ICG fluorescence endoscope for visualization of the placental vascular network

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Abstract
Intrauterine laser photocoagulation for twin-twin transfusion syndrome (TTTS) needs accurate in-situ recognition of placental vascular anastomosis. Because the conventional procedure is highly dependent upon the operators' skill and experience, we developed a new way to visualize the placental vascular network by a rigid-type fluorescence endoscope coupled with intravenous administration of Indocyanine green (ICG). The feasibility of the fluorescence endoscope was examined with monkey placentas and pregnant rats. The ICG fluorescence endoscope can visualize the placental vascular network in detail even in the presence of turbid amniotic fluid. Thus, this method is potentially useful for in-situ definition of the placental vascular anastomoses during the treatment for TTTS. In addition, our rigid-type fluorescence endoscope will also be a useful tool for lymph node dissection using ICG by endoscopic surgery.

Key words: Endoscope, fluorescence imaging, fetal surgery, Indocyanine green, placenta

Introduction
Twin-twin transfusion syndrome (TTTS) is a major complication developed in 6–12% of monochorionic twin pregnancies (1,2). Its etiology is a blood flow imbalance through anastomotic vessels in the shared placenta (3). The donor fetus develops oliguria and oligohydramnios in response to hypovolemic, while the recipient fetus becomes polyuria and polyhydramnios. TTTS is diagnosed relatively easily as the discordance of amniotic fluid between two fetuses. Currently, the effectiveness of intrauterine laser photocoagulation of the placental anastomotic vessels is better than conventional amnioreduction (4–6). However, laser therapy under a fetoscope requires a highly skilled operator to distinguish pathogenic anastomosis, especially when it exists beneath the surface. A careful investigation concluded the involvement of hidden connections in the intertwin transfusion (7). Therefore, a technical assist to recognize blood vessels should be helpful.

In this communication, we present a new fluorescence endoscope to visualize not only superficial but also rather deep blood vessels, in combination with Indocyanine green (ICG) as a test fluorochrome that emits near infrared fluorescence. Using this new technological device, we demonstrate the feasibility of this technique through a study using monkey placentas and pregnant rats in vitro and in vivo.

Material and methods

Instruments
The ICG fluorescence endoscope was developed by remodeling the rigid type endoscopic system with a Xenon light source (Sinko Optical, Tokyo, Japan). A low pass filter (800 nm) was set in the Xenon light source to excite ICG whose best excitation wavelength ranges between 780 and 810 nm. For image visualization, a low pass filter (700 nm) for
the visible imaging and a high pass filter (820 nm) for
the fluorescence imaging were placed in front of
the CCD of a near-infrared camera (Hamamatsu
Photonics, Hamamatsu, Japan). These imaging modes
can be switched instantly by displacing these filters.
The camera was then mounted on a base position
of the endoscope. The endoscope was equipped
with a specific set of lenses that efficiently pass
the near-infrared light. Three different sizes of
endoscopes with a 2.5, 5.0, or 10 mm diameter were
fabricated. The outline of the whole system is shown
in Figure 1.

Materials
ICG (Diagnogreen for injection) was purchased from
Daiichi Sankyo, and bovine serum from Gemini
Bio-Products (fetal bovine serum for cell culture,
West Sacramento, CA, USA). Monkey (Macaca
fascicularis) placentas and amniotic fluid, obtained
via Caesarean section, were generously supplied
by Dr. Sankai of the Tsukuba Primate Center.
The placentas were stored at 4°C and used within
seven days after delivery. Pregnant Wistar rats
(gestation day 19, weighing approximately 500 g)
were purchased from Sankyo Lab (Tokyo, Japan).

In-vitro study
An intravenous catheter (20G) was inserted into one
umbilical artery of monkey placenta and fixed by
ligation. After rinsing with saline, 10% ICG solution
containing 10% bovine serum in saline was injected
until the solution spread in the placental vascular
network. When the ICG solution was diffused
throughout the entire placenta, zoomed images of the
same areas were recorded in both fluorescent and
visible modes for comparison. The placenta was also
placed in the monkey amniotic fluid to examine
visibility of ICG fluorescence imaging.

In-vivo study
A pregnant Wistar rat was laparotomized under
pentobarbital anesthesia, its uterine wall was
carefully incised, and the fetal side of placental
surface was exposed. ICG (0.2 mg/rat) was adminis-
tered via maternal tail vein and a time course change
of fluorescence was chased by video.

Animal ethics
All animal experiments were performed according
to the institutional animal ethics guidelines of the
National Center for Child Health and Development
and the Tsukuba Primate Research Center.

Results
Dynamic analysis of the placental vascular network
in vitro
Figure 2 illustrates the vascular anastomoses between
the major and succenturiate placentas. The ICG
fluorescence infiltrated gradually in each placenta
and provided the stereoscopic image by transmission
of fluorescence. The fluorography clearly showed
the lobe structure of the placenta in situ that is
not observable from the outside by conventional
methods. In addition, a video was available that
would be helpful in identifying the direction of blood
flow in pathological anastomosis of TTTS.

The fluorescence vascular images of the placenta in vitro
In Figure 3 (a and b), the ICG fluorescence images
(right) and visible counterparts (left) of the placenta
clearly demonstrated that the fluorescence mode
was highly superior to the visible mode in defining
not only superficial but also deep blood vessels.
Visible mode images obtained by 2.5, 5.0, and
10 mm-diameter endoscopes were as clear as those obtained by conventional endoscopes (data not shown). Importantly, the fluorescence clearly detected the vascular network even when the placenta was placed within turbid amniotic fluid, while the visible image was poor (Figures 3c and d).

**In-vivo study**

Figure 4 shows the visible and ICG fluorescence images of the rat placenta three minutes after maternal ICG administration. In the right image, the umbilical cord and chorionic fetal vessels are observed as non-luminous dark lines, while the maternal vessels in placental parenchyma were detected clearly in a luminous white pattern.

The test solution used in the *in vitro* experiments (Figures 2 and 3) contained 250 μg/ml of ICG. The concentration was indeed 15–20 times higher than the blood ICG level 30–60 seconds after intravenous administration (our preliminary observation). At first, we adopted this artificial concentration to obtain a clear image and improve the endoscope system. Then, the results obtained here showed that our endoscope provides a good view of circulating ICG for at least three minutes after the usual dosage.

**Discussion**

Along with the development of techniques and devices for diagnosis and surgical procedures, the number of fetal surgeries is increasing and the outcome is becoming hopeful. The present results clearly demonstrate the feasibility of ICG fluorescence for visualizing both the superficial and deep vascular networks in the placenta, and the technique will contribute to the advancement of TTTS treatment. So far, the ICG fluorescence imaging has been performed using a CCD camera with LEDs (8) or a flexible fluorescence endoscope (9). Considering *in utero* and other general use in minimally invasive surgery, rigid-type
endoscopes capable of both visible imaging and ICG fluorescence visualization must be more suitable.

As expected, the penetrating nature of fluorescence allowed the fetal vessels to emerge even in the presence of turbid amniotic fluid. The accuracy of the fluorescence images is degraded due to the scattering in the amniotic fluid, however, the visualization of the placental vascular network would be still useful to identify the placental area having the responsible anastomotic vessels and it would minimize the need for irrigation of saline to clear up the *in utero* view. In this case, the risk of fetomaternal use of ICG must be carefully examined for the clinical application. Alternatively, to avoid potential risk to the fetus, ICG solution may be intravenously administered into the pregnant mother as in our present *in vivo* experiment. ICG remained inside the placenta (Figure 4), probably in the intervillous spaces.

Figure 3. Visible (left) and ICG fluorescence (right) images of the same placental areas. In the left images, the vessels colored red and green represent veins and arteries. (a, b) Examinations in air, (c, d) Examinations within turbid amniotic fluid.
ICG fluorescence endoscope

filled with maternal blood (10), and highlighted the fetal vessels as shadow images.

Apart from clinical operation, the use of fluorescence and a camera will help the postpartum examination of placentas with TTTS. To date, dye-injection (3,11), vascular casting (12), and angiography (7) have been employed for examination of the placental vascular network. Dye injection technique visualizes the superficial anastomoses only, and vascular casting or angiography that discloses the placental angioarchitecture are laborious and time-consuming. Instead, the present method enables in situ observation of vascular configuration, and, if a movie is taken, the investigation of blood flow will be achievable.

Besides in-utero surgery, because the ICG fluorescence is well known to depict the intraperitoneal or axillary lymph flow and its sentinel regional nodes, the endoscope is expected to help in other common surgical procedures for malignant lesions including gastrointestinal and breast cancer cases. Accordingly, our ICG fluorescence endoscope will become a novel lineup to the conventional endoscopic surgery.

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