

Optical method for real-time monitoring of drug concentrations facilitates the development of novel methods for drug delivery to brain tissue

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In the brain, the vascular endothelial cells lining the blood vessels demonstrate unique anatomical characteristics that limit the passage of many molecules, creating in effect a barrier between the contents of the blood and the brain tissue. The blood-brain barrier (BBB) is a membrane that limits passage to molecules that are either very small (<400 Daltons) or highly lipid soluble (such as oxygen, carbon dioxide, ethanol, and steroid hormones). Alternatively, molecules could be transported across the barrier with the aid of specific transport mechanisms (such as sugars and some amino acids). A major challenge for the treatment of most brain disorders is the difficulty of delivering therapeutic agents across the BBB to specific regions of the brain. BBB disruption can be induced biochemically by the use of vasoactive substances, such as mannitol, which is a pharmacologically inert hyperosmotic agent. Mitoxantrone (MTX) is a water-soluble chemotherapy agent that is used in the treatment of some types of cancer, such as breast cancer, leukemia and non-Hodgkin's lymphoma, prostate cancer, and some GI cancers. MTX has a molecular weight of 454 Daltons, has optical absorption peaks at 608 and 671 nm, and does not penetrate the BBB. Recent work by others has shown that chemotherapy agents can be

Abstract. The understanding of drug delivery to organs, such as the brain, has been hampered by the inability to measure tissue drug concentrations in real time. We report an application of an optical spectroscopy technique that monitors *in vivo* the real-time drug concentrations in small volumes of brain tissue. This method will facilitate development of new protocols for delivery of drugs to treat brain cancers. The delivery of many anticancer drugs to the brain is limited by the presence of the blood-brain barrier (BBB). Mitoxantrone (MTX) is a water-soluble anticancer drug that poorly penetrates the BBB. It is preliminarily determined in an animal model that the brain tissue uptake of chemotherapy agents—in this demonstration, MTX—delivered intra-arterially is enhanced when the BBB is disrupted. © 2007 Society of Photo-Optical Instrumentation Engineers. [DOI: 10.1117/1.2744025]

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successfully delivered to the brain when the BBB is disrupted.¹⁻³

The concentration and kinetics of drugs at specific locations in the body are generally difficult to determine given only the administered dosage or blood serum measurements. There are several conventional laboratory techniques for monitoring local pharmacokinetics,⁴⁻⁹ but they lack fast temporal resolution. It has been previously demonstrated that the drug concentrations of optically absorbing drugs can be measured locally in small volumes of tissue for a wide range of tissue scattering properties by the method of optical pharmacokinetics (OP), based on a variation of diffuse reflectance spectroscopy.^{10,11} The goal of this work is to demonstrate that the OP method can measure *in vivo* and in real time the time-concentration profile of an optically absorbing chemotherapy drug in brain tissue. It is established that the concentration of a chemotherapy agent in brain tissue through intra-arterial (IA) administration is enhanced when it is coupled with the temporary disruption of the BBB. The OP technique could be useful for future studies of drug pharmacokinetics in the treatment of brain tumors.

The OP method has been described in detail in previous publications.^{10,11} Briefly, a xenon-arc lamp (Perkin Elmer, LS-1130-3) was used as an illumination light source

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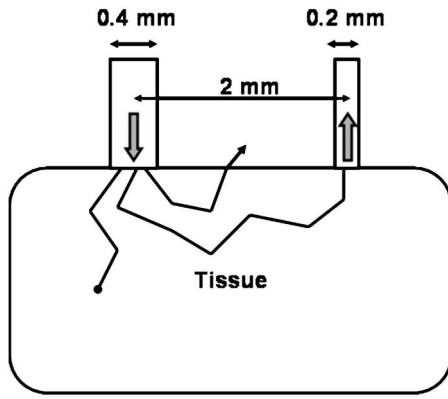


Fig. 1 Schematic diagram of the optical pharmacokinetics system.

(450 to 850 nm). Light was delivered to and collected from the tissue using optical fibers. The delivery and collection fibers had a diameter of 400 μm and 200 μm , respectively, and had a center-to-center separation of 2 mm. Both fibers had a numerical aperture of 0.22. A spectrometer (Ocean Optics, S2000) is used to collect light scattered by the tissue, and the spectrum is analyzed by a computer. A schematic diagram of the system is shown in Fig. 1.

Measurements were taken at several time points before and after drug administration. The data analysis has been previously described by Mourant et al.¹¹ Briefly, the OP system takes a background measurement followed immediately by a measurement while firing the lamp. The background measurement is subtracted from the light measurement to obtain a data point (I). The change in tissue absorption coefficient ($\Delta\mu_a$) is obtained using Eq. (1):

$$-\ln\left[\frac{I(t)}{I(t_0)}\right] = B + \Delta\mu_a L(\mu_a), \quad (1)$$

where $L(\mu_a)$ is the path length, B is a baseline shift due to the changes in the scattering parameters between the two measurements, and $I(t_0)$ and $I(t)$ are measured data points before and at any time after the injection of the drug, respectively. The baseline is defined by Eq. (2):

$$B = c_0(t) + c_1(t)\lambda + c_2(t)\lambda^2, \quad (2)$$

where $c_0(t)$, $c_1(t)$, and $c_2(t)$ are baseline coefficients, and λ is the wavelength. The path length, which is dependent on the total absorption, is determined by Eq. (3):

$$L(\mu_a) = x_0 + x_1 \exp(-x_2\mu_a), \quad (3)$$

where x_0 , x_1 , and x_2 are calibration coefficients specific to the probe geometry, which are obtained by taking measurements on a tissue phantom with known optical properties. Last, μ_a and $\Delta\mu_a$ are defined by Eqs. (4) and (5), respectively:

$$\begin{aligned} \mu_a = & [\Delta c_{HbO}(t) + c_{HbO}(t_0)]\varepsilon_{HbO}(\lambda) + [\Delta c_{Hb}(t) \\ & + c_{Hb}(t_0)]\varepsilon_{Hb}(\lambda) + c_{drug}(t)\varepsilon_{drug}(\lambda), \end{aligned} \quad (4)$$

$$\Delta\mu_a = \Delta c_{HbO}(t)\varepsilon_{HbO}(\lambda) + \Delta c_{Hb}(t)\varepsilon_{Hb}(\lambda) + c_{drug}(t)\varepsilon_{drug}(\lambda). \quad (5)$$

The initial concentrations of oxyhemoglobin and deoxyhemoglobin are represented by $c_{HbO}(t_0)$ and $c_{Hb}(t_0)$, respectively, and the changes in concentration are represented by $\Delta c_{HbO}(t)$ and $\Delta c_{Hb}(t)$, respectively. The concentration of the drug is represented by $c_{drug}(t)$, and the extinction coefficients of oxyhemoglobin, deoxyhemoglobin, and the drug are given by $\varepsilon_{HbO}(\lambda)$, $\varepsilon_{Hb}(\lambda)$, and $\varepsilon_{drug}(\lambda)$, respectively. It is important to note that the path length is wavelength dependent and varies over time as a function of the variation of the total background absorption (μ_a).

The negative log ratio of each resulting spectrum was fitted to Eq. (1) for the wavelengths of 500 to 800 nm. The parameters that were allowed to vary during the fit were Δc_{HbO} , Δc_{Hb} , c_{drug} , c_0 , c_1 , and c_2 . The values of $c_{HbO}(t_0)$ and $c_{Hb}(t_0)$ were approximated by fitting a straight line to the wavelength range of 750 to 800 nm of the initial measurement, and the line was extrapolated over the full wavelength range of interest. Eq. (1) was used, where $I(t_0)$ is the extrapolated line and $I(t)$ is the initial measurement. For this case, $\mu_a = \Delta\mu_a$, which is given by Eq. (6):

$$\mu_a = \Delta\mu_a = c_{HbO}(t_0)\varepsilon_{HbO}(\lambda) + c_{Hb}(t_0)\varepsilon_{Hb}(\lambda). \quad (6)$$

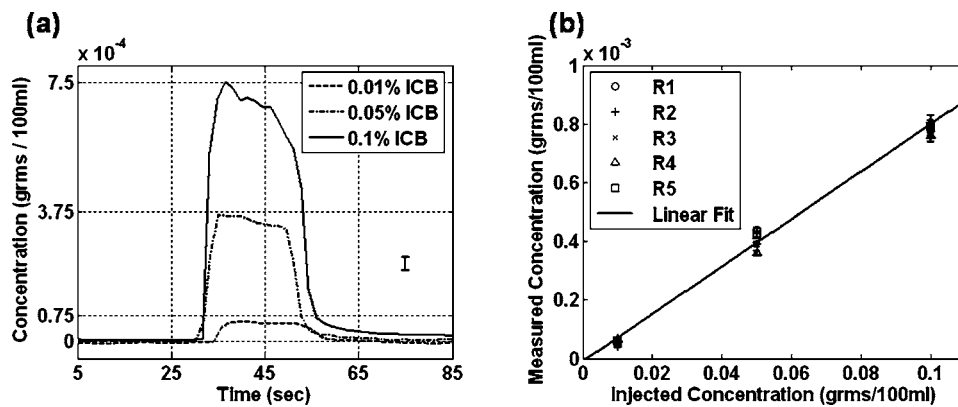


Fig. 2 (a) OP tissue measurements of Indigo Carmine Blue bolus, for 0.01, 0.05, and 0.1 g/100 ml. The error bar represents the maximum fit uncertainty. (b) Maximum peak of ICB concentration measured as a function of the ICB concentration injected. The error bars represents the fit uncertainty.

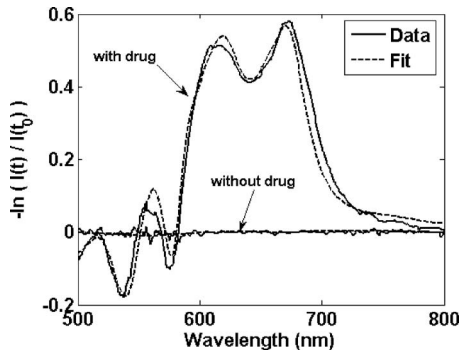


Fig. 3 Example of the negative log ratio and fit of Eq. (1) during (with drug) and after (without drug) drug infusion on an intact BBB.

Five New Zealand white rabbits (3 to 4 lb) were used as the animal models. Rabbits were selected as the experimental model because

- they present a clear, primate-like separation of the external and internal carotid circulations, permitting regionally controlled perfusion for IA administration¹²
- the rabbit's skull has a fairly large size, which permits the setup of a variety of instrumentation
- there are established tumor models in the animal species¹³
- the animal can withstand hemodynamic stress associated with the experimental protocol.¹⁴

The rabbits were anesthetized, and the left internal carotid artery (ICA) was located using the retinal discoloration test.¹⁵ For all animals, the anesthesia was maintained with 0.5-ml boluses of intravenous 1% propofol (Diprivan, Astra Zeneca). At 2 to 5 mm anterior to the bregma and 3 to 5 mm lateral to the midline on the surface of the brain, a small area (~4 mm diam) of the skull was shaved down until a thickness of ~100 μm was obtained, and the optical fiber probe was placed in contact with the remaining thin layer. Optical contact between the fiber probe and the thin layer of skull was assisted by wetting the surface with water. A catheter was inserted into the left branch of the carotid, to control the blood perfusion and to administer the compounds.

Each of the five rabbits was injected through the ICA with three boluses of Indigo Carmine Blue (ICB) (MW: 466.35). Each bolus had a different concentration value of 0.01, 0.05, and 0.1 g per 100 ml of saline. Each bolus consisted of 2 ml of solution manually infused over 20 s. Measurements were taken once every second for a period of 2 minutes. Indigo Carmine Blue does not penetrate the BBB under normal conditions; therefore, the measurements obtained relate to the amount of drug contained within the blood compartment, which occupies a fraction of the small tissue volume probed with the OP system. The boluses were injected 5 min apart. Since blood-borne ICB is rapidly removed by the kidneys, local ICB concentrations in the brain were below the measurement threshold after 5 min. Figure 2(a) depicts the time-concentration profile of the ICB (in the blood compartment) as measured by the OP system in the brain tissue during the infusions of the three dye concentrations in one rabbit. The maximum peak of the ICB concentration measured in each animal is plotted as a function of the injected ICB concentration in Fig. 2(b). The variability of the measured drug concentration from animal to animal is very small. It is observed that the concentration measured by the OP system increases linearly, showing proportionality to the concentration of the dye injected. This demonstrates the linearity of the system during *in vivo* measurements in the model.

The rabbits were separated into two groups. Group 1 consisted of two rabbits for which the BBB remained intact, and Group 2 consisted of three rabbits for which the BBB was disrupted. MTX was injected into all the animals. There was a 1-h waiting period between the end of the ICB injection and the beginning of the MTX experiment to allow enough time for the ICB to clear from the system.

For Group 1, MTX was administered as a single bolus. For Group 2, 10 ml of 25% mannitol was first infused through the IA catheter over 40 s to temporarily disrupt the BBB, followed (within 1 min) by a single bolus of MTX. For both groups, solutions of 0.1% MTX were prepared in a hood as per OSHA guidelines and in compliance with hospital environment health and safety regulations. A 3-ml bolus of MTX solution was manually injected through the ICA during 40 s.

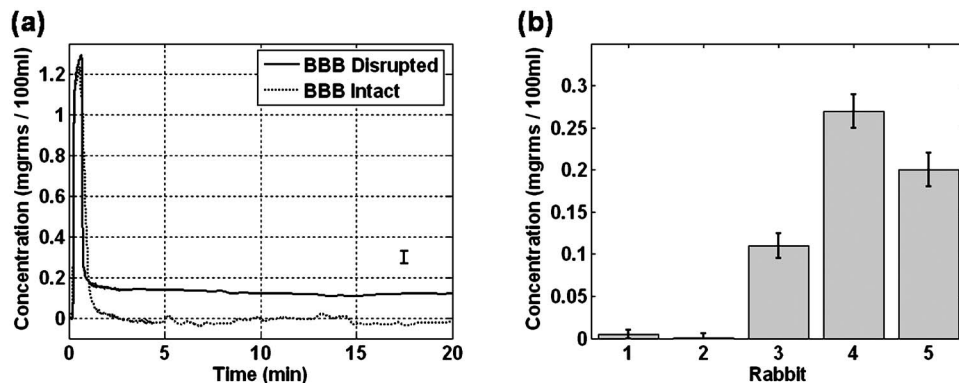


Fig. 4 (a) Concentration time history of MTX for a disrupted BBB (solid line) and an intact BBB (dotted line). The error bar represents the maximum fit uncertainty. (b) Concentration of Mitoxantrone, 20 min after injection on a group with an intact BBB (rabbits 1 and 2) and a disrupted BBB (rabbits 3, 4, and 5). The error bars represents the fit uncertainty.

OP measurements were taken every second for the first 3 min and every 20 s for the following 17 min.

The negative log ratio at each time point was fitted to Eq. (1). Examples of the fit at a time point during drug infusion (“with drug”) and at a time point after drug infusion, when the drug has fully dissipated from the animal’s system (“without drug”) on a rabbit with an intact BBB, are shown in Fig. 3. The spectrum “without drug” is a flat line at a value near 0, indicating that there has not been a significant change in the amount of blood, drug, or scattering between times before and sufficiently long after drug infusion. The spectrum during drug infusion presents negative features in the UV-VIS region due to the reduction of the amount of blood, which was displaced by drug in the blood vessels, and positive features in the VIS-NIR region due to the drug present in the blood vessels. A small baseline shift is noted.

The absorption spectrum of MTX is affected by the ratio of the amount of monomeric and dimeric forms present and by the percentage of drug bound to DNA. The native extinction coefficient of MTX was determined using a spectrophotometer (Varian, Cary-50). The data analysis was performed both with the spectrum obtained from the spectrophotometer and with the spectrum red shifted by 18 nm, which would be indicative of DNA-bound form.¹¹ The R^2 of the fits were smaller with the spectrum obtained by the spectrophotometer; therefore, the data analysis presented in this paper assumes that within 20 min of drug infusion, the amount of DNA-bound MTX in brain tissue is negligible under these conditions.

Two examples of the time-concentration profile of MTX are shown in Fig. 4(a) one with an intact BBB and one with a disrupted BBB. During the first 40 s, the signal increases to a maximum peak. This initial peak lasts 40 s and is due to the bolus of drug contained in the blood compartment while it is being injected through a catheter in the ICA. During infusion, the blood vessels are completely perfused by the drug, resulting in significant blood displacement. After the infusion of the drug is completed, the blood reenters the vessels in the brain and the drug circulates through the body, where it binds with plasma proteins, gets diluted, concentrates in some tissues with high affinity for the drug, and eventually is excreted from the body. After the 40-s bolus has passed, there is a significant amount of drug remaining in the brain of the rabbit with a disrupted BBB, whereas in the rabbit with an intact BBB, the concentration returned to baseline levels, indicating lack of drug retention in the brain tissue.

Figure 4(b) depicts the final concentration of MTX in brain tissue for both groups of rabbits 20 min after drug administration. It is observed that the concentration remaining in the rabbits with a disrupted BBB is significantly higher than the group with an intact BBB. The variation in the drug concentration after 20 min of the group with a disrupted BBB is significant, which is indicative of the drug pharmacokinetic variability from animal to animal.

Real-time measurements of drug concentrations in the brain can facilitate the development of drug delivery protocols aimed at the central nervous system. Conventional techniques for monitoring drug concentration in tissue require many animals and biopsy measurements to obtain a time history, with crude temporal resolution. Optical measurements can provide

a full temporal history, with fine temporal resolution, and with a single animal. A detailed time history of drug concentration in the brain of a single animal would be advantageous during the animal study phases of drug development, since fewer animals would be required and animal-to-animal variability can be monitored. It should be noted, again, that the OP method is limited to drugs that are chromophoric or can be tagged with a chromophore.

It is prudent to mention that there might be errors propagated throughout the measurements. Possible sources of error might include:

- Inaccuracies in the extinction coefficients of hemoglobin and drugs.
- The technique used to measure the initial background absorption of hemoglobin [$c_{Hb}(t_0)$ and $c_{HbO}(t_0)$] is an approximation.
- There could be cross talk between the baseline term of Eq. (1) and the fitting parameters of the blood and drug.

In summary, we have demonstrated the use of a simple optical technique that can monitor real-time drug concentrations *in vivo* in brain tissue, on a fast time scale, and we describe the method’s ability to track local dynamics of tissue concentrations with and without BBB disruption after IA delivery. In this preliminary study, the benefit of BBB disruption has been demonstrated for MTX, although its suitability for treating human brain tumors is not known. Future studies will utilize the OP method to assess the influence of different blood-flow control protocols on the delivery of drugs to the brain.

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