sickle red blood cell (RBC) adhesion to the endothelium, which constitutes a major pathologic event in sickle cell disease (SCD), are not fully understood. Adhesion of sickle RBCs to endothelial cells is believed to be regulated by multiple hematologic and physiologic factors including fetal hemoglobin levels, leukocyte count, oxygen tension, inflammatory cytokines, and nitric oxide (NO) bioavailability, but the extent to which each parameter contributes to sickle RBC adhesion remains unclear. Our objective was to examine how the adhesion of sickle RBCs to endothelium is affected by hypoxia and NO bioavailability using an *in vivo* system.

Materials and Methods

Mice

Mice deficient for endothelial NOS (eNOS-/- mice, strain B6.129P2-Nos3^{tm1Unc}/J, C57BL/6J background).[1] Knockout-transgenic SCD model mice.[2] C57BL/6J mice were used as a control.

Blood cell isolation and labeling

RBC from donor SCD or control mice were isolated and labeled with 7-bis-(carboxyethyl)-5-(and-6)carboxyfluorescein (BCECF).

Surgical procedure

Mice were anesthetized with ketamine/xylazine (0.1 mg/0.015 mg/g bw; IP), given a tracheotomy, connected to a small animal respirator and ventilated with room air (21% O_2) or 12% O_2 with or without 20 ppm NO. NO was delivered using the INOvent delivery system. Polyethylene catheter (PE-10) was inserted into the right common carotid artery for the injection of fluorescent sickle RBC and blood pressure monitoring. The scalp was incised in the midline to expose the fronto-parietal skull. Temperature was maintained at 37°C and constantly monitored.

Intravital video microscopy

The skull bone marrow microvascular network was observed using an intravital microscope equipped with water-immersion objectives, SIT camera, a time-base generator and DVCAM digital videocassette recorder. Images were recorded, digitized and analyzed off-line for RBC velocity and adhesion using Image Pro-Plus 5.0 software.[3]

Results

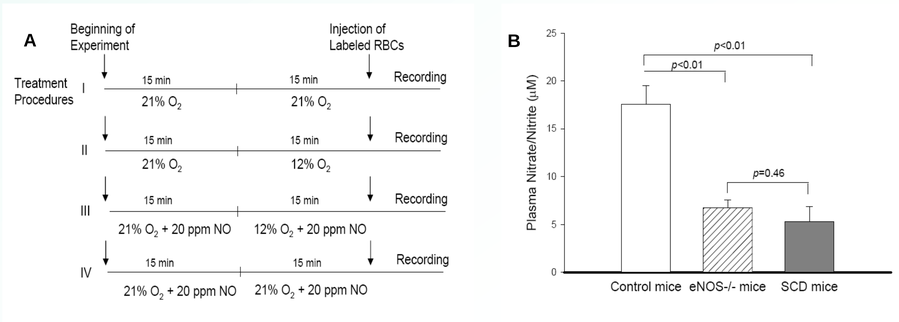

Figure 1. (A) Experimental design. Control and eNOS-/- mice were treated with four different procedures (I–IV). A bolus of BCECF-labeled sickle or control mouse RBCs was injected into sickle or control mice (5–7 in each group). Three minutes later, images of the microcirculation were recorded and evaluated for RBC adhesion using four 1-minute video segments.

(B) Plasma nitrate/nitrite levels in control, eNOS-/-, and SCD mice. Plasma nitrate/nitrite levels were determined using nitrate/nitrite colorimetric assay kit. The NO bioavailability of eNOS-/- mice is comparable to that of SCD mice. Means ± standard error (SE) obtained from 3–4 mice in each group.

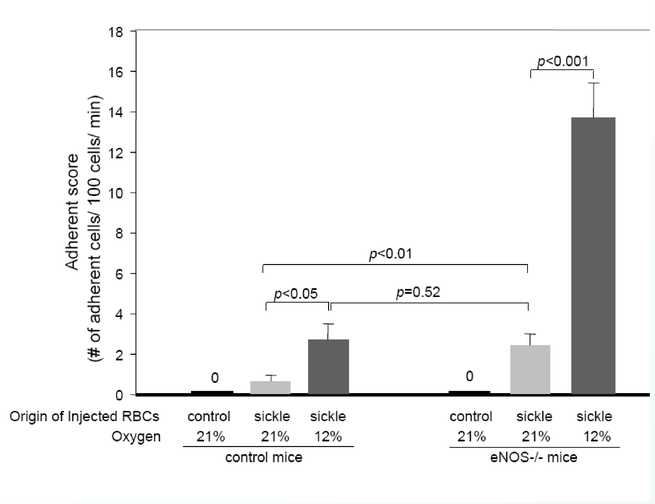

Figure 2. Sickle RBC adhesion in control and eNOS-/- mice under normoxia or hypoxia. BCECF-labeled control RBCs or sickle RBCs were injected into control or eNOS-/- mice (see Figure 1A). Control RBCs did not adhere in either mouse strain under normoxia. In eNOS-/- mice, hypoxia resulted in a significant increase in adhesion scores. Hypoxia-treated control mice showed adhesion scores similar to those observed in eNOS-/- mice under normoxia. Values were means ± SE obtained from 5–7 mice in each group.

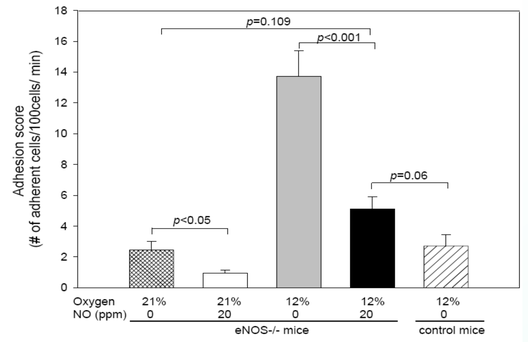

Figure 3. Effect of NO inhalation on sickle RBC adhesion in eNOS-/- mice. Adhesion scores were calculated in venules of eNOS-/- mice under normoxia, hypoxia, and hypoxia/normoxia with NO. Inhalation of 20 ppm NO under hypoxia reduced adhesion scores to levels comparable to normoxic values and indistinguishable from those of control mice under hypoxia. Means ± SE obtained from 5–7 mice in each group.

Summary and Conclusions

In this study we disclosed novel findings on the mechanisms controlling sickle RBC adhesion to endothelial cells. Using a new *in vivo* system that utilized eNOS-/- mice which have NO bioavailability similar to that of SCD model mice we showed:

1. Adhesion of sickle RBCs to the endothelium increases synergistically with hypoxia and low NO bioavailability.
2. Breathing room air (21% O_2) or NO (20 ppm NO) markedly decreases sickle RBC adhesion.
3. Breathing NO gas induces only a marginal change in wall shear rates (data not shown).

Breathing NO gas may reduce the clinical severity of SCD by disrupting the synergy for sickle RBC adhesion created by hypoxia and low NO bioavailability. The recently published results of our clinical trial supports a role for NO breathing in SCD therapy.[4]

References

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[4] Head CA, et al. Am J Hematol 2010;85:800-802