Correlation between light scattering and reduction level of cytochrome oxidase in perfused brains of rats

S. Kawauchi[1], S. Sato[2], H. Ooigawa[3], H. Nawashiro[3], K. Shima[3] and M. Kikuchi[1]

[1] Department of Medical Engineering, National Defense Medical College, Tokorozawa, Japan
[2] Division of Biomedical Information Sciences, National Defense Medical College Research Institute, Tokorozawa, Japan
[3] Department of Neurosurgery, National Defense Medical College, Tokorozawa, Japan

Abstract— **To investigate the correlation between light scattering and tissue viability for brains, we performed multi-wavelength diffuse reflectance measurement with perfused brains of rats, in which the reduction level of CuA in cytochrome oxidase was used as an indicator of brain tissue viability. Diffuse reflectance intensity at 620 nm, an isosbestic point of the oxidation-reduction state of cytochrome oxidase, was detected as a scattering signal, while relative diffuse reflectance intensity at 800 nm to that at 620 nm was measured to monitor the absorption change due to the reduction of CuA. After starting perfusion, the scattering signal showed a drastic, triphasic change (increase, decrease and increase) in the time range of 220 – 310 s. After this triphasic change, the scattering signal increased slowly until the end of the measurement (~ 500 s). The reduction of CuA started and proceeded rapidly during the triphasic scattering change (270 – 310 s). Before and after the triphasic change, we found that light scattering highly correlated with the reduction level of CuA; loss of tissue viability was accompanied by increase in light scattering. These results suggest that the detection of triphasic scattering change is useful to predict loss of tissue viability in brains.**

Keywords— **light scattering, brain, tissue viability, cytochrome oxidase, multiwavelength diffuse reflectance measurement**

I. Introduction

Noninvasive, real-time monitoring of brain tissue viability is important for intraoperative and intensive care management as well as for future regenerative medicine. Since metabolic energy production is required to maintain the structural integrity of cells in brain tissue [1], its viability is thought to be connected with morphological characteristics of cells and organelles in brain tissue. Thus, light scattering, which is sensitive to structural changes in cells and organelles, can provide information on brain tissue viability. It has been demonstrated that for whole brains and brain tissue slices [2,3], light scattering decreases during ischemia or hypoxia; the decrease in light scattering has been attributed to cell swelling. Biphasic scattering change (decrease and increase) has also been reported for brain tissue slices [3,4]; mitochondrial swelling has been suggested as a possible reason for the scattering increase [5]. A recent study with isolated brain mitochondria has shown that the scattering increase during anoxia is caused by mitochondrial matrix shrinkage [6]. Thus, change in light scattering during loss of tissue viability of brains has not been clarified.

In the present study, we investigated the correlation between light scattering and the reduction level of CuA in cytochrome oxidase during loss of tissue viability for brains. Since the reduction level of CuA indicated by change in the absorption at ~ 830 nm has been shown to closely correlate with cerebral ATP level [7], we used the light absorption signal due to the reduction of CuA as an indicator of brain tissue viability. Changes in light scattering and light absorption in brain tissue were examined by multiwavelength diffuse reflectance measurements.

II. Materials and Methods

Figure 1 shows a diagram of the experimental setup. Rats were anesthetized and placed in a stereotactic frame; after a midline scalp incision, the bone was exposed over the parietal cortex of the right hemisphere, in which a cranial window with a diameter of 5 mm was prepared. For diffuse reflectance measurement, a pair of optical fibers (source and detector fibers) was placed on the exposed brain surface; the core diameters and center distance of the fibers were 800 μm and 1.5 mm, respectively. NIR light from a LD (center wavelength, 799 nm; spectral width, 4 nm FWHM) and red light from LED (peak wavelength, 621 nm; spectral width, 16 nm FWHM) were coupled to the source fiber, and the light that traveled through the tissue was collected by the detector fiber and analyzed with a polychromator. Diffuse reflectance intensity at 620 nm, an isosbestic point of cytochrome oxidase [6,8], was detected to examine the change in light scattering and relative diffuse reflectance intensity at 800 nm to that at 620 nm was measured for monitoring change in the reduction level of CuA in cytochrome oxidase. These measurements were started at 30 s after starting brain perfusion for which saline containing heparin at a concentration of 1.0 U/mL was used as a perfusion solution. Such a rapid blood removal enables us

to characterize light scattering and the reduction level of CuA without effects of blood components.

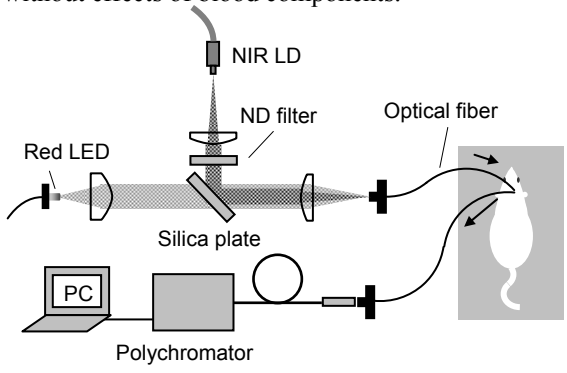

Fig. 1 Diagram of the experimental setup for diffuse reflectance measurement for brains of rats.

III. Results and Discussion

Typical time courses of diffuse reflectances at 620 and 800 nm after starting perfusion are shown in Fig. 2. After starting perfusion, reflectance intensity at 620 nm increased gradually (phase I) and it became constant at ~ 150 s (phase II). Subsequently, reflectance intensity rapidly increased, decreased, and increased again in the time range of 220 – 310 s (phases III - V), after which reflectance intensity increased slowly until the end of the measurement (~ 500 s) (phase VI). The initial increase in reflectance intensity at 620 nm is thought to be due to decrease in blood volume (phase I). Thus, after the beginning of phase II, blood might be completely removed from the brain, indicating that cytochromes and cytochrome oxidase are dominant chromophores in the tissue (phase II - VI). The triphasic reflectance change at 620 nm, *i.e.*, triphasic scattering change in phase III – V is probably due to morphological transition of cells and/or organelles by anoxic depolarization in the tissue. Although reflectance intensity at 800 nm also showed similar change in phases I - III, remarkable difference was seen in phases IV - VI when compared with that at 620 nm. The higher level of reflectance intensity at 800 nm than that at 620 nm in these phases indicates decreased absorption at 800 nm, *i.e.*, the reduction of CuA in cytochrome oxidase. Thus, to show the change in the reduction level of CuA clearly, the reflectance intensity at 800 nm (R_{800}) is normalized by that at 620 nm (R_{620}) in Fig. 3, where an increase in R_{800}/R_{620} represents the progress of the reduction of CuA. The time course of R_{800}/R_{620} showed that the reduction of CuA started and proceeded rapidly in the time range of 270 – 310 s (phases IV and V). This result indicates that the reduction of CuA proceeded during the drastic, triphasic scattering change. Before and after the triphasic scattering change (phase II and VI), we found that the scattering signal was highly correlated with the change in the reduction level of CuA; the loss of brain tissue viability was accompanied by an increase in light scattering. In Fig. 3, it can also be noted that the first increase in the scattering signal (phase III) preceded the start of reduction of CuA, suggesting that a rapid increase in scattering signal can predict the beginning of loss of tissue viability in brains.

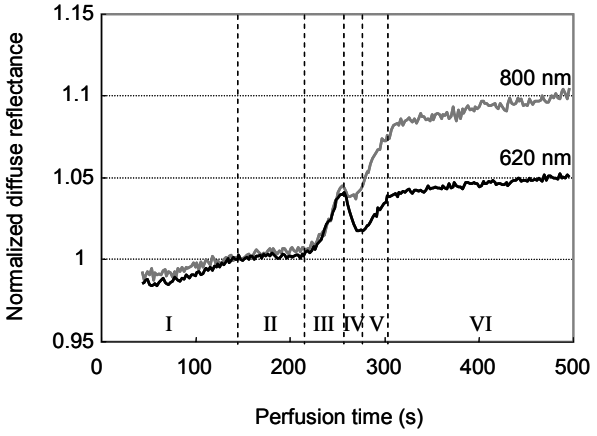

Fig. 1 Typical time courses of diffuse reflectances at 620 and 800 nm during saline perfusion.

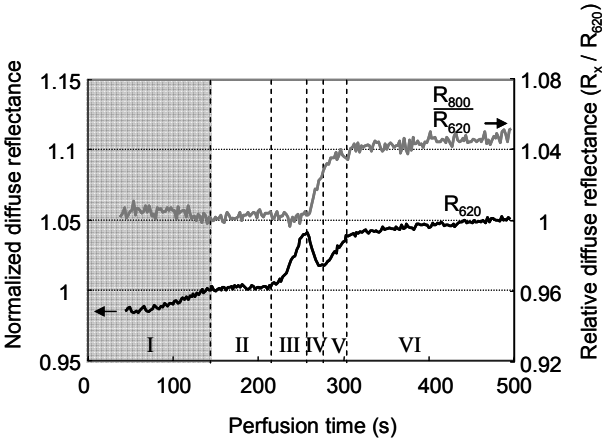

Fig. 3 Time courses of diffuse reflectance at 620 nm and relative diffuse reflectance at 800 nm to that at 620 nm.

IV. Conclusions

Diffuse reflectance measurements for perfused brains in rats showed that triphasic change in light scattering occurred in the perfusion time range of 220 - 310 s. During

this range, the reduction of CuA started and proceeded rapidly. Before and after the triphasic change, there was a close correlation between the scattering signal and the reduction level of CuA, showing that loss of tissue viability was accompanied by increase in light scattering. These findings suggest that light scattering signal becomes an indicator of tissue viability for brain. As a next step, we plan to perform experiments using a brain model with reversible viability, such as an ischemia-reperfusion model.

Acknowledgment

We thank Toshihiko Kiritani for technical support in the use of LED devices.

References

1. Hossmann KA (1994) Viability thresholds and the penumbra of focal ischemia. Ann Neurol 36:557-565
2. Harrison DK, Delpy DT (1997) Oxygen Transport to Tissue XIX. Plenum Press, New York
3. Tao L, Masri D, Hrabetova S et al (2002) Light scattering in rat neocortical slices differs during spreading depression and ischemia. Brain Res 952: 290-300.
4. Muller M, Somjen GG (1999) Intrinsic optical signals in rat hippocampal slices during hypoxia-induced spreading depression-like depolarization. J Neurophysiol 82:1818-1831
5. Bahar S, Fayuk D, Somjen GG et al (2000) Mitochondrial and intrinsic optical signals imaged during hypoxia and spreading depression in rat hippocampal slices. J Neurophysiol 84:311-324.
6. Fujii F, Nodasaka Y, Nishimura G et al (2004) Anoxia induces matrix shrinkage accompanied by an increase in light scattering in isolated brain mitochondria. Brain Res 999:29-39.
7. Matsunaga A, Nomura Y, Kuroda S et al (1998) Energy-dependent redox state of heme a + a3 and copper of cytochrome oxidase in perfused rat brain in situ. Am J Physiol Cell Physiol 275:C1022-1030.
8. Nomura Y, Matsunaga A, Tamura M et al (1998) Optical characterization of heme a + a3 and copper of cytochrome oxidase in blood-free perfused rat brain. J Neurosci Methods 82:135-144.

Address of the corresponding author:

Author:	Satoko Kawauchi
Institute:	National Defense Medical College
Street:	Namiki 3-2
City:	Tokorozawa
Country:	Japan
Email:	skawauch@ndmc.ac.jp