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Investigating viability of intestine using spectroscopy: a pilot study

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ABSTRACT

Background: The differentiation of “viable” from “nonviable” bowel remains a challenge in the treatment of acute mesenteric ischemia. In this study, diffuse reflectance spectroscopy (DRS) was used to investigate the viability of bowel tissue after ischemia and reperfusion in an animal model *in vivo* and in real time.

Methods: A total of 25 females Sprague–Dawley rats were divided into five groups based on different bowel ischemia times. In each study group for four of them, the superior mesenteric artery was occluded using a vascular clamp for a different period (i.e. 30, 45, 60, and 90 min; $n = 5$ for each group). Intestinal reperfusion was accomplished by releasing the clamps after the given occlusion period for each group. Spectra were acquired by gently touching the optical fiber probe to the bowel tissue before the induce ischemia, at the end of the induced ischemia, and after the reperfusion. The data acquired before the ischemia were used as a control group. Without occluding the superior mesenteric artery, the spectra were acquired on the bowel with the same time intervals of the experiments were used as a sham group ($n = 5$). Subsequently, the same bowel segments were sent for histopathologic examination.

Results: Based on the correlation between the spectra acquired from the bowel segments and the results from the histopathologic investigation, DRS is able to differentiate the histopathologic grading that appears when the Chiu/Park score $\geq$5 (i.e., high-level ischemic injury) than Chiu/Park score $<$5. Eight out of nine low-level ischemic injury tissue samples were correctly defined using the spectroscopic classification system. All eleven high-level ischemic injury tissues that were histopathologically assigned grade 5 and above were correctly defined using the spectroscopic classification system in the ischemia–reperfusion groups.

Conclusions: DRS could potentially be used intraoperatively for the assessment of bowel viability in real time. These preliminary findings suggest that DRS has the potential to reduce unnecessary resection of viable tissue or insufficient resection of nonviable tissues may reduce the mortality and morbidity rates of intestinal ischemia–reperfusion as acute mesenteric ischemia.

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1. Surgical relevance

Current approach to mesenteric ischemia comprises the resection of nonviable intestinal segments. But, clinical assessment of it is frequently deficient in certain prediction of bowel viability.

Real time spectroscopic technique has the potential for comprehensive and intraoperative assessment of tissue viability based on alteration of tissue oxyhemoglobin and deooxyhemoglobin contents. This case study was conducted using rats to assess the effectiveness of spectral data in differentiation between necrotic and viable intestine tissues by comparing the results of histopathology. Our findings show that spectroscopic measurements are able to assess the viability of intestinal tissues successfully.

This experiment may be of interest to clinical researchers as the rat acute mesenteric ischemia model provides a step forward for clinical studies in humans. In the future, we believe that diffuse reflectance spectroscopy (DRS) can provide accurate prediction of bowel viability in the surgical treatments of acute mesenteric ischemia in real time intraoperatively.

2. Introduction

Acute mesenteric ischemia is a relatively rare life-threatening emergency with an incidence of 1 in 1000 patient admissions to hospitals, but this condition has a mortality rate of 60%–80%. The etiologic factors that contribute to the formation of acute mesenteric ischemia include emboli (i.e., in 40%–50% of cases), arterial thrombus (in 25%–30% of cases), nonocclusive ischemia (i.e., in 20% of cases), and venous thrombus (i.e., in 10% of cases). The overall survival time of patients with acute mesenteric ischemia is affected by the specific etiologic factors contributing to the development of the condition, the presence of concomitant diseases, and the characteristics of the subsequent surgical excision [1–4]. Current approaches to mesenteric ischemia include the resection of necrotic intestinal tissue and anastomosis to reestablish blood flow through the bowel. However, in some cases, despite the reestablishment of blood flow through the intestinal segments, the viability of a bowel segment is still unclear [5,6]. The previous studies suggested that if viability of the bowel is doubtful, it should not be resected for preserving as much bowel as possible at the initial operation because of avoiding the short bowel syndrome. Surgeons should decide the resection of the bowel segments at the second look within 24–48 h after the first operation [7,8]. Therefore, accuracy of the assessment of bowel viability in the first operation is important to avoid additional surgeries.

Currently, clinical findings (e.g., unaided visual color inspection, peristaltic activation, and arterial pulsations) are used to investigate the viability of the intestinal tissues. In general, two methods have historically been used to intraoperatively assess the viability of bowel tissue. The first method is Doppler ultrasound, which has a low accuracy because of the blood flow that is still present in the ischemic tissue [9]. The second method is a fluorescence technique, which requires the intravenous administration of fluorescent molecules into the blood stream and should be used within washout time of injected fluoresce molecule. Both these current methods have limitations [10–17]. In addition, near infrared spectroscopy has been subject of research interest of several groups to measure tissue oxygen saturation [18–20].

In this study, we investigated correlations between DRS measurements, and pathology results were used to assess intestinal ischemic–reperfusion injury. DRS data were acquired from the ischemic-reperfused intestine tissues without using any extrinsic contrast agent. In the ischemic tissue, the concentration of oxyhemoglobin decreases, whereas the concentration of deoxyhemoglobin increases. The absorption spectrum of oxyhemoglobin differs from that of deoxyhemoglobin as illustrated in Figure 1. Therefore, the back reflection spectrum of ischemic tissue should be different than that of normal tissue. As seen in Figure 1, the difference between the absorption spectra is highest at wavelengths 560 nm and 577 nm (i.e., the two black vertical lines in Fig. 1). As seen in Figure 1, the ratio, $R =$ absorption (560)/absorption (577) is <1 for oxyhemoglobin and >1 for deoxyhemoglobin absorption spectra. In this study, the ratio of the absorption of these two wavelengths in the studied tissue is compared with the results of histopathology to determine the potential for using this ratio as a parameter to assess the viability of intestinal tissues.

3. Materials and methods

This study conformed to the Guide for the Care and Use of Laboratory Animals as published by the US National Institutes of Health (NIH Publication No. 85-23 revised, 1985) and was approved by the local Committee on Animal Research Ethics at the Akdeniz University (No: 2011.09.01) Twenty-five Sprague–Dawley rats with weights of 250 ± 25 g were used in this study. Animals were bred and reared in the Research Animal Facilities of Akdeniz University, School of Medicine, Turkey. The rats were kept in polycarbonate cages under the following conditions: a controlled ambient temperature of 22°C–25°C, automatically adjusted humidity (i.e., 45%–50%), and 12:12 h

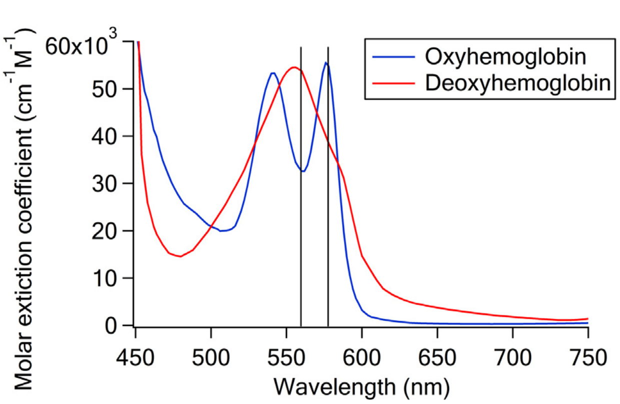

Fig. 1 – Absorption spectra of oxyhemoglobin and deoxyhemoglobin. (Color version of figure is available online.)