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Hypoxia perturbs endothelium by re-organizing cellular actin architecture: Nitric oxide offers limited protection

Akila Swaminathan[a], Dharanibalan Kasiviswanathan[b], Uma Maheswari Balaguru[a], Gopi Krishna Kolluru[d], Geetha SuryaKumar[c,**], Suvro Chatterjee[a,b,*]

[a] Vascular Biology Lab, AU-KBC Research Centre, MIT Campus of Anna University, Chennai, India
[b] Department of Biotechnology, Anna University, Chennai, India
[c] Defence Institute of Physiology and Allied Sciences, DIPAS, Delhi, India
[d] Department of Pathology, LSU Health Sciences Center, Shreveport USA

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ABSTRACT

Exposure to hypoxia causes structural changes in the endothelial cell (EC) monolayer that alter its permeability. There was a report earlier of impairment of nitric oxide (NO) production in endothelium. The intervention of NO in the altered cellular arrangements of actin cytoskeleton in endothelium for rectification of paracellular gaps in endothelium under hypoxia was observed. The present study demonstrates hypoxia inducing paracellular gaps in hypoxia-exposed blood capillaries in chick embryo extravascular model. Phalloidin staining confirmed significant polymerization of actin and unique cellular localization of the F-actin bands under hypoxia treatments. Addition of spermine NONOate (SPNO), a NO donor, or reoxygenation to endothelial monolayer attenuated hypoxia-mediated effects on endothelial permeability with partial recovery of endothelial integrity through actin remodeling. The present study indicates link of hypoxia-induced actin-associated cytoskeletal rearrangements and paracellular gaps in the endothelium with a low NO availability in the hypoxia milieu. The author concludes that NO confers protection against hypoxia-mediated cytoskeletal remodeling and endothelial leakiness.

1. Introduction

Hypoxia extenuates nitric oxide (NO) is produced in the endothelial cell and destabilizes vascular homeostasis. Low oxygen level in blood induces morphological alteration in the endothelial monolayer (Hackett and Roach, 2004) followed by disturbed endothelial integrity, increased permeability and resulting eventually in endothelial barrier dysfunction (Bärtsch et al., 2005; Hackett and Roach, 2004; Wojciak-Stothard et al., 2006; Aslam et al., 2013; Seerapu et al., 2010). There have been reports lately of hypoxia making alterations in the cellular actin dynamics through changes made in the cell shape and adhesion (Misra et al., 2012; Vogler et al., 2013). The formation of polarized actin band in the cellular loci under hypoxia speeds up physical tension at cell-to-cell interface, with contribution to the formation of paracellular gaps in the monolayer (Kolluru et al., 2008). Alteration in the vascular permeability of the ECs in vessel wall through changes in the actin pattern as a result of NO alteration has been demonstrated (Baldwin et al., 1998; Fischer et al., 1999). Inducement of barrier dysfunction from hypoxia-mediated attenuation by Rho-kinase dependent actin modulation in the EC is obvious (Parikh et al., 2012; Wojciak-Stothard et al., 2012).

Inhalation of NO improves oxygenation in hypoxemic patients and offers protection from hypoxia-induced endothelial leakiness by NO/cGMP pathways (Teman et al., 2015; Kolluru et al., 2008). Palmer et al. (1987) and Michelakis et al. (2002) report that NO obviates chances of lungs getting leaky during any injury apart from maintaining vascular integrity in the respiratory system. Inhalation of NO was used for the treatment of high-altitude pulmonary edema (HAPE) (Mundy and Dorrington, 2000; Michelakis et al., 2002; Himashree et al., 2003; Perrin et al., 2006). Liu and Sundqvist (1997) report that NO and cGMP regulating the permeability and actin filament in reactive oxygen species (ROS) stressed ECs.

The author has reported earlier that NO-cGMP analogue rescues

Abbreviations: cGMP, cyclic guanosine monophosphate; DAPI, (4′,6-Diamidino-2-Phenylindole, Dihydrochloride); EC, endothelial cell; ECs, endothelial cells; eNOS, endothelial nitric oxide synthase; FBS, fetal bovine serum; FITC, Fluorescein isothiocyanate; HAPE, high-altitude pulmonary edema; HIF 1α, hypoxia inducible factor 1 α; L-NAME, L-N^G-Nitroarginine methyl ester; MRTF, myocardin-related transcription factor; MRTF-A, myocardin-related transcription factor A; MRTF-B, myocardin-related transcription factor B; NO, Nitric Oxide; PBS, phosphate buffer saline; ROS, reactive oxygen species; SPNO, Spermine NoNoate; SRF, serum response factor; TRITC, Tetramethylrhodamine; TMBH2O2, 3,3′,5,5′-Tetramethylbenzidine

* Corresponding author: Department of Biotechnology, Anna University, Chennai-600025, India.
** Corresponding author.
E-mail addresses: geethasuryakumar@yahoo.com (G. SuryaKumar), soovro@yahoo.ca (S. Chatterjee).

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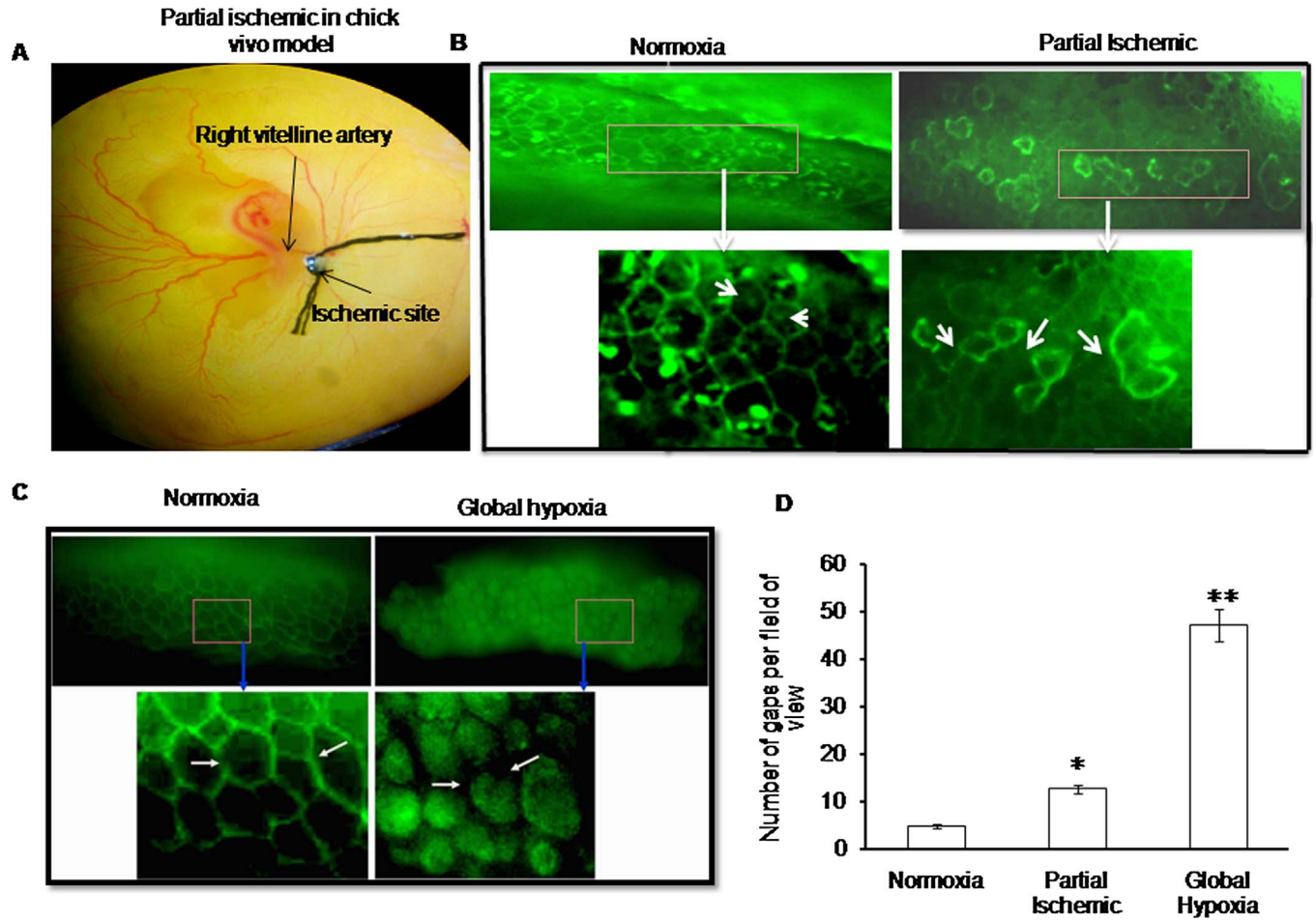

Fig. 1. Effect of low oxygen on endothelial integrity in Chick vascular blood vessel.
1A. Schematic representation of how partial ischemia was created in chick vascular blood vessel 1B. The global hypoxia, partial ischemic and normoxia treated capillaries were isolated and processed for actin staining. The group of ischemic treated blood capillaries showed paracellular gaps (marked in white arrow) compare to normoxic capillaries. The white arrows in the normoxia panel showed the intact monolayer of cells were observed (n = 3). 1C. The global hypoxia treated blood capillaries showed a large number of paracellular gaps compared to normoxic blood capillaries (marked in white arrow) (n = 3). 1D. Graph represents that number of gaps was calculated per field of view of chick vascular bed treated with normoxia, partial ischemic and global hypoxia. **P < 0.001 versus normoxia; *P = 0.045 versus normoxia; n = 3.Values represent the mean for each group SEM (one way ANOVA and LSD).

endothelium from hypoxia-induced vascular leakiness under reduced oxygen milieu (Kolluru et al., 2008). The work now presented establishes inducement of leakiness by hypoxia in the vasculature of chick embryo area vasculose, while exogenous supplementation of NO partially makes a revision in hypoxia-induced leakiness. The study further emphasizes hypoxiamediated actin dependent endothelial remodeling, with eventual proof of the leakiness in the blood vessels.

2. Materials and methods

2.1. Reagents

Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were purchased from PAN-Biotech (Aidenbach, Germany). Spermine NoNoate was purchased from Cayman chemicals (Michigan, USA). DAPI, Phalloidin-Alexa Fluor 568 (phalloidin), Rhodamine Phalloidin, and Texas Red were purchased from Invitrogen Life Technologies. β−Actin and HIF-1 α antibodies were purchased from Abcam (Kolkata, India). All other chemicals were reagent grade.

2.2. Cell line, culture conditions and animals

Immortalized endothelial cell lines, EAhy926, a benign gift from Dr. C.J.S. Edgell, Mayo Clinic (Rochester, Minn., U.S.A.), were used for our study (Edgell et al., 1983). The endothelial cells were cultured in DMEM supplemented with 10% FBS, 1% penicillin–streptomycin (w/v) and maintained in a 5% CO_2 humidified incubator at 37 °C.

Fertilized brown leghorn chicken eggs were purchased from Government poultry station, Potheri, Chennai, India and incubated in sterile humidified incubator at 37 °C. All the experimental manipulations in chick embryos were performed on the 4th day of incubation.

2.3. Hypoxia treatment in cell model

ECs were cultured in a 12-well plate and allowed to grow until reaching 100% confluence. The culture plate was placed into a special chamber system with an inlet and an outlet for purging the required percentage of O_2. For the control sets, the cells were incubated under normoxia conditions. For all the hypoxia experiments, a gas mixture (5% O_2 + 95% N_2) was purged in the hypoxia chamber for 1 h (Seerapu et al., 2010)

2.4. Hypoxia treatment of chick vascular bed

2.4.1. Global hypoxia

The fertilized egg was opened in sterile dishes on the fourth day. The chick vascular bed contained dish was placed in the hypoxic chamber. Then a gas mixture (5% O_2 + 95% N_2) was purged in the hypoxia chamber. The temperature in the hypoxic chamber was maintained at 37 °C for 2 h. For control sets, the vascular beds were