Broadband Near Infrared Spectroscopy Can Detect Cyanide-Induced Cytochrome AA_3 Inhibition in Rats

Presenting Author: Robert H. Thiele MD, Univ. VA
Co-Authors: Keita Ikeda, PhD Uniiv. VA Yunzhen Wang, M.D.Univ. VA Raquel R. Bartz, M.D. DUKE Zhiyi Zuo, M.D., Ph.D. Univ. VA

Introduction: Clinically available near infrared spectroscopy (NIRS) devices are based on the Beer-Lambert law and are designed to measure the hemoglobin saturation in tissue (StO_2). The outcome data to support using these devices is not strong. The oxidation state of cytochrome aa3 (Cyt_{aa3}), the terminal component of the electron transport chain, reflects the state of subcellular energetics directly, and may be a more physiologically relevant chromophore than hemoglobin. Cyt_{aa3} absorbs near infrared radiation (NIR) with a peak around 820 nm, and thus the oxidation state of Cyt_{aa3} (Cyt_{ox}) can be measured using NIRS. Measurement of Cyt_{ox} using NIRS is technically challenging, and several investigators have developed "broadband" techniques to improve signal-to-noise ratios. The purpose of this study was to verify the ability of broadband NIRS to measure Cyt_{ox} independent of changes in hemoglobin saturation by manipulating Cyt_{ox} and StO_2 independently.

Methods: We developed a 301 wavelength broadband NIRS device and algorithm based on the previously-validated UCL*n* algorithm using primarily commercial off-the-shelf components. NIR radiation was provided by a single halogen light source coupled to a single emitting fiber optic cable with an attached prism at the end to transmit near infrared radiation to the subject. Three receiving fiber optic cables were utilized – one was directed at the light source to continuously measure the intensity of light at all wavelengths, while the other two were directed at the base of the skull. Each receiving fiber was connected to an indepdendent spectrometer. Intensity data was exported into MATLAB for analysis.

Results: Twenty two-month-old male 300g Sprague-Dawley rats were anesthetized with isoflurane, intubated, and ventilated with 100% O_2 containing 2% isoflurane. Sodium cyanide (NaCN) was intravenoulsy injected at 5 mg/kg in order to produce cytochrome reduction independent of hemoglobin. Two to three minutes after NaCN injection, oxygen supply was eliminated and 100%-N_2 with isoflurane was used for ventilation (anoxia), in order to induce a reduction in both cytochrome and hemoglobin. Twenty rats completed the study. Ninety seconds after injection of cyanide, Hgb decreased in 80% of animals (median -0.014, range -0.056 to 0.22, $p < 0.001$), Hgb-O_2 increased in 75% of animals (median 0.013, range -0.059 to 0.073, $p < 0.001$), and cytochrome reduction occurred in 100% of animals (median -0.015, range -0.028 to -0.0012, $p < 0.001$). Ninety seconds after initiation of anoxia, Hgb increased in 95% of animals (median 0.13, range -0.026 to 0.21, $p < 0.001$), Hgb-O_2 decreased in 95% of animals (median -0.17, range -0.24 to 0.024, $p < 0.001$), and cytochrome reduction occurred in 100% of animals (median -0.04, range -0.069 to -0.0065, $p < 0.001$).

Conclusions: By demonstrating independent manipulation of Cyt_{ox} and hemoglobin oxidation state, this data demonstrates that accurate trending of changes in Cyt_{ox} is possible

using readily available equipment, lowering the barrier to further development of devices designed to measure electron transport chain dysfunction / subcellular energy derangements in real-time.

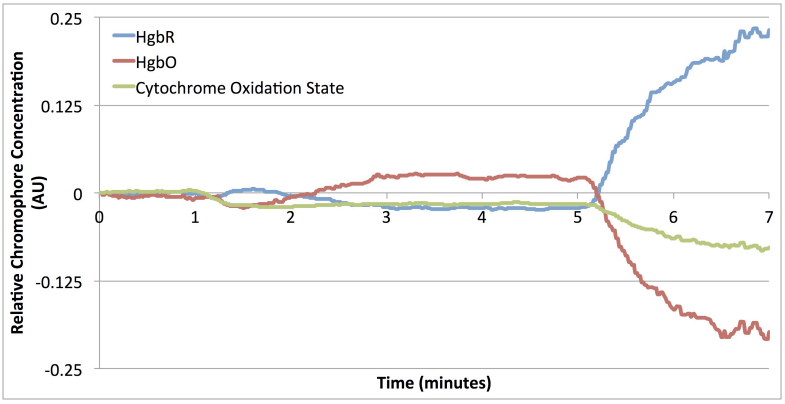

Figure 1. Injection of NaCN (1 min) leads to cytochrome desaturation and increase in tissue oxygen saturation. Subsequent exposure to 100% N2 (anoxia, 5 min) leads to additional cytochrome desaturation and tissue hypoxia.