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Biphasic Tumor Oxygenation during Respiratory Challenge may Predict Tumor Response during Chemotherapy

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Our previous study showed that switching the inhaled gas from hypoxic gas to hyperoxic gas for 10 minutes increased tumor oxygenation and that the magnitude of oxyhemoglobin increase responded earlier than tumor volume change after chemotherapy. During 10 minutes of inhaled-oxygen modulation, oxyhemoglobin concentration first shows a rapid increase and then a slow but gradual increase, which has been fitted with a double-exponential equation in this study. Two amplitude values, amplitudes 1 and 2, respectively represent the magnitudes of rapid and slow increase of oxyhemoglobin. The trends of changes in amplitudes 1 and 2 were different, depending on tumor volume when chemotherapy started. However, both amplitudes 1 and 2 changed earlier than tumor volume, regardless of when chemotherapy was initiated. These results imply that by observing amplitude 1 changes post chemotherapy, we can reduce the time of a respiratory challenge from 10 minutes to less than 2 minutes, to see the chemotherapy response. We believe that by reducing the time of the respiratory challenge, we have taken a step forward to translating our previous study into clinical application.

Keywords : Vascular reactivity, Chemotherapeutic efficacy, Near-infrared spectroscopy, Hemodynamic changes
OCIS codes : (000.1430) Biology and medicine; (170.2655) Functional monitoring and imaging; (170.6510) Spectroscopy, tissue diagnostics; (300.6340) Spectroscopy, infrared

I. INTRODUCTION

Tumor oxygenation is a very important parameter to improve the efficacy of cancer treatment since it is well known that hypoxic tumor cells are highly resistant to radiation therapy [1, 2], photodynamic therapy [3], and chemotherapy [4]. Thus, many studies have been conducted to increase tumor oxygenation, including inhaled-gas modulation using a hyperoxic gas such as carbogen or 100% oxygen [5-12]. Therefore, it has become very important to monitor changes in tumor oxygenation during inhaled-gas modulation.

Liu *et al*. [10] employed near-infrared spectroscopy to monitor the change of tumor oxygenation during hyperoxic gas intervention and showed that tumor-oxygenation change has biphasic characteristics of rapid and then slow increase during hyperoxic gas intervention, from animal models of both breast and prostate tumors. They proposed that this biphasic change of oxyhemoglobin come from the heterogeneity of tumor perfusion. A rapid increase of oxyhemoglobin corresponds to a well-perfused region, while a slow but gradual increase of oxyhemoglobin corresponds to a poorly perfused region in a tumor. They further developed a model and fitted the change of oxyhemoglobin concentration during carbogen inhalation with a double-exponential equation, resulting in two amplitudes and two time constants.

In our previous report, we performed animal experiments and showed that vascular reactivity, defined as the change of oxyhemoglobin concentration during a hyperoxic inhaled-

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Color versions of one or more of the figures in this paper are available online.

gas intervention, corresponds well with tumor growth, and also showed an earlier response compared to tumor-volume change post chemotherapy [13]. However, the duration of gas inhalation was 10 minutes to observe vascular reactivity, which is potentially a hurdle for clinical application, since cancer patients may not be able to endure such a long time for the measurement.

In this study, we hypothesized that well-perfused and poorly perfused regions of tumor would show different changes during tumor growth and chemotherapy, due to the difference in tumor vascular structure. To confirm this, the volume fraction of both well-perfused and poorly perfused regions was monitored daily, before cancer cell inoculation, during tumor growth, and post chemotherapy.

II. METHODS

2.1. Animal Model and Care

Eighteen female Fisher 344 rats (180-200 g) were divided into 3 groups (control, chemo, and early chemo group) for this study. The number of animals in each group was six. The animals were kept at room temperature, with free access to water and feed during the whole experimental period. To conduct this study, we cultured the 13762 MAT B-III (CRL-1666, ATCC, Manassas, Virginia) rat breast cancer cell using combined McCoy's 5A (ATCC, Manassas, Virginia) medium with 10% fetal bovine serum and 1% penicillin/streptomycin (P/S) in a CO_2 incubator under 5% CO_2. Cell viability was checked using Trypan blue and a hemacytometer, before cell inoculation to the left caudal mammary fat pad of the rat. All of our animal protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of the Gwangju Institute of Science and Technology.

2.2. Experimental Setup

The continuous wave near infrared spectroscopy (CWNIRS) system was composed of two broadband light sources (tungsten halogen lamp, HL-2000-HP, Ocean Optics) and two NIR spectrometers (USB4000, Ocean Optics). The source and detector probes were multimode optical fibers with 2 mm core diameter and were placed in direct contact with the tissue. Each pair of source and detector probes were placed 5 mm apart. A gas mixer with an isoflurane vaporizer was used in this study, to perform gas interventions for respiratory challenges and to deliver anesthetic agent (1.5-2% isoflurane) during the experiment. To prevent hypothermia and maintain the body temperature of the animal during anesthesia, a warm water pad was used for the entire duration of each experimental session (Fig. 1).

2.3. Experimental Procedure

For this study, one million cells of the 13762 MAT B-III breast cancer cell line were inoculated into a left mammary fat pad (second from the tail) of each rat. Tumor growth was monitored every day by measuring the diameter with a caliper; then the ellipsoid volume calculation method was applied to estimate tumor volume. For the chemotherapy treatment, we administered to the chemo group a single high dose of cyclophosphamide (100 mg/1 kg body weight) via intraperitoneal (IP) injection 7 days after cell inoculation, when the diameter of the tumor became approximately 8 mm. Otherwise, we performed an experimental procedure similar to that for the chemo group, except for the earlier administration of chemotherapy. For the early chemo group, we conducted chemotherapy 2 days earlier than for the chemo group, to validate the effects of chemotherapy. For the control group, saline was administered instead of chemotherapy. The NIRS data were obtained from both the tumor breast and contralateral normal breast during the whole experimental period with inhaled-gas intervention, shown in Fig. 2. The baseline was taken to be the first 20 min with air (21% oxygen + 79% nitrogen). Thereafter, hypoxic gas (16% oxygen + 84% nitrogen) was supplied for 10 min, followed by 10 min of hyperoxic gas (100% oxygen) inhalation. Air was then given for the next 15 min, to return tissue oxygenation back to baseline. Changes in

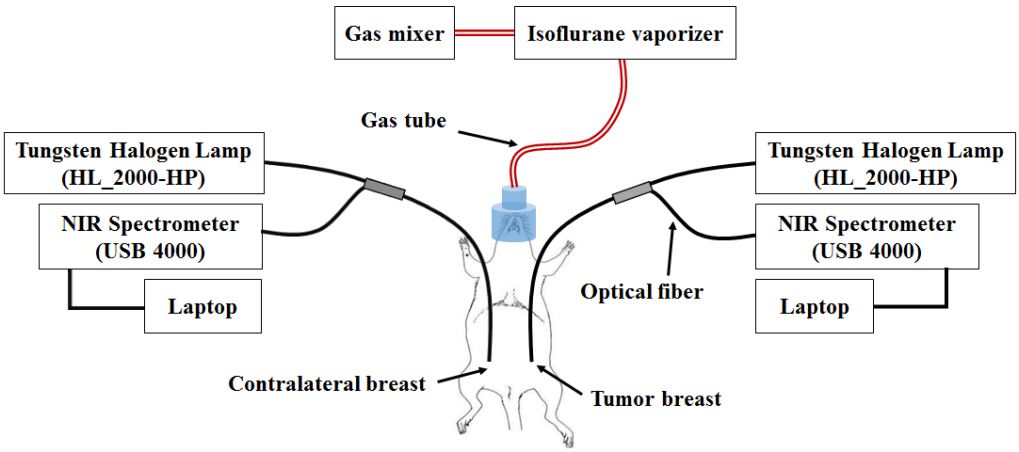


FIG. 1. Experimental setup of the CWNIRS system used for monitoring tumor hemodynamics.

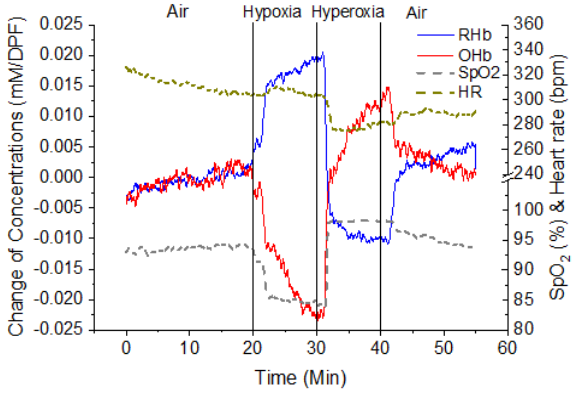
Air_20 min (21% O_2 + 79% N_2)	Hypoxia_10 min (16% O_2 + 84% N_2)	Hyperoxia_10 min (100% O_2)	Air_15 min (21% O_2 + 79% N_2)

FIG. 2. Protocol of inhaled-gas intervention.

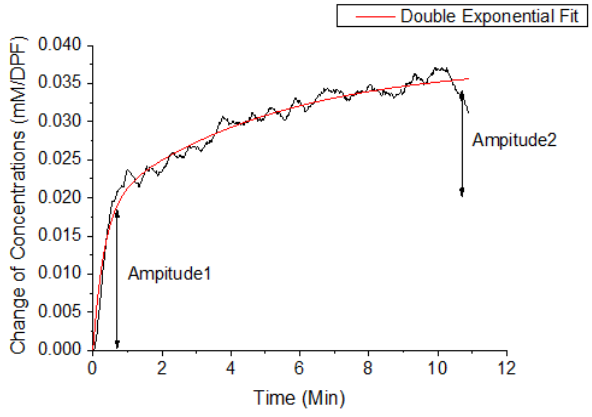
gas concentrations were performed automatically by a gas mixer system (SHGM 3000, Sehwa Hightech, Korea). In addition, pulmonary oxygen saturation (SpO_2) and heart rate (HR) were measured from the hind foot during the whole experiment, using an animal pulse oximeter (MouseOx, USA).

2.4. Data Analysis

To calculate the hemodynamic changes using CWNIRS, we acquired the intensity values at five wavelengths: 730, 750, 800, 830 and 850 nm. The modified Beer-Lambert law was applied to obtain the deoxyhemoglobin (RHb) and oxyhemoglobin (OHb) concentration changes (A detailed description can be found in our previous reports [13, 14]).



(a)



(b)

FIG. 3. (a) Representative set of data showing oxyhemoglobin (OHb), deoxyhemoglobin (RHb), pulmonary oxygen saturation (SpO_2), and heart rate (HR) during the inhaled-gas interventions. (b) A double-exponential equation (Eq. (1)) was fitted to the change of OHb concentration during hyperoxia. Amplitude 1 (A1) corresponds mainly to hemodynamics in the well-perfused region of the tumor, while Amplitude 2 (A2) corresponds to the poorly perfused region.

Figure 3(a) shows representative changes of OHb and RHb concentrations during the protocol of respiratory challenges shown in Fig. 2. Using Origin 2016, we fitted a double-exponential model (Eq. (1), which was derived by Liu *et al.* [10]) to the OHb concentration change during the hyperoxic gas intervention to obtain the values of amplitude 1 (A1) and amplitude 2 (A2) from the tumor breast and contralateral breast.

$$\triangle OHb = A1\left[1-\exp\left(\frac{-t}{\tau 1}\right)\right]+A2\left[1-\exp\left(\frac{-t}{\tau 2}\right)\right] \qquad (1)$$

where $A1$ and $A2$ represent respectively the volume fractions of well-perfused and poorly perfused regions of the tumor, and $\tau 1$ and $\tau 2$ represent perfusion rates for the well-perfused and poorly perfused regions respectively [5]. Figure 3(b) shows a representative oxyhemoglobin change with a double-exponential fit [10, 15, 16].

III. RESULTS

Figure 4 shows the average changes of A1 and A2 and tumor-volume change for the control group (Fig. 4(a)), chemo group (Fig. 4(b)), and early chemo group (Fig. 4(c)), for both the contralateral (A1(c), A2(c)) and tumor breasts (A1(t), A2(t)). A1 and A2 values were measured for 2 days as a baseline, and then tumor cells were inoculated into the tissue. In the contralateral breast, A1(c) and A2(c) kept a relatively constant value during the whole experiment from all groups. On the other hand, A1(t) and A2(t) increased as the tumor grew and decreased after chemotherapy, for all groups. However, a different trend between chemo and early chemo groups has been observed. In the control group, A1(t) increases as the tumor grows, but a drop in A1(t) was observed on day 13 when the tumor grew bigger than 700 mm^3. On the other hand, A2(t) did not increase much until day 9, but started to increase rapidly when tumor volume was greater than 200 mm^3.

A1(t) and A2(t) from chemo group showed an interesting change after chemotherapy. A1(t) significantly decreased on day 1 post chemotherapy, while A2(t) continued to rise. On day 2 post chemotherapy, A1(t) recovered to lower than the level at the start of chemotherapy, while A2(t) fell down to the level of day 0 of chemotherapy.

The early chemo group showed similar trends in A1(t) and A2(t) as those from the chemo group. However, both A1(t) and A2(t) from the early chemo group reached a maximum value 1 day after chemotherapy, and decreased at day 2 post chemotherapy. One difference is that the

early chemo group started chemotherapy when the tumors were small (~40 mm^3), compared to the tumor volumes for the chemo group (~400 mm^3). Both A1(t) and A2(t) showed a one-day earlier response compared to tumor-volume change due to chemotherapy. The tumor regression rate was also faster than that for the chemo group.

IV. DISCUSSION

In this study, we demonstrated the biphasic characteristics of tumor hemodynamics during a modulation of inhaled oxygen gas, before and after chemotherapy. The purpose of using inhaled-oxygen intervention is to cause a hemodynamic contrast between tumor and normal breast, which can be observed with CWNIRS. Indeed, we found that vascular reactivity, defined as the magnitude of tumor oxyhemoglobin concentration change during hyperoxic gas inhalation, showed a big difference between tumorous and normal breasts [13]. The vascular reactivity became larger as the tumor grew, and more importantly it responded one day earlier than did the tumor-volume change after chemotherapy. This result shows the potential of using vascular reactivity during inhaled-gas modulation with CWNIRS as a biomarker to predict tumor response during chemotherapy.

However, we applied each inhaled-gas modulation for 10 minutes in our previous study, which is quite a long time for patients if our approach is to be translated for a clinic. Therefore, we further analyzed our previous results by employing a biphasic model proposed by Liu *et al.* [10]. Tumor oxyhemoglobin increase during hyperoxic gas inhalation was fitted with a double exponential equation (Eq. (1)) and two amplitude values (A1 and A2), representing volume fractions of well-perfused and poorly perfused regions of the tumor, were acquired.

Decomposing the overall magnitude of vascular reactivity into A1 and A2 values enabled us to monitor two differently perfused regions in a tumor as the tumor grows, and during chemotherapy. In our previous report, vascular reactivity decreased once the tumor grew bigger than 700 mm^3. In this study, we also confirmed that A1(t) in a control group decreased on day 13, which may come from the reduction of the well-perfused region of the tumor as the tumor grew bigger than 700 mm^3 (Fig. 4(a)). On the other hand, as we mentioned, A2(t) started to increase rapidly on day 10, when tumor volume was greater than 400 mm^3. From this result, we found that A1, representing the well-perfused region of the tumor, is mainly responsible for the decrease in vascular reactivity at day 13 due to the formation of hypoxic and necrotic regions in the tumor, while the volume of the poorly perfused region (A2) continued to increase (Fig. 4(a)).

Vascular-reactivity changes were similar between the chemo and early chemo groups in our previous study. However, once the signal has been decomposed into A1 and A2 values, the changes in A1 and A2 post chemotherapy showed a difference between the chemo and early chemo groups. The chemo group showed that A1(t) drops 1 day after cyclophosphamide administration, while A2(t) continued to increase on day 1 and then dropped on day 2 post chemotherapy. This result may imply that cyclophosphamide is first effective in the well-perfused region, and then starts

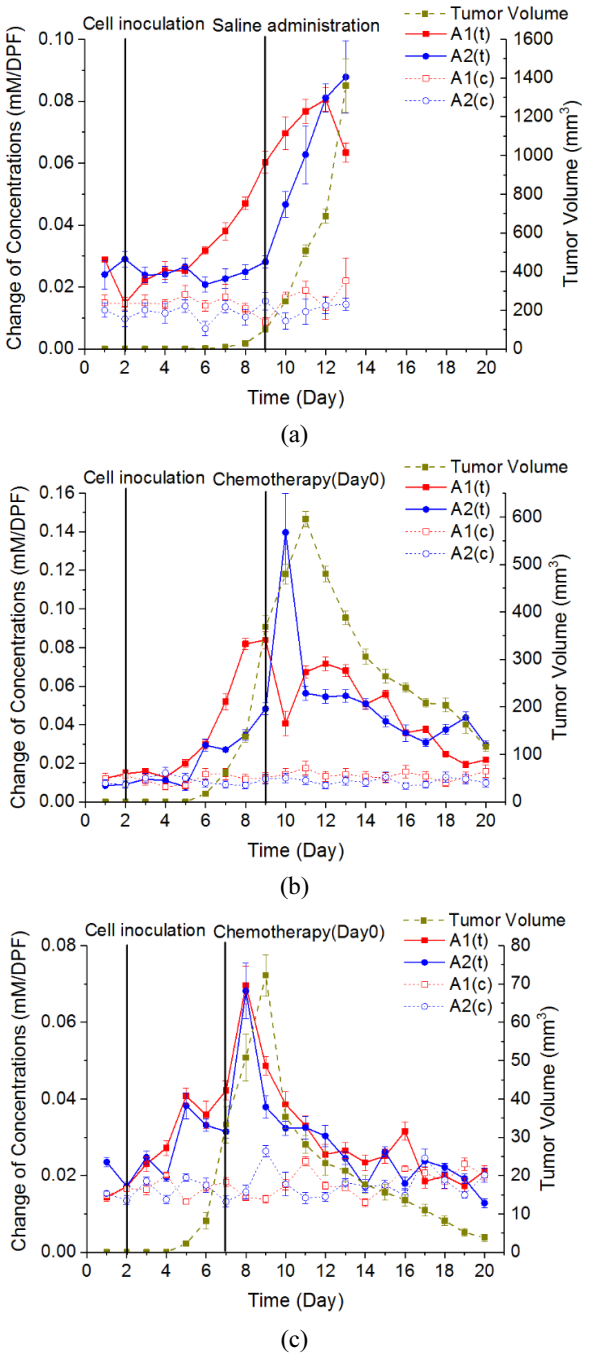


FIG. 4. The changes of A1 and A2 values and tumor-volume change from (a) the control group (n = 6), (b) chemo group (n = 6), and (c) early chemo group (n = 6). A1(t) and A2(t) represent amplitude values from the tumor breast, while A1(c) and 2(c) represent amplitude values from the contralateral normal breast.

to work in the poorly perfused region. Meanwhile, the early chemo group showed both A1(t) and A2(t) decreasing on day 2 post chemotherapy. This could be because tumors in the early chemo group were relatively small at the time of cyclophosphamide administration, so that the difference in perfusion between well-perfused and poorly perfused region was not as great as in the chemo group. This proves that monitoring the biphasic characteristics of tumor hemodynamics provides more insight into tumor response during chemotherapy.

As we described, tumor regression in the early chemo group was faster than in the chemo group, because a tumor may consist mostly of a well-perfused region rather than a poorly perfused region, which allowed cyclophosphamide to be readily delivered to the whole tumor. A common observation from Figs. 4(b) and 4(c) is that A1 changes 1 or 2 days earlier than tumor regression during chemotherapy. This can be important when we translate the results from our previous study into clinical practice, since monitoring A1 requires less than 2 minutes, while our previous vascular-reactivity measurement required 10 minutes of inhaled-gas modulation.

There are a few limitations in this study. First, we measured the biphasic characteristics of tumor hemodynamics using a single-channel CWNIRS system. Therefore, our results may not represent the whole tumor's response during chemotherapy, especially when tumor diameter is larger than the source-detector separation of our probe (5 mm). The heterogeneity of tumor vasculature requires the use of a multichannel system, which can provide information from multiple sites in the tumor. Second, the hemodynamic change measured with CWNIRS does not account for the effect of light scattering in tissue, and therefore the trend of hemoglobin-concentration changes measured in this study could be different, if the scattering properties of the tumor were to change during the respiratory-challenge protocol. Therefore, it will be important to confirm our results with a quantitative NIRS system, such as a time-domain NIRS. Despite these limitations, our results demonstrate that the A1 value, which can be obtained with a relatively short respiratory-challenge protocol, has potential as a biomarker to diagnose and to predict the efficacy of chemotherapeutic treatment of breast tumors.

V. CONCLUSION

In this study, we decomposed the magnitude of vascular reactivity during a hyperoxic inhaled-gas intervention into A1 and A2 values, by fitting the increase of tumor oxyhemoglobin concentration with a double-exponential model. This allowed us to monitor well-perfused and poorly perfused regions in the tumor individually and provided more insight into tumor response during chemotherapy. Since A1 showed an earlier response than did tumor volume post chemotherapy, the respiratory-challenge protocol can be reduced from 10 minutes to less than 2 minutes of hyperoxic gas inhalation. This will allow patients to feel more comfortable when the respiratory-challenge protocol is applied in the clinic. This study demonstrates that CWNIRS with a quick application of respiratory challenges can be a very useful tool to monitor tumor response during chemotherapy.

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