Noninvasive optical imaging of the subarachnoid space and cerebrospinal fluid pathways based on near-infrared fluorescence

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The authors have developed a noninvasive optical method to image the subarachnoid space and cerebrospinal fluid pathways in vivo based on the near-infrared fluorescence of indocyanine green (ICG).

The ICG was bound to purified lipoproteins (ICG–lipoprotein) and injected into the subarachnoid space of neonatal and adult rats. The ICG fluorescence was detected by a cooled charge-coupled device camera. After injection of ICG–lipoprotein into the cerebral subarachnoid space of the neonatal rat, ICG fluorescence was clearly detected at the injection site through the skull and skin. The ICG fluorescence was observed in the cerebellum and the lumbar spinal cord 1 and 8 hours postinjection, respectively. After injection of ICG–lipoprotein into the lumbar spinal subarachnoid space of an adult rat, ICG fluorescence was observed from the injection site to the thoracic levels along the spinal subarachnoid space. In addition, with the rat’s head tilted downward, ICG fluorescence had extended to the cerebral subarachnoid space by 1 hour postinjection. The ICG fluorescence imaging of the cerebral subarachnoid space demonstrated an increase in fluorescence intensity around the lambdoid suture and the forebrain. On dissection of the rat brain the former location was identified as the supracerebellar cistern and the latter as the olfactory cistern.

The results of this study are the first to demonstrate that an optical technique is applicable to imaging of the subarachnoid space and cerebrospinal fluid pathways in vivo. In addition, ICG–lipoprotein provides a sensitive optical tracer for imaging extravascular biological structures. Finally, ICG fluorescence imaging does not require an intricate imaging system because ICG is localized near the surface of the body.

KEY WORDS • cerebrospinal fluid flow • fluorescence • indocyanine green • near-infrared light • subarachnoid space • rat

CEREBROSPINAL fluid (CSF) flow is one of the major factors affecting cerebral functions. Obstruction of CSF pathways leads to an increase in intracranial pressure, resulting in cerebral functional disorders. Recent advances in magnetic resonance (MR) imaging allow us to measure not only morphological changes in the subarachnoid space but also abnormal movement of local CSF, which is demonstrated by a flow void signal. Magnetic resonance imaging is a powerful noninvasive diagnostic tool for CSF flow disorders; however, invasive diagnostic methods such as myelography and radioisotope cisternography are still useful for measuring the pathways and dynamics of CSF flow. In addition, noninvasive diagnostic techniques such as MR imaging require relatively large and expensive equipment. Therefore, the development of other noninvasive methods is necessary to address the deficiencies of the currently available techniques.

Near-infrared light is known to be highly transmittable through biological tissue. In addition, important chromophores such as hemoglobin show characteristic absorption spectra in the near-infrared range. These characteristics of near-infrared light allow noninvasive measurement of the oxygenation state and blood flow of biological tissue. In addition to exploiting the optical properties of chromophores in vivo, administration of optical contrast agents is another method to measure biological structures and functions. For example, indocyanine green (ICG), which is used for clinical examinations such as liver function tests, has been used as a contrast agent for retinal angiography and two-dimensional imaging of cerebral blood flow because of its absorption property in the near-infrared light range. In addition, ICG absorption imaging has recently been used to demonstrate tumor margins in the brain by comparing blood flow in tumor tissue with that in normal brain tissue.

Indocyanine green also emits near-infrared fluorescence when it is excited by near-infrared light. The near-infrared fluorescence of ICG was applied to the imaging of retinal angiogenesis and the detection of experimental tumors by the clearance method. We compared near-infrared fluorescence imaging with absorption imaging and demonstrated that the detectability of ICG in biological tissues by using fluorescence imaging exceeds that...
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![Graph comparing the fluorescence spectrum of ICG dissolved in water and lipoprotein.](image)

**Fig. 1.** Graph comparing the fluorescence spectrum of ICG dissolved in water and lipoprotein.

achievable using absorption imaging. However, ICG must be bound to proteins such as plasma proteins to emit an adequate intensity of fluorescence. Therefore, ICG fluorescence imaging outside blood vessels requires a device that emits adequate fluorescence.

In the present study, we attempted to visualize the subarachnoid space and CSF pathways by exploiting the near-infrared fluorescence of ICG. The ICG was bound to purified lipoproteins and injected into the subarachnoid space of rats. Some of our results have appeared in abstract form.

**Materials and Methods**

**Optical Contrast Agents**

We used ICG as an optical contrast agent. In circulating blood most ICG is bound to lipoprotein; thus we bound ICG to purified human high-density lipoprotein before administration into the subarachnoid space. The ICG was diluted with distilled water to a concentration of 5.54 mM, and 5 μl of 5.54 mM ICG was mixed with 500 μl of human lipoprotein.

Figure 1 compares the fluorescence spectra of ICG dissolved in water with ICG bound to lipoprotein. The fluorescence spectrum was measured by a fluorescence spectrophotometer. Indocyanine green emits fluorescence in lipoprotein; however, in water it does not emit detectable fluorescence.

**Imaging System**

The imaging system is shown schematically in Fig. 2. We used two kinds of light sources for the excitation of ICG: a 150 W xenon bulb with a bandpass filter (central wavelength 740 nm) for the neonatal rat and a laser diode (wavelength 789.8 nm) for the adult rat.

Although the optical power of the laser diode was enough to excite ICG fluorescence in the adult rat, the amount of stray light from the imaging system using the laser diode was slightly more than that using the combination of xenon bulb and optical filter because the wavelength of the laser diode (789.8 nm) was close to the central wavelength of the bandpass filter (840 nm) attached to a cooled charge-coupled device (CCD) camera.

The ICG fluorescence was recorded by a cooled CCD camera (512 × 483 pixels, 16 bit, cooling temperature −34°C) with a camera lens and a bandpass filter (central wavelength 840 nm). An image processor was used for image capture and analysis. The digital images obtained by the CCD camera were accumulated on a frame memory of the image analyzer. The fluorescence images in Figs. 3 to 6 were obtained as a result of 100 frames of accumulation.

We could obtain similar results in as few as 20 frames of accumulation. A subtraction of the fluorescence imaging from the control image was not necessary because of a high signal-to-noise ratio in the present imaging system.

**Animals and ICG Administration**

We used neonatal (5–7 days old, 10 animals) and adult Wistar rats (100 g, 10 animals) in the experiment. Neonatal rats were anesthetized by cooling and 100 μl of ICG–lipoprotein was injected into the right frontoparietal cerebral subarachnoid space. It was impossible to avoid injecting ICG–lipoprotein into the cortex because of the small head size. During recording of ICG fluorescence, the rats were anesthetized by cooling and placed horizontally on a plastic plate.

In the study in which adult rats were used, the animals were anesthetized by intraperitoneal injection of pentobarbital (40 mg/kg), and the hair on the skin surface was shaved off to eliminate unnecessary light scattering. After laminectomy at L-6, 50 μl of ICG–lipoprotein was injected into the L-6 subarachnoid space. During recording of ICG fluorescence, the rat was placed on a plastic plate with its head tilted downward at an approximately 30° angle.

To identify the specific cerebral cisterns detected by the fluorescence imaging, the rat brain was dissected after the injection of ICG. The ICG was diluted with distilled water to a concentration of 5.54 mM, and 300 μl of this preparation was injected into the L-6 subarachnoid space. The brain was dissected after the rat’s head had been tilted downward at an angle of approximately 30° for 1 to 2 hours.
Sources of Supplies and Equipment

The ICG (Dianogreen) was supplied by Daiichi Pharmaceutical Co., Tokyo, Japan and the high-density lipoprotein by Organon Technica, Durham, NC. The fluorescence spectrophotometer (model 850) was manufactured by Hitachi, Tokyo, Japan. The 150 W xenon bulb (Fiber Optic Light Source) and the CCD camera lens (Mikro-Nikkor) were manufactured by Nikon, Tokyo, Japan. The bandpass filter (model IR840) was purchased from Asahi-bunko, Tokyo, Japan; the laser diode (model SLD-304XT) was obtained from Sony, Inc., Tokyo, Japan; and the cooled CCD camera (C-4880-11) and the image processor (Argus-50) were purchased from Hamamatsu Photonics, Hamamatsu, Japan.

Results

Figure 3 shows fluorescence imaging in the neonatal rat after injection of ICG into the right frontoparietal cerebral subarachnoid space. Immediately postinjection, ICG fluorescence was localized around the injection site (Fig. 3B), with ICG fluorescence extending to the posterior portion of the brain, including the cerebellum, 1 hour after the injection (Fig. 3C). In addition, 8 hours postinjection, ICG fluorescence was observed in the brain and the lumbar spinal cord (Fig. 3D). The presence of ICG fluorescence in the brain was caused by migration of ICG–lipoprotein to the brain tissue after injection. The ICG fluorescence intensity was much higher in the spinal cord than in the brain. The presence of ICG fluorescence in the lumbar spinal cord suggests that ICG–lipoprotein was transferred from the injection site to the lumbar spinal subarachnoid space via CSF pathways by diffusion and CSF flow.

Figure 4 shows ICG fluorescence imaging of the spinal subarachnoid space of the adult rat. The ICG fluorescence was clearly detected from the injection site to the lower thoracic levels along the spinal cord (Fig. 4C). The ICG fluorescence that leaked out at the injection site was also noted. As the focus of the imaging was moved to more rostral levels, ICG fluorescence was detected at the throracic (Fig. 4D and E) and cervical levels (Fig. 4F). The outline of the ICG fluorescence image in the cervical subarachnoid space (Fig. 4F) was not as distinct as those obtained in the lumbar and thoracic subarachnoid spaces (Fig. 4D and E).

Figure 5 shows ICG fluorescence imaging of the cerebral subarachnoid space in the adult rat. The rat brain underwent continuous imaging after the injection of ICG at the L-6 subarachnoid space with the head tilted downward. Five minutes postinjection (Fig. 5C), ICG fluorescence was detected around the lambdoid suture and then appeared gradually in the forebrain with an increase in fluorescence intensity at the lambdoid suture (Fig. 5D–F). Finally, these areas of high-intensity fluorescence merged into each other and tended to form an oval (Fig. 5G and H). There were no prominent changes in the fluorescence images approximately 1 hour after injection.

Figure 6 shows the time course of the fluorescence intensity in three regions of interest in the rat head. The changes in fluorescence intensity at the lambdoid suture and the forebrain were measured, with the intensity change at the left ear serving as a control. The fluorescence intensity at the lambdoid suture increased 5 minutes postinjection and gradually reached a plateau. The intensity at the forebrain showed a similar time course after the injection. However, the intensity at the forebrain was less than that at the lambdoid suture. The intensity at the left
ear increased slightly after the injection and stayed at that level.

Figure 7A shows ICG fluorescence imaging of the cerebral subarachnoid space viewed from the left and right lateral oblique directions. High-intensity fluorescence was observed mainly at the frontoparietal area, particularly around the lambdoid suture. In addition, the bilateral temporoparietal area showed a moderate increase in fluo-
cence intensity. However, high-intensity fluorescence was not observed caudal to the lambdoid suture or below the orbitomeatal line.

Dissection of the rat brain demonstrated the presence of ICG in all of the cerebral cisterns 1 to 2 hours after ICG injection into the L-6 subarachnoid space. However, the intensity of ICG in the different cisterns varied. Figure 7B illustrates the distribution of ICG in the cerebral cisterns viewed from parietal and basal directions. From the parietal view, high-intensity ICG was observed in the supra-cerebellar cistern, the olfactory cistern, and the cisterna magna. Indocyanine green was also observed in the interhemispheric and the middle cerebral artery cisterns; however, its intensity in these cisterns was less than in the first three mentioned. From the basal view, high-intensity ICG was observed in all of the basal cisterns. In addition, the coronal section of the brain demonstrated an accumulation of ICG in the lateral ventricle (not shown).

**Discussion**

These results are the first to demonstrate that a noninvasive optical technique is applicable to imaging of the subarachnoid space and CSF pathways based on near-infrared fluorescence of ICG. In addition, ICG bound to lipoprotein provides a sensitive optical tracer for obtaining images of biological structures and functions outside blood vessels, as in the subarachnoid space.

Optical diagnostic techniques have begun to be recognized as new and powerful monitoring methods providing noninvasive continuous assessment of tissue oxygenation and hemodynamics. At present, near-infrared spectroscopy (NIRS) is the most practical among the optical diagnostic methods. However, single-channel NIRS, which is commercially available, cannot image biological functions and structures. To address this disadvantage of NIRS, there have been efforts to develop optical bioimaging methods such as optical computerized tomography (CT). Another hopeful optical method is imaging by CCD camera. Compared with optical CT, imaging by CCD camera has the following advantages: 1) two-dimensional imaging can be performed with a CCD camera; 2) imaging by CCD camera does not require an intricate image analyzing system; and 3) the imaging system of the CCD camera is more compact than that of the optical CT. Therefore, the CCD camera has been used in the experi-
mental study of cerebral blood flow and applied to retinal angiography.

Two methods using a CCD camera to obtain images of ICG accumulated in vivo have been reported: fluorescence and absorption. Recently, we used a CCD camera to compare fluorescence imaging with absorption imaging for their ability to detect ICG accumulated in the mouse brain. The ICG located in the brain could be detected by fluorescence imaging through the skin, the skull, and the brain tissue at a minimum concentration of approximately 2 μmol; however, ICG at much higher concentrations (≥1350 μmol) along with the presence of an iron needle could not be detected at the same depth by absorption imaging. This certainly indicates that the detectability of ICG in biological tissues by using fluorescence imaging exceeds that achievable using absorption imaging.

In absorption imaging, back-scattering of light in tissue and reflection of light from the surface disturb the imaging of an absorbing obstacle. In contrast, fluorescence imaging can decrease both of these effects because the wavelength of excitation light differs from that of fluorescence. In addition, ICG becomes a secondary light source in tissue during fluorescence imaging. Thus, a higher light intensity and a more precise image are made available by fluorescence imaging because there is less light scattering and absorption in tissue.

Indeed, we obtained clear imaging of the rat spinal subarachnoid space, particularly at the lumbar and thoracic levels, in the present experiment. The ICG fluorescence could be detected at the cervical spinal level; however, clear imaging of that space could not be obtained. This may be explained by the fact that the cervical muscles, which are relatively thick, scatter and absorb the light. On the other hand, the structure of the cerebral subarachnoid space is more complex than the spinal subarachnoid space because of the ventricular system. The ICG fluorescence imaging of the cerebral subarachnoid space demonstrated an increase in fluorescence intensity around the lambdoid suture and the forebrain. Dissection of the rat brain identified the former as the supracerebellar cistern and the latter as the olfactory cistern. In addition, an elliptical area of increased fluorescence intensity was observed around the lateral ventricle. The basal cisterns and the cisterna magna were also filled by ICG; however, the fluorescence intensity in these cisterns may be very low because the brain tissue, the muscle, and the soft tissue around the basal cisterns and the cisterna magna are thicker than those around the supracerebellar and olfactory cisterns and the lateral ventricle, resulting in greater light scattering and absorption.

The depth at which ICG is detectable in tissue by means of a CCD camera is limited because of light scattering and absorption. There have been efforts recently to develop a tomographic imaging device to detect fluorescent markers...
in thick tissue; these studies have not progressed beyond in vitro experiments using phantoms. However, the present simple system using a CCD camera suffices to obtain two-dimensional imaging of ICG fluorescence, insofar as ICG locates near the tissue surface.

Possible applications of the ICG fluorescence imaging

**Fig. 7.** A: Indocyanine green fluorescence imaging of the cerebral subarachnoid space of the adult rat viewed from right (upper) and left (lower) oblique directions. Dotted lines indicate the superior sagittal and lambdoid sutures. B: Schematic drawings of the distribution of ICG (gray areas) in the cerebral cisterns viewed from the parietal (left) and basal (right) directions. High-intensity ICG (dark gray area) was observed in the supracerebellar cistern (SCC), the olfactory cistern (OLC), the cisterna magna (CM), and all of the basal cisterns. Moderate-intensity ICG (light gray area) was observed in the interhemispheric cistern (IHC) and the middle cerebral artery cistern (MCAC).
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technique should be considered. This imaging system can be a simple and inexpensive method to study CSF circulation in hydrocephalic rat models, such as HTX rats. Although the CSF pathways in the adult human cannot be examined by the present imaging system, it may be applicable to ventriculoperitoneal shunt studies because ventricular shunt systems are implanted just under the skin. In addition, if ICG can be combined with antibodies that are tumor specific, this optical probe could be used to detect residual tumor tissues that extend deeply into the brain because near-infrared light with ICG fluorescence is highly transmittable through biological tissue. Imaging of a tumor during an operation can increase the removal rate and decrease the risk of invasion in normal brain tissue. Finally, we emphasize that this imaging system can easily be placed on a surgical microscope.

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References


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