

Anoxia/Reoxygenation of Equine Endothelial Cells Produce Reactive Oxygen Species: An Oxymetric and Electron Paramagnetic Resonance Investigation

G. de la Rebière de Pouyade,^{1,2} A. Mouithys-Mickalad,² A. Salciccia,^{1,2} J. Ceusters,² G. Deby-Dupont,² and D. Sertheyn^{1,2}, ¹Department of Clinical Sciences, Large Animal Surgery, Faculty of Veterinary Medicine B41, University of Liège, Sart Tilman, 4000 Liège, Belgium, ²Center for Oxygen Research and Development, Institute of Chemistry B6a, University of Liège, Sart Tilman, 4000 Liège, Belgium

TAKE HOME MESSAGE

Equine endothelial cells (EC) submitted to anoxia/reoxygenation (A/R) produce reactive oxygen species (ROS).

INTRODUCTION

Endothelium participates to the regulation of the inflammatory response and ischemia/reperfusion-induced injuries are suspected in laminitis. Herein, we investigated the effect of anoxia/reoxygenation on ROS production by EC.

MATERIALS AND METHODS

EC cultures were obtained from equine carotid. Detached cells (10^7) were submitted either to 3 cycles of 20min anoxia followed by reoxygenation in presence of POBN (50mM)/ethanol (2%) mixture as spin trap or to 1h of anoxia followed by reoxygenation and immediate addition of 100mM DMPO. Normoxic cells were used as control (n=3). Anoxia and reoxygenation were monitored by high-resolution respirometry and ROS production was evidenced by electron paramagnetic resonance (EPR).

RESULTS

After A/R cycles, high intensity EPR spectra, assigned to the POBN/•CH(OH)CH₃ adducts were observed, but not in normoxic cells (control). After 1h of anoxia followed by reoxygenation, an enhanced production of DMPO-OH adducts was observed compared to control.

DISCUSSION

The EPR spectra were consistent with the trapping of superoxide anion produced by EC. Such a production could derive from either xanthine oxidase activity or mitochondria dysfunction. Production of ROS by EC could be involved in the disruption of the endothelial barrier, the recruitment of neutrophils and participate to the development of laminitis.

CLINICAL RELEVANCE

Drugs (fractionated heparin or pentoxifylline) able to protect endothelium may have therapeutic relevance in preventing laminitis.

CONCLUSION

ROS from EC submitted to A/R might play a crucial role in the oxidative aspect of laminitis.

Contractile Responses of Laminar Blood Vessels Isolated From Horses Given Black Walnut Heartwood Extract, Carbohydrate-Overload or LPS Infusion: Implications for Equine Laminitis

T.P. Robertson,¹ E. Noschka,² M. Lopes,¹ S. Kwon,¹ J.F. Peroni,¹ and J.N. Moore¹, ¹Dept. of Large Animal Medicine, The College of Veterinary Medicine, The University of Georgia, Athens, GA 30605, ²Department of Large Animal Clinical Sciences, Virginia Maryland Regional College of Veterinary Medicine, Duck Pond Drive, Blacksburg, VA, 24071

TAKE HOME MESSAGE

Laminar veins are dysfunctional in horses with laminitis but not those given LPS.

INTRODUCTION

The objective was to determine whether laminar and digital vessels from horses with carbohydrate (CHO) or black walnut heartwood extract (BWHE) -induced laminitis or from horses infused with LPS possess basal tone.

MATERIALS & METHODS

24 horses were divided into 4 groups: 1) BWHE group (via nasogastric tube); 2) CHO group (via nasogastric tube); 3) LPS group (endotoxin, 30ng/kg, over 30min); or 4) Control Group (7L water via nasogastric tube). Horses were euthanized at the following time points: BWHE or CHO at the onset of Obel grade 1 laminitis; LPS and control groups at 12h after LPS or water. Digital and laminar arteries and veins were isolated to assess contractile function.

RESULTS

Laminar veins from horses given BWHE or CHO possessed basal tone (constricted when the bathing solution was switched from 0mM Ca²⁺ to 2mM Ca²⁺). Basal tone was not attributable to capacitative or voltage-gated Ca²⁺ entry, but was reversed by an inhibitor of Rho-kinase.