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Oxygen saturation monitoring using resonance Raman spectroscopy

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ABSTRACT

Background: The knowledge of hemoglobin oxygen saturation (SO_2) and tissue oxygenation is critical to identify the presence of shock and therapeutic options. The resonance vibrational enhancement of hemoglobin allows measurement of oxy- and deoxy species of hemoglobin and resonance Raman spectroscopy (RRS-StO_2) has been successfully used to measure aggregate microvascular oxygenation. We tested the hypothesis that noninvasive oxygen saturation measured by RRS-StO_2 could serve as surrogate of systemic central venous SO_2.

Methods: In anesthetized rats, measurements of RRS-StO_2 made in oral mucosa, skin, muscle, and liver were compared with measurements of central venous SO_2 using traditional multi-wavelength oximetry. Various oxygenation levels were obtained using a stepwise hemorrhage while over 100 paired blood samples and Raman-based measurements were performed. The relationships between RRS-StO_2 and clinically important systemic blood parameters were also evaluated. RRS-StO_2 measurements were made in 3-mm diameter tissue areas using a microvascular oximeter and a handheld probe.

Results: Significant correlations were found between venous SO_2 and RRS-StO_2 measurements made in the oral mucosa ($r = 0.913$, $P < 0.001$), skin ($r = 0.499$, $P < 0.01$), and liver ($r = 0.611$, $P < 0.05$). The mean difference between sublingual RRS-StO_2 and blood sample SO_2 values was 5.4 ± 1.6%. Sublingual RRS-StO_2 also correlated with lactate ($r = 0.909$, $P < 0.01$), potassium ($r = 0.757$, $P < 0.01$), and pH ($r = 0.703$, $P < 0.05$).

Conclusions: Raman-based oxygen saturation is a promising technique for the noninvasive evaluation of oxygenation in skin, thin tissues, and solid organs. Under certain conditions, sublingual RRS-StO_2 measurements correlate with central venous SO_2.

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1. Introduction

The noninvasive determination of tissue oxygenation is critical to identify the presence of shock and the suitability of therapeutic measures aimed at its resolution. The vibrational bands of hemeproteins provide medically relevant information such as hemoglobin oxygen saturation (SO_2). Under proper excitation, the resonance vibrational enhancement of hemoglobin allows simultaneous identification and measurement of the proportion of oxy- and deoxy species of hemoglobin in a concentration-dependent manner with a single excitation wavelength. A chemical fingerprint of the species can be obtained with little or no interference from other compounds [1–3].

Because the profile of the microvasculature and blood volume in tissue is heavily weighted toward a venous composite (80%), aggregate measures of tissue oxygenation are representative of the post-extraction compartment of tissues. In global shock states such as hemorrhage, tissue oxygenation from certain tissues may compare well to central measures such as central venous or mixed venous hemoglobin oxygen saturation. In previous studies from our group [1–3], resonance Raman spectroscopy (RRS-StO_2) has been successfully used to measure aggregate microvascular tissue oxygen saturation in the skin of normotensive rats using two laser excitations (406.7 and 532 nm). Encouraging results were also found in large animal models of hemorrhagic shock [4].

In this study, we tested the hypothesis that noninvasive tissue oxygen saturation measured by RRS-StO_2 could serve as surrogate of systemic central venous SO_2. For this purpose, we compared noninvasive RRS-StO_2 measurements made in several different tissues (oral mucosa, skin, muscle, and liver) with measurements of central venous SO_2 using traditional multi-wavelength CO-oximetry. To provide a wide range of oxygenation levels, a protocol of stepwise hemorrhage was conducted while paired blood samples and Raman measurements were performed. We also evaluated quantitatively the relationship between RRS-StO_2 and clinically important systemic blood parameters such as lactate and potassium.

2. Materials and methods

2.1. Experimental animals

This study was approved by the Institutional Animal Care and Use Committee of Virginia Commonwealth University. We used 13 Sprague–Dawley rats (Harlan, Indianapolis, IN) weighing 241–501 g. The rats were kept on a commercial diet with free access to drinking water, in a facility with constant temperature (21°C–23°C) and humidity (~50%) that rotated through 12-h light/dark cycles.

2.2. Surgical preparation

The animals were anesthetized with 2% isoflurane and kept under constant anesthesia during cannulations (1.5 L/min balance 21% O_2). The core temperature was maintained at 37°C using a thermostatically controlled heating blanket (Harvard Apparatus, Holliston, MA). A tracheostomy was performed to maintain a clear airway. The right jugular vein was cannulated with PE-90 tubing advanced to the entrance of the right atrium, used for the collection of blood samples and for injection of Euthasol at the end of the data acquisition process. The right femoral vein was cannulated with PE-50 tubing for infusion (0.17–0.24 $mg/kg^{-1}h^{-1}$) of alfaxalone/alfadolone acetate (Saffan; Schering-Plough Animal Health, Welwyn Garden City, England) anesthesia by a microprocessor-controlled infusion/withdrawal syringe pump (model PHD 2000; Harvard Apparatus, Holliston, MA) once the experimental procedure began. The right femoral artery was cannulated with phosphate-buffered saline (PBS)–filled PE-50 tubing connected to a pressure transducer to continuously measure arterial blood pressure (AP), for the controlled-hemorrhage process and for collection of blood samples. The AP was monitored using a pressure transducer connected to a computer for continuous data acquisition (DA100C, MP150, Acknowledge 3.9.0, Biopac Systems, Goleta, CA). All lines were flushed with heparinized PBS (10 i.u. mL^{-1} heparin) after each blood collection to inhibit clot formation. The vein and artery on the left side were not cannulated, and this thigh was used for Raman monitoring.

2.3. Resonance Raman spectroscopy–based O_2 saturation measurements

A microvascular oximeter (Pendar Medical, Cambridge, MA) was used to measure tissue oxygenation using RRS-StO_2. The device has a laser (405 nm, 4 mW) coupled to a complex plastic fiber optic cable and a handheld probe that illuminates a tissue area (approximately 3 mm in diameter, less than 1 mm deep). The oxyhemoglobin and deoxyhemoglobin molecules can be excited by the laser light into distinct vibrational states resulting in a differential wavelength shift of the scattered light. The charge-coupled device–based spectrometer of the oximeter captures the spectrum of the scattered light every second, with distinct sharp peaks linearly proportional to the relative concentrations of oxyhemoglobin and deoxyhemoglobin. The device contains dedicated software to calculate the RRS-StO_2 using the spectral peaks as the relative ratio of concentration of oxygenated hemoglobin to that of total hemoglobin. (Fig. 1)

2.4. Animal groups and testing sites

Two groups of animals were studied. In one group of animals, the gracilis muscle, the sublingual surface, and the surface of the paw were prepared for examination, as described in the following section. In the second group of animals, the surface of the liver was prepared. The RRS-StO_2 readings were taken from each site by gently placing the Raman probe directly over each tissue area. To prepare the “muscle” site, the left thigh was shaved and void of hair using a commercial hair removal cream. The skin layer was cut and folded back to expose the gracilis muscle that was immediately covered with Saran wrap to maintain the exposed tissue void of contact with atmosphere. PBS was administered (0.5 mL, i.v.) as a hydration